# Virus Taxonomy

## Classification and Nomenclature of Viruses

**Ninth Report**
**of the**
**International Committee on Taxonomy of Viruses**

Editors

**Andrew M.Q. King, Michael J. Adams, Eric B. Carstens, and Elliot J. Lefkowitz**

International Union of Microbiological Societies
Virology Division

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Amsterdam • Boston • Heidelberg • London • New York • Oxford
Paris • San Diego • San Francisco • Singapore • Sydney • Tokyo

Academic Press is an imprint of Elsevier
32 Jamestown Road, London NW1 7BY, UK
225 Wyman Street, Waltham, MA 02451, USA
525 B Street, Suite 1800, San Diego, CA 92101-4495, USA



*Cover design:* Coccolithoviruses (family *Phycodnaviridae*) released from the ubiquitous, chalk-covered marine alga *Emiliania huxleyi*. Phytoplankton are the foundation of the oceanic food chain and their viruses are an essential component of it. Despite their importance, however, these viruses remain largely uncharacterized. (Based on an illustration by Glynn Gorick in collaboration with Willie Wilson.)



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**British Library Cataloguing-in-Publication Data**
A catalogue record for this book is available from the British Library

**Library of Congress Cataloging-in-Publication Data**
A catalog record for this book is available from the Library of Congress

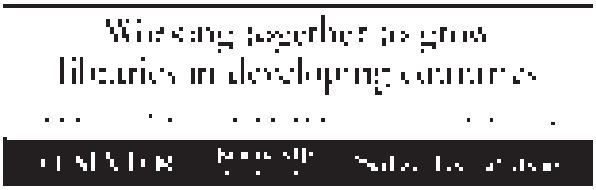
ISBN : 978-0-12-384684-6

For information on all Academic Press publications visit our website at http://www.elsevierdirect.com

Typeset by MPS Limited, a Macmillan Company, Chennai, India
www.macmillansolutions.com

Printed and bound in United States of America

12 13 14 15 10 9 8 7 6 5 4 3 2 1



# Preface and Acknowledgments

The International Committee on Taxonomy of Viruses (ICTV) has the task of developing a single, universal taxonomic scheme for all viruses irrespective of host. The Executive Committee coordinates a series of subcommittees and study groups that draw on the experience of a large number of virologists throughout the world and their collective efforts are represented by this Report.

Part I is an introduction that includes a historical survey of the development of virus taxonomy and explains the operation of the current taxonomic system.

Part II is the main body of the Report and includes descriptions of all the orders, families, subfamilies, and genera of viruses recognized by ICTV as of April 2011 and lists the species associated with each.

Part III provides details of the many people who have served ICTV in various capacities and the rules under which ICTV operates.

Part IV provides two indices. The Virus Index enables the user to locate any of the individual viruses and species that are mentioned in the Report. The Taxonomic Index does the same for the descriptions of orders, families, subfamilies and genera.

Many sections of the Report rely heavily on the relevant sections of earlier ICTV Reports and we express our indebtedness to the work of earlier generations of virologists, as summarized in Part I. We are deeply grateful to the many people who have contributed to this volume and have cooperated patiently with us in its production. Associate Editors and authors are listed in Part I and the authors of the descriptions in Part II are also listed at the end of their respective contributions. The detailed development of taxonomy relies on the work of the Study Groups listed in Part III, many of whom are also authors of the relevant parts of Part II. We are especially grateful to Dr. Elliot Lefkowitz for facilitating the use of ICTVonline by the contributors and editors of the Ninth Report and, with his assistant, Ms Donna Sophronia-Sims, for the editing and redrawing of the figures.

For the Editors

**Eric B. Carstens**

President of the International Committee on Taxonomy of Viruses

*Cover design* Coccolithoviruses (family *Phycodnaviridae*) released from the ubiquitous, chalk-covered marine alga *Emiliania huxleyi*. Phytoplankton are the foundation of the oceanic food chain and their viruses are an essential component of it. Despite their importance, however, these viruses remain largely uncharacterized. (Based on an illustration by Glynn Gorick in collaboration with Willie Wilson.)

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# Part I
# Introduction

# Introduction to Virus Taxonomy

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The first internationally organized attempts to introduce some order in the bewildering variety of viruses took place at the International Congress of Microbiology held in Moscow in 1966. A committee was created, later called The International Committee on Taxonomy of Viruses (ICTV), which was given the task of developing a single, universal taxonomic scheme for all the viruses infecting animals (vertebrates, invertebrates and protozoa), plants (higher plants and algae), fungi, bacteria and archaea. ICTV was created as a committee of the Virology Division of the International Union of Microbiological Societies (IUMS) and is governed by Statutes approved by the Virology Division. These are listed in full in Part III of this Report and define the objectives of ICTV as: (i) to develop an internationally agreed taxonomy for viruses (the term "viruses" for this purpose is taken to include viroids and some important groups of satellite viruses); (ii) to develop internationally agreed names for these taxa; (iii) to communicate taxonomic decisions to the international community of virologists; and (iv) to maintain an Index of virus names. The Statutes also state that classification and nomenclature of viruses will be subject to rules set out in an International Code (the current version of which is also provided in Part III).

Virus taxonomy differs from other types of biological classification because ICTV not only regulates a Code of Nomenclature but also considers and approves the creation of virus taxa (currently orders, families, subfamilies genera and species). Priority of publication is not the determining factor. Species names are usually derived from the common (vernacular) name of the virus (usually in English) used to establish the species. The names of all recognized taxa are written in italics with an initial capital letter.

## ICTV Reports

To communicate its decisions to the international community, ICTV has published a series of reports. Eight of these have been published since 1971, often following formal approval of taxonomic proposals at plenary meetings of ICTV held during International Congresses for Virology. These reports provide milestones by which the progress in virus taxonomy can be tracked.

The Reports of ICTV

| Report | Reference | Reporting ICTV Proceedings at the International Congress of Virology held in: | Content |
|---|---|---|---|
| First | Wildy (1971) | Helsinki, 1968 | 43 families and groups |
| Second | Fenner (1976) | Budapest, 1971 and Madrid, 1975 | 47 families and groups |
| Third | Matthews (1979) | The Hague, 1978 | 50 families and groups |
| Fourth | Matthews (1982) | Strasbourg, 1981 | 54 families and groups |
| Fifth | Francki *et al*. (1991) | Sendai, 1984, Edmonton, 1987, and Berlin, 1990 | 2420 viruses belonging to 73 families or groups |
| Sixth | Murphy *et al*. (1995) | Glasgow, 1993 | 1 order, 50 families, 9 subfamilies, 164 genera and more than 3,600 virus species |
| Seventh | van Regenmortel *et al*. (2000) | Jerusalem, 1996 | 3 orders, 63 families, 9 subfamilies, 240 genera, 1550 species* |
| Eighth | Fauquet *et al*. (2005) | Sydney, 1999 and Paris, 2002 | 3 orders, 73 families, 11 subfamilies, 289 genera and 1898 species |

*With the introduction of the current species definition and the adoption of formal species demarcation criteria in the Seventh Report (see text), many virus strains that had hitherto been listed as separate species were reorganized into new, more broadly defined, species. This explains the apparent reduction in their number between the Sixth and Seventh Reports.

The first report was published in 1971 by the then International Committee on Nomenclature of Viruses (ICNV), which had been established in 1966 (Wildy, 1971). This report, covering the period 1966 to 1970, established five Subcommittees: Bacteriophage (now Prokaryote Virus), Invertebrate Virus, Plant Virus, Vertebrate Virus and Cryptograms. The Subcommittees were responsible for approving taxonomic proposals relevant to their groups of viruses and presenting these proposals for approval by the Executive Committee (EC), the ICNV and "a sizable number of virologists working in the relevant field were to be consulted". This report included the designations Family, Genus (Groups) and Type species, establishing and setting the foundations for viral taxonomy.

By the Second Report (Fenner, 1976), the name change to ICTV had been approved (in 1973) and a formal structure of ICTV officers was reported, consisting of the President, Vice-President, two Secretaries, Chairs of the Subcommittees, Elected Members and Life Members. There was now a Fungal Virus Subcommittee (created from the disbanded Cryptograms Subcommittee) and a Coordination Subcommittee (disbanded 1995) charged with ensuring that Study Groups included virologists with interests in particular virus groups in each class of host affected.

In the Third Report (Matthews, 1979), the viruses were listed on the basis of the kind and strandedness of the nucleic acid making up the viral genome, and the presence or absence of an envelope. It was noted that although ICTV had approved families and genera, there was no such approved "taxon equivalent to species, lying between genus and strain or variant". The problem of defining species for viruses and naming these species was presented and an extensive discussion of the various points of view as presented by representatives of different groups of viruses was outlined for the first time. It was suggested that it might take 10–20 years to provide these taxa, and an appeal was made to Study Groups to put forward species proposals for consideration by ICTV. This was also the first ICTV report to include virus diagrams, grouped according to the major hosts (animal, bacteria or plant). There was also a list of some unclassified viruses and virus-like agents including agents of scrapie, Kuru and Creutzfeldt–Jakob diseases, viroids, and satellite viruses and satellite RNAs in plants.

The same general arrangement was followed in the Fourth (Matthews, 1982) and Fifth (Francki *et al*., 1991) Reports. By the time of the Sixth Report (Murphy *et al*., 1995) ICTV had finally accepted

the controversial category of virus species based on a proposal made in 1990 (van Regenmortel, 1990). A virus species was defined as "a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche". There was also a clear description of the usage of formal taxonomic nomenclature and an attempt to explain the appropriate usage of this formal nomenclature versus informal vernacular usage. More than 15 years later, this issue continues to elicit controversy.

In the Seventh Report (van Regenmortel *et al.*, 2000), there was an extensive discussion of the species concept in virus taxonomy, and an appeal to the virology community to establish species demarcation criteria which, when applied, could be used to discriminate between virus species within the same genus. Some but not all genera in the Seventh Report did include the criteria by which species were differentiated.

The Eighth Report (Fauquet *et al.*, 2005) continued this process and provided an epic compilation of virus taxonomy illustrated with 436 electron micrographs, diagrams of virus particles, diagrams of genome organization and phylogenetic trees in a total of 1259 pages. As this approached the limit for publication of a single volume book, it was becoming obvious that a different vehicle for transmission of virus taxonomy would be required in the near future to meet the needs of the ever-expanding knowledge base of viruses.

## ICTV on the internet

A plan to develop a universal virus database was first discussed around 1990 and led to the development of ICTVdb. The known properties of virus isolates and species were encoded and "translated" for the user in natural language text. Enormous efforts were made to maintain and develop this database and to link with other important databases on biological taxonomy, publications, sequences etc. This was managed by the Virus Data Subcommittee of ICTV and relied heavily on the energy and commitment of Cornelia Büchen-Osmond. The database contains a wealth of important information but it has proved difficult to sustain funding and personnel at a time when taxonomic information is expanding rapidly. At the time of writing, the future of ICTVdb is uncertain.

In recent years, ICTV has also had a web presence providing lists of the currently recognized taxa and information on the Executive Committee, Subcommittees and Study Groups. Templates and other information to assist in writing and submitting taxonomic proposals have also been provided. For some years, this was hosted by Claude Fauquet at the Danforth Center, St Louis, but since 2008 the Virus Data subcommittee has overseen the development and maintenance of an official ICTV website (http://www.ictvonline.org/) that now provides a central point of reference for all ICTV matters. A separate website (http://talk.ictvonline.org/) is used to host taxonomic proposals and allows for comment and discussion to which all virologists are invited to contribute.

## The working of ICTV

To date, the Executive Committee (EC) has established 76 international Study Groups (SGs) covering all major virus families and genera. The Chair of each SG is appointed by the relevant Subcommittee Chair who is a member of the EC. Chairs are responsible for (i) organizing discussions among SG members of emerging taxonomic issues in their field, (ii) for overseeing the submission of proposals for new taxonomy, and (iii) the preparation, or revision, of relevant chapter(s) in ICTV Reports.

## Developing new taxonomy

ICTV welcomes taxonomic proposals from any interested individual although in practice most are prepared by the relevant SG. An all-purpose template and guidance notes are available for downloading from http://talk.ictvonline.org/. Proposals will be forwarded to all interested SGs and are also

made available on the website for public comment. Authors are invited to respond to any comments made. Subcommittee Chairs (or their deputies) then present taxonomic proposals to the Executive Committee for discussion and approval. These meetings are usually held annually. Straightforward proposals to create new species in existing genera can normally be approved at a single meeting if the proposed new species clearly meet the criteria established for species demarcation. The criteria differ between genera and families but are usually specified in the relevant section of this Report. More complex or controversial proposals are made available on the ICTV website for public comment for a further year before being re-considered by the Executive Committee.

Proposals approved by the EC do not become accepted taxonomy until a final "ratification" vote by the full ICTV membership. As specified in the Statutes (see Part III), this includes members of the various Subcommittees (mostly chairs of Study Groups), National Members and Life Members. Lists of members are provided in part III. Ratification is now done by an email vote after which the approved taxonomy is updated at http://www.ictvonline.org/. This site should always be consulted for the most up-to-date ICTV taxonomic information. Summaries of the voting decisions are also prepared and published in an article under the heading Virology Division News in *Archives of Virology*.

### *Virus Taxonomy*, 2011

The current ICTV report lists 2284 virus and viroid species distributed amongst 349 genera, 19 subfamilies, 87 families and 6 orders. There is also a chapter of unassigned viruses that provides information on a number of viruses that have not yet been classified but which are probably representatives of new genera and/or families. The final chapters describe the satellites (and other virus-dependent nucleic acids) and prions (which include the agents of spongiform encephalopathies of humans), which are not formally classified by ICTV but simply listed for historical reasons. The Ninth Report is being published both as a book and also online. ICTV expects to make regular updates to keep the online version in step with the latest taxonomic decisions.

Each genus contains a type species (the representative used in defining the genus) and often a number of other species. For each species, authors have been asked to provide details of a single isolate, a characterized virus that is representative of the species as a whole. Some SGs are working to define official "type isolates" for each species; we believe this is a desirable goal although it has not been adopted as ICTV policy.

Most species are members of a genus. However, the code also allows for species to be created within a family (or subfamily) but unassigned to a particular genus. Similarly, most genera are now members of a family but some genera remain unassigned pending further information on their status and relationships. Wherever possible, genera and families are justified on a phylogenetic basis although we recognize that the use of phylogenetic comparisons in virus classification still has much further to go. The designation of higher levels of taxonomy (only orders are recognized in the current Code) is often made difficult by the mosaicism evident amongst the genomes of many related viruses. This poses one of the major challenges for the future. The other most obvious challenge is the vast amount of sequence data that is being generated from environmental samples through metagenomic surveys. These data are revealing the presence of many viruses that are largely uncharacterized and for which host organisms are often unknown (Suttle, 2005). The lack of biological data makes such viruses difficult to classify using current criteria so processes need to be found to integrate the phylogenetic information they provide into future taxonomic schemes.

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# Glossary of Abbreviations

In addition to universally accepted abbreviations such as DNA and RNA, it is common practice to use abbreviations for virological and technical words in virology. In the Report, we have adopted commonly used abbreviations (e.g. CP for capsid protein and NC for nucleocapsid). These have been approved by the Executive Committee of ICTV for use in the ICTV Report but have no official status. The abbreviations will be used without definition throughout the book, except in a few instances where Study Group conventions (e.g. C in place of CP for capsid protein) dictate different practice. In these instances, abbreviations are defined locally.

## Abbreviations

| | |
|---|---|
| aa | amino acid(s) |
| bp | base pair(s) |
| CF | complement fixing |
| CP | capsid/coat protein (unless otherwise defined) |
| CPE | cytopathic effect |
| D | diffusion coefficient |
| DI | defective interfering |
| DNAse | deoxyribonuclease |
| ds | double stranded |
| gRNA | genomic RNA |
| HE | hemagglutination esterase |
| Hel | helicase |
| HI | hemagglutination inhibition |
| h | hour(s) |
| IRES | internal ribosome entry site |
| kbp | kilobase pairs |
| kDa | kilodalton |
| $5m^7G$ | 7-methylguanosine (the 5′ cap structure of many RNAs) |
| min | minutes(s) |
| MP | movement protein |
| Mr | relative molar mass |
| mRNA | messenger RNA |
| Mtr | methyltransferase |
| N | nucleoprotein |
| NC | nucleocapsid |
| NES | nuclear export signal |
| NLS | nuclear localization signal |
| nt | nucleotide(s) |
| NTR | non-translated region |
| ORF | open reading frame |
| PAGE | polyacrylamide gel electrophoresis |
| PCR | polymerase chain reaction |
| Pol | polymerase |
| Pro | protease |
| RdRp | RNA-dependent RNA polymerase |
| Rep | replication associated protein |
| RF | replicative form |
| RFLP | restriction fragment length polymorphism |
| RI | replicative intermediate |
| RNAse | ribonuclease |

| | |
|---|---|
| RNP | ribonucleoprotein |
| RT | reverse transcriptase |
| sgRNA | subgenomic RNA |
| ss | single stranded |
| T (e.g. T3) | triangulation number |
| T cell | T lymphocyte |
| UTR | untranslated region |
| VPg | genome-linked protein |

# *Families and Genera of Viruses Listed According to the Nature of the Genome*

| Order | Family or unassigned genus | Nature of the genome | Presence of an envelope | Morphology | Virion size | Genome configuration | Genome size (kbp or kb) | Host |
|---|---|---|---|---|---|---|---|---|
| *Caudovirales* | *Myoviridae* | dsDNA | − | icosahedral head with tail | icosahedral heads: 60–145 nm; elongated heads: 80 × 110 nm; tail: 16–20 × 80–455 nm | 1 linear segment | 31–317 | B, Ar |
| *Caudovirales* | *Podoviridae* | dsDNA | − | icosahedral head with short tail | head: 60–70 nm tail: 10–20 nm | 1 linear segment | 16–78 | B |
| *Caudovirales* | *Siphoviridae* | dsDNA | − | icosahedral head with tail | head: 40–80 nm; tail: 5–10 nm × 100–210 nm | 1 linear segment | 21–134 | B, Ar |
| *Herpesvirales* | *Alloherpesviridae* | dsDNA | + | spherical virion, icosahedral core | 160–300 nm | 1 linear segment | 134–295 | V |
| *Herpesvirales* | *Herpesviridae* | dsDNA | + | spherical virion, icosahedral core | 160–300 nm | 1 linear segment | 125–241 | V |
| *Herpesvirales* | *Malacoherpesviridae* | dsDNA | + | icosahedral | 160–300 nm | 1 linear segment | 207 | I |
| Unassigned family | *Adenoviridae* | dsDNA | − | icosahedral | 70–90 nm | 1 linear segment | 26–48 | V |
| Unassigned family | *Ampullaviridae* | dsDNA | + | bottle-shaped | 75 to 4 nm × 230 nm | 1 linear segment | 24 | Ar |
| Unassigned family | *Ascoviridae* | dsDNA | + | bacilliform, ovoidal, allantoid | 130 nm × 200–400 nm | circular genome | 150–190 | I |
| Unassigned family | *Asfarviridae* | dsDNA | + | icosahedral | 200–250 nm | 1 linear segment | 165–190 | V, I |
| Unassigned family | *Baculoviridae* | dsDNA | + | rod-shaped | 30–60 nm × 250–300 nm | circular supercoiled genome | 80–180 | I |
| Unassigned family | *Bicaudaviridae* | dsDNA | + | lemon shaped, two-tailed | 80 nm × 120–400 nm | circular genome | 63 | Ar |
| Unassigned family | *Corticoviridae* | dsDNA | − | icosahedral | 60 nm | circular supercoiled genome | 10 | B |
| Unassigned family | *Fuselloviridae* | dsDNA | + | lemon-shaped | 55–60 nm × 80–100 nm | circular supercoiled genome | 15–18 | Ar |
| Unassigned family | *Globuloviridae* | dsDNA | + | spherical | 70–100 nm | 1 linear segment | 21–28 | Ar |
| Unassigned family | *Guttaviridae* | dsDNA | + | droplet-shaped | 75–90 nm × 110–185 nm | circular genome | 20 | Ar |
| Unassigned family | *Iridoviridae* | dsDNA | +/− | icosahedral, spherical | 120–350 nm | 1 linear segment | 140–303 | V, I |
| Unassigned family | *Lipothrixviridae* | dsDNA | + | filamentous | 24–38 nm × 410–2200 nm | 1 linear segment | 16–42 | Ar |
| Unassigned family | *Mimiviridae* | dsDNA | − | pseudo-icosahedral | 750 nm | 1 linear segment | 1200 | Pr |
| Unassigned family | *Nimaviridae* | dsDNA | + | ovoid, bacilliform | 120–150 nm × 270–290 nm | circular genome | 300 | I |
| Unassigned family | *Papillomaviridae* | dsDNA | − | icosahedral | 55 nm | circular genome | 6.8–8.4 | V |
| Unassigned family | *Phycodnaviridae* | dsDNA | − | icosahedral | 100–220 nm | 1 linear segment | 100–560 | Al |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Unassigned family | *Plasmaviridae* | dsDNA | + | quasi-spherical, pleomorphic | 50–125 nm | circular supercoiled genome | 12 | B |
| Unassigned family | *Polydnaviridae* | dsDNA | + | prolate ellipsoid | bracovirus nucleocapsids: 34–40 nm × 8–150 nm; ichnovirus nucleocapsids: 85 nm × 330 nm | multiple circular supercoiled segments | total genome: 190–600 | I |
| Unassigned family | *Polyomaviridae* | dsDNA | − | icosahedral | 40–45 nm | circular genome | 4.7–5.4 | V |
| Unassigned family | *Poxviridae* | dsDNA | + | pleomorphic | 140–260 nm × 220 nm × 450 nm | 1 linear segment | 130–375 | V, I |
| Unassigned family | *Rudiviridae* | dsDNA | − | rod-shaped | 23 nm × 600–900 nm | 1 linear segment | 25–35 | Ar |
| Unassigned family | *Tectiviridae* | dsDNA | − | icosahedral | 66 nm | 1 linear segment | 15 | B |
| Unassigned genus | *Rhizidiovirus* | dsDNA | − | icosahedral | 60 nm | 1 linear segment | 27 (estimate) | Pr |
| Unassigned genus | *Salterprovirus* | dsDNA | + | lemon-shaped | 44–77 nm | 1 linear segment | 14.5 | Ar |
| Unassigned family | *Anelloviridae* | ssDNA (−) | − | icosahedral | 30 nm | circular genome | 2–4 | V |
| Unassigned family | *Circoviridae* | ssDNA (−) or (+/−) | − | icosahedral | 12–27 nm | circular genome | 1.7-2.3 | V |
| Unassigned family | *Geminiviridae* | ssDNA (+/−) | − | icosahedral | 22 × 38 nm | 1 or 2 circular segments | 2.5-3 per segment | P |
| Unassigned family | *Inoviridae* | ssDNA (+) | − | inoviruses: filamentous; plectroviruses: rod-shaped | inoviruses: 7 nm × 700–3500 nm; plectroviruses: 15 nm × 200–400 nm | circular genome | inoviruses: 5.8–12.4; plectroviruses: 4.5–8.2 | B |
| Unassigned family | *Microviridae* | ssDNA (+) | − | icosahedral | 25–27 nm | circular genome | 4.4–6.1 | B |
| Unassigned family | *Nanoviridae* | ssDNA (+) | − | icosahedral | 18–20 nm | nanoviruses: 8; babuviruses: 6 circular segments | 0.98–1.1 per segment | P |
| Unassigned family | *Parvoviridae* | ssDNA (+/−) | − | icosahedral | 21–26 nm | 1 linear segment | 4–6.3 | V, I |
| Unassigned family | *Caulimoviridae* | dsDNA-RT | − | icosahedral, bacilliform | icosahedral: 50–52 nm; bacilliform: 30 nm × 130–150 nm | non-covalently closed circular genome | 7.2–9.2 | P |
| Unassigned family | *Hepadnaviridae* | dsDNA-RT | + | spherical | 42–50 nm | non-covalently closed circular genome | 3–4 | V |
| Unassigned family | *Metaviridae* | ssRNA-RT (+) | +/− | intracellular virus-like particles and/or enveloped extracellular virions | unknown | 1 linear segment | 4–10 | F, I, P, V |
| Unassigned family | *Pseudoviridae* | ssRNA-RT (+) | − | spherical, icosahedral | 60–80 nm | 1 linear segment | 5–9 | Al, F, I, P, Pr |



# *Families and Genera of Viruses Listed According to the Nature of the Genome (Continued)*

| Order | Family or unassigned genus | Nature of the genome | Presence of an envelope | Morphology | Virion size | Genome configuration | Genome size (kbp or kb) | Host |
|---|---|---|---|---|---|---|---|---|
| Unassigned family | *Retroviridae* | ssRNA-RT (+) | + | spherical | 80–100 nm | 1 linear segment (dimer) | 7–13 | V |
| Unassigned family | *Birnaviridae* | dsRNA | − | icosahedral | 65 nm | 2 linear segments | 3.5, 3 | V, I |
| Unassigned family | *Chrysoviridae* | dsRNA | − | icosahedral | 35–40 nm | 4 linear segments | 3.6, 3.2, 3.0, 2.9 | F |
| Unassigned family | *Cystoviridae* | dsRNA | + | spherical | 85 nm | 3 linear segments | 6.4–7.1, 3.6–4.7, 2.6–3.2 | B |
| Unassigned family | *Endornaviridae* | dsRNA | N/A | pleomorphic RNA-containing vesicles | no virions; viral RNA contained in cytoplasmic vesicles | 1 linear segment | 14–18 | Al, F, P |
| Unassigned family | *Partitiviridae* | dsRNA | − | icosahedral | 30–43 nm | 2 linear segments | 1.4–2.4 per segment | P, F |
| Unassigned family | *Picobirnaviridae* | dsRNA | − | icosahedral | 33–37 nm | 2 linear segments | 2.4–2.6, 1.5–1.9 | V |
| Unassigned family | *Reoviridae* | dsRNA | − | turreted (*Spinareovirinae*) or non-turreted (*Sedoreovirinae*) icosahedral capsid | 60–85 nm | 9–12 linear segments | total genome: 19–32 | F, I, P, V, Pr |
| Unassigned family | *Totiviridae* | dsRNA | − | icosahedral | 33–40 nm | 1 linear segment | 4–7 | F, Pr |
| *Mononegavirales* | *Bornaviridae* | ssRNA (−) | + | spherical | 90–130 nm | 1 linear segment | 9 | V |
| *Mononegavirales* | *Filoviridae* | ssRNA (−) | + | bacilliform, filamentous | 80 nm × 660–800 nm | 1 linear segment | 19 | V |
| *Mononegavirales* | *Paramyxoviridae* | ssRNA (−) | + | spherical, filamentous, pleomorphic | >150 nm | 1 linear segment | 13–19 | V |
| *Mononegavirales* | *Rhabdoviridae* | ssRNA (−) | + | bullet-shaped, bacilliform | 45–100 nm × 100–430 nm | 1 linear segment | 11–15 | V, I, P |
| Unassigned family | *Arenaviridae* | ssRNA (+/−) | + | spherical, pleomorphic | 50–300 nm | 2 linear segments | 7.5, 3.5 | V |
| Unassigned family | *Bunyaviridae* | ssRNA (+/−) | + | spherical | 80–120 nm | 3 linear segments | 6.4–12.3, 3.9–5.4, 0.96–3.0 | V, I, P |
| Unassigned family | *Ophioviridae* | ssRNA (−) | − | filamentous, coiled | 3 nm × >= 760 nm | 3 or 4 linear segments | 7.6–8.2, 1.6–1.8, 1.5, 1.4 | P |
| Unassigned family | *Orthomyxoviridae* | ssRNA (−) | + | spherical, filamentous, pleomorphic | 80–120 nm | 6–8 linear segments | 0.7–2.4 per segment | V, I |
| Unassigned genus | *Deltavirus* | ssRNA (−) | + | spherical | 36–43 nm | circular genome | 1.7 | V |

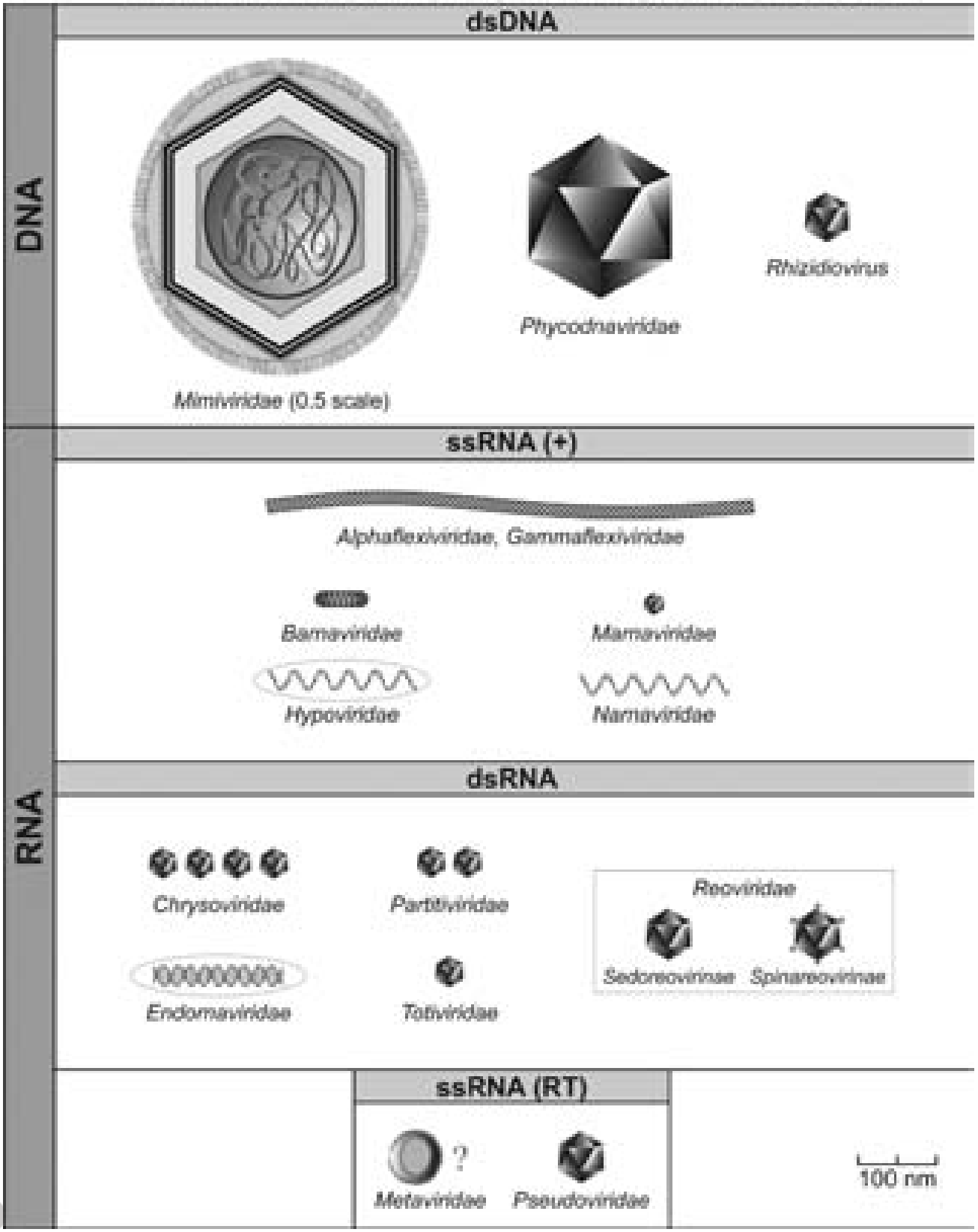
| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Unassigned genus | *Emaravirus* | ssRNA (−) | + | spherical | 80–100 nm | 4 linear segments | 7, 2.3, 1.6, 1.4 | P |
| Unassigned genus | *Tenuivirus* | ssRNA (+/−) | − | filamentous | 3–10 nm × >100 nm | 4–6 linear segments | total genome: 17–23 | P, I |
| Unassigned genus | *Varicosavirus* | ssRNA (−) | − | rod-shaped | 18 nm × 320–360 nm | 2 linear segments | 6.8, 6.1 | P |
| *Nidovirales* | *Arteriviridae* | ssRNA (+) | + | spherical, pleomorphic | 50–74 nm | 1 linear segment | 13–16 | V |
| *Nidovirales* | *Coronaviridae* | ssRNA (+) | + | spherical, bacilliform | spherical: 120–160 nm; bacilliform: 75–90 nm × 170–200 nm | 1 linear segment | 26–32 | V |
| *Nidovirales* | *Roniviridae* | ssRNA (+) | + | bacilliform | 40–60 nm × 150–200 nm | 1 linear segment | 26 | I |
| *Picornavirales* | *Dicistroviridae* | ssRNA (+) | − | icosahedral | 30 nm | 1 linear segment | 8.5–10 | I |
| *Picornavirales* | *Iflaviridae* | ssRNA (+) | − | icosahedral | 26–30 nm | 1 linear segment | 8.8–10 | I |
| *Picornavirales* | *Marnaviridae* | ssRNA (+) | − | icosahedral | 25 nm | 1 linear segment | 8.6 | Al |
| *Picornavirales* | *Picornaviridae* | ssRNA (+) | − | icosahedral | 30 nm | 1 linear segment | 7-9 | V |
| *Picornavirales* | *Secoviridae* | ssRNA (+) | − | icosahedral | 25–30 nm | 1 or 2 linear segments | monopartite: 9.8–12.5; bipartite: 5.8–8.4, 3.3–7.3 | P |
| *Tymovirales* | *Alphaflexiviridae* | ssRNA (+) | − | filamentous | 10–15 nm × 470–800 nm | 1 linear segment | 6–9 | P, F |
| *Tymovirales* | *Betaflexiviridae* | ssRNA (+) | − | filamentous | 10–15 nm × 600–1000 nm | 1 linear segment | 6–9 | P |
| *Tymovirales* | *Gammaflexiviridae* | ssRNA (+) | − | filamentous | 13 × 720 nm | 1 linear segment | 7 | F |
| *Tymovirales* | *Tymoviridae* | ssRNA (+) | − | icosahedral | 30 nm | 1 linear segment | 6–7.5 | P, I |
| Unassigned family | *Astroviridae* | ssRNA (+) | − | icosahedral | 28–30 nm | 1 linear segment | 6–8 | V |
| Unassigned family | *Barnaviridae* | ssRNA (+) | − | bacilliform | 18–20 nm × 48–53 nm | 1 linear segment | 4 | F |
| Unassigned family | *Bromoviridae* | ssRNA (+) | − | icosahedral, bacilliform | icosahedral: 25–35 nm; bacilliform: 18–26 nm × 30–85 nm | 3 linear segments | 3.4, 2.8, 2.3 | P |
| Unassigned family | *Caliciviridae* | ssRNA (+) | − | icosahedral | 35-40 nm | 1 linear segment | 7–8 | V |
| Unassigned family | *Closteroviridae* | ssRNA (+) | − | filamentous | 12 nm × 650–2000 nm | 1–3 linear segments | monopartite: 13–19; bipartite: 8–9, 8–9; tripartite: 8, 5.3, 3.9 | P |
| Unassigned family | *Flaviviridae* | ssRNA (+) | + | spherical | 40–60 nm | 1 linear segment | 9–13 | V, I |
| Unassigned family | *Hepeviridae* | ssRNA (+) | − | icosahedral | 27–34 nm | 1 linear segment | 6.6–7.2 | V |
| Unassigned family | *Hypoviridae* | ssRNA (+) | N/A | pleomorphic RNA-containing vesicles | no virions; viral RNA contained in cytoplasmic vesicles | 1 linear segment | 9–13 | F |
| Unassigned family | *Leviviridae* | ssRNA (+) | − | icosahedral | 26 nm | 1 linear segment | 3.5–4.3 | B |
| Unassigned family | *Luteoviridae* | ssRNA (+) | − | icosahedral | 25–30 nm | 1 linear segment | 5–6 | P |
| Unassigned family | *Narnaviridae* | ssRNA (+) | N/A | no true virions.; intracellular RNP complex | N/A | 1 linear segment | 2–3 | F |

## *Families and Genera of Viruses Listed According to the Nature of the Genome (Continued)*

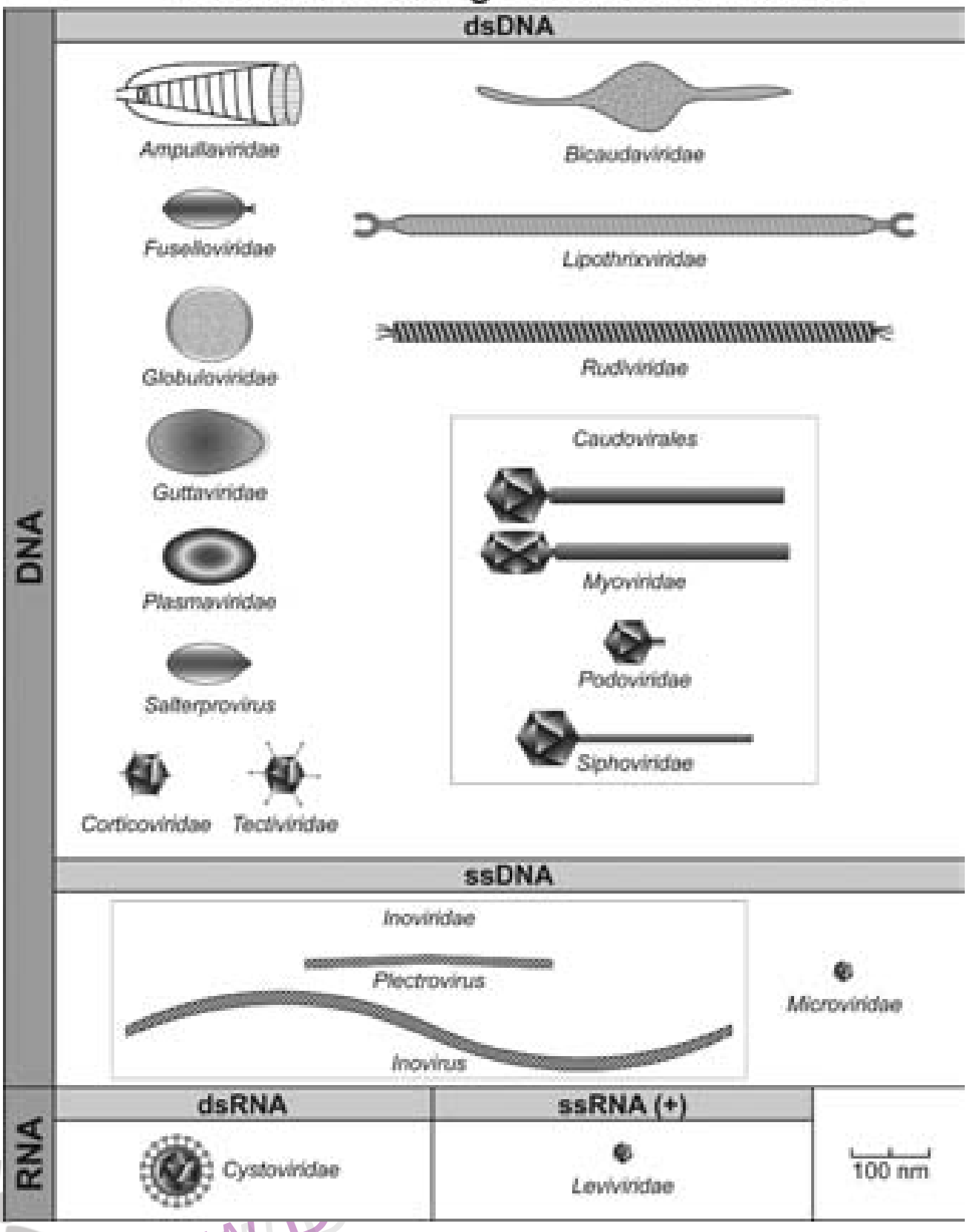
| Order | Family or unassigned genus | Nature of the genome | Presence of an envelope | Morphology | Virion size | Genome configuration | Genome size (kbp or kb) | Host |
|---|---|---|---|---|---|---|---|---|
| Unassigned family | *Nodaviridae* | ssRNA (+) | − | icosahedral | 33 nm | 2 linear segments | 3.1, 1.4 | V, I |
| Unassigned family | *Potyviridae* | ssRNA (+) | − | filamentous | monopartite:11-15nm × 650–900nm; bipartite: 11–15nm × 250–300 and 500–600nm | 1 or 2 linear segments | monopartite: 9.3–10.8; bipartite: 7.3–7.6, 3.5–3.7 | P |
| Unassigned family | *Tetraviridae* | ssRNA (+) | − | icosahedral | 40 nm | 1 or 2 linear segments | monopartite: 5.7–6.6; bipartite: 5.3–5.5, 2.5 | I |
| Unassigned family | *Togaviridae* | ssRNA (+) | + | spherical | 60–70 nm | 1 linear segment | 9.7 to 11.8 | V, I |
| Unassigned family | *Tombusviridae* | ssRNA (+) | − | icosahedral | 28–35 nm | 1 or 2 linear segments | monopartite: 3.7–4.8; bipartite: 3.8, 1.4 | P |
| Unassigned family | *Virgaviridae* | ssRNA (+) | − | rod-shaped | 18–21 nm × 50–310 nm | 1–3 linear segments | monopartite: 6.3–6.6; bipartite: 6–7, 1.8–4.5; tripartite: 3.7–6, 3.0–3.6, 2.5–3.2 | P |
| Unassigned genus | *Benyvirus* | ssRNA (+) | − | rod-shaped | 20 nm × 65–390 nm | 4 or 5 linear segments | 6.7, 4.6, 1.8, 1.4, 1.3 | P |
| Unassigned genus | *Cilevirus* | ssRNA (+) | − | bacilliform | 50–55 nm × 120–130 nm | 2 linear segments | 8.7, 5.0 | P |
| Unassigned genus | *Idaeovirus* | ssRNA (+) | − | icosahedral | 33 nm | 2 linear segments | 5.5, 2.2 | P |
| Unassigned genus | *Ourmiavirus* | ssRNA (+ | − | bacilliform | 18 nm × 30–62 nm | 3 linear segments | 2.8, 1.1, 0.97 | P |
| Unassigned genus | *Polemovirus* | ssRNA (+) | − | icosahedral | 34 nm | 1 linear segment | 4.6 | P |
| Unassigned genus | *Sobemovirus* | ssRNA (+) | − | icosahedral | 25–30 nm | 1 linear segment | 4–5 | P |
| Unassigned genus | *Umbravirus* | ssRNA (+) | N/A | no true virions; intracellular RNP complex | N/A | 1 linear segment | 4.0–4.2 | P |
| Viroids | *Avsunviroidae* | RNA | − | unencapsidated; rod-like | <50 nm | 1 circular (non-coding) segment | 250–400 nts | P |
| Viroids | *Pospiviroidae* | RNA | − | unencapsidated; rod-like | <50 nm | 1 circular (non-coding) segment | 250–375 nts | P |
| Prions | Fungal Prions | Protein | N/A | filamentous fibers, particles, aggregates | variable | N/A | variable number of amino acid residues | F |
| Prions | Vertebrate Prions | Protein | N/A | rod-shaped, filamentous fibers, particles, aggregates | variable | N/A | variable number of amino acid residues | V |

Abbreviations of the virus hosts: Algae, Al; Archaea, Ar; Bacteria, B; Fungi, F; Invertebrates, I; Plants, P; Protozoa, Pr; Vertebrates, V.

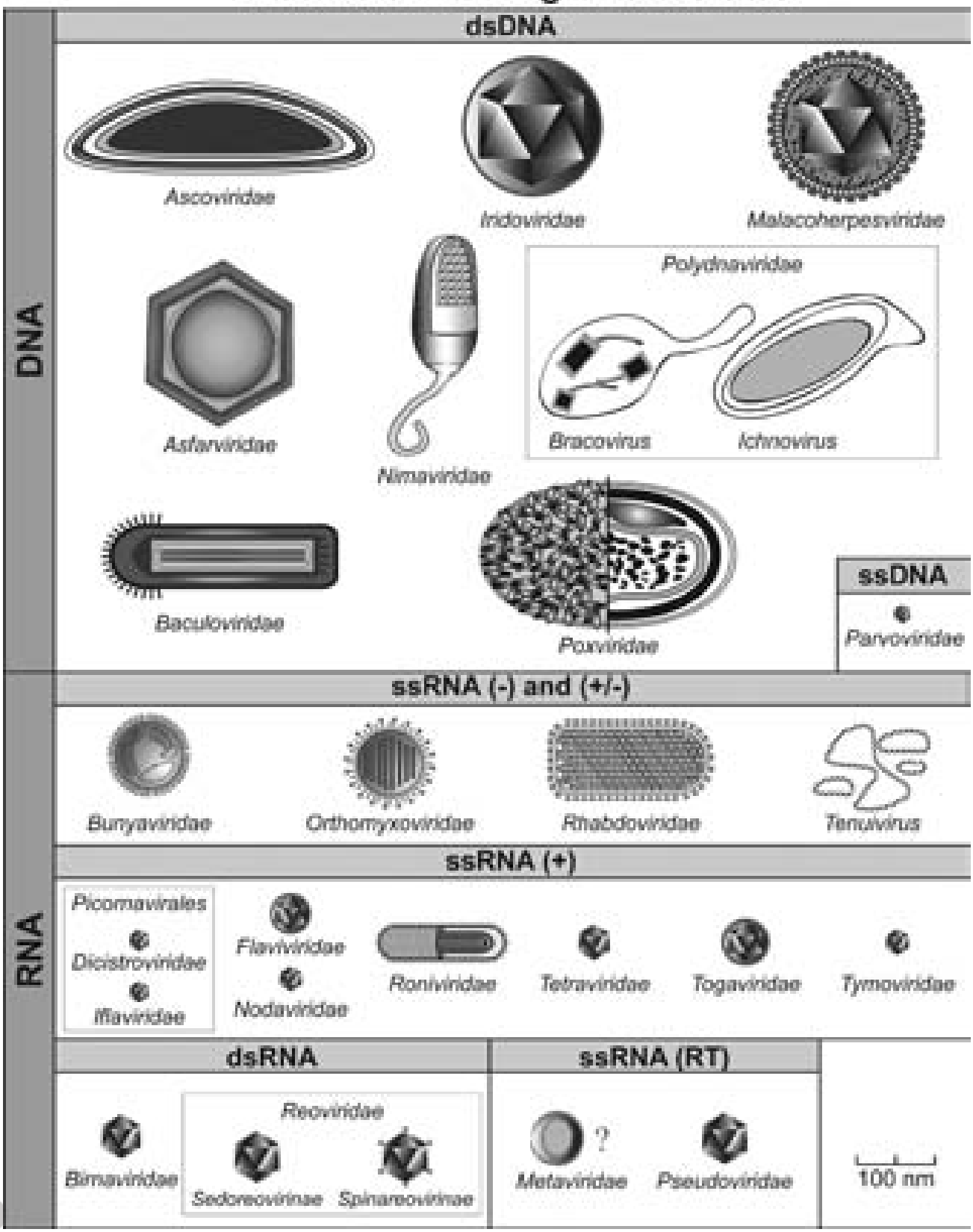
# Virus Taxa Infecting Algae, Fungi, and Protozoa

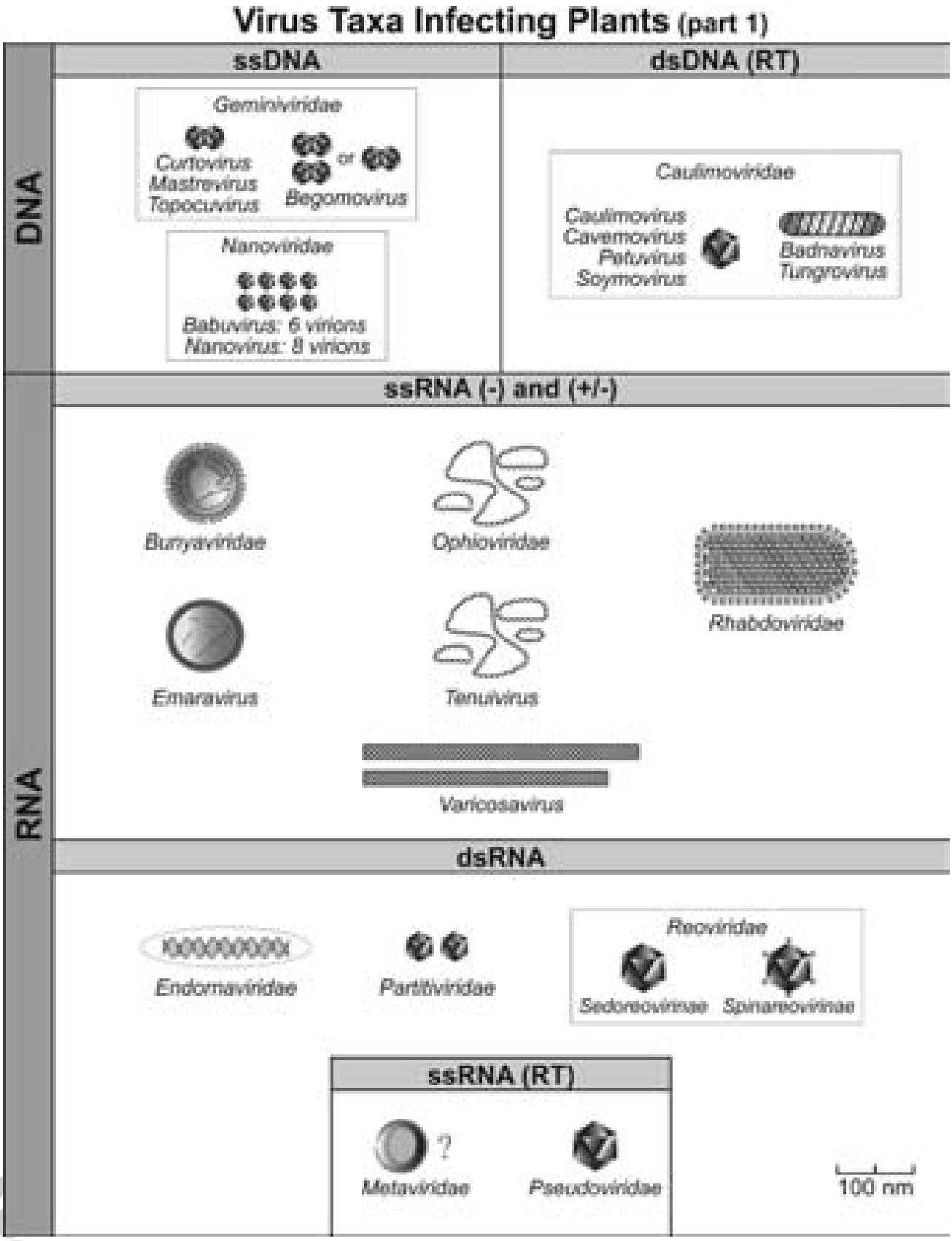


## Virus Taxa Infecting Bacteria and Archaea

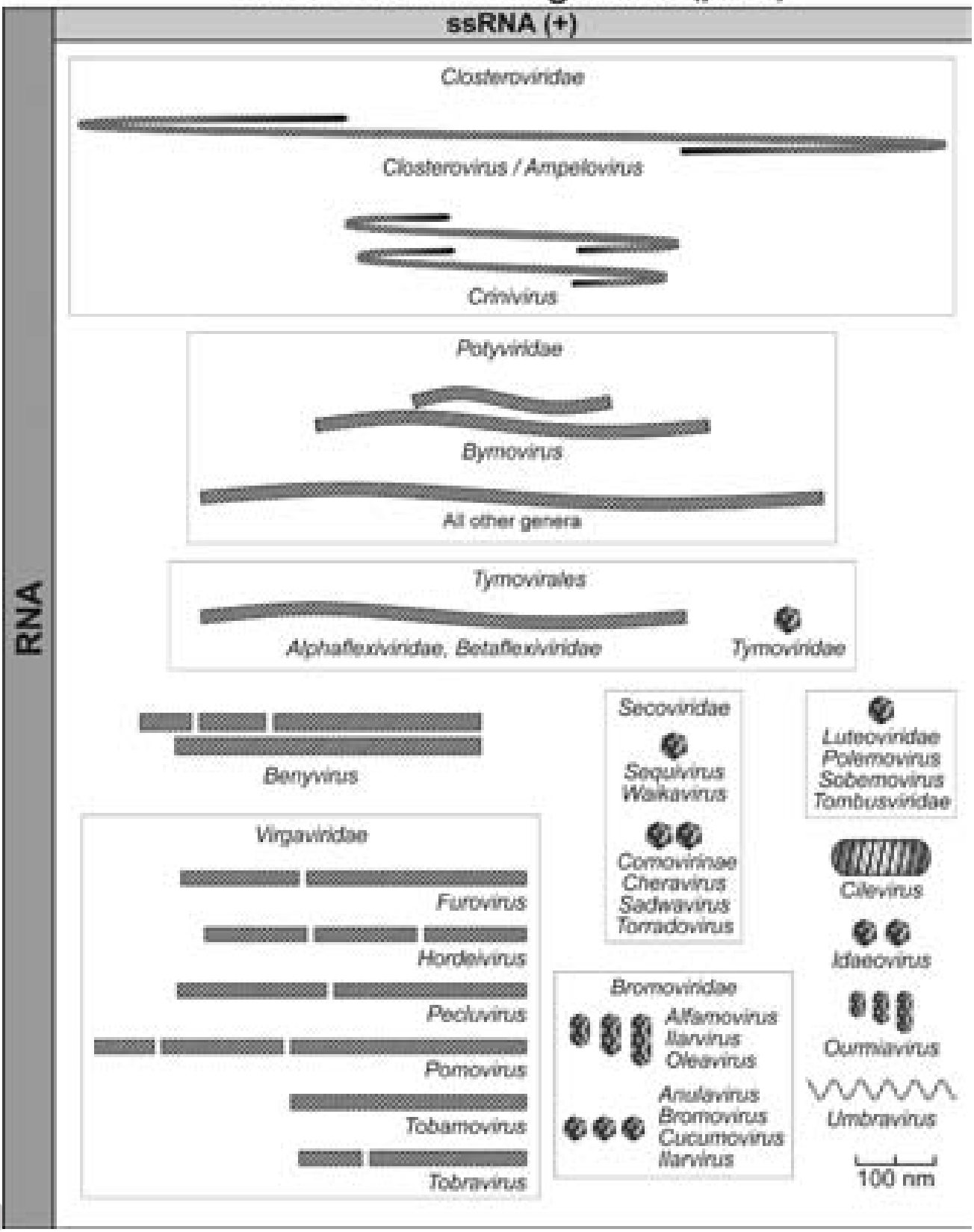


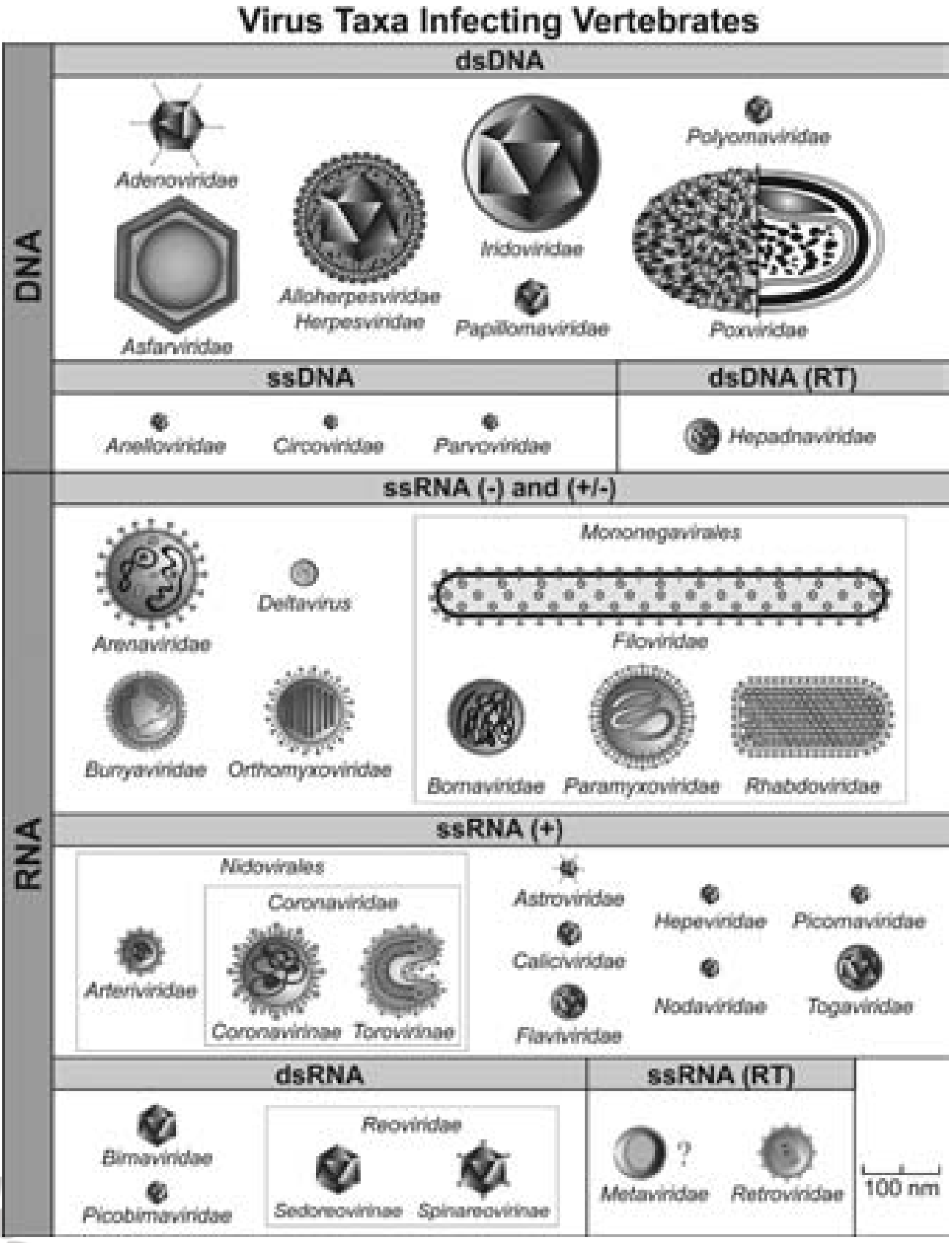
# Virus Taxa Infecting Invertebrates





# Virus Taxa Infecting Plants (part 2)





# Part II
# The Viruses

# Order of Presentation of Virus Taxonomic Descriptions

In this Report, chapters describing the major taxa are presented in groups. The first level of organization groups chapters according to genome composition and structure (dsDNA, ssDNA, etc.). The second level is according to taxonomic rank: first, order, including constituent families and their genera; second, family, including constituent genera; third, genus, if unassigned to a family. Within each group, chapters appear alphabetically. Finally, within a chapter, genera may be presented in order of priority or importance in the family and not necessarily alphabetically. The table below provides the order of presentation of each taxon within this Report.

At the end of each genus description is a list of species. The species names are in italic script. The names of representative isolates within a species follow the species name and are indented and in roman script. Isolate names are aligned with relevant accession numbers (between square brackets in the center column) and a recommended abbreviation (between parenthesis in the rightmost column). Some lists contain extra information such as details of vector and/or host. In some genera, species are clustered into groups or serogroups, while in others the isolates within a species are clustered. These clusters are not formal taxonomic groupings.

| Order | Family | Subfamily | Genus | Type Species | Host | Page |
|---|---|---|---|---|---|---|
| **DNA Viruses dsDNA Viruses** | | | | | | |
| *CAUDOVIRALES* | | | | | | |
| | *Myoviridae* | | "T4-like viruses" | *Enterobacteria phage T4* | B | 46 |
| | | | "P1-like viruses" | *Enterobacteria phage P1* | B | 49 |
| | | | "P2-like viruses" | *Enterobacteria phage P2* | B | 51 |
| | | | "Mu-like viruses" | *Enterobacteria phage Mu* | B | 52 |
| | | | "SPO1-like viruses" | *Bacillus phage SPO1* | B | 54 |
| | | | "PhiH-like viruses" | *Halobacterium phage phiH* | Ar | 55 |
| | | | "PhiKZ-like viruses" | *Pseudomonas phage phiKZ* | B | 56 |
| | | | "I3-like viruses" | *Mycobacterium phage I3* | B | 58 |
| | *Podoviridae* | | "BPP-1-like viruses" | *Bordetella phage BPP-1* | B | 63 |
| | | | "Epsilon15-like viruses" | *Salmonella phage epsilon15* | B | 65 |
| | | | "LUZ24-like viruses" | *Pseudomonas phage LUZ24* | B | 66 |
| | | | "N4-like viruses" | *Escherichia phage N4* | B | 68 |
| | | | "P22-like viruses" | *Enterobacteria phage P22* | B | 69 |
| | | | "Phieco32-like viruses" | *Enterobacteria phage Phieco32* | B | 72 |
| | | *Autographivirinae* | "PhiKMV-like viruses" | *Pseudomonas phage phiKMV* | B | 74 |
| | | | "SP6-like viruses" | *Enterobacteria phage SP6* | B | 75 |
| | | | "T7-like viruses" | *Enterobacteria phage T7* | B | 77 |
| | | *Picovirinae* | "AHJD-like viruses" | *Staphylococcus phage 44AHJD* | B | 80 |
| | | | "Phi29-like viruses" | *Bacillus phage phi29* | B | 81 |
| | *Siphoviridae* | | "Lambda-like viruses" | *Enterobacteria phage lambda* | B | 86 |
| | | | "T1-like viruses" | *Enterobacteria phage T1* | B | 88 |
| | | | "T5-like viruses" | *Enterobacteria phage T5* | B | 89 |
| | | | "L5-like viruses" | *Mycobacterium phage L5* | B | 90 |
| | | | "c2-like viruses" | *Lactococcus phage c2* | B | 91 |
| | | | "PsiM1-like viruses" | *Methanobacterium phage psiM1* | Ar | 93 |
| | | | "PhiC31-like viruses" | *Streptomyces phage phiC31* | B | 94 |
| | | | "N15-like viruses" | *Enterobacteria phage N15* | B | 95 |
| | | | "SPbeta-like viruses" | *Bacillus phage SPbeta* | B | 96 |

| Order | Family | Subfamily | Genus | Type Species | Host | Page |
|---|---|---|---|---|---|---|
| *HERPESVIRALES* | | | | | | |
| | *Alloherpesviridae* | | *Batrachovirus* | *Ranid herpesvirus 1* | V | 108 |
| | | | *Cyprinivirus* | *Cyprinid herpesvirus 3* | V | 108 |
| | | | *Ictalurivirus* | *Ictalurid herpesvirus 1* | V | 109 |
| | | | *Salmonivirus* | *Salmonid herpesvirus 1* | V | 109 |
| | *Herpesviridae* | *Alphaherpesvirinae* | *Iltovirus* | *Gallid herpesvirus 1* | V | 111 |
| | | | *Mardivirus* | *Gallid herpesvirus 2* | V | 112 |
| | | | *Simplexvirus* | *Human herpesvirus 1* | V | 113 |
| | | | *Varicellovirus* | *Human herpesvirus 3* | V | 114 |
| | | *Betaherpesvirinae* | *Cytomegalovirus* | *Human herpesvirus 5* | V | 115 |
| | | | *Muromegalovirus* | *Murid herpesvirus 1* | V | 116 |
| | | | *Proboscivirus* | *Elephantid herpesvirus 1* | V | 117 |
| | | | *Roseolovirus* | *Human herpesvirus 6* | V | 117 |
| | | *Gammaherpesvirinae* | *Lymphocryptovirus* | *Human herpesvirus 4* | V | 118 |
| | | | *Macavirus* | *Alcelaphine herpesvirus 1* | V | 119 |
| | | | *Percavirus* | *Equid herpesvirus 2* | V | 120 |
| | | | *Rhadinovirus* | *Saimiriine herpesvirus 2* | V | 121 |
| | *Malacoherpesviridae* | | *Ostreavirus* | *Ostreid herpesvirus 1* | I | 123 |
| | *Adenoviridae* | | *Mastadenovirus* | *Human adenovirus C* | V | 129 |
| | | | *Aviadenovirus* | *Fowl adenovirus A* | V | 133 |
| | | | *Atadenovirus* | *Ovine adenovirus D* | V | 134 |
| | | | *Siadenovirus* | *Frog adenovirus* | V | 137 |
| | | | *Ichtadenovirus* | *Sturgeon adenovirus A* | V | 138 |
| | *Ampullaviridae* | | *Ampullavirus* | *Acidianus bottle-shaped virus* | Ar | 143 |
| | *Ascoviridae* | | *Ascovirus* | *Spodoptera frugiperda ascovirus 1a* | I | 147 |
| | *Asfarviridae* | | *Asfivirus* | *African swine fever virus* | V,I | 153 |
| | *Baculoviridae* | | *Alphabaculovirus* | *Autographa californica multiple nucleopolyhedrovirus* | I | 167 |
| | | | *Betabaculovirus* | *Cydia pomonella granulovirus* | I | 169 |
| | | | *Gammabaculovirus* | *Neodiprion lecontei nucleopolyhedrovirus* | I | 170 |
| | | | *Deltabaculovirus* | *Culex nigripalpus nucleopolyhedrovirus* | I | 171 |
| | *Bicaudaviridae* | | *Bicaudavirus* | *Acidianus two-tailed virus* | Ar | 175 |
| | *Corticoviridae* | | *Corticovirus* | *Pseudoalteromonas phage PM2* | B | 179 |
| | *Fuselloviridae* | | *Fusellovirus* | *Sulfolobus spindle-shaped virus 1* | Ar | 183 |
| | *Globuloviridae* | | *Globulovirus* | *Pyrobaculum spherical virus* | Ar | 187 |
| | *Guttaviridae* | | *Guttavirus* | *Sulfolobus newzealandicus droplet-shaped virus* | Ar | 191 |

| Order | Family | Subfamily | Genus | Type Species | Host | Page |
|---|---|---|---|---|---|---|
| | *Iridoviridae* | | *Iridovirus* | *Invertebrate iridescent virus 6* | I | 201 |
| | | | *Chloriridovirus* | *Invertebrate iridescent virus 3* | I | 203 |
| | | | *Ranavirus* | *Frog virus 3* | V | 204 |
| | | | *Lymphocystivirus* | *Lymphocystis disease virus 1* | V | 205 |
| | | | *Megalocytivirus* | *Infectious spleen and kidney necrosis virus* | V | 207 |
| | *Lipothrixviridae* | | *Alphalipothrixvirus* | *Thermoproteus tenax virus 1* | Ar | 212 |
| | | | *Betalipothrixvirus* | *Sulfolobus islandicus filamentous virus* | Ar | 213 |
| | | | *Gammalipothrixvirus* | *Acidianus filamentous virus 1* | Ar | 214 |
| | | | *Deltalipothrixvirus* | *Acidianus filamentous virus 2* | Ar | 215 |
| | *Mimiviridae* | | *Mimivirus* | *Acanthamoeba polyphaga mimivirus* | Pr | 223 |
| | *Nimaviridae* | | *Whispovirus* | *White spot syndrome virus* | I | 229 |
| | *Papillomaviridae* | | *Alphapapillomavirus* | *Human papillomavirus 32* | V | 239 |
| | | | *Betapapillomavirus* | *Human papillomavirus 5* | V | 240 |
| | | | *Gammapapillomavirus* | *Human papillomavirus 4* | V | 241 |
| | | | *Deltapapillomavirus* | *European elk papillomavirus* | V | 241 |
| | | | *Epsilonpapillomavirus* | *Bovine papillomavirus 5* | V | 242 |
| | | | *Zetapapillomavirus* | *Equine papillomavirus 1* | V | 242 |
| | | | *Etapapillomavirus* | *Fringilla coelebs papillomavirus* | V | 242 |
| | | | *Thetapapillomavirus* | *Psittacus erithacus timneh papillomavirus* | V | 243 |
| | | | *Iotapapillomavirus* | *Mastomys natalensis papillomavirus* | V | 243 |
| | | | *Kappapapillomavirus* | *Cottontail rabbit papillomavirus* | V | 244 |
| | | | *Lambdapapillomavirus* | *Canine oral papillomavirus* | V | 244 |
| | | | *Mupapillomavirus* | *Human papillomavirus 1* | V | 244 |
| | | | *Nupapillomavirus* | *Human papillomavirus 41* | V | 245 |
| | | | *Xipapillomavirus* | *Bovine papillomavirus 3* | V | 245 |
| | | | *Omikronpapillomavirus* | *Phocoena spinipinnis papillomavirus* | V | 246 |
| | | | *Pipapillomavirus* | *Hamster oral papillomavirus* | V | 246 |

| Order | Family | Subfamily | Genus | Type Species | Host | Page |
|---|---|---|---|---|---|---|
| | *Phycodnaviridae* | | *Chlorovirus* | *Paramecium bursaria Chlorella virus 1* | Al | 253 |
| | | | *Coccolithovirus* | *Emiliania huxleyi virus 86* | Al | 256 |
| | | | *Prasinovirus* | *Micromonas pusilla virus SP1* | Al | 256 |
| | | | *Prymnesiovirus* | *Chrysochromulina brevifilum virus PW1* | Al | 257 |
| | | | *Phaeovirus* | *Ectocarpus siliculosus virus 1* | Al | 258 |
| | | | *Raphidovirus* | *Heterosigma akashiwo virus 01* | Al | 259 |
| | *Plasmaviridae* | | *Plasmavirus* | *Acholeplasma phage L2* | B | 263 |
| | *Polydnaviridae* | | *Bracovirus* | *Cotesia melanoscela bracovirus* | I | 269 |
| | | | *Ichnovirus* | *Campoletis sonorensis ichnovirus* | I | 273 |
| | *Polyomaviridae* | | *Polyomavirus* | *Simian virus 40* | V | 279 |
| | *Poxviridae* | *Chordopoxvirinae* | *Avipoxvirus* | *Fowlpox virus* | V | 297 |
| | | | *Capripoxvirus* | *Sheeppox virus* | V | 298 |
| | | | *Cervidpoxvirus* | *Deerpox virus W-848-83* | V | 299 |
| | | | *Leporipoxvirus* | *Myxoma virus* | V | 299 |
| | | | *Molluscipoxvirus* | *Molluscum contagiosum virus* | V | 300 |
| | | | *Orthopoxvirus* | *Vaccinia virus* | V | 300 |
| | | | *Parapoxvirus* | *Orf virus* | V | 302 |
| | | | *Suipoxvirus* | *Swinepox virus* | V | 303 |
| | | | *Yatapoxvirus* | *Yaba monkey tumor virus* | V | 303 |
| | | *Entomopoxvirinae* | *Alphaentomopoxvirus* | *Melolontha melolontha entomopoxvirus* | I | 305 |
| | | | *Betaentomopoxvirus* | *Amsacta moorei entomopoxvirus 'L'* | I | 306 |
| | | | *Gammaentomopoxvirus* | *Chironomus luridus entomopoxvirus* | I | 307 |
| | *Rudiviridae* | | *Rudivirus* | *Sulfolobus islandicus rod-shaped virus 2* | Ar | 311 |
| | *Tectiviridae* | | *Tectivirus* | *Enterobacteria phage PRD1* | B | 317 |
| | Unassigned | | *Rhizidiovirus* | *Rhizidiomyces virus* | Pr | 323 |
| | | | *Salterprovirus* | *His 1 virus* | Ar | 325 |

| Order | Family | Subfamily | Genus | Type Species | Host | Page |
|---|---|---|---|---|---|---|
| **ssDNA Viruses** | | | | | | |
| | *Anelloviridae* | | *Alphatorquevirus* | *Torque teno virus 1* | V | 333 |
| | | | *Betatorquevirus* | *Torque teno mini virus 1* | V | 334 |
| | | | *Gammatorquevirus* | *Torque teno midi virus 1* | V | 335 |
| | | | *Deltatorquevirus* | *Torque teno tupaia virus* | V | 336 |
| | | | *Epsilontorquevirus* | *Torque teno tamarin virus* | V | 336 |
| | | | *Zetatorquevirus* | *Torque teno douroucouli virus* | V | 337 |
| | | | *Etatorquevirus* | *Torque teno felis virus* | V | 337 |
| | | | *Thetatorquevirus* | *Torque teno canis virus* | V | 338 |
| | | | *Iotatorquevirus* | *Torque teno sus virus 1* | V | 338 |
| | *Circoviridae* | | *Circovirus* | *Porcine circovirus-1* | V | 344 |
| | | | *Gyrovirus* | *Chicken anemia virus* | V | 347 |
| | *Geminiviridae* | | *Mastrevirus* | *Maize streak virus* | P | 352 |
| | | | *Curtovirus* | *Beet curly top virus* | P | 355 |
| | | | *Topocuvirus* | *Tomato pseudo-curly top virus* | P | 357 |
| | | | *Begomovirus* | *Bean golden yellow mosaic virus* | P | 359 |
| | *Inoviridae* | | *Inovirus* | *Enterobacteria phage M13* | B | 379 |
| | | | *Plectrovirus* | *Acholeplasma phage MV-L51* | B | 381 |
| | *Microviridae* | | *Microvirus* | *Enterobacteria phage phiX174* | B | 387 |
| | | *Gokushovirinae* | *Chlamydiamicrovirus* | *Chlamydia phage 1* | B | 390 |
| | | | *Bdellomicrovirus* | *Bdellovibrio phage MAC 1* | B | 391 |
| | | | *Spiromicrovirus* | *Spiroplasma phage 4* | B | 392 |
| | *Nanoviridae* | | *Nanovirus* | *Subterranean clover stunt virus* | P | 399 |
| | | | *Babuvirus* | *Banana bunchy top virus* | P | 410 |
| | *Parvoviridae* | *Parvovirinae* | *Parvovirus* | *Minute virus of mice* | V | 410 |
| | | | *Erythrovirus* | *Human parvovirus B19* | V | 411 |
| | | | *Dependovirus* | *Adeno-associated virus-2* | V | 413 |
| | | | *Amdovirus* | *Aleutian mink disease virus* | V | 415 |
| | | | *Bocavirus* | *Bovine parvovirus* | V | 416 |
| | | *Densovirinae* | *Iteravirus* | *Bombyx mori densovirus* | I | 418 |
| | | | *Brevidensovirus* | *Aedes aegypti densovirus* | I | 419 |
| | | | *Densovirus* | *Junonia coenia densovirus* | I | 420 |
| | | | *Pefudensovirus* | *Periplaneta fuliginosa densovirus* | I | 421 |

| Order | Family | Subfamily | Genus | Type Species | Host | Page |
|---|---|---|---|---|---|---|
| **Reverse Transcribing DNA and RNA Viruses** | | | | | | |
| | *Caulimoviridae* | | *Caulimovirus* | *Cauliflower mosaic virus* | P | 432 |
| | | | *Petuvirus* | *Petunia vein clearing virus* | P | 434 |
| | | | *Soymovirus* | *Soybean chlorotic mottle virus* | P | 435 |
| | | | *Cavemovirus* | *Cassava vein mosaic virus* | P | 437 |
| | | | *Badnavirus* | *Commelina yellow mottle virus* | P | 438 |
| | | | *Tungrovirus* | *Rice tungro bacilliform virus* | P | 441 |
| | *Hepadnaviridae* | | *Orthohepadnavirus* | *Hepatitis B virus* | V | 450 |
| | | | *Avihepadnavirus* | *Duck hepatitis B virus* | V | 453 |
| | *Metaviridae* | | *Metavirus* | *Saccharomyces cerevisiae Ty3 virus* | F, I, P, V | 460 |
| | | | *Errantivirus* | *Drosophila melanogaster Gypsy virus* | I | 462 |
| | | | *Semotivirus* | *Ascaris lumbricoides Tas virus* | I, V | 464 |
| | *Pseudoviridae* | | *Pseudovirus* | *Saccharomyces cerevisiae Ty1 virus* | F, P | 469 |
| | | | *Hemivirus* | *Drosophila melanogaster copia virus* | Al, F, I | 471 |
| | | | *Sirevirus* | *Glycine max SIRE1 virus* | P | 472 |
| | *Retroviridae* | *Orthoretrovirinae* | *Alpharetrovirus* | *Avian leukosis virus* | V | 481 |
| | | | *Betaretrovirus* | *Mouse mammary tumor virus* | V | 482 |
| | | | *Gammaretrovirus* | *Murine leukemia virus* | V | 484 |
| | | | *Deltaretrovirus* | *Bovine leukemia virus* | V | 486 |
| | | | *Epsilonretrovirus* | *Walleye dermal sarcoma virus* | V | 487 |
| | | | *Lentivirus* | *Human immunodeficiency virus 1* | V | 489 |
| | | *Spumaretrovirinae* | *Spumavirus* | *Simian foamy virus* | V | 492 |

| Order | Family | Subfamily | Genus | Type Species | Host | Page |
|---|---|---|---|---|---|---|
| **RNA Viruses**<br>**dsRNA Viruses** | | | | | | |
| | *Birnaviridae* | | *Aquabirnavirus* | *Infectious pancreatic necrosis virus* | V, I | 503 |
| | | | *Avibirnavirus* | *Infectious bursal disease virus* | V | 504 |
| | | | *Blosnavirus* | *Blotched snakehead virus* | V | 505 |
| | | | *Entomobirnavirus* | *Drosophila X virus* | I | 505 |
| | *Chrysoviridae* | | *Chrysovirus* | *Penicillium chrysogenum virus* | F | 509 |
| | *Cystoviridae* | | *Cystovirus* | *Pseudomonas phage phi6* | B | 515 |
| | *Endornaviridae* | | *Endornavirus* | *Vicia faba endornavirus* | Al, F, P | 519 |
| | *Partitiviridae* | | *Partitivirus* | *Atkinsonella hypoxylon virus* | F | 524 |
| | | | *Alphacryptovirus* | *White clover cryptic virus 1* | P | 527 |
| | | | *Betacryptovirus* | *White clover cryptic virus 2* | P | 529 |
| | | | *Cryspovirus* | *Cryptosporidium parvum virus 1* | Pr | 531 |
| | *Picobirnaviridae* | | *Picobirnavirus* | *Human picobirnavirus* | V | 535 |
| | *Reoviridae* | *Spinareovirinae* | *Orthoreovirus* | *Mammalian orthoreovirus* | V | 546 |
| | | | *Aquareovirus* | *Aquareovirus A* | I, V | 554 |
| | | | *Oryzavirus* | *Rice ragged stunt virus* | I, P | 560 |
| | | | *Fijivirus* | *Fiji disease virus* | I, P | 563 |
| | | | *Mycoreovirus* | *Mycoreovirus 1* | F | 567 |
| | | | *Cypovirus* | *Cypovirus 1* | I | 572 |
| | | | *Idnoreovirus* | *Idnoreovirus 1* | I | 580 |
| | | | *Dinovernavirus* | *Aedes pseudoscutellaris reovirus* | I | 584 |
| | | | *Coltivirus* | *Colorado tick fever virus* | I, V | 588 |
| | | *Sedoreovirinae* | *Orbivirus* | *Bluetongue virus* | I, V | 592 |
| | | | *Rotavirus* | *Rotavirus A* | V | 603 |
| | | | *Seadornavirus* | *Banna virus* | I, V | 613 |
| | | | *Phytoreovirus* | *Wound tumor virus* | I, P | 620 |
| | | | *Cardoreovirus* | *Eriocheir sinensis reovirus* | I | 626 |
| | | | *Mimoreovirus* | *Micromonas pusilla reovirus* | Pr | 629 |
| | *Totiviridae* | | *Totivirus* | *Saccharomyces cerevisiae virus L-A* | F | 640 |
| | | | *Victorivirus* | *Helminthosporium victoriae virus 190S* | F | 643 |
| | | | *Giardiavirus* | *Giardia lamblia virus* | Pr | 645 |
| | | | *Leishmaniavirus* | *Leishmania RNA virus 1 - 1* | Pr | 647 |
| **Order** | **Family** | **Subfamily** | **Genus** | **Type Species** | **Host** | **Page** |

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| | | | *Ebolavirus* | *Zaire ebolavirus* | V | 669 |
| | *Paramyxoviridae* | *Paramyxovirinae* | *Rubulavirus* | *Mumps virus* | V | 676 |
| | | | *Avulavirus* | *Newcastle disease virus* | V | 677 |
| | | | *Respirovirus* | *Sendai virus* | V | 678 |
| | | | *Henipavirus* | *Hendra virus* | V | 679 |
| | | | *Morbillivirus* | *Measles virus* | V | 680 |
| | | *Pneumovirinae* | *Pneumovirus* | *Human respiratory syncytial virus* | V | 682 |
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| | | | *Lyssavirus* | *Rabies virus* | V | 696 |
| | | | *Ephemerovirus* | *Bovine ephemeral fever virus* | V,I | 699 |
| | | | *Novirhabdovirus* | *Infectious hematopoietic necrosis virus* | V | 701 |
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| | *Arenaviridae* | | *Arenavirus* | *Lymphocytic choriomeningitis virus* | V | 715 |
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| | | | *Nairovirus* | *Dugbe virus* | V,I | 734 |
| | | | *Phlebovirus* | *Rift Valley fever virus* | V,I | 735 |
| | | | *Tospovirus* | *Tomato spotted wilt virus* | I, P | 737 |
| | *Ophioviridae* | | *Ophiovirus* | *Citrus psorosis virus* | P | 743 |
| | *Orthomyxoviridae* | | *Influenzavirus A* | *Influenza A virus* | V | 753 |
| | | | *Influenzavirus B* | *Influenza B virus* | V | 755 |
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| | Unassigned | | *Deltavirus* | *Hepatitis delta virus* | V | 763 |
| | | | *Emaravirus* | *European mountain ash ringspot-associated virus* | P | 767 |
| | | | *Tenuivirus* | *Rice stripe virus* | P,I | 771 |
| | | | *Varicosavirus* | *Lettuce big-vein associated virus* | P | 777 |

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| | | *Torovirinae* | *Torovirus* | *Equine torovirus* | V | 820 |
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| | | | *Torradovirus* | *Tomato torrado virus* | P | 895 |
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| | | | *Bromovirus* | *Brome mosaic virus* | P | 969 |
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| | | | *Rymovirus* | *Ryegrass mosaic virus* | P | 1082 |
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Abbreviations of the virus hosts: Algae, Al; Archaea, Ar; Bacteria, B; Fungi, F; Invertebrates, I; Plants, P; Protozoa, Pr; Vertebrates, V.

# ORDER *CAUDOVIRALES*

## Taxonomic structure of the order

| | |
|---|---|
| Order | *Caudovirales* |
| Family | *Myoviridae* |
| Genus | **"T4-like viruses"** |
| Genus | **"P1-like viruses"** |
| Genus | **"P2-like viruses"** |
| Genus | **"Mu-like viruses"** |
| Genus | **"SPO1-like viruses"** |
| Genus | **"PhiH-like viruses"** |
| Genus | **"PhiKZ-like viruses"** |
| Genus | **"I3-like viruses"** |
| Family | *Podoviridae* |
| Genus | **"BPP-1-like viruses"** |
| Genus | **"Epsilon15-like viruses"** |
| Genus | **"LUZ24-like viruses"** |
| Genus | **"N4-like viruses"** |
| Genus | **"P22-like viruses"** |
| Genus | **"Phieco32-like viruses"** |
| Subfamily | *Autographivirinae* |
| Genus | **"PhiKMV-like viruses"** |
| Genus | **"SP6-like viruses"** |
| Genus | **"T7-like viruses"** |
| Subfamily | *Picovirinae* |
| Genus | **"AHJD-like viruses"** |
| Genus | **"Phi29-like viruses"** |
| Family | *Siphoviridae* |
| Genus | **"Lambda-like viruses"** |
| Genus | **"T1-like viruses"** |
| Genus | **"T5-like viruses"** |
| Genus | **"L5-like viruses"** |
| Genus | **"c2-like viruses"** |
| Genus | **"PsiM1-like viruses"** |
| Genus | **"PhiC31-like viruses"** |
| Genus | **"N15-like viruses"** |
| Genus | **"SPbeta-like viruses"** |

The order consists of the three families of tailed bacterial viruses infecting Bacteria and Archaea: *Myoviridae* (long contractile tails), *Siphoviridae* (long non-contractile tails) and *Podoviridae* (short non-contractile tails). Tailed bacterial viruses are an extremely large group with highly diverse virion, genome and replication properties. Over 4500 descriptions have been published (accounting for 96% of reported bacterial viruses): 24% in the family *Myoviridae*, 62% in the family *Siphoviridae* and 14% in the family *Podoviridae* (as of November 2001). However, data on virion structure, genome organization and replication properties are available for only a small number of well-studied species. Although extensive horizontal gene transfer between bacterial cells and viruses has obscured phylogenetic relationships amongst some tailed viruses, particularly those which are temperate, enough common features still survive to indicate their fundamental relatedness. Therefore, formal taxonomic names are used for *Caudovirales* at the order and family level, but only vernacular names at the genus level. Since publication of the Eighth Report, some taxonomic revision of the family *Podoviridae* has been accomplished based on genome information. This has led to the introduction of subfamilies to accommodate the wealth and diversity of bacterial viruses being discovered.

## Virion properties

### Morphology

The virion has no envelope and consists of two parts, the head and the tail. The head is a protein shell and contains a single linear dsDNA molecule, and the tail is a protein tube whose distal end binds the surface receptors on susceptible bacterial cells. DNA travels through the tail tube during delivery (often called "injection") into the cell being infected. Heads have icosahedral symmetry or elongated derivatives thereof (with known triangulation numbers of T = 4, 7, 13, 16 and 52). Capsomers are seldom visible: heads usually appear smooth and thin-walled (2–3 nm). When they are visible, morphological features (capsomeres) on the surface of the head commonly form 72 capsomers (T = 7; 420 protein subunits), but known capsomer numbers vary from 42 to 522. Isometric heads are typically 45–170 nm in diameter. Elongated heads derive from icosahedra by addition of equatorial belts of capsomers and can be up to 230 nm long. DNA forms a tightly packed coil (without bound proteins) inside the head. Tail shafts have six-fold or (rarely) three-fold symmetry, and are helical or stacks of disks of subunits from 3 and 825 nm in length. They usually have base plates, spikes, or terminal fibers at the distal end. Some viruses have collars at the head–tail junction, head or collar appendages, transverse tail disks, or other attachments.

### Physicochemical and physical properties

Virion Mr is 20–600 $\times 10^6$; $S_{20,w}$ values are 200 to >1200S. Both upper limits may be underestimates, since these properties have not been determined for the largest tailed viruses. Buoyant density in CsCl is typically about 1.5 g $cm^{-3}$. Most tailed viruses are stable at pH 5–9; a few are stable at pH 2 or pH 11. Heat sensitivity is variable, but many virions are inactivated by heating at 55–75 °C for 30 min. Tailed viruses are rather resistant to UV irradiation. Heat and UV inactivation generally follow first-order kinetics. Most tailed phages are stable to chloroform. Inactivation by nonionic detergents is variable and concentration-dependent. Some virions are sensitive to osmotic shock, and many are sensitive to $Mg^{++}$ chelators.

### Nucleic acid

Virions contain one molecule of linear dsDNA. Genome sizes are 18 to >500 kbp, corresponding to Mr values of 11 to >300 $\times 10^6$. DNA content is 45–55% of the virions. G+C contents are 27–72% and usually resemble those of host DNA. Some viral DNAs contain modified nucleotides which partially or completely replace normal nucleotides (e.g., 5-hydroxymethylcytosine instead of cytosine), and/or are glycosylated or otherwise modified.

### Proteins

There are 7–49 different virion structural proteins. Typical head shells are made up of 60T molecules of a single main building block CP and 12 molecules of portal protein through which DNA enters and leaves, but they can also contain varied numbers of proteins that plug the portal hole, proteins to which tails bind, proteins that bind to the outside of the CP shell (decoration proteins) and other proteins whose roles are not known. Non-contractile tails are made of one major shaft or tube protein and contractile tails have a second major protein, the sheath protein that forms a cylinder around the central tube. Tails also have small numbers of varied specific proteins at both ends. Those at the end distal from the head form a structure called the tail tip (Siphovirus) or baseplate (Myovirus) to which the tail fibers are attached. The tail fibers bind to the first-contact receptors on the surface of susceptible cells. Fibers or baseplates may include proteins with endoglycosidase or peptidoglycan hydrolase activity that aid in gaining access to the cell surface and entry of DNA into the cell. Most virions carry proteins that are injected with the DNA, such as transcription factors, RNA polymerase and others with poorly understood functions.

### Lipids

No well-characterized virions contain lipid.

### Carbohydrates

Glycoproteins, glycolipids, hexosamine and a polysaccharide have been reported in certain virions but these are not well-characterized.

## Genome organization and replication

### Genome organization

The linear dsDNA genomes encode from 27 to over 600 genes that are highly clustered according to function and tend to be arranged in large operons. Complete functional genomic maps are very diverse and available for only a relatively small number of tailed viruses. Virion DNAs may be circularly permuted and/or terminally redundant, have single stranded gaps, or have covalently-bound terminal proteins. The ends of these linear molecules can be blunt or have complementary protruding 5′ or 3′ ends (the "cohesive" or "sticky" ends, which can base-pair to circularize the molecule). Prophages of temperate tailed viruses are either integrated into the host genome or replicate as circular or linear plasmids; these linear plasmids have covalently-closed hairpin telomeres.

### Replication

In typical lytic infections, after entering the host cell, viral DNA may either circularize or remain linear. A few viruses use terminal proteins to prime DNA replication and package progeny viral DNA (φ29 and its relatives) or replicate DNA by a duplicative transposition mechanism (Mu and its relatives). Gene expression is largely time-ordered and groups of genes are sequentially expressed. "Early genes" are expressed first and are largely involved in host cell modification and viral DNA replication. "Late genes" specify virion structural proteins and lysis proteins (Figure 1). The larger

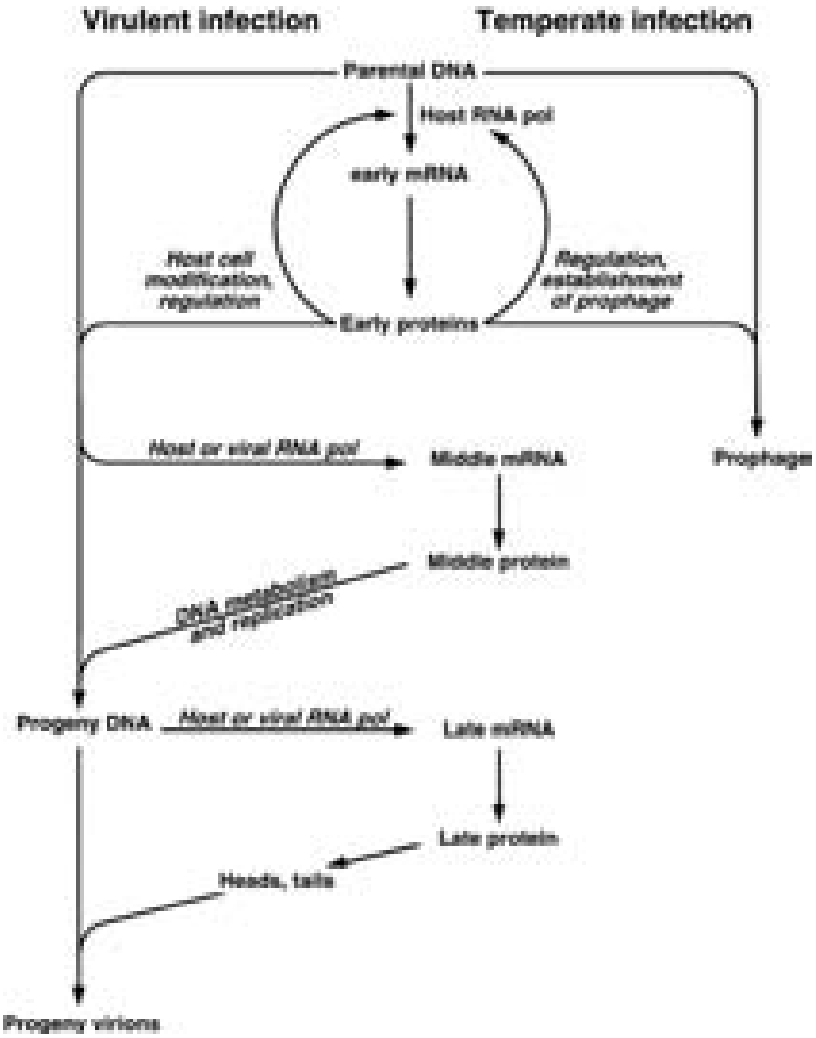


**Figure 1:** Flow chart of tailed phage replication. The chart depicts the replication of "typical" virulent phages such as Enterobacteria phage T4 (T4), Enterobacteria phage T7 (T7), and the temperate phages.

tailed viruses have gene expression cascades that are more complex than this simple scenario. Transcription often requires host RNA polymerase, but many tailed viruses encode RNA polymerases or transcription factors that affect the host RNA polymerase. Translational control is poorly understood and no generalizations are possible at the present state of knowledge. All tailed viruses encode proteins that direct the replication apparatus to the replication origin, but this apparatus may be entirely host-derived, partly virus encoded, or entirely virus encoded. DNA replication is semi-conservative, may be either bidirectional or unidirectional, and usually results in the formation of concatemers (multiple genomes joined head-to-tail) by recombination between phage DNAs or by rolling circle replication. Progeny viral DNA is generated during virion assembly by cleavage from this concatemeric DNA: (i) at unique sites to produce identical DNA molecules with either *cos* sites or blunt-ended, terminally redundant termini, (ii) at *pac* sites to produce circularly permuted, terminal redundant DNAs, or (iii) by a headful mechanism to produce terminally redundant, circularly permuted DNAs.

### Virion assembly and DNA packaging

Assembly of virions from newly made proteins and replicated DNA is complex and generally includes separate pathways for heads, tails and tail fibers. Coat protein shells, called procapsids or proheads, are assembled first, and DNA is inserted into these preformed proteinaceous containers. Assembly of procapsids is poorly understood, but often utilizes an internal scaffolding protein which helps CP assemble correctly and is then released from the shell after its construction. In many, but not all, tailed viruses, proteolytic cleavages (by host or virus-encoded proteases) of some proteins accompany assembly. Virus-specific DNA is recognized for packaging into procapsids by the terminase protein. One end of the DNA is then threaded through the procapsid's portal structure, and DNA is pumped into the head by an ATP hydrolysis-driven motor that is probably made up of the two terminase subunits and portal protein. Unless unit length DNA molecules are the substrate for packaging (such as with Phi29), when the head is full of DNA a "headful sensing device" recognizes this fact and causes the terminase to cleave the DNA to release the full head from the unpackaged remainder of the DNA concatemer. The terminase subunits are usually released from the virion after DNA is packaged. Filled heads then join to tails and tail fibers to form progeny virions. Some viruses form intracellular arrays, and many produce aberrant structures (polyheads, polytails, giant, multi-tailed, or misshapen particles). Progeny viruses are liberated by lysis of the host cell. Cell lysis is caused by phage-encoded peptidoglycan hydrolases; but lysis timing is controlled by holins, phage encoded inner membrane proteins that allow the hydrolases to escape from the cytoplasm.

## Antigenic properties

Viruses are antigenically complex and efficient immunogens, inducing the formation of neutralizing and complement-fixing antigens. The existence of group antigens is likely within species or genera.

## Biological properties

### Infection

Tailed-viruses are lytic or temperate. Lytic infection results in production of progeny viruses and destruction of the host. Phages adsorb tail-first to specific proteins or polysaccharides on the host cell outer cell surface. In a few cases the primary adsorption sites (receptors) are flagella or pili. Upon adsorption to the outside of the cell, virions undergo complex and often poorly understood rearrangements which release the DNA to enter the cell through the tail. Cell walls are often locally digested by a virion-associated peptidoglycan hydrolase and viral DNA enters the cytoplasm by as yet unknown mechanisms. In some cases DNA entry is stepwise and transcription of the first DNA to enter is required for entry of the rest of the DNA. Empty virions remain outside the infected bacterium, however most viruses inject specific proteins with the DNA. Temperate viruses can, upon infection, either enter a lytic growth cycle (above) or establish a lysogenic state (below). Physiological factors in the cell can affect the decision between these two pathways.

### Latency

All three-tailed virus families include genera or species of temperate viruses. Viral genomes in lysogenized cells are called "prophages". Prophages are either integrated into host cell chromosomes

or persist as extrachromosomal elements (plasmids). Integration is usually mediated by recombinases called integrases. The most common are in the tyrosine-active site class and some are in the serine-active site class. For the Mu-like viruses, integration is accomplished by transposases. Integrated prophages typically express only a very small fraction of their genes. The genes that are expressed from the prophage are called "lysogenic conversion" or "cargo" genes, and their products usually alter the properties of the bacterial host. Among these genes is the prophage repressor gene, whose product binds operators in the prophage genome to keep the lytic cascade of gene expression from initiating. Plasmid prophages typically express many of their early genes, some of which are involved in replication of the plasmid (which can be circular or linear). Prophages can often be induced to initiate a lytic growth cycle; DNA damaging agents such as ultraviolet light or mitomycin C cause many prophages to induce.

### Host range

Tailed viruses have been found in over 140 prokaryote genera representing most branches of the bacterial and archaeal phylogenetic trees. The host specificity of these viruses can vary widely; some can infect members of multiple genera, but perhaps more common (especially in the host family Enterobacteriaceae, where the most varieties have been studied) are viruses that are specific for particular isolates or groups of isolates of closely related host species.

### Transmission in nature

Virions are typically carried and transmitted in aqueous environments, although a few are stable to drying. Virus genomes can be carried as prophages inside host bacteria. Such lysogenic bacteria can induce release of virions, either spontaneously or in response to specific environmental signals.

### Geographic distribution

Tailed phages are the most abundant type of organism on Earth; the current best estimates are $10^{31}$ particles in our biosphere. If all these phages were laid end to end the line would extend for $2\times10^{8}$ light years. Data from genome sequence analyses imply that these viruses can move around the globe on a time scale that is short relative to the rate at which they accumulate mutations. They have a worldwide distribution and presumably share the habitats of their hosts. An important habitat is inside lysogenic bacteria as prophages.

## Phylogenetic relationships within the order and the perils of mosaicism

The recent availability of high-throughput DNA sequencing has led to a dramatic increase in the number of complete genome sequences that are available for members of the *Caudovirales*. At the latest count 101 myoviruses, 91 podoviruses and 244 siphoviruses are listed in the RefSeq Genomes section of NCBI. The new data substantially enrich our appreciation of the genetic structure and diversity of the global *Caudovirales* population and of the evolutionary mechanisms within that order. The new data also substantially complicate considerations of how best to represent these viruses in a coherent and easy to use taxonomy.

The hallmark of the genomes of these viruses is that they are genetic mosaics, a property that becomes apparent when two or more genome sequences are compared. The modules of sequence that constitute the mosaic are typically individual genes, but they can also be parts of genes corresponding to protein domains, or small groups of genes such as prohead assembly genes. The mosaicism is evidently the result of non-homologous recombination during the evolution of these viruses. The novel juxtapositions of sequence produced in this way are spread through the population and reassorted with each other by means of homologous recombination. Regardless of mechanism, the overall result is as if each phage had constituted its genome by picking modules from a menu, choosing one module from each of perhaps fifty columns, each of which has alternative choices.

While there is no doubt that recombinational exchange has muddied the relationship between certain phages, recent whole genome comparative proteomics revealed that in many cases clear phylogenetic relationships exist even though DNA sequence similarity is small. Using CoreGenes, a BLASTP-based comparative genomic tool, all fully sequenced members of the *Podoviridae* and *Myoviridae* were analyzed. From these studies it was possible to identify high level relationships between phages that shared ≥40% homologous proteins distributed over the length of their genomes (representing phage within the same genus) and lower level relationships in which only

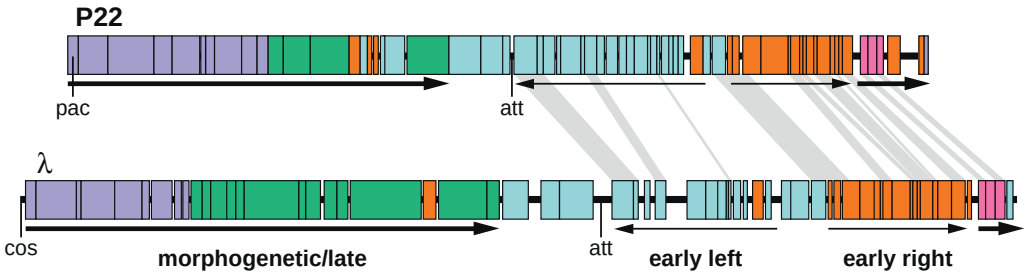


**Figure 2:** The mosaic relationship between the genomes of phages P22 and lambda. The circular maps are opened for linear display between the lysis and head genes. The genes in each genome are represented by rectangles. P22 genes that have sequence similarity to lambda genes are connected by light gray trapezoids. The thin arrows represent transcription of the early operons and thick arrows transcription of the late operons. The circular phage genomes are opened at their attachment (att) sites for insertion of the prophage into the host chromosome in lysogens. DNA packaging initiation sites (called pac and cos in P22 and lambda, respectively) are also indicated below the maps.

20–30% of the proteins were significantly (BLAST score ≥75) similar. This has led, for example, to the creation of three distinct genera within a new subfamily (*Autographivirinae*) for phages previously known as the "T7 superfamily" and has helped classify or re-classify many other members of the families *Podoviridae* and *Myoviridae*. While this approach has significantly reduced the number of unclassified viruses, there are still many that remain genomic orphans. It has not yet been possible to place certain phages which are clearly related but only at a low level. For example, the myoviruses that infect *Prochlorococcus* and *Synechococcus* possess a set of genes which they share with T4-like phages but score very low in CoreGenes. Higher-level relationships will therefore have to be addressed in the future. The tentative classification of phages based purely on morphological grounds and minimal sequence analysis should be discouraged in favor of full sequence analysis on genomes that have been carefully annotated.

In the current ICTV taxonomy, presented here, the division of the order *Caudovirales* into three families is based solely on tail morphology: members of the family *Siphoviridae* have long non-contractile tails, *Myoviridae* have long contractile tails, and *Podoviridae* have short tails. As might be expected from the discussion above, this hierarchical division of phages on the basis of one character leads to many examples of inappropriate divisions of other characters. One well-known and easily illustrated example of this is shown in Figure 2, comparing phages lambda and P22. These two phages are considered by many phage biologists to be closely related, because they share genome organization (including regulation and layout of transcription and functional order of genes), temperate lifestyle, a number of similarities of gene sequences and they can form viable hybrids. Despite these similarities, they are classified into different families (*Siphoviridae* and *Podoviridae* for lambda and P22, respectively) based on their differences in tail morphology. It may be arguable whether the similarities between these two phages are enough for them to be classified in the same family, but it is in any case clear that P22 is much closer to lambda than it is to most other members of the family *Podoviridae*, such as phages T7 and N4, which have essentially no similarity to lambda in sequence, genome organization, or lifestyle.

Where mosaicism is extensive, ICTV will have to come to terms with the fact that not all phages will be simply classifiable in a straightforward hierarchical manner. Because of this, the ICTV considers the taxonomy of this group to be provisional, and this is the reason that the names of the genera are in a non-official vernacular format. Discussions are ongoing both within the ICTV and in the virology community at large, and there may well be significant changes to the *Caudovirales* taxonomy in the future, in response to our new understanding of the biology.

## Similarity with other taxa

Tailed bacterial viruses resemble members of the family *Tectiviridae* by the presence of a dedicated structure for DNA injection, but differ from them by the permanent nature of their tails and lack of a lipid bilayer. Tailed viruses resemble viruses belonging to the family *Herpesviridae* in

morphogenesis (use of scaffolding proteins, packaging of DNA into preformed shells, maturation of procapsids by proteolytic cleavage, and capsid conformational change) and overall strategy of replication. In addition, temperate tailed phages and members of the family *Herpesviridae* are able to establish latent infections.



## Derivation of names

*Caudo*: from Latin *cauda*, "tail".
*Myo*: from Greek my, *myos*, "muscle", referring to the contractile tail.
*Sipho*: from Greek *siphon*, "tube", referring to the long tail.
*Podo*: from Greek *pous*, *podos*, "foot", referring to the short tail.

## Further reading

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## Contributed by

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The authors acknowledge the contribution to the Eighth ICTV Report of Casjens, S.R. and Hendrix, R.W.

# Family *Myoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Myoviridae* |
| Genus | **"T4-like viruses"** |
| Genus | **"P1-like viruses"** |
| Genus | **"P2-like viruses"** |
| Genus | **"Mu-like viruses"** |
| Genus | **"SPO1-like viruses"** |
| Genus | **"PhiH-like viruses"** |
| Genus | **"phiKZ-like viruses"** |
| Genus | **"I3-like viruses"** |

## Distinguishing features of the family

Tails are contractile, more or less rigid, long and relatively thick (80–455 × 16–20 nm). They consist of a central core built of stacked rings of six subunits and surrounded by a helical contractile sheath, which is separated from the head by a neck. During contraction, sheath subunits slide over each other and the sheath becomes shorter and thicker. This brings the tail core in contact with the bacterial plasma membrane and is an essential stage of infection. Heads and tails are assembled in separate pathways. Compared to other tailed phages, myoviruses often have larger heads and higher particle weights and DNA contents, and seem to be more sensitive to freezing and thawing and to osmotic shock. Genera are differentiated by genome organization, mechanisms of DNA replication, and packaging, and the presence or absence of unusual bases and DNA polymerases.

# Genus "T4-like viruses"

Type species *Enterobacteria phage T4*

## Distinguishing features of the genus

Virions have elongated heads and tails with long, kinked fibers. Double stranded DNA genomes are circularly permuted and terminally redundant, and typically code for hydroxymethylcytosine synthesizing enzymes and B-type DNA polymerase. The genome is linear but circularly permuted and the DNA is packaged by a headful mechanism.

## Virion properties

### Morphology

Phage heads are prolate icosahedra (elongated pentagonal bipyramidal antiprisms), measure about 111 × 78 nm, and consist of 152 capsomers (T = 13, elongated). Tails measure 113 × 16 nm and have a collar, base plate, six short spikes and six long fibers (Figure 1). Aberrant head structures (polyheads and isometric heads) are frequent.

### Physicochemical and physical properties

Virion Mr is about $210 \times 10^6$, buoyant density in CsCl is 1.50 g cm$^{-3}$, and $S_{20,w}$ about 1030S. Infectivity is ether and chloroform resistant.

### Nucleic acid

Genomes have a Mr about $120 \times 10^6$, corresponding to 48% of the particle weight. DNA contains 5-hydroxymethylcytosine (HMC) instead of cytosine (these nucleotides are glycosylated), a G+C content of 35%, and is circularly permuted and terminally redundant. The Enterobacteria phage T4 (T4) genome has been sequenced (168,903 bp).

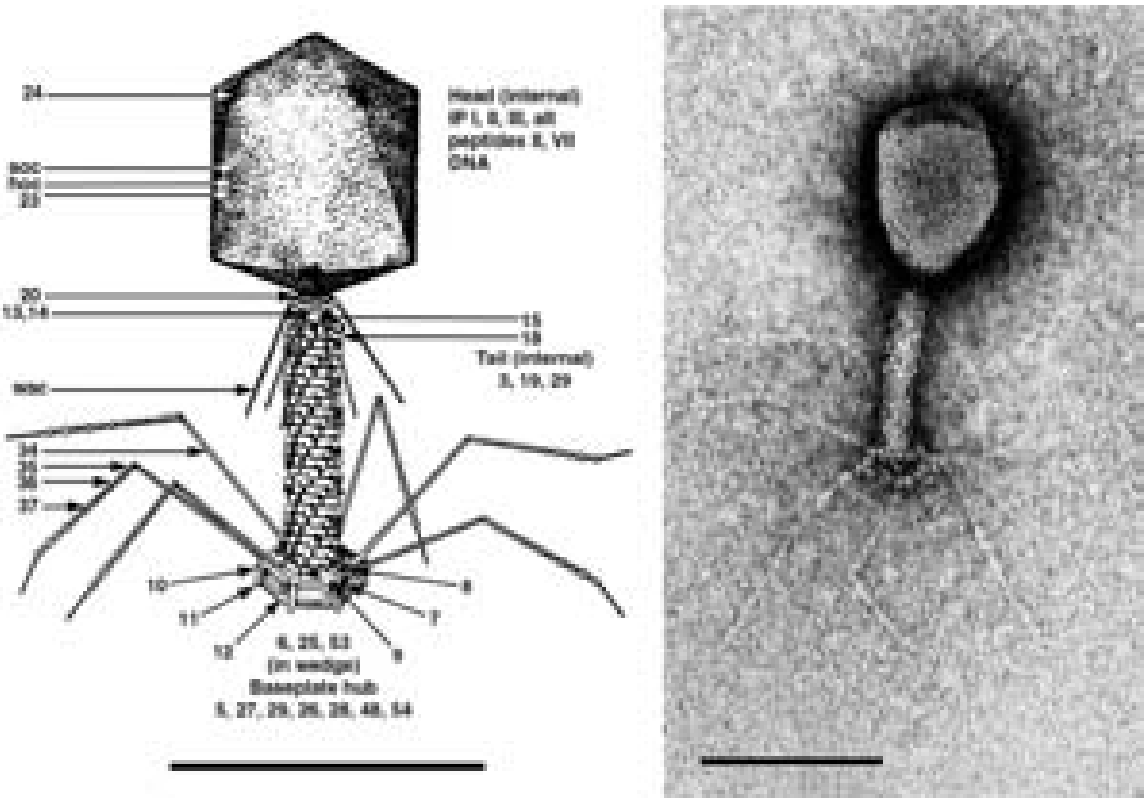


**Figure 1:** (Left) Diagram of Enterobacteria phage T4 (T4) showing detailed location of structural proteins. Head vertices consist of cleaved gp24. Gp20 is located at the head–tail connector. Collar and whiskers appear to be made of the same protein, gp*wac*. Sheath subunits (gp18) fit into holes in the base plate and short tail proteins (gp12) are shown in the quiescent state. The complex base is assembled from a central plug and six wedges. Tail fibers consist of three proteins. (From Eiserling, F.A. (1983). In: *Bacteriophage T4* (C.K. Mathews, E.M. Kutter, G. Mosig and P.B. Berget, Eds.), American Society for Microbiology, Washington, DC; with permission.) (Right) Negative contrast electron micrograph of T4 particle stained with uranyl acetate. The bars represent 100 nm.

### Proteins

T4 virions contain at least 49 proteins (8–155 kDa), including 1600–2000 copies of the major CP (43 kDa) and three proteins located inside the head. Various enzymes are encoded on the viral genome, e.g. type B (*E. coli* Pol II) DNA polymerase, numerous nucleotide metabolism enzymes and lysozyme (Figure 1).

### Lipids

None reported.

### Carbohydrates

Glucose is covalently linked to HMC in phage DNA.

## Genome organization and replication

The genome is linear and comprises about 300 genes. Morphopoietic genes generally cluster together, but this is not true for all cases, suggesting extensive translocation of genes during evolution. The genome is circularly permuted and has 1–3% terminal redundancy. For this reason the genetic map is usually represented as a circle (Figure 2). After infection, the host chromosome breaks down and viral DNA replicates as a concatemer, generating forked replicative intermediates from multiple origins of replication. Transcription is regulated in part by phage-induced modification of host RNA polymerase and proceeds in three temporal waves (early, middle, late). Heads,

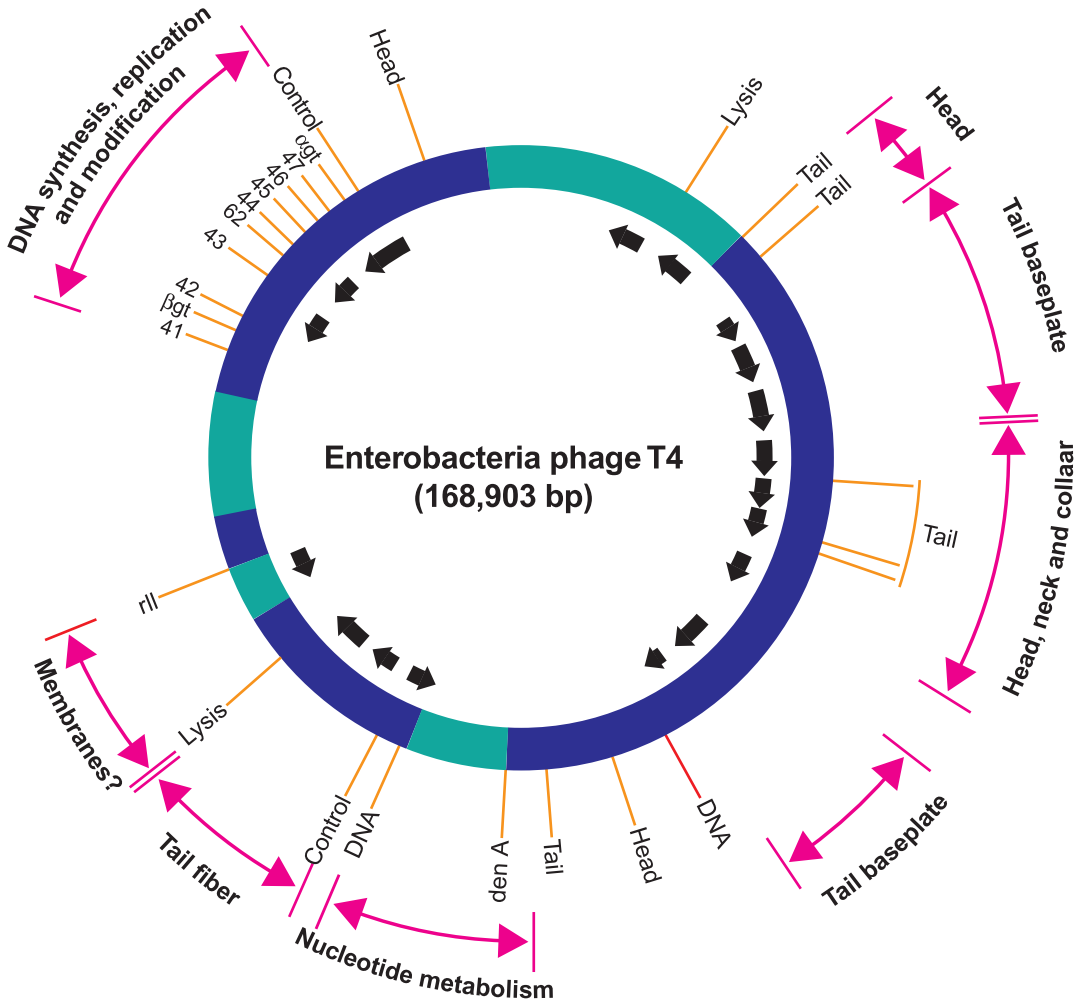


**Figure 2:** Simplified genetic map of Enterobacteria phage T4 (T4) showing clustering of genes with related functions, location of essential genes (solid bars) and direction and origin of transcripts (arrows). (From Freifelder, D. (Ed.) (1983). *Molecular Biology*, Science Books International, Boston, MA, and Van Nostrand Reinhold, New York, p. 614; with permission.)

tails and tail fibers are assembled in three separate pathways. Unique DNA molecules are packaged by a headful mechanism. Virions are assembled at the cell periphery.

## Antigenic properties

A group antigen and antigens defining eight subgroups have been identified by complement fixation.

## Biological properties

Phages are virulent, and infect enteric and related bacteria (Gammaproteobacteria). Their distribution is worldwide.

## Species demarcation criteria in the genus

Species differ in host range, capsid size, serological properties and, insofar as known, DNA sequences.

## List of species in the genus "T4-like viruses"

| | | |
|---|---|---|
| *Acinetobacter phage 133* | | |
| Acinetobacter phage 133 | | (133) |
| *Aeromonas phage 40RR2.8t* | | |
| Aeromonas phage 40RR2.8t | [AY375531] | (40RR2.8t) |
| (Aeromonas phage 40R) | | (40R) |
| *Aeromonas phage 65* | | |
| Aeromonas phage 65 | | (65) |
| *Aeromonas phage Aeh1* | | |
| Aeromonas phage Aeh1 | [AY266303] | (Aeh1) |
| *Enterobacteria phage SV14* | | |
| Enterobacteria phage D2A | | (D2A) |
| *Enterobacteria phage T4* | | |
| Enterobacteria phage C16 | | (C16) |
| *Pseudomonas phage 42* | | |
| Pseudomonas phage 42 | | (42) |
| *Vibrio phage nt-1* | | |
| Vibrio phage KVP20 | | (KVP20) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

## List of other related viruses which may be members of the genus "T4-like viruses" but have not been approved as species

| | | |
|---|---|---|
| Acinetobacter phage E4 | | (E4) |
| Acinetobacter phage E5 | | (E5) |
| Aeromonas phage 1 | | (Aer1) |
| Aeromonas phage 25 | | (25) |
| Aeromonas phage 31 | | (31) |
| Enterobacteria phage 1 (Phage aeI) | | (aeI) |
| Enterobacteria phage 11F | | (11F) |
| Enterobacteria phage 3 | | (3) |
| Enterobacteria phage 3T+ | | (3T+) |
| Enterobacteria phage 50 | | (50) |
| Enterobacteria phage 5845 | | (5845) |
| Enterobacteria phage 66F | | (66F) |
| Enterobacteria phage 8893 | | (8893) |
| Enterobacteria phage 9/0 | | (9/0) |
| Enterobacteria phage alpha1 | | (alpha1) |
| Enterobacteria phage DdVI | | (DdV1) |
| Enterobacteria phage F7 | | (F7) |
| Enterobacteria phage Kl3 | | (K13) |
| Enterobacteria phage RB42 | | (RB42) |
| Enterobacteria phage RB43 | | (RB43) |
| Enterobacteria phage RB49 | | (RB49) |
| Enterobacteria phage RB69 | [AY303349] | (RB69) |
| Enterobacteria phage SMB | | (SMB) |
| Enterobacteria phage SMP2 | | (SMP2) |

# GENUS "P1-LIKE VIRUSES"

Type species ***Enterobacteria phage P1***

## Distinguishing features of the genus

Virions produce head size variants. DNA is circularly permuted and terminally redundant, and is packaged from a pac site. The genome is linear, and phages can carry out generalized transduction. Prophages persist as plasmids.

## Virion properties

### Morphology
Virions have icosahedral heads about 85 nm in diameter; head size variants (ca. 47–65 nm) have been observed. Tails measure 228×18 nm in Enterobacteria phage P1 (P1) and vary in length from 170 to 240 nm in other members of the genus (i.e., Enterobacteria phage P1D (P1D) and Aeromonas phage 43 (43)). Tails have base plates and six 90 nm-long kinked fibers. Particles with contracted tails aggregate side-by-side by means of exposed tail cores.

### Physicochemical and Physical Properties
Phage P1 virion buoyant density is 1.48 g cm$^{-3}$.

### Nucleic Acid
Genomes comprise about 100 kbp and have a G+C content of 46%.

### Proteins
Virions contain 24–28 constitutive proteins (10–220 kDa), including a major coat protein of 44 kDa.

### Lipids
None reported.

### Carbohydrates
None reported.

## Genome organization and replication
The genome is linear and carries about 100 genes; related functions are often distributed over several genome regions. Prophage DNA is circular. The genome is circularly permuted and terminally redundant (8–12%), and includes a recombinational hot spot (*lox-cre*). The genome also has an invertible tail fiber segment of about 4 kbp (C-loop) that is homologous to the G-loop of Enterobacteria phage Mu (Mu). Virion DNA circularizes after injection. Replication starts at a single site and has a phase of theta replication and then a phase of sigma structures, suggesting a rolling-circle mechanism. Progeny DNA is cut from concatemers at a pac site.

## Antigenic properties
Phages P1, P2 and Mu share tail fiber antigens.

## Biological properties
Phages are temperate, can carry out generalized transduction, and infect enteric and related gram-negative bacteria. Prophages are maintained as plasmids (1–2 copies per cell) or integrate (rarely) at specific sites into the bacterial chromosome. Prophages are weakly UV-inducible. The invertible C-loop codes for two sets of tail fiber genes and provides a means of extending host range.

## Species demarcation criteria in the genus
Species differ in host range and tail length (phage P1, 228 nm; phage P1D, 240 nm; and phage 43, 170 nm).

## List of species in the genus "P1-like viruses"

| | | |
|---|---|---|
| *Aeromonas phage 43* | | |
| Aeromonas phage 43 | | (43) |
| *Enterobacteria phage P1* | | |
| Enterobacteria phage P1 | [AF234172] | (P1) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus "P1-like" viruses but have not been approved as species

| | |
|---|---|
| Acetobacter phage pKG-2 | (pKG-2) |
| Acetobacter phage pKG-3 | (pKG-3) |
| Enterobacteria phage D6 | (D6) |
| Enterobacteria phage PhiW39 | (PhiW39) |
| Enterobacteria phage j2 | (j2) |
| Pseudomonas phage PP8 | (PP8) |
| Vibrio phage PhiVP25 | (PhiVP253) |
| Vibrio phage P147 | (P147) |

## GENUS "P2-LIKE VIRUSES"

Type species *Enterobacteria phage P2*

### Distinguishing features

Virion DNA has cohesive ends. Transcription of virion structural genes is divergent.

### Virion properties

#### MORPHOLOGY

Phage heads are icosahedral, measure about 60 nm in diameter, and consist of 72 capsomers (60 hexamers and 12 pentamers; T = 7). Tails measure 135×18nm and have a collar and six short kinked fibers.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virion Mr is $58\times10^6$; buoyant density in CsC1 is 1.43 g $cm^{-3}$; and $S_{20,W}$ is 283S.

#### NUCLEIC ACID

Genomes are about 34 kbp, constitute about 48% of particle weight, and have a G+C content of 52%. The genomes of P2 and the related phages (HP1, HP2, 186, φCTX, Fels-2 and K139) have been sequenced.

#### PROTEINS

Virions contain at least 13 structural proteins (20–94 kDa), including 420 copies of the major CP (39 kDa). Amino acid sequences of the proteins of phages with completely sequenced genomes are available at GenBank and EMBL.

#### LIPIDS

None reported.

#### CARBOHYDRATES

None reported.

### Genome organization and replication

The genome is linear and non-permuted, has *cos* sites and includes about 40 genes. Transcription starts in the right half of the genome, has two phases (early and late) and depends on host RNA polymerase. Replication starts at a single site, is unidirectional and follows a modified rolling-circle mechanism. DNA is cut from concatemers at specific sites during packaging into proheads.

### Antigenic properties

Virions of phages P2, P1 (genus "P1-like viruses") and Mu (genus "Mu-like viruses") share tail fiber antigens.

## Biological properties

Phages are temperate, adsorb to the cell wall and infect enteric and related Gram-negative bacteria. Prophages may integrate at about 10 specific sites of the bacterial chromosome and are not UV-inducible. P2 acts as a "helper" for defective Enterobacteria phage P4 (P4) by providing head and tail genes for its propagation.

## Species demarcation criteria in the genus

Species differ in host range and DNA sequence.

## List of species in the genus "P2-like viruses"

| | | |
|---|---|---|
| *Enterobacteria phage P2* | | |
| Enterobacteria phage P2 | [AF063097] | (P2) |
| *Haemophilus phage HP1* | | (HP1) |
| Haemophilus phage HP1 | [U24159] | (HP1) |
| Haemophilus phage S2 | | (S2) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus "P2-like viruses" but have not been approved as species

| | | |
|---|---|---|
| Aeromonas phage 29 | | (29) |
| Aeromonas phage 37 | | (37) |
| Agrobacterium phage PIIBNV6 | | (PIIBNV6) |
| Caulobacter phage PhiCr24 | | (PhiCr24) |
| Enterobacteria phage 186 | [U32222] | (186) |
| Enterobacteria phage 299 | | (299) |
| Enterobacteria phage Beccles | | (Beccles) |
| Enterobacteria phage Pk2 | | (Pk2) |
| Enterobacteria phage W-Phi | | (W-Phi) |
| Haemophilus phage HP2 | [AY027935] | (HP2) |
| Pasteurella phage AU | | (AU) |
| Pseudomonas phage PhiCTX | [AB008550] | (PhiCTX) |
| Pseudomonas phage PsP3 | | (PsP3) |
| Rhizobium phage Phi-gal-1/R | | (Phi-gal-1/R) |
| Rhizobium phage WT1 | | (WT1) |
| Salmonella phage Fels-2 | | (Fels-2) |
| Vibrio phage X29 | | (X29) |
| Vibrio phage K139 | [AF125163] | (K139) |

# GENUS "MU-LIKE VIRUSES"

Type species ***Enterobacteria phage Mu***

## Distinguishing features

The viral genome contains two terminal, variable sequences of host DNA. It is able to integrate at virtually any site of the host chromosome and generate a wide range of mutations due to its unique mode of DNA replication (replicative transposition). Integration is required for establishment of lysogeny and for DNA replication during lytic development.

## Virion properties

### Morphology
Virions have icosahedral heads about 60 nm in diameter, contractile tails about 120 × 18 nm, a baseplate and six short fibers.

### Physicochemical and Physical Properties
Virion buoyant density in CsC1 is 1.49 g $cm^{-3}$.

### Nucleic Acid
The phage Mu genome is about 36–40 kbp, corresponding to about 40% of particle weight, has a G+C content of 50–51% and has been sequenced.

### Proteins
Particles have 12 structural proteins (20–76 kDa), including the major coat protein (33 kDa).

### Lipids
None reported.

### Carbohydrates
None reported

## Genome organization and replication
The phage Mu genome is linear and includes 55 genes. Related functions cluster together. The genome is non-permuted and heterogeneous, consisting of 36,717 bp of phage-specific DNA flanked at both ends by 0.5–3 kbp of covalently bound segments of host DNA. It contains an invertible segment of about 3 kbp (the G-loop) that is homologous to the invertible C-segment of Enterobacteria phage P1 (P1) DNA. Infecting DNA undergoes either lytic or lysogenic development. Both modes require (random) integration of phage DNA into host DNA, mediated by a phage-encoded transposase. Transcription starts at the left end of the genome and depends on host RNA polymerase. Replication may start at either end of the genome, is semi-conservative and occurs during transposition into new integration sites. Phage heads package integrated, non-concatemeric phage DNA and adjacent host DNA by an atypical headful mechanism. Progeny phage DNA is cut out of the host DNA 100–200 bp away from a phage-coded pac site.

## Antigenic relationships
Enterobacteria phages Mu, D108, P1 and P2 have some common tail fiber antigens.

## Biological properties
Viruses are temperate and can carry out generalized transduction. They infect enteric and (possibly) other related gram-negative bacteria. The invertible G-loop codes for two sets of tail fibers which provides a means of extending host range. Prophages are not inducible by irradiation with UV light.

## Species demarcation criteria in the genus
Not applicable.

## List of species in the genus "Mu-like viruses"

| | | |
|---|---|---|
| *Enterobacteria phage Mu* | | |
| Enterobacteria phage D108 | | (D108) |
| Enterobacteria phage Mu | [AF083977] | (Mu) |
| (Enterobacteria phage Mu-1) | | |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus "Mu-like viruses" but have not been approved as species

| | | |
|---|---|---|
| Pseudomonas phage B3 | [AF232233] | (B3) |
| Pseudomonas phage B39 | | (B39) |
| Pseudomonas phage D3112 | [AY394005] | (D3112) |
| Pseudomonas phage PM69 | | (PM69) |
| Vibrio phage VcA3 | | (VcA3) |

## GENUS "SPO1-LIKE VIRUSES"

Type species *Bacillus phage SPO1*

### Note on nomenclature

The "O" in the name SPO1 derives from Osaka, where the phage was isolated. It is therefore properly the letter "O" (oh) and not the numeral "0" (zero). However, in the published literature and earlier versions of this taxonomy, the names "SPO1" and SP01" are used interchangeably to refer to the same virus. As a consequence, database searches for SPO1 should always be done with both forms of the name.

### Distinguishing features

Members of this genus are large lytic phages. Heads show conspicuous capsomers. DNA is terminally redundant (but not circularly permuted), contains 5-hydroxymethyluracil and codes for a type A (*E. coli* Pol I) DNA polymerase.

### Virion properties

#### MORPHOLOGY

Virions have isometric, icosahedral heads of about 94 nm in diameter with conspicuous capsomers. Contractile tails measure 150×18 nm and have a small collar and a 60 nm-wide baseplate.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

SPO1 virion Mr is about $180\times10^6$; buoyant density in CsC1 is 1.54 g cm$^{-3}$; and $S_{20,w}$ is 794S.

#### NUCLEIC ACID

Genomes are linear, carry about 140–160 kbp and those that have been studied have a G+C content of 42%. Thymine is replaced by 5-hydroxymethyluracil in SPO1 DNA.

#### PROTEINS

Virions carry about 53 proteins (16 in the head and 28 in the tail and baseplate). Type A DNA polymerase is encoded in the phage genome.

#### LIPIDS

None reported.

#### CARBOHYDRATES

None reported.

### Genome organization and replication

The genome is linear and may contain as many as 200 genes. Related functions cluster together. The genome has a terminally redundancy of about 12 kbp, but is not circularly permuted. After infection, host syntheses are shut off and replication starts at two SPO1 DNA sites. Phage-encoded sigma factors are used to modify and appropriate host RNA polymerase for phage synthesis.

### Biological properties

Phages are virulent and so far have been characterized only from *Bacillus* and *Lactobacillus*. Distribution is worldwide.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus "SPO1-like viruses"

*Bacillus phage SPO1*
Bacillus phage SPO1 [FJ230960] (SPO1)

Species names are in italic script; names of isolates are in roman script. Sequence accessions [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus "SPO1-like viruses" but have not been approved as species

| | |
|---|---|
| Bacillus phage AR1 | (AR1) |
| Bacillus phage GS1 | (GS1) |
| Bacillus phage I9 | (I9) |
| Bacillus phage NLP-1 | (NLP-1) |
| Bacillus phage SP5 | (SP5) |
| Bacillus phage SW | (SW) |
| Bacillus phage Phi-e | (Phi-e) |
| Bacillus phage Phi25 | (Phi25) |
| Bacillus phage 2C | (2C) |
| Lactobacillus phage 222a | (222a) |

## GENUS "PHIH-LIKE VIRUSES"

Type species *Halobacterium phage PhiH*

### Distinguishing features

The host is an archaeon. Phage DNA has a pac site, and is circularly permuted and terminally redundant.

### Virion properties

#### MORPHOLOGY

Virions have isometric heads 64 nm in diameter, tails of 170×18 nm and short tail fibers.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Not known.

#### NUCLEIC ACID

Genomes are linear, about 59 kbp in size and have a G+C content of 64%. Cytosine is replaced by 5-methylcytosine.

#### PROTEINS

Virions have three major proteins (20, 45 and 70 kDa) and 10 minor components.

#### LIPIDS

None reported.

#### CARBOHYDRATES

None reported.

### Genome organization and replication

Genomes are partially circularly permuted and about 3% terminally redundant and have a pac site. *Halobacterium* phage ΦH DNA is markedly variable. All DNAs harbor one or more insertion

elements, and also include ordinary deletion and insertion variants. Early transcription is regulated by viral antisense mRNA. Replication results in formation of concatemers. Cutting of concatemers at pac sites is inaccurate and produces DNA molecules with imprecisely defined ends.

### Antigenic properties

Not known.

### Biological properties

Phages are temperate, specific for halobacteria and require the presence of 3.5 M NaCl. Prophages persist as plasmids and are not UV-inducible.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus "PhiH-like viruses"

*Halobacterium phage phiH*
Halobacterium phage phiH (PhiH)

Species names are in italic script; names of isolates are in roman script. Assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus "PhiH-like viruses" but have not been approved as species

Halobacterium phage Hs1 (Hs1) (Hs1)

## GENUS "PHIKZ-LIKE VIRUSES"

Type species ***Pseudomonas phage phiKZ***

### Distinguishing features

Originally isolated in 1975 in Kazakhstan, phage φKZ represents a genus of unusually large and complex virulent phages specifically infecting *Pseudomonas* species. Virions are very large and have tails surrounded by fibers, while the phage heads contain an inner body of which the function is not yet known. These phages typically have a broad host spectrum and form a very distant branch in the family *Myoviridae*. Their genomes (>280 kb) are circularly permuted and terminally redundant.

### Virion properties

#### MORPHOLOGY

Virions have extraordinarily large icosahedral heads of about 1445 Å in diameter and a 1600 Å long tail (Figure 3), which contracts to half of its original length upon infection. Ultrastructural studies of both the head and tail of φKZ revealed that the major capsid protein of φKZ (gp120) is organized into a surface lattice of hexamers with T = 27 triangulation. These are similarly shaped and sized as the hexametric building blocks of bacteriophages T4, ϕ29, P22 and HK97. A complex of several proteins was shown to occupy 11 pentameric vertices of the φKZ capsid.

The tail sheath is assembled around the tail tube and is composed of about 44 rings. Each 36.2 Å-thick ring consists of six gp29 subunits and is rotated by 22° with respect to the previous

**Figure 3:** Cryo-EM-based reconstruction of bacteriophage φKZ. Spiralling tail sheath vertices are highlighted. (Reconstruction courtesy of Andrei Fokine.)

ring. Analogously, despite the fact that the φKZ tail is much longer, the helical parameters of their contractile sheaths which surround their tail tubes are comparable to the T4 tail. The φKZ baseplate is significantly larger than that of T4 and has a rather flat, hexagonal shape with a diameter of 800 Å and a thickness of 350 Å. Nevertheless, φKZ, like T4, has a cell-puncturing device in the middle of its baseplate, which is likely composed of gp181. Six tail fibers are attached to the φKZ baseplate, each with an approximate length of 500 Å.

Finally, two discs with a radius of 160 Å are present in the neck part of the virus. The φKZ neck, like the neck of T4, may be composed of different proteins whose genes have not yet been identified.

### Physicochemical and Physical Properties

Phage 201$\phi$2-1 virion buoyant density is 1.37 g cm$^{-3}$. The infectivity is chloroform-resistant and heat-sensitive.

### Nucleic Acid

Their circularly permuted genomes (280–316 kb) are packed into a highly condensed series of layers, separated by 24 Å, that follow the contour of the inner wall of the capsid.

### Proteins

Virions of different genus members contain between at least 64 (φKZ) and 76 (phage 201$\phi$2-1) different particle proteins, several of which are subjected to proteolytic processing. Several proteins with unanticipated functions, including an RNA polymerase $\beta$ subunit, a helicase, a ligase, and an exonuclease, are encoded among the virion-associated genes and were identified as virion proteins.

## Genome organization and replication

Phages belonging to the "phiKZ-like viruses" have extraordinarily large genomes. The two fully sequenced members, *P. aeruginosa* phage φKZ and *P. chlororaphis* phage 201$\phi$2-1, carry genomes

of 280 and 316 kb, respectively encoding 306 and 461 genes. Comparison between the genomes of 201ϕ2-1 and φKZ revealed substantial conservation of the genome plan, and a large region with an especially high rate of gene replacement.

They have a remarkable low GC content (36% for φKZ) and only a minor fraction of the encoded genes exhibit similarity to proteins of known function. Most of these conserved gene products, such as dihydrofolate reductase, ribonucleoside diphosphate reductase, thymidylate synthase, thymidylate kinase and deoxycytidine triphosphate deaminase are involved in nucleotide metabolism. However, no virus-encoded DNA polymerase, DNA replication-associated proteins, or single stranded DNA-binding protein were found based on amino acid homology, and they may therefore be strongly divergent from known homologous proteins. The phiKZ genome also encodes six tRNAs specific for Met (AUG), Asn (AAC), Asp (GAC), Leu (TTA), Thr (ACA) and Pro (CCA). In contrast, only a single tRNA (Leu; anticodon = UAA) is encoded in 201ϕ2-1.

### Antigenic properties

None reported.

### Biological properties

Phages are virulent general transducers and specific for *Pseudomonas* bacteria, occur in water and soil, and have a worldwide distribution. Although there are only two sequenced members, over 20 similar giant phages with various geographic origins and infecting *P. aeruginosa*, *P. fluorescens*, *P. chlororaphis* and *P. stutzeri* have been reported. Plaques are clear and very small (0.1 mm in diameter).

### Species demarcation criteria in the genus

The identified species share limited or no DNA homology.

### List of species in the genus "phiKZ-like viruses"

| | | |
|---|---|---|
| *Pseudomonas phage phiKZ* | | |
| Pseudomonas phage phiKZ | [AF399011] | (phiKZ) |
| *Pseudomonas phage 201phi2-1* | | |
| Pseudomonas phage 201phi2-1 | [EU197055] | (201phi2-1) |
| *Pseudomonas phage Lin68* | | |
| Pseudomonas phage Lin68 | | (Lin68) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus "phiKZ-like viruses" but have not been approved as species

| | | |
|---|---|---|
| Pseudomonas phage EL | [AJ697969] | (EL) |

## GENUS "I3-LIKE VIRUSES"

Type species ***Mycobacterium phage I3***

### Distinguishing features

Mycobacteriophage I3 was isolated in 1970 from a soil sample using *Mycobacterium smegmatis* strain SN2 as host organism. This phage represents a genus of generally transducing myoviruses which specifically infect mycobacteria. They have an unusual morphology with a characteristic short tail, not resembling any other myoviruses. Their genomes (153–164 kb) are significantly larger than any other mycobacteriophage genome, and are circularly permuted. They form a very distinct branch in the *Myoviridae* family.

## Virion properties

### Morphology

The phage head of "I3-like viruses" has regular isometric symmetry with a diameter that varies between 74.7 (Bxz1) and 95.4 (Rizal) nm (Figure 4). As a consequence, the capsid volume fluctuates between 163690 and 340961 $nm^3$. Head capsomers can be seen on the edges of the triangular faces. The tail is distinctly shorter compared to other myoviruses, with an average length of 53.4 nm. A typical hollow tail tube running the length of the tail is apparent after contraction. Other components of the tail are a double collar at the neck of the virus particle and a cup-shaped baseplate.

### Physicochemical and physical properties

Like many other mycobacteriophage, phage I3 is sensitive to organic solvents. Maximum inactivation of 6 (LOG10 units) orders was observed with butanol, followed by 3 LOG10 inactivation with methanol. Less than 1 LOG10 inactivation was observed with chloroform, ether and benzene. Starting from these observations, Gope and Gopinathan (1982) demonstrated the presence of lipids in phage I3 comprises 42% DNA, 43% proteins and 15% lipids. Total lipid is composed of 69% phospholipids and 31% neutral lipids. The fatty acid composition of the phage differs markedly from that of its host, both in chain length and the degree of saturation. The phage lipid is mostly composed of saturated fatty acids of which more than 50% are short chain fatty acids.

### Nucleic acid

Members of this genus have circularly permuted genomes (153–164 kb). Phage I3 was shown to have modified bases (5-methylcytosine) and 13 to 14 single stranded gaps of about 10 nucleotides long.

### Proteins

An analysis of the phage protein content is not yet reported.

### Lipids

Unconfirmed.

### Carbohydrates

None reported.

## Genome organization and replication

Phage I3 is the first isolated phage of this genus, but its genome has not yet been fully sequenced. The first sequenced "I3-like virus" phage genome was that of Bxz1, which was recently followed by genomes of six other related phages. Phages of this genus have genomes with GC content varying between 64.7 and 65.4% and which encode between 218 and 229 ORFs. Maximal 8% (Bxz1) of the genome is noncoding sequence.

These phages have very few genes in common with other mycobacteriophages, and only 15% of the ORFs have a predicted function. One of the most striking genomic features is the presence of 26–31 tRNA genes, which is among the highest number identified in any bacteriophage sequenced thus far. The tRNAs in Bxz1 carry anticodons for 15 amino acids. In contrast to phage T4, the genes for tRNAs are not clustered, but are rather scattered in sets of small groups. It was shown that the Bxz1-specific tRNAs modulate the optimal expression of its proteins during development.

Other genomic features include the presence of adenylosuccinate synthase homologs among the Bxz1 subgroup (gp250) and its absence in the genome of Myrna. The latter possesses several proteins not present in the Bxz1 group, including the large hypothetical proteins gp187 (YP_002225066.1) and gp243 (YP_002225120.1), a putative nicotinate phosphoribosyltransferase (gp263, YP_002225140.1) and ATP-dependent protease (gp262, YP_002225139.1).

Bxz1 gp220 encodes a homolog of the human Ro protein, a major target of the autoimmune response in Lupus and Sjögren's diseases. This suggests that bacteriophages could act in concert with their hosts to stimulate autoimmunity.

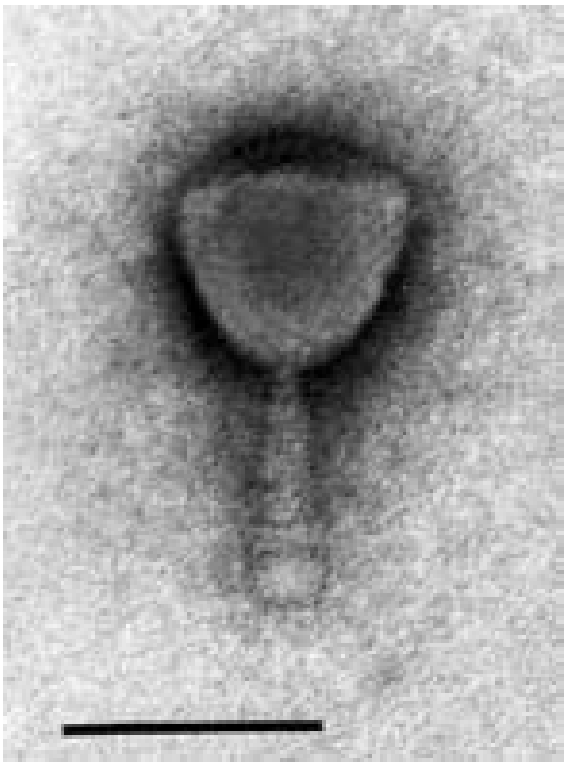

**Figure 4:** Electron micrographs of *Mycobacterium* phage I3. Phage particles were positively stained with uranyl acetate. The bar represents 100 nm. (Image courtesy of H. Ackermann.)

## Antigenic properties

None reported.

## Biological properties

Phages are virulent general transducers and specific for mycobacteria, occur in water and soil and have a worldwide distribution. Phage Bxz1 and its relatives form hazy plaques, although it is unclear whether the cellular survivors are uninfected cells, resistant mutants, or lysogens. Phage I3 was shown to be specific for cell-wall-associated glycopeptidolipid. A single methylated rhamnose was critical for phage binding. The presence of lipids may facilitate the phage–host interaction.

## Species demarcation criteria in the genus

Within the "I3-like viruses", two species can be delineated based on sequence similarity. Phages Cali, Catera, Rizal, ScottMcG show >90% protein similarity to Bxz1 and can be considered as one species (see below). Mycobacteriophage Myrna, with a genome of 164 kb, shares approximately 45% of proteins with the Bxz1 subgroup phages.

## List of species in the genus "I3-like viruses"

*Mycobacterium phage I3*

| | | |
|---|---|---|
| Mycobacterium phage I3 | | (I3) |
| Mycobacterium phage Bxz1 | [AY129337] | (Bxz1) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus "I3-like viruses" but have not been approved as species

None reported.

## List of unassigned species in the family *Myoviridae*

| | | |
|---|---|---|
| *Bacillus phage G* | | |
| Bacillus phage G | | (G) |
| *Bacillus phage PBS1* | | |
| Bacillus phage PBS1 | | (PBS1) |
| *Microcystis aeruginosa phage Ma-LMM01* | | |
| Microcystis aeruginosa phage Ma-LMM01 | [AB231700] | (Ma-LMM01) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the family *Myoviridae* but have not been approved as species

| | | |
|---|---|---|
| Acinetobacter phage A3/2 | | (A3/2) |
| Acinetobacter phage A10/45 | | (A10/45) |
| Acinetobacter phage BS46 | | (BS46) |
| Acinetobacter phage E14 | | (E14) |
| Actinomycetes phage SK1 | | (SK1) |
| Actinomycetes phage 108/016 | | (108/016) |
| Aeromonas phage Aeh2 | | (Aeh2) |
| Aeromonas phage 29 | | (29) |
| Aeromonas phage 51 | | (51) |
| Aeromonas phage 59.1 | | (59.1) |
| Alcaligenes phage A6 | | (A6) |
| Bacillus phage Bace-11 | | (Bace-11) |
| Bacillus phage CP-54 | | (CP-54) |
| Bacillus phage MP13 | | (MP13) |
| Bacillus phage SP3 | | (SP3) |
| Bacillus phage SP10 | | (SP10) |
| Bacillus phage SP15 | | (SP15) |
| Bacillus phage SP50 | | (SP50) |
| Bacillus phage Spy-2 | | (Spy-2) |
| Bacillus phage Spy-3 | | (Spy-3) |
| Bacillus phage SST | | (SST) |
| Clostridium phage HM3 | | (HM3) |
| Clostridium phage CE-beta | | (CE-beta) |
| Coryneform phage A19 | | (A19) |
| Cyanobacteria phage AS-1 | | (AS-1) |
| Cyanobacteria phage N1 | | (N1) |
| Cyanobacteria phage S-6(L) | | (S-6(L)) |
| Enterobacteria phage FC3-9 | | (FC3-9) |
| Enterobacteria phage Kl9 | | (Kl9) |
| Enterobacteria phage PhiP27 | [AJ298298] | (PhiP27) |
| Enterobacteria phage 01 | | (01) |
| Enterobacteria phage ViI | | (ViI) |
| Enterobacteria phage Phi92 | | (Phi92) |
| Enterobacteria phage 121 | | (121) |
| Enterobacteria phage 16-19 | | (16-19) |
| Enterobacteria phage 9266 | | (9266) |
| Halorubrum phage HF2 | [AF222060] | (HF2) |
| Lactobacillus phage fri | | (fri) |
| Lactobacillus phage hv | | (hv) |
| Lactobacillus phage hw | | (hw) |
| Listeria phage A511 | | (A511) |
| Listeria phage 4211 | | (4211) |

| | | |
|---|---|---|
| Mollicutes phage Br1 | | (Br1) |
| Pseudomonas phage PB-1 | | (PB-1) |
| Pseudomonas phage PS17 | | (PS17) |
| Pseudomonas phage PhiW-14 | | (PhiW-14) |
| Pseudomonas phage 12S | | (12S) |
| Rhizobium phage CM1 | | (CM1) |
| Rhizobium phage CT4 | | (CT4) |
| Rhizobium phage m | | (m) |
| Shigella phage SfV | [AF339141] | (SfV) |
| Xanthomonas phage XP5 | | (XP5) |
| Vibrio phage kappa | | (kappa) |
| Vibrio phage 06N-22P | | (06N-22P) |
| Vibrio phage VP1 | | (VP1) |
| Vibrio phage II | | (II) |

## Phylogenetic relationships within the family

No information available.

## Similarity with other taxa

See Order *Caudovirales*.

## Derivation of name

*Myo*: from Greek *my*, *myos*, "muscle", referring to the contractile tail.

## Further reading

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## Contributed by

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The authors acknowledge the contribution to the Eighth ICTV Report of Hendrix, R.W. and Casjens, S.R.

# Family *Podoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Podoviridae* |
| Genus | **"BPP-1-like viruses"** |
| Genus | **"Epsilon15-like viruses"** |
| Genus | **"LUZ24-like viruses"** |
| Genus | **"N4-like viruses"** |
| Genus | **"P22-like viruses"** |
| Genus | **"Phieco32-like viruses"** |
| Subfamily | *Autographivirinae* |
| Genus | **"PhiKMV-like viruses"** |
| Genus | **"SP6-like viruses"** |
| Genus | **"T7-like viruses"** |
| Subfamily | *Picovirinae* |
| Genus | **"AHJD-like viruses"** |
| Genus | **"Phi29-like viruses"** |

## Distinguishing features

Virions have short, non-contractile tails about 20 × 8 nm. Heads are assembled first and tail parts are added to them sequentially. The classification of the family has recently been revised and is based on available proteomic data, linked to biological properties that include morphology, genome organization, mechanisms of DNA packaging and presence of DNA or RNA polymerase genes on the genome. Phages were clustered in groups using various clustering algorithms and careful review of available literature and experimental data. Analysis and biological interpretation of the molecular correlations among all tailed phages (*Caudovirales*) with known genome sequence, allowed the relationship between two phages to be summarized in a single correlation score (= the relative number of homologous proteins between two sequenced phages). Using a cut-off score of 40% homologous proteins between two phages, phages cluster correctly within existing genera. Higher level relationships (20% correlation) supported the introduction of subfamilies. Subfamilies emphasize commonalities between related genera and prevent excessive subdivision during classification.

# Genus "BPP-1-like viruses"

Type species ***Bordetella phage BPP-1***

## Distinguishing features

The "BPP1-like viruses" include temperate viruses (infecting members of the Betaproteobacteria *Bordetella* [BPP-1, BMP-1 and BIP-1] and *Burkholderia* [BcepC6B]). They share the presence of genes specifying DNA polymerase I, a helicase and repressors.

BPP-1-like phages share some common gene products with the temperate VP2-related and "Epsilon15-like" phages. However, this homology is largely restricted to the proteins involved in morphogenesis and these phages have not therefore been grouped into a single subfamily.

## Virion properties

### Morphology

Phage BPP-1 particles have a 60 nm icosahedral capsid, a short tubular tail with a collar, and six tail fibers with bilobed globular ends (Figure 1).

### Physicochemical and physical properties

No information available.

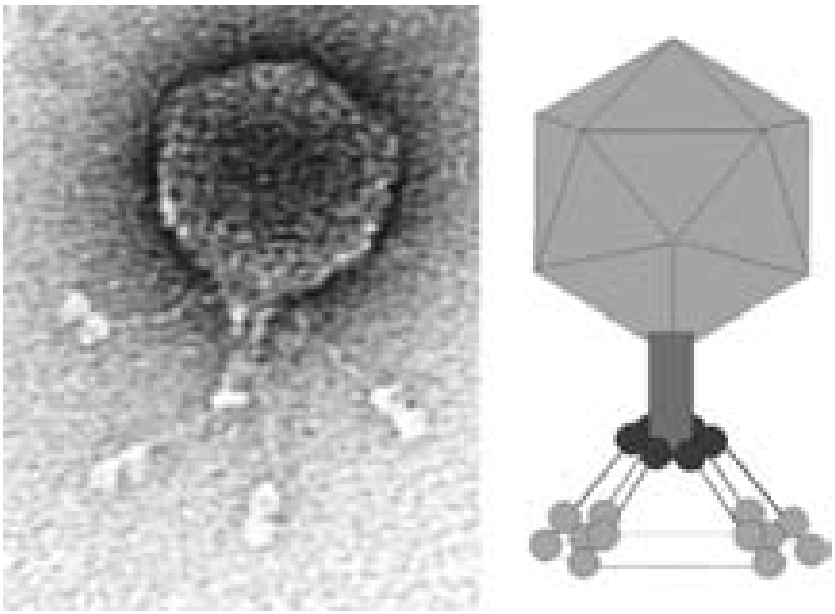

**Figure 1:** Phage BPP-1 negatively stained with 1% uranyl acetate, and an interpretive diagram. (From Liu *et al.* (2004). *J. Bacteriol.*, **186**, 1503-1517; with permission.)

### Nucleic acid
Linear doubled stranded DNA genomes of 42.4–42.7 kb with a G+C content of 65.2–65.4%.

### Proteins
Genomes encode 46–49 proteins. The *Bordetella* phages code for a 38.0 kDa protein Brt (gp5) with a homology to RNA-directed DNA polymerases (reverse transcriptase) which plays a role in tropism.

### Lipids
None known.

### Carbohydrates
None known.

## Genome organization and replication
The *Bordetella* phages integrate into host his-tRNA genes (attP TGGGGTGGCTGATG-GGACTCGAACCCA). The nature of the *Burkholderia* phage attP site is not known.

## Biological properties
No information available.

## Species demarcation criteria in the genus
The species have different hosts and only about 40% of the predicted gene products show proteomic correlation to other species. There is no genome-wide DNA homology between species.

## List of species in the genus "BPP-1-like viruses"

| | | |
|---|---|---|
| *Bordetella phage BPP-1* | | |
| Bordetella phage BPP-1 | [AY029185 = NC_005357] | (BPP-1) |
| *Burhkolderia phage BcepC6B* | | |
| Burhkolderia phage BcepC6B | [AY605181 = NC_005887] | (BcepC6B) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed

### List of other related viruses which may εbe members of the genus "BPP-1-like viruses" but have not been approved as species

None reported. BMP-1 was derived from BPP-1 through repeated passage on an alternative strain selecting for tropism switching. BPP-1 was isolated from a different lysogen of *Bordetella bronchiseptica.*



## Genus "Epsilon15-like viruses"

Type species *Salmonella phage epsilon15*

### Virion properties

**Morphology**
Heads are icosahedra with 60 hexamers and 11 pentamers, and measure about 70 nm in diameter in cryo-EM images. Tails are about 15 nm in length and there are six tailspikes (gp20) surrounding an external tail hub (17 × 14 nm; Figure 2).

**Physicochemical and physical properties**
No information available.

**Nucleic acid**
Genomes are about 39 kbp, linear and have a G+C content of 48–50%.

**Proteins**
Only the virions of ε15 have been studied in depth. They possess six structural proteins as shown by SDS-PAGE and mass spectrometry. These include 420 copies of the major capsid protein (gp7; 36.8 kDa, but maybe cleaved and cross-linked), 140 copies of the putative head stabilization protein gp10 (12.2 kDa), 12 copies of the portal protein (gp4; 61.7 kDa), putative hub-associated proteins gp15 (91 kda), gp16 (67.4 kDa) and gp17 (100 kDa); and, tailspike protein gp20 (115.7 kDa; possesses endorhamnosidase activity). Both ε15 and φV10 carry O-antigen modification cassettes.

**Lipids**
None known.

**Carbohydrates**
None known.

### Genome organization and replication

The genomes are linear, terminally redundant and circularly permuted with approximately 40 kbp (48–50% G+C). They encode 53 proteins, and no tRNAs. Simplistically speaking, the genes are arrayed in two transcriptional units, one on the negative strand involved in regulation and recombination, and the other on the positive strand, involved in packaging, morphogenesis, lysis and integration. The attachment sites of these phages are a subset of ATTGAGTGGGAATGATT which overlap the 3′ end of *guaA*. This site is located upstream of the integrase gene. Both phages carry lipopolysaccharide-modifying cassettes.

### Biological properties

Phages are temperate and infect *Salmonella* and *Escherichia coli* strains. Distribution is unknown.

### Species demarcation criteria in the genus

The species have different hosts and only about 40% of the predicted gene products show proteomic correlation to other species. There is no genome-wide DNA homology between species.

### List of species in the genus "Epsilon15-like viruses"

*Salmonella phage epsilon15*
Salmonella phage ε15 [AY150271 = NC_004775] (Eta15)

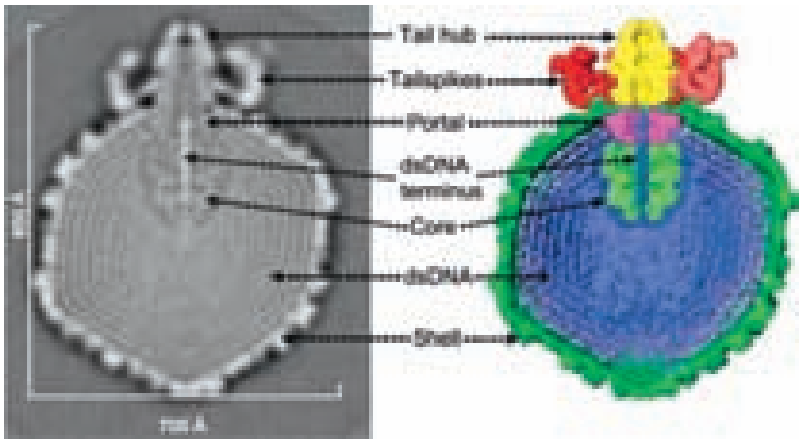


**Figure 2:** Electron micrograph of *Salmonella* phage ε15 stained with phosphotungstic acid. The bar represents 100 nm. (Courtesy of Wen Jiang and Wah Chiu.)

*Escherichia phage PhiV10*
Escherichia phage V10 [DQ126339 = NC_007804] (V10 or φV10)

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed
It is quite likely that φV10 is identical to phage V10 which is part of the O157 typing set. φV10 and ε15 are related to a genomic island designated as PHIAP20 in *E. coli* APEC O1.

### List of other related viruses which may be members of the genus "Epsilon15-like viruses" but have not been approved as species

None reported.

## Genus "LUZ24-like viruses"

Type species ***Pseudomonas phage LUZ24***

### Virion properties

#### Morphology

Virions carry an icosahedral head with a 63 nm diameter and a short tail (12 × 8 nm). Decoration proteins protruding from the phage capsid can be distinguished (Figure 3).

#### Physicochemical and physical properties

No information available.

#### Nucleic acid

The "LUZ24-like viruses" contain linear dsDNA of around 45 kb. DNA of *Pseudomonas* phage LUZ24 was shown to have terminal direct repeats of 184 bp.

#### Proteins

Only a limited number of protein functions could be predicted by similarity searches. Using mass spectrometry, nine virion-associated proteins have been identified, delineating the structural region of LUZ24 from gene product 49 to gene product 65.

#### Lipids

None known.

#### Carbohydrates

None known.

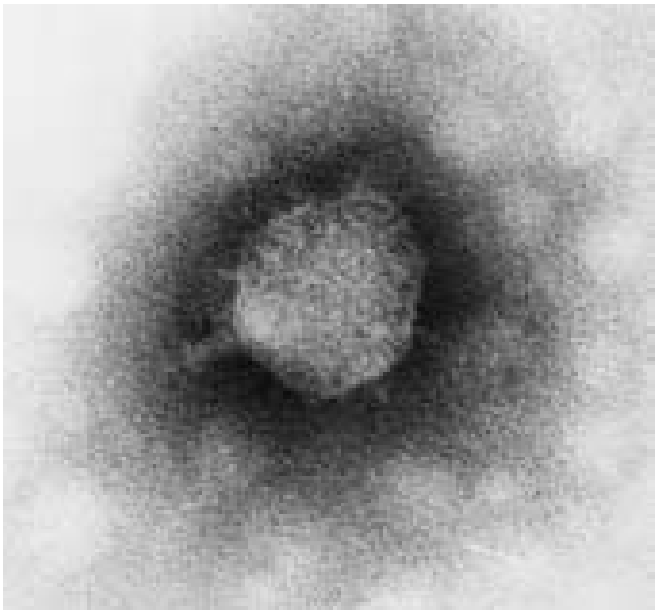

**Figure 3:** Electron micrograph of *Pseudomonas* phage LUZ24. (Courtesy Hans-Wolfgang Ackermann.)

## Genome organization and replication

The genome of LUZ24 and PaP3 share an overall nucleotide sequence identity of 71%. Eight insertions/deletions are present and 88% of the encoded gene products are related. Type species LUZ24 encodes 68 proteins, 47 genes are arranged in rightward orientation while 21 are leftward. Two tRNA genes (tRNAAsn and tRNAPro) are present at the right end of the LUZ24 genome, compared to four in the genome of PaP3. LUZ24 shares its bidirectional genome organization with a vast number of virulent phages like cyanophages P60, Pf-WMP3 and Pf-WMP4, Roseophage SIO1 and vibriophage VpV262 in the subfamily *Autographinae*. These phage also share a strikingly conserved order of structural genes suggesting that these features are of very ancient origin.

## Biological properties

Host range screenings on environmental and clinical *P. aeruginosa* strains revealed that LUZ24 lyses 36 out of 123 (29%) strains. Small and turbid plaques (1 mm) arise after infection of *P. aeruginosa* PAO1, although the phage extracted from such plaques appears not to differ from normal in latent period or burst size. Stable lysogenic *P. aeruginosa* clones could not be isolated. No phages could be induced, and no integrated LUZ24 DNA sequences could be demonstrated by PCR or restriction analysis. These data suggest that the phages are lytic.

## Species demarcation criteria in the genus

The overall DNA sequence similarity between LUZ24 and PaP3 genomes is 71%; 88% of the encoded gene products are homologous.

## List of species in the genus "LUZ24-like viruses"

| | | |
|---|---|---|
| *Pseudomonas phage LUZ24* | | |
| Pseudomonas phage LUZ24 | [AM910650 = NC_010325] | (LUZ24) |
| *Pseudomonas phage PaP3* | | |
| Pseudomonas phage PaP3 | [AY078382 = NC_004466] | (PaP3) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus "LUZ24-like viruses" but have not been approved as species

None reported.

## Genus "N4-like viruses"

Type species *Escherichia phage N4*

### Virion properties

#### Morphology

Virions have icosahedral heads about 70 nm in diameter and short tails 10 nm in length, with several short fibers originating from the junction between the head and tail (Figure 4).

#### Physicochemical and physical properties

Particle weight is about $84\times10^6$. Buoyant density in CsCl is 1.500 g $cm^{-3}$. Lipids have not been detected in the established species.

#### Nucleic acid

Virions contain a single molecule of ds DNA of 70,153 bp of unique sequence flanked by 390–440 bp direct terminal repeats (variable lengths within the population). There are also short 3′ ssDNA extensions at both ends. G+C content is 41.3%.

#### Proteins

Virions contain at least 10 structural proteins. The major CP (ca. 500 molecules per phage) has a subunit mass of 44.0 kDa.

#### Lipids

Lipids (2.4%) have been reported in phage Sd, a putative member of this genus.

#### Carbohydrates

None reported.

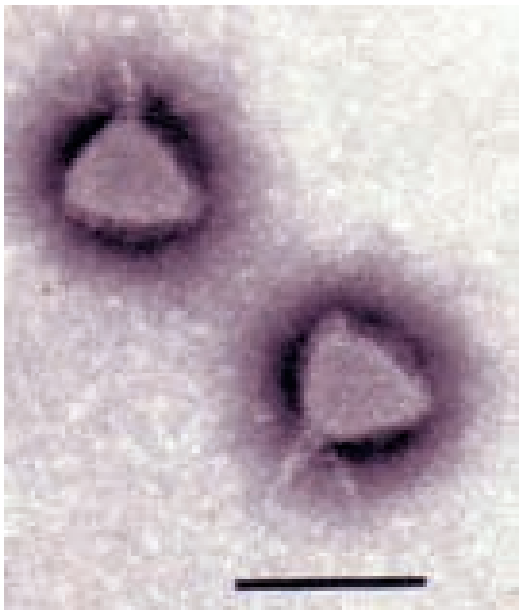

**Figure 4:** Electron micrograph of N4 stained with phosphotungstate. The bar represents 100 nm. (Courtesy Hans-Wolfgang Ackermann.)

### Genome organization and replication

The physical map is linear, but a genetic linkage map has not been determined for technical reasons. There are 72 protein-coding genes identified which occupy 94% of the DNA sequence. Transcription of N4 DNA is carried out by sequential activity of three different RNA polymerases. Early transcription is performed by the viral RNA polymerase, a large (3500 aa) single subunit enzyme that is present in 1–2 copies in the virion and injected with the DNA during infection. Early proteins include a two-subunit RNA polymerase responsible for middle transcription. Middle proteins include a 98 kDa DNA polymerase and other DNA replication functions that replicate phage DNA in cooperation with some host functions. Late transcription is carried out by the host (*E. coli*) RNA polymerase. Early and middle genes occupy a contiguous block in the left half of the genome and are transcribed rightwards. Late genes occupy the right half of the genome and are transcribed leftwards. Other noteworthy genes include three tRNA genes (Asn, Thr, Pro), a gene encoding a dCTP deaminase, homologs of the *rIIA* and *rIIB* genes of phage T4, and a homolog of the gene *17* of temperate phage P22. Assembling particles form intracellular crystal-like arrays of phage heads.

### Biological properties

Phages are virulent. Infection is lytic. Host range was originally thought to be restricted to enterobacteria (*E. coli*), but related *Pseudomonas* phages have been isolated recently.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus "N4-like viruses"

*Escherichia phage N4*
Escherichia coli bacteriophage N4 [EF056009 = NC_008720] (N4)

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus "N4-like viruses" but have not been approved as species

| | | |
|---|---|---|
| Silicibacter phage DSS3Phi2 | [FJ591093 = NC_012697] | (DSS3Phi2) |
| Sulfitobacter phage EE36Phi1 | [FJ591094 = NC_012696] | (EE36Phi1) |
| Pseudomonas phage LIT1 | [FN422399 = NC_013692] | (LIT1) |
| Pseudomonas phage LUZ7 | [FN422398 = NC_013691] | (LUZ7) |
| Enterobacteria phage PEV2 | | (PEV2) |
| Enterobacteria phage Sd | | (Sd) |

## GENUS "P22-LIKE VIRUSES"

Type species ***Enterobacteria phage P22***

### Distinguishing features

Virions have short tails which have six prominent tail spikes. DNA is circularly permuted, terminally redundant, and packaged from a pac site by a headful mechanism. Phages can carry out generalized transduction. The genetic map is circular.

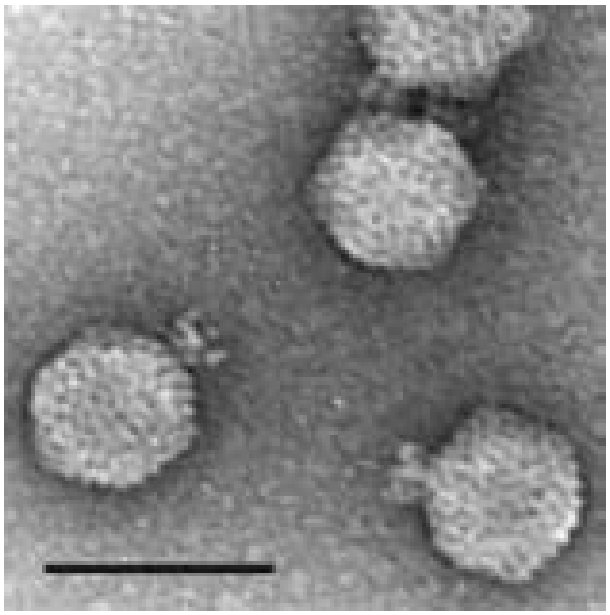

**Figure 5:** Phage P22 stained with uranyl acetate. The bar represents 100 nm bar. (Courtesy Sherwood Casjens.)

## Virion properties

### Morphology

P22 virions have isometric icosahedral heads with 72 faintly visible capsomers (60 hexamers, 12 pentamers; T = 7) and 59 to 63 nm (three-fold and five-fold axes, respectively) in diameter (Figure 5). Tails are 18 nm long and have six tail spikes.

### Physicochemical and physical properties

Virion buoyant density in CsC1 is about 1.50 g $cm^{-3}$ and $S_{20,w}$ is 500S. Infectivity is ether- and chloroform-resistant.

### Nucleic acid

The P22 genome is linear double stranded DNA of 41,754 bp. The chromosome in virions ranges from 42.7 to 44.1 kbp, corresponds to about 55% of particle weight, and has a G+C content of 47%. Other members of the genus have genome sequences of 38–42 kbp with similar organization and similar transcriptional programs.

### Proteins

P22 virions contain nine structural proteins: 415 copies of the major capsid protein (gp5; 46.7 kDa), 12 copies of the portal protein gp1 (82.7 kDa), two protein, gp4 (18.0 kDa) and gp10 (52.5 kDa), which are part of the hub, gp7 (23.4 kDa), gp16 (64.4 kDa) and gp20 (50.1 kDa) which are pilot/injection proteins, the tail needle protein gp26 (three copies, 24.7 kDa) and 18 copies of the tailspike/endorhamnosidase protein gp9 (71.9 kDa). Other members of the genus are similar.

### Lipids

None known.

### Carbohydrates

None known.

## Genome organization and replication

The P22 genetic map is circularly permuted, has terminal repeats of about 1600 bp (3.8% of the genome), and comprises at least 65 genes. The genome sequence is partially (13.5%) similar to that

of coliphage λDNA and common sequences are scattered across the right half of the genome. Other genus members have similar but different relationships with other "lambda-like viruses". The integration system is dispensable for lytic growth. Transcription starts with regulatory genes and proceeds in three partly overlapping waves that are very similar to those of the lambda-like viruses. Replication starts at a single site and involves replication by a θ (theta) structure mechanism that switches at late times to a rolling-circle mechanism (σ, sigma replication). Progeny DNA is cut from concatemers at a pac site and packaged by a headful mechanism.

## Antigenic properties

No group antigens are reported.

## Biological properties

Phages are temperate and can carry out generalized transduction with lysogenic conversion ability. Members of the genus infect *Enterobacteria* (*Escherichia*, *Salmonella* and other Gammaproteobacteria) and have, under suitable conditions, very high (up to 500) burst sizes. Phage genomes integrate at specific sites in the bacterial genome and are UV-inducible.

## Species demarcation criteria in the genus

Lack of DNA homology between species.

## List of species in the genus "P22-like viruses"

| | | |
|---|---|---|
| *Enterobacteria phage P22* | | |
| Enterobacteria phage P22 | [BK000583 = NC_002371] | (P22) |
| Salmonella phage ε34 | [EU570103 = NC_011976] | (ε34) |
| Enterobacteria phage ST104 | [AB102868 = NC_005841] | (ST104) |
| *Salmonella phage HK620* | | |
| Salmonella phage HK620 | [AF335538 = NC_002730] | (HK620) |
| Salmonella phage ST64T | | |
| Salmonella phage ST64T | [AY052766 = NC_004348] | (ST64T) |
| *Shigella phage Sf6* | | |
| Shigella phage Sf6 | [AF547987 = NC_005344] | (Sf6) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus "P22-like viruses" but have not been approved as species

| | |
|---|---|
| Aeromonas phage Aa-1 | (Aa-1) |
| Azotobacter phage A12 | (A12) |
| Enterobacteria phage L | (L) |
| Enterobacteria phage LP7 | (LP7) |
| Enterobacteria phage MG40 | (MG40) |
| Enterobacteria phage PSA78 | (PSA78) |
| Hyphomicrobium phage HyPhi30 | (HyPhi30) |
| Pseudomonas phage 525 | (525) |
| Vibrio phage O6N-72P | (06N-72P) |

Other highly likely members of this genus include phages: CUS-3 (CP000711), c341 (FJ000341 = NC_013059), SE1 (DQ003260 = NC_011802), SETP1, SETP8, SETP10, SETP14, SETP15, SETP16 and MG178.

# Genus "Phieco32-like viruses"

Type species *Enterobacteria phage Phieco32*

## Distinguishing features

A comparatively rare morphotype among the *Podoviridae*, PhiEco32 possesses an elongated head and a unique mechanism of transcription.

## Virion properties

### Morphology

This virus displays C3 type morphology with an elongated head (ca. 145 × 44 nm) and a short tail (ca. 13 × 8 nm) which may carry short kinked tail fibers (Figure 6).

### Physicochemical and physical properties

Not known.

### Nucleic acid

The linear double stranded DNA genome has 77,554 bp with 193 bp terminal direct repeats (42.3% G+C). It comprises 128 ORFs and a gene for arginyl-tRNA (codon: AGA).

### Proteins

Ten virion proteins were identified by SDS-PAGE and mass spectrometry: gp8 (portal), gp11 (major capsid), gp14 and gp15 (tail fibres) and gp13, gp18-19, gp25-26 and gp58. The capsid protein may also exist as a larger frameshift derivative.

### Lipids

None known.

### Carbohydrates

None known.

## Genome organization and replication

The phiEco32 genome encodes enzymes for nucleotide metabolism (gp34, deoxynucleoside monophosphate kinase; gp37, high-affinity ADP-ribose binding protein (Macro_Poa1p_like); gp64, thymidylate synthase; gp65 a thioredoxin-like protein, and gp72 a dCTP deaminase) and DNA replication (gp62, a DNA-binding protein; gp33, 5′-3′ exonuclease; gp74, 3′-5′ exonuclease; gp75, primase/helicase; gp62, DNA ligase and a Family A (pfam00476) DNA polymerase (gp53).

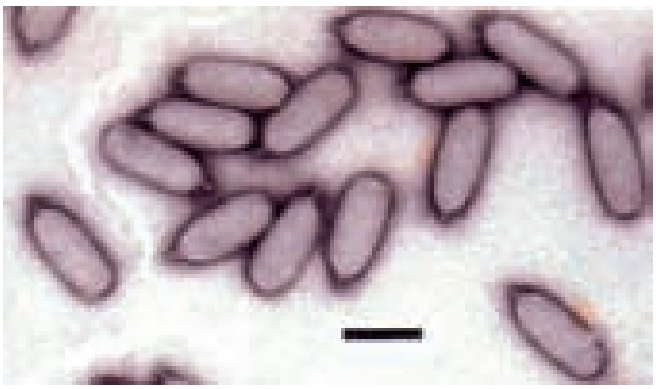

**Figure 6:** Electron micrograph of phiEco32 stained with phosphotungstate in the presence of bacitracin. The bar represents 100 nm. (Courtesy Hans-Wolfgang Ackermann.)

This phage genome possesses several sequences bearing strong sequence similarity to the consensus sigma-70 RNA polymerase promoters of the host bacterium (TTGACA-N15-17-TATAAT). It encodes an ECF-like sigma factor (gp36, 25.8 kDa) which is found associated with RNA polymerase (RNP) isolated from phage-infected cells, along with phage protein gp79 (9.5 kDa). The promoters recognized by this modified RNP are not known.



### Biological properties

None reported.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus "Phieco32-like viruses"

*Enterobacteria phage Phieco32*
Enterobacteria phage PhiEco32 [EU330206 = NC_010324] (phiEco32)

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus "Phieco32-like viruses" but have not been approved as species

Recently *Salmonella* phage 7-11 was sequenced and suggested as a member of this genus.

## SUBFAMILY *AUTOGRAPHIVIRINAE*

### Taxonomic structure of the subfamily

Subfamily *Autographivirinae*
Genus **"PhiKMV-like viruses"**
Genus **"SP6-like viruses"**
Genus **"T7-like viruses"**

### Distinguishing features

The T7, SP6 and PhiKMV-like viruses have long been recognized as being related and are often termed members of a "supergroup". Based on comparative proteomics data, this grouping was established as a subfamily. The origin of the name for this subfamily refers to the "auto-graphein" or "self-transcribing" phages which encode their own (single subunit) RNA polymerase, a common characteristic among the phages of this subfamily. More importantly, these phages share a common general genome organization, with genes encoded solely on the Watson strand.

Within this subfamily, distinctive features warrant a separation into different genera. Phages SP6 and K1-5 have been considered an "estranged subgroup of the T7-like viruses". Also, the "phiKMV-like viruses" carry their single-subunit RNA polymerase gene adjacent to the structural protein region of the genome instead of in the early (host conversion) region, and they lack readily identifiable phage promoters. This suggests a different transcription scheme for "phiKMV-like viruses" compared to the "T7-like viruses".

It is possible that the cyanophages P60, Syn5 and P-SSP7 represent one or more additional genera in this subfamily; however, a reevaluation of the P60 genome is necessary to perform an accurate analysis. *Synechococcus* phage syn5 and *Prochlorococcus* phage P-SSP7, share a correlation to P60 (36% and 35% respectively). These phage are therefore treated as unassigned species within the subfamily.

Bacteriophage SIO1 is related to this subfamily from an evolutionary perspective, but falls outside it because of the absence of a phage-encoded RNA polymerase and greater differences at the genome organization level. The same argument can be given for marine bacteriophage VpV262 infecting *Vibrio parahaemolyticus*. Genomic data indicates that an ancestral component of a T7 viral supergroup is conserved in the marine environment, but overall genomic differences and the lack of a phage-encoded RNA polymerase places this virus outside the subfamily.

Phage KSY1 does contain a phage-encoded RNA polymerase and a T7-like transcription scheme, but its overall morphology, genome size and proteome make-up does not warrant a classification within this subfamily.

# Genus "PhiKMV-like viruses"

Type species ***Pseudomonas phage phiKMV***

## Distinguishing features

The members of this genus share no DNA sequence homology whatsoever to other members of the *Autographivirinae*. While the general genome organization is conserved, the CoreGenes algorithm only detects nine clear homologous gene products between ϕKMV and T7 (http://binf.gmu.edu:8080/CoreGenes2.0/).

## Virion properties

### Morphology

Phage morphology is conserved compared to "T7-like viruses" and consists of a short tail and an icosahedral capsid of about 60 nm in diameter. Preliminary cryo EM reconstructions of ϕKMV does suggest the presence of head-spike proteins associated with the main capsid proteins.

### Physicochemical and physical properties

φKMV virion Mr is about $48 \times 10^6$, buoyant density in CsCl is $1.50\,g\,cm^{-3}$, and $S_{20,w}$ is about 510S. Infectivity is ether and chloroform resistant.

### Nucleic acid

The double-stranded DNA genome varies around 42 kb in size, with direct terminal repeats (between 200 and 500 bp). The genome has few type II restriction endonuclease recognition sites but does contain site-specific nicks, also observed in *Escherichia coli* phage T5.

### Proteins

The structural proteome of the type species φKMV has been determined experimentally by mass spectrometry, as have several of its enzymes (DNA ligase, lysins). The structural (virion-associated) peptidoglycan hydrolase domain, located at the C-terminus of the predicted internal protein gp36, has a high refolding capacity and is thermoresistant. This property may be of relevance upon DNA ejection into the host cell, as proposed for phage T7 (folding carpenter's rule model).

### Lipids

None known.

### Carbohydrates

None known.

## Genome organization and replication

While the "T7-like viruses" and the "phiKMV-like viruses" share a common, general genome organization, typical of the *Autographivirinae*, individual protein homology is limited to a core set of proteins. In addition, φKMV and LKA1 carry a single-subunit RNA polymerase gene adjacent to

the structural genome region (instead of in the early region in "T7-like viruses"), and they lack conserved phage promoters. These features suggest major differences in the transcription scheme for "phiKMV-like viruses".



### Biological properties

Phages are highly virulent with a short infection cycle, and infection typically leads to the formation of large (ca. 5 mm), clear plaques. Similar to many *Pseudomonas* phages, infection by φKMV, LKD16 is pili-dependent.

### Species demarcation criteria in the genus

Phages φKMV and LKA1 have similar genome organization and are similar in 45% of their gene products. Based on DNA sequence analysis, phages φKMV, LKD16, φkF77 and LUZ19 are clearly related. Although LKD16 is closely related to φKMV and shows strong similarity in 90% of its predicted ORFs, differences in host range and infectivity parameters have been shown, albeit insufficient to warrant the establishment as different species . Partial/complete genome sequencing on bacteriophages PT2, PT5 and PS028, MMA1, MMA2, FMV, PBK, LUZ24 show these six phages are also close relatives of φKMV.

### List of species in the genus "PhiKMV-like viruses"

| | | |
|---|---|---|
| *Pseudomonas phage phiKMV* | | |
| Pseudomonas phage φKMV | [AJ505558 = NC_005045] | (PhiKMV) |
| *Pseudomonas phage LKA1* | | |
| Pseudomonas phage LKA1 | [AM265639 = NC_009936] | (LKA1) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus "PhiKMV-like viruses" but have not been approved as species

| | | |
|---|---|---|
| Pseudomonas phage LKD16 | [AM265638 = NC_009935] | (LKD16) |
| Pseudomonas phage phikF77 | [FN263372 = NC_012418] | (PhikF77) |
| Pseudomonas phage phi-2 | [FN594518 = NC_013638] | (Phi-2) |
| Pseudomonas phage PT2 | [EU236438 = NC_011107] | (PT2) |
| Pseudomonas phage PT5 | [EU056923 = NC_011105] | (PT5) |
| Pseudomonas phage LUZ19 | [AM910651 = NC_010326] | (LUZ19) |
| Caulobacter phage Cd1 | [GU393987] | (Cd1) |
| Ralstonia phage RSB1 | [AB451219 = NC_011201] | (RSB1) |
| Klebsiella phage KP34 | [GQ413938 = NC_013649] | (KP34) |
| Vibrio phage VP93 | [FJ896200 = NC_012662] | (VP93) |

## GENUS "SP6-LIKE VIRUSES"

Type species ***Enterobacteria phage SP6***

### Distinguishing features

This group of viruses is distinguished from other members of the *Caudovirales* by tailspike-like proteins possessing hydrolytic activities which are encoded at the extreme right end of the genome. Although there are substantial similarities to the T7-like viruses, there are key proteomic differences shown by CoreGenes analysis.

## Virion properties

### Morphology
Bacteriophages SP6 and K1-5 possess icosahedral heads (65–68 nm in diameter). The tail is short and stubby (like T7) and has been described as possessing an "irregular bushy structure". Additional appendages are apparent, perhaps reflecting the cell envelope hydrolytic activities carried by both SP6 and K1-5 virions.

### Physicochemical and physical properties
Infectivity is ether- and chloroform-resistant.

### Nucleic acid
Genomes are, on average, 44.7 kbp (43769 bp for SP6), have a G+C content of approximately 47%, and are non-permuted and terminally redundant (the redundancies range from 174–288 bp).

### Proteins
Structural proteins have been predicted based on the relationship to other phage in the subfamily *Autographivirinae.*

### Lipids
None known.

### Carbohydrates
None known.

## Genome organization and replication
SP6-like viruses are not fundamentally different from the T7-like viruses in their gene order: RNA polymerase, helicase/primase, DNA polymerase, exonuclease, portal, scaffold, major capsid, tail, internal proteins, terminase subunits. The SP6-like viruses are recognized as a separate genus on the basis of CoreGenes analysis, showing several distinct differences. They lack T7gp2.5 (single-stranded DNA-binding protein) homologs; the ligase homolog (SP6 gp24) is located immediately upstream of the morphogenesis genes (in T7-like viruses gp1.3 is an early gene); and, the packaging proteins (SP6 gp39 and gp40) are contiguous on the genomes while in the T7-like viruses the holin-lysin complex is found between them (T7 gp18 & gp19). The DNA polymerase of SP6 (gp13) is significantly larger (849 amino acids) than that of T7 (gp5; 704 amino acids).

## Biological properties
Infect *Salmonella*, *Escherichia coli* and *Erwinia* strains.

## Species demarcation criteria in the genus
Species are separated by host range and supported by proteomic analyses.

## List of species in the genus "SP6-like viruses"

| | | |
|---|---|---|
| *Enterobacteria phage SP6* | | |
| Enterobacteria phage SP6 | [AY288927 = NC_004831] | (SP6) |
| *Enterobacteria phage K1-5* | | |
| Enterobacteria phage K1-5 | [AY370674 = NC_008152] | (K1-5) |
| *Enterobacteria phage K1E* | | |
| Enterobacteria phage K1E | [AM084415 = NC_007637] | (KE1) |
| *Enterobacteria phage K5* | | |
| Enterobacteria phage K5 | | (K5) |
| *Erwinia amylovora phage Era103* | | |
| Erwinia phage Era103 | [EF160123 = NC_009014] | (Era103) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

**List of other related viruses which may be members of the genus "SP6-like viruses" but have not been approved as species**

None reported.



# GENUS "T7-LIKE VIRUSES"

Type species *Enterobacteria phage T7*

## Distinguishing features

Medium-sized lytic phages, with nonpermuted terminally redundant DNA that codes for both DNA and RNA polymerases. Heads contain a unique eight-fold symmetric core structure. DNA is injected stepwise rather than all at once.

## Virion properties

### MORPHOLOGY

T7 phage heads are icosahedra, measure about 60 nm in diameter, and consist of 72 capsomers (60 hexamers and 12 pentamers; T = 7). Tails measure 17 × 8 nm and have six short fibers (Figure 7).

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

T7 virion Mr is about $48 \times 10^6$, buoyant density in CsCl is 1.50 g cm$^{-3}$, and $S_{20,w}$ is about 510S. Infectivity is ether and chloroform resistant.

### NUCLEIC ACID

Genomes are about 40 kbp (39,936 bp for T7), corresponding to 50% of virion particle weight, have a G+C content of 50%, and are non-permuted and terminally redundant.

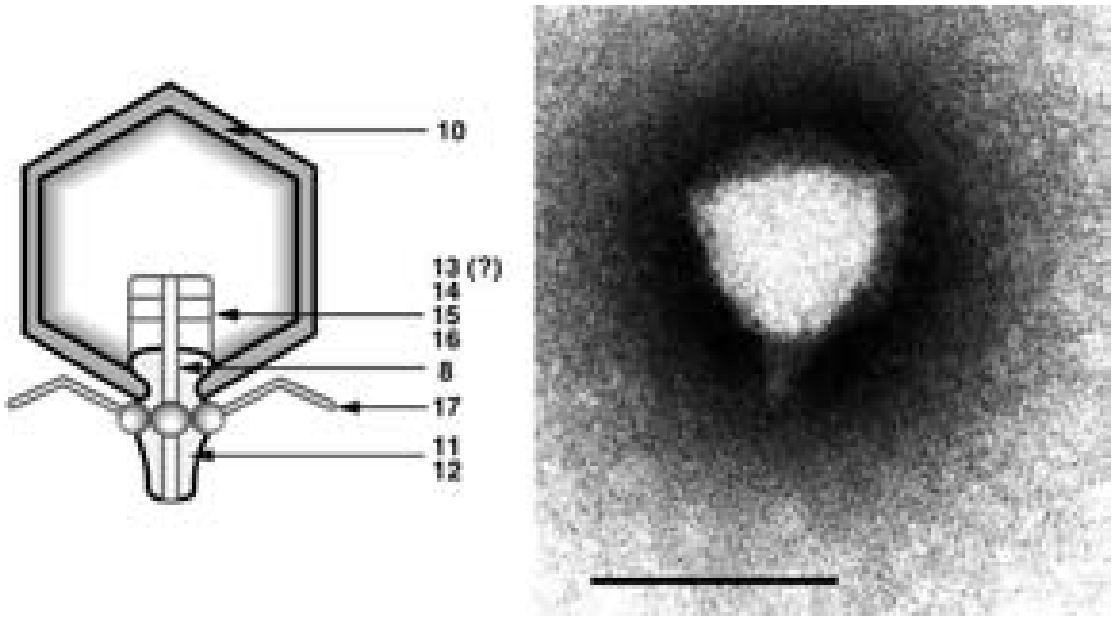


**Figure 7:** (Left) Diagram of particle of Enterobacteria phage T7 (T7) in section. The bar represents 50 nm. (Modified from Eiserling, F.A. (1979). Bacteriophage structure. In: *Comprehensive Virology* (H. Fraenkel-Conrat and R.R. Wagner, Eds.), vol. 13, Plenum Press, New York, p. 543; with permission.) (Right) Negative contrast electron micrograph of a particle of phage T7, stained with phosphotungstate. The bar represents 100 nm.

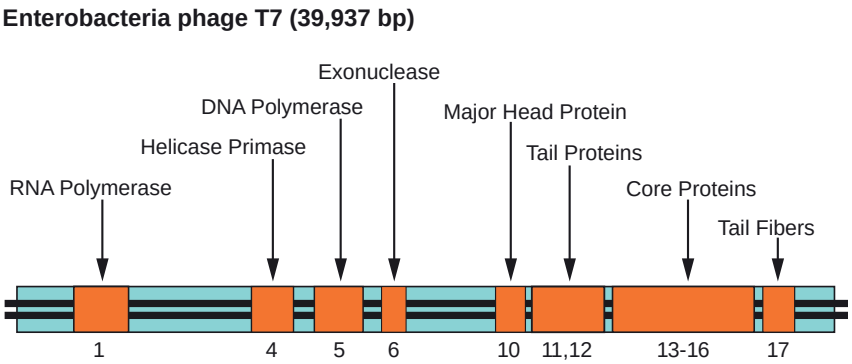


**Figure 8:** Simplified genetic map of Enterobacteria phage T7 (T7). (Redrawn after Freifelder, D. (Ed.) (1983). *Molecular Biology*, Science Books International, Boston, and Van Nostrand Reinhold, New York, p. 630.)

### Proteins
Particles have at least nine structural proteins (13–150 kDa), including about 420 copies of the major capsid protein (in T7, 38 kDa).

### Lipids
None known.

### Carbohydrates
None known.

## Genome organization and replication
The T7 genetic map is linear, non-permuted and terminally redundant, and comprises about 55 genes, several of which overlap. Related functions cluster together (Figure 8). The genome encodes a type B (*E. coli* Pol II) DNA polymerase and RNA polymerase. Infection results in shut-off of host syntheses and a breakdown of the host genome. The start of replication requires phage-encoded DNA and RNA polymerase and has multiple origins. Transcription proceeds in three waves. Only one strand is transcribed. Replication is bidirectional and produces concatemers by end-to-end joining of intermediate forms. Irregular polyheads are frequently observed. Packaged DNA is cut at fixed sites.

## Biological properties
Phages are virulent and are specific for enterics and related Gram-negative bacteria.

## Species demarcation criteria in the genus
Species differ by host range and, insofar as known, DNA sequence similarity.

## List of species in the genus "T7-like viruses"

| | | |
|---|---|---|
| *Enterobacteria phage T7* | | |
| Enterobacteria phage T7 | [V01146 = NC_001604] | (T7) |
| *Kluyvera phage Kvp1* | | |
| Kluyvera phage Kvp1 | [FJ194439 = NC_011534] | (Kvp1) |
| *Pseudomonad phage gh-1* | | |
| Pseudomonas phage gh-1 | [AF493143 = NC_004665] | (gh-1) |
| Vibrio phage VP4 | [DQ029335 = NC_007149] | (VP4) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus "T7-like viruses" but have not been approved as species

| | | |
|---|---|---|
| Enterobacteria phage 285P | [GQ468526] | (285P) |
| Enterobacteria phage 13a | [EU734174 = NC_011045] | (13a) |
| Enterobacteria phage EcoDS1 | [EU734172 = NC_011042] | (EcoDS1) |
| Enterobacteria phage BA14 | [EU734171 = NC_011040] | (BA14) |
| Enterobacteria phage T3 | [AY318471 = NC_003298] | (T3) |
| Klebsiella phage KP32 | [GQ413937 = NC_013647] | (KP32) |
| Klebsiella phage K11 | [EU734173 = NC_011043] | (K11) |
| Morganella phage MmP1 | [EU652770 = NC_011085] | (MnP1) |
| Salmonella phage phiSG-JL2 | [EU547803 = NC_010807] | (phiSG-JL2) |
| Vibrio phage N4 | [FJ409640 = NC_013651] | (N4) |
| Yersinia phage Yepe2 | [EU734170 = NC_011038] | (Yepe2) |
| Enterobacteria phage H | | (H) |
| Enterobacteria phage W31 | | (W31) |
| Enterobacteria phage WPK | | (WPK) |
| Enterobacteria phage PhiI | | (PhiI) |
| Enterobacteria phage PhiII | | (PhiII) |
| Enterobacteria phage BA14 | | (Ba14) |
| Enterobacteria phage Phi1.2 | | (Phi1.2) |
| Enterobacteria phage IV | | (IV) |
| Enterobacteria phage K11 | | (K11) |
| Enterobacteria phage PTB | | (PTB) |
| Enterobacteria phage R | | (R) |
| Enterobacteria phage Y | | (Y) |
| Enterobacterial phage ViIII | | (ViIII) |
| Escherichia phage K1F | [DQ111067 = NC_007456] | (K1F) |
| Pseudomonas phage PhiPLS27 | | (PhiPLS27) |
| Pseudomonas phage PhiPLS743 | | (PhiPLS743) |
| Pseudomonas phage Psy9220 | | (Psy9220) |
| Rhizobium phage 2 | | (2) |
| Rhizobium phage S | | (III) |
| Vibrio phage III | | (III) |
| Yersinia phage PhiA1122 | [AY247822 = NC_004777] | (PhiA1122) |
| Yersinia Berlin | [AM183667 = NC_008694] | (Berlin) |
| Yersinia phage PhiYeO3-12 | [AJ251805 = NC_001271] | (PhiYeO3-12) |

### List of unassigned species in the subfamily *Autographivirinae*

| | | |
|---|---|---|
| *Prochlorococcus phage P-SSP7* | | |
| Prochlorococcus phage P-SSP7 | [AY939843 = NC_006882] | (P-SSP7) |
| *Synechococcus phage P60* | | |
| Synechococcus phage P60 | [AF189021 = NC_003390] | (P60) |
| *Synechococcus phage syn5* | | |
| Synechococcus phage syn5 | [EF372997 = NC_009531] | (syn5) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## SUBFAMILY *PICOVIRINAE*

### Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Picovirinae*** |
| Genus | **"AHJD-like viruses"** |
| Genus | **"Phi29-like viruses"** |

### Distinguishing features

All these phages share unique properties, which differentiate them from other *Podoviridae*: a similar, special tail structure, their relatively small size and genome (with DNA with inverted terminal repeats), a similar gene number (20–29), a protein-primed DNA polymerase which, among phages, is found elsewhere only in the family *Tectiviridae*. Several genomic relationships to φ29 shown by the CoreExtractor/CoreGenes analysis have previously been observed for phages 44AJHD, P68 and C1. These relatives of the φ29-like phages were previously listed in the VIIIth ICTV Report. Here, φ29 and its relatives are upgraded from a genus to a subfamily with two genera: the "Phi29-like viruses" and the "AHJD-like viruses". *Picovirinae* refers to the small (Pico-) virion and genome sizes of the viruses within this subfamily, which represent the smallest tailed phages known.

The evolutionary link to the "Phi29-like viruses" is clearly present throughout the subfamily, both morphologically and molecularly, since all these phages also contain a type B polymerase, apart from other similar gene products and overall genome size. From this perspective, phages *Actinomyces* phage Av-1, *Streptococcus* phage Cp-1 are included within this subfamily but not assigned to a particular genus.

Mycoplasma phage P1 occupies a distinct and unclear position. Its genome has 11 structural genes, the same type of DNA polymerase as the other "phi29-like viruses", and a genome size of only 11,660 bp. This needs confirmation since we may be observing a case of genome size reduction (as shown by Mycoplasma hosts themselves).

## Genus "AHJD-like viruses"

Type species ***Staphylococcus phage 44AHJD***

### Distinguishing features

The "AHJD-like viruses" include *Staphylococcus* phages 44AHJD, P68, SAP-2 and 66, as well as *Streptococcus* phage C1. Evidence that distinguishes them from "phi29-like viruses" comes from their genome analysis which reveals that the 44AHJD and P68 lysis genes (amidases) are located within the morphogenesis genes rather than downstream, as is observed with φ29. Furthermore, these phages lack a classical holin-lysin cassette. Lastly, the gene for the major capsid protein (gp8) of φ29 and its close relatives is located in the left third of the genome, while the analogous genes in 44AHJD and P68 (gp20) are near the right end of the genome.

### Virion properties

#### Morphology
Isometric phages, with short, non-contractile tails and a pre-neck appendage.

#### Physicochemical and physical properties
No information available.

#### Nucleic acid
These dsDNA viruses have genomes of between 16 and 19 kbp. The terminal DNA fragments of P68 DNA and P68 DNA protein complex suggest the presence of a terminal protein at either DNA end.

#### Proteins
These phages encode between 20 (phages C1 and SAP-2) and 27 (phage 66) proteins.

#### Lipids
None known.

#### Carbohydrates
None known.

### Genome organization and replication

Phage 44AHJD and phage P68 genomes are predicted to contain 21 and 22 ORFs, respectively. Metabolism-related genes are encoded on the Watson strand of the genome, whereas genes encoding structural proteins are located on the Crick strand, separated by a bidirectional terminator. Evidence for the presence of a terminal protein in P68 has been published.

### Biological properties

Phages 44AHJD, P68, phage 66 and SAP-2 are lytic phages infecting *Staphylococcus aureus*. Phage C1 infects *Streptococcus*.

### Species demarcation criteria in the genus

The presence of unique genes.

### List of species in the genus "AHJD-like viruses"

| | | |
|---|---|---|
| *Staphylococcus phage 44AHJD* | | |
| Staphylococcus phage 44AHJD | [AF513032 = NC_004678] | (44AHJD) |
| Staphylococcus phage phiP68 | [AF513033 = NC_004679] | (phiP68) |
| Staphylococcus phage 66 | [AY954949 = NC_007046] | (66) |
| Staphylococcus phage SAP-2 | [X99260 = NC_004165] | (SAP2) |
| *Streptococcus phage C1* | | |
| Streptococcus phage C1 | [AY212251 = NC_004814] | (C1) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus "AHJD-like viruses" but have not been approved as species

None reported.

## GENUS "PHI29-LIKE VIRUSES"

Type species ***Bacillus phage phi29***

### Virion properties

#### MORPHOLOGY

Heads are prolate icosahedra (T = 3 with 30 hexamers and 11 pentamers) and measure about 54 × 42 nm. Some members, including *Bacillus* phage φ29, have about 55 fibers on the head (Figure 9). Tails measure 46 × 8 nm; have a distal thickening, and a collar with 12 appendages.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

φ29 virion Mr is $29 \times 10^7$, buoyant density in CsC1 is 1.46 g cm$^{-3}$, and $S_{20,w}$ is 254S. Infectivity is chloroform-resistant.

#### NUCLEIC ACID

Genomes are 16–20 kbp, correspond to about 50% of particle weight, and have a G+C content of 35–38%.

#### PROTEINS

Virions have nine structural proteins (13-86 kDa), including 235 copies of the major capsid protein (49.6 kDa in φ29 and 42 kDa in Cp-1). Type B (*E. coli* Pol II) DNA polymerase is coded for.

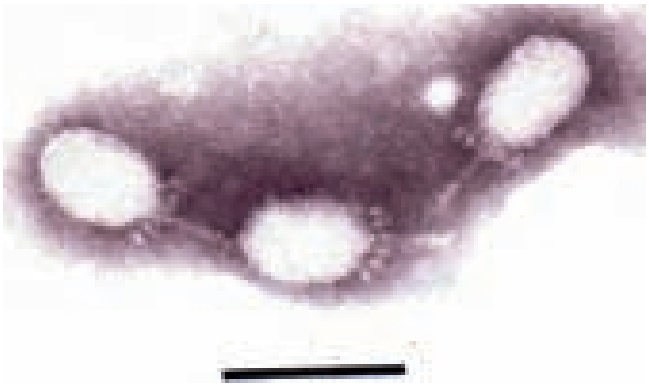

**Figure 9:** Electron micrograph of the φ29-related virus GA-1 stained with phosphotungstate. The bar represents 100 nm. (Courtesy Hans-Wolfgang Ackermann.)

**LIPIDS**
None known.

**CARBOHYDRATES**
None known.

## Genome organization and replication

The genetic map is linear and includes 20–29 ORFs, one of which codes for a type B (*E. coli* Pol II) DNA polymerase. Genomes are non-permuted and have inverted terminal repeats from 6 to 8 bp (Phi29) to 230–240 bp (Cp-1). Both 5′ ends are covalently linked to a terminal protein. Infecting DNA does not circularize. Transcription proceeds in two waves. Early genes are transcribed from right to left on the standard map (except in Cp-1 where some are from left to right); late genes are transcribed from left to right. Replication is primed by the terminal protein and starts at both DNA ends, and proceeds by strand displacement. The terminal protein is essential in DNA packaging. The packaging substrate is non-concatemeric DNA. Packaging requires phage-encoded RNA.

## Antigenic properties

No group antigens are reported.

## Biological properties

Phages are virulent and infect Gram-positive bacteria with low G+C contents. Distribution is worldwide.

## Species demarcation criteria in the genus

All species differ in host range. Phages φ29 and GA-1 differ in serological properties and protein molecular weights. Phage φ29, PZA, GA-1, B103, 44AHJD, P68, C1 and Cp-1 DNAs differ in DNA sequence and in the length of inverted terminal repeats (6–8 to 236–247 bp). In addition, phages φ29 and Cp-1 have opposite directions for transcription of early genes at the left end of the chromosome.

## List of species in the genus "Phi29-like viruses"

*Bacillus phage phi29*
Bacillus phage φ29 [EU771092 = NC_011048] (Phi29)
*Bacillus phage GA-1*
Bacillus phage GA-1 [X96987 = NC_002649] (GA-1)

| | | |
|---|---|---|
| *Bacillus phage B103* | | |
| Bacillus phage B103 | [X99260 = NC_004165] | (B103) |
| *Kurthia phage 6* | | |
| Kurthia phage 6 | | (K6) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus "Phi29-like viruses" but have not been approved as species

| | | |
|---|---|---|
| Bacillus phage Nf | [EU622808] | (Nf) |
| Bacillus phage BS32 | [B32RGHSEQ] | (BS32) |
| Bacillus phage Phi15 | | (Phi15) |
| Bacillus phage AR13 | | (AR13) |
| Bacillus phage MY2 | | (MY2) |
| Bacillus phage M2 | | (M2) |
| Bacillus phage N Phi | | (N Phi) |
| Bacillus phage SF5 | | (SF5) |
| Kurthia phage 7 | | (K7) |
| Bacillus phage PZA | [9626384] | (PZA) |

About 45 additional, poorly characterized phages may belong to this genus.

## List of unassigned species in the subfamily *Picovirinae*

| | | |
|---|---|---|
| *Actinomyces phage Av-1* | | |
| Actinomyces phage Av-1 | [DQ123818 = NC_009643] | (Av-1) |
| *Mycoplasma phage P1* | | |
| Mycoplasma phage P1 | [AF246223 = NC_002515] | (P1) |
| *Streptococcus phage Cp-1* | | |
| Streptococcus phage Cp-1 | [Z47794 = NC_001825] | (Cp-1) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of unassigned species in the family *Podoviridae*

| | | |
|---|---|---|
| *Endosymbiont phage APSE-1* | | |
| Endosymbiont phage APSE-1 | [AF157835 = NC_000935] | (APSE-1) |
| *Lactococcus phage KSY1* | | |
| Lactococcus phage KSY1 | [DQ535032 = NC_009817] | (KSY1) |
| *Phormidium phage Pf-WMP3* | | |
| Phormidium phage Pf-WMP3 | [EF537008 = NC_009551] | (Pf-WMP3) |
| *Phormidium phage Pf-WMP4* | | |
| Phormidium phage Pf-WMP4 | [DQ875742 = NC_008367] | (Pf-WMP4) |
| *Pseudomonas phage 119X* | | |
| Pseudomonas phage 119X | [DQ163914 = NC_007807] | (119X) |
| *Pseudomonas phage F116* | | |
| Pseudomonas phage F116 | [AY625898 = NC_006552] | (F116) |
| *Roseobacter phage SIO1* | | |
| Roseobacter phage SIO1 | [AF189021 = NC_002519] | (SIO1) |
| *Vibrio phage VpV262* | | |
| Vibrio phage VpV262 | [AY095314 = NC_003907] | (VpV262) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the family *Podoviridae* but have not been approved as species

| | | |
|---|---|---|
| Acinetobacter phage A36 | | (A36) |
| Bacillus phage PhiBa1 | | (PhiBa1) |
| Brucella phage Tb | | (Tb) |
| Clostridium phage HM2 | | (HM2) |
| Coryneforms phage 7/26 | | (7/26) |
| Coryneforms phage AN25S-1 | | (AN25S-1) |
| Cyanobacteria phage A-4(L) | | (A-4(L) |
| Cyanobacteria phage AC-1 | | (AC-1) |
| Cyanobacteria phage LPP-1 | | (LPP-1) |
| Cyanobacteria phage SM-1 | | (SM-1) |
| Enterobacteria phage 7-11 | [HM997019] | (7-11) |
| Enterobacteria phage 7480b | | (7480b) |
| Enterobacteria phage Esc-7-11 | | (Esc-7-11) |
| Enterobacteria phage W8 | | (W8) |
| Lactococcus phage PO34 | | (PO34) |
| Mollicutes phage C3 | | (C3) |
| Mollicutes phage L3 | | (L3) |
| Mycobacterium phage Phi17 | | (Phi17) |
| Pasteurella phage 22 | | (22) |
| Rhizobium phage Phi2042 | | (Phi2042) |
| Streptococcus phage 182 | | (182) |
| Streptococcus phage 2BV | | (2BV) |
| Streptococcus phage Cvir | [AF33467] | (Cvir) |
| Streptococcus phage H39 | | (H39) |
| Vibrio phage 4996 | | (4996) |
| Vibrio phage I | | (I) |
| Vibrio phage OX N-100P | | (OXN-100P) |
| Xanthomonas phage RR66 | | (RR66) |

## Phylogenetic relationships within the family

No information available.

## Similarity with other taxa

"Phi29-like viruses", adenoviruses and tectiviruses have proteins linked to DNA ends and protein-primed replication, and code for type B (*E. coli* Pol II) DNA polymerase. See "Similarity with other taxa" in the *Caudovirales* description for further details.

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## Supplementary reading

A supplementary list of reading is available online on Science Direct®, www.sciencedirect.com.

## Contributed by

Lavigne, R. and Kropinski, A.M.

# Family *Siphoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Siphoviridae* |
| Genus | **"Lambda-like viruses"** |
| Genus | **"T1-like viruses"** |
| Genus | **"T5-like viruses"** |
| Genus | **"L5-like viruses"** |
| Genus | **"c2-like viruses"** |
| Genus | **"PsiM1-like viruses"** |
| Genus | **"PhiC31-like viruses"** |
| Genus | **"N15-like viruses"** |
| Genus | **"SPbeta-like viruses"** |

## Distinguishing features

Virions have long, non-contractile, thin tails (65–570×7–10 nm) which are often flexible. Tails are built of stacked disks of six subunits. Heads and tails are assembled separately. Genera are differentiated by genome organization, mechanisms of DNA packaging and presence or absence of DNA polymerases.

# Genus "Lambda-like viruses"

Type species *Enterobacteria phage lambda*

## Distinguishing features

The DNA has cohesive ends and is packaged as a unit-size filament.

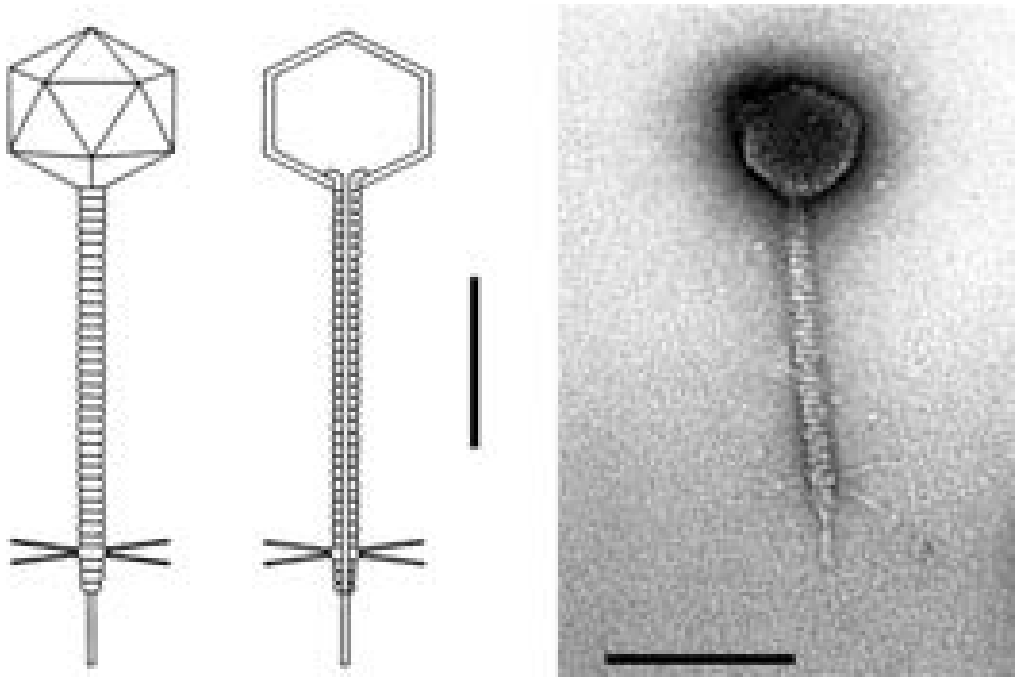

**Figure 1:** Enterobacteria phage λ (λ): (left) representative diagram of a phage λ particle; (right) electron micrograph of phage λ particles with negative staining. The bar represents 100 nm.

## Virion properties

### Morphology

Phage heads are icosahedra, about 60 nm in diameter, and consist of 72 capsomers (60 hexamers, 12 pentamers, T = 7). Tails are flexible, 150×8 nm, and have a short terminal fiber and four long, jointed fibers attached subterminally (Figure 1). The latter fibers are absent in most laboratory strains of the phage.

### Physicochemical and physical properties

Virion Mr is about $60 \times 10^6$, buoyant density in CsCl is $1.50\,g\,cm^{-3}$, and the $S_{20,w}$ is about 390S. Infectivity is chloroform- and ether-resistant.

### Nucleic acid

The phage λ genome is 48,503 bp in size, corresponding to 54% of particle weight, has 52% G+C. The DNA has cohesive ends and is non-permuted.

### Proteins

Virions contain about 14 structural proteins (11–130 kDa), including 415 copies each of major capsid proteins E and D (38 and 11 kDa, respectively).

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

The genome includes about 70 genes and has cohesive ends (Figure 2). Related functions cluster together. The infecting DNA circularizes and either replicates or integrates into the host genome. Transcription starts in the immunity region and proceeds in three waves. Bidirectional DNA replication as a Θ (theta) structure, starting from a single site, is followed by unidirectional replication via a rolling-circle mechanism. There is no breakdown of host DNA. Proheads are frequent in lysates.

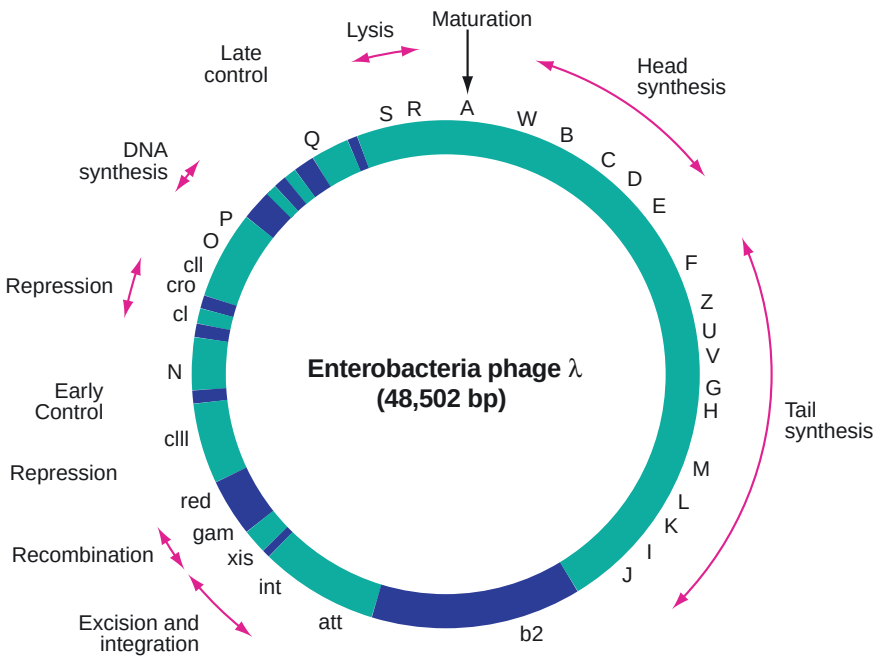


**Figure 2:** Simplified genetic map of Enterobacteria phage λ (λ). Dark portions of the genome indicate non-essential regions. (From Freifelder, D. (Ed.) (1983). *Molecular Biology*. Science Books International, Boston, and Van Nostrand Rinehold, New York, p. 639; with permission.)

### Biological properties

Phages are temperate and apparently specific for enterobacteria. Phages generally integrate at specific sites and are UV-inducible.

### Species demarcation criteria in the genus

Species are distinguished by different combinations of alleles of genes encoding head proteins, homologous recombination proteins, and DNA replication proteins, in the context of very similar genome organization.

### List of species in the genus "Lambda-like viruses"

| | | |
|---|---|---|
| *Enterobacteria phage HK022* | | |
| Enterobacteria phage HK022 | [AF069308] | (HK022) |
| *Enterobacteria phage HK97* | | |
| Enterobacteria phage HK97 | [AF069529] | (HK97) |
| *Enterobacteria phage lambda* | | |
| Enterobacteria phage λ | [J02459] | (λ) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus "Lambda-like viruses" but have not been approved as species

| | |
|---|---|
| Enterobacteria phage PA-2 | (PA-2) |
| Enterobacteria phage FD328 | (FD328) |
| Enterobacteria phage φ80 | (φ80) |
| Rhizobium phage 16-6-2 | (16-6-2) |

## GENUS "T1-LIKE VIRUSES"

Type species *Enterobacteria phage T1*

### Distinguishing features

Tails are extremely flexible. Phage DNA has pac sites and is terminally redundant and circularly permuted.

### Virion properties

#### MORPHOLOGY

Virions have icosahedral heads of about 60 nm and extremely flexible tails of 151 × 8 nm, with four short, kinked, terminal fibers. The flexible nature of the tail is best seen after phosphotungstate staining.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virion buoyant density in CsC1 is 1.5 g cm$^{-3}$. Virion infectivity is stable to drying.

#### NUCLEIC ACID

Genomes are about 49 kbp and have a G+C content about 48%.

#### PROTEINS

Virions contain at least 14 proteins, including two major head proteins (26 and 33 kDa).

#### LIPIDS

None reported.

#### CARBOHYDRATES

None reported.

## Genome organization and replication

The genetic map is linear and comprises 36–41 genes; related functions cluster together. The genome is circularly permuted and terminally redundant (2.8 kbp or 6% of genome), and includes a recombinational hot spot. Host syntheses are inhibited after infection. Little is known about the mechanism of Enterobacteria phage T1 replication. Progeny DNA is cut from concatemers at pac sites and packaged by a headfull mechanism.

## Biological properties

Phages are virulent, can carry out generalized transduction and infect enterobacteria.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus "T1-like viruses"

*Enterobacteria phage T1*
Enterobacteria phage T1 [AY216660] (T1)

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus "T1-like viruses" but have not been approved as species

Enterobacteria phage 102 (102)
Enterobacteria phage 103 (103)
Enterobacteria phage 150 (150)
Enterobacteria phage 168 (168)
Enterobacteria phage 174 (174)
Enterobacteria phage b4 (b4)
Enterobacteria phage D20 (D20)
Enterobacteria phage fg (fg)
Enterobacteria phage Hi (Hi)
Enterobacteria phage UC-1 (UC-1)

# GENUS "T5-LIKE VIRUSES"

Type species *Enterobacteria phage T5*

## Distinguishing features

Virions have large heads and long, kinked tail fibers. The DNA has five single-stranded gaps, large terminal repetitions, codes for a type A (*E. coli* Pol I) DNA polymerase, and is injected in two steps.

## Virion properties

### MORPHOLOGY

Virions have icosahedral heads about 80 nm in diameter. Tails measure 180×9 nm in Enterobacteria phage T5 (T5) and 160×9 nm in Vibrio phage 149 (type IV), have a subterminal disk with three kinked fibers about 120 nm in length, and a conical tip with a single, straight, 50 nm long fiber. Vibrio phage 149 tail fibers have terminal knobs.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virion Mr is $114\times10^6$, buoyant density in CsC1 is 1.53 g cm$^{-3}$, and $S_{20,w}$ is 608S.

### NUCLEIC ACID

Genomes are about 121 kbp, corresponding to 62% of particle weight, and have a G+C content of 44%.

#### Proteins
Virions contain at least 15 structural proteins (15.5–125 kDa), including about 775 copies of the major capsid protein (44 kDa). Type A (*E. coli* Pol I) DNA polymerase is encoded.

#### Lipids
None reported.

#### Carbohydrates
None reported.

### Genome organization and replication
The genetic map is linear. The genome includes at least 80 genes, is divided into five regions, is non-permuted, and has a large terminal repetition of about 10 kbp (8.5% of genome) and five single stranded gaps at specific sites. It has neither cos nor pac sites. Pre-early, early and late genes cluster together. Only 8% of DNA is injected immediately after adsorption; the rest follows after 3–4 minutes. Transcription involves modification of host RNA polymerase by phage gene products. DNA replication follows a bidirectional or a rolling-circle mechanism or both. Concatemers are produced.

### Biological properties
Infection is virulent. Known phages of the genus infect enterobacteria and vibrios.

### Species demarcation criteria in the genus
Species differ by host range.

### List of species in the genus "T5-like viruses"

| | | |
|---|---|---|
| *Enterobacteria phage T5* | | |
| Enterobacteria phage T5 | [AY543070] | (T5) |
| *Vibrio phage 149 (type IV)* | | |
| Vibrio phage 149 (type IV) | | (φ149) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus "T5-like viruses" but have not been approved as species

| | |
|---|---|
| Enterobacteria phage BF23 | (BF23) |
| Enterobacteria phage PB | (PB) |
| Enterobacteria phage San 2 | (San2) |

## Genus "L5-like viruses"

Type species *Mycobacterium phage L5*

### Distinguishing features
Phage DNA has cos sites and codes for a type A (*E. coli* Pol I) DNA polymerase.

### Virion properties

#### Morphology
Virions have isometric heads about 60 nm in diameter and flexible tails of 135×8 nm with a terminal knob and a single short fiber.

### Physicochemical and physical properties

Virion Mr is 116×106, buoyant density in CsC1 is 1.51 g cm$^{-3}$, and $S_{20,w}$ is 410 (data from Mycobacterium phage phlei). Chloroform sensitivity has been reported in possibly related phages.

### Nucleic acid

Genomes are about 52 kbp. The *Mycobacterium* phage L5 (L5) genome has been sequenced and has 52,297 bp. The G+C content is about 63%.

### Proteins

Virions contain at least six structural proteins (19–22 to 250 kDa), including a major capsid protein of 35 kDa. Type A (*E. coli* Pol I) DNA polymerase is encoded.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

The genetic map is linear. Related genes cluster together. The genome includes 88 genes and has cohesive ends. Host syntheses are shut off during replication. Transcription of structural genes is unidirectional.

## Biological properties

Phages are temperate and specific for mycobacteria. Prophages integrate at specific sites in the bacterial genome.

## Species demarcation criteria in the genus

Species differ by relative insertions and deletions in the context of otherwise similar sequence and gene organization.

## List of species in the genus "L5-like viruses"

| | | |
|---|---|---|
| *Mycobacterium phage D29* | | |
| Mycobacterium phage D29 | [AF022214] | (D29) |
| *Mycobacterium phage L5* | | |
| Mycobacterium phage L5 | [Z18946] | (L5) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus "L5-like viruses" but have not been approved as species

| | | |
|---|---|---|
| Mycobacterium phage Bxb1 | [AF271693] | (Bxb1) |
| Mycobacterium phage Leo | | (Leo) |
| Mycobacterium phage minetti | | (minetti) |
| Mycobacterium phage phlei | | (GS4E) |

# Genus "c2-like viruses"

Type species *Lactococcus phage c2*

## Distinguishing features

Heads are prolate; phage DNA has cos sites and codes for a putative type B DNA polymerase.

## Virion properties

### Morphology
Virions have prolate heads about 56×41 nm and tails of 98×9 nm, with a collar (inconstant) and small base plate, and produce rare morphological aberrations (two heads joined by a bridge).

### Physicochemical and physical properties
Virion buoyant density in CsC1 is 1.46 g cm$^{-3}$.

### Nucleic acid
Genomes are about 22 kbp (22,163–22,195 bp) and have a G+C content of 35–40%. The genomes of *Lactococcus* phage c2 (c2) and *Lactococcus* phage bIL67 (bIL67) have been fully sequenced.

### Proteins
Virions have at least six structural proteins (19.2–175 kDa): major proteins are 29, 90 and 175 kDa. Type B (*E. coli* Pol II) DNA polymerase is apparently encoded.

### Lipids
None reported.

### Carbohydrates
None reported.

## Genome organization and replication
The genetic map is linear. The genome includes 37–38 genes in two clusters and has cohesive ends. Early and late genes are separated by an intergenic region. Late genes are all transcribed in the same direction.

## Biological properties
Phages are temperate and specific for lactococci.

## Species demarcation criteria in the genus
Phages bIL67 and c2 share 80% nucleotide sequence identity but comparisons also show several DNA deletions or insertions which corresponded to the loss or acquisition of specific ORFs.

## List of species in the genus "c2-like viruses"

| | | |
|---|---|---|
| *Lactococcus phage bIL67* | | |
| Lactococcus phage bIL67 | [L33769] | (bIL67) |
| *Lactococcus phage c2* | | |
| Lactococcus phage c2 | [L48605] | (c2) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus "c2-like viruses" but have not been approved as species

| | |
|---|---|
| Lactococcus phage c6A | (PBc6A) |
| Lactococcus phage P001 | (P001) |
| Lactococcus phage φvML3 | (φvML3) |
| (Lactococcus phage ML3) | (ML3) |
| (Lactococcus phage ml3) | (ml3) |
| (Lactococcus phage 3ML) | (3ML) |

About 200 additional, poorly characterized lactococcal phages have been reported that are morphologically indistinguishable from c2.

# GENUS "PSIM1-LIKE VIRUSES"

Type species *Methanobacterium phage psiM1*



## Distinguishing features

The host is an archaeon. Viral genomes are circularly permuted and terminally redundant.

## Virion properties

### MORPHOLOGY

Virions have isometric heads 55 nm in diameter and tails of 210×10 nm with a terminal knob.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Not known.

### NUCLEIC ACID

Genomes are about 30 kbp in size.

### PROTEINS

Not known.

### LIPIDS

None reported.

### CARBOHYDRATES

None reported.

## Genome organization and replication

Genomes are circularly permuted and terminally redundant.

## Biological properties

Phages are lytic and infect members of the genus *Methanobacterium*.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus "PsiM1-like viruses"

*Methanobacterium phage psiM1*

| | | |
|---|---|---|
| Methanobacterium phage ΨM1 | [AF065411, AF065412] | (ΨM1) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus "PsiM1-like viruses" but have not been approved as species

| | |
|---|---|
| Methanobacterium phage FF3 | (FF3) |
| Methanobrevibacter phage PG | (PG) |

# Genus "PhiC31-like viruses"

Type species *Streptomyces phage phiC31*

## Distinguishing features

Phage DNA has cos ends, codes for a type A DNA polymerase and has a serine site-specific recombinase.

## Virion properties

### Morphology

Virions have isometric heads about 53 nm in diameter and flexible tails 100 nm long and 5 nm wide, a base plate of 15 nm and four tail fibers with terminal knobs ("toes").

### Physicochemical and physical properties

Virion bouyant density is 1.493 g $cm^{-3}$, and virions are chloroform-sensitive.

### Nucleic acid

Genomes are about 43 kbp. Two genomes have been completely sequenced: phages φC31 and φBT1 are 41,491 and 41,832 bp respectively. G+C content is 63.6%.

### Proteins

Virions contain 10 structural proteins, visible by Coomassie staining, of about 10–70 kDa.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

The genetic map is linear and related genes cluster together. The phage φC31 genome encodes 54 genes and has cohesive ends with 10 nt protruding at the 3′ end. Transcription of all except one gene is unidirectional. One tRNA is encoded. The mode of replication is unknown, but the genome encodes a DNA polymerase, phage P4-like primase-helicase, D29-like dCMP deaminase and T4-like nucleotide kinase. Head assembly genes most closely resemble those of Pseudomonas phage D3 and Enterobacteria phage HK97. The putative tail fiber gene contains a collagen motif. Lytic growth occurs via transcription from multiple conserved promoters in the early region and a single operon in the late region. A repressor gene encodes three nested N-terminally different in-frame proteins which bind to multiple highly conserved operators. The integrase belongs to the serine recombinase family of site-specific recombinases.

## Biological properties

Phages are temperate and specific for *Streptomyces* spp. Phages integrate at a specific site in the host genome and are not UV inducible. Phages homoimmune to φC31 are susceptible to a phage resistance mechanism (Pgl; phage growth limitation) in *S. coelicolor* A3(2). Lytic growth switches off host transcription.

## Species demarcation criteria in the genus

Not applicable.

### List of species in the genus "PhiC31-like viruses"

*Streptomyces phage phiC31*
Streptomyces phage φC31 [AJ006589] (φC31)

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus "PhiC31-like viruses" but have not been approved as species

| | | |
|---|---|---|
| Streptomyces phage φBT1 | [AJ550940] | (φBT1) |
| Streptomyces phage TG1 | | (TG1) |
| Streptomyces phage SEA | | (SEA) |
| Streptomyces phage R4 | | (R4) |
| Streptomyces phage VP5 | | (VP5) |
| Streptomyces phage RP2 | | (RP2) |
| Streptomyces phage RP3 | | (RP3) |



## GENUS "N15-LIKE VIRUSES"

Type species *Enterobacteria phage N15*

### Distinguishing features

Prophage DNA is present as a linear plasmid with covalently closed hairpin telomeres. Virion DNA has cohesive ends and is packaged as a unit-size molecule.

### Virion properties

#### MORPHOLOGY

Phage heads are hexagonal in outline (probable icosahedra), about 60 nm in diameter. Tails are non-contractile, flexible, measure 140×8 nm, and have short brush-like terminal fibers.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Not characterized in detail.

#### NUCLEIC ACID

The genome is 46,363 bp, has a G+C content of 51.2%, 12 nt 5′-protruding cohesive ends, and is non-permuted. The genome has been sequenced.

#### PROTEINS

Virion proteins have not been studied, but their high level of similarity to those of phage λ heads and phage HK97 tails suggests that they are very like those phages.

#### LIPIDS

None reported.

#### CARBOHYDRATES

None reported.

### Genome organization and replication

The virion genome includes about 50 genes and has cohesive ends. Related functions cluster together. The infecting DNA circularizes and replicates, or becomes established as a linear plasmid which is a circular permutation of the virion DNA. Details of DNA replication have not been studied. Organization and sequences of the late expressed virion structural protein and lysis genes are similar to lambda, but the early expressed genes are very different. An anti-repressor system and putative DNA polymerase gene (primase type) have been identified in the early left operon. Unique to this phage type is the presence of a protelomerase gene that encodes an enzyme that resolves a

circular genome molecule at the telRL site into the linear molecule with covalently closed hairpin telomeres. An unusually large number of phage genes are expressed from the prophage.

### Biological properties

Phages are temperate and mitomycin C inducible. Prophages are linear plasmids with covalently closed hairpin telomeres.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus "N15-like viruses"

*Enterobacteria phage N15*
Enterobacteria phage N15 [AF064539] (N15)

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus "N15-like viruses" but have not been approved as species

Yersinia phage PY54 (PY54)

## Genus "SPbeta-like viruses"

Type species ***Bacillus phage SPbeta***

### Distinguishing features

The virions have the largest heads and longest tails in the family *Siphoviridae* (Figure 3).

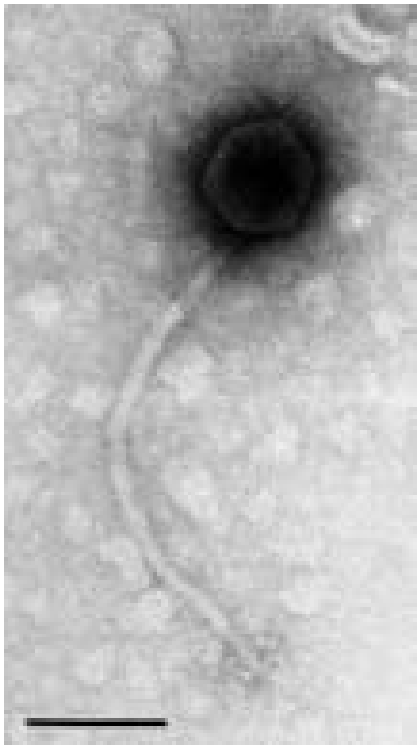

**Figure 3:** Electron micrograph of *Bacillus* phage SPβ particles with negative staining. The bar represents 100 nm.

## Virion properties

### Morphology
Heads are icosahedra of 81 nm in diameter. Tails measure 355×10 nm, are relatively rigid, have no collar and possess six club-shaped terminal spikes.

### Physicochemical and physical properties
Virion buoyant density in CsCl is 1.52 g cm$^{-3}$.

### Nucleic acid
The genome of SPβ is 134.416 kb in size, consists of 187 ORFs, and has a G+C content of 34–35%. Thymidylate synthetase genes are absent in SPβ but present in other members of the group (φ3T, φ11 isolates).

### Proteins
Virions have 6–7 major proteins.

### Lipids
None reported.

### Carbohydrates
None reported.

## Genome organization and replication
The genetic map is linear. Based on the origin of transcription, the genome is divided into three main clusters, the second of which may correspond to late genes. The genome is mosaic and contains bacterial elements, especially in *B. subtilis*, and elements of eight morphologically unrelated phages or prophages. Only 25% of ORFs have significant homology to known sequences.

## Biological properties
These are temperate phages.

## Species demarcation criteria in the genus
Not applicable.

## List of species in the genus "SPbeta-like viruses"

| | | |
|---|---|---|
| *Bacillus phage SPbeta* | | |
| Bacillus phage SPbeta | | (SPbeta) |
| Bacillus phage SPBc | [AF020713] | (SPBc2) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus "SPbeta-like viruses" but have not been approved as species
None reported.

## List of unassigned species in the family *Siphoviridae*
None reported.

## Phylogenetic relationships within the family
Refer to the discussion of this topic under Order *Caudovirales*.

## Similarity with other taxa

Refer to the discussion of this topic under Order *Caudovirales*.

## Derivation of name

*Sipho*: from Greek *siphon*, "tube", referring to the long tail.

## Further reading

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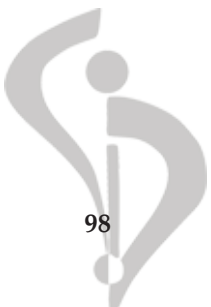
## Contributed by

Hendrix, R.W., Casjens, S.R. and Lavigne, R.



# ORDER HERPESVIRALES

## Taxonomic structure of the order

| | |
|---|---|
| Order | *Herpesvirales* |
| Family | *Alloherpesviridae* |
| Genus | *Batrachovirus* |
| Genus | *Cyprinivirus* |
| Genus | *Ictalurivirus* |
| Genus | *Salmonivirus* |
| Family | *Herpesviridae* |
| Subfamily | *Alphaherpesvirinae* |
| Genus | *Iltovirus* |
| Genus | *Mardivirus* |
| Genus | *Simplexvirus* |
| Genus | *Varicellovirus* |
| Subfamily | *Betaherpesvirinae* |
| Genus | *Cytomegalovirus* |
| Genus | *Muromegalovirus* |
| Genus | *Proboscivirus* |
| Genus | *Roseolovirus* |
| Subfamily | *Gammaherpesvirinae* |
| Genus | *Lymphocryptovirus* |
| Genus | *Macavirus* |
| Genus | *Percavirus* |
| Genus | *Rhadinovirus* |
| Family | *Malacoherpesviridae* |
| Genus | *Ostreavirus* |



## Virion properties

### MORPHOLOGY

Virions have complex and characteristic structures consisting of both symmetrical and non-symmetrical components. The spherical virion is comprised of the core, capsid, tegument and envelope (Figure 1). The core consists of the viral genome packaged as a single, linear, dsDNA molecule into a preformed capsid. DNA is packed in a liquid-crystalline array that fills the entire internal volume of the capsid. The mature capsid is a T = 16 icosahedron. In virions of human herpesvirus 1 (HHV-1), the 16 nm thick protein shell has an external diameter of 125 nm. The 11 pentons and 150 hexons (a total of 161 capsomers) are composed primarily of five and six copies, respectively, of the same protein and are joined by masses, termed triplexes, which are made of two smaller proteins present in a 2:1 ratio (Figure 1). The twelfth pentonal position is occupied by a ring-like structure consisting of 12 copies of the capsid portal protein.

Capsids assemble by co-condensation around a protein scaffold to form a procapsid in which the subunits are weakly connected. Proteolytic cleavage of the scaffolding protein triggers loss of scaffold and reorganization of the shell into the characteristic capsid form. The structure of the tegument is poorly defined, with evidence of symmetry only in the region immediately adjacent to the capsid. Electron tomography indicates that there are inner (capsid-associated) and outer (envelope-associated) tegument layers, and that capsids may be non-symmetrically situated within the envelope. The tegument contains many proteins, not all of which are required for the formation of virions. Individual tegument proteins can vary markedly in abundance. Enveloped tegument structures lacking capsids can assemble and are released from cells along with virions. The envelope is a lipid bilayer that is intimately associated with the outer surface of the tegument. It contains a number (at least 11 in HHV-1) of different integral viral glycoproteins that form a network of closely spaced spikes (mean of 659 per HHV-1 virion) of at least three distinct morphologies.

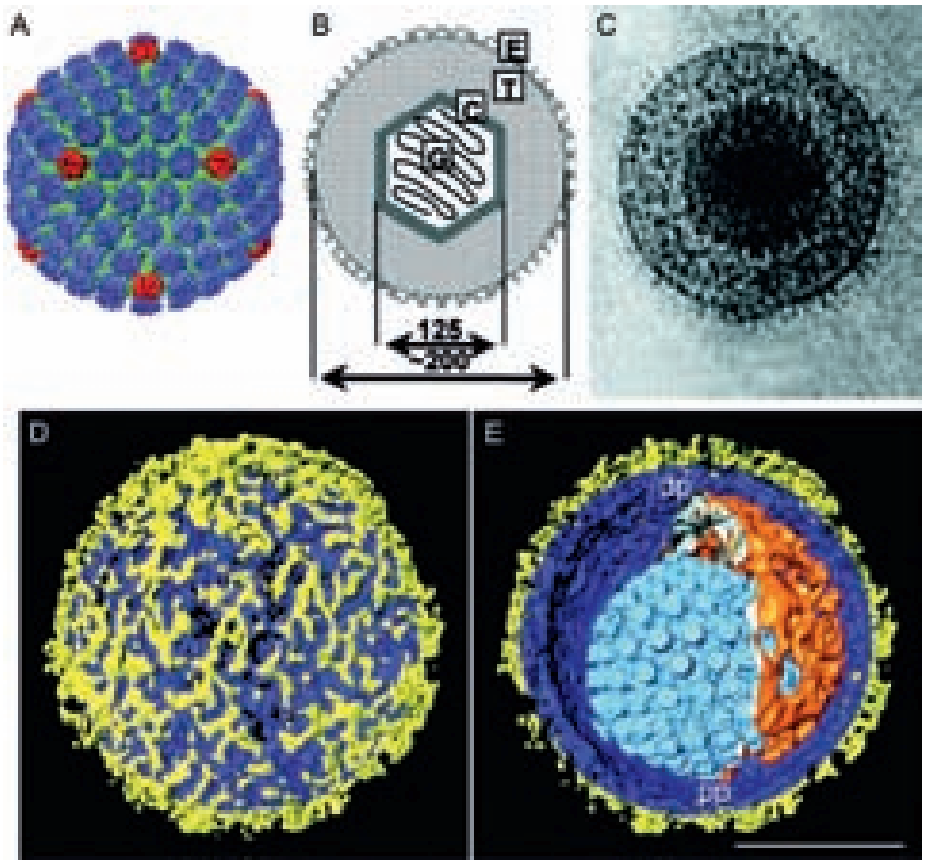



**Figure 1:** Herpesvirus morphology. (A) Reconstruction of a human herpesvirus 1 (HHV-1) capsid generated from cryo-electron microscope images, viewed along the two-fold axis. The hexons are shown in blue, the pentons in red and the triplexes in green (courtesy of W. Chiu and H. Zhou; Zhou, Z.H., Dougherty, M., Jakana, J., He, J., Rixon, F.J. and Chiu, W. (2000). Seeing the herpesvirus capsid at 8.5 Å. *Science*, **288**, 877–880; reprinted with permission from AAAS). (B) Schematic representation of a virion with diameters in nm. G: genome; C: capsid; T: tegument; E: envelope. (C) Cryo-electron microscope image of a HHV-1 virion (from Rixon, F.J. (1993). Structure and assembly of herpesviruses. *Semin. Virol.*, **4**, 135–144; with permission from Elsevier). (D and E) Segmented surface rendering of a single virion tomogram after denoising. (D) Outer surface showing the distribution of glycoprotein spikes (yellow) protruding from the membrane (blue). (E) Cutaway view of the virion interior, showing the capsid (light blue) and the tegument "cap" (orange) inside the envelope (blue and yellow). pp, proximal pole; dp, distal pole. Bar = 100 nm (from Grünewald, K., Desai, P., Winkler, D.C., Heyman, J.B., Belnap, D.M., Baumeister, W. and Steven, A.C. (2003). Three-dimensional structure of herpes simplex virus from cryo-electron tomography. *Science*, **302**, 1396–1398; reprinted with permission from AAAS).

## Physicochemical and physical properties

The mass of the HHV-1 virion is about $13\times10^{-16}$ g, of which the DNA comprises about 10%. The mass of a full capsid is about $5\times10^{-16}$ g. The buoyant density of virions in CsCl is 1.22–1.28 g cm$^{-3}$. The stability of different herpesviruses varies considerably, but they are generally unstable to desiccation and low pH. Infectivity is destroyed by lipid solvents and detergents.

## Nucleic acid

The genomes are composed of linear dsDNA ranging from 125 to 295 kbp in size and from 32 to 75% in G+C content. The genomes examined in sufficient detail contain a single nucleotide extension at the 3′-ends, and no terminal protein has been identified. The arrangement of reiterated sequences (direct or inverted, at the termini or internally) results in a number of different genome structures (Figure 2), including isomers that result from recombination between inverted terminal and internal reiterations.

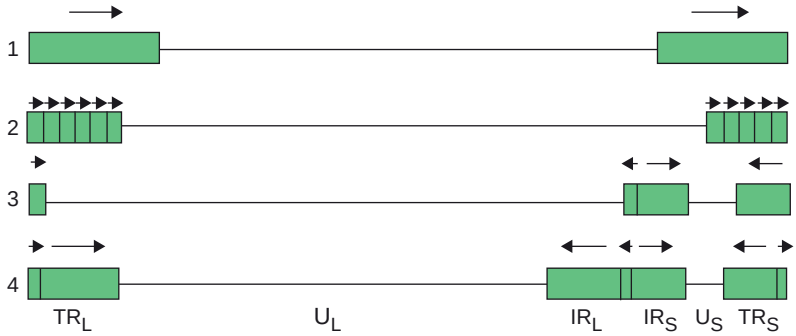



**Figure 2:** Simplified illustration of herpesvirus genome structures. Unique and repeated sequences are shown as solid lines and rectangles, respectively. The orientations of repeated sequences are indicated by arrows. The nomenclature used to describe regions of type 3 and 4 genomes is shown: $U_L$ = unique long; $U_S$ = unique short. The repeated sequences flanking the unique regions are named "terminal repeat short" ($TR_S$) and "internal repeat short" ($IR_S$), etc.

In Figure 2, structure 1 shows a unique sequence flanked by a direct repeat that may be larger than 10 kbp (human herpesvirus 6; HHV-6) or as short as 30 bp (murid herpesvirus 1; MuHV-1). Structure 2 also contains a single unique sequence but in this case it is flanked by a variable number of repeated sequences at each terminus (e.g. human herpesvirus 8; HHV-8). Structure 3 contains different elements at each terminus, which are present internally in inverted orientation. The genome is thus divided into two unique regions (one "long" and one "short"), which are flanked by inverted repeats. The repeated sequence flanking the long unique sequence is very short (88 bp in human herpesvirus 3; HHV-3) or absent (e.g. suid herpesvirus 1 [SuHV-1] and equid herpesvirus 1 [EHV-1]). Homologous recombination in replicated concatemeric DNA results in inversion of the two regions, and cleavage largely or entirely at one of the two junction regions results in unit length genomes that are one or the other of two isomers differing in the orientation of the short unique sequence. Structure 4 is the most complex. Like structure 3, it contains long and short unique regions, but in this case, both are flanked by large inverted repeat sequences. Homologous recombination and cleavage with equal probability at either of the two junction regions results in the formation of four isomers differing in the orientations of the unique sequences, with each isomer equimolar in virion populations (e.g. HHV-1). In addition, structure 4 genomes contain a short terminal repeat, which is present internally in inverse orientation in the junction region. The different isomers of type 3 and 4 genomes appear to be functionally equivalent.

It should be emphasized that Figure 2 is a simplified depiction of herpesvirus genome structures. Some herpesviruses contain large repetitive elements within the genome that are unrelated to those found at the termini (e.g. human herpesvirus 4; HHV-4), and a more complex set of structures can be cataloged if these are included. Particular genome structures are associated with certain herpesvirus taxa. Thus, the presence of multiple repeated elements at both termini (structure 2) is associated with the subfamily *Gammaherpesvirinae* (though not all members have this structure) while structure 3 is associated with the genus *Varicellovirus*. However, distantly related viruses may have equivalent genome structures, which have presumably evolved independently. For example, HHV-6 (family *Herpesviridae*) and ictalurid herpesvirus 1 (IcHV-1; family *Alloherpesviridae*) share structure 1, while HHV-1 (family *Herpesviridae*, subfamily *Alphaherpesvirinae*) and human herpesvirus 5 (HHV-5; family *Herpesviridae*, subfamily *Betaherpesvirinae*) share structure 4.

## Proteins

The polypeptide composition of the mature virion varies greatly among herpesviruses. More than 30 different polypeptides have been identified in HHV-1 virions; others likely remain to be found. The mature capsid is composed of four major and several minor proteins, while the tegument contains at least 15 different polypeptides, many of which are dispensable *in vitro* and are therefore not required for virion morphogenesis. The viral envelope contains at least 10 (and in some viruses many more) integral membrane proteins, a subset of which is required for adsorption and penetration of the host cell. Host proteins can be present in virions, but their functional significance has not been demonstrated.

### Lipids

The lipid composition of few herpesvirus envelopes has been reported. The lipid composition of the HHV-1 envelope is reported to resemble that of Golgi membranes more closely than that of other cellular membranes.

### Carbohydrates

Virion envelopes contain multiple proteins that carry N-linked and O-linked glycans. Mature, cell-free virions contain complex glycans, whereas a proportion of intracellular virions contain N-linked glycans of the immature high mannose type.

## Genome organization and replication

The number of ORFs contained within herpesvirus genomes that potentially encode proteins ranges from about 70 to more than 200. In addition to proteins, herpesvirus genomes also harbor varying numbers of microRNA genes (none yet identified for some viruses and 33 for HHV-4), and express numerous putatively non-translated transcripts of unknown function. A subset of about 40 protein-coding genes is conserved among the viruses of mammals and birds (family *Herpesviridae*), arranged into six gene blocks (Figure 3). The conserved gene blocks have different orders and

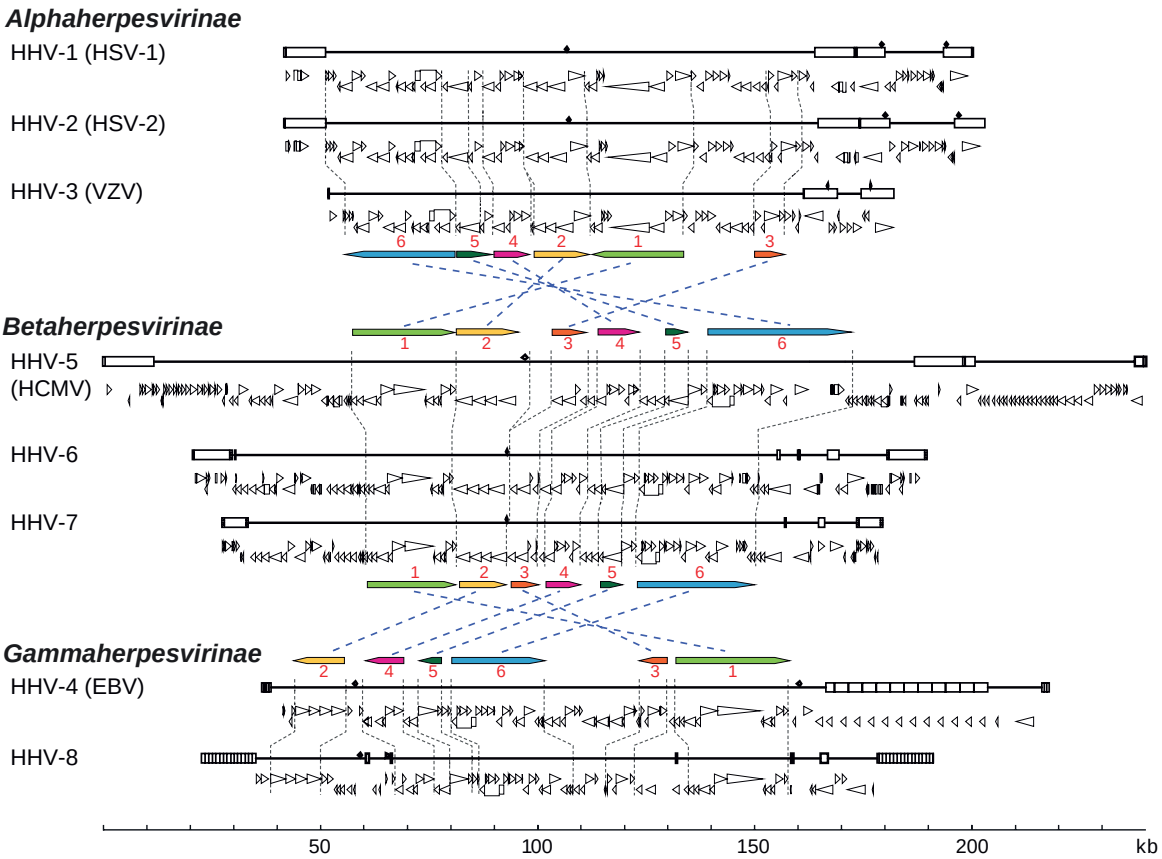



**Figure 3:** Genomic and genetic architectures of the human herpesviruses. Major repeat elements are indicated on each genomic schematic as boxes. Beneath each genome, ORFs considered likely to encode expressed proteins are indicated as triangles that are oriented to show their direction of transcription. 5′-exons of spliced genes are indicated as boxes that are connected by bars to 3′-exons. The six conserved herpesvirus sequence blocks (Block 1 through Block 6) are diagrammed to show their relative locations and orientations in the three major lineages. Diagrams are based on coordinates in GenBank accession numbers NC_001806 (HSV-1 strain 17), NC_001798 (HSV-2 strain HG52), NC_001348 (VZV strain Dumas), NC_001347 (HCMV), NC_000898 (HHV-6B strain Z29), U43400 and NC_001716 (HHV-7 strains JI and RK), NC_007605 (EBV strain B95-8) and U75698 (HHV-8, strain BC-1). (Adapted from Pellett, P.E. and Roizman, B. (2007). The *Herpesviridae*: a brief introduction In: *Fields Virology*, 5th edn (D.M. Knipe and P.M. Howley, Eds.), Lippincott, Williams, & Wilkins, Philadelphia, vol. 2, ch. 66, pp. 2479–2499; with permission of Lippincott Williams & Wilkins.)

orientations in different herpesvirus subfamilies, but genes within a block generally maintain order and transcriptional polarity. The conserved genes encode capsid proteins, components of the DNA replication and packaging machinery, nucleotide modifying enzymes, membrane proteins and tegument proteins, and to a lesser extent control proteins. This reinforces the view that, despite their genetic diversity, these viruses share common features in many aspects of their replication strategies. Members of the three families in the order *Herpesvirales* are phylogenetically very distant from each other, detectably sharing only two genes (encoding DNA polymerase and the putative ATPase subunit of terminase) derived from a common ancestor, plus a few additional genes that were probably captured independently. The unifying feature across the order *Herpesvirales* is virion morphology rather than genetic content.

Given the genetic diversity of members of the order *Herpesvirales*, it is probable that the details of their replication strategy vary, perhaps substantially. What follows, therefore, is a brief description based on well-studied members of the group, HHV-1 in particular (Figure 4). Adsorption and penetration involve the interaction of multiple virion envelope proteins with multiple cell surface receptors. Entry takes place by membrane fusion either at the cell surface or following endocytosis of the attached virion. The nucleocapsid is transported to the region of a nuclear pore by retrograde microtubule transport, while tegument proteins, many of whose functions are unknown, are thought to modify cellular metabolism. For HHV-1, one tegument protein (the UL41 gene product, vhs) acts in the cytoplasm to inhibit host protein synthesis while another (the UL48 gene product, VP16) is a transcription factor that enters the nucleus and activates viral immediate early genes. In permissive cells, entry of the genome into the nucleus is followed by a transcriptional cascade. Immediate early (α) genes, which are largely distinct among the various families and subfamilies, regulate subsequent gene expression by transcriptional and post-transcriptional mechanisms. Early (β) genes encode the DNA replication complex and a variety of enzymes and proteins involved in modifying host cell metabolism, while the structural proteins of the virus are encoded primarily by late (γ) genes. Immediate early genes can be transcribed in the absence of *de novo* protein synthesis. Early gene transcription is dependent on expression of immediate early proteins. Late gene transcription is dependent on viral DNA synthesis. With the exception of a small number of non-translated RNAs expressed by specific herpesviruses, transcription is by host RNA polymerase II.

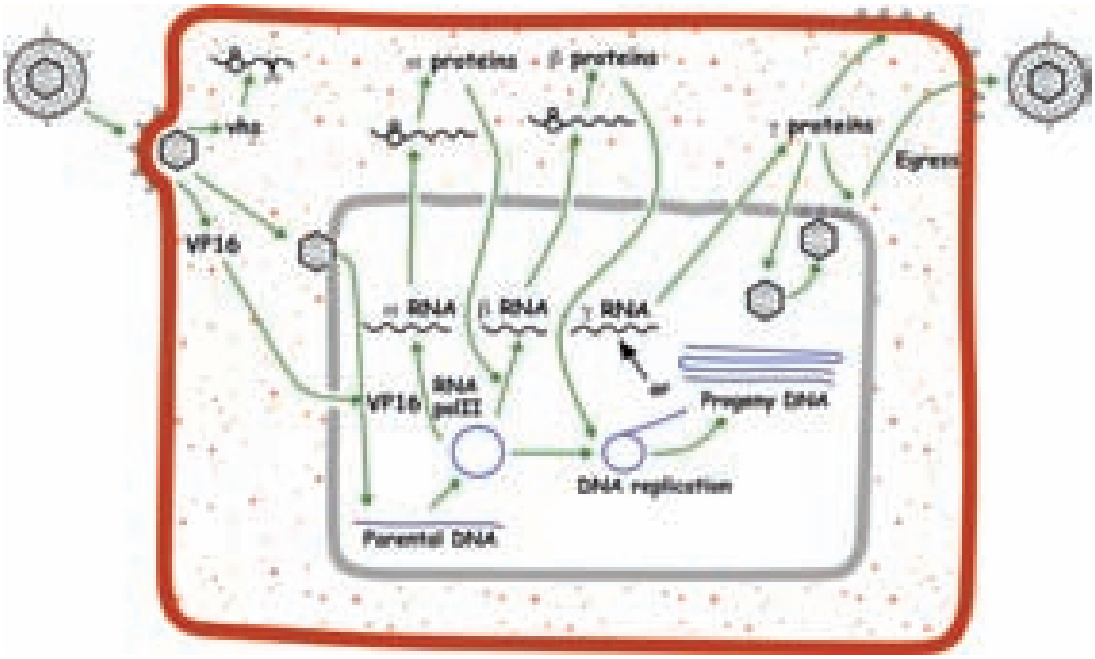



**Figure 4:** Schematic representation of the lytic replication cycle of human herpesvirus 1 (HHV-1) in permissive cells. (From Roizman, B. and Knipe, D.M. (2001). Herpes simplex viruses and their replication. In: *Fields Virology*, 4th edn (D.M. Knipe and P.M. Howley, Eds.), Lippincott Williams & Wilkins, Philadelphia, vol. 2, ch. 72, pp. 2399–2459; with permission of Lippincott Williams & Wilkins.)

Viral DNA synthesis occurs from one or more origins of replication, probably by a rolling circle mechanism. In HHV-1, DNA replication requires seven gene products: an origin-binding protein, an ssDNA-binding protein, a DNA polymerase composed of two subunits and a helicase-primase complex composed of three gene products. Homologs of all but the origin-binding protein have been identified in members of all three subfamilies in the family *Herpesviridae*. Newly synthesized DNA is packaged from the concatemer into preformed immature capsids within the nucleus by processes that involve several viral proteins. Immature capsids contain a core of scaffolding proteins, which are expelled after proteolytic cleavage during maturation. The subsequent steps in morphogenesis of secreted enveloped virions are a subject of ongoing experimentation and debate. Nucleocapsids are observed budding through the inner nuclear membrane into the perinuclear space. One line of evidence favors the view that the enveloped particles in the perinuclear space become "de-enveloped" by fusion with the outer nuclear membrane and that the resulting nucleocapsids are re-enveloped in a Golgi or post-Golgi compartment. Alternatives include (i) envelopment at the inner nuclear membrane and subsequent transit to the plasma membrane in transport vesicles, and (ii) nuclear egress via dilated nuclear pore complexes, followed by envelopment at a cytoplasmic vesicle that is transported to the plasma membrane for virion release. Only a subset of herpesvirus genes is required to achieve this basic replication cycle *in vitro*. Almost half the genes of HHV-1 are not individually required for replication in cultured cells; the products of these auxiliary genes (sometimes described as being non-essential for replication in cell culture) have diverse and often significant functions *in vivo*.

The alternative to the productive cycle, and consequent cell death, is latent infection. The establishment and maintenance of the latent state is not thoroughly understood, but the weight of evidence favors a "default" mechanism in which failure of immediate early gene expression leads to maintenance of the input genome as a circular episomal element. It has been reported that some latently-infected neurons contain large numbers of HHV-1 DNA copies, suggesting that a latent state can be established after initiation of the productive cycle. Changes in the transcription factor milieu of the latently infected cell, due to external stimuli or cell differentiation, lead to immediate early gene expression and entry into the productive cycle.

Like other large eukaryotic DNA viruses, herpesviruses are used as vectors for gene therapy.

## Antigenic properties

Infected hosts produce antibodies and cell-mediated immune responses to many structural and non-structural virus proteins. Individual antigenic proteins can harbor multiple epitopes to which antibody and cell mediated responses are elicited. Some of the envelope glycoproteins are particularly immunogenic and are targets for neutralizing antibodies. Efficient cross-neutralization is observed only between closely related viruses within a genus.

## Biological properties

The range of host species is very wide. It is probable that all vertebrates carry multiple herpesvirus species, and a herpesvirus has also been identified in invertebrates (molluscs). As a general rule, the natural host range of individual viruses is highly restricted, and most herpesviruses are thought to have evolved in association with single host species. However, there are many examples of cross-species transmission that sometimes result in severe disease and even death. This can lead to misidentification of the natural host species. Cross-species transmission is most likely among related hosts, e.g. among equids. SuHV-1 has an exceptional and remarkably wide host range in the wild, causing fatal disease in unrelated species following natural modes of transmission.

Host range varies considerably in experimental animal systems: some members of the subfamily *Alphaherpesvirinae* can infect a wide variety of animal species, whereas members of the subfamilies *Betaherpesvirinae* and *Gammaherpesvirinae* exhibit a very restricted experimental animal host range. Host range *in vitro* also varies considerably, though the same general rule holds true: members of the subfamily *Alphaherpesvirinae* will often infect a variety of cells of diverse mammalian species *in vitro*, whereas members of the subfamilies *Betaherpesvirinae* and *Gammaherpesvirinae* exhibit greater restriction. The basis of host restriction both *in vivo* and *in vitro* is poorly understood. In a few

instances (e.g. HHV-4), cell surface receptors play an important part in determining host range, but more commonly the virion is capable of entering a wide variety of cells, with intracellular factors determining susceptibility (e.g. HHV-5). Natural transmission routes range from highly contagious aerosol spread (HHV-3, equid herpevirus 4 [EHV-4] and felid herpesvirus 1 [FHV-1]) to intimate oral contact (HHV-4) or sexual contact (human herpesvirus 2 [HHV-2]); HHV-6 can be transmitted rarely via the host germ line as an integrated viral genome. Unusually, the alphaherpesvirus gallid herpesvirus 2 (GaHV-2) is shed predominantly from the base of chicken feather follicles and transmitted as an airborne, inhaled dander. Vector-mediated transmission has not been reported.

Herpesviruses are highly adapted to their hosts, and severe infection is usually observed only in the very young, the fetus, the immunocompromised, or following infection of an alternative host. Most herpesviruses establish a systemic infection, a cell-associated viraemia being detectable during primary infection. Some members of the genus *Simplexvirus* appear to be an exception to the rule: in the normal host, infection is limited to epithelium at the site of infection and sensory nerves innervating the site. A variety of immune evasion mechanisms have been identified in different viruses, including those operating against complement, antibody, MHC class I presentation and NK cell killing. The key to survival of herpesviruses is their ability to establish life-long latent infection, a feature that is assumed to be the hallmark of all herpesviruses. The cell type responsible for harboring latent virus has been established in relatively few instances. Nevertheless, the emerging picture is that for the family *Herpesviridae*, members of the subfamily *Alphaherpesvirinae* establish latent infection in neurons, members of the subfamily *Betaherpesvirinae* establish latent infection in cells of the monocyte series, and members of the subfamily *Gammaherpesvirinae* establish latent infection in lymphocytes. It should be emphasized, however, that this general picture is based on a very limited number of examples and that there are reports of latent infection at other sites. Little detail is known about latency for the families *Alloherpesviridae* and *Malacoherpesviridae*.

## Phylogenetic relationships within the order

Members of the order *Herpesvirales* comprise a diverse collection of viruses that share characteristic virion morphology. The protein that comes nearest to being herpesvirus-specific is the putative ATPase subunit of the terminase (a complex that is responsible for packaging viral DNA into nascent capsids), which is conserved in all herpesviruses and has more distant relatives in T4-like bacteriophages of the family *Myoviridae*. The diversity of the order has meant that criteria such as serology or nucleic acid hybridization have limited value in determining relationships between different viruses, and the construction of a satisfactory taxonomic structure has been a significant challenge. Historically, members of the family *Herpesviridae* were grouped into subfamilies on the basis of broad biological criteria. Division of the subfamilies into genera was based on antigenic cross-reactivity and molecular criteria, primarily the size and structure of the genome. In retrospect, these assignments were found to be generally consistent with sequence-based phylogeny. Modern classification of herpesviruses is based primarily on genetic content.

At all levels from order to species, herpesvirus taxa are described as corresponding to "distinct genetic lineages"; these lineages are defined by two criteria: (a) comparison of nucleotide or predicted amino acid sequences of conserved herpesvirus genes, and (b) identification of particular genes or genetic properties that are unique to a virus subset.

Phylogenetic relationships within the family *Herpesviridae* are illustrated in Figure 5.

## Species demarcation criteria within the order

A herpesvirus may be classified as a species if it has distinct epidemiological or biological characteristics and a distinct genome that represents an independent replicating lineage.

Sequence information is required for formal recognition of new herpesvirus species. Replicating lineages of herpesviruses are now identified primarily on the basis of information derived from genomic sequences. Sequence information sufficient to demonstrate that a novel virus represents a replicating lineage distinct from known herpesvirus species is taken as evidence that the virus in

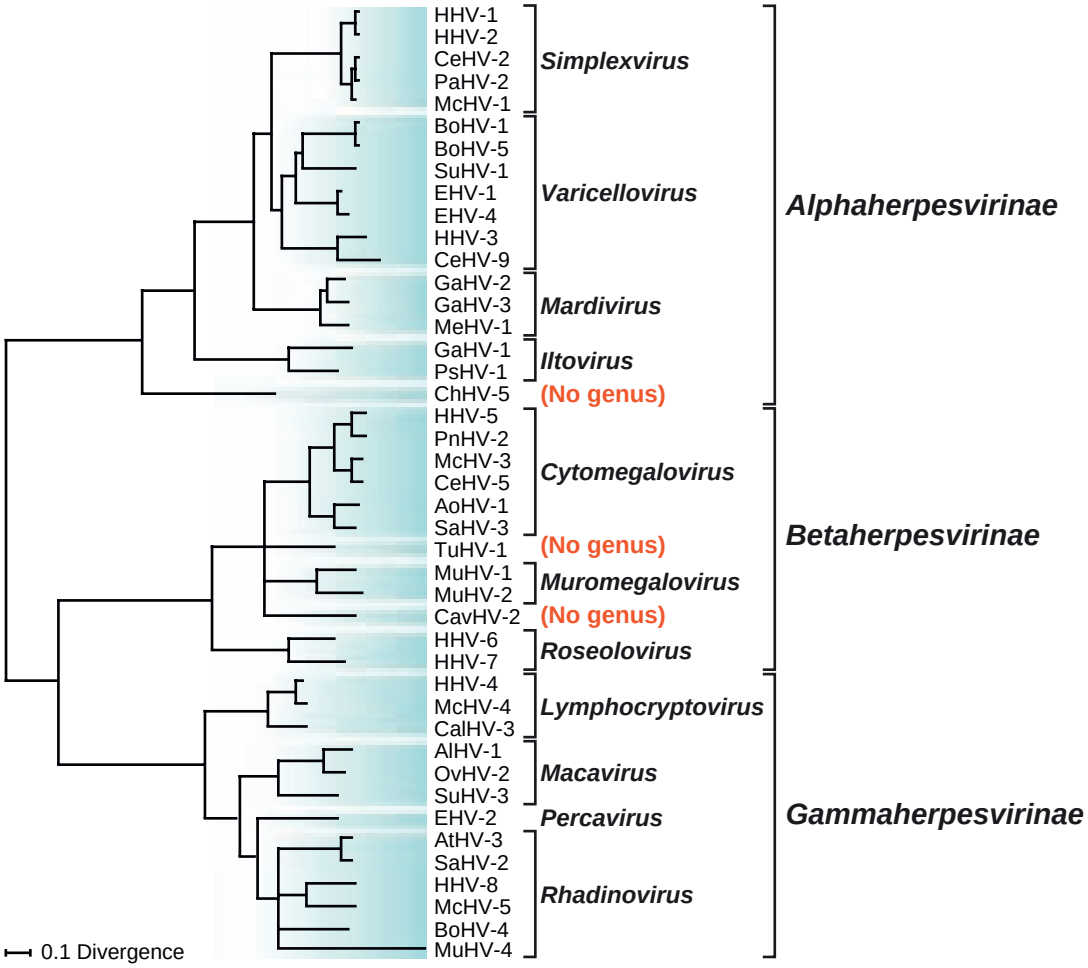



**Figure 5:** Phylogenetic relationships within the family *Herpesviridae*. The unrooted Bayesian tree is based on amino acid sequence alignments for the orthologs of HHV-1 genes UL15, UL19, UL27, UL28, UL29 and UL30. The scale indicates the number of amino acid substitutions per site. (Adapted from McGeoch, D.J., Davison, A.J., Dolan, A., Gatherer, D. and Sevilla-Reyes, E.E. (2008). Molecular evolution of the *Herpesvirales*. In: *Origin and Evolution of Viruses*, 2nd edn (E. Domingo, C.R. Parrish and J.J. Holland, Eds.), Elsevier, London, pp. 447–475; used with permission from Elsevier.)

question exists in nature, occupies a distinct ecological niche and thus can be recognized as a herpesvirus species. For some well-studied genes, there are levels of sequence difference beyond which there are no instances in which the viruses in question do not have distinct epidemiological and biological properties; such viruses can be reliably recognized as species on the basis of limited sequence information. There are also closely related viruses that have relatively small differences in the sequences of individual genes, but genetic differences extend across the respective genomes in a manner indicative of them representing independent replicating lineages. These viruses also have distinct epidemiological and biological characteristics (e.g. host identity, pathogenic and epidemiological properties, and the lack of occurrence of natural recombinants) and thus meet the definition of herpesvirus species.

## Similarity with other taxa

Herpesviruses possess several genes (e.g. encoding enzymes or immunomodulatory factors) that are related to cellular genes and are assumed to have been gained by capture. The equivalent feature in certain other virus families results in genetic similarities that probably indicate independent capture events rather than direct evolutionary relationships. One speculative exception is the putative ATPase subunit of the DNA packaging terminase complex in T4 and related dsDNA bacteriophages, which has distant counterparts in all herpesviruses.

## Nomenclature of herpesvirus species

A herpesvirus species name consists of three parts.

(i) A term derived from a taxon of the host that in its natural setting harbors the virus. The default taxon employed is that of family, and, except for the species of humans, it ends in '-id'. Exceptions are species from the family Bovidae, which are designated by host subfamily or genus, and nonhuman primates (host genus); these names end in '-ine'.
(ii) The word "herpesvirus".
(iii) An Arabic numeral, which, in combination with (i), provides a unique name.

## Derivation of names

*Allo*: from Greek *allo*, "other, different".
*Alpha*: Greek α, "a".
*Batracho*: from Greek *batrachos*, "frog".
*Beta*: Greek β, "b".
*Cyprini*: from Latin *cyprinus*, "carp".
*Cytomegalo*: from Greek *kytos*, "cell", and *megas*, "large".
*Gamma*: Greek γ, "g".
*Herpes*: from Greek *herpes*, "creeping".
*Ictaluri*: from *Ictaluri*dae.
*Ilto*: from *i*nfectious *l*aryngo*t*racheitis
*Lymphocrypto*: from Latin *lympha*, "water", and Greek *kryptos*, "concealed".
*Maca*: from *ma*lignant *ca*tarrhal fever.
*Malaco*: from Greek *malaco,* "soft", as applied to molluscs.
*Mardi*: from *Mar*ek's *di*sease.
*Muromegalo*: from Latin *mus*, "mouse", and Greek *megas*, "great".
*Ostrea*: from Greek *ostreo* or Latin *ostrea*, "oyster".
*Perca*: from *per*issodactyl and *ca*rnivore.
*Probosci*: from Greek and Latin *proboscis*, "elephant's trunk".
*Rhadino*: from Greek *rhadinos*, "slender, taper".
*Roseolo*: from Latin *rose*, "rose, rosy".
*Simplex*: from Latin *simplex*, "simple".
*Varicello*: from Latin *varius*, "spotted", and its diminutive *variola*, "smallpox".

## Further reading

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## Contributed by

Pellett, P.E. (Chair), Davison, A.J., Eberle, R., Ehlers, B., Hayward, G.S., Lacoste, V., Minson, A.C., Nicholas, J., Roizman, B., Studdert, M.J. and Wang, F.

# Family *Alloherpesviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Alloherpesviridae*** |
| Genus | ***Batrachovirus*** |
| Genus | ***Cyprinivirus*** |
| Genus | ***Ictalurivirus*** |
| Genus | ***Salmonivirus*** |

For details of Virion properties, Genome organization and replication, Antigenic properties and Species demarcation criteria, refer to Order *Herpesvirales*.

## Biological properties

The family *Alloherpesviridae* currently incorporates herpesviruses of fishes and frogs. The cyprinid herpesvirus genomes are the largest in the order *Herpesvirales*, up to 295 kbp.

# Genus *Batrachovirus*

Type species ***Ranid herpesvirus 1***

## List of species in the genus *Batrachovirus*

| | | |
|---|---|---|
| *Ranid herpesvirus 1* | | |
| Ranid herpesvirus 1 (Lucké tumor herpesvirus) | [DQ665917 = NC_008211] | (RaHV-1) |
| *Ranid herpesvirus 2* | | |
| Ranid herpesvirus 2 (Frog virus 4) | [DQ665652 = NC_008210] | (RaHV-2) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Batrachovirus* but have not been approved as species

None reported.

# Genus *Cyprinivirus*

Type species ***Cyprinid herpesvirus 3***

## List of species in the genus *Cyprinivirus*

| | | |
|---|---|---|
| *Cyprinid herpesvirus 1* | | |
| Cyprinid herpesvirus 1 (Carp pox herpesvirus) | | (CyHV-1) |
| *Cyprinid herpesvirus 2* | | |
| Cyprinid herpesvirus 2 (Goldfish haemotopoietic necrosis herpesvirus) | | (CyHV-2) |
| *Cyprinid herpesvirus 3* | | |
| Cyprinid herpesvirus 3 (Koi herpesvirus) | [DQ657948 = NC_009127] | (CyHV-3) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Cyprinivirus* but have not been approved as species

None reported.

## GENUS *ICTALURIVIRUS*

Type species ***Ictalurid herpesvirus 1***

### List of species in the genus *Ictalurivirus*

| | | |
|---|---|---|
| *Acipenserid herpesvirus 2* | | |
| Acipenserid herpesvirus 2 (White sturgeon herpesvirus 2) | | (AciHV-2) |
| *Ictalurid herpesvirus 1* | | |
| Ictalurid herpesvirus 1 (Channel catfish virus) | [M75136 = NC_001493] | (IcHV-1) |
| *Ictalurid herpesvirus 2* | | |
| Ictalurid herpesvirus 2 (Black bullhead herpesvirus) | | (IcHV-2) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Ictalurivirus* but have not been approved as species

None reported.

## GENUS *SALMONIVIRUS*

Type species ***Salmonid herpesvirus 1***

### List of species in the genus *Salmonivirus*

| | |
|---|---|
| *Salmonid herpesvirus 1* | |
| Salmonid herpesvirus 1 (Herpesvirus salmonis) | (SalHV-1) |
| *Salmonid herpesvirus 2* | |
| Salmonid herpesvirus 2 (Oncorhynchus masou herpesvirus) | (SalHV-2) |
| *Salmonid herpesvirus 3* | |
| Salmonid herpesvirus 3 (Epizootic epitheliotropic disease virus) | (SalHV-3) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Salmonivirus* but have not been approved as species

None reported.

## List of other related viruses which may be members of the family *Alloherpesviridae* but have not been approved as species

| | | |
|---|---|---|
| Acipenserid herpesvirus 1<br>(White sturgeon herpesvirus 1) | | (AciHV-1) |
| Anguillid herpesvirus 1<br>(Eel herpesvirus ) | [FJ940765 = NC_013668] | (AngHV-1) |
| Clupeid herpesvirus 1<br>(Pilchard herpesvirus) | | (ClHV-1) |
| Esocid herpesvirus 1<br>(Northern pike herpesvirus) | | (EsHV-1) |
| Percid herpesvirus 1<br>(Walleye epidermal hyperplasia herpesvirus) | | (PeHV-1) |
| Pleuronectid herpesvirus 1<br>(Turbot herpesvirus) | | (PlHV-1) |

## List of unassigned species in the family *Alloherpesviridae*

None reported.

## Phylogenetic relationships within the family

Members of the family *Alloherpesviridae* are more divergent than members of the family *Herpesviridae*, and share only approximately 13 detectably conserved genes that were putatively derived from an ancestral herpesvirus.

For Derivation of names, Further reading and Contributors, refer to Order *Herpesvirales*.

# Family *Herpesviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Herpesviridae*** |
| Subfamily | ***Alphaherpesvirinae*** |
| Genus | ***Iltovirus*** |
| Genus | ***Mardivirus*** |
| Genus | ***Simplexvirus*** |
| Genus | ***Varicellovirus*** |
| Subfamily | ***Betaherpesvirinae*** |
| Genus | ***Cytomegalovirus*** |
| Genus | ***Muromegalovirus*** |
| Genus | ***Proboscivirus*** |
| Genus | ***Roseolovirus*** |
| Subfamily | ***Gammaherpesvirinae*** |
| Genus | ***Lymphocryptovirus*** |
| Genus | ***Macavirus*** |
| Genus | ***Percavirus*** |
| Genus | ***Rhadinovirus*** |

For details of Virion properties, Genome organization and replication, Antigenic properties and Species demarcation criteria, refer to Order *Herpesvirales*.

## Biological properties

Current members of family *Herpesviridae* have hosts that include reptiles, birds and mammals.

# Subfamily *Alphaherpesvirinae*

## Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Alphaherpesvirinae*** |
| Genus | ***Iltovirus*** |
| Genus | ***Mardivirus*** |
| Genus | ***Simplexvirus*** |
| Genus | ***Varicellovirus*** |

## Distinguishing features

The nucleotide sequences or predicted amino acid sequences of subfamily members form a distinct lineage within the family. A region of the genome comprising the unique short sequence ($U_S$) and flanking internal and terminal inverted repeats ($IR_S$ and $TR_S$) contains genes homologous to those found in HHV-1 and characteristic of the subfamily. The viruses productively infect fibroblasts in culture and epithelial cells *in vivo*. Many members cause overt, usually vesicular epithelial lesions in their natural hosts.

# Genus *Iltovirus*

Type species ***Gallid herpesvirus 1***

## Distinguishing features

Predicted amino acid sequences of the members of this genus place them in a lineage distinct from other genera within the subfamily. Members have birds as hosts.

### List of species in the genus *Iltovirus*

| | | |
|---|---|---|
| *Gallid herpesvirus 1* | | |
| Gallid herpesvirus 1 (Infectious laryngotracheitis virus) | [NC_006623] | (GaHV-1) |
| *Psittacid herpesvirus 1* | | |
| Psittacid herpesvirus 1 (Pacheco's disease virus) | [AY372243 = NC_005264] | (PsHV-1) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Iltovirus* but have not been approved as species

None reported.

## GENUS *MARDIVIRUS*

Type species ***Gallid herpesvirus 2***

### Distinguishing features

Nucleotide sequences or predicted amino acid sequences of members of the genus form a distinct lineage within the subfamily. Members have been found only in birds and are the only members of the subfamily associated with malignancy. For at least GaHV-2, production of infectious extracellular virus appears to be limited to feather-follicle epithelium. Members may cross-react serologically.

### List of species in the genus *Mardivirus*

| | | |
|---|---|---|
| *Columbid herpesvirus 1* | | |
| Columbid herpesvirus 1 (Pigeon herpesvirus) | | (CoHV-1) |
| *Gallid herpesvirus 2* | | |
| Gallid herpesvirus 2 (Marek's disease virus type 1) | [AF243438 = NC_002229] | (GaHV-2) |
| *Gallid herpesvirus 3* | | |
| Gallid herpesvirus 3 (Marek's disease virus type 2) | [AB049735 = NC_002577] | (GaHV-3) |
| *Meleagrid herpesvirus 1* | | |
| Meleagrid herpesvirus 1 (Turkey herpesvirus) | [AF291866 = NC_002641] | (MeHV-1) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Mardivirus* but have not been approved as species

None reported.

# GENUS *SIMPLEXVIRUS*

Type species ***Human herpesvirus 1***

## Biological properties

Nucleotide sequences or predicted amino acid sequences of members of the genus form a distinct lineage within the subfamily. The viruses have a broad host cell range *in vitro* with a relatively rapid cytolytic productive cycle. Latent infection is established in neurons. Members of the genus are viruses of mammals, mostly primates. Members may have serological cross-reaction with other members.

## List of species in the genus *Simplexvirus*

| | | |
|---|---|---|
| *Ateline herpesvirus 1* | | |
| Ateline herpesvirus 1 | | (AtHV-1) |
| (Spider monkey herpesvirus) | | |
| *Bovine herpesvirus 2* | | |
| Bovine herpesvirus 2 | | (BoHV-2) |
| (Bovine mammillitis virus) | | |
| *Cercopithecine herpesvirus 2* | | |
| Cercopithecine herpesvirus 2 | [AY714813 = NC_006560] | (CeHV-2) |
| (Simian agent 8 (SA8)) | | |
| *Human herpesvirus 1* | | |
| Human herpesvirus 1 | [X14112 = NC_001806] | (HHV-1) |
| (Herpes simplex virus [type] 1) | | |
| *Human herpesvirus 2* | | |
| Human herpesvirus 2 | [Z86099 = NC_001798] | (HHV-2) |
| (Herpes simplex virus [type] 2) | | |
| *Leporid herpesvirus 4* | | |
| Leporid herpesvirus 4 | | (LeHV-4) |
| *Macacine herpesvirus 1* | | |
| Macacine herpesvirus 1 | [AF533768 = NC_004812] | (McHV-1) |
| (Cercopithecine herpesvirus 1) | | |
| (B-virus; herpesvirus simiae) | | |
| *Macropodid herpesvirus 1* | | |
| Macropodid herpesvirus 1 | | (MaHV-1) |
| (Parma wallaby herpesvirus) | | |
| *Macropodid herpesvirus 2* | | |
| Macropodid herpesvirus 2 | | (MaHV-2) |
| (Dorcopsis wallaby herpesvirus) | | |
| *Papiine herpesvirus 2* | [DQ149153 = NC_007653] | |
| Papiine herpesvirus 2 | | (PaHV-2) |
| (Cercopithecine herpesvirus 16) | | |
| (Herpesvirus papio 2) | | |
| *Saimiriine herpesvirus 1* | | |
| Saimiriine herpesvirus 1 | [HM615781 = NC_014567] | (SaHV-1) |
| (Herpesvirus tamarinus) | | |
| (Marmoset herpesvirus) | | |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Simplexvirus* but have not been approved as species

None reported.

# Genus *Varicellovirus*

Type species ***Human herpesvirus 3***

## Distinguishing features

Nucleotide sequences or predicted amino acid sequences of members of the genus form a distinct lineage within the subfamily. Latent infection is established in cells of the sensory nervous system, though latent infection of other sites has also been reported. Members have been found in a wide range of mammalian hosts. Some members cross-react serologically.

## List of species in the genus *Varicellovirus*

| | | |
|---|---|---|
| *Bovine herpesvirus 1* | | |
| Bovine herpesvirus 1 | [AJ004801 = NC_001847] | (BoHV-1) |
| (Infectious bovine rhinotracheitis virus) | | |
| *Bovine herpesvirus 5* | | |
| Bovine herpesvirus 5 | [AY261359 = NC_005261] | (BoHV-5) |
| (Bovine encephalitis herpesvirus) | | |
| *Bubaline herpesvirus 1* | | |
| Bubaline herpesvirus 1 | | (BuHV-1) |
| (Water buffalo herpesvirus) | | |
| *Canid herpesvirus 1* | | |
| Canid herpesvirus 1 | | (CaHV-1) |
| (Canine herpesvirus) | | |
| *Caprine herpesvirus 1* | | |
| Caprine herpesvirus 1 | | (CpHV-1) |
| (Goat herpesvirus) | | |
| *Cercopithecine herpesvirus 9* | | |
| Cercopithecine herpesvirus 9 | [AF275348 = NC_002686] | (CeHV-9) |
| (Simian varicella virus) | | |
| (Liverpool vervet herpesvirus) | | |
| (Patas monkey herpesvirus) | | |
| (Medical Lake macaque herpesvirus) | | |
| *Cervid herpesvirus 1* | | |
| Cervid herpesvirus 1 | | (CvHV-1) |
| (Red deer herpesvirus) | | |
| *Cervid herpesvirus 2* | | |
| Cervid herpesvirus 2 | | (CvHV-2) |
| (Reindeer herpesvirus) | | |
| *Equid herpesvirus 1* | | |
| Equid herpesvirus 1 | [AY665713 = NC_001491] | (EHV-1) |
| (Equine abortion virus) | | |
| *Equid herpesvirus 3* | | (EHV-3) |
| (Equine coital exanthema virus) | | |
| *Equid herpesvirus 4* | | |
| Equid herpesvirus 4 | [AF030027 = NC_001844] | (EHV-4) |
| (Equine rhinopneumonitis virus) | | |
| *Equid herpesvirus 8* | | |
| Equid herpesvirus 8 | | (EHV-8) |
| (Asinine herpesvirus 3) | | |
| *Equid herpesvirus 9* | | |
| Equid herpesvirus 9 | [AP010838 = NC_011644] | (EHV-9) |
| (Zebra herpesvirus) | | |
| (Gazelle herpesvirus) | | |
| *Felid herpesvirus 1* | | |
| Felid herpesvirus 1 | [FJ478159 = NC_013590] | (FeHV-1) |
| (Feline rhinotracheitis virus) | | |
| *Human herpesvirus 3* | | |
| Human herpesvirus 3 | [X04370 = NC_001348] | (HHV-3) |
| (Varicella-zoster virus) | | |

*Phocid herpesvirus 1*
Phocid herpesvirus 1 (PhoHV-1)
(Harbour seal herpesvirus)
*Suid herpesvirus 1*
Suid herpesvirus 1 [BK001744 = NC_006151] (SuHV-1)
(Pseudorabies virus)

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.



### List of other related viruses which may be members of the genus *Varicellovirus* but have not been approved as species

Equid herpesvirus 6 (EHV-6)
(Asinine herpesvirus 1)

### List of unassigned species in the subfamily *Alphaherpesvirinae*

*Chelonid herpesvirus 5*
Chelonid herpesvirus 5 (ChHV-5)
(Chelonid fibropapilloma-associated herpesvirus)
*Chelonid herpesvirus 6*
Chelonid herpesvirus 6 (ChHV-6)
(Lung-eye-trachea disease-associated virus)

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Assigned abbreviations ( ) are also listed.

## SUBFAMILY *BETAHERPESVIRINAE*

### Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Betaherpesvirinae*** |
| Genus | ***Cytomegalovirus*** |
| Genus | ***Muromegalovirus*** |
| Genus | ***Proboscivirus*** |
| Genus | ***Roseolovirus*** |

### Distinguishing features

The nucleotide or predicted amino acid sequences of the subfamily members form a distinct lineage within the family. Genes corresponding to the HHV-5 US22 gene family are characteristic of the subfamily. The viruses tend to be species-specific and cell-type-specific in culture. The growth cycle is slow and virus tends to remain cell-associated. Infection is often clinically non-apparent in immune-competent hosts. In some instances, latent infection has been associated with cells of the monocyte series.

## GENUS *CYTOMEGALOVIRUS*

Type species ***Human herpesvirus 5***

### Biological properties

The nucleotide or predicted amino acid sequences of members of the genus form a distinct lineage within the subfamily. Cytomegalovirus genomes are large ( > 200 kbp). Members of the genus have

mammals as hosts. Infections with these viruses generally result in a marked increase in cell volume (cytomegalia) and development of prominent and distinctive nuclear and cytoplasmic inclusions.

### List of species in the genus *Cytomegalovirus*

| | | |
|---|---|---|
| *Cercopithecine herpesvirus 5* | | |
| Cercopithecine herpesvirus 5<br>(African green monkey cytomegalovirus)<br>(Simian cytomegalovirus) | [FJ483968 = NC_012783] | (CeHV-5) |
| *Human herpesvirus 5* | | |
| Human herpesvirus 5<br>(Human cytomegalovirus) | [AY446894 = NC_006273] | (HHV-5) |
| *Macacine herpesvirus 3* | | |
| Macacine herpesvirus 3<br>(Cercopithecine herpesvirus 8)<br>(Rhesus monkey cytomegalovirus) | [AY186194 = NC_006150] | (McHV-3) |
| *Panine herpesvirus 2* | | |
| Panine herpesvirus 2<br>(Pongine herpesvirus 4)<br>(Chimpanzee cytomegalovirus) | [AF480884 = NC_003521] | (PnHV-2) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Cytomegalovirus* but have not been approved as species

| | | |
|---|---|---|
| Aotine herpesvirus 1<br>(Herpesvirus aotus type 1)<br>(Owl monkey cytomegalovirus) | [FJ483970] | (AoHV-1) |
| Aotine herpesvirus 2<br>(Herpesvirus aotus type 2) | | (AoHV-2) |
| Aotine herpesvirus 3<br>(Herpesvirus aotus type 3) | | (AoHV-3) |
| Saimiriine herpesvirus 3<br>(Squirrel monkey cytomegalovirus) | [FJ483967] | (SaHV-3) |
| Papiine herpesvirus 3<br>(Baboon cytomegalovirus) | [AC090446] | (PaHV-3) |

## Genus *Muromegalovirus*

Type species ***Murid herpesvirus 1***

### Biological properties

The nucleotide or predicted amino acid sequences of members of the genus form a distinct lineage within the subfamily. Muromegalovirus genomes are large (>200 kbp). Infected cells become enlarged (cytomegalic). Members of the genus have rodents as hosts.

### List of species in the genus *Muromegalovirus*

| | | |
|---|---|---|
| *Murid herpesvirus 1* | | |
| Murid herpesvirus 1<br>(Mouse cytomegalovirus) | [U68299 = NC_004065] | (MuHV-1) |
| *Murid herpesvirus 2* | | |
| Murid herpesvirus 2<br>(Rat cytomegalovirus, Maastricht strain) | [AF232689 = NC_002512] | (MuHV-2) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Muromegalovirus* but have not been approved as species

Murid herpesvirus 8 (MuHV-8)
(Rat cytomegalovirus, England strain)



## GENUS *PROBOSCIVIRUS*

Type species ***Elephantid herpesvirus 1***

### Biological properties

The nucleotide or predicted amino acid sequences of members of the genus form a distinct lineage within the subfamily. Elephants are the only known hosts of members of the genus.

### List of species in the genus *Proboscivirus*

*Elephantid herpesvirus 1*
Elephantid herpesvirus 1 (ElHV-1)
(Elephant endotheliotropic herpesvirus)

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Proboscivirus* but have not been approved as species

None reported.

## GENUS *ROSEOLOVIRUS*

Type species ***Human herpesvirus 6***

### Biological properties

The nucleotide or predicted amino acid sequences form a distinct lineage within the subfamily and the genomes are smaller than those of members of the other genera within the subfamily (<200 kbp). The viruses productively infect T lymphocytes. Current members are serologically related.

### List of species in the genus *Roseolovirus*

| | | |
|---|---|---|
| *Human herpesvirus 6* | | |
| Human herpesvirus 6 | | (HHV-6) |
| (Human herpesvirus 6, variant A) | [X83413 = NC_001664] | (HHV-6A) |
| (Human herpesvirus 6, variant B) | [AF157706 = NC_000898] | (HHV-6B) |
| *Human herpesvirus 7* | | |
| Human herpesvirus 7 | [AF037218 = NC_001716] | (HHV-7) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Roseolovirus* but have not been approved as species

None reported.

### List of unassigned species in the subfamily *Betaherpesvirinae*

| | | |
|---|---|---|
| *Caviid herpesvirus 2* | | |
| Caviid herpesvirus 2 (Guinea pig cytomegalovirus) | [FJ355434 = NC_011587] | (CavHV-2) |
| *Suid herpesvirus 2* | | (SuHV-2) |
| Suid herpesvirus 2 (Pig cytomegalovirus) | | |
| *Tupaiid herpesvirus 1* | | |
| Tupaiid herpesvirus 1 (Tree shrew herpesvirus) | [AF281817 = NC_002794] | (TuHV-1) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## SUBFAMILY *GAMMAHERPESVIRINAE*

### Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Gammaherpesvirinae*** |
| Genus | ***Lymphocryptovirus*** |
| Genus | ***Macavirus*** |
| Genus | ***Percavirus*** |
| Genus | ***Rhadinovirus*** |

### Distinguishing features

The nucleotide or predicted amino acid sequences of the subfamily members form a distinct lineage within the family. Certain genes may be unique to members of the subfamily. These include BNRF-1, BTRF-1 and BRLF-1 of HHV-4 (the corresponding ORFs of saimiriine herpesvirus 2 are ORFs 3, 23 and 50, respectively). Many members of the subfamily infect lymphocytes *in vitro*, and "carrier" cultures can be established in which a minority of cells is productively infected. Latent infection *in vivo* occurs in lymphocytes or lymphoid tissue. Acute infection is frequently associated with lymphoproliferative disorders and many members of the subfamily are associated with malignancies of lymphoid and non-lymphoid origin.

## GENUS *LYMPHOCRYPTOVIRUS*

Type species ***Human herpesvirus 4***

### Biological properties

The nucleotide or predicted amino acid sequences of members of the genus form a distinct lineage within the subfamily. The Epstein–Barr nuclear antigen (EBNA) genes of HHV-4 and their homologs appear to be unique to members of the genus. The viruses infect B lymphocytes in culture but infection is usually non-productive and can result in immortalization. B cells or their precursors are thought to be the site of latent infection *in vivo*. Current members of the genus have been found only in primates.

## List of species in the genus *Lymphocryptovirus*

| | | |
|---|---|---|
| *Callitrichine herpesvirus 3* | | |
| Callitrichine herpesvirus 3 | [AF319782 = NC_004367] | (CalHV-3) |
| (Marmoset lymphocryptovirus) | | |
| *Cercopithecine herpesvirus 14* | | |
| Cercopithecine herpesvirus 14 | | (CeHV-14) |
| (African green monkey EBV-like virus) | | |
| *Gorilline herpesvirus 1* | | |
| Gorilline herpesvirus 1 | | (GoHV-1) |
| (Pongine herpesvirus 3) | | |
| (Gorilla herpesvirus) | | |
| *Human herpesvirus 4* | | |
| Human herpesvirus 4 | | (HHV-4) |
| (Epstein–Barr virus) | | |
| (Epstein–Barr virus, type 1) | [AJ507799 = NC_007605] | |
| (Epstein–Barr virus, type 2) | [DQ279927 = NC_009334] | |
| *Macacine herpesvirus 4* | | |
| Macacine herpesvirus 4 | [AY037858 = NC_006146] | (McHV-4) |
| (Cercopithecine herpesvirus 15) | | |
| (Rhesus EBV-like herpesvirus) | | |
| (Rhesus lymphocryptovirus) | | |
| *Panine herpesvirus 1* | | |
| Panine herpesvirus 1 | | (PnHV-1) |
| (Pongine herpesvirus 1) | | |
| (Herpesvirus pan) | | |
| *Papiine herpesvirus 1* | | |
| Papiine herpesvirus 1 | | (PaHV-1) |
| (Cercopithecine herpesvirus 12) | | |
| (Herpesvirus papio) | | |
| (Baboon herpesvirus) | | |
| *Pongine herpesvirus 2* | | |
| Pongine herpesvirus 2 | | (PoHV-2) |
| (Orangutan herpesvirus) | | |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Lymphocryptovirus* but have not been approved as species

None reported.

# GENUS *MACAVIRUS*

Type species ***Alcelaphine herpesvirus 1***

## Biological properties

The nucleotide or predicted amino acid sequences of members of the genus form a distinct lineage within the subfamily. Members of the genus have mammals as hosts.

## List of species in the genus *Macavirus*

| | | |
|---|---|---|
| *Alcelaphine herpesvirus 1* | | |
| Alcelaphine herpesvirus 1 | [AF005370 = NC_002531] | (AlHV-1) |
| Malignant catarrhal fever virus | | |

| | | |
|---|---|---|
| *Alcelaphine herpesvirus 2* | | |
| Alcelaphine herpesvirus 2 (Hartebeest malignant catarrhal fever virus) | | (AlHV-2) |
| *Bovine herpesvirus 6* | | |
| Bovine herpesvirus 6 (Bovine lymphotropic herpesvirus) | | (BoHV-6) |
| *Caprine herpesvirus 2* | | |
| Caprine herpesvirus 2 | | (CpHV-2) |
| *Hippotragine herpesvirus 1* | | |
| Hippotragine herpesvirus 1 (Roan antelope herpesvirus) | | (HiHV-1) |
| *Ovine herpesvirus 2* | | |
| Ovine herpesvirus 2 (Sheep-associated malignant catarrhal fever virus) | [AY839756 = NC_007646] | (OvHV-2) |
| *Suid herpesvirus 3* | | |
| Suid herpesvirus 3 (Porcine lymphotropic herpesvirus 1) | | (SuHV-3) |
| *Suid herpesvirus 4* | | |
| Suid herpesvirus 4 (Porcine lymphotropic herpesvirus 2) | | (SuHV-4) |
| *Suid herpesvirus 5* | | |
| Suid herpesvirus 5 (Porcine lymphotropic herpesvirus 3) | | (SuHV-5) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Macavirus* but have not been approved as species

None reported.

## GENUS *PERCAVIRUS*

Type species ***Equid herpesvirus 2***

### Biological properties

The nucleotide or predicted amino acid sequences of members of the genus form a distinct lineage within the subfamily. Members of the genus have mammals as hosts.

### List of species in the genus *Percavirus*

| | | |
|---|---|---|
| *Equid herpesvirus 2* | | |
| Equid herpesvirus 2 (Equine herpesvirus 2) | [U20824 = NC_001650] | (EHV-2) |
| *Equid herpesvirus 5* | | |
| Equid herpesvirus 5 (Equine herpesvirus 5) | | (EHV-5) |
| *Mustelid herpesvirus 1* | | |
| Mustelid herpesvirus 1 (Badger herpesvirus) | | (MusHV-1) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Percavirus* but have not been approved as species

None reported.



## GENUS *RHADINOVIRUS*

Type species ***Saimiriine herpesvirus 2***

### Biological properties

The nucleotide or predicted amino acid sequences of members of the genus form a distinct lineage within the subfamily. Members of genus *Rhadinovirus* have mammals as hosts.

### List of species in the genus *Rhadinovirus*

| | | |
|---|---|---|
| *Ateline herpesvirus 2* | | |
| Ateline herpesvirus 2 | | (AtHV-2) |
| (Herpesvirus ateles strain 810) | | |
| *Ateline herpesvirus 3* | | |
| Ateline herpesvirus 3 | [AF083424 = NC_001987] | (AtHV-3) |
| (Herpesvirus ateles strain 73) | | |
| *Bovine herpesvirus 4* | | |
| Bovine herpesvirus 4 | [AF318573 = NC_002665] | (BoHV-4) |
| (Movar virus) | | |
| *Human herpesvirus 8* | | |
| Human herpesvirus 8 | [AF148805 = NC_009333] | (HHV-8) |
| (Kaposi's sarcoma-associated herpesvirus) | | |
| *Macacine herpesvirus 5* | | |
| Macacine herpesvirus 5 | [AF083501 = NC_003401] | (McHV-5) |
| (Cercopithecine herpesvirus 17) | | |
| (Rhesus rhadinovirus) | | |
| *Murid herpesvirus 4* | | |
| Murid herpesvirus 4 | [U97553 = NC_001826] | (MuHV-4) |
| (Mouse herpesvirus strain 68) | | |
| (Murine gammaherpesvirus 68) | | |
| *Saimiriine herpesvirus 2* | | |
| Saimiriine herpesvirus 2 | [X64346 = NC_001350] | (SaHV-2) |
| (Herpesvirus saimiri) | | |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Rhadinovirus* but have not been approved as species

| | |
|---|---|
| Leporid herpesvirus 1 | (LeHV-1) |
| (Cottontail rabbit herpesvirus) | |
| Leporid herpesvirus 2 | (LeHV-2) |
| (Herpesvirus cuniculi) | |
| Leporid herpesvirus 3 | (LeHV-3) |
| (Herpesvirus sylvilagus) | |
| Marmodid herpesvirus 1 | (MarHV-1) |
| (Woodchuck herpesvirus) | |
| (Herpesvirus marmota) | |
| Murid herpesvirus 7 | (MuHV-7) |
| (Wood mouse herpesvirus) | |

## List of unassigned species in the subfamily *Gammaherpesvirinae*

*Equid herpesvirus 7*
Equid herpesvirus 7 (EHV-7)
(Asinine herpesvirus 2)
*Phocid herpesvirus 2*
Phocid herpesvirus 2 (PhoHV-2)
*Saguinine herpesvirus 1*
Saguinine herpesvirus 1 (SgHV-1)
(Callitrichine herpesvirus 1)
(Herpesvirus saguinus)

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Assigned abbreviations ( ) are also listed.

## List of unassigned species in the family *Herpesviridae*

*Iguanid herpesvirus 2*
Iguanid herpesvirus 2 (IgHV-2)
(Iguana herpesvirus)

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Assigned abbreviations ( ) are also listed.

**Note:** A supplementary list of other related viruses which may be members of the family *Herpesviridae* but have not been approved as species is available online on Science Direct®, www.sciencedirect.com.

## Phylogenetic relationships within the family

Members of the *Herpesviridae* can be readily and reliably assigned to one of the three subfamilies on the basis of relationships among any of several conserved proteins, e.g. the DNA polymerase. Many elements of gene content and gene order are conserved at the subfamily level.

For Derivation of names, Further reading and Contributors, refer to Order *Herpesvirales*.

# FAMILY *MALACOHERPESVIRIDAE*

## Taxonomic structure of the family

Family ***Malacoherpesviridae***
Genus ***Ostreavirus***

For details of Virion properties, Genome organization and replication, Antigenic properties and Species demarcation criteria, refer to Order *Herpesvirales*.

## Biological properties

The family *Malacoherpesviridae* contains the single known herpesvirus species with an invertebrate host.

# GENUS *OSTREAVIRUS*

Type species ***Ostreid herpesvirus 1***

## List of species in the genus *Ostreavirus*

*Ostreid herpesvirus 1*
Ostreid herpesvirus 1 [AY509253 = NC_005881] (OsHV-1)
(Oyster herpesvirus)

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Ostreavirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Malacoherpesviridae*

None reported.

For Derivation of names, Further reading and Contributors, refer to Order *Herpesvirales*.

# Family *Adenoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Adenoviridae* |
| Genus | *Mastadenovirus* |
| Genus | *Aviadenovirus* |
| Genus | *Atadenovirus* |
| Genus | *Siadenovirus* |
| Genus | *Ichtadenovirus* |



## Virion properties

### Morphology

Virions are non-enveloped, 70–90 nm in diameter. The icosahedral capsid consists of 240 non-vertex capsomers (hexons), 8–10 nm in diameter, and 12 vertex capsomers (penton bases), each with a fiber protruding from the virion surface giving the characteristic morphology (Figure 1). Penton base and fiber together make up the penton. The length of fibers examined so far ranges between 9 and 77.5 nm. Human adenoviruses 40 and 41 have fibers of two different lengths that occur alternately on the vertexes. Members of the genus *Aviadenovirus* have two fiber proteins per vertex. The 240 hexons are formed by the interaction of three identical polypeptides (designated II) and consist of two distinct parts: a triangular top with three "towers", and a pseudohexagonal base with a central cavity. The hexon bases are tightly packed, forming a protein shell that protects the inner components. In members of the genus *Mastadenovirus*, 12 copies of polypeptide IX are found between nine hexons in the centre of each facet. Polypeptide IX is not present in the other four genera. Two monomers of IIIa are located underneath the vertex region. Multiple copies of protein VI form a ring underneath the peripentonal hexons. The 12 penton bases are each formed by the interaction of five polypeptides (III) and are tightly associated with one or two (only in aviadenoviruses) fibers, each consisting of three polypeptides (IV) that interact to form a shaft of characteristic length with a distal knob. The 12 pentons (III and IV) are less tightly associated with the neighbouring (peripentonal) hexons. Polypeptide VIII has been assigned to the inner surface of the hexon capsid.

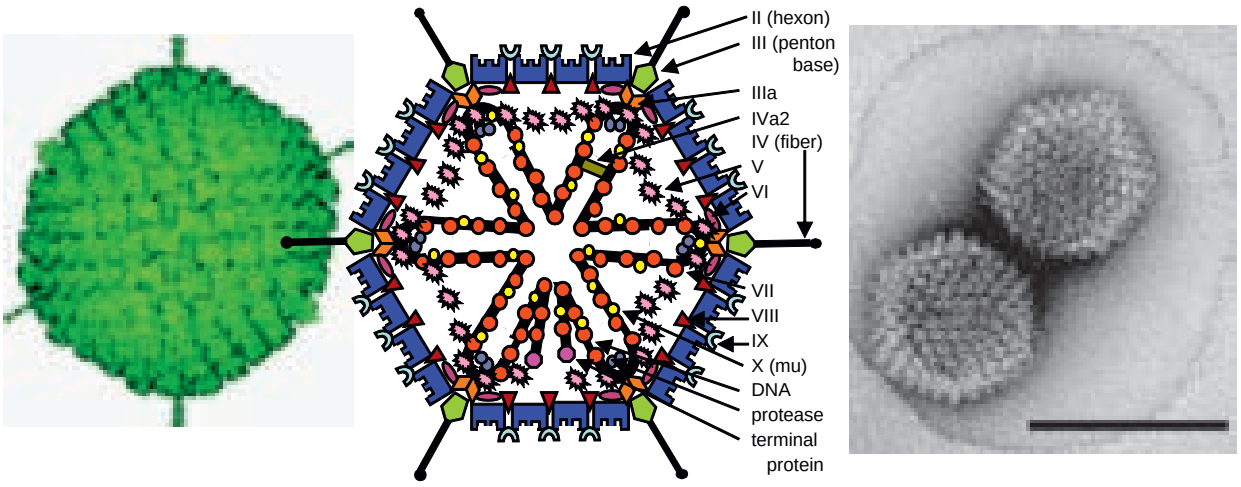


**Figure 1:** (Left) Cryo-EM reconstruction of a human adenovirus 2 particle (Stewart, P.L. *et al.* (1991). *Cell*, **67**, 145–154). (Centre) Stylized section of a mastadenovirus particle. For a description of the capsid proteins (II, III, IIIa, IV, VI, VIII and IX) and core proteins (V, VII, X and TP), see text. As the structure of the nucleoprotein core has not been established, the polypeptides associated with the DNA are shown in hypothetical locations. (Adapted from Stewart, P.L. and Burnett, R.M. (1993). *Jpn J. Appl. Phys.*, **32**, 1342–1347). (Right) Fowl adenovirus 9 particle negatively stained with uranyl acetate, showing the characteristic double fibers of fowl adenoviruses. (From Gelderblom, H. and Maichle-Lauppe, I. (1982). *Arch. Virol.* **72**, 289–298; with permission.) The bar represents 100 nm.

Other polypeptides (monomers of IIIa, trimers of IX and multimers of VI) are in contact with hexons, completing a continuous protein shell. Polypeptides VI and VIII appear to link the capsid to the virus core. The core consists of the DNA genome complexed with four polypeptides (V, VII, X, also known as mu or μ, and terminal protein). Protein V is found only in mastadenoviruses.

### Physicochemical and physical properties

Virion Mr is 150–180 $\times 10^6$; buoyant density in CsCl is 1.31–1.36 g cm$^{-3}$. Viruses are stable on storage in the frozen state. They are stable to mild acid and insensitive to lipid solvents. Heat sensitivity varies in the different genera.

### Nucleic acid

The genome is a single, linear molecule of dsDNA and contains an inverted terminal repetition (ITR). A virus-coded terminal protein (TP) is covalently linked to the 5′ end of each DNA strand. The size of genomes fully sequenced to date ranges between 26,163 and 48,395 bp, with ITRs of 36 to 371 bp. The G+C content of DNA varies between 33.6% and 66.9%. The central part of the genome is well conserved throughout the family, whereas the two ends show large variations in length and gene content (Figure 2).

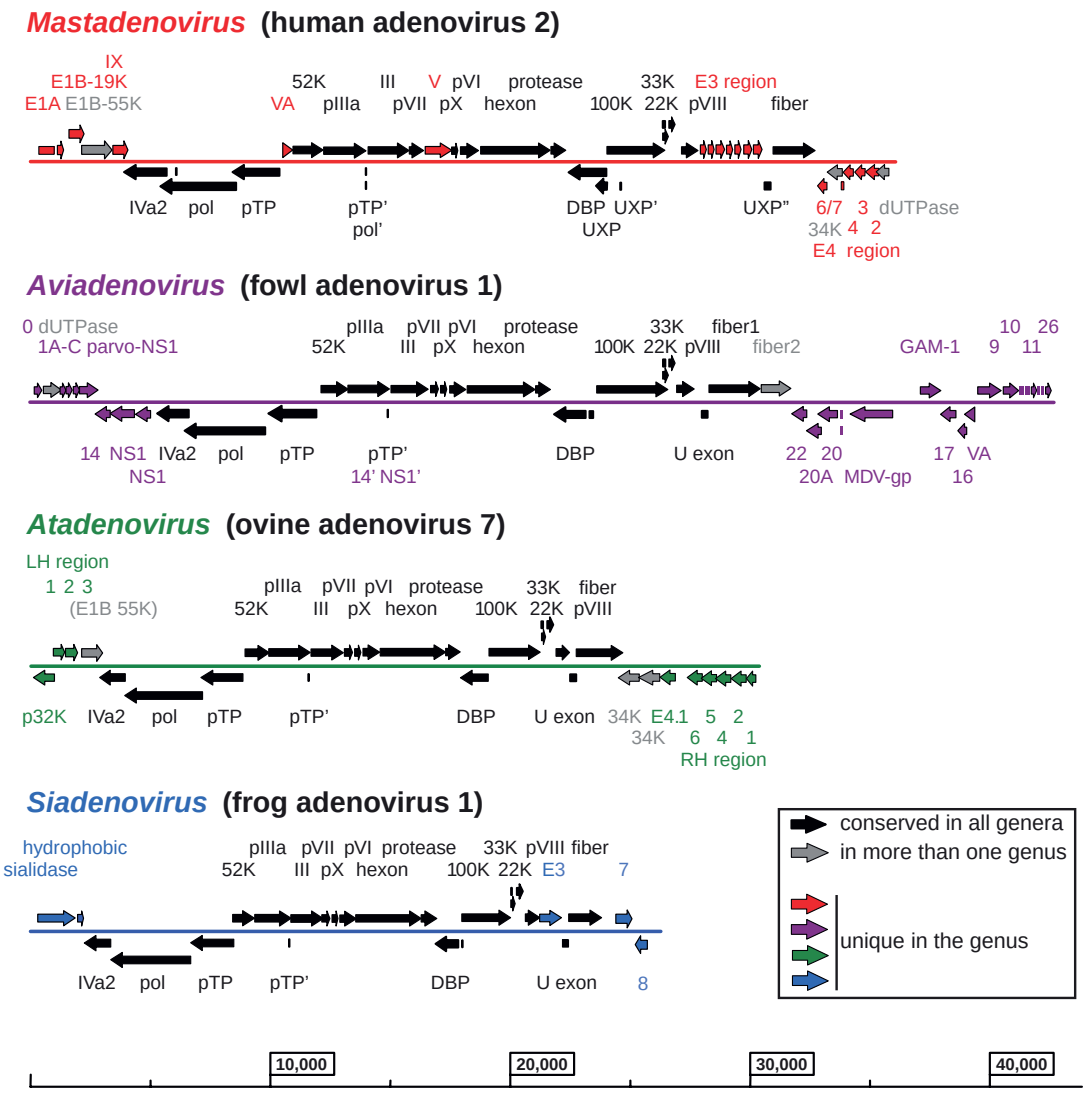


**Figure 2:** Schematic illustration of the various genome organizations found in members of four adenovirus genera. Black arrows depict genes conserved in every genus, grey arrows show genes present in more than one genus, and coloured arrows show genus-specific genes.

### Proteins

About 40 different polypeptides are produced, mostly via complex splicing mechanisms (Figure 2, Table 1). Almost a third compose the virion, including a virus-encoded cysteine protease (23 kDa), which is necessary for the processing of some precursor proteins (marked with p). With the exception of proteins V and IX, the other structural proteins are well conserved in every genus. Products of the four early regions (E1 to E4; E1 is often considered as two regions, E1A and E1B) facilitate extensive modulation of the host cell's transcriptional machinery (E1 and E4), comprise the virus DNA replication complex (E2) and provide means for subverting host defense mechanisms (E3). E2 is well conserved throughout the family, while the length and gene content of E1, E3 and E4 show great variability even within genera. Intermediate (IX and IVa2) and late gene products (L1–L5) are concerned with virion assembly and maturation.

### Lipids

None reported.

### Carbohydrates

Fiber proteins and some of the nonstructural proteins are glycosylated.

**Table 1:** Virus proteins as deduced from genome sequence of human adenovirus 2

| kDa | Transcription class | Description | Note |
|---|---|---|---|
| 13, 27, 32 | E1A | NS | Only in mastadenoviruses |
| 16, 21 | E1B | NS | Only in mastadenoviruses |
| 55 | E1B | NS | Only in mastadenoviruses |
| 59 | E2A | NS; 72 kDa* DBP | |
| 120 | E2B | NS; 140 kDa* DNA pol | |
| 75 | E2B | S; Term, 87 kDa* pTP† | |
| 4, 7, 8, 10, 12 13, 15, 15, 19 | E3 | NS | Only in mastadenoviruses |
| 7, 13, 13, 14 | E4 | NS | Only in mastadenoviruses |
| 15 | E4 | NS; 31 kDa* dUTPase | Only in some mast- and aviadenoviruses |
| 17 | E4 | NS; 34 kDa* | Only in mast- and atadenoviruses |
| 47 | L1 | NS; scaffolding 52/55 kDa* | |
| 64 | L1 | S (pIIIa);† p-protein | |
| 63 | L2 | S (III); penton base* | |
| 22 | L2 | S (pVII);† major core | |
| 42 | L2 | S (V); minor core | Only in mastadenoviruses |
| 10 | L2 | S (pX);† X/μ | |
| 27 | L3 | S (pVI)† | |
| 109 | L3 | S (II); hexon | |
| 23 | L3 | S; protease | |
| 90 | L4 | NS; 100 kDa* | |
| 25 | L4 | NS; 33 kDa* p-protein | |
| 25 | L4 | S (pVIII)† | |
| 62 | L5 | S (IV); fiber | |
| 14 | Intermediate | S (IX) | Only in mastadenoviruses |
| 51 | Intermediate | S (IVa2) | |

Molecular masses are rounded to nearest 1000, and are presented as unmodified and uncleaved gene products. NS = non-structural; S = structural; p = precursor; p-protein = phosphoprotein; DBP = DNA-binding protein, DNA pol = DNA polymerase; TP = terminal protein; * = Mr values are significantly different from those obtained by SDS-PAGE; † = cleaved by viral protease.

## Genome organization and replication

Virus entry occurs by attachment via the fiber knob to different receptors on the surface of susceptible cells, and subsequent internalization via interaction between the penton base and cellular $\alpha_v$ integrins. Protein VI mediates the release of virions from the endosomes, allowing dynein-mediated transport on microtubules to nuclear pores. After uncoating, the virus core is delivered to the nucleus, which is the site of virus RNA transcription, DNA replication and assembly. Virus infection mediates the early shut-off of host DNA synthesis and, later, synthesis of host mRNA and protein are also shut off. Transcription by host RNA polymerase II involves both DNA strands of the virus genome, and initiates (in human adenovirus 2, HAdV-2) from five early (E1A, E1B, E2, E3 and E4), two intermediate (IX and IVa2), the major late (L) and the U exon protein (UXP) late promoter in the pattern shown in Figure 3. All primary transcripts are capped and polyadenylated. There are complex splicing patterns to produce families of mRNAs. In primate adenoviruses, there are one or two virus-associated (VA) RNA genes, which are transcribed by cellular RNA polymerase III. These encode RNA products that facilitate translation of late mRNAs and blocking of the cellular interferon response. Similar VA RNA genes have not been identified in other adenoviruses. In some fowl adenoviruses, the existence of one VA RNA gene at a different genome position has been described, but these VA RNAs are not homologous to mastadenovirus VA RNAs.

## Antigenic properties

Adenovirus serotypes are differentiated on the basis of neutralization assays. A serotype is defined as one that either exhibits no cross-reaction with others, or shows a homologous:heterologous titer ratio greater than 16 (in both directions). For homologous:heterologous titer ratios of 8 or 16, a serotype assignment is made if either the viral hemagglutinins are unrelated (as shown by lack of cross-reaction in hemagglutination-inhibition tests), or if substantial biophysical, biochemical or phylogenetic differences exist. Antigens at the surface of the virion are mainly type-specific. Hexons

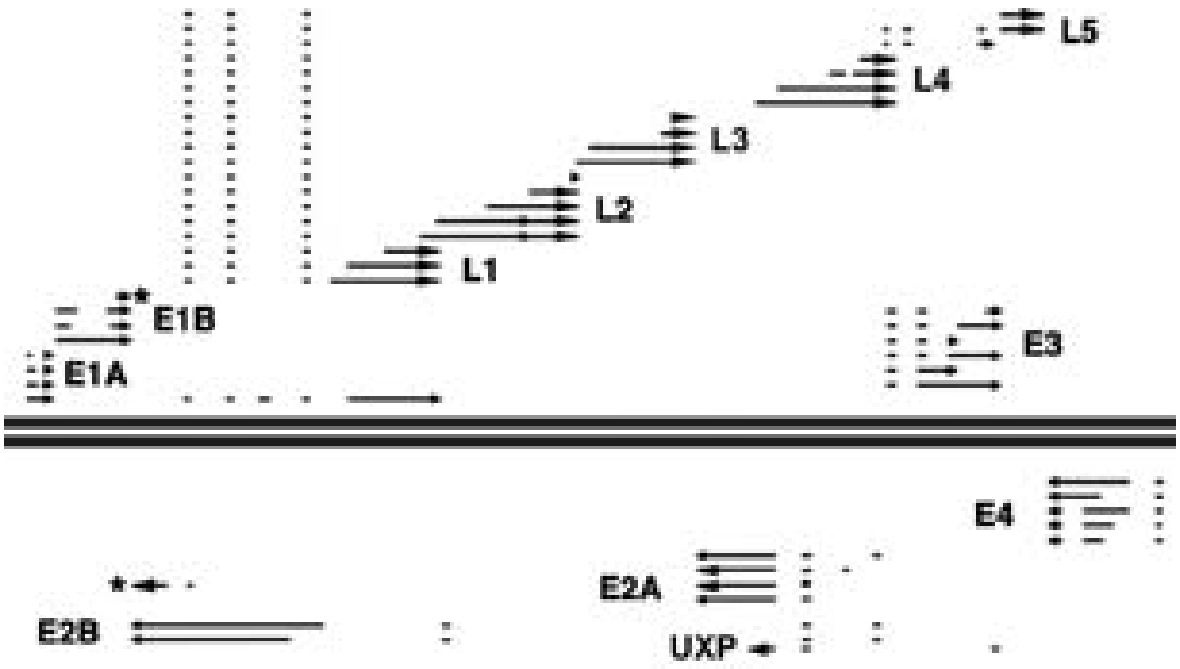


**Figure 3:** Schematic illustration of the transcription pattern of human adenovirus 2. The parallel lines indicate the linear dsDNA genome of 36 kbp. The dots, broken lines and split arrows indicate the spliced structures of the mRNAs. E1A, E1B, E2A, E2B, E3 and E4 refer to early transcription units. Most (but not all) late genes are in the major late transcription unit which initiates after the E1B and protein IX genes of the *r* strand (transcribed rightward), and which includes the L1, L2, L3, L4 and L5 families of mRNAs. Intermediate genes (of protein IX and protein IVa2) are marked with a star. (Adapted from Wold, W.S. and Gooding, L.R. (1991). *Virology*, **184**, 1–8.)

are involved in neutralization, and fibers in neutralization and hemagglutination-inhibition. Soluble antigens associated with virus infections include surplus capsid proteins that have not been assembled. As defined using monoclonal antibodies, hexons and other soluble antigens carry numerous epitopes that can be genus-, species- or type-specific. Free hexon protein reacts mainly as a genus-specific antigen. The genus-specific antigen is located on the basal surface of the hexon, whereas serotype-specific antigens are located mainly on the tower region.

### Biological properties

The natural host range of adenovirus types is usually confined to a single species, or to closely related species. This also applies for cell cultures. Some human adenoviruses (HAdV) (mainly from members of the species *Human adenovirus C*) can cause productive infection in different animal cells (e.g. rodent or ruminant). Several human adenoviruses cause tumours in newborn hamsters. The majority of adenovirus infections in humans are subclinical. Direct or indirect transmission occurs from throat, faeces, eye or urine, depending on the virus type. Certain HAdV types (below in parentheses) are predominantly associated with specific pathology, such as adenoidal–pharyngeal conjunctivitis (3, 4, 7, 14), acute respiratory outbreaks (4, 7, 14, 21), epidemic keratoconjunctivitis (8, 19, 37, 53, 54) or venereal disease (37). HAdV-40 and HAdV-41 can be isolated in high yield from faeces of young children with acute gastroenteritis and are second only to rotaviruses as a major cause of infantile viral diarrhoea. HAdV-11, HAdV-34, and HAdV-35 cause persistent interstitial infection in the kidney and hemorrhagic cystitis, occurring most frequently in immuno-suppressed patients after organ transplantation. HAdV-42 to HAdV-51 were all isolated from AIDS patients. In other mammals, mastadenovirus infections are common, but manifest disease usually appears only if predisposing factors (e.g. management problems, crowding, shipping or concurrent bacterial infections) are present. Canine adenovirus (CAdV) seems to be an exception. CAdV-1 is the causative agent of infectious canine hepatitis (Rubarth disease), a life-threatening disease of puppies, and of encephalitis in numerous other carnivore species such as foxes, raccoons, bears and skunks. CAdV-2 causes infectious laryngotracheitis (kennel cough) in dogs, and this is common among breeder stocks. Adenoviruses infecting susceptible cells cause similar gross pathology, i.e. early rounding of cells and aggregation or lysis of chromatin, followed by the later appearance of characteristic basophilic or eosinophilic nuclear inclusions. HAdV-5 has been engineered and is used extensively as a gene vector. Other (including non-human) serotypes are being developed to overcome the problem posed by pre-existing neutralizing antibodies in the population, and also to achieve better targeting of specific organs and tissues.

## Genus *Mastadenovirus*

Type species ***Human adenovirus C***

### Distinguishing features

Mastadenoviruses infect mammals only, and can be distinguished from members of other adenovirus genera traditionally by serology (genus members share complement-fixing antigen) and more recently (and preferably) by genome organization characteristics and phylogenetic distances. Virus infectivity is inactivated after heating at 56 °C for more than 10 min. Mastadenovirus genomes fully sequenced to date range between 30,536 bp (CAdV-1) and 37,860 bp (simian adenovirus 31.2; SAdV-31.2). The G+C content of the DNA varies between 43.6% (bovine adenovirus 2; BAdV-2) and 63.9% (porcine adenovirus 3; PAdV-3). The ITRs of mastadenoviruses are in general longer (93–371 bp) and more complex (containing a variety of cellular factor binding sites) than in members of the other genera. HAdV-2 comprises 35,937 bp and its ITR is 103 bp long.

Unique proteins of mastadenoviruses are proteins V and IX, and most of those coded by the E1A, E1B, E3 and E4 regions. As well as cementing the hexons on the outer surface of the capsid, protein IX also acts as a transcriptional activator and takes part in nuclear re-organization. Protein V is a core protein that, in association with cellular protein p32, seems to be involved in transport of viral DNA into the nucleus of the infected cell. The E3 and E4 proteins also often differ substantially between different mastadenovirus species.

Genome organization and replication have been most extensively studied for isolates of the species *Human adenovirus C* (Figure 3), and the findings seem to be generally applicable to all mastadenoviruses, except in the E3 and E4 regions. These early regions are also different in the non-primate mastadenoviruses. In the E4 region, a single homolog of the HAdV-2 34K protein exists in all mastadenoviruses and is duplicated in bovine adenovirus 3 and porcine adenovirus 5. The E3 region is also considerably shorter and less complex in the non-primate mastadenoviruses. The simplest E3 region, comprising a single gene, occurs in murine adenovirus 1 (MAdV-1) and MAdV-3.

## Species demarcation criteria in the genus

Species demarcation is based on evolutionary distance as reflected by phylogenetic distances and genome organizational differences. The species contain similar types (designated by Arabic numbers) that were traditionally distinguished serologically (by virus neutralization). The serological type demarcation criterion is currently being replaced by criteria similar to those used for species demarcation. Species designation depends on several of the following characteristics:

- Phylogenetic distance (>5–15%, based primarily on distance matrix analysis of the DNA polymerase amino acid sequence)
- Genome organization (characteristically in the E3 region)
- Nucleotide composition (G+C%)
- Oncogenicity in rodents
- Host range
- Cross-neutralization
- Ability to recombine
- Number of VA RNA genes
- Hemagglutination

For example, if virus neutralization data are available, lack of cross-neutralization combined with a phylogenetic distance of more than 15% separates two types into different species. If the phylogenetic distance is less than 5%, any additional common grouping criteria from the list above may classify separate types into the same species even if they were isolated from different hosts. As an example, the most numerous types from the same host, the human adenoviruses, can be clearly separated into seven species supported by phylogenetic analysis, ability to recombine (e.g. between HAdV-1, 2, 5 and 6), growth characteristics (HAdV-40 and 41 show similar restricted capacity), oncogenicity and nucleotide composition (HAdV-12, 18 and 31, which are members of the species *Human adenovirus A*, share high oncogenicity in rodents and low G+C percentage in their genome). Adenoviruses isolated from chimpanzees resemble certain HAdVs to such an extent that they are classified into "human" adenovirus species. For example, simian adenoviruses (SAdVs) 22 to 25 belong to the species *Human adenovirus E*, and SAdV-21 belongs to the species *Human adenovirus B*.

## List of species in the genus *Mastadenovirus*

| | | |
|---|---|---|
| *Bovine adenovirus A* | | |
| Bovine adenovirus 1 | [BD269513 = NC_006324] | (BAdV-1) |
| *Bovine adenovirus B* | | |
| Bovine adenovirus 3 | [AF030154 = AC_000002] | (BAdV-3) |
| *Bovine adenovirus C* | | |
| Bovine adenovirus 10 | [AF027599] | (BAdV-10) |
| *Canine adenovirus* | | |
| Canine adenovirus 1 | [Y07760 = AC_000003] | (CAdV-1) |
| Canine adenovirus 2 | [U77082 = AC_000020] | (CAdV-2) |
| *Equine adenovirus A* | | |
| Equine adenovirus 1 | [L79955] | (EAdV-1) |
| *Equine adenovirus B* | | |
| Equine adenovirus 2 | [L80007] | (EAdV-2) |

| | | |
|---|---|---|
| *Human adenovirus A* | | |
| Human adenovirus 12 | [X73487 = NC_001460] | (HAdV-12) |
| Human adenovirus 18 | [GU191019] | (HAdV-18) |
| Human adenovirus 31 | [AM749299] | (HAdV-31) |
| *Human adenovirus B* | | |
| Human adenovirus 3 | [DQ086466 = NC_011203] | (HAdV-3) |
| Human adenovirus 7 | [AY495969 = AC_000018] | (HAdV-7) |
| Human adenovirus 11 | [AY163756 = NC_011202] | (HAdV-11) |
| Human adenovirus 14 | [AY803294] | (HAdV-14) |
| Human adenovirus 16 | [AY601636] | (HAdV-16) |
| Human adenovirus 21 | [AY601633] | (HAdV-21) |
| Human adenovirus 34 | [AY737797] | (HAdV-34) |
| Human adenovirus 35 | [AY271307] | (HAdV-35) |
| Human adenovirus 50 | [AJ272612] | (HAdV-50) |
| Simian adenovirus 21 | [AR101858 = AC_000010] | (SAdV-21) |
| *Human adenovirus C* | | |
| Bovine adenovirus 9 | | (BAdV-9) |
| Human adenovirus 1 | [AF534906] | (HAdV-1) |
| Human adenovirus 2 | [J01917 = NC_001405] | (HAdV-2) |
| Human adenovirus 5 | [M73260 = AC_000008] | (HAdV-5) |
| Human adenovirus 6 | [HC492785] | (HAdV-6) |
| Simian adenovirus 31 | [FJ025904] | (SAdV-31) |
| *Human adenovirus D* | | |
| Human adenovirus 8 | [AB448767] | (HAdV-8) |
| Human adenovirus 9 | [AJ854486 = NC_010956] | (HAdV-9) |
| Human adenovirus 10 | [DQ149615] | (HAdV-10) |
| Human adenovirus 13 | [DQ149616] | (HAdV-13) |
| Human adenovirus 15 | [AB562586] | (HAdV-15) |
| Human adenovirus 17 | [AF108105 = AC_000006] | (HAdV-17) |
| Human adenovirus 19 | [EF121005] | (HAdV-19) |
| Human adenovirus 20 | [DQ149619] | (HAdV-20) |
| Human adenovirus 22 | [FJ404771] | (HAdV-22) |
| Human adenovirus 23 | [DQ149621] | (HAdV-23) |
| Human adenovirus 24 | [DQ149622] | (HAdV-24) |
| Human adenovirus 25 | [DQ149623] | (HAdV-25) |
| Human adenovirus 26 | [EF153474] | (HAdV-26) |
| Human adenovirus 27 | [DQ149625] | (HAdV-27) |
| Human adenovirus 28 | [FJ824826] | (HAdV-28) |
| Human adenovirus 29 | [AB562587] | (HAdV-29) |
| Human adenovirus 30 | [DQ149628] | (HAdV-30) |
| Human adenovirus 32 | [DQ149629] | (HAdV-32) |
| Human adenovirus 33 | [DQ149630] | (HAdV-33) |
| Human adenovirus 36 | [GQ384080] | (HAdV-36) |
| Human adenovirus 37 | [DQ900900] | (HAdV-37) |
| Human adenovirus 38 | [DQ149633] | (HAdV-38) |
| Human adenovirus 39 | [DQ149634] | (HAdV-39) |
| Human adenovirus 42 | [DQ149635] | (HAdV-42) |
| Human adenovirus 43 | [DQ149636] | (HAdV-43) |
| Human adenovirus 44 | [DQ149637] | (HAdV-44) |
| Human adenovirus 45 | [DQ149638] | (HAdV-45) |
| Human adenovirus 46 | [AY875648] | (HAdV-46) |
| Human adenovirus 47 | [DQ149640] | (HAdV-47) |
| Human adenovirus 48 | [EF153473] | (HAdV-48) |
| Human adenovirus 49 | [DQ393829] | (HAdV-49) |
| Human adenovirus 51 | [DQ149642] | (HAdV-51) |
| Human adenovirus 53 | [FJ169625] | (HAdV-53) |
| Human adenovirus 54 | [AB333801 = NC_012959] | (HAdV-54) |
| *Human adenovirus E* | | |
| Human adenovirus 4 | [AY487947 = NC_003266] | (HAdV-4) |
| Simian adenovirus 22 | [AY530876] | (SAdV-22) |
| Simian adenovirus 23 | [AY530877] | (SAdV-23) |
| Simian adenovirus 24 | [AY530878] | (SAdV-24) |
| Simian adenovirus 25 | [AR101859 = AC_000011] | (SAdV-25) |

| | | |
|---|---|---|
| *Human adenovirus F* | | |
| Human adenovirus 40 | [L19443 = NC_001454] | (HAdV-40) |
| Human adenovirus 41 | [DQ315364] | (HAdV-41) |
| *Human adenovirus G* | | |
| Human adenovirus 52 | [DQ923122] | (HAdV-52) |
| Simian adenovirus 1 | [AY771780 = NC_006879] | (SAdV-1) |
| Simian adenovirus 2 | [SAU03008] | (SAdV-2) |
| Simian adenovirus 7 | [DQ792570] | (SAdV-7) |
| Simian adenovirus 11 | [SAU03014] | (SAdV-11) |
| Simian adenovirus 12 | | (SAdV-12) |
| Simian adenovirus 15 | [SAU03016] | (SAdV-15) |
| *Murine adenovirus A* | | |
| Murine adenovirus 1 | [NC_000942] | (MAdV-1) |
| *Murine adenovirus C* | | |
| Murine adenovirus 3 | [EU835513 = NC_012584] | (MAdV-3) |
| *Ovine adenovirus A* | | |
| Bovine adenovirus 2 | [AF252854 = AC_000001] | (BAdV-2) |
| Ovine adenovirus 2 | [DQ630755] | (OAdV-2) |
| Ovine adenovirus 3 | [DQ630756] | (OAdV-3) |
| Ovine adenovirus 4 | [DQ630757] | (OAdV-4) |
| Ovine adenovirus 5 | [DQ630758] | (OAdV-5) |
| *Ovine adenovirus B* | | |
| Goat adenovirus 2 | [DQ630760] | (GAdV-2) |
| Ovine adenovirus 1 | [DQ630754] | (OAdV-1) |
| *Porcine adenovirus A* | | |
| Porcine adenovirus 1 | [L43364] | (PAdV-1) |
| Porcine adenovirus 2 | [L43365] | (PAdV-2) |
| Porcine adenovirus 3 | [AF083132 = NC_005869] | (PAdV-3) |
| *Porcine adenovirus B* | | |
| Porcine adenovirus 4 | [U13893] | (PAdV-4) |
| *Porcine adenovirus C* | | |
| Porcine adenovirus 5 | [AF289262 = NC_002702] | (PAdV-5) |
| *Simian adenovirus A* | | |
| Simian adenovirus 3 | [AY598782 = NC_006144] | (SAdV-3) |
| Simian adenovirus 4 | [SAU03010] | (SAdV-4) |
| Simian adenovirus 6 | [CQ982401] | (SAdV-6) |
| Simian adenovirus 9 | [SAU03012] | (SAdV-9) |
| Simian adenovirus 10 | [SAU03013] | (SAdV-10) |
| Simian adenovirus 14 | [SAU03016] | (SAdV-14) |
| Simian adenovirus 48 | [HQ241818] | (SAdV-48) |
| *Tree shrew adenovirus* | | |
| Tree shrew adenovirus 1 | [AF258784 = NC_004453] | (TSAdV-1) |

Species names are in italic script; names of types are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed. Full genome sequences are available from 19 further novel chimpanzee, eight gorilla and six bonobo adenoviruses. These belong to the species *Human adenovirus B*, *C* and *E*, with representatives in each. Due to lack of space and confirmation of their type demarcation, they are not listed.

## List of other related viruses which may be members of the genus *Mastadenovirus* but have not been approved as species

| | | |
|---|---|---|
| Alpaca adenovirus 1 | [GQ499375] | (AlAdV-1) |
| Bat adenovirus 1 (FBV1) | [AB303301] | (BtAdV-1) |
| Bat adenovirus 2 (PPV1) | [FJ983127] | (BtAdV-2) |
| Bat adenovirus 3 (TJM) | [GU226970] | (BtAdV-3) |
| Guinea pig adenovirus 1 | [X95630] | (GPAdV-1) |
| Murine adenovirus 2 | [HM049560 = NC_014899] | (MAdV-2) |
| Ovine adenovirus 6 | [DQ630759] | (OAdV-6) |
| Simian adenovirus 5, 8, 13, 16–20 | [18: FJ025931] | (SAdV-5, 8, 13, 16–20) |
| Squirrel adenovirus 1 | [GU735084] | (SqAdV-1) |

# Genus *Aviadenovirus*

Type species ***Fowl adenovirus A***

## Distinguishing features

Aviadenoviruses are serologically distinct from members of the other adenovirus genera and they only infect birds. The virions contain two fibers per vertex. Fowl adenovirus 1 (FAdV-1), FAdV-4 and turkey adcnovirus 1 (TAdV-1) have two fiber genes, and two projections (in case of FAdV-1, of considerably different lengths) on each penton base. Other FAdVs also have two fibers per vertex, but apparently only one fiber gene, and the fiber shafts are of similar lengths. The long fiber of FAdV-1 uses the coxsackievirus and adenovirus receptor (CAR) for attachment to the cell.

Aviadenovirus genomes are considerably larger (20–45%) than those of mastadenoviruses. Five aviadenovirus [FAdV-1, FAdV-4, "FAdV-8", FAdV-9 and TAdV-1] genomes have been fully sequenced, and range between 43,804 bp (FAdV-1) and 45,667 (FAdV-4). These are thought to represent the longest adenovirus DNA molecules after that of white sturgeon adenovirus. The G+C content of partial or complete sequences of aviadenovirus genomes varies between 53.8 and 66.9%. The size of sequenced aviadenovirus ITRs are between 54 and 95 bp long. The genomic organization of aviadenoviruses is also different from that of adenoviruses in other genera (Figure 2). The genes of proteins V and IX, as well as genes in mastadenovirus early regions E1 and E3, are missing. The E4 region may be translocated from its position in mastadenovirus genomes, resulting in the gene encoding dUTP pyrophosphatase (dUTPase, not present in every mastadenovirus) being on the left end of the genome, rather than the right end. (Alternatively, this gene may have been captured independently by ancestors of aviadenoviruses and mastadenoviruses.) The organization of the central part of the genome containing the late genes and the E2 region is similar to that of mastadenoviruses. The right end of the genome contains several transcription units, which are unique to aviadenoviruses. The majority of genes and proteins from this region have not yet been characterized in detail. A novel protein GAM-1 of FAdV-1 has been demonstrated to have an anti-apoptotic effect, and to activate the heat-shock response in the infected cell. GAM-1, in synergy with another novel protein encoded by ORF22, binds the retinoblastoma protein and can activate the E2F pathway. Additional, and as yet uncharacterized predicted gene products, exhibit sequence homology to proteins of other viruses, such as the nonstructural protein NS1 (also known as Rep) of parvoviruses, and a triacylglycerol lipase, a homolog of which also occurs in an avian herpesvirus (Marek's disease virus). Aviadenoviruses possess no complement-fixing antigen in common with the members of the other genera. There are isolates where serum neutralization cannot differentiate clearly between the serotypes. The introduction of FAdV-8a and 8b was deemed necessary because of the inconsistency in the type-numbering scheme used in different countries and continents over the years. Avian adenoviruses have been associated with diverse disease patterns, including inclusion body hepatitis, bronchitis, pulmonary congestion and oedema in different bird species. Hydropericardium syndrome is caused by FAdV-4 in chickens, mainly in Asia. Falcon adenovirus 1 has caused fatalities in different falcon species. FAdV-1 (CELO virus), 9 and 10 are being studied for their feasibility as gene delivery vectors.

## Species demarcation criteria in the genus

Species designation depends on several of the following characteristics:

- Phylogenetic distance (>5–15%, based primarily on distance matrix analysis of the DNA polymerase amino acid sequence)
- Genome organization (characteristically in the region at the right end of the genome)
- RFLP analysis
- Host range
- Pathogenicity
- Cross-neutralization
- Ability to recombine

For example, the fowl adenovirus serotypes can be grouped into five species on the basis of phylogeny, genome organization, RFLP profiles and the lack of significant cross-neutralization.

## List of species in the genus *Aviadenovirus*

Note: A specific problem that has been addressed but not resolved is the lack of consensus in the numbering of the individual fowl adenovirus serotypes. Strains deposited in the American Type Culture Collection are numbered inconsistently in relation to the majority of newer publications. For this reason, one representative strain of each serotype is also listed (in parentheses).

| | | |
|---|---|---|
| *Falcon adenovirus A* | | |
| Falcon adenovirus 1 | [AY683541] | (FaAdV-1) |
| *Fowl adenovirus A* | | |
| Fowl adenovirus 1 (CELO) | [U46933 = AC_000014] | (FAdV-1) |
| *Fowl adenovirus B* | | |
| Fowl adenovirus 5 (340) | [AF508952] | (FAdV-5) |
| *Fowl adenovirus C* | | |
| Fowl adenovirus 4 (ON1) | [GU188428 = NC_015323] | (FAdV-4) |
| Fowl adenovirus 10 (CFA20) | [AF160185] | (FAdV-10) |
| *Fowl adenovirus D* | | |
| Fowl adenovirus 2 (P7-A) | [AF339915] | (FAdV-2) |
| Fowl adenovirus 3 (75) | [AF508949] | (FAdV-3) |
| Fowl adenovirus 9 (A2-A) | [AF083975 = AC_000013] | (FAdV-9) |
| Fowl adenovirus 11 (380) | [AF339925] | (FAdV-11) |
| *Fowl adenovirus E* | | |
| Fowl adenovirus 6 (CR119) | [AF508954] | (FAdV-6) |
| Fowl adenovirus 7 (YR36) | [AF508955] | (FAdV-7) |
| Fowl adenovirus 8a (CFA40) | [AF155911] | (FAdV-8a) |
| Fowl adenovirus 8b (764) | [AF508958] | (FAdV-8b) |
| *Goose adenovirus* | | |
| Goose adenovirus 1 | | (GoAdV-1) |

Species names are in italic script; names of types and isolates ( ) are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Aviadenovirus* but have not been approved as species

| | | |
|---|---|---|
| Duck adenovirus 2 | | (DAdV-2) |
| Meyer's parrot adenovirus 1 | [AY644731] | |
| Pigeon adenovirus 1 | [FN824512] | (PiAdV-1) |
| Psittacine adenovirus 1 | [EF442329] | (PsAdV-1) |
| Turkey adenovirus 1 | [GU936707 = NC_014564] | (TAdV-1) |
| Turkey adenovirus 2 | [GU936708] | (TAdV-2) |

# GENUS *ATADENOVIRUS*

Type species ***Ovine adenovirus D***

## Distinguishing features

Atadenoviruses are serologically distinct from viruses in the other adenovirus genera, and their genomic organization and capsid protein complement also differ. Atadenoviruses have been detected in a broad range of hosts, including scaled reptiles (order Squamata) and species ranging from birds to ruminants and a marsupial. Virions are relatively heat stable and retain substantial infectivity after treatment for 30 min at 56 °C, conditions that inactivate mastadenovirions. The genome size of sequenced isolates ranges from 29,576 (ovine adenovirus 7, OAdV-7) to 33,213 bp (duck adenovirus 1, DAdV-1) with ITRs of 46 (OAdV-7) to 118 bp (snake adenovirus 1, SnAdV-1). For ruminant, marsupial and avian atadenoviruses, the G+C content of the DNA is low and varies between 33.6 (OAdV-7) and 43.0% (DAdV-1). The corresponding high A+T content was deemed sufficiently characteristic to be used as the basis of the name of the genus, though atadenoviruses originating from scaled reptiles have a non-biased nucleotide composition

(50.0% G+C in SnAdV-1). The proteins encoded by an atadenovirus genome are summarized in Table 2. The central part of the atadenovirus genome is similar to that of mastadenoviruses (except that there are no protein V and IX genes), whereas the extremities differ markedly. Atadenoviruses have several unique proteins, including some that may be distant homologs of proteins in other adenovirus genera. The left end of the genome carries a gene for p32K, a unique structural protein. At this end, gene LH1 is also unique to the genus but not present in all members. The right end of the genome contains genes that are related to each other, suggestive of gene duplication. There are two E4 34K gene homologs (E4.2 and E4.3), and one to five gene RH homologs. At this end, genes E4.1 and RH1–6 are also unique to the genus but are not present in all members. The proteins encoded by genes LH3 and E4.3 (and its paralog, E4.2) show limited similarity to mastadenovirus proteins E1B 55K and E4 34K, respectively. However, LH3 is a structural protein that forms prominent "knobs" on the virion surface that distinguish atadenoviruses structurally from other known AdVs (Figure 4). LH3 is located in the same relative position among the hexon subunits as protein IX (present only in mastadenoviruses) but sits on top of the "central" (so called H3) hexon trimers, appearing to hold the outer capsid together. Particles that lack the LH3 or p32K proteins are viable although less stable. (Mastadenoviruses that lack proteins V or IX are also viable.) No immunomodulatory genes such as those found in the mastadenovirus E3 region have yet been identified. DAdV-1 has a unique genome region at the far right end with seven uncharacterized ORFs that may be host-specific in function. ORF5 and ORF6 are related to each other. This unique region of DAdV-1 also contains a VA RNA gene that is supposedly homologous to that of FAdV-1.

Certain atadenoviruses can cause hemorrhagic epizooty in free-living ruminants. DAdV-1 is also associated with a specific disease of hens that is characterized globally by sharp decreases in egg production (egg drop syndrome). Due to the lack of pre-existing immunity in humans and its biosafety profile, OAdV-7 has been developed as a gene delivery vector intended for human vaccine and gene therapy applications.

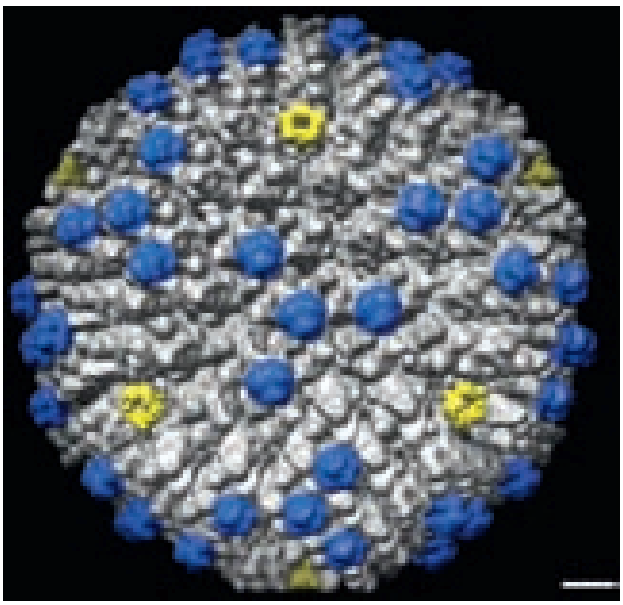

**Figure 4:** Cryo-EM image of ovine atadenovirus 7 at 10.6 Å resolution. The capsid surface is oriented around one of the three-fold axes. Penton complexes are marked in yellow, indicating the approximate bounds of the icosahedral facet. A major capsid protein (LH3) that is a key distinguishing feature of the virus is marked in blue. The bar represents 10 nm. (Reproduced with permission from Pantelic, R.S. *et al.* (2008), *J. Virol.*, **82**, 7346–7356.)

**Table 2:** Virus proteins as deduced from the genome sequence of ovine adenovirus 7

| kDa | Transcription class | Description | Note |
|---|---|---|---|
| 32 | Timing not known | S (p32K†) | Unique to atadenoviruses |
| 13 | LH1 | NS | ORF not in DAdV-1 |
| 14.7 | LH2 | NS | |
| 42.8 | LH3 | S | Distant homolog of mastadenovirus |
| | Timing not known | | E1B 55K |
| 43 | E2 | NS; DBP | |
| 123 | E2 | NS; DNA pol | |
| 67.1 | E2 | S; Term, pTP† | |
| 12.9, 20.9, 19.8, 19.8 | RH1, RH2, RH4, RH6, early | NS | F-box proteins unique for atadenoviruses Homology to each other |
| 22.6 | RH5, early | NS | ORF not in DAdV-1 |
| 17.1 | E4.1, early | NS | ORF not in DAdV-1 |
| 25.6, 30.8 | E4.2, E4.3, early | NS | Distant homologs of mastadenovirus E4 34K |
| 38.2 | Early and late | NS; scaffolding | |
| | | 52/55 kDa* | |
| 58.4 | Late | S (pIIIa);† p-protein | |
| 51 | Late | S (III); penton | |
| | | base* | Lacks integrin binding motif |
| 12.9 | Late | S (pVII);† major core | |
| 7.3 | Late | S (pX);† X/μ | |
| 24.5 | Late | S (pVI)† | |
| 102 | Late | S (II); hexon | |
| 23 | Late | S; protease | |
| 72 | Late | NS; 100 kDa* | Hexon assembly protein |
| 15.7 | Late | NS; 33 kDa* | p-protein not found in DAdV-1 |
| 24.7 | Late | S (pVIII)† | |
| 58.2 | Late | S (IV); fiber | Cell attachment protein |
| 37.5 | Timing not known | S (IVa2) | |

Molecular masses are presented as unmodified and uncleaved gene products. S = structural; NS = non-structural; LH = left-hand end [genes]; RH = right-hand end [genes]; p = precursor; p-protein = phosphoprotein; DBP = DNA-binding protein; DNA pol = DNA polymerase; Term = terminal protein; * = Mr values are significantly different from those obtained by SDS-PAGE; † = cleaved by viral protease. All NS proteins are hypothetical until characterized.

## Species demarcation criteria in the genus

Species designation depends on several of the following characteristics:

- Phylogenetic distance (>5–15%, based primarily on distance matrix analysis of the DNA polymerase amino acid sequence)
- Host range
- DNA hybridization
- Nucleotide composition (G+C%)
- Cross-neutralization
- Gene organization of the right end of the genome

## List of species in the genus *Atadenovirus*

| | | |
|---|---|---|
| *Bovine adenovirus D* | | |
| Bovine adenovirus 4 | [AF036092 = NC_002685] | (BAdV-4) |
| Bovine adenovirus 5 | [AF207658] | (BAdV-5) |
| Bovine adenovirus 8 | [AF238233] | (BAdV-8) |
| Bovine adenovirus strain Rus | [AF238880] | (BAdV-Rus) |
| *Duck adenovirus A* | | |
| Duck adenovirus 1 | [Y09598 = AC_000004] | (DAdV-1) |
| *Ovine adenovirus D* | | |
| Goat adenovirus 1 | [AF207660] | (GAdV-1) |
| Ovine adenovirus 7 | [U40839 = NC_004037] | (OAdV-7) |
| *Possum adenovirus* | | |
| Possum adenovirus 1 | [AF338822] | (PoAdV-1) |
| *Snake adenovirus A* | | |
| Snake adenovirus 1 | [DQ106414 = NC_009989] | (SnAdV-1) |

Species names are in italic script; names of types and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Atadenovirus* but have not been approved as species

| | | |
|---|---|---|
| Asp viper adenovirus | [EU914209] | |
| Bearded dragon adenovirus 1 (agamid adenovirus 1) | [AY576678] | (BDAdV-1) |
| Blue-tongued skink adenovirus | [AY576682] | |
| Bovine adenovirus 6 | [AF207659] | (BAdV-6) |
| Bovine adenovirus 7 | [AF238232] | (BAdV-7) |
| Chameleon adenovirus 1 | [AY576679] | (ChAdV-1) |
| Emerald monitor adenovirus | [EU914208] | |
| Gecko adenovirus 1 | [AY576677] | (GeAdV-1) |
| Gila adenovirus 1 | [AY576680] | |
| Hagen's pit viper adenovirus | [EU851415] | |
| Mexican beaded lizard adenovirus 1 | [EU914207] | |
| Odocoileus adenovirus 1 | [AF198354] | (OdAdV-1) |
| Snake adenovirus 2 | [FJ012163] | (SnAdV-2) |
| Snake adenovirus 3 | [FJ012164] | (SnAdV-3) |
| Tokay gecko adenovirus | [AY576681] | |

# GENUS *SIADENOVIRUS*

Type species ***Frog adenovirus***

## Distinguishing features

Siadenoviruses are serologically and phylogenetically distinct from members of the other adenovirus genera. This genus comprises only three accepted members, frog adenovirus 1 (FrAdV-1), turkey adenovirus 3 (TAdV-3) and raptor adenovirus 1 (RAdV-1). FrAdV-1 was isolated from an amphibian (frog) and TAdV-3 from birds (turkey, pheasant and chicken). RAdV-1 was detected in captive raptors by PCR. The RAdV-1 genome was the first adenovirus genome to be fully sequenced solely by PCR-based methods without virus isolation. The genomes of the three siadenovirus types represent the shortest adenovirus genomes known to date. Their lengths are between 26,163 and 26,283 bp, with G+C contents of 34.9 to 38.5%, and ITRs of 29–39 bp. The genomic organization of siadenoviruses is also characteristic and different from that of other genera. The genes of proteins V and IX, and homologs in mastadenovirus early regions E1, E3 and E4 are absent. Apart from the proteins conserved in all adenoviruses, there are only five ORFs potentially encoding novel proteins. At the left end of the genome, the first putative gene encodes a protein that is related to sialidases. Adjacent to it is a novel ORF predicted to code for a highly hydrophobic protein. The gene named "E3" solely because of its position between the pVIII and fiber genes is not homologous to any of the mastadenovirus E3 genes (or to any other known genes). The right end

of the genome harbours ORF7 and ORF8. TAdV-3 has no common complement-fixing antigen with other adenoviruses isolated from birds and classified in the aviadenovirus or atadenovirus genera. FrAdV-1 is supposedly non-pathogenic. TAdV-3 is associated with specific diseases in different hosts (hemorrhagic enteritis in turkey, marble spleen disease in pheasants and splenomegaly in chickens).

### Species demarcation criteria in the genus

Species designation depends on the following characteristics:

- Phylogenetic distance (>5–15%, based primarily on distance matrix analysis of the DNA polymerase amino acid sequence)
- Host range

### List of species in the genus *Siadenovirus*

| | | |
|---|---|---|
| *Frog adenovirus* | | |
| Frog adenovirus 1 | [AF224336 = NC_002501] | (FrAdV-1) |
| *Raptor adenovirus A* | | |
| Raptor adenovirus 1 | [EU715130 = NC_015455] | (RAdV-1) |
| *Turkey adenovirus A* | | |
| Turkey adenovirus 3 | [AF074946 = AC_000016] | (TAdV-3) |

Species names are in italic script; names of types are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Siadenovirus* but have not been approved as species

| | | |
|---|---|---|
| Budgerigar adenovirus 1 | [AB485763] | |
| Great tit adenovirus 1 | [FJ849795] | (GTAdV-1) |
| Psittacine adenovirus 2 | [EU056825] | (PsAdV-2) |
| Sulawesi tortoise adenovirus 1 | [EU056826] | (STAdV-1) |

## GENUS *ICHTADENOVIRUS*

Type species ***Sturgeon adenovirus A***

### Distinguishing features

The single known member of this genus, white sturgeon adenovirus 1 (WSAdV-1), is the only confirmed fish adenovirus. While the host is very divergent from those of other AdVs, phylogenetic calculations and a unique genome organization further distinguish this virus from all other adenoviruses. The WSAdV-1 genome is 48,395 bp, and thus it is longer than the genome of any known adenovirus. The G + C content is 42.7%. The fiber gene was not found in its usual position towards the right end of the genome, but fiber gene homologs were discovered at the left end. The virus seems to be non-pathogenic for fish.

### List of species in the genus *Ichtadenovirus*

| | | |
|---|---|---|
| *Sturgeon adenovirus A* | | |
| White sturgeon adenovirus 1 | [AJ495768] | (WSAdV-1) |

Species names are in italic script; names of types are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Ichtadenovirus* but have not been approved as species

None reported.



## Phylogenetic relationships within the family

Consistent with specific characteristics of genome organization, phylogenetic calculations result in clear separation of five different clusters corresponding to the five genera (*Mastadenovirus*, *Aviadenovirus*, *Atadenovirus*, *Siadenovirus* and *Ichtadenovirus*; Figure 5). Within the genera, evolutionary distances among adenoviruses resemble those of their host. There are some exceptions, where very distantly related viruses infect the same host. Adenovirus types isolated from cattle, sheep or chicken appear on very distant branches, and even in separate clusters corresponding to different genera. Beside the co-evolution that has been hypothesized for animals and their adenoviruses, multiple host switches may also have occurred. For example, adenoviruses from scaled reptiles switched to ruminants and birds.

## Similarity with other taxa

A dsDNA bacteriophage, PRD1 (in the family *Tectiviridae*), shares a similar virion architecture (an icosahedral capsid with fiber-like projections) with adenoviruses. The 15 kbp genome of PRD1 has ITRs and contains genes encoding terminal protein and DNA polymerase arranged in the same order as in adenoviruses. The terminal protein also acts as primer in PRD1 DNA replication. A study on the resolution of the main capsid proteins (P3 of PRD1 and hexon of HAdV-2) revealed a very similar arrangement and structure, and again suggested an evolutionary link between the two viruses. Fungi and plants also have a linear plasmid (killer plasmid in yeast) either in the cytoplasm or in the mitochondria that has adenovirus-like features (an ITR and a terminal protein gene adjacent to a DNA polymerase gene). *Sulfolobus* turreted icosahedral virus, which infects a crenarchaeal host (in domain Archaea), and also two archaeal proviruses (TKV4 and MVV), which are integrated into the 5′- and 3′-distal regions of tRNA genes of the euryarchaeal species *Thermococcus kodakaraensis* KOD1 and *Methanococcus voltae* A3, respectively, could also be placed into the PRD1-adenovirus lineage based on established or predicted three-dimensional structures of their major capsid protein and on sharing a characteristic ATPase.

Adenoviruses also show some similarity to other viruses. The fibers of many adenovirus types use the same cellular receptor (CAR) for attachment as coxsackie B viruses. Adenovirus fibers were reported to show structural similarity with reovirus attachment protein sigma 1, which binds the JAM (junction adhesion molecule) receptor. Adenoviruses may occur together with dependent parvoviruses, for which they may provide helper functions. Similarity was observed between certain proteins of the E3 region of human adenoviruses and the RL11 gene family of human cytomegalovirus (a herpesvirus). The primary structure of the p32K protein, which is characteristic of atadenoviruses, has similarity to bacterial small acid soluble proteins (SASPs) commonly found in various spore-forming bacteria.

## Derivation of names

*Adeno*: from Greek *aden*, *adenos*, "gland"; in recognition of the fact that adenoviruses were first isolated from human adenoid tissue.
*At*: from *a*denine and *t*hymine, in recognition that the genome of the first recognized members of the genus (from ruminant, avian and marsupial hosts) has a remarkably high A+T content.
*Avi*: from Latin *avis*, "bird".
*Icht*: from Greek *ichthys*, "fish".
*Mast*: from Greek *mastos*, "breast".
*Si*: from *si*alidase, in recognition that members of the genus have a putative sialidase homolog.

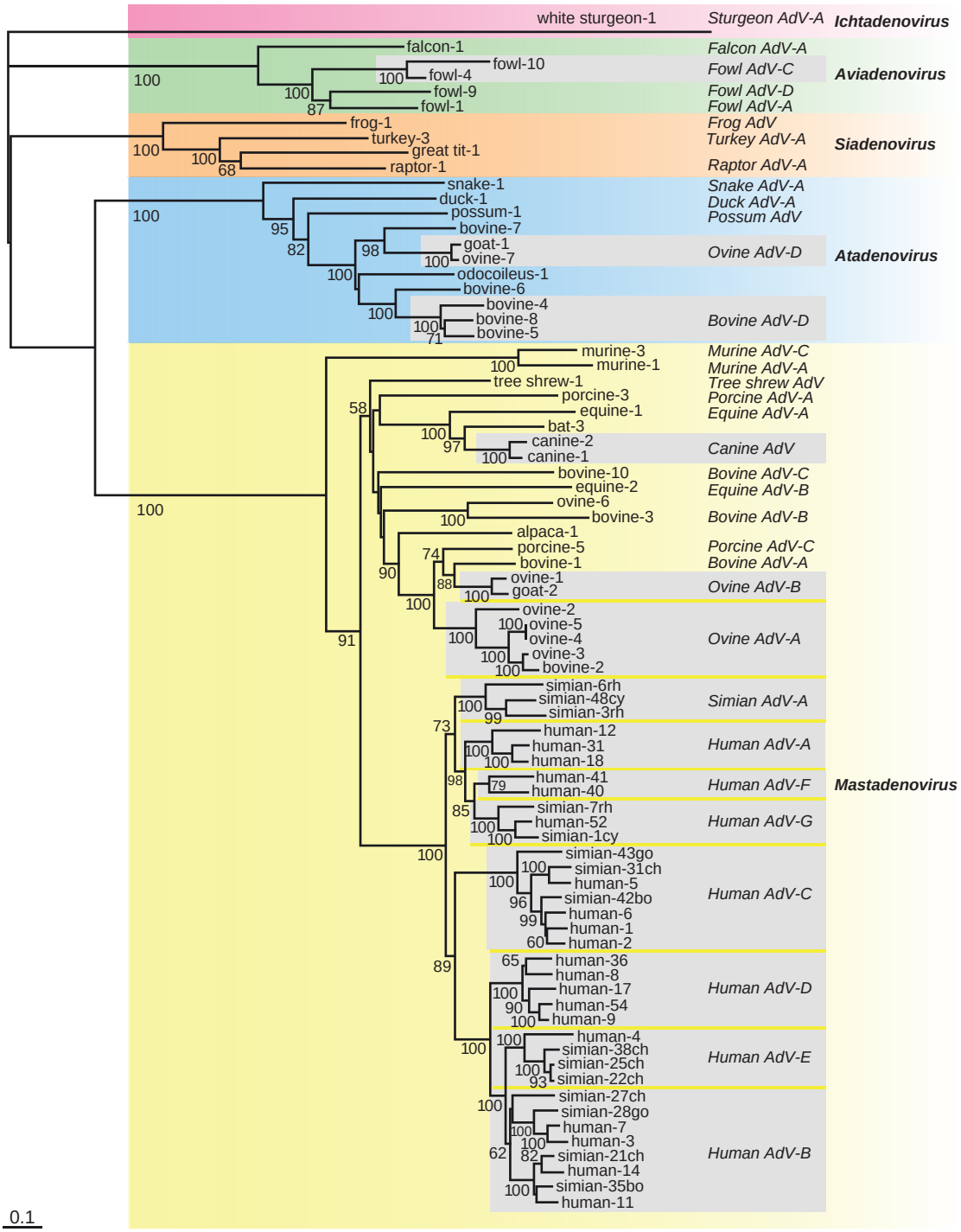


**Figure 5:** Phylogenetic tree of adenoviruses based on a distance matrix analysis of hexon amino acid sequences. Primate adenovirus hexons known to have resulted from homologous recombination were excluded from the analysis. Only selected members of primate adenovirus species with more than three members are shown (boxed in grey). The Seqboot (bootstrap), Protdist (categories matrix), Fitch (global rearrangements), and Consense programs of the PHYLIP 3.68 package were used. The tree was generated by unrooted calculation, and is shown with white sturgeon adenovirus 1 chosen as outgroup. Species names are indicated, but for reasons of presentation the word "adenovirus" is abbreviated to AdV followed by a hyphen. Abbreviations after the type numbers of simian adenoviruses: bo, bonobo; ch, chimpanzee; go, gorilla; cy, cynomolgus macaque; rh, rhesus macaque. Bootstrap values higher than 50 (from 100 re-samplings) are shown for every confirmed branching.

## Further reading

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# Family *Ampullaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Ampullaviridae* |
| Genus | *Ampullavirus* |

Since only one genus is currently recognized, the family description corresponds to the genus description.



# Genus *Ampullavirus*

Type species *Acidianus bottle-shaped virus*

## Virion properties

### Morphology

The virion is enveloped, resembles in its shape a bottle and has an overall length of about 230 nm and a width varying from about 75 nm, at the broad end, to 4 nm, at the pointed end (Figure 1). The broad end of the virion exhibits 20 (±2) thin rigid filaments, 20 nm long and 3 nm in width, which appear to be interconnected at their bases and regularly distributed around, and inserted into, a disc or ring. The 9-nm-thick envelope encases a funnel-shaped core formed by a torroidally supercoiled nucleoprotein filament, 7 nm in width. The core structure shows striations running perpendicularly to the long axis, with periodicities of 13 $nm^{-1}$ and 4.3 $nm^{-1}$, indicative of helical arrangement of subunits.

### Physicochemical and physical properties

Virion buoyant density in sucrose is about 1.3 $g\,cm^{-3}$. The virions are fragile and disrupted by high speed ultracentrifugation, as well as by prolonged storage in buffers. The pointed end of the virion, rather than the broad end, is likely to be involved in adsorption.

### Nucleic acid

Virions contain one molecule of dsDNA of 23,814 bp, with a GC content of 35%.

### Proteins

The virions carry six major proteins in the size range 15 to 80 kDa.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

The linear genome carries 590-bp-long inverted terminal repeats. It encodes 57 predicted proteins and contains two large non-coding regions, of about 600 and 300 bp (Figure 2). Fifteen pairs of genes show small overlaps. Three genes contain putative internal start codons with ribosome-binding sites. The DNA polymerase, putative glycosyltransferase and thymidylate kinase are encoded on the viral genome. Several predicted proteins show recognisable structural motifs. The RNA has predicted secondary structure highly similar to that of the prohead RNA of bacteriophage φ29 and may be involved in genome packaging. Viral DNA polymerase is apparently responsible for genome replication. Its properties, predicted from sequence analysis, imply a protein-primed replication model.

## Antigenic properties

No information available.

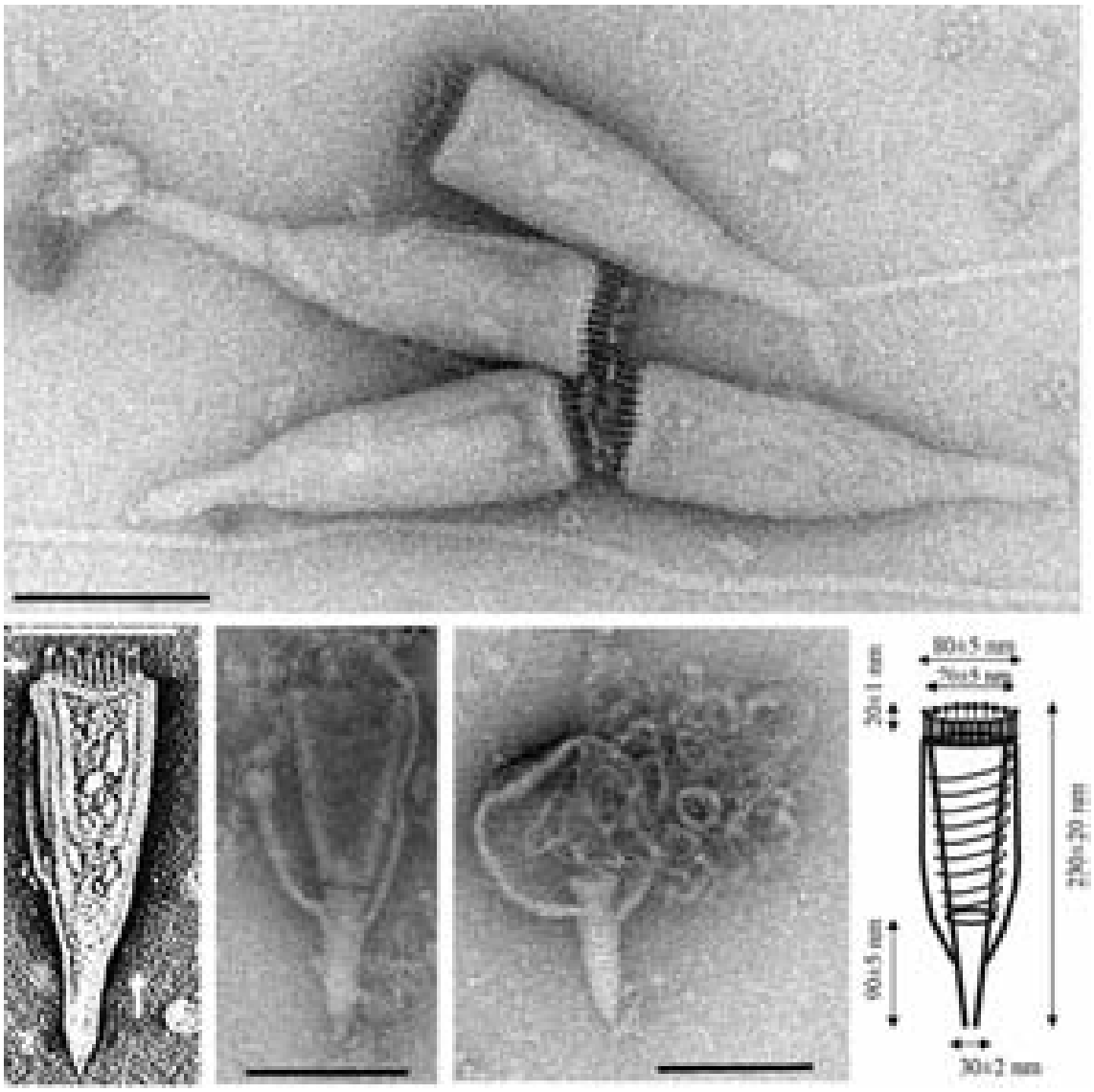

**Figure 1:** Negative contrast electron micrographs of virions of an isolate of *Acidianus bottle-shaped virus*. (Top) Intact virions. (Bottom, left) Horizontal slice (0.7 nm) though the three dimensional data set of the 3D reconstruction of the virion. (Bottom, center) partially disrupted virions. (Bottom, right) Schematic representation of the virion. The scale bars represent 100 nm. (Modified from Häring et al. (2005). *J. Virol.*, **79**, 9904–9911.)

## Acidianus bottle-shaped virus, ABV (23,814 bp)

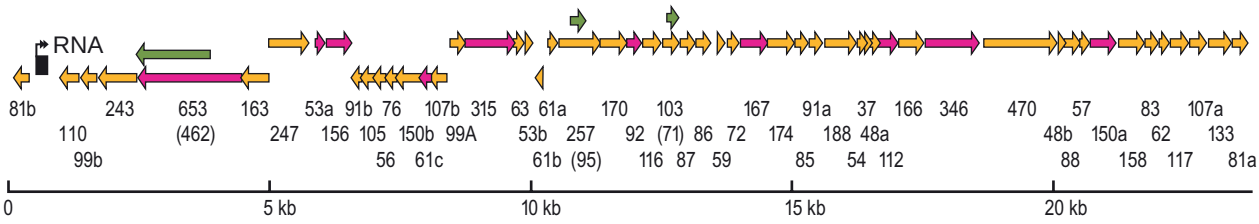


**Figure 2:** Genome organisation of Acidianus bottle-shaped virus, showing location, sizes and direction of putative genes. The square indicates the position of the RNA gene. Red arrows indicate genes with assigned functions or recognizable structural motifs, while putative genes of unknown structure and function are denoted by yellow arrows. Three internal ORFs are denoted by green arrows and their amino acid sizes are given in brackets. (Modified from Peng *et al*. (2007). *Virology* **364**, 237–243.)

## Biological properties

The virus was isolated from a hot acidic spring (87–93 °C, pH 1.5–2.0) in Pozzuoli, Italy. The host range is limited to autochthonous species of hyperthermophilic archaea from the genus *Acidianus*. Host lysis is not observed. Virus infection increases a generation time of the host from about 24 hours to about 48 hours. Release of particles is observed only in the stationary growth phase of the host culture.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the *genus Ampullavirus*

*Acidianus bottle-shaped virus*
Acidianus bottle-shaped virus [EF432053] (ABV)

Species names are in italic script; names of strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Ampullavirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Ampullaviridae*

None reported.

## Phylogenetic relationships within the family

Not applicable.

## Similarity with other taxa

Not known.

## Derivation of name

*Ampulla*: From Latin *ampulla*, for "bottle".

## Further reading

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## Contributed by

Prangishvili, D.

# Family *Ascoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Ascoviridae* |
| Genus | *Ascovirus* |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Ascovirus*

Type species *Spodoptera frugiperda ascovirus 1a*

## Virion properties

### Morphology

Virions of ascoviruses are either bacilliform, ovoidal or allantoid in shape, depending on the species. Ascovirus virions have complex symmetry, and are large, measuring approximately 130 nm in diameter by 200–400 nm in length. The virion consists of an inner particle surrounded by an outer envelope. The inner particle typically measures 80 × 300 nm and contains a DNA/protein core bounded by an apparent lipid bilayer, the external surface of which bears a distinctive layer of protein subunits. The virion, therefore, appears to contain two lipid membranes, one associated with the inner particle and the other forming the envelope. In negatively stained preparations, virions have a distinctive reticulate appearance thought to result from superimposition of protein subunits on the surface of the internal particle with those in the external envelope (Figure 1).

### Physicochemical and physical properties

Virions are sensitive to organic solvents and detergents. Other properties are not known.

### Nucleic acid

The inner particle contains a single molecule of circular dsDNA ranging in size from 150 to 190 kbp. G+C content ranges from 42% to 60% depending on the species.

### Proteins

Virions contain at least 21 polypeptides ranging in size from 6 to 200 kDa. Ascovirus genomes contain from 117 to 180 genes, of which 40 are common with each other.

### Lipids

Ultrastructural evidence and detergent sensitivity indicate the presence of lipid in both the outer envelope and inner particle of the virion. Specific lipid composition is not known.

### Carbohydrates

None reported.

## Genome organization and replication

The genomes of ascoviruses are composed of circular dsDNA. The genomes of three ascoviruses, Heliothis virescens ascovirus 3e (HvAV-3e), Spodoptera frugiperda ascovirus 1a (SfAV-1a) and Trichoplusia ni ascovirus 6a (TnAV-6a; previously named TnAV2c) have been sequenced. The recent sequencing of the Diadromus pulchellus ascovirus 4a (DpAV-4a) genome has revealed that this virus is not an ascovirus, but originated from a sibling family.

Ascoviruses initiate replication in the nucleus. The nucleus enlarges and ruptures, after which the plasmalemma invaginates, forming internal membranous folds that cleave the cell into a cluster of virion-containing vesicles. Virion assembly becomes apparent after the nucleus ruptures. The first recognizable structural component of the virion to form is the multilaminar layer of the inner particle. Based on its ultrastructure, this layer consists of a unit membrane and an exterior layer of

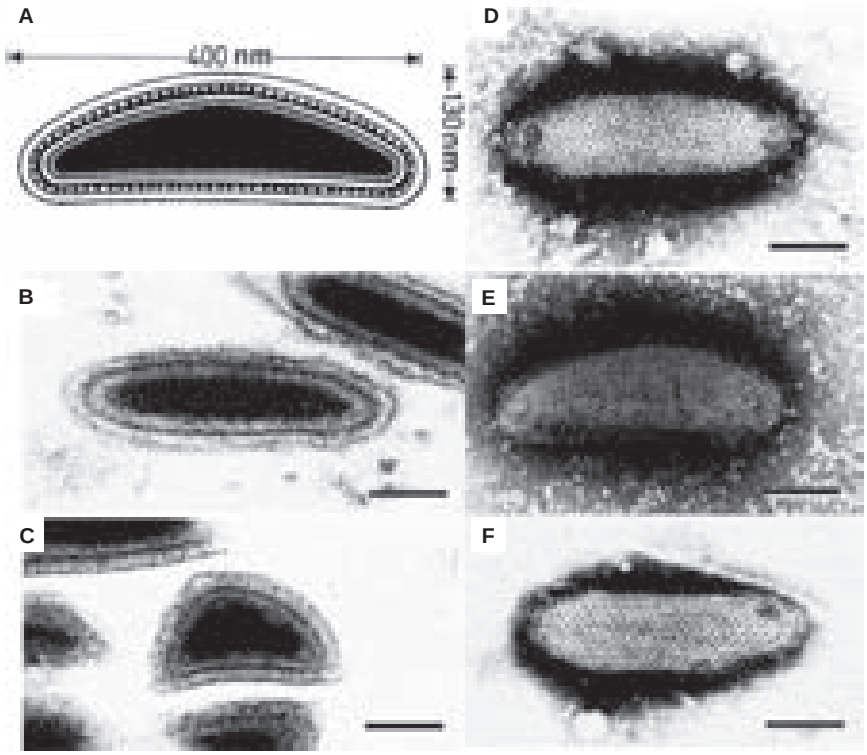


**Figure 1:** Morphology of ascovirus virions. (A) Schematic illustration of the structure of a typical ascovirus virion. The virion consists of an inner particle and an outer envelope. The inner particle is complex and contains a DNA/protein core surrounded by an apparent unit membrane, the external surface of which bears a layer of distinctive protein subunits. (B,C) Respectively, ultrathin longitudinal- and cross-sections through typical ascovirus virions. The dense inner layer corresponds with the distinctive layer of subunits shown in top left. (D) Negatively stained preparations of virions from isolates of three different ascovirus species: top right, *Spodoptera frugiperda ascovirus 1a* (SfAV-1a); E, *Trichoplusia ni ascovirus 2a* (TnAV-2a), and (F), *Heliothis virescens ascovirus 3a* (HvAV-3a). The reticulate appearance of the virion is thought to be due to the superimposition of top and bottom layers of the inner particle and outer envelope. The bar represents 50 nm.

protein subunits. As the multilaminar layer forms, the dense DNA/protein core assembles along the inner surface. As this process continues, the allantoid, ovoidal or bacilliform shape of the inner particle becomes apparent. After the inner particle is assembled, it is enveloped by a membrane, synthesized *de novo*, or elaborated from cell membranes, within the cell or vesicle. In SfAV-1a, the virions are occluded in an occlusion body composed of minivesicles and protein.

## Antigenic properties

No information available.

## Biological properties

### Host range

Ascoviruses cause disease in lepidopteran larvae and pupae, and have been reported most commonly from species of the family Noctuidae, including *Trichoplusia ni*, *Heliothis virescens*, *Helicoverpa zea*, *Spodoptera frugiperda* and *Autographa precationis*. TnAV-2a and HvAV-3a have been shown to have a broad experimental host range among larvae of the lepidopteran family Noctuidae, but the host range of SfAV-1a is restricted primarily to species of *Spodoptera*. However, ascoviruses could have an expanded host range that includes non-noctuid insects. For example, recent studies have

demonstrated that HvAV-3e is able to productively propagate in *Crocidomia pavonana* and *Plutella xylostella* larva, lepidopteran species belonging to families, respectively, *Crambidae* and *Plutellidae*.

### Transmission

Ascoviruses are difficult to transmit *per os*, and experimental studies as well as field observations indicate most are transmitted horizontally by endoparasitic wasps (Hymenoptera), many species of which belong to the families Braconidae and Ichneumonidae. During egg-laying, the ovipositor of female wasps becomes contaminated with virions and virion-containing vesicles that circulate in the blood (hemolymph) of infected caterpillars. Wasps contaminated in this manner subsequently transmit ascovirus virions to new caterpillar hosts during oviposition.

### Geographical distribution

Ascoviruses are known from the United States, Europe, Australia, Indonesia and Mexico, and likely occur worldwide, that is, wherever species of Lepidoptera and their hymenopteran parasites occur. Ascoviruses cause a chronic, fatal disease that markedly retards larval development, but which typically exhibits little other gross pathology. This lack of easily recognizable gross pathology probably accounts for the lack of host records from many geographical regions.

### Cytopathic effects

Ascoviruses vary in tissue tropism, with some attacking most host tissues, such as TnAV-2a and HvAV-3a, whereas others, such as the type species SfAV-1a, are restricted to the fat body. The unique property of ascovirus infection is a novel cytopathology in which host cells cleave to form virion-containing vesicles by a developmental process utilizing a modified form of apoptosis. Infection results in nuclear hypertrophy followed by lysis. The anucleate cell enlarges 5–10-fold and then cleaves into 10–30 virion-containing vesicles. Membranes delimiting vesicles form by invagination of the plasmalemma and membrane synthesis. Millions of vesicles (ca. $10^7$–$10^8$ vesicles $ml^{-1}$) accumulate in the hemolymph, turning it milky white. Opaque white hemolymph containing refractile virion vesicles is unique and diagnostic for ascovirus disease.

## Species demarcation criteria in the genus

The following list of characters is used in combination to differentiate species in the genus:

- Virion morphology
- Phylogenetic position of genes encoding the major capsid protein and the DNA polymerase and their homologs in other characterized ascoviruses and invertebrate iridoviruses
- Presence or absence of occlusion bodies
- Lack of DNA/DNA hybridization with other species at low stringency
- Restriction enzyme fragment length polymorphisms (RFLPs)
- Host of isolation and experimental host range
- Tissue tropism
- Association with specific hymenopteran parasites, if apparent

Ascoviruses can have broad host ranges among the larvae of lepidopteran species, and the fat body tissue is a major site of replication for most species. In addition, the virions of most isolates are similar in size and shape. The above characters are therefore used in combination to distinguish existing and new ascovirus species from one another. Hybridization studies have proven particularly useful, and when combined with RFLPs and phylogenetics can also be used to distinguish variants within a species.

For example, SfAV-1a DNA does not hybridize with HvAV-3a or TnAV-2a DNAs under conditions of low stringency. TnAV-2a DNA hybridizes to some extent with HvAV-3a DNA, but not as strongly as it does with homologous DNA. In addition, TnAV-2a replicates in a range of larval tissues including the fat body, tracheal matrix and epidermis, but SfAV-1a and HvAV-3a appear to replicate, respectively, only or primarily in the fat body tissue of most hosts. SfAV-1a virions are bacilliform and are occluded in vesiculate occlusion bodies, whereas TnAV-2a virions are allantoid and are not occluded in occlusion bodies. HvAV-3a virions vary from allantoid to bacilliform, and are not occluded in occlusion bodies.

When the genome of a new isolate cross-hybridizes with that of an existing species member, RFLPs can be used to distinguish variants. Numerous ascovirus isolates, for example, have been obtained from larvae of different noctuid species, including *Heliothis virescens*, *Helicoverpa zea*, *Autographa precationis* and *Spodoptera exigua* in the United States, as well as from *Helicoverpa* and *Spodoptera* species in Australia and Indonesia. The DNA of many of these isolates shows strong reciprocal hybridization with HvAV-3a DNA under conditions of high stringency. RFLP profiles of these isolates, however, often show variations from HvAv-3a that range from minor to major. Because these isolates cross-hybridize strongly with HvAV-3a, they are considered variants of this viral species. Moreover, experimentally these isolates have been shown to have host ranges that overlap with HvAV-3a, providing additional evidence that they are variants of the same species. A similar situation occurs with isolates of TnAV-2a and TnAV-2b.

## List of species in the genus *Ascovirus*

| | | |
|---|---|---|
| *Heliothis virescens ascovirus 3a* | | |
| Heliothis virescens ascovirus 3a | [AJ279817*] | (HvAV-3a) |
| Heliothis virescens ascovirus 3b | | (HvAV-3b) |
| Heliothis virescens ascovirus 3c | [AJ312696*, AJ312697*, AJ312704*, AJ312698*] | (HvAV-3c) |
| Heliothis virescens ascovirus 3d | [AJ279820*] | (HvAV-3d) |
| Heliothis virescens ascovirus 3e | [EF133465 = NC_009233] | (HvAV-3e) |
| Heliothis virescens ascovirus 3f | [DQ015956*] | (HvAV-3f) |
| Heliothis virescens ascovirus 3g | [AJ620611*, AJ620613*] | (HvAV-3g) |
| (Spodoptera exigua ascovirus 5a) | | |
| *Spodoptera frugiperda ascovirus 1a* | | |
| Spodoptera frugiperda ascovirus 1a | [AM398843 = NC_008361] | (SfAV-1a) |
| Spodoptera frugiperda ascovirus 1b | | (SfAV-1b) |
| Spodoptera frugiperda ascovirus 1c | [AJ279824*] | (SfAV-1c) |
| *Trichoplusia ni ascovirus 2a* | | |
| Trichoplusia ni ascovirus 2a | [AJ312707*, AJ279826*] | (TnAV-2a) |
| Trichoplusia ni ascovirus 2b | [AJ279827*] | |
| *Diadromus pulcehllus ascovirus 4a*** | | |
| Diadromus pulchellus ascovirus 4a | [CU469068 = NC_011335] | (DpAV-4a) |

Species names are in italic script; names of strains are in roman script; synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.
**Evidence obtained by Y. Bigot et al. (2009) shows that the species *Diadromus pulcehllus ascovirus 4a* does not belong to the same lineage as other ascoviruses and should be moved into either the, related, *Iridoviridae* family (see Figure 3) or an entirely new family.

## List of other related viruses which may be members of the genus *Ascovirus* but have not been approved as species

| | | |
|---|---|---|
| Helicoverpa armigera ascovirus 7a | | (HaAV-7a) |
| Helicoverpa punctigera ascovirus 8a | | (HpAV-8a) |
| Spodoptera exigua ascovirus 9a | | (SeAV-9a) |
| Trichoplusia ni ascovirus 6a | [DQ517337 = NC_008518] | (TnAV-6a) |
| (Trichoplusia ni ascovirus 2c) | | |

## Phylogenetic relationships within the family

Phylogenetic relationships within the family have been inferred from the full length or partial peptide or DNA sequences of the DNA polymerase and major capsid protein. To date, the family appears to have evolved as a homogeneous lineage. Nevertheless, it must be noted that the current phylogenetic scheme is based on ascoviruses isolated from noctuid hosts. Whether ascoviruses occur in other lepidopteran or insect families in nature is unknown, but it is possible that such isolates could have dramatically different phylogenetic properties.

## Similarity with other taxa

Comparison of the gene content of three ascoviruses (HvAV-3e, SfAV-1a, TnAV-6a), DpAV-4a, two invertebrate iridoviruses (Chilo iridescent virus type [CIV] and mosquito iridovirus [MIV]) and eight vertebrate iridoviruses have demonstrated that these viruses share 28 core gene homologs and orthologs. The data also revealed that 67 core gene homologs are shared within the genus *Ascovirus*, with 29 and 42 of these being core genes in, respectively, invertebrate iridoviruses and DpAV4.

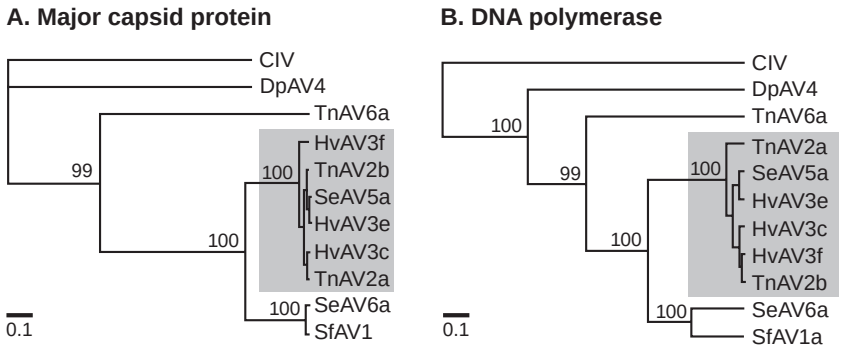


**Figure 2:** Phylogenetic relationships within the family *Ascoviridae*. Consensus neighbor-joining trees (PHYLIP) were constructed using alignments of ascovirus DNA polymerase and major capsid protein sequences. Trees were rooted using corresponding sequences from the Chilo iridescent virus type 6 (CIV) and DpAV4a. Bootstrap values are shown at branch nodes in the trees and branch lengths are proportional to genetic distances. In shaded boxes are located a lineage of ascovirus that were previously considered as different species but which, in the light of the most recent data, belong to a master species in which some features, such as the presence of occlusion bodies, can vary significantly.

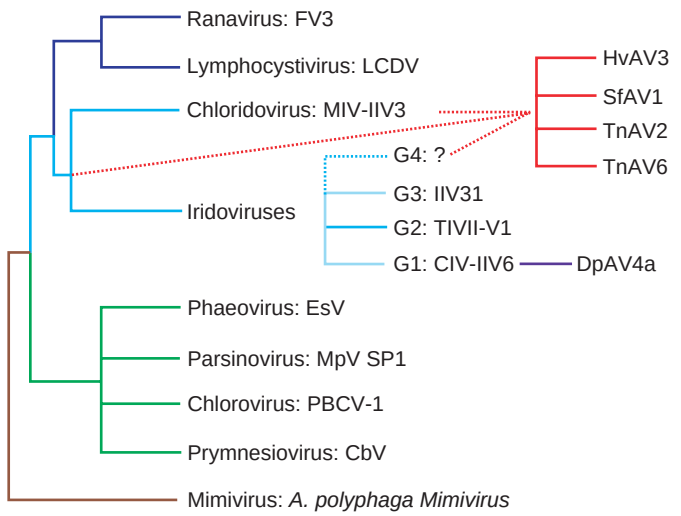


**Figure 3:** Synthesis of the evolutionary relationships among various genera of *Mimiviridae* (brown), *Phycodnaviridae* (green), *Iridoviridae* (invertebrate genera are in blue and vertebrate genera in dark blue), *Ascoviridae* (red) and DpAV4a (purple). The name of the principal virus representative of each genus is indicated. This synthetic tree was determined from results obtained with analyses of the 28 core genes. Plain lines represent verified relationships. Dotted lines indicate (putative) evolutionary pathways among virus families that require further support for confirmation. Phylogenetic analyses of major capsid protein (MCP) sequences revealed that at least three groups (G1, G2, G3) occurred within the iridovirus genus. A hypothetical fourth one, G4, was used to suggest the putative origin of the *Ascoviridae*.

Based on phylogenetic analyses of the 28 core genes, the following alternative theories for the origin of ascoviruses have been proposed:

(i) Ascoviruses and DpAV4 have different origins and their common molecular and biological features are due to the proximity of their ancestor and to convergences probably resulting from evolutionary processes occurring in similar environments.
(ii) *Ascoviridae* and DpAV4 are two virus families that originated from invertebrate iridoviruses. Although the molecular clock varies significantly between core genes, *Ascoviridae* appear to have emerged early during the evolutionary radiation of invertebrate iridoviruses. Moreover, DpAV4 represents a more recent virus lineage that originated from a close iridovirus ancestor of Chilo iridescent virus (CIV, IIV6).

Further sequencing and phylogenetic analyses of invertebrate iridovirus genomes is required to definitively assess the origin of the *Ascoviridae* family among invertebrate iridoviruses.

## Derivation of name

*Asco*: from the Greek for "sac", referring to the virion-containing vesicles produced by cleavage of host cells, which are characteristic for all known viruses of this family.

## Further reading

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## Contributed by

Bigot, Y., Asgari, S., Bideshi, D.K., Cheng X.W., Federici, B.A. and Renault, S.

# Family *Asfarviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Asfarviridae* |
| Genus | *Asfivirus* |

Since only one genus is currently recognized, the family description corresponds to the genus description.



# Genus *Asfivirus*

Type species *African swine fever virus*

## Virion properties

### Morphology

African swine fever virus (ASFV) virions consist of a nucleoprotein core structure, 70–100 nm in diameter, surrounded by an internal lipid layer and an icosahedral capsid, 170–190 nm in diameter, and an external lipid-containing envelope. The capsid exhibits icosahedral symmetry (T = 189–217) corresponding to 1892–2172 capsomers (each capsomer is 13 nm in diameter and appears as a hexagonal prism with a central hole; intercapsomeric distance is 7.4–8.1 nm). Extracellular enveloped virions have a diameter of 175–215 nm (Figure 1).

### Physicochemical and physical properties

Virion buoyant density is 1.095 g cm$^{-3}$ in Percoll, 1.19–1.24 g cm$^{-3}$ in CsCl; $S_{20,W}$ is about 3500S. Virions are sensitive to ether, chloroform and deoxycholate, and are inactivated at 60 °C within 30 min, but survive for years at 20 °C or 4 °C. Infectivity is stable over a wide pH range. Some infectious virus may survive treatment at pH4 or pH13. Infectivity is destroyed by some disinfectants (1% formaldehyde in 6 d, 2% NaOH in 1 d); paraphenylphenolic disinfectants are very effective. Virus is sensitive to irradiation.

### Nucleic acid

The genome consists of a single molecule of linear, covalently close-ended, dsDNA 170–190 kbp in size (varying among isolates). The end sequences are present as two flip-flop forms that are inverted and complementary with respect to each other, and adjacent to both termini are identical tandem repeat arrays about 2.1 kbp long. The complete nucleotide sequences of 11 isolates have been determined. These include the tissue culture-adapted Ba71V isolate (ASFV-Ba71V) and 10 field isolates from Europe and Africa.

### Proteins

Virions contain more than 50 proteins, including a number of enzymes and factors needed for early mRNA transcription and processing (Table 1). Enzymes packaged into virions include the multisubunit RNA polymerase, polyA polymerase, guanylyl transferase and protein kinase. The inhibitor of apoptosis (IAP) homolog protein is also packaged in virions. Virion structural proteins characterized include on the outer envelope the CD2v protein (EP402R), p22 (pKP177R), p12 (pO61R), on the capsid shell the major capsid protein p72 (pB646L), p49 (pB438L), in the internal envelope p17 (pD117L), p54 or j13L (pE183L) and probably j18L (pE199L), j5R (pH108R). The products of a 220 kDa protein (pCP2475L) that is cleaved to give four structural proteins (p150, p37, p14 and p34), and the products of a 62 kDa protein (pCP530R) that is cleaved to give two structural proteins (p35 and p15) are localized in the matrix or inner core shell. A virus-encoded protease related to the SUMO-1-specific protease family is involved in cleavage of these polyproteins. Two DNA binding proteins, p10 (pA78R) and p14.5 (pE120R), are present in virions. The virus encodes components of a redox pathway, including the pB119L (or 9GL), pE248R and pA151R proteins, of which the pA151R and pB119L proteins are non-structural. The pB602L protein is a chaperone required for assembly of the p72 capsid protein into virions.

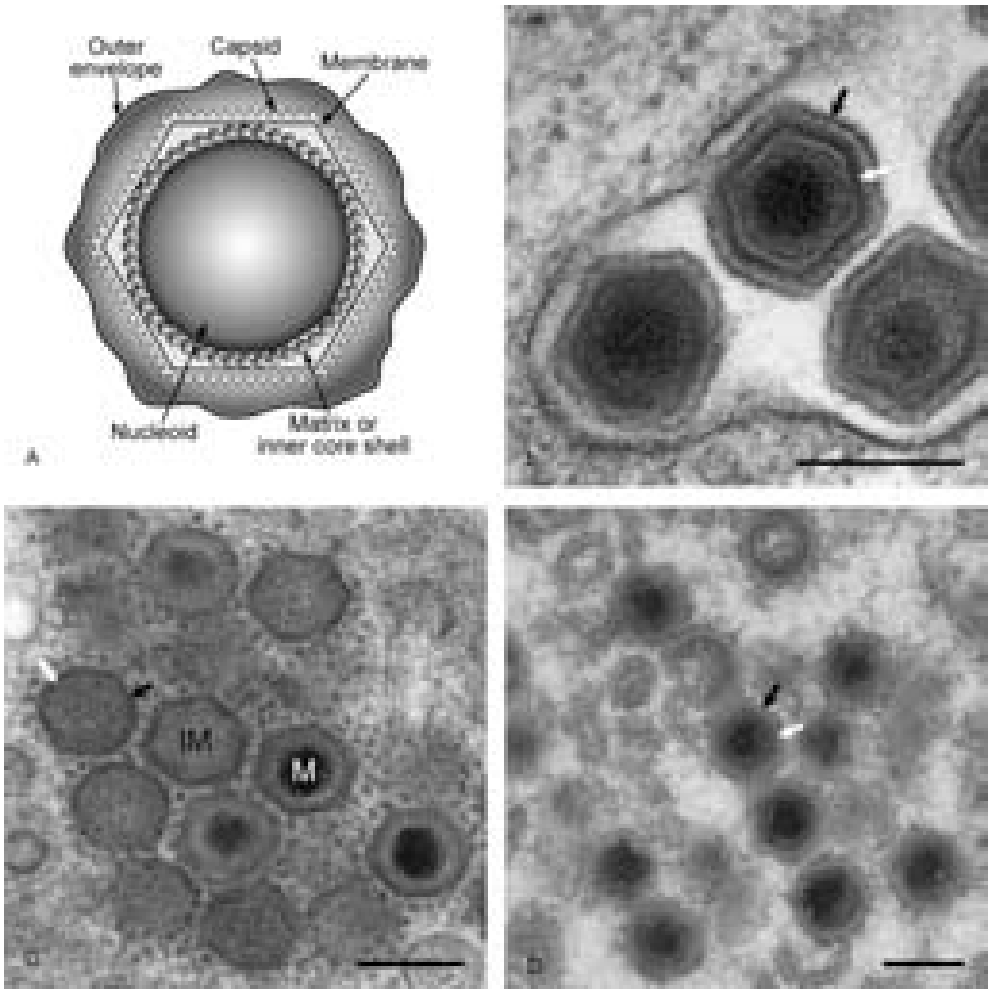


**Figure 1:** (a) Diagram of extracellular ASFV virions showing nucleoid, matrix or inner core shell, capsid and lipid envelopes. (b) EM image of extracellular virions. Black arrow is outer envelope, white arrow is virus membrane. Bar = 200 nm. The preparation method was standard chemical fixation for EM. (c) EM image of intracellular virions. IM = immature virion, M = mature virion. Black arrow is capsid protein, white arrow is virus membrane. Bar = 200 nm. Preparation method was high pressure freezing followed by freeze substitution. (d) EM image of intracellular virions. Black arrow is capsid protein, white arrow is virus membrane. Bar = 200 nm. Preparation method was thawed cryo-sections stained with uranyl acetate. (Images kindly provided by Pippa Hawes, Institute for Animal Health, UK.)

Other predicted proteins encoded by the virus include enzymes involved in nucleotide metabolism (ribonucleotide reductase, thymidine kinase, thymidylate kinase and deoxyuridine triphosphatase), DNA replication and repair or transcription (DNA polymerase, DNA polymerase X, DNA ligase, topoisomerase II, guanylyl transferase, three members of DNA helicase superfamily II, and AP endonuclease). Deletion of the thymidine kinase, AP endonuclease and deoxyuridine triphosphatase genes does not affect virus replication in tissue culture cell lines but reduces virus replication in fully differentiated non-dividing macrophages and reduces virulence of the virus in pigs. Two enzymes involved in post-translational protein modification (a ubiquitin-conjugating enzyme and a serine/threonine protein kinase) and an enzyme involved in synthesis of isoprenoid compounds (trans-prenyltransferase) are encoded by the virus.

Five different multigene families (MGF 110, MGF 360, MGF 530/505, MGF 300 and MGF 100) are found in genome regions close to the termini. Large length variations between genomes of different isolates are due to gain or loss of members of these multigene families. MGF 110 contains 14 members and individual isolates contain between 5 and 11 of these. MGF 360 has 22 members

**Table 1:** Functions of African swine fever virus (ASFV) encoded proteins

| Gene function | Gene name | Predicted protein size (kDa) |
|---|---|---|
| **Nucleotide metabolism, transcription, replication and repair** | | |
| Thymidylate kinase | A240L | 27.8 |
| Thymidine kinase | K196R | 22.4 |
| dUTPase* | E165R | 18.3 |
| Ribonucleotide reductase (small subunit) | F334L | 39.8 |
| Ribonucleotide reductase (large subunit) | F778R | 87.5 |
| DNA polymerase α-like | G1211R | 139.8 |
| DNA topoisomerase type II* | P1192R | 135.5 |
| Proliferating cell nuclear antigen (PCNA)-like | E301R | 35.3 |
| DNA polymerase X-like* | O174L | 20.3 |
| DNA ligase* | NP419L | 48.2 |
| AP endonuclease class II* | E296R | 33.5 |
| RNA polymerase subunit 2 | EP1242L | 139.9 |
| RNA polymerase subunit 6 | C147L | 16.7 |
| RNA polymerase subunit 1 | NP1450L | 163.7 |
| RNA polymerase subunit 3 | H359L | 41.3 |
| RNA polymerase subunit 5 | D205R | 23.7 |
| RNA polymerase subunit 10 | CP80R | |
| TFIIB like | C315R | |
| Helicase superfamily II | A859L | 27.8 |
| Helicase superfamily II | F1055L | 123.9 |
| Helicase superfamily II | B962L | 109.6 |
| Helicase superfamily II | D1133L | 129.3 |
| Helicase superfamily II | Q706L | 80.4 |
| Helicase superfamily II | QP509L | 58.1 |
| Transcription factor SII | I243L | 28.6 |
| Guanylyl transferase* | NP868R | 29.9 |
| PolyA polymerase large subunit | C475L | 54.8 |
| FTS J-like methyl transferase domain | EP424R | 49.3 |
| ERCC4 nuclease domain | EP364R | 40.9 |
| Lambda-like exonuclease | D345L | 39.4 |
| VV A2L-like transcription factor | B385R | 45.3 |
| VV A7L-like transcription factor | G1340L | 155.0 |
| VV VLTF2-like late transcription factor | B175L | 20.3 |
| FCS-like finger DNA primase | C962R | 111.3 |
| **Other enzymes** | | |
| Prenyltransferase* | B318L | 35.9 |
| Serine protein kinase* | R298L | 35.1 |
| Ubiquitin conjugating enzyme* | I215L | 24.7 |
| Nudix hydrolase* | D250R | 29.9 |
| **Host cell interactions** | | |
| IAP apoptosis inhibitor* | A224L | 26.6 |
| Bcl 2 apoptosis inhibitor* | A179L | 21.1 |
| Inhibitor of host gene transcription* | A238L | 28.2 |
| C-type lectin-like* | EP153R | 18.0 |

(*Continued*)

**Table 1:** Functions of African swine fever virus (ASFV) encoded proteins *(Continued)*

| Gene function | Gene name | Predicted protein size (kDa) |
|---|---|---|
| Similar to HSV ICP34.5 neurovirulence factor | DP71L | 8.5 |
| Nif S-like | QP383R | 42.5 |
| ERV 1-like. Involved in redox metabolism* | B119L | 14.4 |
| Phosphoprotein binds to ribonucleoprotein-K | CP204L | 30.0 |
| **Structural proteins and proteins involved in morphogenesis** | | |
| P22 | KP177R | 20.2 |
| Histone-like | A104R | 11.5 |
| P11.5 | A137R | 21.1 |
| P10 | A78R | 8.4 |
| pA151R. Contains CXXC motif similar to that in thioredoxins. Binds to E248R protein. Not incorporated into virions. Possible component of redox pathway. | A151R | 17.5 |
| P72 major capsid protein. Involved in virus entry | B646L | 73.2 |
| P49. Required for formation of vertices in icosahedral capsid | B438L | 49.3 |
| Chaperone. Involved in folding of capsid. Not incorporated into virions | B602L | 45.3 |
| SUMO-1-like protease. Involved in polyprotein cleavage | S273R | 31.6 |
| pp220 polyprotein precursor of p150, p37, p14 and p34. Required for packaging of nucleoprotein core | CP2475L | 281.5 |
| P32 (P30) phosphoprotein. Involved in virus entry | CP204L | 23.6 |
| pp62 (pp60) polyprotein precursor of p35 and p15 | CP530R | 60.5 |
| P12 attachment protein | O61R | 6.7 |
| P17. Required for progression of precursor membranes to icosahedral intermediates | D117L | 13.1 |
| J5R. Transmembrane domain | H108R | 12.5 |
| P54 (j13L). Binds to LC8 chain of dynein, involved in virus entry. Required for recruitment of envelope precursors to the factory | E183L | 19.9 |
| J18L. Transmembrane domain | E199L | 22.0 |
| P14.5. DNA-binding. Required for movement of virions to plasma membrane | E120R | 13.6 |
| E248R (k2R). Possible component of redox pathway required disulfide bond formation. Structural protein | E248R | 27.5 |
| XP124L. Multigene family 110 member. Contains KDEL ER retrieval sequence and transmembrane domain | MGF 110-4L (XP124L) | 14.2 |
| EP402R. Similar to host CD2 protein. Required for binding red blood cells to infected cells and extracellular virus particles. Glycoprotein inserted into external virus envelope* | EP402R | 45.3 |
| **Multigene family members** | | |
| Multigene family 360 | MGF 360-1L (KP360L) | 41.7 |
| | MGF 360-2L (KP362L) | 42.6 |
| | MGF 360-3L (L356L) | 41.7 |
| | MGF 360-4L (LIS382) | 44.9 |
| | MGF 360-5L (UP60L) | 7.0 |
| | MGF 360-6L (LIS375) | 43.9 |
| | MGF 360-7L (LIS375a) | 44.1 |
| | MGF 360-8L (J319L) | 31.3 |
| | MGF 360-9L (A125L) | 14.5 |
| | MGF 360-10L | 41.6 |

(*Continued*)

**Table 1:** Functions of African swine fever virus (ASFV) encoded proteins *(Continued)*

| Gene function | Gene name | Predicted protein size (kDa) |
|---|---|---|
| | MGF 360-11L | 41.6 |
| | MGF 360-12L | 41.1 |
| | MGF 360-13L | 41.0 |
| | MGF 360-14L | 41.3 |
| | MGF 360-15R (A276R) | 31.6 |
| | MGF 360-16R (DP311R) | 35.6 |
| | MGF 360-17R (DP63R) | 8.4 |
| | MGF 360-18R (DP148R) | 17.2 |
| | MGF 360-19R (DP363R) | 42.4 |
| | MGF 360-20R (DP42R) | 4.9 |
| | MGF 360-21R | 42.0 |
| | MGF 360-22R | 41.7 |
| Multigene family 110 | MGF 110-1L (L270L) | 32.4 |
| | MGF 110-2L (U104L) | 12.2 |
| | MGF 110-3L (LIS124-1) | 14.3 |
| | MGF 110-4L (XP124L) | 14.2 |
| | MGF 110-5L (V82L) | 9.4 |
| | MGF 110-6L (Y118L) | 13.9 |
| | MGF 110-7L (LIS137) | 15.9 |
| | MGF 110-8L (LIS124-2) | 14.9 |
| | MGF 110-9L (LIS290) | 34.8 |
| | MGF 110-10L (190-2) | 22.7 |
| | MGF 110-11L (LIS119-1) | 32.5 |
| | MGF 110-12L (LIS 119-2) | 12.5 |
| | MGF 110-13L (LIS 117) | 18.3 |
| | MGF 110-14L (LIS121-2) | 14.7 |
| Multigene family 300 | MGF 300-1L (J268L) | 31.3 |
| | MGF 300-2R (J154R) | 17.6 |
| | MGF 300-3L (J104L) | 12.5 |
| | MGF 300-4L (J182L) | 21.7 |
| Multigene family 505 | MGF 505-1R | 62.6 |
| | MGF 505-2R (A489R) | 57.7 |
| | MGF 505-3R (A280R) | 32.5 |
| | MGF 505-4R (A505R) | 59.2 |
| | MGF 505-5R (A498R) | 58.7 |
| | MGF 505-6R (A518R) | 61.8 |
| | MGF 505-7R (A528R) | 61.7 |
| | MGF 505-8R | 61.7 |
| | MGF 505-9R (A506R) | 59.4 |
| | MGF 505-10R (A542R) | 59.4 |
| | MGF 505-11L (DP542L) | 63.1 |
| Multigene family 100 | MGF 100-1R | 15.3 |
| | MGF 100-2L (DP141L) | 16.8 |
| | MGF 100-3L (DP146L) | 17.2 |

*The table lists functions of ASFV-encoded proteins. Functions have been demonstrated experimentally for those proteins marked with an asterisk.

and between 11 and 18 copies are encoded by different isolates. MGF 530/505 has 11 members and between 8 and 10 copies are present. MGF 300 has 4 copies with 3 or 4 present and MGF 100 has 3 copies with 2 or 3 present. Members of families MGF 360 and 530 have been implicated as macrophage host range determinants, and deletion of 6 members of MGF 360 and 2 of MGF 530 results in an increase in type I interferon production. These 6 copies of MGF 360 and 1 or 2 copies of MGF 530 are deleted from the genome of an attenuated field isolate OURT88/3 and the tissue-culture adapted BA71V isolate.

Virus-encoded proteins that modulate the host response to virus infection include homologs of the apoptosis inhibitors Bcl2 and IAP. Both of these proteins inhibit apoptosis; the IAP homolog inhibits caspase 3 activity. A C-type lectin (pEP153R) has also been reported to inhibit apoptosis. Although the virus encodes proteins that inhibit apoptosis, apoptotic cells are observed at late stages of infection. The A238L protein inhibits transcriptional activation dependent on a number of different host transcription factors, including NFAT, NFkB and cJun, by inhibiting their transactivation mediated by p300. This protein also binds to and inhibits the host calcineurin phosphatase and is therefore predicted to inhibit calcineurin-dependent pathways. The A238L protein may therefore inhibit transcriptional activation in infected macrophages of a wide range of host immunomodulatory genes that are dependent on these factors. A virus protein, EP402R, which is similar to the host T cell adhesion protein CD2, is required for the hemadsorption of red blood cells around virus-infected cells and is also thought to mediate the adhesion of extracelluar virions to red blood cells. Deletion of the EP402R gene reduces virus dissemination in infected pigs and *in vitro* abrogates the ability of ASFV-infected cells to inhibit proliferation of bystander lymphocytes in response to mitogens. One protein (designated pNL-S, pl14L or pDP71L) is similar over a conserved C-terminal domain to a herpes simplex virus-encoded neurovirulence factor (ICP34.5) and host protein GADD34. These proteins all act to recruit cellular protein phosphatase 1 to dephosphorylate translation initiation factor eIF2$\alpha$ and inhibit global shut-off of translation. GADD34 and ICP34.5 are larger and have other demonstrated roles. One of the major ASFV-induced proteins, encoded by gene CP204L, interacts with the heterogeneous nuclear ribonucleoprotein K (hnRNP-K) with potential implications in the downregulation of host cell mRNA translation.

### Lipids

Enveloped virions contain lipids, including glycolipids and phospholipids such as phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol.

### Carbohydrates

One virion protein is glycosylated (pEP402R), and glycolipids are also incorporated into virions. The virus encodes several predicted transmembrane proteins that contain putative N-linked glycosylation sites.

## Genome organization and replication

The genomes of different isolates vary in length between 165,795 and 191,036 bp, excluding the terminal inverted repeat sequences, and encode between 151 and 167 protein-encoding ORFs. The ORFs are closely spaced with intergenic distances generally less than 200 bp, and read from both DNA strands. A few intergenic regions contain short tandem repeat arrays.

The primary cell types infected by the virus include those of the mononuclear-phagocytic system, including fixed tissue macrophages and specific lineages of reticular cells. Virus replicates *in vitro* in macrophages and endothelial cells, and several isolates have been adapted to replicate in tissue culture cell lines. Virus enters cells primarily by clathrin- and dynamin-dependent receptor-mediated endocytosis, and is transported to perinuclear areas associated with the microtubular motor light chain dynein. Early mRNA synthesis begins in the cytoplasm immediately following entry using enzymes and factors packaged in the virus core. Virus DNA replication and assembly take place in perinuclear factory areas. At early times post-infection, virus DNA is detected in the nucleus, suggesting a possible role for nuclear enzymes in initial stages of DNA replication. Head-to-head virus DNA concatemers, which are thought to be replicative intermediates, are detected in the cytoplasm from 6 h post-infection. The mechanism of DNA replication in the cytoplasm is similar to that of viruses in the family *Poxviridae*.

Virus transcripts are 3′-polyadenylated and 5′-capped. Genes are expressed in an ordered cascade. Early genes are expressed prior to DNA replication; expression of late genes is dependent on the onset of DNA replication. Synthesis of some early genes continues throughout infection. Intermediate genes are expressed late but their expression does not depend on the onset of DNA replication. Promoter elements are relatively short and located immediately upstream from ORFs; transcription start sites are generally a short distance from start codons. Both early and late gene transcripts are of defined length; sequences of seven or more consecutive thymidylate residues in the coding strand are signals for mRNA 3′-end formation.

Several structural proteins are expressed as polyproteins and cleaved at the sequence GlyGlyX. The polyprotein with Mr 220 $\times 10^3$ is myristylated. Other virus-encoded proteins are modified by phosphorylation (p10 and p32) and N-linked glycosylation. Virus morphogenesis takes place in perinuclear virus factories. Virus factories are surrounded by a vimentin cage and increased numbers of mitochondria. A single lipid membrane thought to be derived from the endoplasmic reticulum is incorporated as an internal lipid membrane in virus particles. The p17 protein is essential for the progression of viral membrane precursors toward icosahedral intermediates. The p54 protein (pE183L) is required for intracellular virus transport and for recruiting envelope precursors to assembly sites. This protein binds to the LC8 component of the dynein motor complex, and this interaction is involved in recruitment to the assembly sites. Formation of the icosahedral capsid occurs on the internal membrane. Assembly of the major capsid protein p72 (pB646L) requires a virus-encoded chaperone pB602L. The pB438L protein is required for formation of the vertices of the icosahedral capsid. The virus genome and enzymes required to initiate infection are packaged into a nucleoprotein core. Processing of the virus polyproteins pp62 and pp220 is essential for core development. Extracellular virus has a loose-fitting external lipid envelope derived by budding through the plasma membrane. Virus is transported to and from sites of assembly on microtubules. The pE120R virion protein is required for virus transport from assembly sites to the plasma membrane.

## Antigenic properties

Antibodies induced in pigs that recover from infection with less virulent isolates can neutralize virus infection in pig macrophages and cell lines. This neutralization is effective using virus with a low number of passages in tissue culture but is not as effective against virus with a high number of passages. Serotyping of virus isolates by neutralization has not been carried out since most virulent isolates kill pigs before an effective antibody response is mounted. Virus targets for neutralization in cell culture have been identified using antibodies against proteins p72, p12, p30 and p54, those against p30 inhibiting virus internalization rather than attachment. Virus isolates have been separated into genotypes by sequencing of several genes, but there is no evidence to relate genotype to cross-reactive groups or serotypes.

## Biological properties

ASFV infects domestic and wild swine (*Sus scrofa domesticus* and *S. s. ferus*), warthogs (*Phacochoerus africanus*) and bushpigs (*Potamochoerus porcus*). Disease signs are apparent only in domestic and wild swine. Soft ticks of the genus *Ornithodoros* are also infected, *O. moubata* acts as a vector in parts of Africa south of the Sahara and *O. erraticus* acted as a vector in SW Spain and Portugal. Virus can be transmitted in ticks trans-stadially, and sexual and transovarial transmission has also been demonstrated in *O. moubata*. Warthogs, bushpigs and swine can be infected by bites from infected ticks. Neither vertical nor horizontal transfer of virus between warthogs is thought to occur. However, transmission between domestic swine can occur by direct contact, by ingestion of infected meat, by fomites, or mechanically by biting flies. Warthogs, bushpigs, wild swine and ticks act as reservoirs of virus. Disease is endemic in domestic swine in many African countries and in Europe in Sardinia. In 2007, African swine fever was introduced to Georgia in the Trans-Caucasus and from there spread to neighbouring countries, including the Russian Federation. ASFV was first reported in Madagascar in 1998 and remains endemic there. Disease was first introduced into Europe in Portugal in 1957 and was endemic in parts of the Iberian peninsula from 1960 until 1995. Sporadic outbreaks have occurred in, and been eradicated from, Belgium, Brazil, Cuba, the Dominican Republic, France, Haiti, Holland and Malta.

ASFV causes hemorrhagic fever in domestic pigs and wild boar. Virus isolates differ in virulence and may produce a variety of disease signs ranging from acute to chronic to inapparent. Virulent isolates may cause 100% mortality in 5–10 days. Moderately virulent isolates have reduced mortality and up to 50% of pigs may recover from infection. Attenuated isolates cause few disease signs. Recovered pigs can remain persistently infected and are protected against challenge with related virulent isolates. CD8+ T cells are required for this protection. Viruses replicate in cells of the mononuclear phagocytic system and reticuloendothelial cells in lymphoid tissues and organs of domestic swine. Cell surface markers expressed from intermediate stages of monocyte–macrophage differentiation are indicators of cell susceptibility to infection. Widespread cell death caused by apoptosis occurs in both T and B lymphocytes in lymphoid tissues and endothelial cells in arterioles and capillaries. This accounts for the lesions seen in acute disease. Disseminated intravascular coagulation develops during the late phase of acute infections, and this may lead to the characteristic hemorrhagic syndrome.

## Species demarcation criteria in the genus *Asfarviridae*

Not applicable.

## List of species in the genus *Asfivirus*

| | | |
|---|---|---|
| *African swine fever virus* | | |
| African swine fever virus Benin97/1 | [AM712239] | (ASFV-Benin97) |
| ASFV-BA71V | [U18466 = NC_001659] | |
| ASFV-Ken | [AY261360] | |
| ASFV-Mal | [AY261361] | |
| ASFV-Mku | [AY261362] | |
| ASFV-OurT88/3 | [AM712240] | |
| ASFV-Pret | [AY261363] | |
| ASFV-Teng | [AY261364] | |
| ASFV-War | [AY261366] | |
| ASFV-Warm | [AY261365] | |
| ASFV-E75 | [FN557520] | |

Species names are in italic script; names of isolates and strains are in roman script. Full genome sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Asfivirus* but have not been approved as species

None reported.

## Phylogenetic relationships within the family

Not applicable.

## Similarity with other taxa

Earlier, ASFV was listed as a member of the family *Iridoviridae*. As more information was obtained, it was removed from this family. Analysis of the replication strategies and genes encoded have shown that ASFV is related to other viruses in the nucleo-cytoplasmic large DNA virus superfamily, which also includes the families *Poxviridae*, *Iridoviridae*, *Phycodnaviridae* and *Mimiviridae* (Figure 2). Metagenomic sequencing projects have identified sequences related to ASFV in virus fractions from oceans, sewage and human serum.

## Derivation of name

*Asfar*: from *A*frican *s*wine *f*ever *a*nd *r*elated viruses.

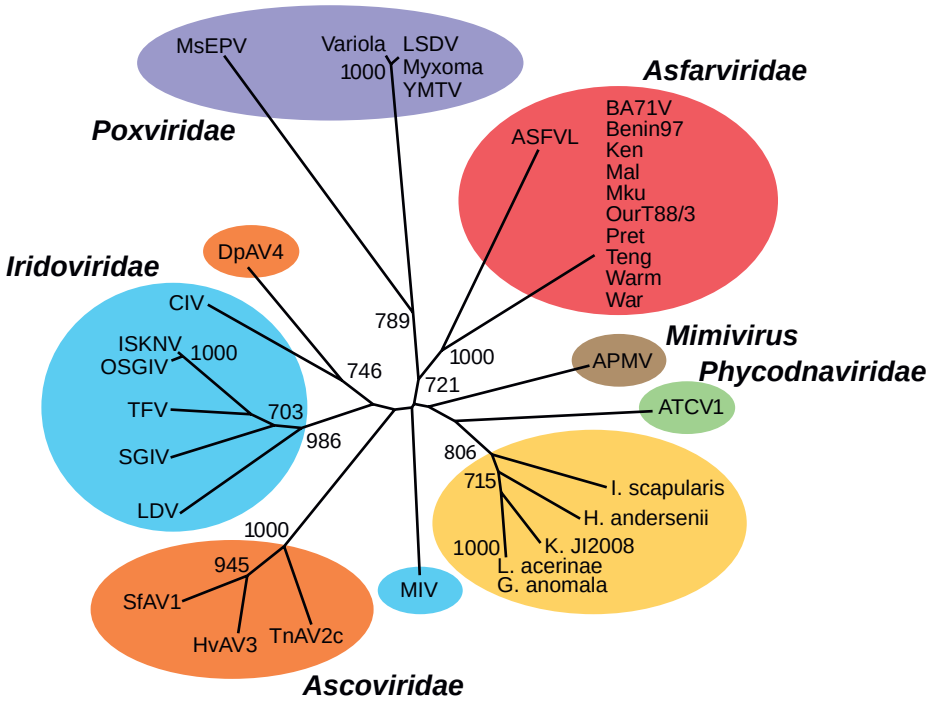


**Figure 2:** Phylogenetic analysis of ASFV sequences for RNA polymerase, compared to corresponding sequences from dsDNA viruses and non-viral sequences. Sequences are shown in color as follows: asfarviruses, red; mimivirus, brown; poxviruses, purple; phycodnaviruses, green; ascoviruses, orange; iridoviruses, blue; non-viral Blast matches, yellow. Bootstrap values over 65% (>650) are shown. Abbreviations: APMV, Acanthamoeba polyphaga mimivirus; ATCV1, Acanthocystis turfacea chlorella virus 1; CIV, Chilo iridescent virus; DpAV4, Diadromus pulchellus ascovirus 4; G. anomala, Glugea anomala; H. andersenii, Hemiselmis andersinii; HvAV3, Heliothis virescens ascovirus 3; I. scapularis, Ixodes scapularis; ISKNV, infectious spleen and kidney necrosis virus; K. JI2008, Kabatana sp. strain JI2008; L. acerinae, Loma acerinae; LDV, lymphocystis disease virus; LSDV, lumpy skin disease virus; MsEPV, Melanoplus sanguinipesentomopoxvirus; OSGIV, orange-spotted grouper iridovirus; SfAV1, Spodoptera frugiperda ascovirus 1; SGIV, Singapore grouper iridovirus; TFV, tiger frog virus; TnAV2c, Trichoplusia ni ascovirus 2c; YMTV, Yaba monkey tumor virus, Variola, ASFVL African swine fever virus-like sequence. (Figure redrawn from Loh, J., Zhao, G., Presti, R.M., Holtz, L.R., Finkbeiner, S.R., Droit, L., Villasana, Z., Todd, C., Pipas, J.M., Calgua, B., Girone, R., Wang, D. and Virgin, H.W. (2009). Detection of novel sequences related to African swine fever virus in human serum and sewage. *J. Virol.*, **83**, 13019–13025.)

## Further reading


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## Contributed by

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# FAMILY *BACULOVIRIDAE*



## Taxonomic structure of the family

| | |
|---|---|
| Family | *Baculoviridae* |
| Genus | *Alphabaculovirus* |
| Genus | *Betabaculovirus* |
| Genus | *Gammabaculovirus* |
| Genus | *Deltabaculovirus* |

## Virion properties

### MORPHOLOGY

One or two virion phenotypes are involved in baculovirus infections. Infection is initiated in the gut epithelium by a virus phenotype occluded in a crystalline protein matrix which may be: (a) polyhedral in shape, typically ranging in size from 0.5 to 5 µm and containing many virions for the genera *Alphabaculovirus*, *Gammabaculovirus*, *Deltabaculovirus* (Figure 1 and 2), or (b) ovicylindrical (about 0.3 × 0.5 µm) and containing only one, or rarely two or more virions (genus *Betabaculovirus*) (Figure 1). Virions within occlusions consist of one or more rod-shaped nucleocapsids that have a distinct structural polarity and are enclosed within an envelope. For occluded virions, nucleocapsid envelopment occurs within the nucleus (genus *Alphabaculovirus*) or in the nuclear-cytoplasmic milieu after loss of the nuclear membrane (genus *Betabaculovirus*). Nucleocapsids average 30–60 nm in diameter and 250–300 nm in length. Spike-like structures have not been reported on envelopes of the occlusion-derived virions (ODV). Virions of the second phenotype (termed budded virions or BV) are generated when nucleocapsids bud through the plasma membrane at the surface of infected cells. BVs typically contain a single nucleocapsid. Their envelopes are derived from the cellular plasma membrane and characteristically appear as a loose-fitting membrane that contains an envelope fusion glycoprotein (EFP), such as the GP64 and F proteins at one end of the virion (see "Proteins", below) (Figure 1).

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

ODV buoyant density in CsCl is 1.18–1.25 g cm$^{-3}$, and that of the nucleocapsid is 1.47 g cm$^{-3}$. BV buoyant density in sucrose is 1.17–1.18 g cm$^{-3}$. Virions of both phenotypes are sensitive to organic solvents and detergents. BV is marginally sensitive to heat and pH 8–12, inactivated by pH 3.0, and stable in $Mg^{++}$ ($10^{-1}$ M to $10^{-5}$ M).

### NUCLEIC ACID

Nucleocapsids contain one molecule of circular supercoiled dsDNA, 80–180 kbp in size.

### PROTEINS

Genomic analyses suggest that baculoviruses encode 100–200 proteins. Virions may contain 40 or more different polypeptides. Nucleocapsids from both virion phenotypes (ODV and BV) contain a major capsid protein, a basic DNA binding protein complexed with the viral genome, and at least 2–3 additional proteins. BVs contain an envelope fusion protein (EFP). The EFPs identified to date include the major envelope glycoprotein (the peplomer protein) GP64, which is present in Autographa californica multiple nucleopolyhedrovirus (AcMNPV) and close relatives within the alphabaculoviruses. Most of the alphabaculoviruses and betabaculoviruses encode and appear to utilize F proteins (homologs of the Ld130 protein from Lymantria dispar MNPV (LdMNPV) and the Se8 protein from Spodoptera exigua MNPV (SeMNPV)) as EFP. Several ODV envelope proteins have been identified. Six ODV proteins, including P74, PIF-1, PIF-2, PIF-3, AC96 (PIF-4) and ODV-E56 (PIF-5), are essential for oral infectivity of ODV. The major protein of the occlusion body matrix is a virus-encoded polypeptide of 25–33 kDa. This protein is called polyhedrin for nucleopolyhedroviruses (the former name for the alpha-, delta- and gammabaculoviruses) and granulin for betabaculoviruses. The occlusion body is surrounded by an envelope that contains at least one major protein. The polyhedrin protein of deltabaculoviruses is serologically and genetically unrelated to occlusion body proteins of the alpha-, beta- and gammabaculoviruses.

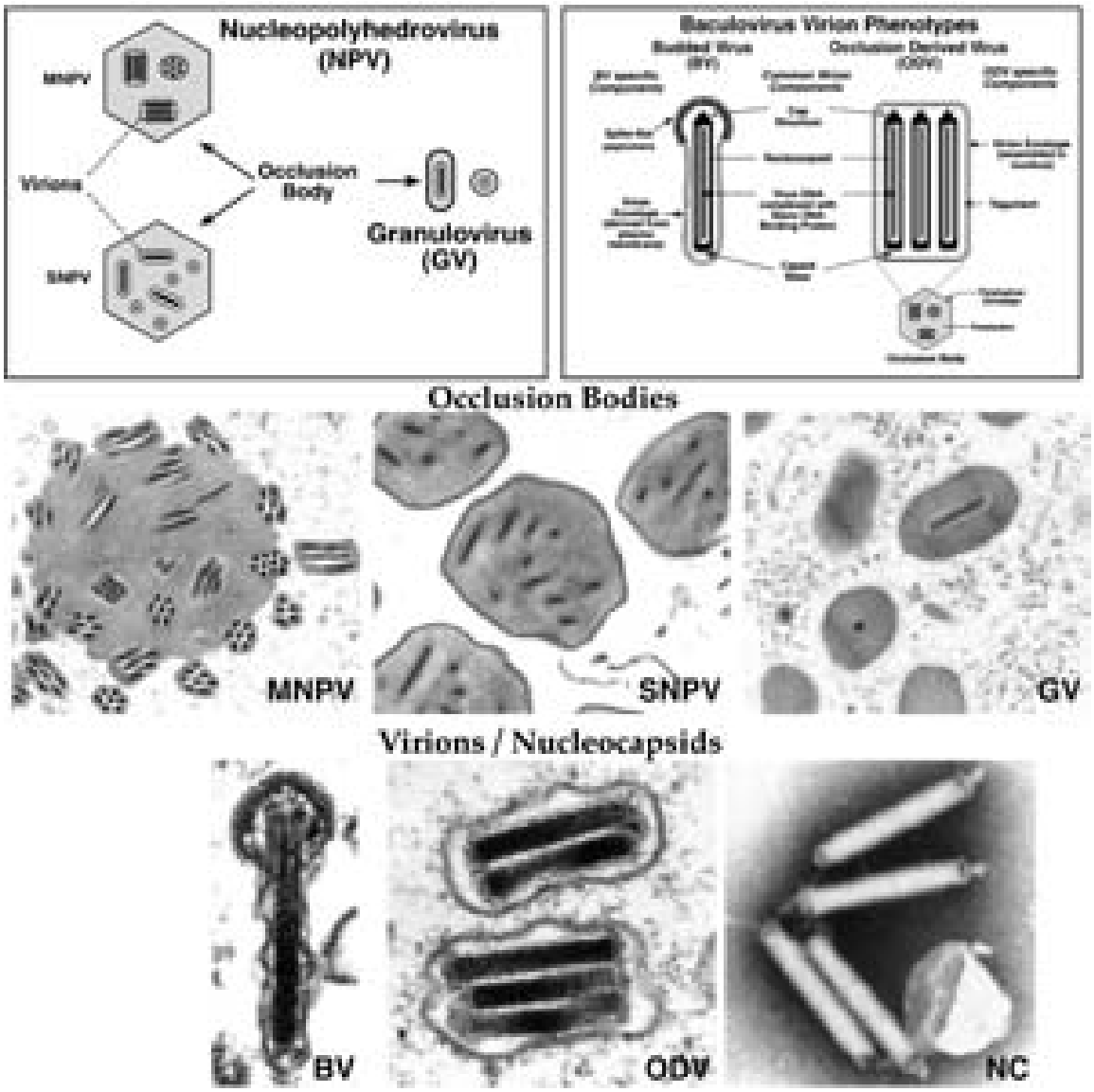


**Figure 1:** Baculovirus occlusion bodies, virions and nucleocapsids. (Upper left) The structures of occlusion bodies from baculoviruses in the genera *Alphabaculovirus* (nucleopolyhedrovirus, NPV) and *Betabaculovirus* (granulovirus, GV) are illustrated. Virions embedded in nucleopolyhedrovirus occlusion bodies may contain multiple nucleocapsids (MNPV) or single nucleocapsids (SNPV). (Upper right) The two baculovirus virion phenotypes are illustrated as diagrams with shared and phenotype-specific components (from Blissard, 1996). (Bottom) Transmission electron micrographs of occlusion bodies (MNPV, SNPV and GV), virion phenotypes BV (budded virions), ODV (occlusion-derived virions) and nucleocapsids (NC). Nucleopolyhedrovirus occlusion bodies of the MNPV (Autographa californica MNPV, top left) and SNPV (Trichoplusia ni SNPV, top middle) types are compared to granulovirus occlusion bodies (Estigmine acrea GV, top right). Transmission electron micrographs of virions of the BV (Lymantria dispar MNPV, bottom left) and ODV (Autographa californica MNPV, bottom center) phenotypes are shown beside negatively stained nucleocapsids (Autographa californica MNPV, bottom right). (Electron micrographs courtesy of J.R. Adams [LdMNPV BV virion] and R. Granados [all others].)

### Lipids

Lipids are present in the envelopes of ODV and BV. Lipid composition differs between the two virion phenotypes.

### Carbohydrates

Carbohydrates are present as glycoproteins and glycolipids.

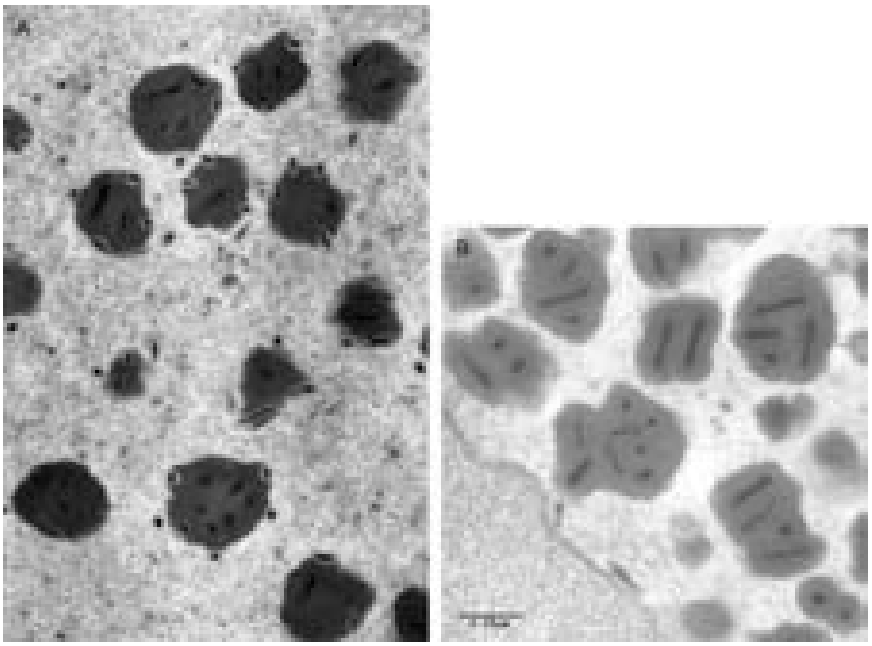

**Figure 2:** Transmission electron micrographs of occlusion bodies from the genera (A) *Gammabaculovirus* (Neodiprion abietis nucleopolyhedrovirus, courtesy of C.J. Lucarotti) and (B) *Deltabaculovirus* (Culex nigripalpus nucleopolyhedrovirus, courtesy of J.J. Becnel).

## Genome organization and replication

Circular genomic DNA is infectious, suggesting that after cellular entry and uncoating, no virion-associated proteins are not essential for infection. Genomes encode 100–200 proteins (Figure 3). Thirty gene homologs, the so-called baculovirus core genes, are shared among alpha-, beta-, gamma- and deltabaculoviruses. The alphabaculoviruses appear to share 60 homologs, comprising a core group of alphabaculovirus genes. These conserved genes are involved in various functions, including DNA replication, late gene transcription and virion structure. In some cases, larger genome sizes may result from the presence of families of repeated genes. Transcription of baculovirus genes is temporally regulated, and two main classes of genes are recognized: early and late. Late genes may be further subdivided as late and very late. Gene classes (early, late and very late) are not clustered on the baculovirus genome, and both strands of the genome are involved in coding functions. Early genes are transcribed by host RNA polymerase II, while late and very late genes are transcribed by an alpha-amanitin-resistant viral RNA polymerase. RNA splicing occurs, but appears to be rare since only two instances have been identified. Transient early and late gene transcription and DNA replication studies suggest that at least three virus encoded proteins regulate early gene transcription, while approximately 19 viral encoded proteins known as late expression factors (LEFs) are necessary for late gene transcription. Of the approximately 19 LEFs, half appear to be involved in DNA replication. Late gene transcription initiates within or near a highly conserved 5′-TAAG-3′ sequence, which appears to be an essential core element of the baculovirus late promoter. Putative replication origins consist of repeated sequences found at multiple locations within the baculovirus genome. These sequences, termed homologous repeat (*hr*) regions, do not appear to be highly conserved among different baculovirus species. Single copy, non-*hr* putative replication origins have also been identified. DNA replication is required for late gene transcription. Most virion structural proteins are encoded by late genes. While transcription of late and very late genes appears to begin immediately after DNA replication, some very late genes that encode occlusion body-specific proteins are transcribed at extremely high levels at a later time. BV production occurs primarily during the late phase, and occlusion body production occurs during the very late phase.

In infected animals, viral replication begins in the insect midgut. Following ingestion, occlusion bodies are solubilized in the gut lumen, releasing the ODVs, which are thought to enter the target

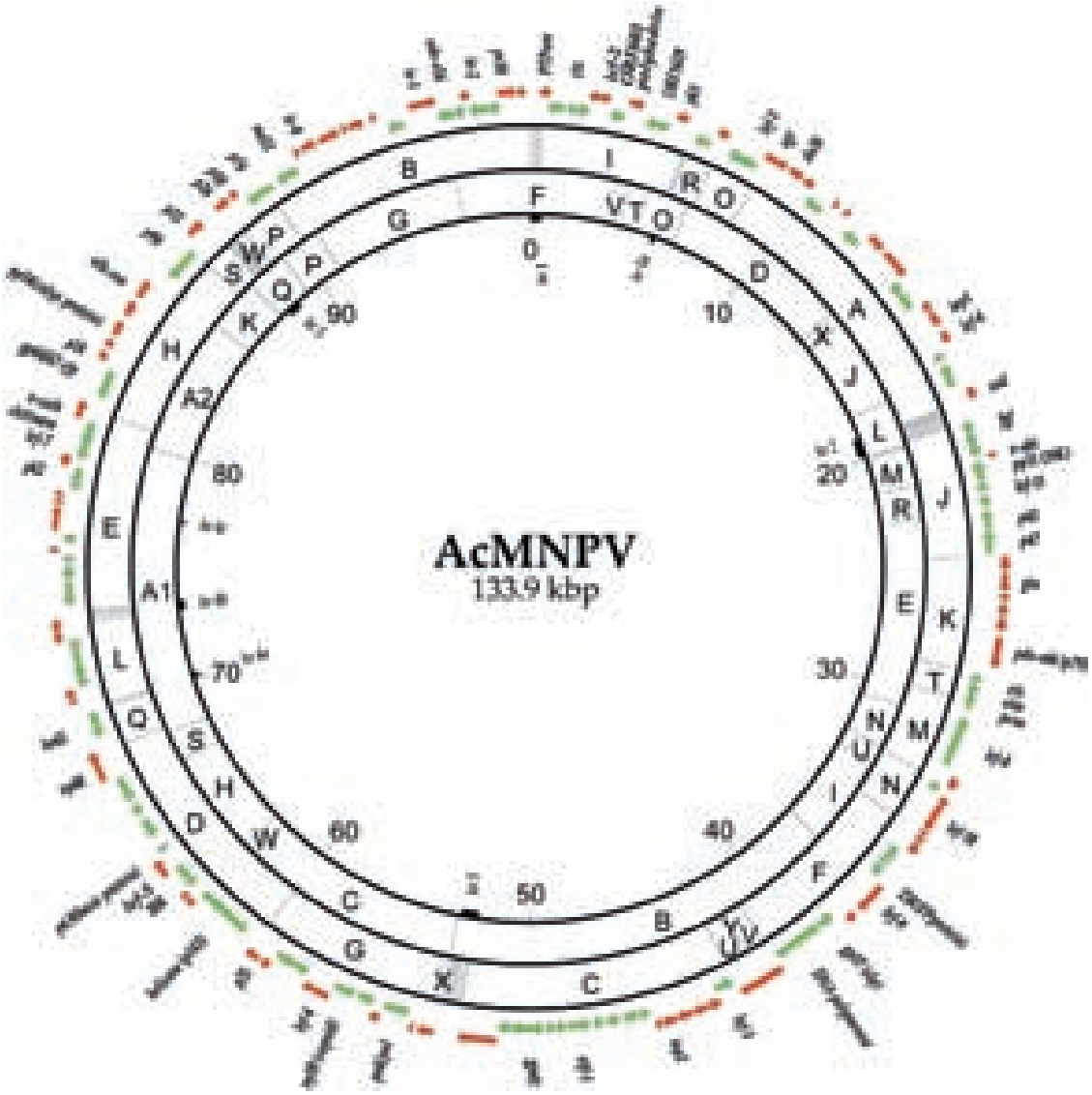


**Figure 3:** The covalently closed circular genome of Autographa californica multiple nucleopolyhedrovirus (AcMNPV) is illustrated with locations and orientations of known and predicted ORFs (arrows). Restriction maps for EcoRI and HindIII are indicated by letters on outer and inner rings, respectively. Locations of homologous repeat (*hr*) sequences are indicated on the inside of the circle as small filled boxes. Map units are indicated on the inside of the map (1 map unit = 1.339 kbp) (redrawn from Ayres *et al.* (1994). *Virology*, **202**, 586–605). (See Rohrmann, 2011 for details.)

epithelial cells via fusion with the plasma membrane at the cell surface. In lepidopteran insects, viral entry into midgut cells occurs in an alkaline environment, up to pH 12. Infection of the midgut is required for initiation of infection in the animal. In most cases, the virus is believed to undergo one round of replication in the midgut epithelium prior to transmission of infection to secondary tissues within the hemocoel. A mechanism for direct movement from the midgut to the hemocoel has also been proposed. DNA replication takes place in the nucleus. In betabaculovirus-infected cells, the integrity of the nuclear membrane is lost during the replication process. With some baculoviruses, replication is restricted to the gut epithelium and progeny virions become enveloped and occluded within these cells, and may be shed into the gut lumen with sloughed epithelium, or released upon death of the host. In other baculoviruses, the infection is transmitted to internal organs and tissues. The second virion phenotype, BV, which buds from the basolateral membrane of infected gut cells

is required for transmission of the infection into the hemocoel. In secondarily infected tissues, BV is produced during the late phase and ODV is produced during the very late phase of the infection. Infected fat body cells are the primary location of occluded virus production in lepidopteran insects. Occluded virus matures within nuclei of infected cells for alpha-, gamma- and deltabaculoviruses and within the nuclear-cytoplasmic milieu for betabaculoviruses. Occlusion bodies containing infectious ODV virions are released upon death, and usually liquefaction, of the host.

### Antigenic properties

Antigenic determinants that cross-react between different baculoviruses exist on virion proteins and on the major occlusion body polypeptide: polyhedrin or granulin. Neutralizing antibodies react with the major surface glycoprotein of BV.

### Biological properties

Baculoviruses have been isolated from insects only; primarily from insects of the order *Lepidoptera*, but also *Hymenoptera*, and *Diptera*. Transmission: naturally (i) horizontal transmission by contamination of food, egg surface, etc. with occlusion bodies; (ii) vertical transmission within the egg either from infected female or male adults; experimentally (iii) by injection of intact hosts with BV; (iv) by infection or transfection of cell cultures. Typically the infection process in a permissive insect host requires approximately one week, and as an end result, the diseased insect liquefies releasing infectious occlusion bodies into the environment. Occlusion bodies represent an environmentally stable form of the virus with increased resistance to chemical and physical decay as well as inactivation by UV light.

## Genus *Alphabaculovirus*

Type species ***Autographa californica multiple nucleopolyhedrovirus***

### Distinguishing features

Virions of the ODV phenotype are embedded within an occlusion body composed of a crystalline matrix of a single viral protein (polyhedrin). Each occlusion body measures 0.15 to 15 µm in size, matures within nuclei of infected cells and characteristically contains many enveloped virions. The occluded virions are packaged with either single (S) or multiple (M) nucleocapsids within a single viral envelope. Some virus species manifest both phenotypes. Factors that regulate nucleocapsid packaging are unknown and, for some species, packaging may be variable. S and M designations in common usage have been retained for species where variability has not been reported and for distinct viruses that would otherwise have identical designations under the current nomenclature. Nucleocapsids are rod-shaped (30–60 nm × 250–300 nm) and contain a single molecule of circular supercoiled dsDNA of 110–170 kbp in size. Nucleocapsid length appears to be proportional to genome size. During viral entry, nucleocapsids are believed to be transported through the nuclear membrane and into the nucleus, where uncoating and viral replication occur. Hosts include one order of insects, the Lepidoptera.

### Species demarcation criteria in the genus

Because detailed comparative data are lacking in most cases, species parameters are not well defined. However, species distinctions indicated here are broadly based on host range and specificity, DNA restriction profiles, DNA sequences from various regions of the genome, and predicted protein sequence similarities.

### List of species in the genus *Alphabaculovirus*

*Adoxophyes honmai nucleopolyhedrovirus*
Adoxophyes honmai nucleopolyhedrovirus (ADN001) [AP006270 = NC_004690] (AdhoNPV)
*Agrotis ipsilon multiple nucleopolyhedrovirus*
Agrotis ipsilon nucleopolyhedrovirus (Illinois) [EU839994 = NC_011345] (AgipNPV)

| | | |
|---|---|---|
| *Anticarsia gemmatalis multiple nucleopolyhedrovirus* | | |
| Anticarsia gemmatalis multiple nucleopolyhedrovirus (2D) | [DQ813662 = NC_008520] | (AgMNPV) |
| *Autographa californica multiple nucleopolyhedrovirus* | | |
| Autographa californica multiple nucleopolyhedrovirus (C6) | [L22858 = NC_001623] | (AcMNPV) |
| *Galleria mellonella multiple nucleopolyhedrovirus* | | (GmMNPV) |
| Plutella xylostella multiple nucleopolyhedrovirus (CL3) | [DQ457003 = NC_008349] | |
| *Spodoptera exempta multiple nucleopolyhedrovirus* | | (SpexNPV) |
| Trichoplusia ni multiple nucleopolyhedrovirus | | (TnMNPV) |
| *Bombyx mori nucleopolyhedrovirus* | | |
| Bombyx mori nucleopolyhedrovirus (T3) | [L33180 = NC_001962] | (BmNPV) |
| Bombyx mandarina nucleopolyhedrovirus (S1) | [FJ882854 = NC_012672] | (BomaNPV) |
| *Buzura suppressaria nucleopolyhedrovirus* | | |
| Buzura suppressaria nucleopolyhedrovirus (S13) | [U61154.1 = GI_2138112] | (BuzuNPV) |
| *Choristoneura fumiferana DEF multiple nucleopolyhedrovirus* | | |
| Choristoneura fumiferana DEF multiple nucleopolyhedrovirus | [AY327402 = NC_005137] | (CfDefNPV) |
| *Choristoneura fumiferana multiple nucleopolyhedrovirus* | | |
| Choristoneura fumiferana multiple nucleopolyhedrovirus (Ireland) | [AF512031 = NC_004778] | (CfMNPV) |
| *Choristoneura rosaceana nucleopolyhedrovirus* | | |
| Choristoneura rosaceana nucleopolyhedrovirus | | (ChroNPV) |
| *Ecotropis obliqua nucleopolyhedrovirus* | | |
| Ecotropis obliqua nucleopolyhedrovirus (A1) | [DQ837165 = NC_008586] | (EcobNPV) |
| *Epiphyas postvittana nucleopolyhedrovirus* | | |
| Epiphyas postvittana nucleopolyhedrovirus | [AY043265 = NC_003083] | (EppoNPV) |
| *Heliocoverpa armigera nucleopolyhedrovirus* | | |
| Heliocoverpa armigera nucleopolyhedrovirus (C1) | [AF303045 = NC_003094] | (HearNPV-C1) |
| Heliocoverpa armigera nucleopolyhedrovirus (NNG1) | [AP010907 = NC_011354] | (HearNPV-NNG1) |
| Heliocoverpa armigera nucleopolyhedrovirus (G4) | [AF271059 = NC_002654] | (HearNPV-G4) |
| *Helicoverpa zea single nucleopolyhedrovirus* | | |
| Helicoverpa zea single nucleopolyhedrovirus | [AF334030 = NC_003349] | (HzSNPV) |
| *Lymantria dispar multiple nucleopolyhedrovirus* | | |
| Lymantria dispar multiple nucleopolyhedrovirus | [AF081810 = NC_001973] | (LdMNPV) |
| *Mamestra brassicae multiple nucleopolyhedrovirus* | | |
| Mamestra brassicae multiple nucleopolyhedrovirus (Oxford) | | (MbMNPV) |
| *Mamestra configurata nucleopolyhedrovirus A* | | |
| Mamestra configurata nucleopolyhedrovirus A (90/2) | [U59461 = NC_003529] | (MacoNPV-A) |
| Mamestra configurata nucleopolyhedrovirus A (90/4) | [AF539999] | |
| *Mamestra configurata nucleopolyhedrovirus B* | | |
| Mamestra configurata nucleopolyhedrovirus B (96B) | [AY126275 = NC_004117] | (MacoNPV-B) |
| *Orgyia pseudotsugata multiple nucleopolyhedrovirus* | | |
| Orgyia pseudotsugata multiple nucleopolyhedrovirus | [U75930 = NC_001875] | (OpMNPV) |
| *Spodoptera exigua multiple nucleopolyhedrovirus* | | |
| Spodoptera exigua multiple nucleopolyhedrovirus (US) | [AF169823 = NC_002169] | (SeMNPV) |
| *Spodoptera frugiperda multiple nucleopolyhedrovirus* | | |
| Spodoptera frugiperda multiple nucleopolyhedrovirus (3AP2) | [EF035042 = NC_009011] | (SfMNPV) |
| *Spodoptera littoralis nucleopolyhedrovirus* | | |
| Spodoptera littoralis nucleopolyhedrovirus (M2) | | (SpliNPV) |
| *Spodoptera litura nucleopolyhedrovirus* | | |
| Spodoptera litura nucleopolyhedrovirus G2 | [AF325155 = NC_003102] | (SpltNPV) |
| *Thysanoplusia orichalcea nucleopolyhedrovirus* | | |
| Thysanoplusia orichalcea nucleopolyhedrovirus A28 | | (ThorNPV) |
| *Trichoplusia ni single nucleopolyhedrovirus* | | |
| Trichoplusia ni single nucleopolyhedrovirus | [DQ017380 = NC_007383] | (TnSNPV) |
| *Wiseana signata nucleopolyhedrovirus* | | |
| Wiseana signata nucleopolyhedrovirus | | (WisiNPV) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses, which may be members of the genus *Alphabaculovirus* but have not been approved as species

| | | |
|---|---|---|
| Adoxophyes orana nucleopolyhedrovirus | [EU591746 = NC_011423] | (AdorNPV) |
| Agrotis segetum nucleopolyhedrovirus A | [DQ123841 = NC_007921] | (AgseNPV) |
| Anagrapha falcifera multiple nucleopolyhedrovirus | | (AnfaNPV) |
| Antheraea pernyi nucleopolyhedrovirus | [DQ486030 = NC_008035] | (AnpeNPV) |
| Chrysodeixis chalcites nucleopolyhedrovirus | [AY864330 = NC_007151] | (ChchNPV) |
| Clanis bilineata nucleopolyhedrovirus | [DQ504428 = NC_008293] | (ClbiNPV) |
| Euproctis pseudoconspersa nucleopolyhedrovirus | [DQ837165 = NC_008586] | (EupsNPV) |
| Hyphantria cunea nucleopolyhedrovirus | [AP009046 = NC_007767] | (HycuNPV) |
| Leucania separata nucleopolyhedrovirus | [AY394490 = NC_008348] | (LeseNPV) |
| Maruca vitrata nucleopolyhedrovirus | [EF125867 = NC_008725] | (MaviNPV) |
| Orgyia leucostigma nucleopolyhedrovirus | [EU309041 = NC_010276] | (OrleNPV) |
| Orgyia pseudotsugata single nucleopolyhedrovirus | | (OpSNPV) |
| Panolis flammea nucleopolyhedrovirus | | (PaflNPV) |
| Rachiplusia ou multiple nucleopolyhedrovirus | [AY145471 = NC_004323] | (RoMNPV) |

## GENUS *BETABACULOVIRUS*

Type species ***Cydia pomonella granulovirus***

### Distinguishing features

Two virion phenotypes (BV and ODV) may be characteristic of a virus species. One (ODV) is occluded within an ovicylindrical occlusion body composed mainly of a single protein (granulin), which is a homolog (ortholog) to polyhedrin of alpha- and gammabaculoviruses. Each occlusion body measures approximately 0.13 × 0.50 µm in size and characteristically contains one virion. Each ODV virion typically contains a single nucleocapsid within a single envelope. Occluded virions may mature among nuclear-cytoplasmic cellular contents after loss of the nuclear membrane of infected cells. Nucleocapsids are rod-shaped (30–60 nm × 250–300 nm) and contain a single molecule of circular supercoiled dsDNA, approximately 110–180 kbp in size. Uncoating is thought to occur by a mechanism in which viral DNA is extruded into the nucleus through the nuclear pore while the capsid remains in the cytoplasm. Species of this genus have been isolated only from the insect order Lepidoptera.

### Species demarcation criteria in the genus

Refer to genus *Alphabaculovirus*.

### List of species in the genus *Betabaculovirus*

| | | |
|---|---|---|
| *Adoxophyes orana granulovirus* | | |
| Adoxophyes orana granulovirus | [AF547984 = NC_005038] | (AdorGV) |
| *Artogeia rapae granulovirus* | | |
| Artogeia rapae granulovirus | | (ArGV) |
| Pieris brassicae granulovirus (384) | | (PbGV) |
| *Choristoneura fumiferana granulovirus* | | |
| Choristoneura fumiferana granulovirus (Bonaventure) | | (ChfuGV) |
| *Cryptophlebia leucotreta granulovirus* | | |
| Cryptophlebia leucotreta granulovirus (CV3) | [AY229987 = NC_005068] | (CrleGV) |
| *Cydia pomonella granulovirus* | | |
| Cydia pomonella granulovirus (M1) | [U53466 = NC_002816] | (CpGV) |
| *Harrisina brillians granulovirus* | | |
| Harrisina brillians granulovirus (M2) | | (HabrGV) |
| *Helicoverpa armigera granulovirus* | | |

| | | |
|---|---|---|
| Helicoverpa armigera granulovirus | [EU255577 = NC_010240] | (HearGV) |
| *Lacanobia oleracea granulovirus* | | |
| Lacanobia oleracea granulovirus (S1) | | (LaolGV) |
| *Phthorimaea operculella granulovirus* | | |
| Phthorimaea operculella granulovirus | [AF499596 = NC_004062] | (PhopGV) |
| *Plodia interpunctella granulovirus* | | |
| Plodia interpunctella granulovirus (B3) | | (PiGV) |
| *Plutella xylostella granulovirus* | | |
| Plutella xylostella granulovirus (K1) | [AF270937 = NC_002593] | (PlxyGV) |
| *Pseudalatia unipuncta granulovirus* | | |
| Pseudalatia unipuncta granulovirus (Hawaiian) | [EU678671 = NC_013772] | (PsunGV) |
| *Trichoplusia ni granulovirus* | | |
| Trichoplusia ni granulovirus (M10-5) | | (TnGV) |
| *Xestia c-nigrum granulovirus* | | |
| Xestia c-nigrum granulovirus (alpha4) | [AF162221 = NC_002331] | (XecnGV) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses, which may be members of the genus *Betabaculovirus* but have not been approved as species

| | | |
|---|---|---|
| Agrotis segetum granulovirus (Xinjiang) | [AY522332 = NC_005839] | (AgseGV) |
| Choristoneura occidentalis granulovirus | [DQ333351 = NC_008168] | (ChocGV) |
| Spodoptera litura granulovirus (K1) | [DQ288858 = NC_009503] | (SpliGV) |

## GENUS *GAMMABACULOVIRUS*

Type species ***Neodiprion lecontei nucleopolyhedrovirus***

### Distinguishing features

The virions are occluded singly into the viral occlusion bodies. The virus is restricted to the host midgut and causes what was previously described in the literature as "infectious diarrhea". Genome sequencing analyses from three viruses (NeleNPV, NeseNPV, NeabNPV) revealed that these viruses do not encode typical envelope fusion proteins found in other baculoviruses. This raised the question of whether the budded virus phenotype plays a role in *Gammabaculovirus* biology. Also, in comparison to other baculoviruses, the genomes of members of the genus *Gammabaculovirus* are relatively high in A+T content (on the order of 67%). The genomes of the three sequenced gammabaculoviruses are collinear except for a large non-syntenic region between the DNA polymerase and polyhedrin genes. This region contains genes and ORFs not shared among the three characterized genomes.

### Species demarcation criteria in the genus

Refer to genus *Alphabaculovirus*.

### List of species in the genus *Gammabaculovirus*

| | | |
|---|---|---|
| *Neodiprion lecontei nucleopolyhedrovirus* | | |
| Neodiprion lecontei nucleopolyhedrovirus | [AY349019 = NC_005906] | (NeleNPV) |
| *Neodiprion sertifer nucleopolyhedrovirus* | | |
| Neodiprion sertifer nucleopolyhedrovirus | [AY430810 = NC_005905] | (NeseNPV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses, which may be members of the genus *Gammabaculovirus* but have not been approved as species

| | | |
|---|---|---|
| Gilpinia hercyniae nucleopolyhedrovirus (i7) | | (GiheNPV) |
| Neodiprion abietis nucleopolyhedrovirus | [DQ317692 = NC_008252] | (NeabNPV) |

# Genus *Deltabaculovirus*

Type species ***Culex nigripalpus nucleopolyhedrovirus***

## Distinguishing features

Replication of Culex nigripalpus nucleopolyhedrovirus (CuniNPV) is restricted to host midgut epithelium, primarily in larval stages but rarely in adults. Two virion phenotypes may be characteristic of a virus species. Virions of the ODV phenotype are embedded within an occlusion body composed of a crystalline matrix of a single viral protein with no homology to polyhedrin or granulin of other baculovirus genera. Each occlusion body ranges in size from 0.5 to 15 µm and contains few (1–4) or many (50+) singly enveloped virions depending on the strain of virus, lacks the polyhedron envelope of other baculoviruses and matures within nuclei of infected cells. Nucleocapsids are rod-shaped (30–60 nm × 200–250 nm) and contain a single molecule of circular supercoiled dsDNA. Transmission of CuniNPV to larval mosquitoes is strongly influenced by divalent cations: $Mg^{2+}$ is a potent enhancer of transmission while $Ca^{2+}$ is a strong inhibitor. The CuniNPV genome is 108,252 bp and encodes at least 109 putative proteins, some of which have sequence homology with those from other baculoviruses. Homologous proteins are involved in early and late gene expression, DNA replication, as well as structural and auxiliary functions. Gene orientation and order in the genome of CuniNPV is different from other baculovirus genera. The CuniNPV genome lacks genes with homologs for several essential and stimulatory genes for DNA replication and transcription found in other baculovirus genera and also lacks homologs for other conserved structural genes involved in the formation of nucleocapsids and occlusion bodies. Only 36 of the 109 putative CuniNPV predicted proteins demonstrate clear homology to proteins from other baculoviruses, and 72 of the CuniNPV ORFs show no homology to any other known baculovirus ORFs. Hosts include at least three genera of mosquitoes but other mosquito genera and families of *Diptera* are likely hosts.

## Species demarcation criteria in the genus

Refer to genus *Alphabaculovirus*.

## List of species in the genus *Deltabaculovirus*

*Culex nigripalpus nucleopolyhedrovirus*
Culex nigripalpus nucleopolyhedrovirus (Florida 1997) [AF403738 = NC_003084] (CuniNPV)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses, which may be members of the genus *Deltabaculovirus* but have not been approved as species

Aedes sollicitans nucleopolyhedrovirus (AesoNPV)
Uranotaenia sapphrinia nucleopolyhedrovirus (UrsaNPV)

## List of unassigned species in the family

None reported.

## Phylogenetic relationships within the family

Phylogenetic analysis based on the 30 baculovirus core genes shows that the family comprises four monophyletic groups (Figure 3), which can also be discriminated based on the insect orders of their hosts and on their morphology. Thus the new family structure classifies baculoviruses into four genera *Alphabaculovirus*, *Betabaculovirus*, *Gammabaculovirus*, *Deltabaculovirus*.

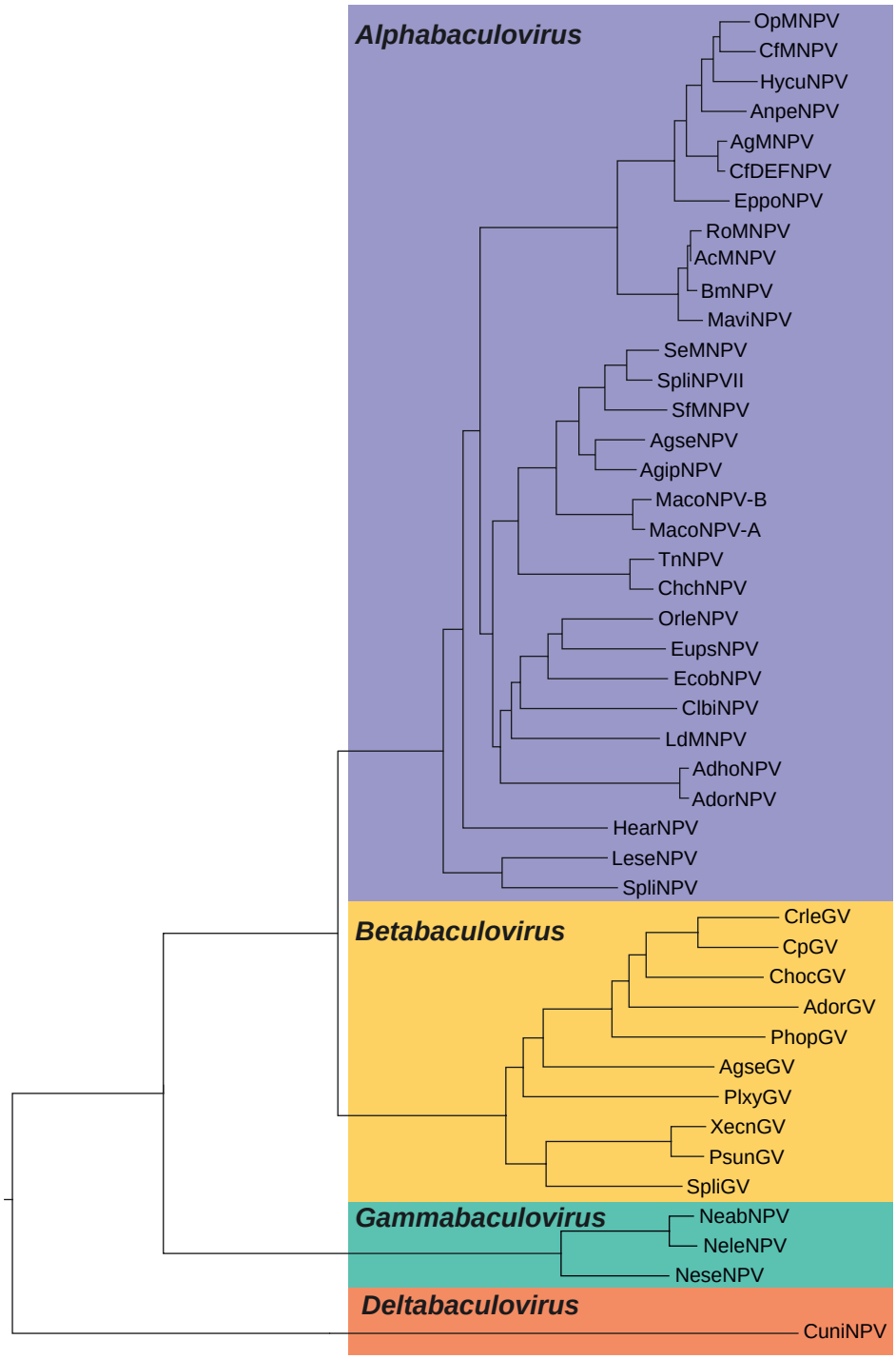


**Figure 4:** Phylogeny of the *Baculoviridae*. The maximum likelihood tree, based on the alignment of 30 genes, shows the relationships of the 39 species for which a completely annotated genome was available at time of analyses. Abbreviations are defined in lists of species and related viruses above.

## Similarity with other taxa

Members of the family *Baculoviridae* share structural and biological characters with the unassigned Nudiviruses, which formerly were called "non-occluded" baculoviruses. The nudiviruses share at least 20 core genes with baculoviruses. Baculoviruses also share at least 10 core genes with members of the genus *Bracovirus*, family *Polydnaviridae*.

## Derivation of names

*Baculo*: from *baculum*, meaning "rod", referring to the morphology of the nucleocapsid.
*Granulo*: from "granule", referring to the relatively small size and granular appearance of GV occlusion bodies in infected cells.
*Polyhedro*: from "polyhedron", referring to the multifaceted shape of occlusion bodies.

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Rohrmann, G.F. (2011). *Baculovirus Molecular Biology* (2nd edn.). Bethesda, MD: National Library of Medicine, NCBI.
van Oers, M.M. and Vlak, J.M. (2007). Baculovirus genomics. *Curr. Drug Targets*, **8**, 1051–1068.

## Contributed by

Herniou, E.A., Arif, B.M., Becnel, J.J., Blissard, G.W., Bonning, B., Harrison, R., Jehle, J.A., Theilmann, D.A. and Vlak, J.M.

# Family *Bicaudaviridae*

## Taxonomic structure of the family

Family *Bicaudaviridae*
Genus *Bicaudavirus*

Since only one genus is currently recognized, the family description corresponds to the genus description.



# Genus *Bicaudavirus*

Type species *Acidianus two-tailed virus*

## Virion properties

### Morphology

Virions are released from host cells as lemon-shaped particles, about 120 × 80 nm, and thereafter develop long tails, one at each of two pointed ends (Figure 1). The tails are heterogeneous in length, reaching 400 nm. They have a tube-like structure with a wall about 6 nm thick. The tube ends in a narrow channel 2 nm in width and a terminal anchor-like structure formed by two furled filaments, each with a width of 4 nm.

### Physicochemical and physical properties

Extracellular morphological development of the virion takes place specifically at temperatures above 75 °C, close to that of the natural habitat, and it does not require the presence of the host cells, an exogenous energy source and any co-factors. The average volume of the tailless virion is about 1.4 $nm^3$, and that of the two-tailed virion 6.2 $nm^3$. Conversely, the total surface areas for the two types of virions are similar, about 6 $\times 10^3$ $nm^2$. The mechanism of tail development is unknown.

### Nucleic acid

Virions contain one molecule of circular dsDNA of 62,730 bp, with a GC content of 41.2%.

### Proteins

Protein patterns of tailless and two-tailed virions are identical. The virions carry 11 major structural proteins (90, 80, 70, 60, 48, 45, 38, 22, 16, 15 and 12 kDa), five of which are modified at their N-termini. Several of the larger proteins are rich in coiled coil and/or low complexity sequence domains. The 80 kDa protein appears to be modified in two-tailed but not in tail-less virions.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

The genome encodes 72 predicted proteins and carries four putative transposable elements, which constitute 8% of the genome (Figure 2). Forty-three genes are estimated to produce leader-less transcripts and 35 genes are organized in 12 putative operons. Several examples of gene duplication and duplication within the gene occur. Putative integrase, AAA-ATPase and an acyltransferase are encoded in the viral genome. No information is available on genome replication.

## Antigenic properties

No information available.

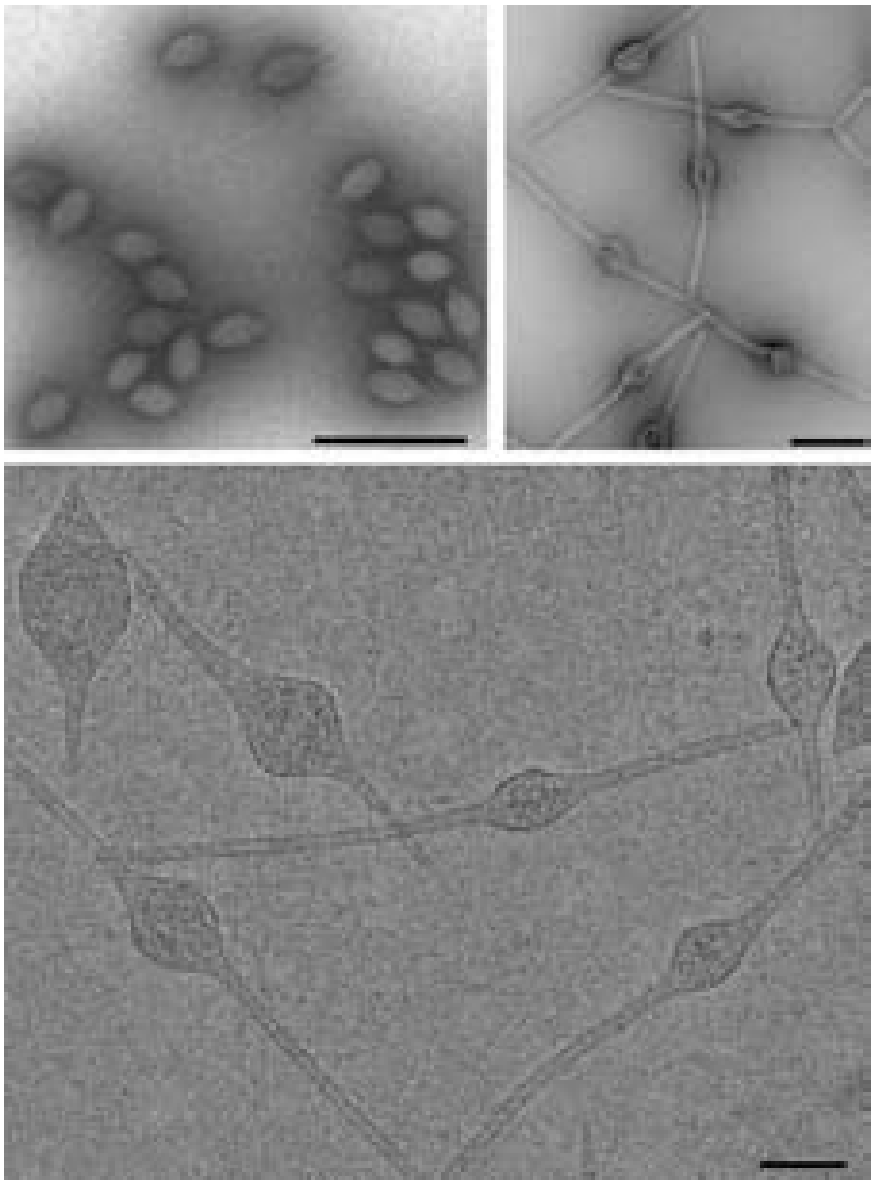

**Figure 1:** Electron micrographs of different forms of virions of an isolate of *Acidianus two-tailed virus*. (Top, left) Negative contrast electron micrograph of virions in tailless form. (Top, right) Negative contrast electron micrographs of virions in two-tailed form. (Bottom) Cryo-electron micrograph of two-tailed virions at different stages of extracellular tail development. The bars represent 100 nm (A,B) and 200 nm (C). (From Prangishvili, D. *et al.* (2006). *J. Mol. Biol.*, **359**, 1203–1216.)

## Biological properties

The virus was isolated from a hot acidic spring (87–93 °C, pH 1.5–2.0) in Pozzuoli, Italy. The host range is limited to autochthonous species of hyperthermophilic archaea from the genus *Acidianus* The infection leads either to viral replication and subsequent cell lysis or conversion of infected cell into a lysogene. The lysogenic cycle involves integration of the viral genome into the host chromosome, probably facilitated by a virus-encoded integrase. The lysogeny can be interrupted by stress factors, e.g. UV-irradiation, decrease of temperature.

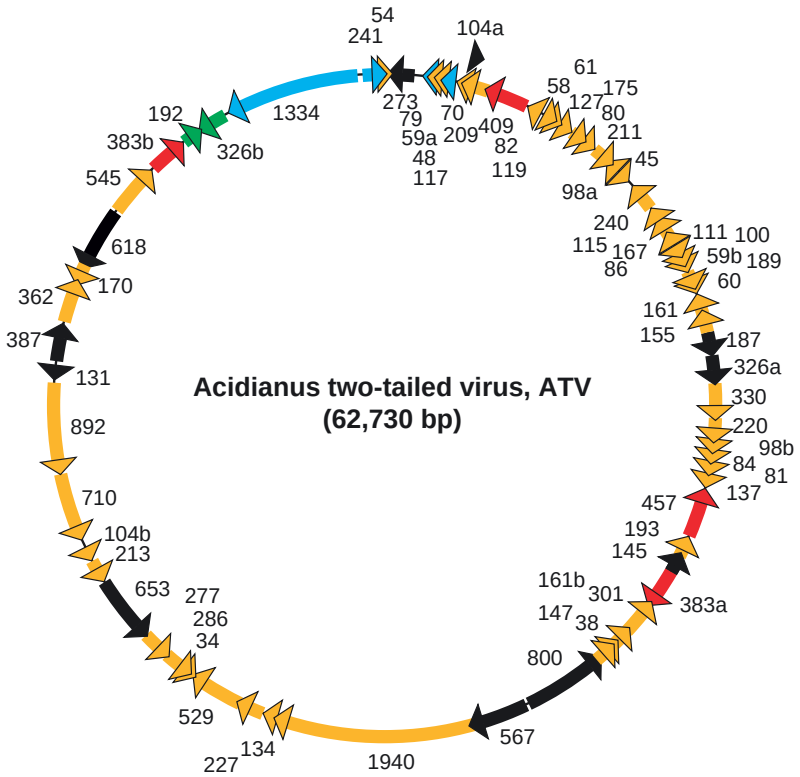


**Figure 2:** Genome organization of Acidianus two-tailed virus showing location, sizes and direction of the putative genes. Colour-coded ORFs correspond to: black, virion proteins; blue, homologous ORFs present in other archaeal hyperthermophilic viruses; green, homologous ORFs occurring in conjugative plasmids of the hyperthermophilic archaeon *Sulfolobus*; red, transposases. (Modified from Prangishvili, D. *et al.* (2006). *J. Mol. Biol.*, **359**, 1203–1216.)

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Bicaudavirus*

*Acidianus two-tailed virus*
Acidianus two-tailed virus [AJ888457] (ATV)

Species names are in italic script; names of strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Bicaudavirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Bicaudaviridae*

None reported.

## Phylogenetic relationships within the family

Not applicable.

## Similarity with other taxa

Not known.

## Derivation of name

*Bicauda*: from Latin *bi*, "two", and *cauda*, "tail".

## Further reading

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Prangishvili, D., Garrett, R.A. and Koonin, E.V. (2006). Evolutionary genomics of archaeal viruses: Unique viral genomes in the third domain of life. *Virus Res.*, **117**, 52–67.

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## Contributed by

Prangishvili, D.

# Family *Corticoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Corticoviridae*** |
| Genus | ***Corticovirus*** |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Corticovirus*

Type species ***Pseudoalteromonas phage PM2***

## Distinguishing features

PM2 is a virulent virus infecting gram-negative *Pseudoalteromonas* species. The characteristic feature of the corticovirus is the highly supercoiled circular double stranded DNA genome. The virions are composed of an icosahedral protein capsid and an inner protein-rich membrane enclosing the genome.

## Virion properties

### Morphology

Icosahedral virions consist of an internal membrane and an outer protein capsid that has a diameter of 57 nm between facets (Figure 1). The capsid consists of 200 major capsid protein P2 trimers that are organized on a pseudo T = 21 lattice. Protein P2 is composed of two beta-barrels disposed normal to the capsid surface. The P2 trimers have pseudo-six-fold symmetry and the structure is stabilized by calcium ions. Spikes protrude about 8 nm from the capsid surface at the five-fold vertices. The spikes are homopentamers and formed of protein P1. P1 is composed of three beta-barrel domains arranged end to end. The distal C-terminal domains of P1 are used for receptor recognition. The N-termini of P1 form pentagonal assemblies at the vertices. The inner membrane (47 nm in diameter) contains host plasma membrane-derived phospholipids and virus-encoded proteins P3 to P10. Transmembrane proteins P3 and P6 are organized into a lattice on the membrane vesicle surface, on which the outer protein capsid is assembled.

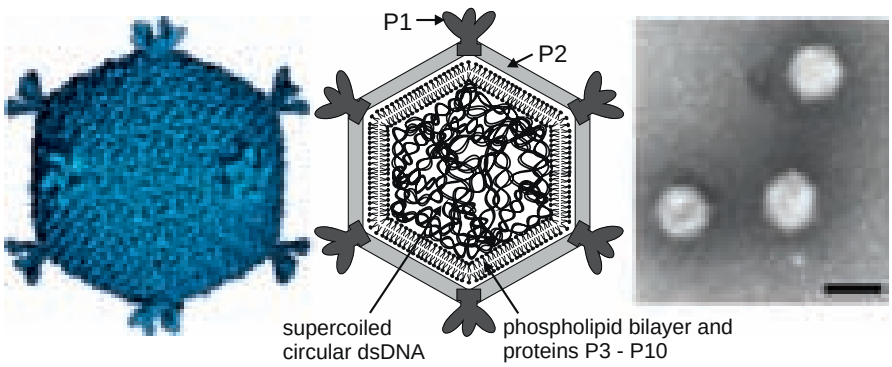


**Figure 1:** (Left) X-ray crystallographic structure of a virion of Pseudoalteromonas phage PM2 at 7 Å resolution, viewed along two-fold axis of symmetry (courtesy of N.G.A. Abrescia). (Middle) A schematic presentation and (right) negative stain electron micrograph of Pseudoalteromonas phage PM2 particles. The bar represents 50 nm.

### Physicochemical and physical properties

The mass of the virion is about $45 \times 10^6$. The buoyant density in CsCl is $1.28\,g\,cm^{-3}$ and in sucrose $1.26\,g\,cm^{-3}$, and the $S_{20,w}$ is 293S. Virions are stable at pH 6–8, and are very sensitive to ether, chloroform and detergents. The virion stability is strongly dependent on sodium and calcium ions. Virions are unstable when frozen.

### Nucleic acid

The genome is a highly supercoiled circular dsDNA of 10,079 bp (Mr of $6.6 \times 10^6$). DNA comprises about 14% of the virion weight and the G + C content is 42.2%. The phage PM2 genome has been sequenced (AF155037).

### Proteins

The genome has 21 putative genes, 17 of which have been shown to code for structural proteins (P1–P10) and nonstructural proteins (P12–P18; Table 1).

### Lipids

Particles are about 14% lipid by weight. The membrane contains about 34% phosphatidyl ethanolamine and about 66% phosphatidyl glycerol and trace amounts of phosphatidic acid and acyl-phosphatidyl glycerol. The lipids are derived from the host plasma membrane, but their composition deviates from that of the host bacterium. Lipids form an internal membrane with virus-specific integral membrane proteins.

### Carbohydrates

None reported.

## Genome organization and replication

To infect and replicate, PM2 delivers its genome across the cell envelope of two known marine host strains: gram-negative *Pseudoalteromonas* species ER72M2 and BAL-31. Virions adsorb via the distal tips of the spike proteins to uncharacterized receptors. The internal membrane mediates

**Table 1:** Pseudoalteromonas phage PM2 proteins

| Protein[a] | Mass (kDa) | Location/function[b] |
|---|---|---|
| P1 | 37.5 | Spike protein, receptor binding |
| P2 | 30.2 | Major capsid protein |
| P3 | 10.8 | Major membrane protein |
| P4 | 4.4 | Membrane |
| P5 | 17.9 | Membrane |
| P6 | 14.3 | Major membrane protein |
| P7 | 3.6 | Membrane |
| P8 | 7.3 | Membrane |
| P9 | 24.7 | Putative packaging ATPase |
| P10 | 29.0 | Membrane |
| P12 | 73.4 | Replication initiation protein (N) |
| P13 | 7.2 | Transcription factor (N) |
| P14 | 11.0 | Transcription factor (N) |
| P15 | 18.1 | Regulative function (N) |
| P16 | 10.3 | Regulative function (N) |
| P17 | 6.0 | Lysis factor (N) |
| P18 | 5.7 | Lysis factor (N) |

[a]P is for protein; Arabic numeral corresponds to the Roman numeral of the gene.
[b]N is for non-structural protein.

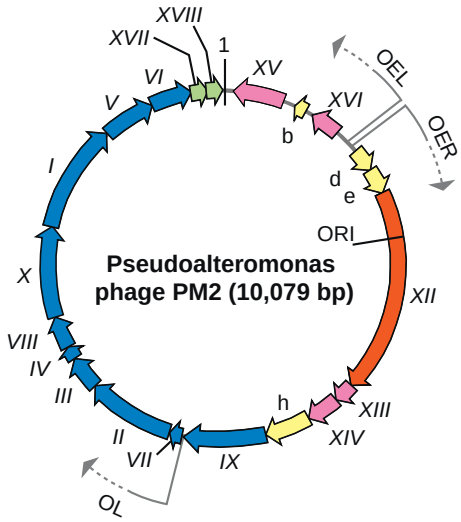


**Figure 2:** Genome organization of Pseudoalteromonas phage PM2 (PM2). The genome is a 10,079 bp highly supercoiled circular dsDNA molecule containing 21 ORFs (as arrows showing their orientations) and three operons (OER, OEL and OL). ORFs shown to code for functional proteins are classified as genes and given a Roman numeral. The different colors indicate the ORFs (yellow), a gene for replication initiation protein (orange) and the following groups of genes: transcriptional regulation (magenta), structural proteins (blue) and lysis (green).

the translocation of the supercoiled genome across the host cell envelopes via fusion. Replication of the PM2 genome, most probably by a rolling circle mechanism, takes place in proximity to the host cytoplasmic membrane. The genome is organized in three operons (Figure 2). Operons OEL and OER encode early function gene products: the replication initiation protein P12 and transcription regulatory proteins P13, P14, P15 and P16. Expression of the genes for structural proteins is under the control of the late promoter (OL), which is activated by the phage-encoded transcription factors P13 and P14. The release of mature virions from the cell occurs by a novel lysis mechanism.

## Antigenic properties

Not known.

## Biological properties

Phages are virulent and replicate in two known strains of marine host bacteria of the genus *Pseudoalteromonas*. Although PM2 is virulent and the sole member of the family *Corticoviridae*, using a comparative genomic approach, integrated corticoviral genetic elements have been identified to commonly reside within aquatic bacterial chromosomes.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Corticovirus*

*Pseudoalteromonas phage PM2*
Pseudoalteromonas phage PM2 [AF155037] (PM2)

Species names are in italic script; names of strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Corticovirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Corticoviridae*

None reported.

## Phylogenetic relationships within the family

Not applicable.

## Similarity with other taxa

Corticoviruses resemble tectiviruses in having a lipid bilayer underneath the isometric protein capsid. These viruses appear to differ by the genome organization and the infection mechanism, since no tectivirus-specific tail-like membrane tube is seen upon corticoviral infection. PM2 capsid architecture and capsid protein fold (trimeric protein with two beta-barrels forming hexagonal capsomers) have also been described in bacteriophages PRD1 and Bam35 (family *Tectiviridae*), the archaeal Sulfolobus turreted icosahedral virus (STIV; family not assigned), human adenovirus (family *Adenoviridae*), and in large eukaryotic viruses Paramecium bursaria Chlorella virus 1 (family *Phycodnaviridae*) and Chilo iridescent virus (family *Iridoviridae*).

## Derivation of name

*Cortico*: from Latin *cortex*, "crust", "bark".

## Further reading

Abrescia, N.G., Grimes, J.M., Kivelä, H.M, Assenberg, R., Sutton, G.C., Butcher, S.J., Bamford, J.K.H., Bamford, D.H. and Stuart, D.I. (2008). Insights into virus evolution and membrane biogenesis from the structure of the marine lipid-containing bacteriophage PM2. *Mol. Cell*, **31**, 749–761.

Bamford, J.K.H. and Bamford, D.H. (2006). Lipid-containing bacteriophage PM2, the type organism of *Corticoviridae*. In R. Calendar (Ed.), *The Bacteriophages* (pp. 171–174) (2nd edn.). New York: Oxford University Press.

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Männistö, R.H., Kivelä, H.M., Paulin, L., Bamford, D.H. and Bamford, J.K.H. (1999). The complete genome sequence of PM2, the first lipid-containing bacterial virus to be isolated. *Virology*, **262**, 355–363.

## Contributed by

Oksanen, H.M and Bamford, J.K.H.

# Family *Fuselloviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Fuselloviridae*** |
| Genus | ***Fusellovirus*** |

Since only one genus is currently recognized, the family description corresponds to the genus description.



# Genus *Fusellovirus*

Type species ***Sulfolobus spindle-shaped virus 1***

## Virion properties

### Morphology

Virions are lemon-shaped, with short tail fibers attached to one pole, and slightly heterogeneous in size. Virions are 55–60 nm in their short dimension and 80–100 nm in their long dimension. A small fraction of the Sulfolobus spindle-shaped virus 1 (SSV-1) population (up to 1%) is larger, with a particle length of about 300 nm. Some other fuselloviruses, particularly SSV-K1, have more elongated virions. Purified SSV-1 virions contain host lipids and three virus encoded proteins, two of which are associated with the coat and the other is DNA-associated.

### Physicochemical and physical properties

Virion buoyant density in CsCl is 1.24 g $cm^{-3}$. The particles are stable at up to 97 °C and are insensitive to urea, ether and pH 2. However, low pH (below 5) reduces viability due to degradation of the DNA, and virions are sensitive to pH above 11 and trichloromethane.

### Nucleic acid

Virions contain circular dsDNA, from 14.8 to 17.8 kbp. In SSV1 virions, DNA is positively supercoiled and associated with polyamines and a virus-coded basic protein.

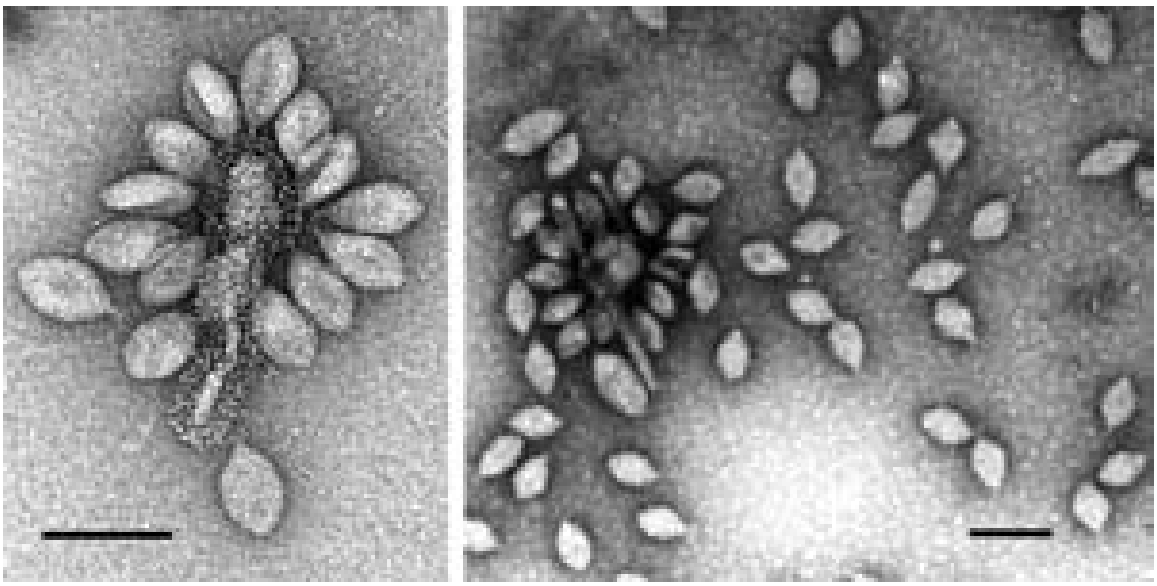

**Figure 1:** Negative contrast electron micrograph of Sulfolobus spindle-shaped virus 1 (SSV-1) virions, bound to a membrane vesicle (left) or isolated (right) (Stedman *et al.*, 1999). The bars represent 100 nm.

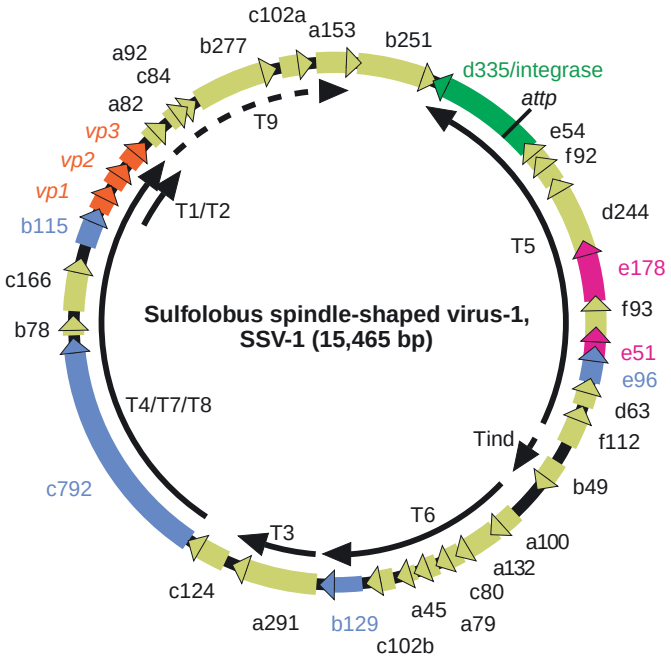


**Figure 2:** Genome organization of Sulfolobus spindle-shaped virus 1 (SSV-1). ORFs are shown as arrows and labeled with gene names. The major virion protein genes (VP1, VP3 and VP2) are labeled in red. The viral integrase gene, ORF d335, is labeled in green. ORFs shown to be essential for virus function are labeled in blue. ORFs shown to be non-essential for virus function are labeled in pink. The viral attachment site is labeled as *attP*. Transcripts are shown as solid arrows and labeled. Transcripts T1 and T2 start at the same promoter and overlap, and transcripts T4, T7 and T8 similarly overlap. The termination site of transcript T9 is not known.

### Proteins

The main constituents of the SSV-1 viral envelope are two basic proteins (VP1 and VP3). In SSV-1, these proteins are 73 and 92 aa, respectively, as deduced from the DNA sequence and from N-terminal protein sequencing. The VP1 protein is post-translationally proteolytically processed. In SSV-1, a very basic protein (VP2, 74 aa) is attached to the viral DNA. However, this gene is lacking in all other sequenced fusellovirus genomes. The genes encoding these structural proteins are closely linked in the fusellovirus genomes, in the order VP1, VP3 and VP2 in SSV-1 (Figure 2). The second largest ORF of SSV-1 (ORF d335, 335 aa) is similar to the integrase family of site-specific tyrosine recombinases. This protein has been expressed in *E. coli* and recombines DNA fragments sequence-specifically *in vitro*. The gene is conserved in all sequenced fuselloviruses. Four ORFs in the virus genome have been shown to be essential for virus function and two have been shown to be non-essential for virus function in SSV-1 (see Figure 2).

### Lipids

It has been reported that 10% of the SSV-1 virion envelope consists of host lipids.

### Carbohydrates

None reported.

## Genome organization and replication

The virus genome is present in cells as circular dsDNA and is site-specifically integrated into a tRNA gene of the host chromosome. In SSV-1 the integrated copy is flanked by a 44 bp direct repeat (attachment core) that occurs once in the SSV-1 circular DNA genome. Upon integration, the viral integrase gene is disrupted. Eleven somewhat overlapping transcripts originating from seven promoters have been detected and mapped. They almost completely cover the SSV-1 genome (Figure 2). UV-irradiation stimulates virus production and progeny virions are released without host cells lysis. A small transcript ($T_{ind}$) is strongly induced upon ultraviolet induction. Particles appear to be assembled and are produced by extrusion at the cell membrane.

## Antigenic properties

Not known.

## Biological properties

The host range of the fuselloviruses is limited to extremely thermophilic Archaea: *Sulfolobus shibatae*, *Sulfolobus solfataricus* strains P1 and P2 and *Sulfolobus islandicus* strains. Very few phage particles are produced in cultures of SSV-1 lysogens. UV-irradiation strongly induces SSV-1 production without evident lysis of the host. Fuselloviruses have been found in about 8% of *Sulfolobus* isolates from Icelandic solfataric fields. They have also been found in solfataric hot springs in Yellowstone National Park in the USA and on the Kamchatka peninsula in the Russian Federation. An infectious shuttle vector that also replicates in *E. coli* has been constructed.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Fusellovirus*

| | | |
|---|---|---|
| *Sulfolobus spindle-shaped virus 1* | | |
| Sulfolobus spindle-shaped virus 1 | | |
| Sulfolobus shibatae | [X07234] | (SSV-1-Ss) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Fusellovirus* but have not been approved as species

| | | |
|---|---|---|
| Sulfolobus spindle-shaped virus 2 | [AY370762] | (SSV-2) |
| Sulfolobus spindle-shaped virus 3 | | (SSV-3) |
| Sulfolobus spindle-shaped virus 4 | [EU030938] | (SSV-4) |
| Sulfolobus spindle-s haped virus 5 | [EU030939] | (SSV-5) |
| Sulfolobus spindle-shaped virus 6 | [FJ870915] | (SSV-6) |
| Sulfolobus spindle-shaped virus 7 | [FJ870916] | (SSV-7) |
| Sulfolobus spindle-shaped virus - Kamchatka 1 | [AY423772] | (SSV-K1) |
| Sulfolobus spindle-shaped virus - Yellowstone 1 | [AY388628] | (SSV-Y1) |
| Acidianus spindle-shaped virus 1 | [FJ870917] | (ASV-1) |

## List of unassigned species in the family *Fuselloviridae*

None reported.

## Phylogenetic relationships within the family

Phylogenetic relationships within the family *Fuselloviridae* are unclear. Different homologous genes give different phylogenetic trees.

## Similarity with other taxa

One ORF in fusellovirus genomes is similar to a gene present in the Sulfolobus viruses of the families *Lipothrixviridae* and *Rudiviridae*. The virus of extreme halophiles, His1, originally suggested to be a fusellovirus, on further characterization has been found to be very dissimilar and a novel genus (*Salterprovirus*) has been established for this group of viruses.

## Derivation of name

*Fusello*: from the Latin *fusello*, "little spindle".

## Further reading

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Stedman, K.M., Schleper, C., Rumpf, E. and Zillig, W. (1999). Genetic requirements for the function of the archaeal virus SSV1 in Sulfolobus solfataricus: Construction and testing of viral shuttle vectors. *Genetics*, **152**, 1397–1406.

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## Contributed by

Prangishvili, D.

The author acknowledges the contribution to the Eighth ICTV Report of Stedman, K.M.

# Family *Globuloviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Globuloviridae* |
| Genus | *Globulovirus* |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Globulovirus*

Type species *Pyrobaculum spherical virus*

## Virion properties

### Morphology

Virions are spherical, 70–100 nm in diameter, with spherical protrusions, about 15 nm in diameter (Figure 1a). Virions carry a lipid-containing envelope. It encases a superhelical core, consisting of linear dsDNA and nucleoproteins (Figure 1b).

### Physicochemical and physical properties

Virion buoyant density in sucrose is about 1.3 g cm$^{-3}$. Virions are highly heat-stable. Prolonged exposure to oxygen does not affect the efficiency of infection of the strictly anaerobic hosts.

### Nucleic acid

Virion contains a single molecule of linear dsDNA, comprising 28,337 bp for Pyrobaculum spherical virus.

### Proteins

Virions contain a major structural protein with molecular mass of about 33 kDa and two minor proteins of about 16 and 20 kDa. About 80% of amino acids of the major structural protein are hydrophobic. There are 38–48 predicted proteins encoded on viral genomes, none of which shows any sequence similarity to proteins in extant databases.

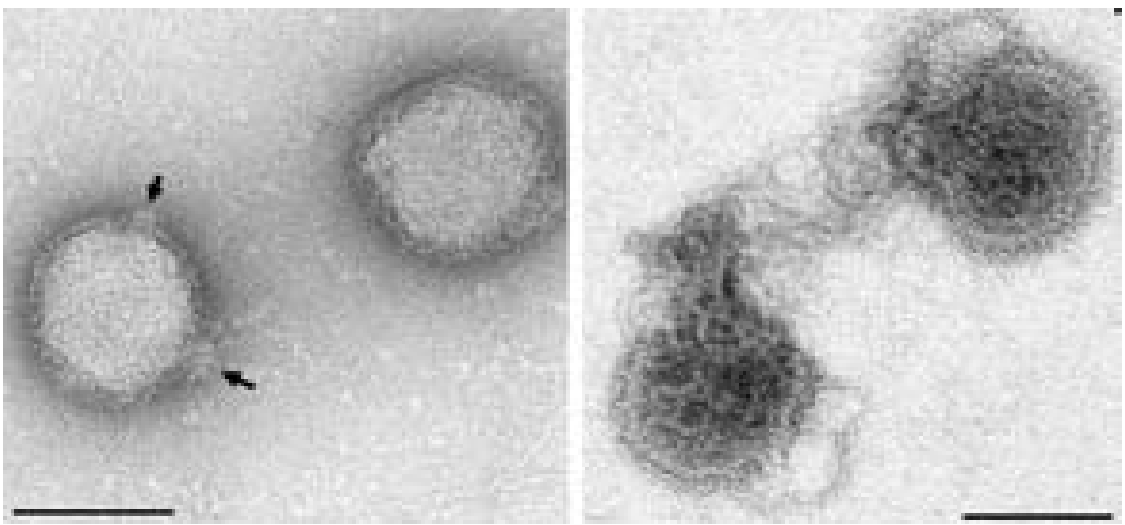

**Figure 1:** Negative contrast electron micrographs of virions of Pyrobaculum spherical virus. (Left) Intact virions; arrows indicate spherical protrusions. (Right) Partially disrupted virions extruding disordered nucleoprotein core. The bars represent 100 nm. (Modified from Häring *et al.* (2004). *Virology*, **323**, 232–242.)

**Pyrobaculum spherical virus, PSV (28,337 bp)**

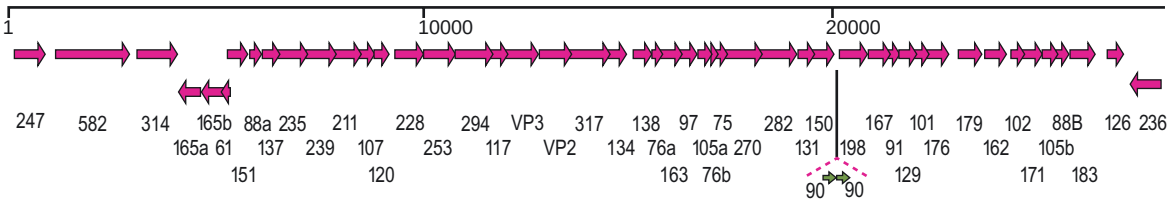


**Figure 2:** Genome organization of Pyrobaculum spherical virus showing location, sizes and direction of putative genes. *VP2* and *VP3* encode the minor and major structural proteins of the virion respectively. (Modified from Häring *et al.* (2004). *Virology*, **323**, 232–242.)

### Lipids
Virion contains modified host lipids.

### Carbohydrates
None reported.

## Genome organization and replication
The genome is linear dsDNA (Figure 2). The ends of the linear genome carry inverted repeat sequences (190 bp long for Pyrobaculum spherical virus), which contain multiple copies of short direct repeats. Almost all recognizable genes are located on one DNA strand. Several examples of gene duplication occur. No information is available about genome replication.

## Antigenic properties
Not known.

## Biological properties
The hosts are members of the hyperthermophilic archaeal genera *Pyrobaculum* and *Thermoproteus*. They are strict aerobes thriving in extreme geothermal environments with temperatures around 85 °C, pH 6. The infection does not cause lysis of host cells and is noncytocidal. The viral genome does not integrate into the host chromosome.

## Species demarcation criteria in the genus
Species in the genus differ in virion size, host range, size and nucleotide sequence of the genome.

## List of species in the genus *Globulovirus*

| | | |
|---|---|---|
| *Pyrobaculum spherical virus* | | |
| Pyrobaculum spherical virus | [AJ635161] | (PSV) |
| *Thermoproteus tenax spherical virus 1* | | |
| Thermoproteus tenax spherical virus 1 | [AY722806] | (TTSV1) |

Species names are in italic script; names of strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Globulovirus* but have not been approved as species
None reported.

## List of unassigned species in the family *Globuloviridae*

None reported.

## Phylogenetic relationships within the family

Not applicable.

## Similarity with other taxa

Not known.

## Derivation of name

*Globulo*: from Latin *globulus*, "small ball".

## Further reading

Ahn, D.-G., Kim, S.-I., Rhee, J.-K., Kim, K.P., Pan, J.-G. and Oh, J.-W. (2006). TTSV1, a new virus-like particle isolated from the hyperthermophilic crenarchaeote *Thermoproteus tenax*. *Virology*, **351**, 280–290.

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## Contributed by

Prangishvili, D.

# Family *Guttaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Guttaviridae*** |
| Genus | ***Guttavirus*** |

Since only one genus is currently recognized, the family description corresponds to the genus description.



# Genus *Guttavirus*

Type species ***Sulfolobus newzealandicus droplet-shaped virus***

## Virion properties

### Morphology

Sulfolobus newzealandicus droplet-shaped virus (SNDV) virions are somewhat pleiomorphic, with a droplet shape 75–90 × 110–185 nm with a beard of dense filaments at the pointed end.

### Physicochemical and physical properties

Virions are unstable in CsCl and lyse.

### Nucleic acid

The genome is covalently-closed circular dsDNA about 20 kbp. The genome cannot be cut by many restriction endonucelases, but can be cut by the *dam*-methylation dependent restriction endonuclease *Dpn*I, indicating that it is extensively methylated by a *dam*-like methylase.

### Proteins

By SDS-PAGE analysis, there is one major virion protein, 17.5 kDa, and two minor virion proteins of 13.5 and 13 kDa.

### Lipids

Not known.

### Carbohydrates

Not known.

## Genome organization and replication

Not known.

## Antigenic properties

Not known.

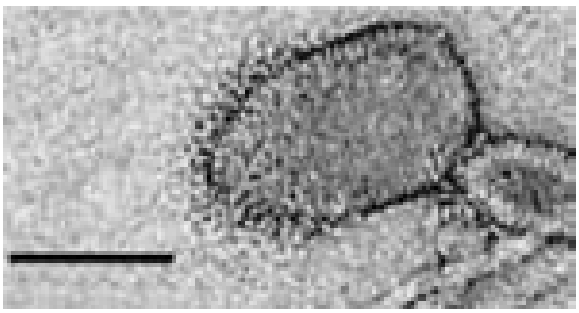

**Figure 1:** Negative contrast electron microscopy of particles of Sulfolobus newzealandicus droplet-shaped virus (SNDV). The bar represents 100 nm. (Courtesy of W. Zillig.)

## Biological properties

SNDV exclusively infects *Sulfolobus* isolates from New Zealand, including the strain STH1/3. Virus production starts in the early stationary phase.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Guttavirus*

*Sulfolobus newzealandicus droplet-shaped virus*
Sulfolobus newzealandicus droplet-shaped virus-NZ (SNDV-NZ)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Guttavirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Guttaviridae*

None reported.

## Phylogenetic relationships within the family

Not applicable.

## Similarity with other taxa

Not known.

## Derivation of name

*Gutta*: from Latin *gutta*, "droplet".

## Further reading

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## Contributed by

In reproducing this chapter from the Eighth ICTV Report, the editors gratefully acknowledge the contribution of the author, Stedman, K.M.

# FAMILY *IRIDOVIRIDAE*



## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Iridoviridae*** |
| Genus | ***Iridovirus*** |
| Genus | ***Chloriridovirus*** |
| Genus | ***Ranavirus*** |
| Genus | ***Lymphocystivirus*** |
| Genus | ***Megalocytivirus*** |

## Virion properties

### MORPHOLOGY

Iridovirus particles consist, from inside out, of an inner DNA/protein core, an internal limiting membrane, a viral capsid, and, in the case of those particles that bud from the plasma membrane, an outer viral envelope (Figure 1). Virions display icosahedral symmetry and are usually 120–200 nm in diameter, but may be up to 350 nm (e.g. genus *Lymphocystivirus*). The vitrified virion of invertebrate iridescent virus 6 (IIV-6) has diameters of 162, 165 and 185 nm along the two-, three- and five-fold axes of symmetry, respectively (Figure 2A, B). The virion core is an electron-dense entity consisting of a nucleoprotein filament surrounded by a lipid membrane containing transmembrane proteins of unknown function (Figure 1; Figure 2). Each particle is formed of 12 pentasymmetrons and 20 trisymmetrons arranged in an icosahedral, quasi-equivalent symmetry with triangulation number T = 147 ($h = 7$, $k = 7$). Both types of structure predominantly comprise hexavalent capsomers, a total of 1460 per virion, that are composed of the major capsid protein (MCP). Each hexavalent capsomer is formed by a non-covalent MCP trimer on the outer surface and a second MCP trimer linked by disulfide bonds on the inner surface. Each MCP monomer contains two beta-barrel domains of the viral jelly-roll type. The 55 capsomers in a trisymmetron are uniformly packed with an intercapsomer distance of 7.5 nm and in a single shared orientation, which is rotated by about 60° compared to the capsomers of the neighbouring trisymmetron. In addition, each pentasymmetron comprises 30 hexavalent (trimeric) capsomers and a single pentavalent capsomer at its centre, at the vertex of each pentasymmetron, for a total of 12 in each virion. The pentavalent capsomer is significantly larger than the trimeric capsomers and has a five-bladed propeller-shaped external appearance and a small central pore that opens into a flask-shaped cavity (Figure 2D, E, F).

A number of additional proteins have recently been identified in the capsid shell and in association with the lipid membrane (Figure 3). These have been named zip monomers, zip dimers, finger proteins and anchor proteins. The zip dimers appear as two halves of a clasp connecting the trimer capsomers along the edges of adjacent trisymmetrons, whereas zip monomers appear to be involved in linking trisymmetron capsomers with those of neighbouring pentasymmetrons. Three sets of nine inward-pointing finger proteins bind the capsomers along the edges of each trisymmetron. Finally, an anchor protein connects each pentasymmetron with the lipid membrane at a distance of two capsomers from the pentavalent vertex (Figure 2G; Figure 3). Other transmembrane proteins are present, but only the anchor protein can be visualized in image reconstruction studies due to its invariable position with respect to the symmetry of the particle. Volume estimates using the MCP as a reference suggest molecular masses of 11.9, 19.7 and 32.4 kDa for the zip, finger and anchor proteins, respectively.

The outer surface of the capsid is covered by flexible fibrils or fibres. Conventional EM studies have suggested that these fibrils are often rather short (ca. 2.5 nm in length) and may have terminal knobs, but in IIV-6 a single fibril of 2 nm width and about 35 nm in length extends outwards from the centre of each trimeric capsomer (Figure 2F; Figure 3). The role of the fibrils remains unknown.

Iridovirids (a generic term describing all members of the family) may acquire an envelope by budding through the host cell membrane. The envelope increases the specific infectivity of virions, but is not required for infectivity as naked particles are also infectious.

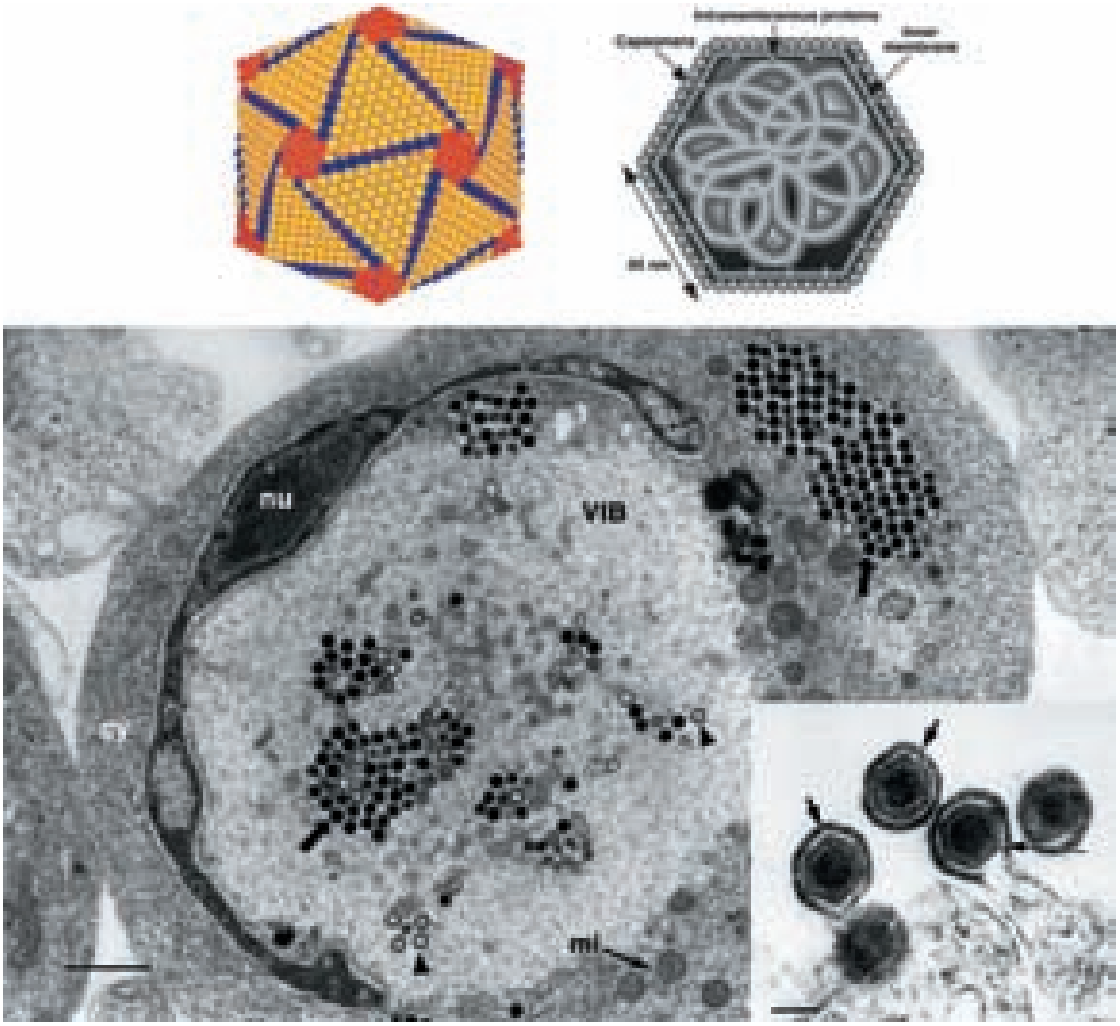


**Figure 1:** (Top left) Outer shell of invertebrate iridescent virus 2 (IIV-2) (from Wrigley *et al.* (1969). *J. Gen. Virol.*, **5**, 123; with permission). (Top right) Schematic diagram of a cross-section of an iridovirus particle, showing capsomers, transmembrane proteins within the lipid bilayer, and an internal filamentous nucleoprotein core (from Darcy-Tripier *et al.* (1984). *Virology*, **138**, 287; with permission). (Bottom left) Transmission electron micrograph of a fat head minnow cell infected with an isolate of *European catfish virus*. Nucleus (nu); virus inclusion body (VIB); paracrystalline array of non-enveloped virus particles (arrows); incomplete nucleocapsids (arrowheads); cytoplasm (cy); mitochondrion (mi); the bar represents 1 µm (from Hyatt *et al.* (2000). *Arch. Virol.*, **145**, 301; with permission). (insert) Transmission electron micrograph of particles of frog virus 3 (FV-3), budding from the plasma membrane. Arrows and arrowheads identify the viral envelope; the bar represents 200 nm (from Devauchelle *et al.* (1985). *Curr. Topics Microbiol. Immunol.*, **116**, 1; with permission.)

### Physicochemical and physical properties

The Mr of virions is 1.05–2.75 $\times 10^9$, their sedimentation coefficient ($S_{20,w}$) is 2020–4460S, and their density is 1.26–1.60 g cm$^{-3}$. Virions are stable in water at 4 °C for extended periods. Sensitivity to pH varies, whereas sensitivity to ether and chloroform depends on the assay system employed. All viruses are inactivated within 30 min at >55 °C. Frog virus 3 (FV-3), infectious spleen and kidney necrosis virus (ISKNV), invertebrate iridescent virus 6 (IIV-6) and likely other members of the family are inactivated by UV-irradiation. Some ranaviruses remain infectious after desiccation, e.g., Bohle iridovirus (BIV) survives desiccation at temperatures up to 42 °C for up to 6 weeks, whereas others are sensitive to drying.

### Nucleic acid

The virion core contains a single linear dsDNA molecule of 140–303 kbp, a value that includes both unique and terminally redundant sequences. However, when considering only the non-redundant

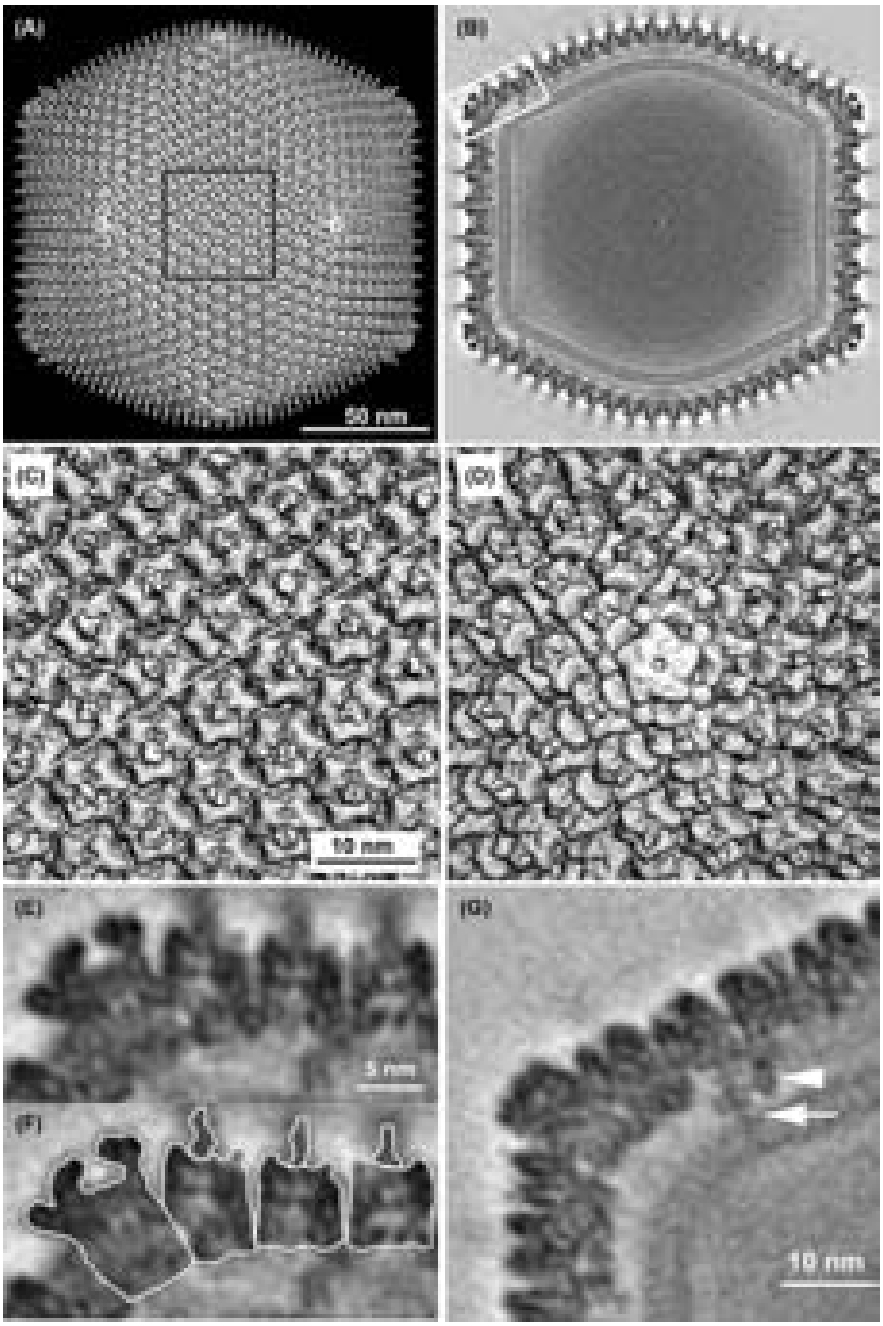


**Figure 2:** Three-dimensional reconstruction of an IIV-6 particle at a resolution of 1.3 nm. (A) 3-D density map of particle viewed along an icosahedral two-fold axis. The external fringe of filaments has been blurred away due to their varying position in relation to the symmetry of the capsid. (B) Central cross-section, one pixel thick. A lipid bilayer follows the inner contour and icosahedral symmetry of the capsid shell whereas the core appears to lack any structures that are arranged following icosahedral symmetry. (C) Magnified view of the central region outlined in (A). The diagonal white line indicates the cleavage plane between adjacent trisymmetrons. (D) Magnified view of the propeller-like pentamer complex at the five-fold vertex. This complex differs from the trimeric capsomers in that it is larger, has a small axial hole, and lacks an external fibre. (E) Magnified view of boxed region in (B) showing transverse section of the pentamer complex and adjacent trisymmetrons. (F) Same as (E) but with the pentamer complex, three capsomers, and their fibres individually outlined. (G) Transmembrane anchor proteins beneath the pentasymmetrons showing two sticklike entities. The longer of the two (long arrow) crosses both leaflets of the bilayer, whereas the other (short arrow) stops at the outer leaflet of the internal lipid membrane. (From Yan *et al*. (2009). *J. Mol. Biol.*, **385**, 1287–1299; with permission.)

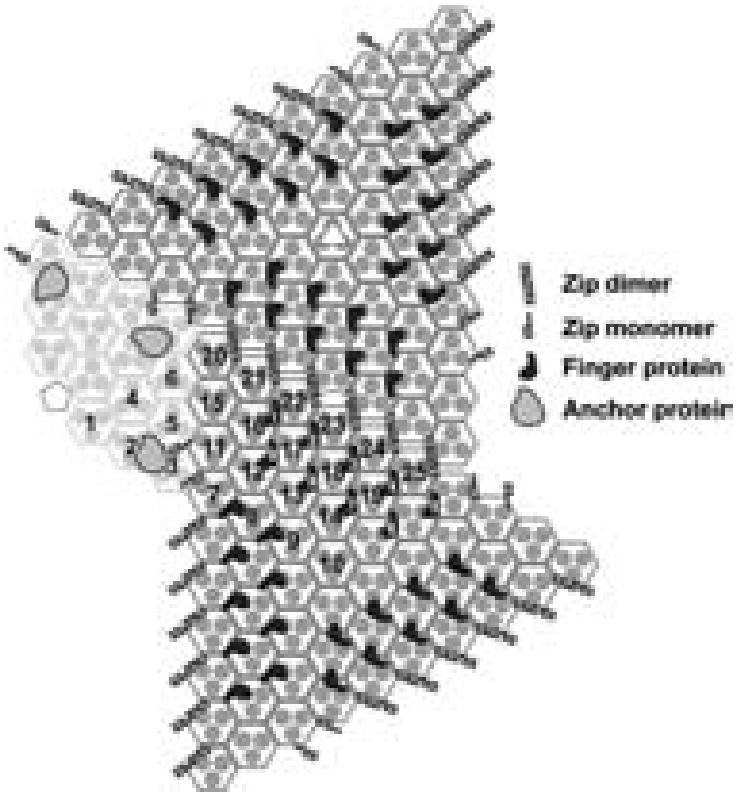


**Figure 3:** Schematic planar diagram of the IIV-6 capsid structure with the central pentamer complex (white pentagon) omitted for clarity. The centrally located white ellipse, triangle and pentagon symbols highlight the positions of 2-, 3-, and 5-fold icosahedral axes, respectively. All major capsid protein trimers are shown as three small grey disks enclosed by a hexagon. The icosahedral asymmetric unit contains 24 and one-third of these capsomers (numbered 1–25). Each trisymmetron contains 55 capsomers, all oriented similarly and rotated by 60° relative to those in the adjacent trisymmetron. Finger proteins bind to each trisymmetron (nine within one asymmetric unit are numbered in white), a total of 27 in each trisymmetron. A total of 18 Zip dimers are present at the interface between one trisymmetron and its adjacent trisymmetrons. Zip monomers are located at the interface between a trisymmetron and its neighbouring pentasymmetrons. The transmembrane anchor proteins (shown in Figure 2G) are located under capsomers 2 and 3 beneath the pentasymmetron. (From Yan *et al*. (2009). *J. Mol. Biol.*, **385**, 1287–1299; with permission.)

portion, genome sizes range from 105 to 212 kbp (Table 1). An isolate of invertebrate iridescent virus 1 (IIV-1) has been reported to have an additional genetic component of 10.8 kbp which exists as a free molecule in the particle core. DNA comprises 12–16% of the particle weight, and the G + C content ranges from about 28 to about 55%. All viruses within the family possess genomes that are circularly permuted and terminally redundant. However, the DNA of vertebrate iridoviruses is highly methylated, whereas little to no methylation is found within the genomes of the invertebrate iridoviruses. The complete genomic sequence is known for 15 iridoviruses, with representative sequence information available from every genus in the family *Iridoviridae*. The iridoviruses whose genomes have been completely sequenced include IIV-6, IIV-3, lymphocystis disease virus 1 (LCDV-1), LCDV-China (LCDV-C), ISKNV, orange spotted grouper iridovirus (OSGIV), rock bream iridovirus (RBIV), red seabream iridovirus (RSIV), grouper iridovirus (GIV), Singapore-GIV (SGIV), tiger frog virus (TFV), frog virus 3 (FV-3), epizootic hematopoietic necrosis virus (EHNV), soft-shelled turtle iridovirus (STIV) and Ambystoma tigrinum virus (ATV). Although naked genomic DNA is not infectious, non-genetic reactivation of viral DNA can be achieved in the presence of viral structural proteins.

### Proteins

Iridoviruses are structurally complex, and up to 36 polypeptides, ranging from about 5 to 250 kDa, have been detected by two-dimensional PAGE of virus particles. Sequence analysis has identified

**Table 1:** Summary of completely sequenced iridovirid genomes

| Genus/Virus | Genome size (kb) | GC content (%) | Number of potential genes | Accession No. |
|---|---|---|---|---|
| ***Ranavirus*** | | | | |
| ATV | 106,332 | 54 | 92 | AY150217 |
| EHNV | 127,011 | 54 | 100 | FJ433873 |
| FV-3 | 105,903 | 55 | 97 | AY548484 |
| TFV | 105,057 | 55 | 103 | AF389451 |
| STIV | 105,890 | 55 | 105 | EU627010 |
| SGIV | 140,131 | 48 | 139 | AY521625 |
| GIV | 139,793 | 49 | 139 | AY666015 |
| ***Megalocytivirus*** | | | | |
| ISKNV | 111,362 | 55 | 117 | AF371960 |
| OSGIV | 112,636 | 54 | 116 | AY894343 |
| RBIV | 112,080 | 53 | 116 | AY532606 |
| RSIV | 112,415 | 53 | 116 | BD143114 |
| ***Lymphocystivirus*** | | | | |
| LCDV-1 | 102,653 | 29 | 108 | L63545 |
| LCDV-C | 186,247 | 27 | 178 | AY380826 |
| ***Iridovirus*** | | | | |
| IIV-6 | 212,482 | 29 | 211 | AF303741 |
| ***Chloriridovirus*** | | | | |
| IIV-3 | 190,132 | 48 | 126 | DQ643392 |

For definitions of abbreviations, please refer to lists of species names, below.

more than 100 ORFs (Tables 1 and 2, Figure 4). The major capsid protein (MCP), 48–55 kDa, comprises 40% of the total virion protein, and its complete amino acid (aa) sequence is known for several viruses. The MCP is highly conserved and shares aa sequence identity with the MCPs of African swine fever virus (ASFV, family *Asfarviridae*), several members of the family *Ascoviridae*, and Paramecium bursaria Chlorella virus 1 (PBCV-1, family *Phycodnaviridae*). At least six DNA-associated polypeptides have been identified in the core of IIV-6, with a major species of 12.5 kDa. A virion-associated protein elicits the shutdown of host macromolecular synthesis, whereas other virion-associated proteins transactivate FV-3 early viral transcription. A number of virion-associated enzymatic activities have been detected, including a protein kinase, nucleotide phosphohydrolase, a ss/dsRNA-specific ribonuclease, pH 5 and pH 7.5 deoxyribonucleases, and a protein phosphatase. In addition to these polypeptides, various other proteins have been identified by BLAST analysis of recently sequenced viral genomes (Table 2; Figure 4).

### Lipids

Non-enveloped particles contain 5–17% lipid, predominantly as phospholipid. Virions possess an internal lipid membrane that lies between the DNA core and the viral capsid, and, in some virions, an outer viral envelope. The origin of the internal lipid membrane is unclear. On one hand, the composition of the internal lipid membrane suggests that this membrane is not derived from host membranes but is produced *de novo*. However, by analogy to African swine fever virus, it has been suggested that the internal lipid membrane is derived from fragments of the endoplasmic reticulum and plays a key role in virion assembly. Viruses released from cells by budding acquire their outer envelope from the plasma membrane.

### Carbohydrates

Carbohydrates are not present in purified virions.

**Table 2:** Partial listing of putative gene products encoded by viruses within the genera *Iridovirus*, *Chloriridovirus*, *Ranavirus*, *Lymphocystivirus* and *Megalocytivirus*

| Category | Gene product | | | | | | |
|---|---|---|---|---|---|---|---|
| | IIV-6 | IIV-3 | ATV | TFV | LCDV-1 | LCDV-C | ISKNV |
| **Enzymes associated with nucleic acid replication and metabolism** | | | | | | | |
| DNA polymerase | + | + | + | + | + | + | + |
| RNA polymerase II, α subunit | + | + | + | + | + | + | + |
| RNA polymerase II, β subunit | + | + | + | + | + | + | + |
| Transcription factor-like protein | + | + | + | + | + | + | |
| RAD-2, DNA repair enzyme | + | + | + | + | + | + | + |
| Cytosine DNA methyltransferase | | | + | + | + | + | + |
| Type II restriction enzyme *Msp* I of *Moraxella* sp. | | | | | + | | |
| RNAse III | + | + | + | + | + | + | + |
| Ribonucleotide reductase, large subunit | + | + | + | + | + | + | |
| Ribonucleotide reductase, small subunit | + | + | + | + | + | + | + |
| DUTPase | + | | + | + | | | |
| Thymidylate synthase | + | | + | + | | + | |
| Thymidine kinase | + | + | | | + | | |
| Thymidylate kinase | + | | + | | | | |
| Topoisomerase II-like protein | + | + | | | | | |
| Helicase | + | + | + | + | | + | |
| PCNA protein | + | + | + | + | + | + | + |
| DNA ligase | + | + | | | | | |
| **Additional enzymatic activities** | | | | | | | |
| Tyrosine phosphatase | + | | | | | | |
| Tyrosine kinase | + | + | + | + | + | + | + |
| Thiol oxidoreductase | + | + | + | + | + | + | + |
| Serine/threonine protein kinase | + | + | + | + | + | + | + |
| ATPase | + | | + | + | + | + | + |
| Matrix metalloproteinase | + | + | | | | | |
| mRNA capping enzyme | | | | | | | + |
| Cathepsin B-like protein | + | | | | + | | |
| Acetyl-coenyme A hydrolase | | | | | | + | |
| **Putative immune evasion proteins** | | | | | | | |
| TNF receptor-associated factor | | | | | | + | + |
| Growth factor/cytokine receptor family signature | + | | | | | | |
| TNFR/NGFR family proteins | | | | | + | | |
| PDGF/VEGF-like protein | | | | | | | + |
| Apoptosis inhibitor (IAP) of Cydia pomonellla granulosis virus, Bir repeat profile | + | | | | | | |
| IAP-like protein of African swine fever virus | + | | | | | | |
| CARD, caspase recruitment domain | | | + | | | + | |

**Table 2:** Partial listing of putative gene products encoded by viruses within the genera *Iridovirus*, *Chloriridovirus*, *Ranavirus*, *Lymphocystivirus* and *Megalocytivirus* (*Continued*)

| Category | Gene product | | | | | | |
|---|---|---|---|---|---|---|---|
| | IIV-6 | IIV-3 | ATV | TFV | LCDV-1 | LCDV-C | ISKNV |
| 3β-hydroxy-$\Delta^5$-$C_{27}$-steroid oxidoreductase | | | + | + | + | + | |
| eIF-2 α homolog | | | + | + | | | |
| Src homology domain, suppressor of cytokine signaling | | | | | | | + |
| **Structural proteins** | | | | | | | |
| Major capsid protein | + | + | + | + | + | + | + |
| Myristylated membrane protein | + | + | + | + | + | + | + |

The presence of a homolog of the indicated gene is indicated by a plus sign (+).

## Ambystoma tigrinum virus, ATV (106,332 bp)

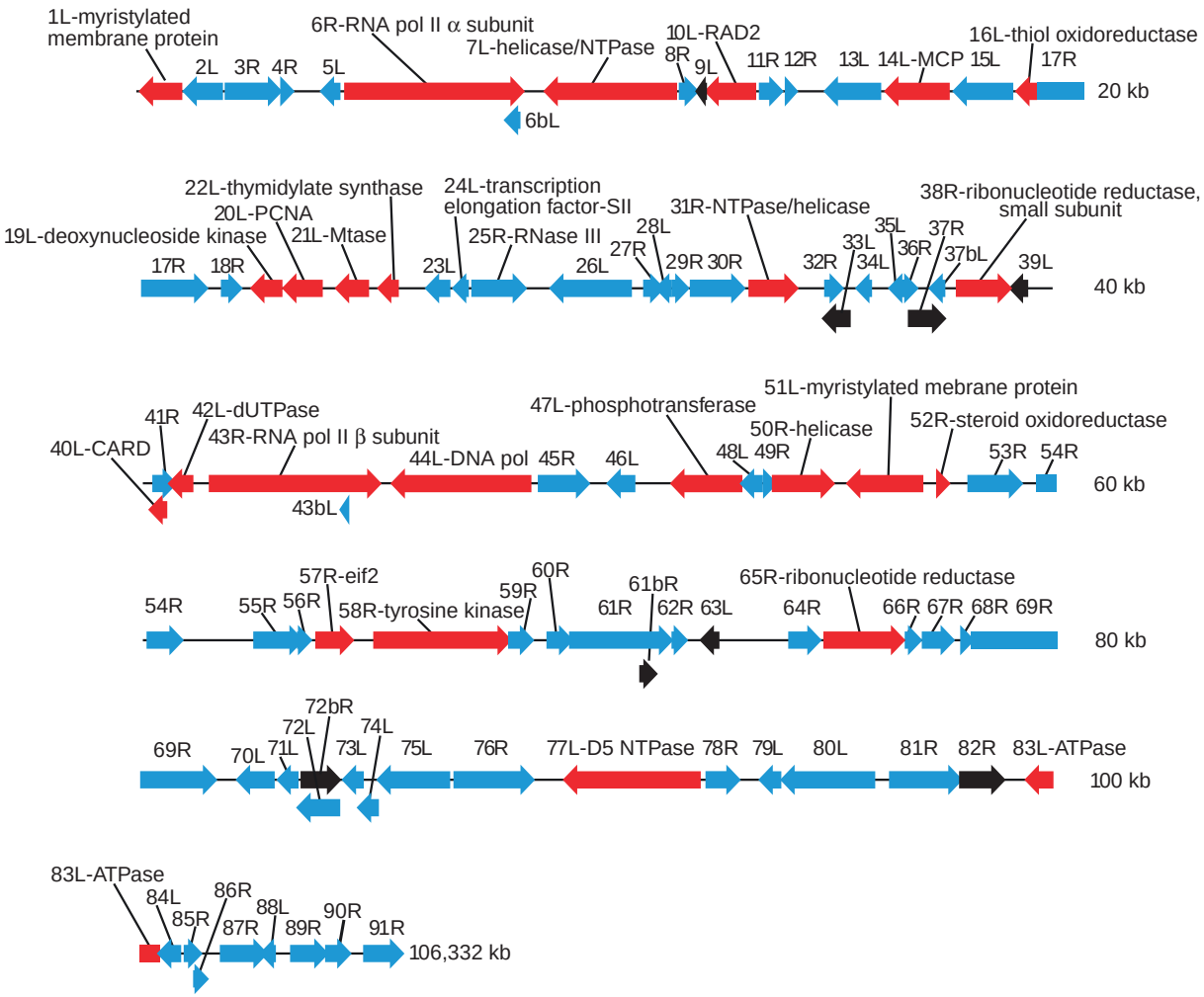


**Figure 4:** Genomic structure of Ambystoma tigrinum virus (ATV). Arrows represent viral ORFs with their size, position and orientation shown. ORFs of known function are colored in red and their putative proteins identified; ORFs with known homology to tiger frog virus (TFV) are in blue; and those of unknown function or with no homology to TFV are indicated in black. (From Jancovich *et al.* (2003). *Virology*, **316**, 90–103; with permission.)

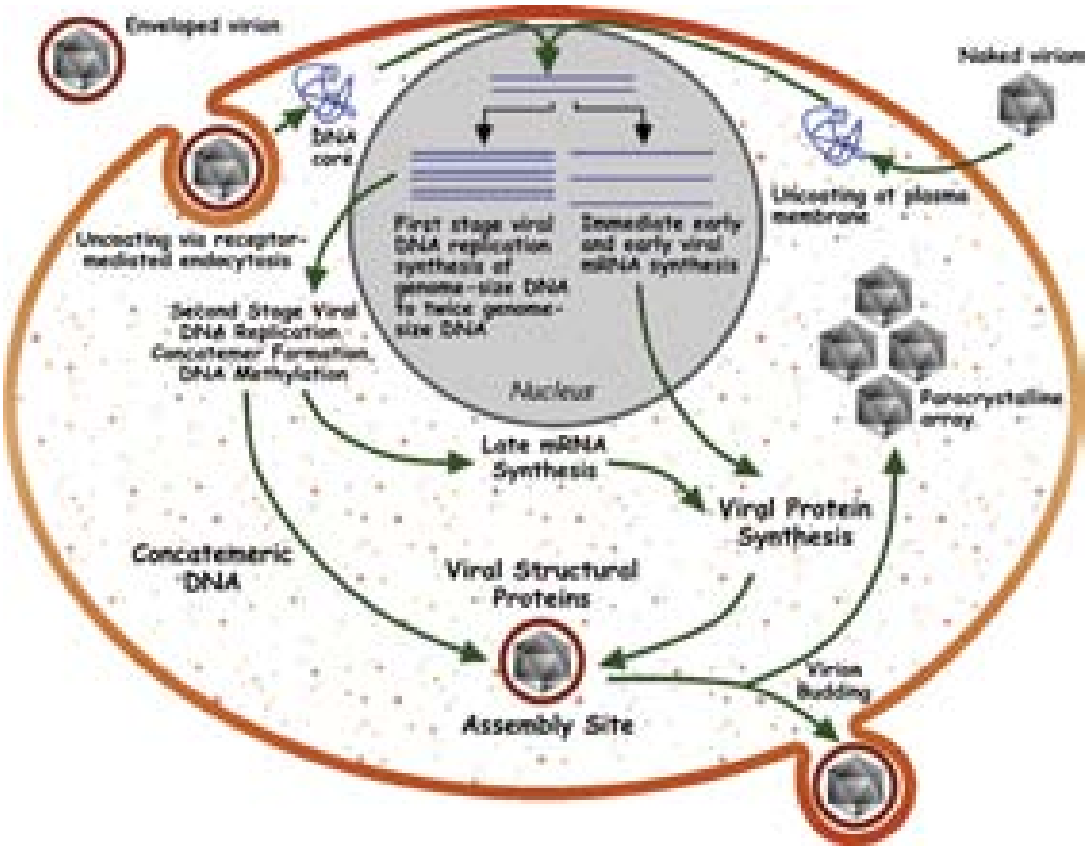


**Figure 5:** Replication cycle of frog virus 3 (FV-3). (From Chinchar *et al.*, (2002). *Arch. Virol.*, **147**, 447; with permission.)

## Genome organization and replication

The replication strategy of iridoviruses is novel and has been elucidated primarily through the study of FV-3, the type species of the genus *Ranavirus* (Figure 5). Virion entry occurs by either receptor-mediated endocytosis (enveloped particles) or by uncoating at the plasma membrane (naked virions). Following uncoating, viral cores enter the nucleus where first stage DNA synthesis and the synthesis of immediate early (IE) and delayed early (DE) viral transcripts takes place. In a poorly understood process, one or more virion associated proteins act as transactivators and re-direct host RNA polymerase II to synthesize IE and DE viral mRNAs using the methylated viral genome as template. Gene products encoded by IE and DE viral transcripts include both regulatory and catalytic proteins. One of these gene products, the viral DNA polymerase, catalyzes the first stage of viral DNA synthesis. In this process, the parental viral genome serves as the template and progeny DNA that is genome-length, to at most twice genome length, is produced. Newly-synthesized viral DNA may serve as the template for additional rounds of DNA replication or early transcription, or it may be transported to the cytoplasm where the second stage of viral DNA synthesis occurs. In the cytoplasm, viral DNA is synthesized into large, branched concatamers that serve as the template for DNA packaging.

Viral DNA methylation also likely occurs in the cytoplasm and, although its precise role is uncertain, it is thought to protect viral DNA from endonucleolytic attack. Synthesis of late (L) viral transcripts occurs in the cytoplasm and full L gene transcription requires prior DNA synthesis. Homologs of the two largest subunits of RNA polymerase II are encoded by all iridoviruses. Whether these function only in the cytoplasm to transcribe L viral transcripts, or whether they also play a role in continued early transcription has not yet been determined. Virion formation takes place in the cytoplasm within morphologically distinct virus assembly sites. Within assembly sites concatameric viral DNA is packaged into virions, it is believed, via a "headful" mechanism that results in the generation of circularly permuted and terminally redundant genomes similar to those

seen with the Enterobacteria phages T4 or P22. The degree of terminal redundancy varies from approximately 5 to 50%. Following assembly, virions accumulate in the cytoplasm within large paracrystalline arrays or acquire an envelope by budding from the plasma membrane. In the case of most vertebrate iridoviruses, the majority of virions remain cell-associated (Figure 5).

### Antigenic properties

The genera are serologically distinct from one another. In the genus *Iridovirus* there exists one main group of serologically interrelated species and others which have little sero-relatedness. Several amphibian isolates (e.g., Rana esculenta iridovirus, REIR) and piscine isolates (e.g. EHNV) show serological cross-reactivity with FV-3 (genus *Ranavirus*). Although antibodies prepared against virions are often non-neutralizing *in vitro*, studies with FV3-infected *Xenopus laevis* indicate a marked antibody response following secondary infection and the generation of protective neutralizing antibodies. Moreover, consistent with a protective antibody response, inactivated virus vaccines protect against disease mediated by RSIV.

### Biological properties

Iridoviruses have been isolated from only poikilothermic animals, usually associated with damp or aquatic environments, including marine habitats. Iridovirus species vary widely in their natural host range and in their virulence. Transmission mechanisms are poorly understood for the majority of these viruses. Invertebrate iridoviruses may be transmitted by cannibalism, endoparasitic wasps or parasitic nematodes. Viruses may be transmitted experimentally by injection or bath immersion, and naturally by co-habitation, feeding, or wounding. While many of these viruses cause serious, life-threatening infections, subclinical infections are common.

## Genus *Iridovirus*

Type species ***Invertebrate iridescent virus 6***

### Distinguishing features

Particle diameter is 120–130 nm in ultrathin section. IIV-1 and IIV-2 are assumed to contain 1472 capsomers arranged in 20 trimers and 12 pentamers. A detailed description of the morphology of IIV-6 is presented in the introductory section on virion morphology.

Virions have an Mr of approximately $1.28 \times 10^9$, a buoyant density of 1.30–1.33 g cm$^{-3}$, and a sedimentation coefficient $S_{20,w}$ of 2020–2250S. IIV-6 is sensitive to chloroform, SDS, sodium deoxycholate, ethanol, pH3 and pH11, but is not sensitive to trypsin, lipase, phospholipase A2 or EDTA. The sensitivity of IIV-6 to ether differs depending on whether an *in vivo* or *in vitro* assay is used to determine residual infectivity.

The unit length genome size of IIV-6 is 212,482 bp. The G+C content is typically 29–32%. Comparative genomic analysis of the IIV-6 and IIV-3 genomes shows no co-linearity between these two viral isolates.

Genetic studies have indicated the presence of discrete complexes of inter-related viruses within this genus: one large complex containing 10 viruses that may be candidates for new species, and two smaller complexes. Serological relationships follow a similar pattern.

Iridoviruses have been isolated from a wide range of arthropods, particularly insects in aquatic or damp habitats. Patently infected animals and purified viral pellets display violet, blue or turquoise iridescence. Non-apparent, non-lethal infections may be common in certain hosts. No evidence exists for transovarial transmission and where horizontal transmission has been demonstrated, it is usually by cannibalism or predation of infected invertebrate hosts. Following experimental injection, many members of the genus can replicate in a large number of insects. In nature, the host range appears to vary but there is evidence, for some viruses, of natural transmission across insect orders and even phyla. Invertebrate iridescent viruses have a global distribution.

## Species demarcation criteria in the genus

The following species-defining characteristics and associated limits are preliminary in nature. The following definitions assume that all material being compared has been grown under near identical conditions and prepared for examination following identical protocols. It is recommended that members of both recognized virus species be included in all characterization studies of novel isolates.

- Amino acid sequence analysis of the MCP: PCR primers have been designed for conserved regions of this gene. Although the complete IIV-6 genome has been determined and the sequence of a number of other proteins from different isolates has been ascertained, this information has not been used for species differentiation and quantitative limits of similarity have not been established.
- DNA-DNA dot-blot hybridization: Hybridization values should be less than 50% for members of distinct species.
- RFLP: Using a panel of not fewer than four restriction endonucleases (both rare and frequent cutters) distinct species should show completely distinct restriction endonuclease profiles.
- Serology: Antisera from members of strains within a species should exhibit high levels of cross reactivity. Within and among species, comparison by Western blot analysis using antibodies raised against disrupted virions is the preferred method. Comparisons should be performed simultaneously wherever possible and reference species should be included in each determination.

The MCP of IIV-1 shows 66.4% aa sequence identity to that of IIV-6 and approximately 50% or lower aa sequence identity to iridoviruses in other genera. Less than 1% DNA–DNA hybridization for genomic DNA was detected by dot-blot method between IIV-1 and IIV-6 (stringency: 26% mismatch). Restriction endonuclease profiles (*Hind*II, *Eco*RI, *Sal*I) showed a coefficient of similarity of <66% between IIV-1 and IIV-6. These species did not share common antigens when tested by tube precipitation, infectivity neutralization, reversed single radial immunodiffusion or enzyme-linked immunosorbent assay. Genome and protein size differences are not useful in differentiating these species. The size of the MCP is well conserved among species.

## List of species in the genus *Iridovirus*

| | | |
|---|---|---|
| *Invertebrate iridescent virus 1* | | |
| Invertebrate iridescent virus 1 | [M33542, M62953] | (IIV-1) |
| (Tipula iridescent virus) | | (TIV) |
| *Invertebrate iridescent virus 6* | | |
| Invertebrate iridescent virus 6 | [AF303741] | (IIV-6) |
| (Chilo iridescent virus) | | (CIV) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Iridovirus* but have not been approved as species

| | | |
|---|---|---|
| Anticarsia gemmatalis iridescent virus | [AF042343] | (AGIV) |
| Invertebrate iridescent virus 2 (Sericesthis iridescent virus) | [AF042335] | (IIV-2) |
| Invertebrate iridescent virus 9 (Wiseana iridescent virus) | [AF025774] | (IIV-9) |
| Invertebrate iridescent virus 16 (Costelytra zealandica iridescent virus) | [AF025775] | (IIV-16) |
| Invertebrate iridescent virus 21 (Heliothis armigera iridescent virus) | | (IIV-21) |
| Invertebrate iridescent virus 22 (Simulium sp. iridescent virus) | [AF042341; M32799] | (IIV-22) |
| Invertebrate iridescent virus 23 (Heteronychus arator iridescent virus) | [AF042342] | (IIV-23) |
| Invertebrate iridescent virus 24 (Apis iridescent virus) | [AF042340] | (IIV-24) |

| | | |
|---|---|---|
| Invertebrate iridescent virus 29 (Tenebrio molitor iridescent virus) | [AF042339] | (IIV-29) |
| Invertebrate iridescent virus 30 (Helicoverpa zea iridescent virus) | [AF042336] | (IIV-30) |
| Invertebrate iridescent virus 31 (Isopod iridescent virus) | [AF042337; AJ279821] | (IIV-31) |



# GENUS *CHLORIRIDOVIRUS*

Type species ***Invertebrate iridescent virus 3***

## Distinguishing features

Particle diameter is approximately 180 nm in ultrathin section. The trimers and pentamers of invertebrate iridescent virus 3 (IIV-3) are larger than the corresponding structures of the genus *Iridovirus*, with probably 14 capsomers to each edge of the trimer. Particle size has historically been used to define viruses that are members of this genus, but the validity of that characteristic is uncertain.

Virions have a Mr of approximately 2.49–2.75 $\times 10^9$, a buoyant density of approximately 1.354 g cm$^{-3}$ in CsCl, and a $S_{20,w}$ of 4440–4460S. Infectivity is believed not to be sensitive to ether.

The genome size of IIV-3 is 190,132 bp with a G+C content of 48%. Of the 126 predicted genes, 27 have homologs in other sequenced iridovirids. IIV-3 and IIV-6 share 52 predicted genes that are not found in vertebrate iridovirids, including DNA topoisomerase II, NAD-dependent DNA ligase, SF1 helicase, IAP and a BRO protein. Thirty-three IIV-3 genes lack homologs in other iridovirids. No colinearity has been observed between IIV-3 and the genome of any other iridovirid sequenced to date.

The low levels of amino acid identity of predicted proteins to iridovirid homologs and phylogenetic analyses of conserved proteins indicate that IIV-3 is only distantly related to other iridovirid genera.

IIV-3 is serologically distinct from members of other genera.

IIV-3 is the only virus characterized from this genus, although more than 20 host species were reported with latent infections world-wide. Chloriridovirus-like infections have only been reported from Diptera with aquatic larval stages, mainly mosquitoes. There is evidence for transovarial transmission in mosquitoes infected by IIV-3. Horizontal transmission is achieved by cannibalism or predation of infected mosquitoes of other species. Patently infected larvae and purified pellets of virus iridesce, usually with a yellow-green color, although orange and red infections are known. IIV-3 appears to have a narrow host range compared to most members of the genus *Iridovirus*.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Chloriridovirus*

| | | |
|---|---|---|
| *Invertebrate iridescent virus 3* | | |
| Invertebrate iridescent virus 3 (Mosquito iridescent virus) | [AJ312708] | (IIV-3) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Chloriridovirus* but have not been approved as species

None reported.

# Genus *Ranavirus*

Type species ***Frog virus 3***

## Distinguishing features

Ranaviruses infect one or more members of the classes Reptilia, Amphibia and Osteichthyes. With the exception of SGIV and GIV they encode a cytosine DNA methyltransferase.

Particle diameter is approximately 150 nm in ultrathin section. Enveloped virions, released by budding, measure 160–200 nm in diameter. The capsid has a skewed symmetry with T = 133 or 147. The internal lipid bilayer likely contains transmembrane proteins. The nucleoprotein core consists of a long coiled filament 10 nm wide.

Buoyant density is 1.28 g cm$^{-3}$ for enveloped particles and 1.32 g cm$^{-3}$ for nonenveloped particles. Infectivity is rapidly lost at pH 2.0–3.0 and at temperatures above 50 °C. Particles are inactivated by treatment with ether, chloroform, sodium deoxychlorate, and phospholipase A.

Virions contain a single linear dsDNA molecule. The genome is circularly permuted and approximately 30% terminally redundant. The unit genome size is approximately 105 kbp with a G+C content of about 54% (Table 1). Cytosines within the dinucleotide sequence CpG are methylated by a virus-encoded cytosine DNA methyltransferase. DNA methylation likely occurs in the cytoplasm and may be important in protecting DNA from viral endonucleases.

The replication cycle of FV-3 serves as the model for the family and has been discussed above (Figure 5). The complete genomes of seven ranaviruses (SGIV, GIV, EHNV, STIV, TFV, FV-3 and ATV) have been sequenced and, while possessing homologous proteins, they are not, for the most part, co-linear (Table 1, Figure 4). Based on whole genome dot plot comparisons there are three genomic phenotypes among the seven completely sequenced ranaviruses (FV3/TFV/STIV, ATV/EHNV, SGIV/GIV). Dot plot analyses shows that the FV3- and ATV-like viruses are much more closely related to each other than are either of these two types to SGIV-like viruses.

Ranaviruses such as FV-3 are serologically and genetically distinct from members of other genera. However, several piscine, reptilian and amphibian ranavirus isolates show serological cross-reactivity with FV-3. Serological cross-reactivity likely reflects the marked amino acid sequence conservation (i.e., >90% identity) within the MCP and other viral proteins.

Viral transmission occurs by feeding, parenteral injection, or environmental exposure. Ranaviruses grow in a wide variety of cultured fish, amphibian and mammalian cells at temperatures up to 32 °C. Infection causes marked cytopathic effects culminating in cell death, likely by apoptosis, such as the mitochondrion-mediated apoptosis observed in fish cells infected with Rana grylio virus (RGV). In contrast to their marked pathogenicity *in vitro*, their effect in animals depends on the viral species, and on the identity and age of the host animal. For example, largemouth bass virus (LMBV) shows evidence of widespread infection in the wild, but is only rarely linked to serious disease. Likewise, FV-3 infection leads to death in tadpoles, but often causes only non-apparent infections in adults that resolve within two weeks. In contrast, RGV causes a lethal syndrome in *Rana gyrlio* tadpoles and young adults. It is likely that environmental stress leading to immune suppression increases the pathogenicity of a given ranavirus. Ranavirus infections are often not limited to a single species or taxonomic class of animals. For example, EHNV has been reported to infect at least 13 species of fish. In addition, BIV, a highly virulent pathogen of the burrowing frog *Lymnodynastes ornatus*, can be experimentally transmitted to fish. Therefore, isolation of a ranavirus from a new host species does not necessarily identify a new viral species. In their most severe clinical manifestations, ranaviruses such as FV-3, ATV, European catfish virus (ECV) and EHNV are associated with systemic disease and show marked hemorrhagic involvement of internal organs such as the liver, spleen, kidneys and gut.

## Species demarcation criteria in the genus

Ranaviruses cause systemic disease in fish, amphibians and reptiles. Members of the six viral species are differentiated from one another by multiple criteria: RFLP profiles, virus protein profiles,

DNA sequence analysis and host specificity. PCR primers have been designed to amplify 3′ and 5′ regions within the MCP gene for identification purposes. Definitive quantitative criteria based on the above features have not yet been established to delineate different viral species. Generally, if a given isolate shows a distinct RFLP profile, possesses a distinctive host range and is markedly different from other viruses at the aa sequence level, it is considered a distinct viral species. For example, ranavirus DNA digested with *Kpn*I can be ordered into several groups based on RFLP profiles. Strains within the same species shared >70% of their bands in common and showed >95% aa sequence identity within the MCP or other key genes (e.g., ATPase, eIF-2α homolog).

### List of species in the genus *Ranavirus*

| | | |
|---|---|---|
| *Ambystoma tigrinum virus* | | |
| Ambystoma tigrinum virus | [AY150217] | (ATV) |
| (Regina ranavirus) | | |
| *Bohle iridovirus* | | |
| Bohle iridovirus | [AF157650, AF157651] | (BIV) |
| *Epizootic hematopoietic necrosis virus* | | |
| Epizootic hematopoietic necrosis virus | [FJ433873] | (EHNV) |
| *European catfish virus* | | |
| European catfish virus | [AF157678, AF127911] | (ECV) |
| (European sheatfish virus) | | (ESV) |
| *Frog virus 3* | | |
| Frog virus 3 | [AY548484] | (FV-3) |
| (Tiger frog virus) | [AF389451] | (TFV) |
| *Santee-Cooper ranavirus* | | |
| Santee-Cooper ranavirus | [AF080250] | (SCRV) |
| (Largemouth bass virus) | | (LMBV) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Ramavirus* but have not been approved as species

| | | |
|---|---|---|
| Rana esculenta iridovirus | | (REIR) |
| Singapore grouper iridovirus | [AY521625] | (SGIV) |
| Grouper iridovirus | | (GIV) |
| Testudo iridovirus | | (ThIV) |
| Rana catesbeiana virus-Z | | (RCV-Z) |

## GENUS *LYMPHOCYSTIVIRUS*

Type species ***Lymphocystis disease virus 1***

### Distinguishing features

Particle size varies from 198 to 227 nm for lymphocystis disease virus 1 (LCDV-1) and 200 nm for LCDV-2. The capsid may show a fringe of fibril-like external protrusions about 2.5 nm in length and a double-layered outer envelope.

Virions are heat labile. Infectivity is sensitive to treatment with ether or glycerol.

The genome length of LCDV-1 is 102.6 kbp and that of LCDV-C is 186 kbp. LCDV-C possesses the largest genome among known members within the three genera of vertebrate iridoviruses. Contour length measurements for LCDV-1 were determined by electron microscopy and indicate the DNA molecule to be 146 kbp; the degree of terminal redundancy is approximately 50% but varies considerably among virions. The G+C content is 29.1% for LCDV-1 and 27.2% for LCDV-C. Like FV-3, the genome is highly methylated. The presence of 5-methylcytosine occurs at 74% of CpG, 1% of CpC and 2–5% of CpA giving an overall level of methylation of 22%. The complete DNA sequence is known for LCDV-1 and LCDV-C.

The LCDV-1 genome contains 108 largely non-overlapping ORFs, 38 of which show significant homology to proteins of known function. These 38 ORFs represent 43% of the coding capacity of the genome. The presence of a DNA methyltransferase and a methyl-sensitive restriction endonuclease with specificity for a CCGG target site may be indicative of a restriction-modification system capable of degrading host genomic DNA while protecting viral DNA by specific methylation. LCDV-1 DNA contains numerous short direct, inverted and palindromic repetitive sequence elements. The LCDV-C genome contains 178 non-overlapping ORFs, 103 of which are homologs to the corresponding ORFs of LCDV-1 and 75 potential genes that were not found in LCDV-1 or in other iridovirids. Among these 75 genes, there are eight genes that contain conserved domains of cellular genes and 67 novel genes that do not show any significant homology with the sequences in public databases. Although LCDV-1 and LCDV-C possess some genes in common, their genomic organization (i.e. gene order) is markedly different. Furthermore, a large number of tandem and overlapping repeated sequences were observed in the LCDV-C genome. As expected, LCDV-C MCP is most similar to the MCP of LCDV-1. Lack of a suitable cell line has hindered studies of LCDV replication. Virus assembly occurs in and around virogenic stroma within viral assembly sites. Crescent-shaped capsid precursors develop into fully-formed capsids followed by condensation of the core structures.

LCDV-1 and LCDV-C infect flounder and plaice, whereas LCDV-2 infects dab. Infection results in benign, wart-like lesions comprising grossly hypertrophied cells occurring mostly in the skin and fins. The disease has been observed in over 100 teleost species although virus species other than LCDV may cause a similar clinical disease. The duration of infected cell growth and viral proliferation is highly variable (5 days to 9 months) and is likely temperature-dependent. Virions are released following degeneration of the lesions. Transmission is achieved by contact; external sites, including the gills, are the principal portals of entry. High host population densities and external trauma are believed to enhance transmission. Implantation and injection are also effective routes of transmission. The incidence of disease may be higher in the presence of certain fish ectoparasites. LCDV is generally not considered to be of major economic importance. However, although infections are usually benign and self-limiting, there may be commercial concerns because the warty appearance of infected fish leads to market rejection. Mortalities may occur, especially when infections involve the gills or when there is debilitation or secondary bacterial infection. LCDV-1, LCDV-C and LCDV-2 are difficult to culture *in vitro* although limited growth has been reported in several fish cell lines.

## Species demarcation criteria in the genus

Definitive criteria have not yet been established to delineate the viral species. The species are distinguished from one another by host specificity, histopathology and molecular criteria: viral protein profiles, DNA sequence analysis and PCR. PCR primers targeted to regions within the MCP and ORF167L can be used to distinguish between species.

## List of species in the genus *Lymphocystivirus*

| | | |
|---|---|---|
| *Lymphocystis disease virus 1* | | |
| Lymphocystis disease virus 1 | [L63545] | (LCDV-1) |
| (Flounder lymphocystis disease virus) | | (FLDV) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Lymphocystivirus*

| | | |
|---|---|---|
| Lymphocystis disease virus 2 | | (LCDV-2) |
| Lymphocystis disease virus-China | [AY380826] | (LCDV-C) |
| Lymphocystis disease rockfish virus | [AB213004, AB213005, AB213006] | (LCDV-RF) |
| Dab lymphocystis disease virus | | |

# GENUS *MEGALOCYTIVIRUS*

Type species ***Infectious spleen and kidney necrosis virus***



## Distinguishing features

Virons are similar in appearance to those of ranaviruses and genomes are highly methylated. Infections *in vivo* lead to the appearance of enlarged cells (inclusion body bearing cells) that are characteristic of megalocytivirus infections.

Virions possess icosahedral symmetry and are about 140–200 nm in diameter.

Virions are sensitive to heat (56 °C for 30 min), sodium hypochlorite, UV irradiation, chloroform and ether, and are variably inactivated by exposure to pH3 and pH11.

The complete genomes of ISKNV, RBIV, RSIV and orange spotted grouper iridovirus (OSGIV) have been sequenced. ISKNV virions contain a single, linear dsDNA molecule of 111,362 bp with a G+C content of 54.8%. As with other members of the family, genomic DNA is circularly permuted, terminally redundant and highly methylated.

Genomic sequence comparisons show co-linearity among all of the completely sequenced members of the genus. However, they do not share sequence co-linearity with other members of the family.

No serotypes are reported and all megalocytiviruses analyzed to date appear to be members of the same viral species or several closely related species. Polyclonal anti-RSIV serum shows cross-reactivity with ESV- and EHNV-infected cells, whereas monoclonal anti-RSIV antibodies react only with RSIV-infected cells. Megalocytiviruses show high levels (i.e. >93%) of aa sequence identity among the proteins characterized to date.

Iridoviruses infecting red seabream, mandarin fish and over 20 other species of marine and tropical fish have been known since the late 1980s. Isolates from red seabream (RSIV) and mandarin fish (ISKNV) have been studied extensively. Viral infection is characterized by the formation of inclusion body-bearing cells (IBC). IBCs may be derived from virus-infected macrophages and enlarge by the growth of a unique inclusion body that may be sharply delineated from the host cell cytoplasm by a limiting membrane. When a limiting membrane is seen, the inclusions contain the viral assembly site and possess abundant ribosomes, rough ER and mitochondria. IBCs frequently appear in the spleen, hematopoietic tissue, gills and digestive tract. Necrotized splenocytes are also observed. Transmission has been demonstrated by feeding, parenteral injection and by environmental exposure. Megalocytiviruses naturally infect and cause significant mortality in freshwater and marine fish in aquaculture facilities in China, Japan and SE Asia. A partial list of susceptible fish species includes mandarin fish (*Siniperca chuatsi*), red seabream (*Pagrus major*), grouper (*Epinephelus* spp.), yellowtail (*Seriola quinqueradiata*), striped beakperch (*Oplegnathus fasciatus*), red drum (*Sciaenops ocellata*) and African lampeye (*Aplocheilichthys normani*). The virus grows in several cultured piscine cell lines and causes a characteristic enlargement of infected cells. Outbreaks of disease caused by ISKNV occur only in fish cultured at temperatures >20 °C. A vaccine targeted to RSIV has been developed suggesting that infection/immunization is capable of eliciting protective antibodies.

## Species demarcation criteria in the genus

Megalocytiviruses are distinguished from ranaviruses and lymphocystiviruses by their cytopathological presentation (i.e., inclusion body-bearing cells) and sequence analysis of key viral genes, e.g., ATPase and MCP, for which PCR primers have been developed. Megalocytiviruses show >94% sequence identity within these genes, whereas sequence identity with ranaviruses and lymphocystiviruses is <50%. Based on sequence analysis and serological studies, all megalocytiviruses isolated to date appear to be strains of the same or several closely-related viral species.

## List of species in the genus *Megalocytivirus*

*Infectious spleen and kidney necrosis virus*
Infectious spleen and kidney necrosis virus [AF371960 = NC_003494] (ISKNV)

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Megalocytivirus* but have not been approved as species

| | | |
|---|---|---|
| Red seabream iridovirus | [BD143114] | (RSIV) |
| Sea bass iridovirus | [AB043977] | (SBIV) |
| African lampeye iridovirus | [AB043979] | (ALIV) |
| Grouper sleepy disease iridovirus | [AB043978] | (GSDIV) |
| Dwarf gourami iridovirus | | (DGIV) |
| Taiwan grouper iridovirus | | (TGIV) |
| Rock bream iridovirus | [AY532606] | (RBIV) |
| Orange-spotted grouper iridovirus | [AY894343] | (OSGIV) |
| Turbot iridovirus | | (TBIV) |
| Spotted knifejaw iridovirus | [GQ202216, GQ202217] | (SKIV) |

## List of other related viruses which may be members of the family *Iridoviridae* but have not been approved as species

| | |
|---|---|
| White sturgeon iridovirus | (WSIV) |
| Erythrocytic necrosis virus | (ENV) |

## Phylogenetic relationships within the family

Phylogenetic analysis using the 26 core iridovirus genes from the 14 completely sequenced iridoviruses shows a clear division between the genera within the family *Iridoviridae* (Figure 6). Members of genera *Ranavirus*, *Lymphocystivirus* and *Megalocytivirus* are in three separate clades with a common ancestor, while members of the genera *Iridovirus* and *Chloriridovirus* form two clades that are more distantly related. In addition, the phylogeny shows a distinct lineage for each genus within

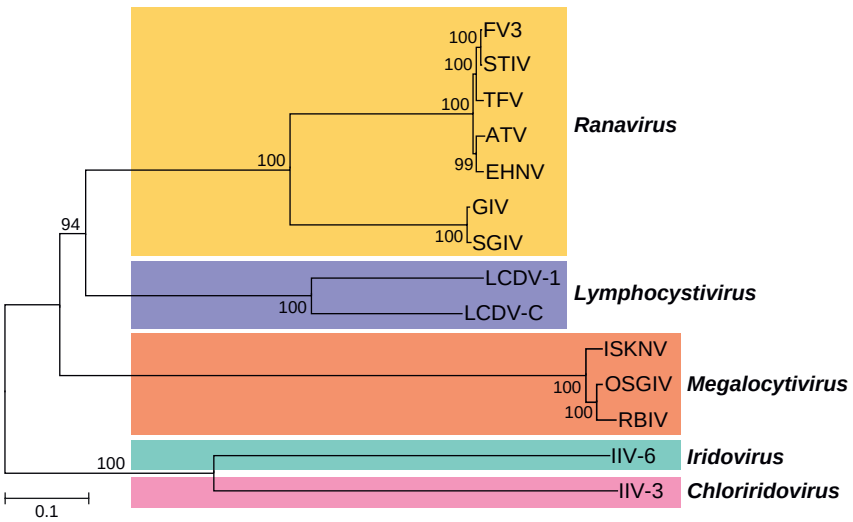


**Figure 6:** Concatenated phylogeny of 26 conserved iridovirid sequences. Phylogenetic relationship of 26 conserved ORFs from the 14 completely sequenced iridovirid genomes. The neighbor-joining tree obtained using MEGA4 is shown with the statistical support indicating the robustness of the inferred branching pattern as assessed using the bootstrap test. (Modified from Jancovich *et al.* (2010). *J. Virol.*, **84**, 2636–2647; with permission.)

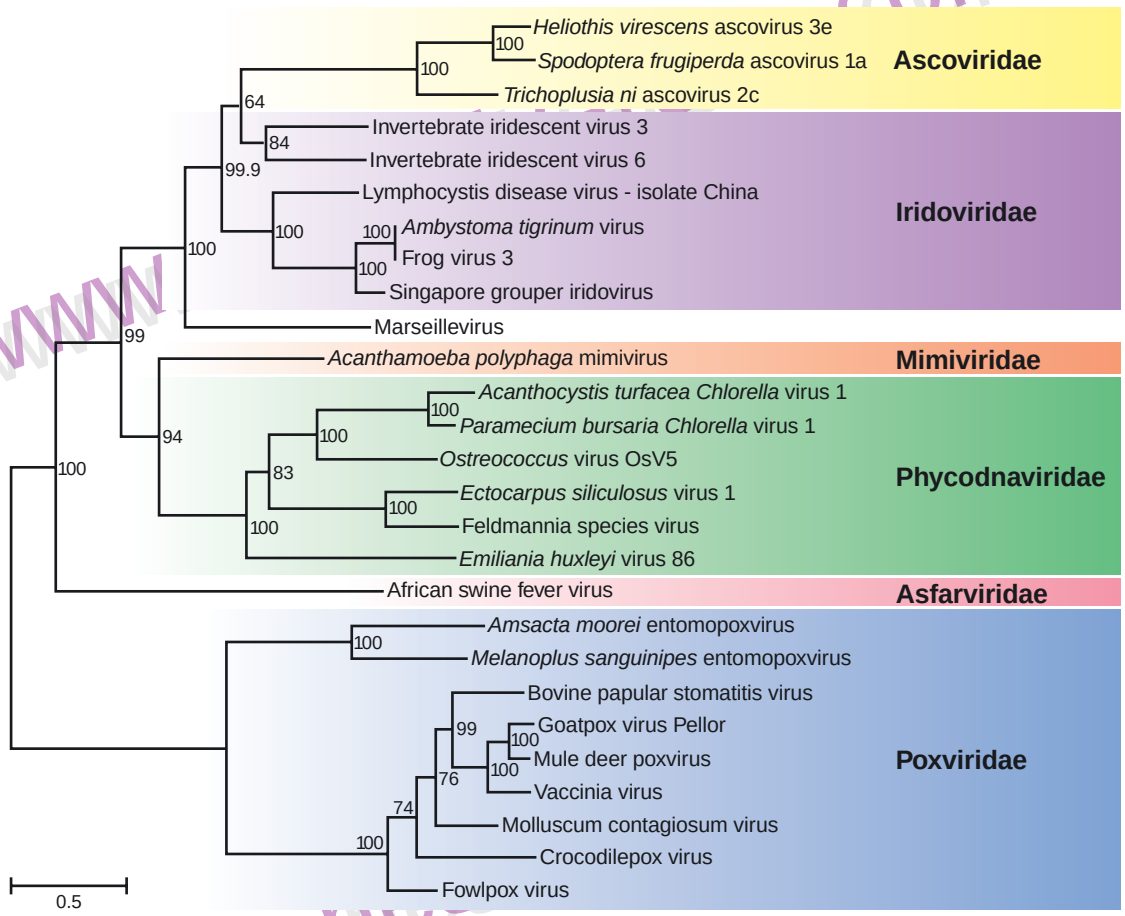


**Figure 7:** A maximum-likelihood tree based on concatenated alignments (1849 positions) of five nucleocytoplasmic large DNA viruses (NCLDV) core proteins: D5 type ATPase, DNA polymerase B, A32 ATPase, major capsid protein and A1L/VLTF2 transcription factor. The tree was built using TreeFinder. (From Boyer *et al.* (2009). *PNAS*, **106**, 21848–21853; with permission.)

the family *Iridoviridae*, with the vertebrate genera being more closely related to each other as compared to the invertebrate genera.

## Similarity with other taxa

The iridovirus D5 ATPase, A32 ATPase, A1L/VLTF2 transcription factor, MCP and viral DNA polymerase B genes share aa sequence similarities to other nucleocytoplasmic large DNA viruses (NCLDV) such as *African swine fever virus* (*Asfarviridae*), species within the family *Ascoviridae*, *Paramecium bursaria Chlorella virus 1* (*Phycodnaviridae*), Marseillevirus (*Mimivirus*), and poxviruses (*Poxviridae*) (Figure 7). Although not shown here, more distant relatedness to other NCLDV species including herpesviruses, adenoviruses and baculoviruses has also been noted.

## Derivation of names

*Chloro*: from Greek *chloros*, "green".
*Cysti*: from Greek *kystis*, "bladder/sac".
*Irido*: from Greek *iris*, *iridos*, goddess whose sign was the rainbow, hence iridescent, "shining like a rainbow", from the appearance of patently infected invertebrates and centrifuged pellets of virions.
*Lympho*: from Latin *lympha*, "water".
*Megalocyti*: from the Greek, meaning "enlarged cell".
*Rana*: from Latin *rana*, "frog".

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## Contributed by

Jancovich, J.K., Chinchar, V.G., Hyatt, A., Miyazaki, T., Williams, T. and Zhang, Q.Y.

# FAMILY *LIPOTHRIXVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Lipothrixviridae*** |
| Genus | ***Alphalipothrixvirus*** |
| Genus | ***Betalipothrixvirus*** |
| Genus | ***Gammalipothrixvirus*** |
| Genus | ***Deltalipothrixvirus*** |



## General properties

Virions are flexible filaments that vary from 410 to 2200 nm in length and 24 to 38 nm in diameter. They are enveloped, and the envelope consists of viral proteins and host derived lipids. The helical nucleoprotein core contains a single molecule of linear dsDNA, 15.9–56 kbp long. Virion ends have specific structures that vary between genera (Figure 1).

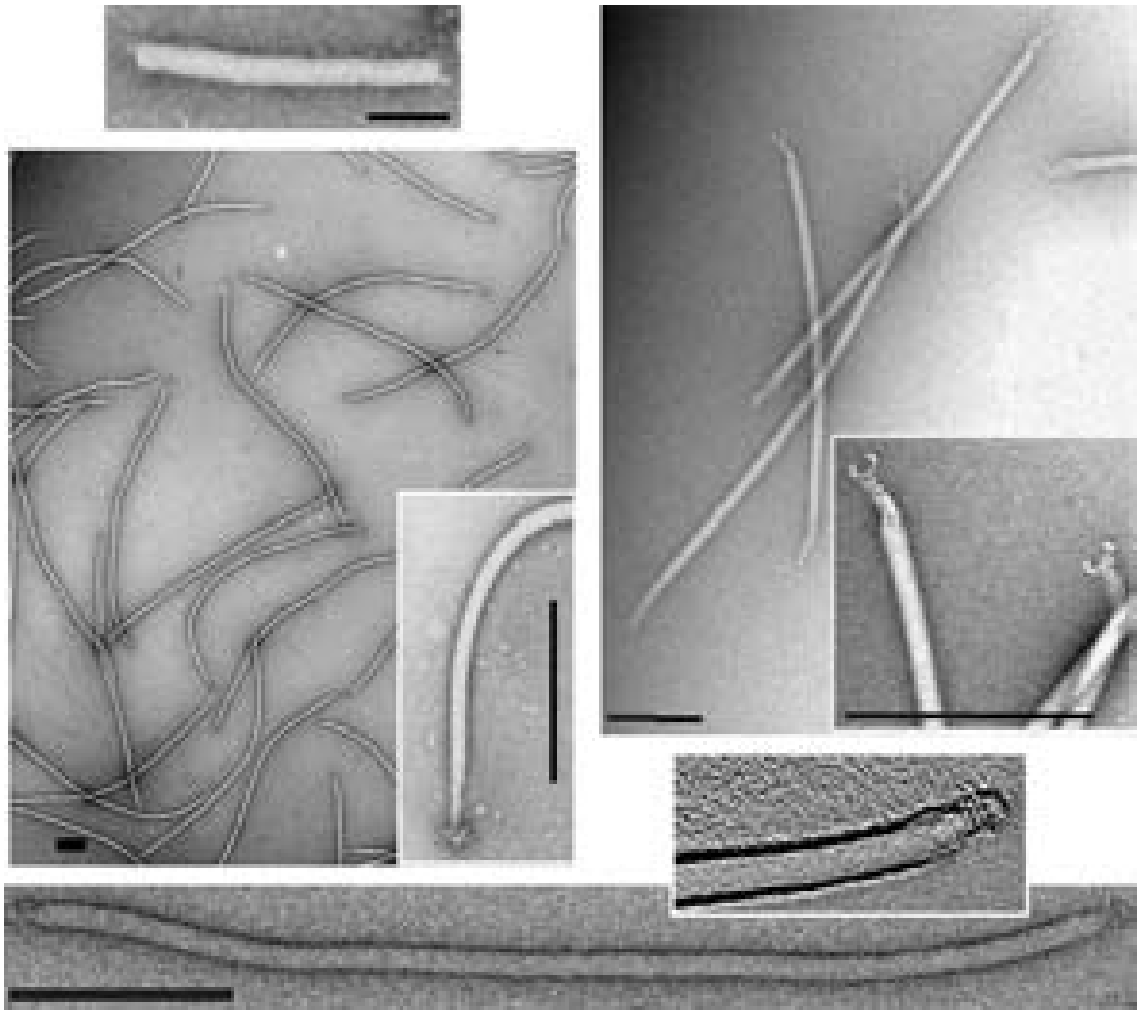

**Figure 1:** Negative contrast electron micrographs of virions of representatives of four genera of the family *Lipothrixviridae*. (Top, left) Thermoproteus tenax virus 1 from the genus *Alphalipothrixvirus*; (center; left) Sulfolobus islandicus filamentous virus from the genus *Betalipothrixvirus*; (top, right) Acidianus filamentous virus 1 from the genus *Gammalipothrixvirus*; (bottom) Acidianus filamentous virus 2 from the genus *Deltalipothrixvirus*. The bars represent 100 nm. (Modified from Arnold *et al*., 2000; Bettstetter *et al*., 2003, Häring *et al*., 2005; Vestergaard *et al*. 2008.)

# Genus *Alphalipothrixvirus*

Type species ***Thermoproteus tenax virus 1***

## Distinguishing features

The width of the virion significantly exceeds that of other members of the family, most likely reflecting exceptional properties of the helical core. The terminal structures are also exceptional: both ends are rounded and do not taper, as in case of members of the other genera. None of the putative genes of the sole member of the genus shows any sequence similarity to sequences in extant databases.

## Virion properties

### Morphology

The virion is a rigid rod, about 410 nm long and 38 nm in diameter, with apparently asymmetric extensions at each end. The envelope encloses a helical core consisting of dsDNA and two DNA-binding proteins (Figures 1, 2).

### Physicochemical and physical properties

Virion Mr is $3.3 \times 10^8$ and buoyant density in CsCl is $1.25\,g\,cm^{-3}$. The virions are stable at 100 °C and a fraction remains viable after autoclaving at 120 °C. Particles maintain their structure in 6M urea and 7M guanidine hydrochloride. Detergents (e.g. Triton X-100 and octylglycoside), dissociate virions into viral cores, containing the DNA and DNA-binding proteins, and viral envelopes, containing isoprenyl ether lipids and capsid proteins (Figure 2). The virion consists of about 3% (w/w) DNA, 75% protein and 22% isoprenyl ether lipids.

### Nucleic acid

The virion contains one molecule of linear dsDNA of 15.9 kbp. The ends of the molecule are masked in an unknown manner.

### Proteins

The virion contains at least four proteins with molecular masses of 12.9, 16.3, 18.1 and 24.5 kDa. The two smallest form the virion core and highly hydrophobic 18.1 kDa protein is present in the envelope. Additional minor proteins may be present.

### Lipids

The virion envelope carries the same lipids as the host membrane, essentially diphytanyl tetraether lipids. The envelope has a bilayer structure. The phosphate residues of the phospholipids are oriented towards the inside and the glycosyl residues towards the outside of the particles.

### Carbohydrates

No information available.

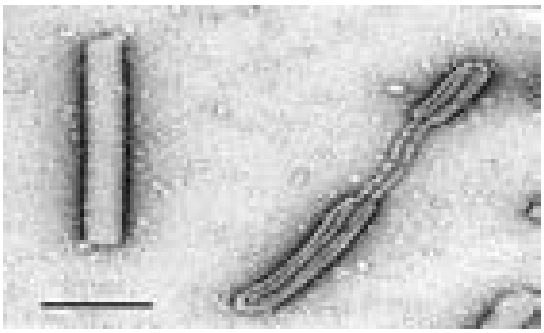

**Figure 2:** Negative contrast electron micrograph of intact and partially deteriorated virions of Thermoproteus tenax virus 1, exhibiting the envelope and the core. The bar represents 100 nm.

### Genome organization and replication

About 85% of the total genome sequence has been determined. The genome contains several transcription units. So far, the function of only a few genes is known, among them those encoding the four structural proteins. These genes are not linked. The genome carries two low complexity sequence regions consisting of the repetitive sequence CCNACN (where N is any nucleotide), one located within the tpx gene and the other in a noncoding region. Frequent recombination appears to occur between these two regions which generates multiple variants of the TPX protein, which is present in infected cells in high amounts, however, is absent among virion proteins. No information is available on genome replication.

### Antigenic properties

No information available.

### Biological properties

Host range is limited to the hyperthermophilic archaeaon *Thermoproteus tenax*. Adsorption and infection appear to proceed via interaction of the terminal protrusions of the virion with the host pili. The virus is temperate and virions are released by cell lysis. The viral genome is present in the host cells in a linear non-integrated form, and only its fragments were found to be integrated in the host chromosome. Mature virions assemble in host cell lumen prior to their release.

### List of species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Alphalipothrixvirus*

*Thermoproteus tenax virus 1*
Thermoproteus tenax virus 1 [X14855] (TTV-1)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### Other related viruses which may be members of the genus *Alphalipothrixvirus* but have not been approved as species

None reported.

## Genus *Betalipothrixvirus*

Type species ***Sulfolobus islandicus filamentous virus***

### Distinguishing features

The virions are the longest in the family. Their terminal structures, as well as DNA arrangement in the virion core, differ from those of members of the other genera. The genomes show a limited sequence similarity to the genomes of members of the other genera.

### Virion properties

#### Morphology

The virion is a flexuous filament, about 2000 nm long and 23 nm wide (Figures 1, 3). It consists of a cylindrical 3.1 nm thick envelope which encases an inner core consisting of DNA and DNA-binding proteins. Each end of the virion is tapered and carries short filaments; their number is either three or six, dependent on the species.

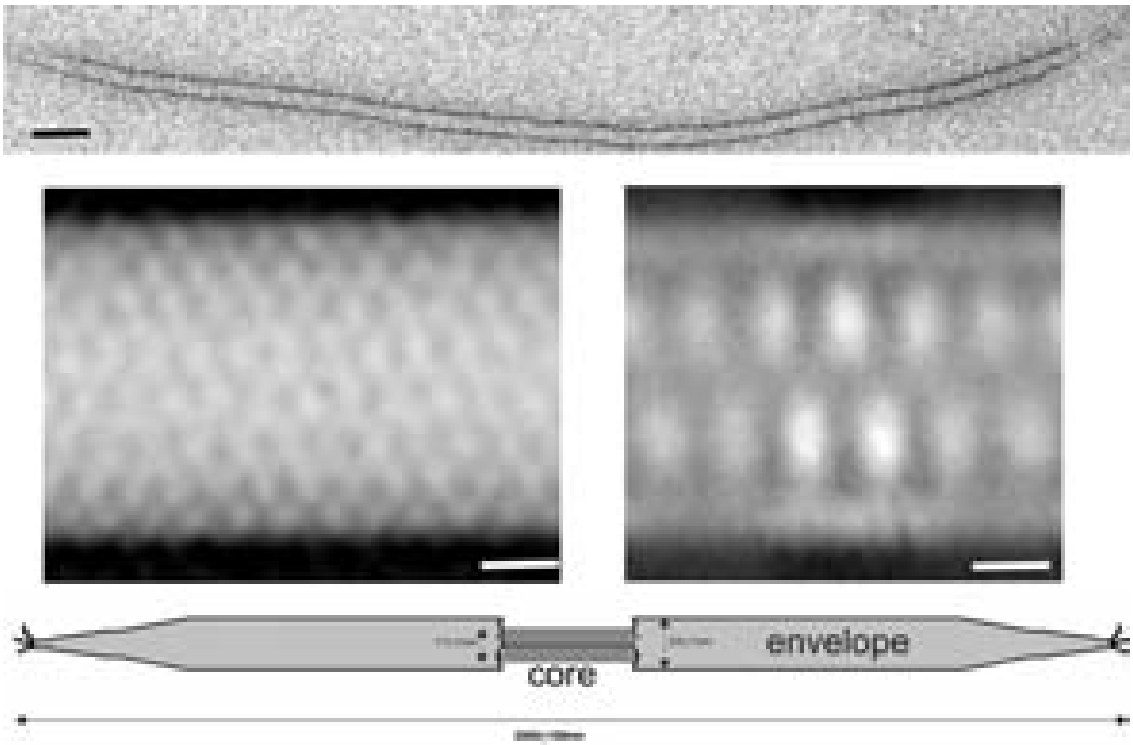


**Figure 3:** (Top) Negative contrast electron micrograph of AFV3 virion. The bar represents 100 nm. (Middle row) Image processing of AFV3 virion: (left) average 2D map computed from a set of the negatively stained sample, and (right) average 2D map computed from a set of images taken from cryo-EM micrographs; the contrast is inverted. The bars represent 5 nm. (Bottom) Schematic model of the virion of the Acidianus filamentous virus 3.

### Physicochemical and physical properties

The buoyant density of the virions is about 1.3 g cm$^{-3}$. Short treatment with detergents removes the terminal structures and the envelope and uncovers a filament about 17 nm in width. The cylindrical envelope contains small globular subunits arranged in a helical formation. The envelope encases an inner core with two parallel rows, producing a zigzag pattern, which is likely to be the projection of a double row of nucleosome-like particles (Figure 3). The linear dsDNA appears to wrap around the nucleosome-like particles.

### Nucleic acid

The genome is linear dsDNA, varying is size from 36.895 to 41.050 kbp. The termini of the two strands are not covalently linked, and are strongly associated with protein(s).

### Proteins

The virion carries two major proteins, of about 17.0–18.5 and 22.5–25.0 kDa, in equal amounts, which are involved in formation of the virion core. Among six minor proteins with molecular masses of in the range of 8.0–19.0 and 30.5 kDa; four are conserved in all known species and two are exclusive to only two viruses.

### Lipids

In the virion, presumably in the envelope, modified host lipids were detected.

### Carbohydrates

No information available.

## Genome organization and replication

The genomes of different species reveal a high level of conservation in both gene content and gene order over large regions (Figure 4). Other shared features of the genomes are: (i) large inverted

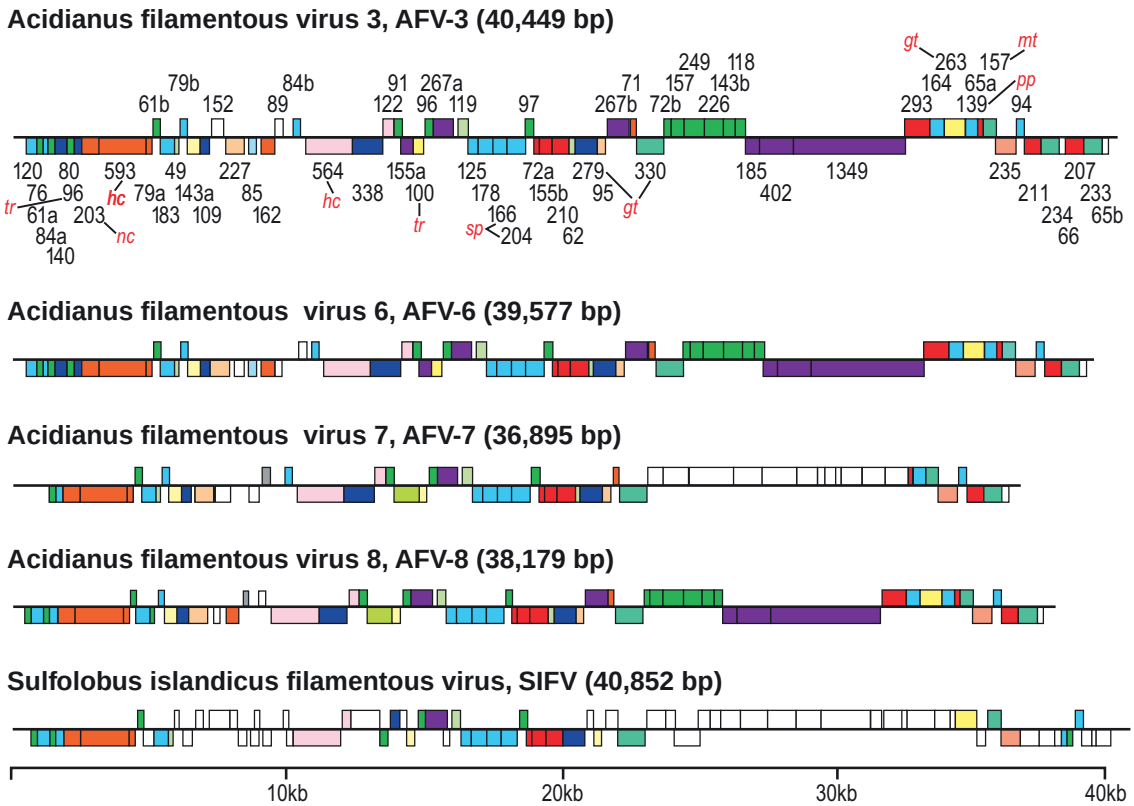


**Figure 4:** Genome maps aligned for the betalipothrixviruses, showing the relative size, location and orientation of the predicted genes. Homologous genes are coded with identical colors. Homologous operons carrying three or more genes are displayed as a consecutive set of genes with the same color. White boxes indicate that no homologous genes were detected in the other genomes. The ORFs of AFV-3 are labelled according to their amino acid lengths. Predicted protein functions are shown as follows: hc, helicase; gt, glycosyltransferase; mt, SAM-dependent methyltransferase; nc, nuclease; pp, protein phosphatase; sp, structural protein; and tr, transcriptional regulator.

terminal repeats exhibiting conserved, regularly spaced direct repeats; (ii) a highly conserved operon encoding the two major structural proteins; and (iii) multiple overlapping ORFs which may be indicative of gene recoding. A few predicted gene products of each species, in addition to the structural proteins, could be assigned functions, including a putative helicase, a nuclease, a protein phosphatase, glycosyltransferase and transcription regulators. No information is available on DNA replication.

## Antigenic properties

No information available.

## Biological properties

The host range is restricted to hyperthermophilic archaea from the genera *Sulfolobus* and *Acidianus*. The virus genome does not integrate into the host chromosome. Host cell lysis was not observed. A population of infected host cells continuously produces the virus. Details of virus–host interactions are not known.

## List of species demarcation criteria in the genus

Species in the genus differ in the length and the terminal structure of the virion, the size and nucleotide sequence of the genomes and their host range.

## List of species in the genus *Betalipothrixvirus*

| | | |
|---|---|---|
| *Acidianus filamentous virus 3* | | |
| Acidianus filamentous virus 3 | [AM087120] | (AFV-3) |
| *Acidianus filamentous virus 6* | | |
| Acidianus filamentous virus 6 | [AM087121] | (AFV-6) |
| *Acidianus filamentous virus 7* | | |
| Acidianus filamentous virus 7 | [AM087122] | (AFV-7) |
| *Acidianus filamentous virus 8* | | |
| Acidianus filamentous virus 8 | [AM087123] | (AFV-8) |
| *Acidianus filamentous virus 9* | | |
| Acidianus filamentous virus 9 | [EU545650] | (AFV-9) |
| *Sulfolobus islandicus filamentous virus* | | |
| Sulfolobus islandicus filamentous virus | [AF440571] | (SIFV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## Other related viruses which may be members of the genus *Betalipothrixvirus* but have not been approved as species

| | |
|---|---|
| Desulfurolobus ambivalens filamentous virus | (DAFV) |
| Thermoproteus tenax virus 2 | (TTV-2) |
| Thermoproteus tenax virus 3 | (TTV-3) |

# Genus *Gammalipothrixvirus*

Type species ***Acidianus filamentous virus 1***

## Distinguishing features

The virion has distinctive claw-like structures at its termini (Figures 1, 5). The virion is the shortest in the family, and the genome is the smallest and significantly differs in sequence from the genomes of other members of the family. Moreover the genome does not carry long ITRs, as other members of the family.

## Virion properties

### Morphology

The filamentous virion is 900 nm long and 24 nm wide (Figures 1, 5). Terminal structures at both ends of the linear virion are identical and resemble claws. They are 20 nm in diameter. Each claw is connected to the virion body by a 60 nm long tapering appendage and a collar (Figure 5). The body of the virion is covered with an envelope about 3.4 nm thick (Figure 6).

### Physicochemical and physical properties

The buoyant density is about 1.3 g cm$^{-3}$. The virion envelope is partially degraded by treatment with 0.3% Triton X-100 and completely removed by treatment with 0.1% SDS. Concomitant with the removal of the envelope, the terminal structures are removed. Conformational changes occur in the


**Figure 5:** Schematic model of the virion of the Acidianus filamentous virus 1.

virion termini at an initial phase of adsorption: the claw-like terminal structures fold and keep the virion tightly attached to the host cell pili.

### Nucleic acid

The genome is linear dsDNA of 20.869 kbp. The ends are modified in an unknown manner. The short inverted terminal repeat $CG_{10}$ is present at the termini, preceded by about 350-bp-long sequence which contains clusters of short direct repeats of the sequence TTGTT or close variants thereof. The structural organisation resembles the telomeric ends of linear eukaryotic chromosomes.

### Proteins

The virion carries two major proteins P132 and P140, and several minor proteins. They bind DNA and form filaments when incubated with linear dsDNA. A C-terminal domain is identified in their crystal structure with a four-helix-bundle fold. In the topological model, the genomic dsDNA super-helix wraps around P132 basic core, and the P140 basic N-terminus binds genomic DNA, while its lipophilic C-terminal domain is embedded in the lipidic outer shell (Figure 6). The four-helix bundle fold of P132 and P140 is identical to that of the coat protein of the members of the family *Rudiviridae.*

### Lipids

In the virion, presumably in the envelope, modified host lipids were detected.

### Carbohydrates

No information available.

## Genome organization and replication

From 40 putative genes, seven have homologs in betalipothrixviruses and rudiviruses (Figure 7). Three of these, ORFs 135, 426 and 223, are ordered similarly to their respective homologs on the genomes of the rudiviruses SIRV1 and SIRV2.

## Antigenic properties

No information available.

## Biological properties

The host range is restricted to hyperthermophilic archaea of the genus *Acidianus*. Virions adsorb to the pili of the host cell, their adsorption to cell surface was never observed. The unusual termini of

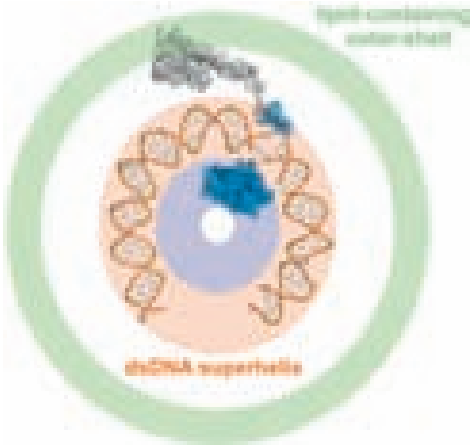

**Figure 6:** Topological model of the virion of Acidianus filamentous virus 1, indicating location of the two major structural proteins, P132 and P140. The P132 patch is represented by the blue surface in the center. The dsDNA backbone is orange and the P140 is represented by the grey surface. P140 contacts both the lipid-containing outer shell (in green) and the genomic DNA. (From Goulet *et al*., 2009.)

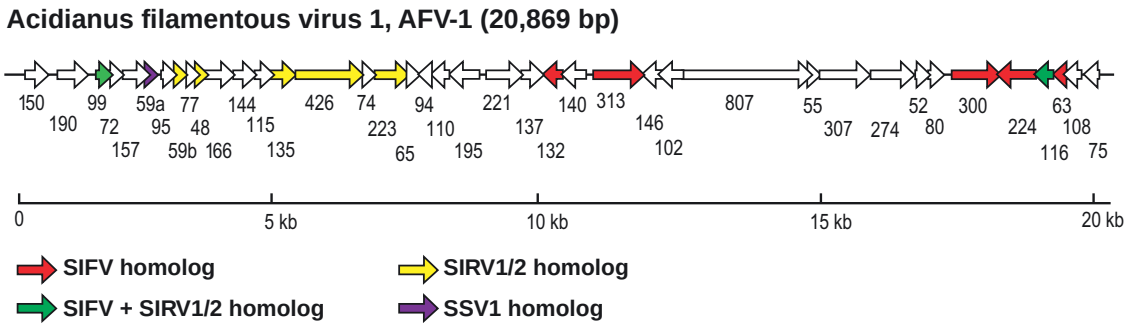


**Figure 7:** Genome map of Acidianus filamentous virus 1, showing relative size, location, and orientations of the predicted genes. ORFs with significant sequence similarities to ORFs in other viruses are shaded and labeled. (From Bettstetter *et al*., 2003.)

the virion appear to have a special function in the process of adsorption: the claw folds and keeps the virion firmly attached to a pilus.

During a cycle of productive infection neither formation of any significant amounts of cell debris, nor decrease in cell density were observed. Latent period is about 4 h. Infected host cell culture was not cured of the virus after several successive transfers into fresh medium and prolonged, continuous growth. Integration of viral genome into the host chromosome was not observed.

### List of species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Gammalipothrixvirus*

*Acidianus filamentous virus 1*
Acidianus filamentous virus 1 [AJ567472] (AFV-1)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### Other related viruses which may be members of the genus *Gammalipothrixvirus* but have not been approved as species

None reported.

## Genus *Deltalipothrixvirus*

Type species *Acidianus filamentous virus 2*

### Distinguishing features

The virion carries exceptional structures at the termini, not observed in the virions of other members of the family. Moreover, the virion core does not exhibit any regular structure on its surface and, thereby, differs from both the nucleoprotein complex of the alphalipothrixvirus TTV1 and the nucleosome-like arrangement of the betalipothrixvirus virion cores.

### Virion properties

#### Morphology

The virion is a flexuous filament, about 1100 nm in length and 24 nm in width (Figures 1, 8). It is enveloped with a 3–4 nm thick envelope. At the two ends of the virion identical structures are


**Figure 8:** Schematic model of the virion of the Acidianus filamentous virus 2.

present, constituting a complex collar with two sets of filaments, resembling a bottle brush with a solid cap 17 nm in diameter on its top (Figure 8).

### Physicochemical and physical properties

The buoyant density of the virions is about 1.3 g cm$^{-3}$. Short treatment with detergents removes the envelope and uncovers a thinner filament about 17 nm in width.

### Nucleic acid

The genome is linear dsDNA of 31.787 kbp. No information is available on modification of the ends of the molecule.

### Proteins

The virion carries seven proteins with apparent molecular masses of 65, 50, 45, 40, 35, 26 and 6 kDa.

### Lipids

Lipids could not be detected.

### Carbohydrates

None reported.

## Genome organization and replication

From 34 putative genes, eight have homologs in betalipotrixviruses and rudiviruses. The genome contains an unusual 1.008 kbp region close to the centre, which carries two large 46-bp direct repeats and multiple imperfect short repeats throughout the region (Figure 9). Its base composition is strongly biased, with one DNA strand containing only 7% guanosines. The region is bordered by short inverted repeats and may constitute a replication initiation site. In the central part of the genome is present also a tRNA$^{Lys}$ gene, and it contains a 12-bp archaeal intron. No information is available on genome replication.

## Antigenic properties

No information available.

## Biological properties

The host range is restricted to hyperthermophilic archaea of the genus *Acidianus*. The virus host is devoid of pilus-like structures and virions attach directly to the cell surface. The terminal structures

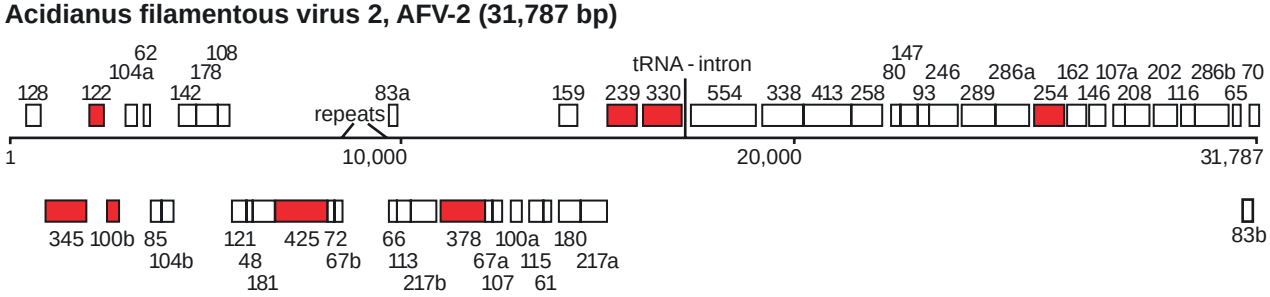


**Figure 9:** Genome map of Acidianus filamentous virus 2, showing relative size and location of the predicted genes. Genes are expressed from left to right in the upper row and from right to left in the lower row. ORFs shared with the beta- and gammalipothrixviruses, and with the rudiviruses, are represented by filled rectangles.

of the virion appear to be involved in adsorption. No conformational changes could be detected in these structures upon adsorption.

During a cycle of productive infection neither formation of any significant amounts of cell debris, nor decrease in cell density was observed. Infected host cell culture was not cured of the virus after several successive transfers into fresh medium and prolonged, continuous cultivation. Integration of the viral genome into the host chromosome was not observed.

## List of species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Deltalipothrixvirus*

*Acidianus filamentous virus 2*
Acidianus filamentous virus 2 [AJ854042] (AFV-2)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## Other related viruses which may be members of the genus *Deltalipothrixvirus* but have not been approved as species

Not applicable.

## List of unassigned species in the family *Lipothrixviridae*

None reported.

## Phylogenetic relationships within the family

The alphalipothrixvirus does not share any homologous gene with the members of the three other genera. There is limited similarity in gene content between beta-, gamma- and deltalipothrixviruses, and only a few ORFs are highly conserved in all of them. A dendrogram constructed by alignment of the sequence of ORF235 of the betalipothrixviruses and its homologs in the gamma- and deltalipothrixviruses reflects relationships within the family (Figure 10).

## Similarity with other taxa

Originally, the two families of dsDNA viruses with linear genomes, the *Rudiviridae* and the *Lipothrixviridae*, were distinguished by differences in virion structure and this was later supported by comparative genomics. Nevertheless, a substantial fraction of orthologous genes, including some encoding glycosyl transferases and transcriptional regulators, are shared by the rudiviruses and lipothrixviruses. For example, of the 45 predicted genes of the rudivirus Sulfolobus islandicus rod-shaped virus 1, nine share orthologs with the lipothrixvirus Sulfolobus islandicus filamentous virus. Moreover, the structure of the major virion protein of the rudiviruses turned out to be identical to the structures of the two major virion proteins of the lipothrixvirus Acidianus filamentous virus 1.

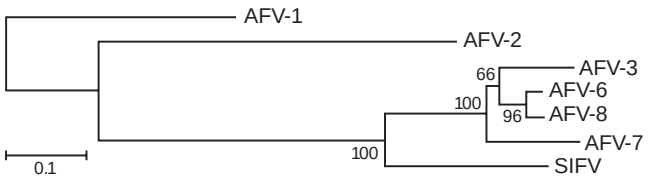


**Figure 10:** Dendrogram for genera *Betalipothrixvirus*, *Gammalipothrixvirus*, and *Deltalipothrixvirus*, derived from comparison of sequences of the only well-conserved putative gene shared by all members of the genera. (Modified from Vestergaard *et al.*, 2008.)

These observations indicate that the known linear dsDNA viruses, all of which infect hyperthermopilic members of the domain Archaea, share a common ancestor. One can suggest a sequence of evolutionary events in which the major virion protein of a "simpler" non-enveloped virion of the *Rudiviridae* has been duplicated and evolved so as to facilitate interactions with a hydrophobic envelope, producing the more complex virion of the *Lipothrixviridae*. Therefore, it has been suggested that the two the families *Rudiviridae* and *Lipothrixviridae* should be placed into a new order.



## Derivation of names

*Lipo*: from Greek *lipos*, "fat".
*Thrix*: from Greek *thrix*, "hair".

## Further reading

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## Contributed by

Prangishvili, D.

# Family *Mimiviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Mimiviridae* |
| Genus | *Mimivirus* |

Since only one genus is currently recognized, the family description corresponds to the genus description.



# Genus *Mimivirus*

Type species *Acanthamoeba polyphaga mimivirus*

## Virion properties

### Morphology

Mimivirus particles are comprised of an icosahedral core protein capsid with a diameter of 0.5 m. This large capsid is uniformly covered with a 0.125 m thick layer of closely packed fibers, forming a roughly spherical object 0.75 m in diameter that is easily visible under the light microscope with Nomarski optics (differential interference contrast microscopy). The mimivirus particle is not entirely symmetrical as it has a five-fold star-shaped structure at a single icosahedral vertex. This structure, coined the stargate, extends along the whole length of the five icosahedral edges surrounding this unique vertex (Figure 1). The central electron-dense nucleocapsid of the particle appears to be contained within two 40 Å-thick lipid membranes surrounded by a 70 Å-thick protein shell (Figure 2).

### Physicochemical and physical properties

Using ultracentrifugation in a CsCl gradient, the particle density was estimated to be about 1.36 g cm$^{-3}$. In addition to their unprecedented size and hairy appearance, mimivirus particles are highly resistant to extreme conditions (pH, temperature, reducing agents) or enzymatic treatments (proteases, glycosidases). The resilience of the particles may be due to their distinctive peripheral fiber layer thought to be made of a dense mesh of a complex (lipo-) polysaccharide biopolymer akin to the outer coat of bacterial spores. The presence of this outer layer, not seen in other families of large nucleocytoplasmic DNA viruses, together with the overall size of the particle, might be linked

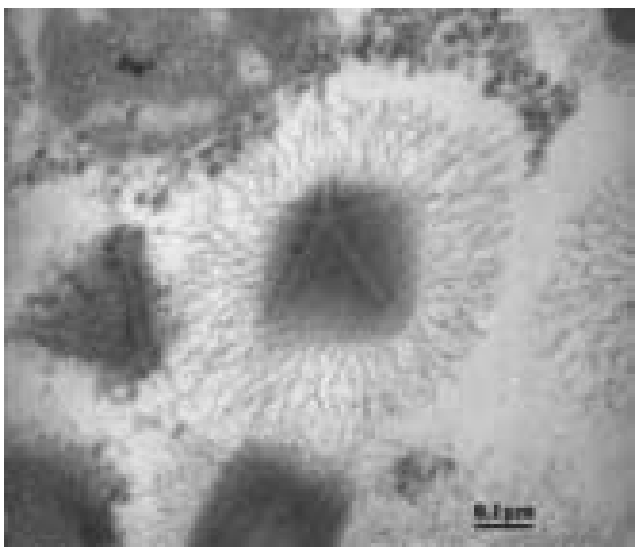

**Figure 1:** Mimivirus particle exhibiting the distinctive stargate structure (transmission electron microscopy).

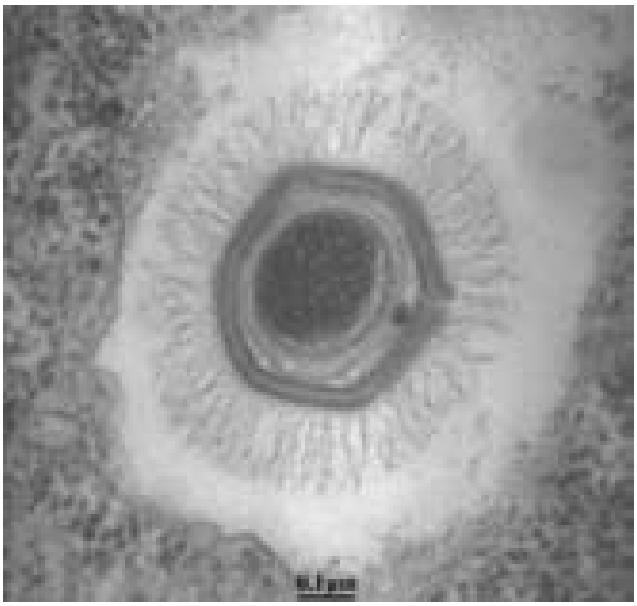

**Figure 2:** The complex internal structure of a mimivirus particle (transmission electron microscopy).

to the heterotrophic nature of the host acanthamoeba; mimivirus particles mimic the bacteria on which they usually feed (Figure 2).

### Nucleic acid

The mimivirus genome is a single linear dsDNA molecule of 1,181,549 bp with an overall nucleotide composition of 72% (A+T). Several virus-encoded mRNAs are associated with the purified particle, but the functional significance of these findings is unknown. Mimivirus genome termini lack the large terminal inverted repeats (up to 2 kb) found in other large DNA viruses such as phycodnaviruses (chlorovirus) or poxviruses. In place of inverted terminal repeats, the mimivirus genome has an inverted repeat of a 617-bp segment approximately 22 kb from one end of its genome and near the other end. Pairing these regions can lead to a putative Q-like form for the mimivirus genome, with a long (22,514 bp) and a short (259 bp) tail. The short tail does not contain any ORFs.

### Proteins

A comprehensive proteomic study of purified virions led to the identification of the products of at least 137 mimivirus genes. Over half of these virion-associated proteins have unknown functions. The 2D gels revealed numerous isoforms, probably due to posttranslational modifications such as glycosylation, acetylation and phosphorylation. In addition to the expected major structural components (e.g., the major capsid protein L425 and core L410 protein), transcription enzymes and factors (12 gene products) constitute the largest functional category associated with the viral particles. This set includes all five predicted DNA-directed RNA polymerase subunits, two helicases (R350, L540), the mRNA capping enzyme and four transcription factors (L377, L538, L544, R563), including a TATA box-like binding protein. The presence of a complete transcription machinery in mimivirus particles is reminiscent of poxviruses. The next largest functional group contains nine gene products associated with oxidative pathways. Protein/lipid modification enzymes are also well represented, including a phosphoesterase and a lipase, which are eventually used for digesting the cell (vacuole) membrane, two protein kinases and a protein phosphatase. Finally, seven proteins associated with DNA topology, damage repair and replication are in the virion, including topoisomerases IA and IB (R194, L221), a DNA UV damage repair endonuclease (L687) and two types (X and B) of DNA polymerases (L318, R322).

### Lipids

The interior of the particle appears to exhibit two lipid membranes, one encircling a dark core (nucleic acid) and one in direct contact with the interior of the protein capsid. However, this membrane organization might also result from the tight folding of a single membrane. Once the infecting virion is located within a phagocytic vacuole, the particle stargate opens allowing fusion of at least one of the mimivirus lipid membranes with the vacuole membrane. This fusion directly delivers the core of the particle into the host cytoplasm where it serves as a seed to initiate the formation of a

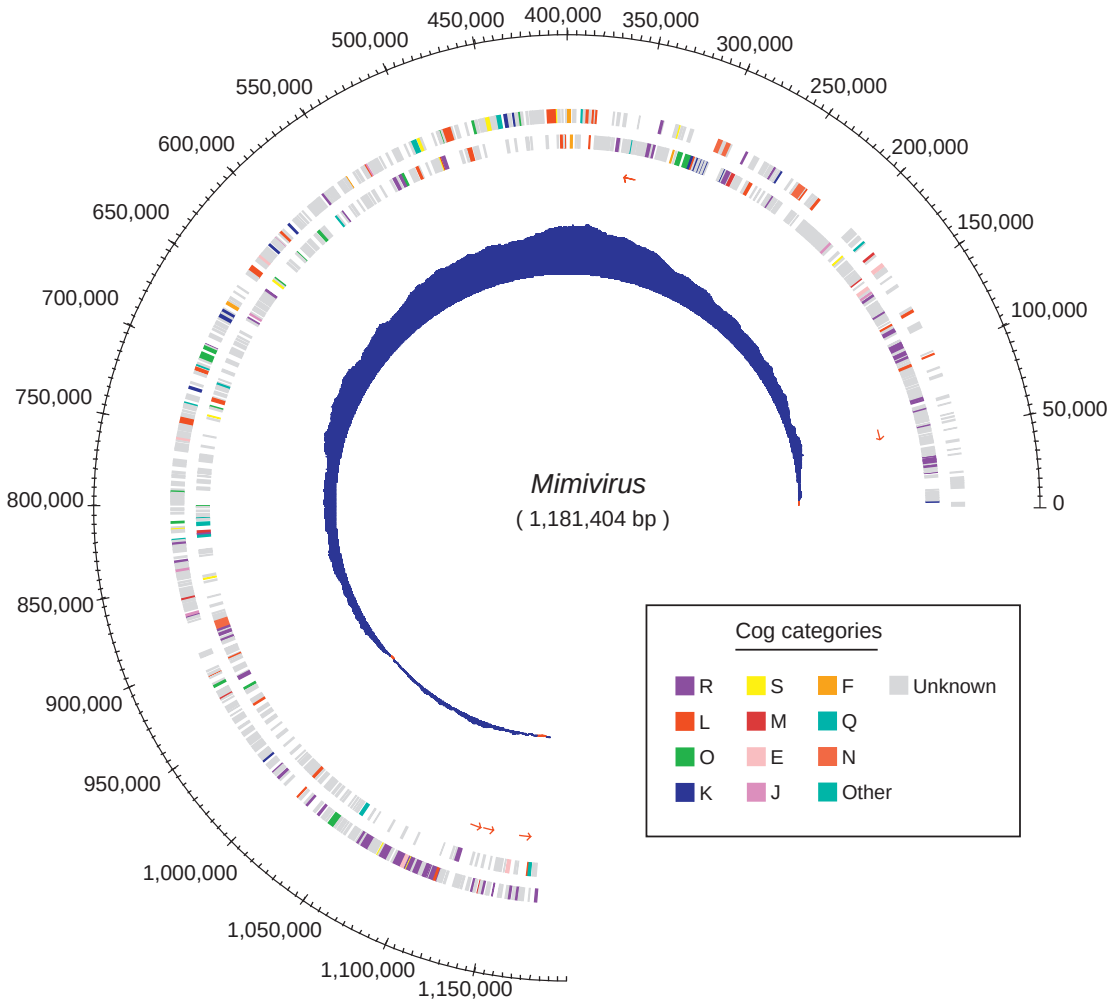


**Figure 3:** Map of the mimivirus chromosome. The predicted protein coding sequences are shown on both strands and colored according to the function category of their matching COG. Genes with no COG match are shown in gray. Abbreviations for the COG functional categories are as follows: E, amino acid transport and metabolism; F, nucleotide transport and metabolism; J, translation; K, transcription; L, replication, recombination, and repair; M, cell wall/membrane biogenesis; N, cell motility; O, posttranslational modification, protein turnover, and chaperones; Q, secondary metabolites biosynthesis, transport, and catabolism; R, general function prediction only; S, function unknown. Small red arrows indicate the location and orientation of tRNAs. The A+C excess profile is shown on the innermost circle, exhibiting a peak around position 380,000. (From Raoult *et al.* (2004). *Science*, **306**, 1344–1350; with permission of AAAS.)

virion factory. As expected from the crucial role likely to be played by the internal lipid membranes of the particle in the infection process, mimivirus virions are readily inactivated by treatment with lipophilic compounds (such as DMSO).

### Carbohydrates

The ultra-structure of the mature particle (as observed by electron or atomic-force microscopy) as well as preliminary biochemical and mass spectrometry analyses suggest that the outer fiber layer might consist of a biopolymer similar to peptidoglycan. Accordingly, the mimivirus genome encodes a number of proteins homologous to enzymes key to the synthesis of bacterial cell walls. Among those proteins, the functions of a UDP-D-glucose 4,6-dehydratase (R141) and of a bifunctional UDP-4-keto-6-deoxy-D-glucose epimerase/reductase (L780) have been experimentally validated.

## Genome organization and replication

The initial mimivirus genome annotation predicted 911 protein-coding genes and 6 tRNAs (Figure 3). More recent data obtained through transcriptome sequencing (RNA-Seq) and deep genome

resequencing allowed the identification of a total of 1018 genes, including 979 protein-coding genes, 6tRNAs and 33 non-coding mRNAs. The latest genome sequence and the most current annotation (including the location of identified promoter signals and known 5′-end and 3′-end transcript boundaries) is available in the RefSeq database under accession number NC_014649.1, and in GenBank under accession number HQ336222.

The penetration of the particle inner core within the host cytoplasm is followed by a complete eclipse phase that lasts approximately two hours in *Acanthamoeba castellanii* (ATCC 30010), after which time mimivirus virion factories become visible. Mimivirus replication entirely takes place in the cytoplasm of the host *Acanthamoeba* cell, through the successive expression of early (from 0 to 3 h post infection), intermediate (from 3 h to 6 h post-infection) and late (after 6 h post-infection) transcripts, each gene class representing approximately one-third of the mimivirus genome. The virion factories develop from the core of individual uncoated virus particles (seeds). The earliest viral transcripts are detected as soon as 15 minutes post infection, most likely produced by the viral transcription machinery within the uncoated particles. Most of the genes involved in nucleotide synthesis and DNA replication are transcribed from 3 h to 6 h post-infection. Late genes (after 6 h) include virion structural components, as well as most of the virally-encoded transcription apparatus components. This expression pattern suggests that the early and intermediate mimivirus transcripts detected before the appearance of fully mature cytoplasmic virion factories are generated by the transcription apparatus associated with the virion core. Mimivirus particles (at least one thousand per infected cell) are continually produced for up to 12 h by the growing virion factories (up to 6 µm in diameter) (Figure 4). Mature mimivirus particles increasingly fill the host cytoplasm and are progressively released from the dying cell. No budding or sudden cell bursts are seen.

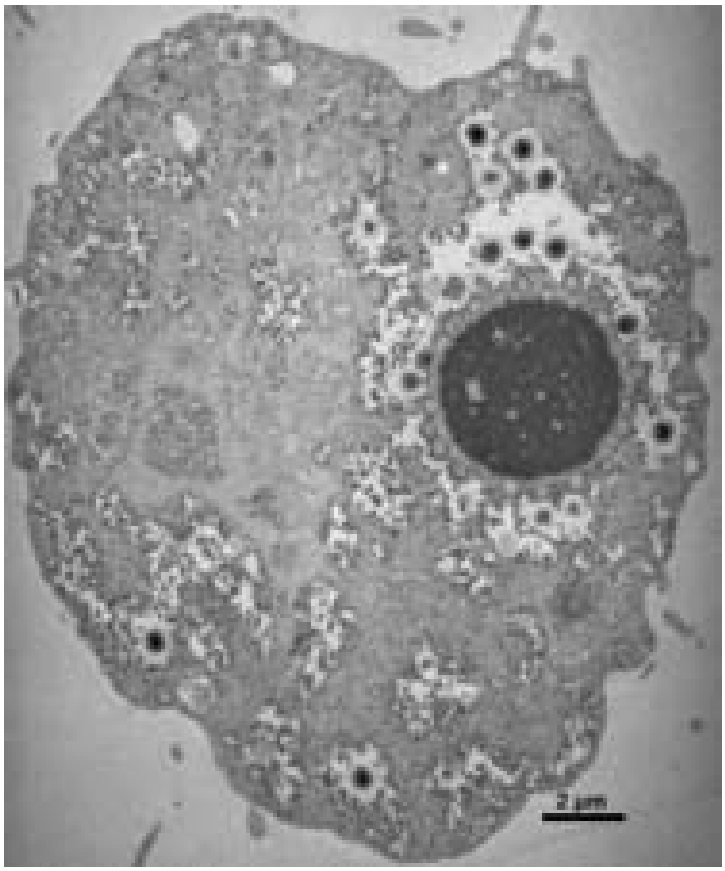


**Figure 4:** The distinctive giant mimivirus virion factory in full production (8 h post infection in *Acanthamoeba castellanii*). The dark circle (about 4.5 µm in diameter) is the virion factory from which mimivirus particles can be seen emerging, first empty, then filled with a dense core, then covered with their outer fiber layer (transmission electron microscopy).

## Antigenic properties and pathogenicity

Acanthamoeba polyphaga mimivirus was isolated from the water of a cooling tower in Bradford, England. Mimivirus readily infects many *Acanthamoeba* strains, including its preferred laboratory host *Acanthamoeba castellanii*. Metagenomic surveys indicate that close relatives of the *Mimiviridae* family are prevalent in the sea, where they probably infect marine heterotrophic protists and regulate plankton populations.

Although mimivirus was isolated in the context of a pneumonia epidemic and initially thought to be an emerging human pathogen based on positive serology, subsequent more specific PCR-based studies failed to detect mimivirus in large numbers of pneumonia patients. As expected from the size of its particle, mimivirus is internalized by various professional phagocytic cells, including human macrophages *in vitro*. However, the recent demonstration that mimivirus particles are recognized by the sera of patients infected by *Francisella tularensis*, casts serious doubt on previously published serological evidence for mimivirus infection of humans. At the moment, there is little evidence that mimivirus is a human pathogen.

## Biological properties

The presence of many proteins never seen in any other virus is one of the unique features of mimivirus. In addition to the full DNA replication and transcription apparatus usually found in large eukaryotic DNA viruses (such as the poxviruses), mimivirus encodes many enzymes central to the translation apparatus such as four aminoacyl-tRNA synthase: Tyr-RS, Met-RS, Arg-RS and Cys-RS. Mimivirus also possesses the first virally-encoded nucleotide diphosphate kinase (NDK), mismatch repair MutS-homolog, and tRNA (Uracil-5-)-methyltransferase. The mimivirus genome also encodes several sugar-manipulating enzymes likely to be involved in the biosynthesis of the particle outer layer. Crystallographic 3D structures have been obtained for the following mimivirus proteins: Tyr-RS (L124), NDK (R418), formamidopyrimidine-DNA glycosylase (L315), sulfhydryl oxidase (R596), mRNA-capping enzyme (R382), and mimivirus cyclophilin (L605). A new type of satellite virus (called the Sputnik virophage) has been isolated in association with a new strain of mimivirus (called mamavirus).

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Mimivirus*

| | | |
|---|---|---|
| *Acanthamoeba polyphaga mimivirus* | | |
| Acanthamoeba polyphaga mimivirus (Rowbotham-Bradford) | [HQ336222 = NC_014649] | (APMV) |
| Acanthamoeba polyphaga mamavirus (La Scola-Paris) | [EU827539, EU827540, EU827541] | (APMV2) |
| *Megavirus chilensis* | | |
| Megavirus chilensis (Claverie-Las Cruces) | [JN258408] | (McV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Mimivirus* but have not been approved as species

Terravirus
Courdovirus
Moumouvirus

## Derivation of name

*Mimi*: for *mimi*cking microbe.

## Further reading

### Journals and books

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### Websites

Results for Mimivirus at http://www.igs.cnrs-mrs.fr

## Contributed by

Claverie, J-M. and Abergel, C.

# Family *Nimaviridae*

## Taxonomic structure of the family

Family *Nimaviridae*
Genus *Whispovirus*

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Whispovirus*

Type species *White spot syndrome virus*

## Virion properties

### Morphology

Virions are ovoid or ellipsoid to bacilliform in shape, have a regular symmetry, and measure 120–150 nm in diameter and 270–290 nm in length. Most notable is the thread- or flagellum-like extension (appendage) at one end of the virion. The virion consists of an inner, rod-shaped nucleocapsid with a tight-fitting capsid layer, an intermediate tegument layer and an outer lipid-containing trilaminar envelope. The isolated nucleocapsid typically measures 65–70 nm in diameter and 300–350 nm in length. It contains a DNA-protein core bounded by a distinctive capsid layer, giving it a cross-hatched or striated appearance (Figure 1).

### Physicochemical and physical properties

Virions have a buoyant density of 1.22 g cm$^{-3}$ in CsCl, whereas the nucleocapsids have a buoyant density of 1.31 g cm$^{-3}$. The virions are sensitive to detergents. Other properties are not known.

### Nucleic acid

The nucleocapsid contains a single molecule of circular dsDNA with an approximate size of 300 kbp. The G+C ratio of white spot syndrome virus (WSSV) is about 41%.

### Proteins

The virions contain at least six major and at least 35 medium or minor polypeptides ranging in size from 14 to 664 kDa. The three structural elements of the virion each contain two major proteins: VP28 and VP19 in the envelope, VP26 and VP24 in the tegument, and VP664 and VP15 in the nucleocapsid. VP15 is a very basic, histone-like DNA-binding nucleoprotein. The three major proteins, VP28, VP26 and VP24, are phylogenetically related.

### Lipids

Lipids are present in the envelope. The composition of lipids is known for virions purified from the crayfish *Procambarus clarkii*. The lipids are of host origin and are derived from host-cell nuclei.

### Carbohydrates

None of the major virion structural proteins is glycosylated.

## Genome organization and replication

The WSSV genome has, depending on the isolate, about 181 non-overlapping ORFs and almost equally distributed over both DNA strands (WSSV-CN; Figure 2). Only a few genes (20%) have been identified. These genes encode virion proteins (see below), proteins involved in DNA replication (DNA polymerase, ribonucleotide reductase subunits, dUTPase, thymidylate synthase, thymidine-thymidylate kinase) and protein-modifying proteins (protein kinase). The virion protein genes are not clustered, but are dispersed on both strands of the WSSV genome. The WSSV genome includes a very large gene (*vp664*) that encodes a major nucleocapsid protein of about 664 kDa. Most ORFs are unassigned. WSSV has also been shown to contain genes homologous to *Marsupenaeus*

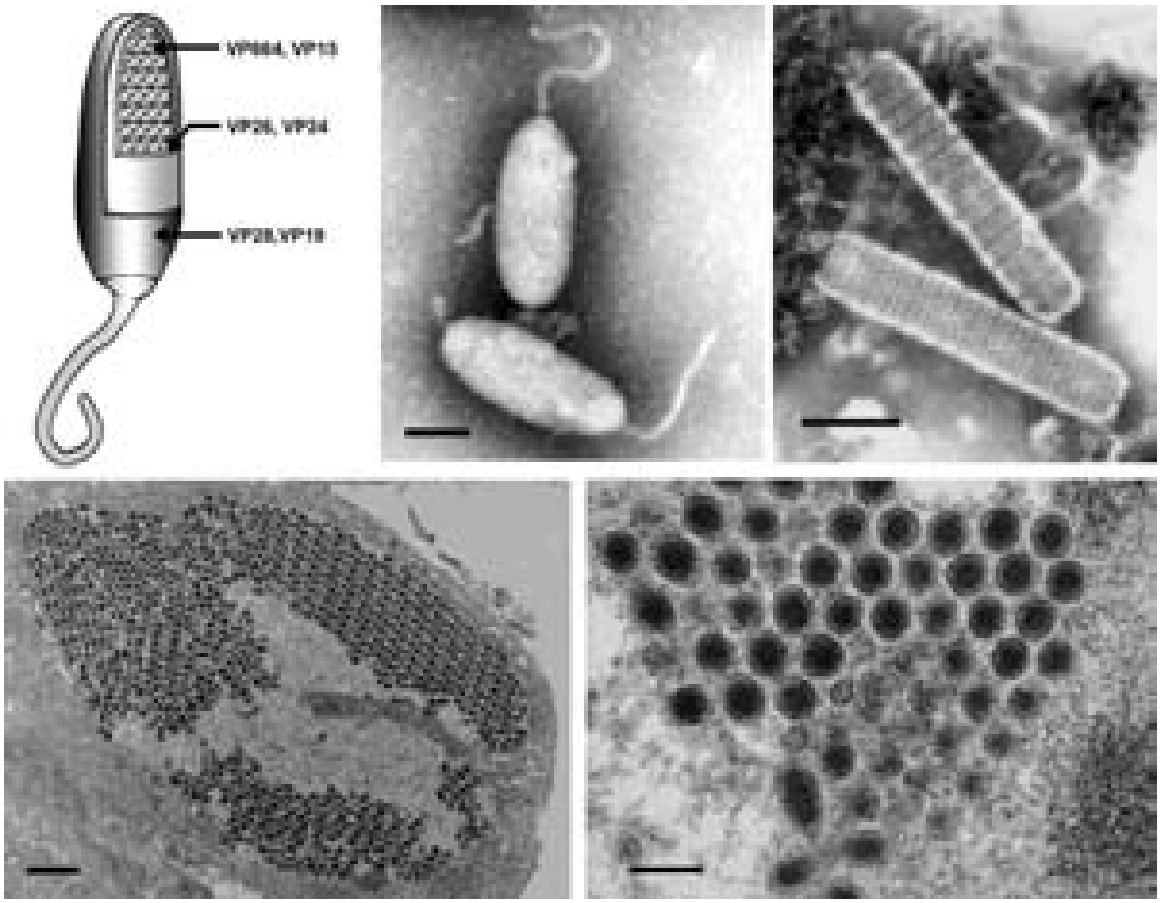


**Figure 1:** (Top) Morphology of virions of white spot syndrome virus (WSSV). (Left) Schematic illustration of the structure of a typical whispovirus virion. (Top center and right) Negative contrast electron micrographs of WSSV virions (center, courtesy of Marielle van Hulten) and nucleocapsids (right, courtesy of Don Lighter) from hemolymph of infected *Penaeus monodon*. The bars represent 100 nm. (Bottom left) Thin section of WSSV-infected stomach epithelium showing the parallel arrangement of virions in the nucleoplasm (the bar represents 1 µm) and (bottom right) a cross-section of virions (the bar represents 1 µm) (courtesy of Don Lightner).

*japonicus* and to *Penaeus monodon*. These may exemplify mimicry of the structure and function of host genes, e.g. to avoid detection and destruction by the host immune system, a stratagem used by other dsDNA viruses. Alternatively they may be degenerated proviral remnants.

The WSSV genome is further characterized by the presence of nine homologous repeat regions (Hr1-9; Figure 2). The number of imperfect palindromic repeats (250 bp in size) within an hr varies per isolate.

Viral transcripts are often but not always polyadenylated and they are usually capped. There is no evidence to suggest the occurrence of RNA splicing. Three immediate early genes have been identified in WSSV, and WSSV early genes and one of the immediately early genes has a promoter motif (-[a,c][a,c]TCAxT-) that matches the RNA polII core promoter of the Arthropods. The WSSV late genes' consensus promoter (-A[a,t][a,t,g]AC-) is usually less than 100 nt (nucleotides) from the transcriptional start site. Consensus TATA box sequences have been found at about 25 bp upstream of early gene transcription initiation sites. Late transcripts seem to start 25 nt downstream of an A/T-rich region. Structural protein genes widely use polycistronic mRNAs and internal ribosome entry site elements to regulate their tranlsation.

Replication of WSSV occurs in the nucleus, where the virions are also assembled (Figure 1).

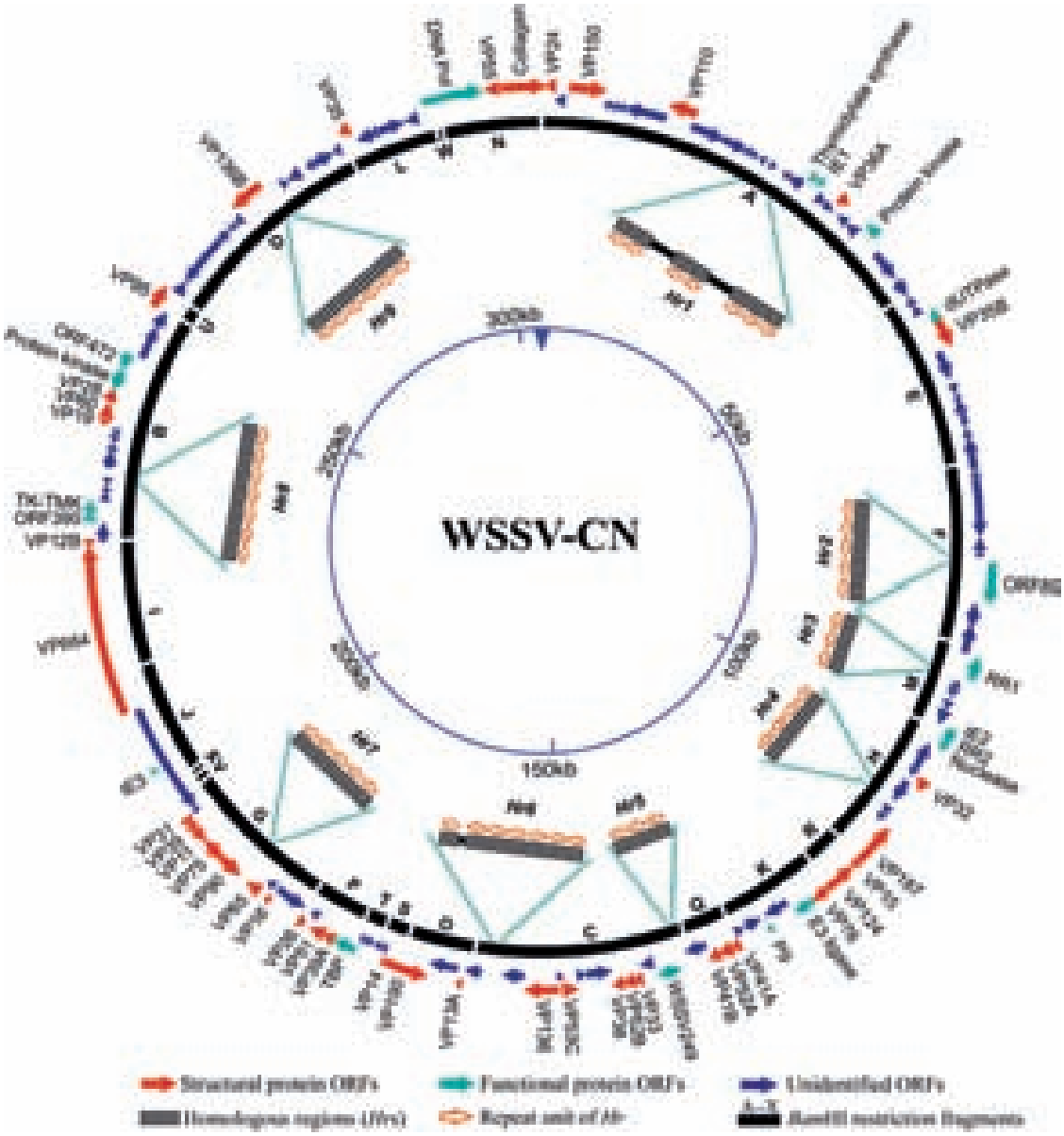


**Figure 2:** Schematic map of the circular dsDNA white spot syndrome virus type strain CN (WSSV-CN) genome showing the genomic organization. The solid arrows indicate the positions and direction of transcription of corresponding genes. The G at the start (GGATCC) of the largest *Bam*HI fragment is designated position 1.

Genotypic variants exist that can be distinguished by restriction length polymorphism and on the basis of genomic sequence. Three WSSV isolates have been sequenced and they vary in size from 297,967 nt (WSSV-TH) to 305,107 nt (WSSV-CN) and 307,287 nt (WSSV-TW). Major differences can be attributed to deletions in WSSV-TH between 275,238 and 287,285, and between 267,203 and 268,046 and to the insertion of a 1.3 kb transposase sequence in WSSV-TW at 204,978–204,979 (Figure 2).

## Antigenic properties

Polyclonal and monoclonal antibodies have been raised against WSSV virions, and they can be used as diagnostic tools. In recent years, researchers have found that the virus infection can be

neutralized with antisera against the envelope proteins VP28, VP31, VP33 (VP36B) and VP12B (VP68). From this, it can be inferred that these proteins are involved in WSSV infection.

## Biological properties

### Host range

WSSV can infect a wide range of aquatic crustaceans including salt, brackish and fresh water penaeids, crabs and crayfish.

### Transmission

The virus is transmitted by mouth by cannibalism of diseased individuals or via water through the gills. The virus can also be transmitted vertically from adults to offspring, either by being released from non-viable WSSV-infected eggs or from the supporting cells in ovarian tissue.

### Geographical distribution

WSSV has been identified from crustaceans in China, Japan, Korea, Southeast Asia, South Asia, the Middle East and the Americas. An unusual property of the virus is the extremely wide host range despite a very low level of genetic polymorphism. The latter suggests that white spot disease (WSD) is a relatively recent epizootic.

### Cytopathic effects

The major targets of WSSV infection are tissues of ectodermal and mesodermal embryonic origin such as subcuticular epithelial cells (including those of the shrimp stomach), gills, lymphoid organ and connective tissue. Although WSSV infects the underlying connective tissue in the shrimp hepatopancreas and midgut, the columnar epithelial cells of these two organs are of endodermal origin, and they do not become infected. WSSV can grow in primary cultures of lymphoid organ and ovary. Infection sometimes causes disease and sometimes not, depending upon factors as yet poorly understood but related to species tolerance and environmental triggers. Shrimp and other crustaceans susceptible to disease from this virus show gross signs of lethargy, such as lack of appetite and slow movement, and often a reddish coloration of the whole body together with "white spots" embedded within the exoskeleton. These spots are the result of calcified deposits that range in size from a few millimeters to 1 or more centimeters in diameter. However, in some cases such as acute experimental infections resulting from injected viral preparations, there may be no gross signs of infection other than lethargy and lack of appetite. Most affected animals die within 3–10 days after infection. With an appropriate infection dose to allow sufficient time before mortality, animals susceptible to disease show large numbers of virions circulating in the hemolymph, but this may also occur for tolerant species that show no mortality. Thus, high viral loads *per se* do not cause disease or mortality for all susceptible species.

## Species demarcation criteria in the genus

Only a single species within the genus has been identified to date (*White spot syndrome virus*). Various isolates with small genetic polymorphisms have been identified (variants). It should be realized, however, that as the *Nimaviridae* is a newly recognized family, the species concept is subject to change after existing and new isolates have been studied in more detail.

## List of species in the genus *Whispovirus*

*White spot syndrome virus*

| | | |
|---|---|---|
| White spot syndrome virus - CN | [AF332093] | (WSSV-CN) |
| White spot syndrome virus -TH | [AF369029] | (WSSV-TH) |
| White spot syndrome virus -TW | [AF440570] | (WSSV-TW) |
| White spot syndrome virus | | |
| Chinese baculo-like virus | | |
| Hypodermic and hemotopoietic necrosis baculovirus | | |
| Penaeus monodon non-occluded baculovirus II | | |
| Penaeus monodon non-occluded baculovirus III | | |
| Penaeus monodon rod-shaped nuclear virus | | |

Systemic ectodermal and mesodermal baculovirus
White spot bacilliform virus
White spot baculovirus

Species names are in italic script; names of strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.



## List of other related viruses which may be members of the genus *Whispovirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Nimaviridae*

None reported.

## Phylogenetic relationships within the family

Not applicable.

## Similarity with other taxa

Morphologically, the WSSV virions and rod-shaped nucleocapsids resemble the budded virus particles of the baculoviruses, polydnaviruses and the unassigned Oryctes rhinoceros virus. The presence of repeat regions dispersed over the DNA genome is a property shared with members of the families *Baculoviridae* and *Ascoviridae*, the proposed *Hytrosaviridae* family and the unassigned nudiviruses. WSSV is phylogenetically distinct from other large DNA viruses (Figure 3).

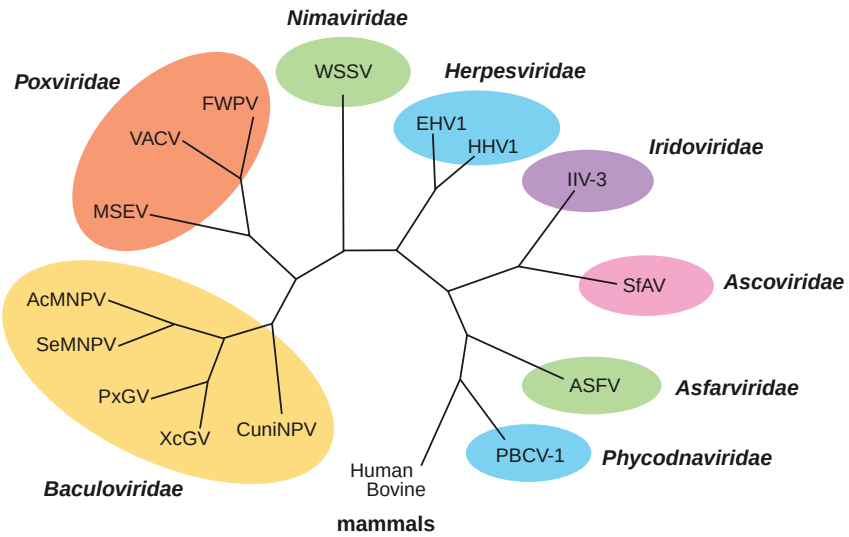


**Figure 3:** Cladogram based on genetic distances between aa sequences derived from the DNA polymerase gene of WSSV and a number of other large DNA viruses, as well as two mammalian representatives.

## Derivation of name

*Nima*: Latin for "thread"; referring to the thread- or tail-like polar extension (appendage) on the virus particle.

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## Contributed by

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# Family *Papillomaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Papillomaviridae*** |
| Genus | ***Alphapapillomavirus*** |
| Genus | ***Betapapillomavirus*** |
| Genus | ***Gammapapillomavirus*** |
| Genus | ***Deltapapillomavirus*** |
| Genus | ***Epsilonpapillomavirus*** |
| Genus | ***Zetapapillomavirus*** |
| Genus | ***Etapapillomavirus*** |
| Genus | ***Thetapapillomavirus*** |
| Genus | ***Iotapapillomavirus*** |
| Genus | ***Kappapapillomavirus*** |
| Genus | ***Lambdapapillomavirus*** |
| Genus | ***Mupapillomavirus*** |
| Genus | ***Nupapillomavirus*** |
| Genus | ***Xipapillomavirus*** |
| Genus | ***Omikronpapillomavirus*** |
| Genus | ***Pipapillomavirus*** |

## Virion properties

### Morphology

Virions are non-enveloped, 55 nm in diameter. The icosahedral capsid is composed of 72 capsomers in skewed (T = 7) arrangement (Figure 1). Filamentous and tubular forms are observed as a result of aberrant maturation.

### Physicochemical and physical properties

The virion Mr is $47 \times 10^6$. Buoyant density of virions in sucrose and CsCl gradients is 1.20 and 1.34–1.35 g cm$^{-3}$, respectively. Virion $S_{20,W}$ is 300. Virions are resistant to ether, acid and heat treatment (50 °C, 1 h).

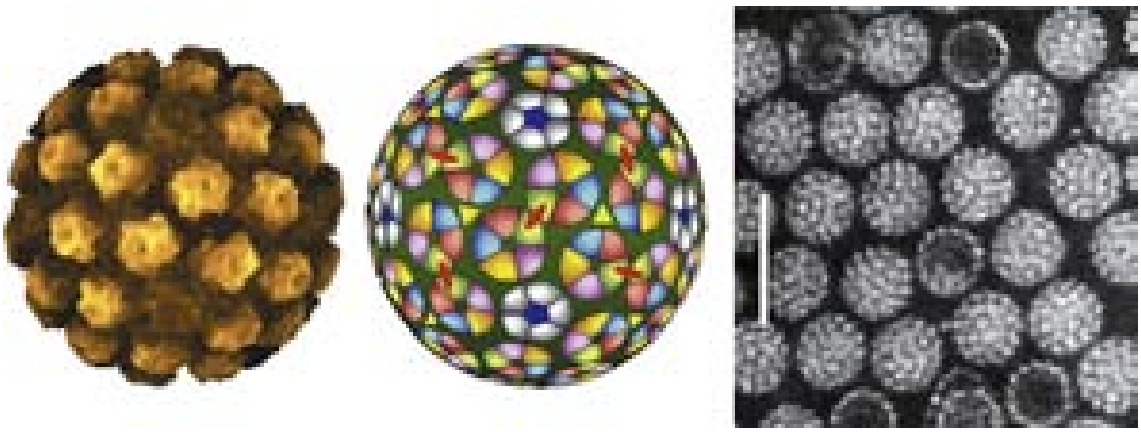

**Figure 1:** (Left) Atomic rendering of a papillomavirus capsid. Derived from an image reconstruction from electron cryomicroscopy of bovine papillomavirus (BPV) at 9 Å resolution combined with coordinates from the crystal structure of small virus-like particles of the human papillomavirus 16 (HPV-16) L1 protein (from Modis *et al.* (2002). *EMBO J.*, **21**, 4754–4762). (Centre) Schematic diagram representing the 72 capsomers in a T = 7 arrangement of a papillomavirus capsid. The icosahedral structure includes 360 VP1 subunits arranged in 12 pentavalent and 60 hexavalent capsomers. (Right) Negative contrast electron micrograph of human papillomavirus 1 (HPV-1) virions. The bar represents 100 nm.

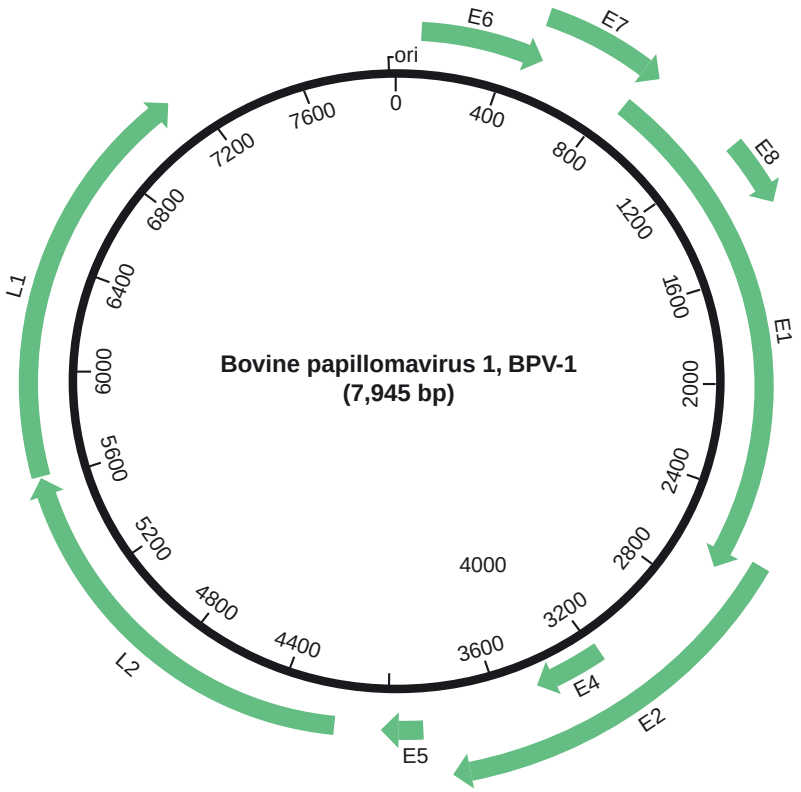


**Figure 2:** Diagram of the bovine papillomavirus 1 (BPV-1) genome. The viral dsDNA (size in bp, origin of replication: ori) is indicated. The outer arrows indicate the protein-coding ORFs and their direction of transcription.

### Nucleic acid

Virions contain a single molecule of circular dsDNA. The genomic size ranges between 6800 and 8400 bp. The DNA constitutes about 10–13% of the virion by weight. The G+C content is 40–60%. In the mature virion, the viral DNA is associated with host cell histone proteins H2a, H2b, H3 and H4 in a chromatin-like complex.

### Proteins

The virus genomes encode 8–10 proteins with sizes ranging from 7 to 73 kDa (Table 1). L1 and L2 make up the capsid. E1 and E2 are involved in replication and in intragenomic regulation (E2). E5, E6 and E7 induce cellular DNA replication. E4 may represent a late function and binds to specific cytoskeleton structures. Genetic evidence has not been presented that associates specific viral proteins with the E3 and E8 ORFs.

### Lipids

None present.

### Carbohydrates

None present.

## Genome organization and replication

Virions that attach to cellular receptors are engulfed by the cell, and the DNA is uncoated and transported to the nucleus. During productive infection, transcription of the viral genome is divided into early and late stages.

Transcription of the early and late ORFs occurs from the same strand in one direction only. Precursor mRNAs undergo post-transcriptional processing, which includes capping and

**Table 1:** Deduced molecular masses of papillomavirus proteins (kDa)

| Virus | CRPV | BPV-1 | HPV-1 |
|---|---|---|---|
| Structural proteins | | | |
| L1 | 57.9 | 55.5 | 59.6 |
| L2 | 52.8 | 50.1 | 50.7 |
| Nonstructural proteins | | | |
| E1 | 67.9 | 68.0 | 73.0 |
| E2 | 44.0 | 48.0 | 41.8 |
| E4 | 25.8 | 12.0 | 10.4 |
| E5 | 11.3 | 7.0 | 9.4 |
| E6 | 29.7 | 15.1 | 19.2 |
| E7 | 10.5 | 14.0 | 11.0 |

Abbreviations: CRPV, cottontail rabbit papillomavirus; BPV, bovine papillomavirus; HPV, human papillomavirus.

polyadenylation of the 5′ and 3′ termini, respectively, as well as splicing. Efficient use of coding information involves differential splicing of the RNAs and utilization of overlapping ORFs. Early mRNAs encode regulatory proteins that may exhibit trans-activating properties. These include proteins that are required for DNA replication. Their expression leads to depression of some host cell enzymes and may also stimulate host cell DNA synthesis. Prior to the start of late events, viral DNA replication is initiated in the nucleus. Translation of the late transcripts produces structural proteins that are involved in capsid assembly. Post-translational modifications of some early and late viral proteins include phosphorylation, N-acetylation, ADP ribosylation and other events. Several of the viral proteins contain sequences, termed nuclear localization signals, which facilitate transport of the proteins to the host cell nucleus where virion maturation occurs. Virions are released by lysis of the virus-producing cells.

The genomes of most members of the family *Papillomaviridae* that have been sequenced contain 9–10 ORFs, labelled E1–E8 and L1–L2 (Figure 3). Some members lack the E3 and E8 ORFs. Proteins encoded by the E ORFs, with the possible exception of E4, represent non-structural polypeptides involved in transcription, DNA replication and transformation, whereas those encoded by the L ORFs represent structural proteins. Replication of the viral genome is initiated bi-directionally by specific binding of the E1 and E2 proteins at a unique origin of replication.

## Antigenic properties

The L1 protein contains type-specific domains, and the L2 protein contains group-specific epitopes (see section on species demarcation for descriptions of groups and types). The availability of papillomavirus-like particles, resulting from the expression of L1 or L1 and L2 in baculovirus, vaccinia virus or yeast systems, has permitted a detailed analysis of antigenic characteristics.

## Biological properties

Papillomaviruses are highly host species-specific and tissue-restricted. All known human papillomaviruses (HPVs) require terminal differentiation for replication and virion production. Infection appears to occur mainly via microlesions of proliferating basal layer cells. Except for inefficient replication of HPVs in raft cultures of human keratinocytes, or more efficiently in human skin or mucosal xenografts in immunocompromised rodents, HPV replication has not been achieved in cell culture systems.

Virus spread occurs by release of virions from the surface of warts and papillomatous lesions, which frequently contain large quantities of viral particles within the superficial differentiated

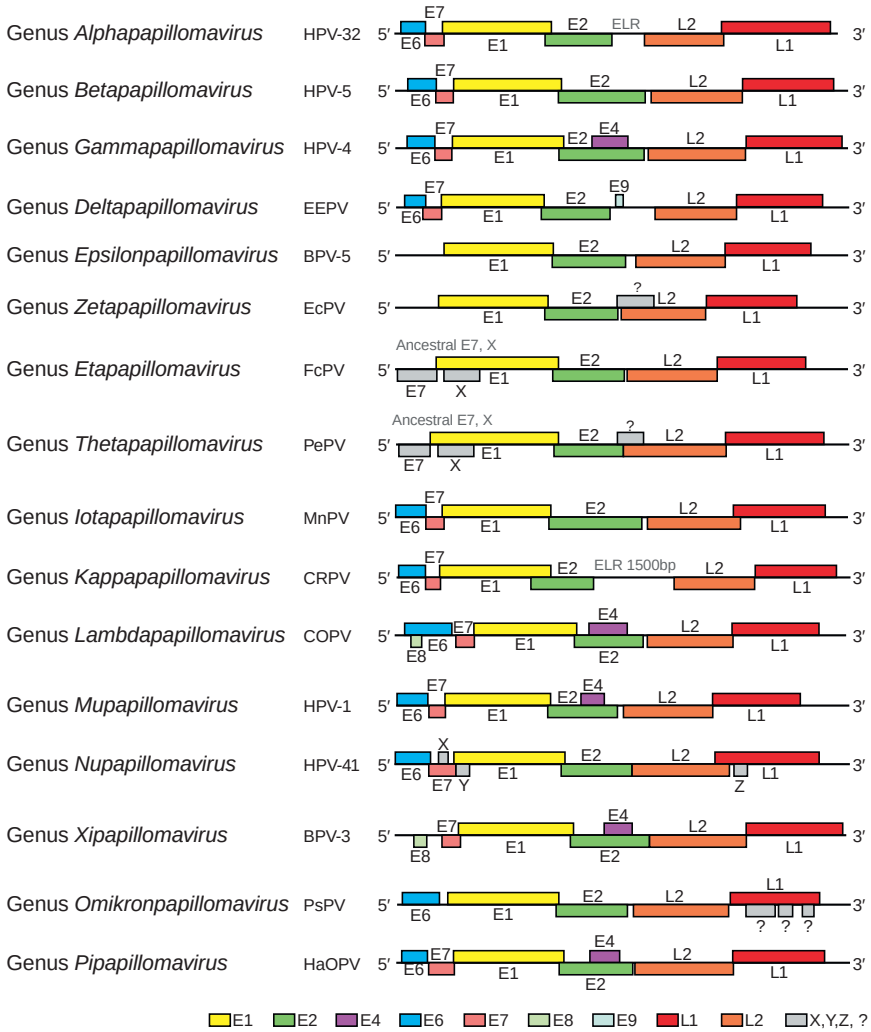


**Figure 3:** Comparison of genome organization for the viruses corresponding to the type species of each genus in the family *Papillomaviridae*. The circular dsDNA genomes have been linearized, with ori as the opening site (see Figure 2). Similar ORFs are indicated in similar colors. For abbreviations, see species lists in text.

layers. Virus reactivation is particularly frequent under conditions of immunosuppression. The mode of viral DNA persistence and possible clearance of HPV infections by immunological interference are still poorly investigated.

Transmission of viral infections occurs by close contacts. Papillomavirus types are distributed worldwide. They cause benign tumors (warts and papillomas) in their natural host and occasionally in related species. Frequently the infection leads to microlesions, which are barely or not at all visible without optical aid. Papillomas are induced in the skin and in mucous membranes, often at specific sites of the body. Some papillomatous proliferations induced by specific types of papillomaviruses bear a high risk for malignant progression. Specific human cancers (e.g. cervical carcinoma, anal, vulval and penile cancers, and specific squamous cell carcinomas of the skin) have been linked to certain types of HPV infection (e.g. by HPV-16 and HPV-18, HPV-5 and HPV-8, and several others). Viral DNA is often, but not always, present in an integrated form, particularly in cervical cancers, whereas skin carcinomas appear to harbor the viral genome in an episomal state. Cancer-linked anogenital HPV types efficiently immortalize a wide variety of human cells in tissue culture. Immortalization results from functions of the E6 and E7 genes, which act cooperatively, although both genes are able to immortalize human cells independently at low efficiency. E6 binds and degrades the cellular p53 protein and stimulates the telomerase enzyme, whereas E7 interacts

with the cellular pRB and some related proteins, and directly activates cyclins E and A. Interactions of the viral oncoproteins with cellular cyclin-dependent kinase inhibitors (p16, p21 and p27) also emerge as important events in immortalization.



### Species demarcation criteria in the family

The demarcation of papillomavirus species by phenotypic criteria similar to those applied to other families of viruses is problematic for a variety of reasons. One is that papillomaviruses do not elicit consistent humoral immune responses in infected human or other mammalian individuals, and it is therefore not possible to develop a taxonomy based on serology. The lack of reliable cell culture and laboratory animal host systems represents further limitations. Moreover, the coverage of different papillomaviruses in the scientific literature is very heterogenous, ranging from scattered single publications addressing individual, rare papillomaviruses to the body of 20,000 biological, medical and epidemiological publications addressing HPV-16 and HPV-18, the causes of cervical cancer. From the beginning of papillomavirus nomenclature in the 1930s, researchers were confronted with the problem of providing succinct names and distinguishing criteria for viruses that share many characteristics, such as similar genome sizes, similar target tissue properties (e.g. mucosal and cutaneous), and similar etiologies ranging from latent infections to various forms of neoplasia. In spite of these limitations, two principal pillars for papillomavirus taxonomy emerged. (1) All known papillomaviruses are strictly host species-specific, and this restriction needs to be reflected in the taxonomy. (2) DNA sequence comparisons led to refined phylogenetic studies, which show that all papillomavirus genomes are monophyletic in origin, that they evolve more slowly than virtually any other group of viruses, and that they do not recombine. The topology of phylogenetic trees is an indispensable criterion for taxonomic evaluation of this virus family.

From their roots nearly 80 years ago as recognized agents of disease, papillomaviruses have been described as "types". This universal usage could potentially have led to the types being defined as species. However, the very large number of types prompted species to be set at a higher level, with the result that many species contain more than one type, with the species name derived from a prominent type in the species. The various species are also well supported by distinct biological properties. Similarly, genera were defined by phylogenetic considerations, relationships between host species and major differences in genome organization. More details on these issues, including the quantitative criteria utilized to define types, species and genera, are discussed in de Villiers *et al.* (2004) and Bernard *et al.* (2010). The latter paper contains extensive proposals to recognize additional species and genera, and to rationalize papillomavirus taxonomy generally. These proposals are currently under consideration by the ICTV.

## Genus *Alphapapillomavirus*

Type species ***Human papillomavirus 32***

### Distinguishing features

Members of this genus preferentially infect the oral or anogenital mucosa in humans and primates. Members of certain species (e.g. *Human papillomavirus 2* and *Human papillomavirus 10*) are also found in lesions of cutaneous sites. Members of some species (e.g. *Human papillomavirus 16* and *Human papillomavirus 18*) are considered as high-risk in view of their regular presence in malignant tissue and their *in vitro* transforming activities. Members of other species (e.g. *Human papillomavirus 53*, *Human papillomavirus 26* and *Human papillomavirus 34*) cause malignant or benign lesions, whereas low-risk viruses (in *Human papillomavirus 61*, *Human papillomavirus 7*, *Human papillomavirus 6*, *Human papillomavirus 54*, *Human papillomavirus cand90* and *Human papillomavirus 71*) mainly cause benign lesions. Genome organization: an E5 ORF is conserved between the early and late coding regions.

### List of species in the genus *Alphapapillomavirus*

*Human papillomavirus 2*
Human papillomavirus 2 [X55964] (HPV-2)
*Human papillomavirus 6*
Human papillomavirus 6

| | | |
|---|---|---|
| *Human papillomavirus 7* | | |
| Human papillomavirus 7 | [X74463] | (HPV-7) |
| *Human papillomavirus 10* | | |
| Human papillomavirus 3 | [X74462] | (HPV-3) |
| Human papillomavirus 78 | | (HPV-78) |
| Human papillomavirus 94 | [AJ620021] | (HPV-94) |
| *Human papillomavirus 16* | | |
| Human papillomavirus 16 | [K02718] | (HPV-16) |
| *Human papillomavirus 18* | | |
| Human papillomavirus 18 | [X05015] | (HPV-18) |
| *Human papillomavirus 26* | | |
| Human papillomavirus 26 | | |
| *Human papillomavirus 32* | | |
| Human papillomavirus 32 | | |
| *Human papillomavirus 34* | | |
| Human papillomavirus 34 | [X74476] | (HPV-34) |
| *Human papillomavirus 53* | | |
| Human papillomavirus 30 | [X74474] | (HPV-30) |
| *Human papillomavirus 54* | | |
| Human papillomavirus 54 | [U37488] | (HPV-54) |
| *Human papillomavirus 61* | | |
| Human papillomavirus 61 | | |
| *Human papillomavirus 71* | | |
| Human papillomavirus 71 | [AB040456] | (HPV-71) |
| *Human papillomavirus cand90* | | |
| Human papillomavirus cand90 | [AY057438] | (HPV-cand90) |
| *Rhesus monkey papillomavirus 1* | | |
| Rhesus monkey papillomavirus 1 | [M60184] | (RhPV-1) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Alphapapillomavirus* but have not been approved as species

None reported.

## GENUS *BETAPAPILLOMAVIRUS*

Type species ***Human papillomavirus 5***

### Distinguishing features

Members of this genus preferentially infect the skin of humans. These infections are latent in the general population, but are activated under conditions of immunosuppression. Members of the species *Human papillomavirus 5*, *Human papillomavirus 9* and *Human papillomavirus 49* are also associated with the disease epidermodysplasia verruciformis. Genome organization: E5 ORF is absent.

### List of species in the genus *Betapapillomavirus*

| | | |
|---|---|---|
| *Human papillomavirus 5* | | |
| Human papillomavirus 5 | [M17463] | (HPV-5) |
| *Human papillomavirus 9* | | |
| Human papillomavirus 9 | [X74464] | (HPV-9) |
| *Human papillomavirus 49* | | |
| Human papillomavirus 49 | [X74480] | (HPV-49) |
| *Human papillomavirus cand92* | | |
| Human papillomavirus cand92 | [AF531420] | (HPV-cand92) |
| *Human papillomavirus cand96* | | |
| Human papillomavirus cand96 | | |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Betapapillomavirus* but have not been approved as species

None reported.

## GENUS *GAMMAPAPILLOMAVIRUS*

Type species ***Human papillomavirus 4***

### Distinguishing features

Members of this genus cause cutaneous lesions in their host and are histologically distinguishable by intracytoplasmic inclusion bodies that are species specific. Genome organization: E5 ORF is absent.

### List of species in the genus *Gammapapillomavirus*

| | | |
|---|---|---|
| *Human papillomavirus 4* | | |
| Human papillomavirus 4 | [X70827] | (HPV-4) |
| *Human papillomavirus 48* | | |
| Human papillomavirus 48 | [U31790] | (HPV-48) |
| *Human papillomavirus 50* | | |
| Human papillomavirus 50 | [U31790] | (HPV-50) |
| *Human papillomavirus 60* | | |
| Human papillomavirus 60 | [U31792] | (HPV-60) |
| *Human papillomavirus 88* | | |
| Human papillomavirus 88 | | (HPV-88) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Gammapapillomavirus* but have not been approved as species

None reported.

## GENUS *DELTAPAPILLOMAVIRUS*

Type species ***European elk papillomavirus***

### Distinguishing features

These viruses induce fibropapillomas in their respective ungulate hosts. Trans-species transmission occurs, where it induces sarcoids. Genome organization: ORFs located in the region between the early and late genes have transforming properties.

### List of species in the genus *Deltapapillomavirus*

| | | |
|---|---|---|
| *Bovine papillomavirus 1* | | |
| Bovine papillomavirus 1 | [X02346] | (BPV-1) |
| *Deer papillomavirus* | | |
| Deer papillomavirus | | |
| *European elk papillomavirus* | | |
| European elk papillomavirus | [M15953] | (EEPV) |
| *Ovine papillomavirus 1* | | |
| Ovine papillomavirus 1 | [U83594] | (OvPV-1) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Deltapapillomavirus* but have not been approved as species

None reported.

## GENUS *EPSILONPAPILLOMAVIRUS*

Type species ***Bovine papillomavirus 5***

### Distinguishing features

Infections cause cutaneous papillomas in cattle.

### List of species in the genus *Epsilonpapillomavirus*

*Bovine papillomavirus 5*
Bovine papillomavirus 5 [AF457465] (BPV-5)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Epsilonpapillomavirus* but have not been approved as species

None reported.

## GENUS *ZETAPAPILLOMAVIRUS*

Type species ***Equine papillomavirus 1***

### Distinguishing features

Infections cause cutaneous lesions in horses. Genome organization: an ORF of unknown functional relevance overlaps with the L2 ORF.

### List of species in the genus *Zetapapillomavirus*

*Equine papillomavirus 1*
Equus caballus papillomavirus 1 [AF498323] (EcPV)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Zetapapillomavirus* but have not been approved as species

None reported.

## GENUS *ETAPAPILLOMAVIRUS*

Type species ***Fringilla coelebs papillomavirus***

### Distinguishing features

Avian papillomaviruses causing cutaneous lesions in their host. Genome organization: an ancestral E7 ORF exists which has partial E6 characteristics. Typical E6 ORF is absent.

### List of species in the genus *Etapapillomavirus*

*Fringilla coelebs papillomavirus*
Chaffinch papillomavirus [AY957109] (FcPV)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Etapapillomavirus* but have not been approved as species

None reported.



## GENUS *THETAPAPILLOMAVIRUS*

Type species ***Psittacus erithacus timneh papillomavirus***

### Distinguishing features

Avian papillomaviruses causing cutaneous lesions in their host. Genome organization: an ancestral E7 ORF exists which have partial E6 characteristics. Typical E4, E5 and E6 ORFs are absent.

### List of species in the genus *Thetapapillomavirus*

*Psittacus erithacus timneh papillomavirus*
Psittacus erithacus timneh papillomavirus [AF420235] (PePV)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Thetapapillomavirus* but have not been approved as species

None reported.

## GENUS *IOTAPAPILLOMAVIRUS*

Type species ***Mastomys natalensis papillomavirus***

### Distinguishing features

Rodent papillomavirus causing cutaneous lesions. Genome organization: the E2 ORF is considerably larger than in other genera and the E5 ORF is absent.

### List of species in the genus *Iotapapillomavirus*

*Mastomys natalensis papillomavirus*
Mastomys natalensis papillomavirus [U01834] (MnPV)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Iotapapillomavirus* but have not been approved as species

None reported.

# Genus *Kappapapillomavirus*

Type species ***Cottontail rabbit papillomavirus***

## Distinguishing features

Members of this genus cause cutaneous and mucosal lesions in rabbits. Genome organization: the E6 ORF is larger than in other genera. An uncharacterized E8 ORF is present in the early region.

## List of species in the genus *Kappapapillomavirus*

| | | |
|---|---|---|
| *Cottontail rabbit papillomavirus* | | |
| Cottontail rabbit papillomavirus | [K02708] | (CRPV) |
| *Rabbit oral papillomavirus* | | |
| Rabbit oral papillomavirus | [AF227240] | (ROPV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Kappapapillomavirus* but have not been approved as species

None reported.

# Genus *Lambdapapillomavirus*

Type species ***Canine oral papillomavirus***

## Distinguishing features

Members of this genus infect cats and dogs, causing mucosal and cutaneous lesions. Genome organization: the region between the early and late coding regions is exceptionally large, ranging between 1200 and 1500 bp.

## List of species in the genus *Lambdapapillomavirus*

| | | |
|---|---|---|
| *Canine oral papillomavirus* | | |
| Canine oral papillomavirus | [L22695] | (COPV) |
| *Feline papillomavirus* | | |
| Feline papillomavirus | [AF377865] | (FdPV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Lambdapapillomavirus* but have not been approved as species

None reported.

# Genus *Mupapillomavirus*

Type species ***Human papillomavirus 1***

## Distinguishing features

Human papillomaviruses causing cutaneous lesions in their host that are histologically distinguishable by species-specific intracytoplasmic inclusion bodies. Genome organization: the control region is larger than in other genera.

### List of species in the genus *Mupapillomavirus*

| | | |
|---|---|---|
| *Human papillomavirus 1* | | |
| Human papillomavirus 1 | [V01116] | (HPV-1) |
| *Human papillomavirus 63* | | |
| Human papillomavirus 63 | [X70828] | (HPV-63) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Mupapillomavirus* but have not been approved as species

None reported.



## GENUS *NUPAPILLOMAVIRUS*

Type species ***Human papillomavirus 41***

### Distinguishing features

Human papillomaviruses causing benign and malignant cutaneous lesions. Genome organization: several larger ORFs are located in the L1 ORF region. The E2 binding sites in the control region all deviate from the typical consensus sequences, ACCGNNNNCGGT.

### List of species in the genus *Nupapillomavirus*

| | | |
|---|---|---|
| *Human papillomavirus 41* | | |
| Human papillomavirus 41 | [X56147] | (HPV-41) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Nupapillomavirus* but have not been approved as species

None reported.

## GENUS *XIPAPILLOMAVIRUS*

Type species ***Bovine papillomavirus 3***

### Distinguishing features

Infections cause true papillomas on the cutaneous or mucosal surfaces of cattle. Genome organization: a characteristic E6 ORF is absent and the E8 ORF located in this region displays transforming properties similar to that of viruses in the species *Bovine papillomavirus 1*.

### List of species in the genus *Xipapillomavirus*

| | | |
|---|---|---|
| *Bovine papillomavirus 3* | | |
| Bovine papillomavirus 3 | [AF486184] | (BPV-3) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed. Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Xipapillomavirus* but have not been approved as species

None reported.

## GENUS *OMIKRONPAPILLOMAVIRUS*

Type species ***Phocoena spinipinnis papillomavirus***

### Distinguishing features

These papillomaviruses have been isolated from genital warts in cetaceans. Genome organization: several larger ORFs are located in the L1 ORF region. True E7 ORF is absent.

### List of species in the genus *Omikronpapillomavirus*

*Phocoena spinipinnis papillomavirus*
Phocoena spinipinnis papillomavirus [AJ238272] (PsPV)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Omikronpapillomavirus* but have not been approved as species

None reported.

## GENUS *PIPAPILLOMAVIRUS*

Type species ***Hamster oral papillomavirus***

### Distinguishing features

Infections with these papillomaviruses cause mucosal lesions in hamsters. The E2 and L2 ORFs are partially overlapping.

### List of species in the genus *Pipapillomavirus*

*Hamster oral papillomavirus*
Hamster oral papillomavirus [E15110] (HaOPV)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Pipapillomavirus* but have not been approved as species

None reported.

### List of other related viruses which may be members of the family *Papillomaviridae* but have not been approved as species

Trichosurus vulpecula papillomavirus [AF181682] (TvPV)
Possum papillomavirus (PoPV)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

Putative papillomaviruses of a variety of different species have been identified from partial sequences. More than 300 such sequences are presently available in the databanks.

### Phylogenetic relationships within the family

Phylogenetic relationships within the family are illustrated in Figure 4.

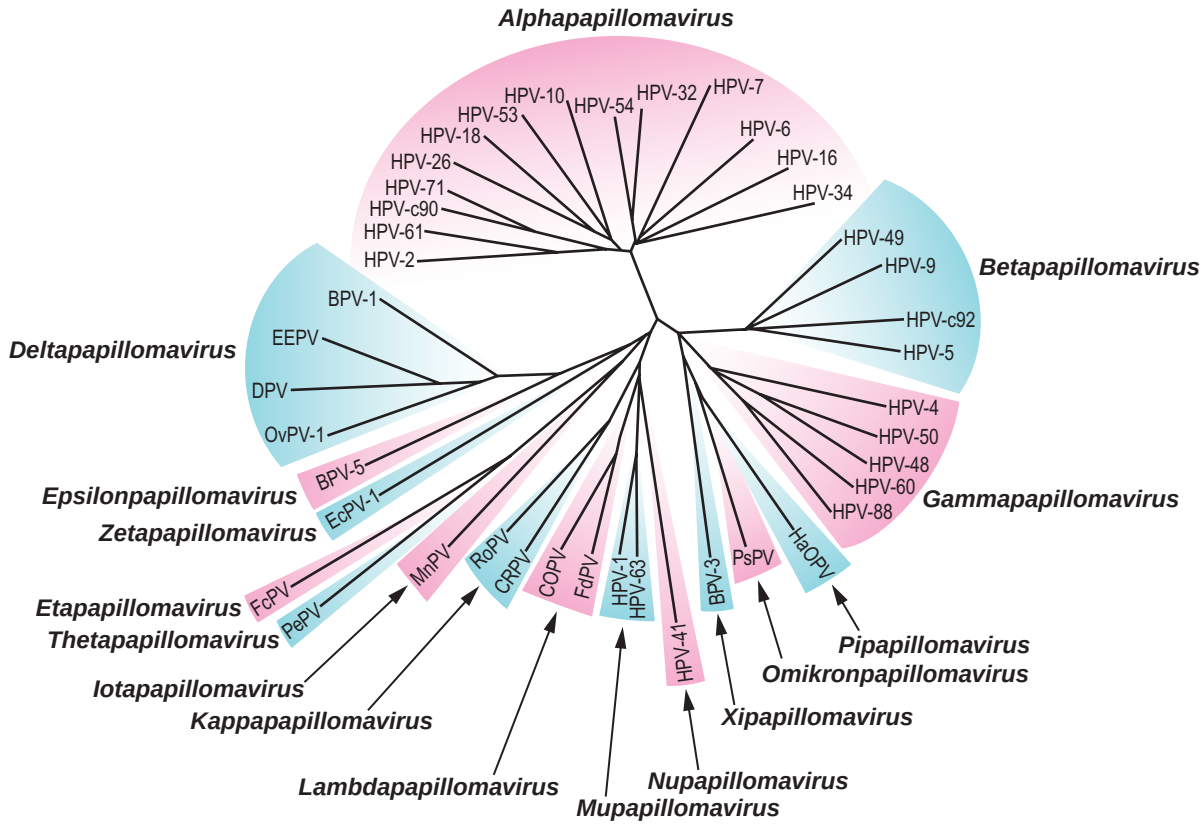


**Figure 4:** Phylogenetic tree representing the sequences of 118 papillomaviruses. The phylogenetically informative region of the L1 ORF was used in a modified version of the Phylip version 3.572 and based on a weighted version of the neighbor-joining analysis. Accession numbers are listed in the tables. The tree was constructed using the Treeview program (R. Page, University of Glasgow).

## Similarity with other taxa

The families *Papillomaviridae* and *Polyomaviridae* share some similarities in morphology and nucleic acid composition, as well as in *in vitro* transforming activities of specific proteins.

## Derivation of name

*Papilloma*: from Latin *papilla*, "nipple, pustule", and Greek suffix *-oma*, used to form nouns denoting "tumors".

## Further reading

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## Contributed by

Bernard, H.-U., Burk, R.D., deVilliers, E.-M. and zur Hausen, H.

# FAMILY *PHYCODNAVIRIDAE*



## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Phycodnaviridae*** |
| Genus | ***Chlorovirus*** |
| Genus | ***Coccolithovirus*** |
| Genus | ***Prasinovirus*** |
| Genus | ***Prymnesiovirus*** |
| Genus | ***Phaeovirus*** |
| Genus | ***Raphidovirus*** |

## Virion properties

### MORPHOLOGY

Originally, all phycodnavirus virions were assumed to be large icosahedral structures (120–220 nm in diameter) with a multilaminate shell surrounding an electron dense core and lacking an external membrane. However, recent experiments indicate that not all virus structures are identical. Five-fold symmetry averaging 3D reconstruction experiments revealed that one of the vertices in the chlorella virus paramecium bursaria Chlorella virus 1 (PBCV-1) has a cylindrical spike 250 Å long and 50 Å wide (Figure 1A). A possible spike or tail structure is also clearly visible in Emiliania huxleyi virus 86 (EhV-86) during infection (Figure 1B) and tail stubs are often visible in EhV-86 virions. External fibers extend from some of the trisymmetron capsomes of PBCV-1 (probably one per trisymmetron) and may facilitate attachment to the host (Figure 1C). EhV-86 may have an external membrane surrounding the polyhedral capsule (Figure 1D).

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

The Mr of PBCV-1 is about $1 \times 10^9$ and the $S_{20,w}$ is more than 2,000S. Some virions in the genus *Chlorovirus* are disrupted in CsCl. The infectivity of chloroviruses is not affected by non-ionic detergents but they are inactivated by organic solvents.

### NUCLEIC ACID

Virions contain large dsDNA genomes, ranging from 100 to 560 kbp. The G+C content of the viral genomes range from 40 to 52%. The genomic DNA in many of the viruses contains methylated bases, both 5-methylcytosine (m5C) and N6-methyladenine (m6A). The percent of methylated bases in the chloroviruses ranges from no m6A and 0.1% m5C to 37% m6A and 47% m5C.

### PROTEINS

Purified virions contain as many as 100 or more proteins ranging in size from <10 to >200 kDa. The chlorovirus PBCV-1 has three glycoproteins, three myristylated proteins [the major capsid protein, Vp54, is both glycosylated and myristylated] and several phosphoproteins. The Vp54 protein consists of two eight-stranded, antiparallel-beta-barrel, "jelly-roll" domains related by a pseudo six-fold rotation. At least four proteins, including Vp54, are located on the surface of PBCV-1. Proteomic analysis determined that the virion of EhV-86 is composed of at least 28 virus encoded proteins, 23 of which are predicted to be membrane proteins. Besides the major capsid protein, putative function can be assigned to four other components of the virion: two lectin proteins, a thioredoxin and a serine/threonine protein kinase.

### LIPIDS

The chlorovirus PBCV-1 virion contains 5–10% lipid. The lipid is in a bilayer membrane located inside the glycoprotein shell and is required for virus infectivity. The coccolithovirus EhV-86 has an external lipid membrane and may also have an internal membrane (Figure 1D).

### CARBOHYDRATES

At least three of the chlorovirus PBCV-1 proteins are glycosylated including the major CP Vp54. The glycan portion of Vp54, which consists of seven neutral sugars, is on the external surface of

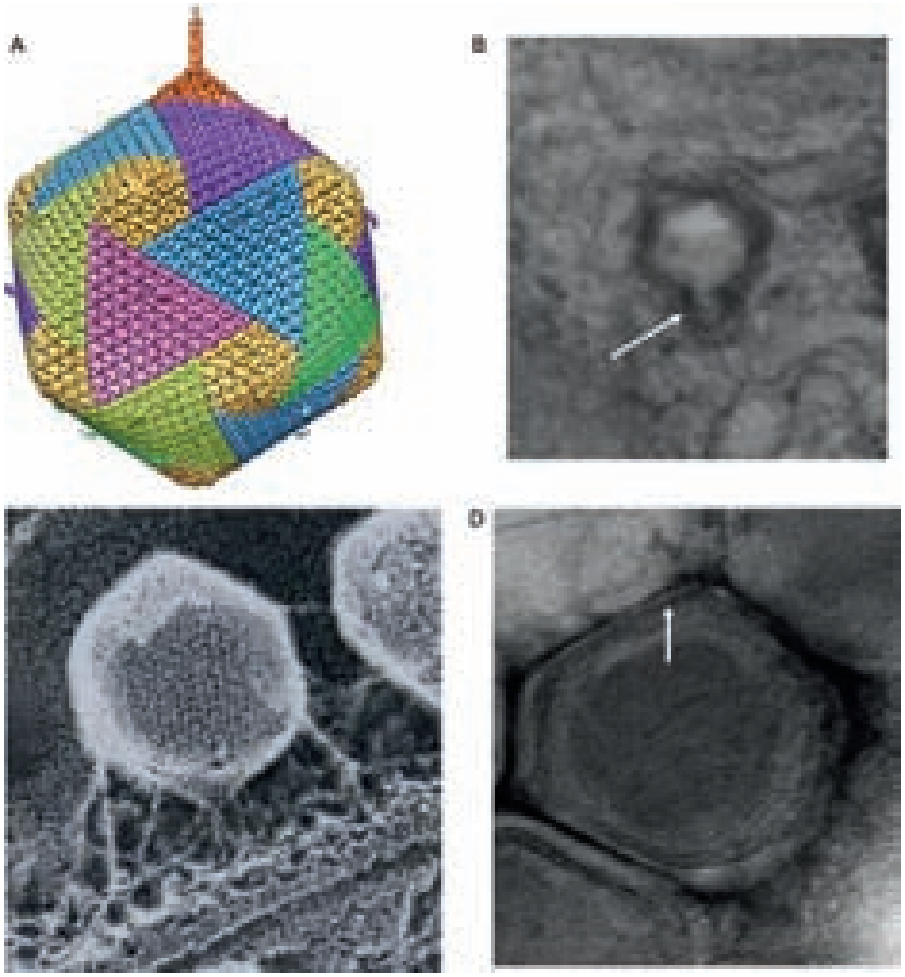

**Figure 1:** (A) Fivefold averaged cryo-electron micrographs of Paramecium bursaria chlorella virus PBCV-1 reveal a long, thin, cylindrical spike structure at one vertex and protrusions (fibers) extending from one unique capsomer per trisymmetron (from Cherrier *et al.* (2009). *Proc. Natl Acad. Sci., U S A*, **106**, 11085-89; with permission). (B) Putative tail structure (arrowed) can be observed in Emiliania huxleyi virus EhV-86 in the cytoplasm of infected *Emiliania huxleyi* before release of progeny virions (approx. 3 h p.i.) (adapted from Mackinder *et al.* (2010). *J. Gen. Virol.*, **90**, 2306-2316; with permission). (C) PBCV-1 attached to the cell wall of its host as viewed by the quick-freeze, deep-etch procedure. Note: fibers attach the virus to the wall (photo courtesy of John Heuser). (D) EhV-86 virion showing the putative internal lipid membrane (arrowed) (photo courtesy of Willie Wilson).

the virion. Unlike other glycoprotein-containing viruses, PBCV-1 encodes most, if not all, of the machinery required to glycosylate its proteins.

## Genome organization and replication

By definition, all phycodnaviruses have large dsDNA genomes. These genomes range in size from 100 kb to over 550 kb with G+C contents ranging from 40 to 52%. Complete genome sequences are available for members in three genera: six chloroviruses, PBCV-1, PBCV-NY2A, PBCV-AR158, PBCV-MT325, PBCV-FR483 and Acanthocystis turfacea chlorella virus 1, two phaeoviruses Ectocarpus siliculosus virus 1 (EsV-1), Feldmannia irregularis virus and the coccolithovirus Emiliania huxleyi virus EhV-86. Partial sequence (approximately 80%) is available for a second coccolithovirus, EhV-163. Additional phycodnavirus genomes are being sequenced, but are not yet publicly available.

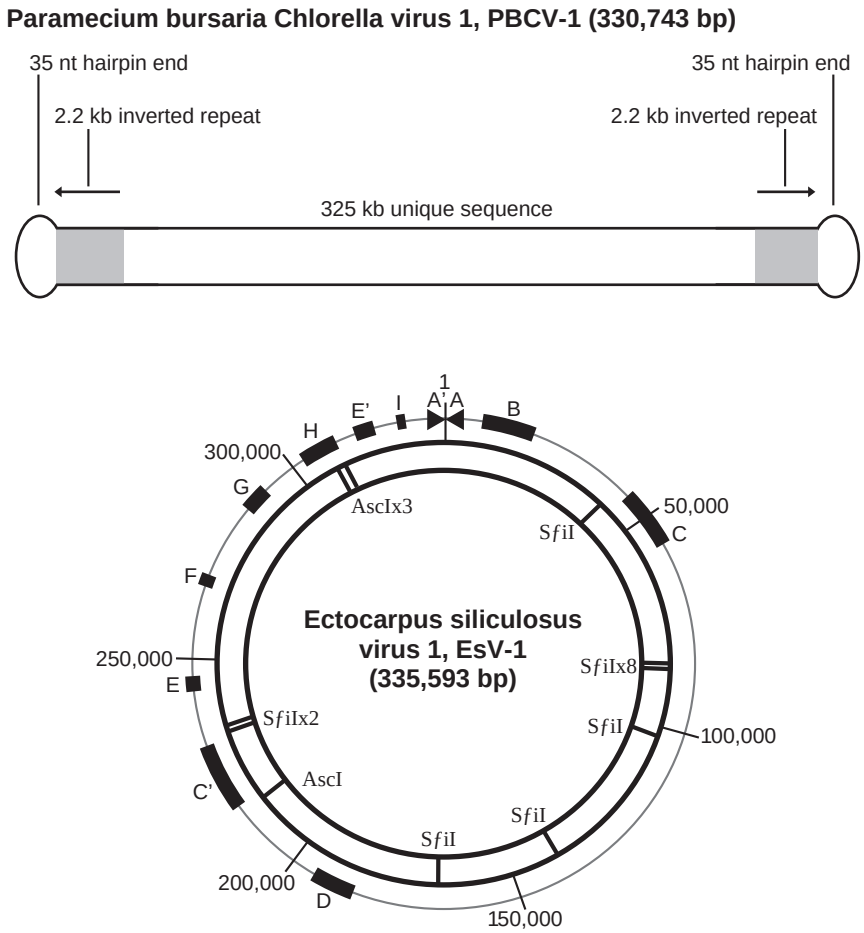


**Figure 2:** (Top) Genome organization of PBCV1. (Bottom) Circular map of the EsV-1 genome. Inner circle, sites for restriction endonucleases *Asc*I and *Sfi*I. Outer circle, nucleotide, coordinates and position of repeat regions (block rectangles: B, C, C′, etc). Triangles, the inverted terminal repeats, ITRs A and A′.

There is considerable variation in genome structure among the phycodnaviruses. The PBCV-1 genome is a linear 330 kb, nonpermuted dsDNA molecule with covalently closed hairpin termini (Figure 2, top). The termini consist of 35 nucleotide-long covalently closed hairpin loops that exist in one of two forms; the two forms are complementary when the 35-nucleotide sequences are inverted (flip-flop). Identical 2221-bp inverted repeats are adjacent to each hairpin end. The remainder of the PBCV-1 genome contains primarily single-copy DNA. EsV-1 has a linear dsDNA genome with almost perfect inverted repeats at each end allowing for circularization of the genome (Figure 2, bottom). It is proposed that the inverted repeats anneal with each other to form a cruciform structure that effectively circularizes the genome.

EhV-86 was originally proposed to have a linear genome. However, PCR amplification over the termini revealed a random A/T single nucleotide overhang (50% A, 50% T) suggesting the virus genome has both linear and circular phases. The detection of a DNA ligase and four endonucleases in EhV-86 hints that a linear genome may be packaged in the virions that circularizes during DNA replication.

Repetitive DNA occurs in the PBCV-1, EsV-1 and EhV-86 genomes. Both EsV-1 and PBCV-1 contain about 2 kb inverted repeats adjacent to the terminal ends. In addition to the terminal repeats, tandem repeats are located throughout the EsV-1 genome and comprise approximately 12% of the total

genome size. A similar proportion of the Feldmannia sp. virus (FsV) genome also consists of repetitive DNA. EhV-86 has three repeat families (none of which is located at the ends of the genome); one family is postulated to act as an origin of replication (adding credence to the circular mode of replication model), another family is postulated to contain immediate early promoter elements and the last family has a large repetitive proline-rich domain. The repetitive regions in these genomes, while hindering sequencing projects, may play a role in recombination between viruses that allows genetic information to be exchanged with themselves and with their hosts.

## Antigenic properties

Four distinct antigenic variants of the chlorovirus PBCV-1 can be isolated that are resistant to polyclonal antibody prepared against wildtype PBCV-1. These variants occur at a frequency of about $1 \times 10^{-6}$. The antibodies react primarily with the glycan portion of the major CP. Additional variants of these viruses can easily be isolated from natural sources.

## Biological properties

The phycodnaviruses, depending on whether they infect freshwater algae or marine algae, are ubiquitous in freshwater or seawater collected throughout the world. Some viruses are host-specific and only infect single isolates or species of algae. For example, chloroviruses only attach to cell walls of certain unicellular, eukaryotic, chlorella-like green algae. Virus attachment is followed by dissolution of the host wall at the point of attachment and entry of the viral DNA and associated proteins into the cell, leaving an empty capsid on the host surface. Beginning about 4 h post infection (p.i.), progeny virions are assembled in the cytoplasm of the host. Infectious virions can be detected inside the cell about 30–40 min prior to virus release; virus release occurs by cell lysis. Coccolithoviruses, prymnesioviruses and raphidoviruses have wider host ranges, where individual viruses can infect a range of host isolates within specific algal species; however they do not cross the species barrier.

The phaeoviruses infect the wall-less spore or gamete stage of filamentous brown algae, followed by fusion of adjacent host and particle surfaces. Empty particles remain on the cell surface following the release of core contents. An eclipse period of approximately 3 h follows the attachment stage. The virus growth cycle is complete after approximately 14 h. During the replication cycle, particles appear in the cytoplasm and are associated with the production of cytoplasmic fibrils (ca. 5–8 nm in diameter) and clusters of membrane-bound vesicles that are absent in healthy cells. Particles are released into the medium via localized ruptures in the cell membrane; ruptures often appear at several locations on the same cell.

Coccolithoviruses attach to exposed membranes of their host and the viruses enter into the host intact via either an endocytotic or an envelope fusion mechanism, after which they rapidly disassemble (Figure 3).

Less is known about the replication of prymnesioviruses and raphidoviruses. Virus formation is observed in the cytoplasm and the nucleus remains intact and separate from the viroplasm that consists of a fibrillar matrix. Ultimately, viral production results in the disruption of organelles, lysis of the cell and release of the virus particles.

The hosts for some of the chloroviruses and coccolithoviruses can easily be grown in the laboratory and the viruses can be plaque-assayed. The hosts for some of the other viruses are either cultured axenically (e.g., prymnesiovirus hosts, *P. globosa*) or non-axenically in uni-alga cultures (e.g. hosts for the prasinoviruses and raphidoviruses). The brown algal viruses, which only appear in mature gametangia or sporangia cells of their hosts, can also be grown in the laboratory.

The chloroviruses, coccolithoviruses, prasinoviruses, prymnesioviruses and raphidoviruses are transmitted horizontally. The phaeoviruses are transmitted both horizontally and vertically.

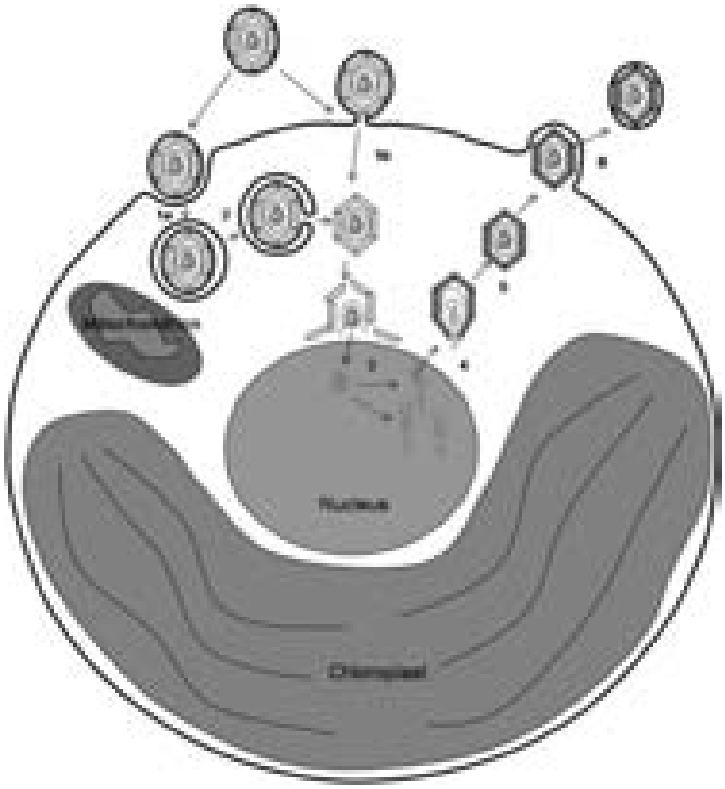


**Figure 3:** Schematic of the proposed life cycle of EhV-86. Enveloped EhV-86 enters *E. huxleyi* with an intact capsid and nucleoprotein core either by an endocytotic mechanism (step 1a) followed by fusion of its envelope with the vacuole membrane (step 2) or by fusion of its envelope with the host plasma membrane (step 1b). The viral capsid encapsulated nucleoprotein core rapidly targets the nucleus where capsid breakdown releases the viral genome (step 3). The viral genome enters the host nucleus where early promoter sequences are expressed by host RNA polymerase. Mid-late genes are expressed by viral RNA polymerase within the cytoplasm where capsid assembly takes place, possibly by filling of a pro-capsid with viral DNA and core proteins (step 4). Early assembled viruses are transported to the plasma membrane (step 5) where they are released by a budding mechanism (step 6) (from Mackinder *et al.* (2010). *J. Gen. Virol.*, **90**(9), 2306-2316; with permission).

## GENUS *CHLOROVIRUS*

Type species ***Paramecium bursaria Chlorella virus 1***

### Distinguishing features

The chloroviruses, which are ubiquitous in freshwater throughout the world, infect certain unicellular, eukaryotic, exsymbiotic chlorella-like green algae. The viruses have linear, non-permuted dsDNA genomes with cross-linked hairpin ends. The DNA termini contain identical inverted 1–2.2 kb repeats. The remainder of the genome is primarily single copy DNA. Viruses in this group can be distinguished from each other by DNA restriction digests, the levels of methylated bases in their genomes, serology and host specificity.

Originally the chloroviruses were believed to be simple polyhedral structures (ca. 190 nm in diameter) with a multilaminate shell surrounding an internal bilayered membrane. However, five-fold symmetry averaging 3D reconstruction experiments of PBCV-1 revealed that one of the vertices in

the chlorella viruses has a cylindrical spike 250 Å long and 50 Å wide (Figure 1A). The spike is too narrow for DNA to pass through it. External fibers extend from some of the trisymmetron capsomes (probably one per trisymmetron) and probably facilitate attachment to the host (Figure 1B). PBCV-1 initiates infection by attaching rapidly and specifically to the host cell wall, probably by the fibers mentioned above. Following host cell wall degradation by virus-packaged enzyme(s), the PBCV-1 internal membrane presumably fuses with the host membrane, facilitating entry of the viral DNA and virion-associated proteins into the cell, leaving an empty capsid attached to the surface. PBCV-1 lacks a recognizable RNA polymerase gene, and so circumstantial evidence suggests that the virus DNA and DNA-associated proteins quickly move to the nucleus, where early transcription begins within a few minutes. PBCV-1 DNA replication begins 60–90 min p.i. and is followed by transcription of late genes. Approximately 2–3 h p.i. assembly of virus capsids begin in localized regions in the cytoplasm, which become prominent 3–4 h p.i. Five to six hours p.i. the cytoplasm fills with infectious progeny virus particles and localized lysis of the host cell releases progeny at 6–8 h p.i.

The 330 kb PBCV-1 genome, along with five other chloroviruses, have been sequenced. The viruses encode as many as 800 ORFs, 40 codons or longer, of which about 50% are predicted to encode proteins. About 40% of these putative protein-encoding ORFs match proteins in the databases. Two of the PBCV-1 genes are interrupted by introns: a transcription factor TFIIS-like gene has a self-splicing type I intron and the DNA polymerase gene has a spliceosomal processed type of intron. The PBCV-1 genome also has 11 tRNA genes, one of which is predicted to contain a small intron. In addition, one of the chloroviruses, NY-2A, contains two inteins. The chloroviruses encode many interesting and unusual proteins including DNA restriction endonucleases, polyamine biosynthetic enzymes, sugar metabolizing enzymes, and ion channel and transporting proteins.

## Species demarcation criteria in the genus

Three groups of viruses are delineated based on host specificity:

Group 1. Paramecium bursaria Chlorella NC64A viruses (NC64A viruses)
Group 2. Paramecium bursaria Chlorella Pbi viruses (Pbi viruses)
Group 3. Hydra viridis Chlorella viruses (HVC viruses)

Chlorella strains NC64A, ATCC 30562 and N1A (originally symbionts of the protozoan *P. bursaria*), collected in the United States, are the only known host for NC64A viruses. Chlorella strain Pbi (originally a symbiont of a European strain *of P. bursaria*) collected in Germany, is the only known host for Pbi viruses. Pbi viruses do not infect Chlorella strains NC64A, ATCC 30562 and N1A. Chlorella strain Florida (originally a symbiont of *Hydra viridis*) is the only known host for Hydra viridis Chlorella virus (HVCV). NC64A viruses are grouped into 16 species based on plaque size, serological reactivity, resistance of the genome to restriction endonucleases, virus encoded restriction endonucleases and nature and content of methylated bases.

## List of species in the genus *Chlorovirus*

**Group 1: Paramecium bursaria Chlorella NC64A virus group**

| | | |
|---|---|---|
| *Paramecium bursaria Chlorella virus 1* | | |
| Paramecium bursaria Chlorella virus 1 | [U42580] | (PBCV-1) |
| *Paramecium bursaria Chlorella virus AL1A* | | |
| Paramecium bursaria Chlorella virus AL1A | | (PBCV-AL1A) |
| *Paramecium bursaria Chlorella virus AL2A* | | |
| Paramecium bursaria Chlorella virus AL2A | | (PBCV-AL2A) |
| *Paramecium bursaria Chlorella virus AR158* | | |
| Paramecium bursaria Chlorella virus AR158 | [NC_009899] | (PBCV-AR158) |
| *Paramecium bursaria Chlorella virus BJ2C* | | |
| Paramecium bursaria Chlorella virus BJ2C | | (PBCV-BJ2C) |
| *Paramecium bursaria Chlorella virus CA4A* | | |
| Paramecium bursaria Chlorella virus CA4A | | (PBCV-CA4A) |
| *Paramecium bursaria Chlorella virus CA4B* | | |
| Paramecium bursaria Chlorella virus CA4B | | (PBCV-CA4B) |

| | | |
|---|---|---|
| *Paramecium bursaria Chlorella virus IL3A* | | |
| Paramecium bursaria Chlorella virus IL3A | | (PBCV-IL3A) |
| *Paramecium bursaria Chlorella virus NC1A* | | |
| Paramecium bursaria Chlorella virus NC1A | | (PBCV-NC1A) |
| *Paramecium bursaria Chlorella virus NE8A* | | |
| Paramecium bursaria Chlorella virus NE8A | | (PBCV-NE8A) |
| *Paramecium bursaria Chlorella virus NY2A* | | |
| Paramecium bursaria Chlorella virus NY2A | [DQ491002] | (PBCV-NY2A) |
| *Paramecium bursaria Chlorella virus NYs1* | | |
| Paramecium bursaria Chlorella virus NYs1 | | (PBCV-NYs1) |
| *Paramecium bursaria Chlorella virus SC1A* | | |
| Paramecium bursaria Chlorella virus SC1A | | (PBCV-SC1A) |
| *Paramecium bursaria Chlorella virus XY6E* | | |
| Paramecium bursaria Chlorella virus XY6E | | (PBCV-XY6E) |
| *Paramecium bursaria Chlorella virus XZ3A* | | |
| Paramecium bursaria Chlorella virus XZ3A | | (PBCV-XZ3A) |
| *Paramecium bursaria Chlorella virus XZ4A* | | |
| Paramecium bursaria Chlorella virus XZ4A | | (PBCV-XZ4A) |
| *Paramecium bursaria Chlorella virus XZ4C* | | |
| Paramecium bursaria Chlorella virus XZ4C | | (PBCV-XZ4C) |
| **Group 2: Paramecium bursaria Chlorella Pbi virus group** | | |
| *Paramecium bursaria Chlorella virus A1* | | |
| Paramecium bursaria Chlorella virus A1 | | (PBCV-A1) |
| *Paramecium bursaria Chlorella virus FR483* | | |
| Paramecium bursaria Chlorella virus FR483 | [NC_008603] | (PBCV-FR483) |
| *Paramecium bursaria Chlorella virus MT325* | | |
| Paramecium bursaria Chlorella virus MT325 | [DQ491001] | (PBCV-MT325) |
| **Group 3: Hydra viridis Chlorella virus group** | | |
| *Hydra viridis Chlorella virus 1* | | |
| Hydra viridis Chlorella virus 1 | | (HVCV-1) |

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Chlorovirus* but have not been approved as species

| | | |
|---|---|---|
| Acanthocystis turfacea chlorella virus 1 | [EF101928] | (ATCV-1) |
| Paramecium bursaria Chlorella virus AL2C | | (PBCV-AL2C) |
| Paramecium bursaria Chlorella virus CA1A | | (PBCV-CA1A) |
| Paramecium bursaria Chlorella virus CA1D | | (PBCV-CA1D) |
| Paramecium bursaria Chlorella virus CA2A | | (PBCV-CA2A) |
| Paramecium bursaria Chlorella virus IL2A | | (PBCV-IL2A) |
| Paramecium bursaria Chlorella virus IL2B | | (PBCV-IL2B) |
| Paramecium bursaria Chlorella virus IL3D | | (PBCV-IL3D) |
| Paramecium bursaria Chlorella virus IL5-2s1 | | (PBCV-IL5-2s1) |
| Paramecium bursaria Chlorella virus MA1D | | (PBCV-MA1D) |
| Paramecium bursaria Chlorella virus MA1E | | (PBCV-MA1E) |
| Paramecium bursaria Chlorella virus NC1B | | (PBCV-NC1B) |
| Paramecium bursaria Chlorella virus NC1C | | (PBCV-NC1C) |
| Paramecium bursaria Chlorella virus NC1D | | (PBCV-NC1D) |
| Paramecium bursaria Chlorella virus NE8D | | (PBCV-NE8D) |
| Paramecium bursaria Chlorella virus NY2B | | (PBCV-NY2B) |
| Paramecium bursaria Chlorella virus NY2C | | (PBCV-NY2C) |
| Paramecium bursaria Chlorella virus NY2F | | (PBCV-NY2F) |
| Paramecium bursaria Chlorella virus NYb1 | | (PBCV-NYb1) |
| Paramecium bursaria Chlorella virus SC1B | | (PBCV-SC1B) |
| Paramecium bursaria Chlorella virus SH6A | | (PBCV-SH6A) |
| Paramecium bursaria Chlorella virus XZ5C | | (PBCV-XZ5C) |
| Paramecium bursaria Chlorella virus CVBII | | (PBCV-CVBII) |
| Paramecium bursaria Chlorella virus CVK2 | | (PBCV-CVK2) |
| Paramecium bursaria Chlorella virus CVU1 | | (PBCV-CVU1) |

# Genus *Coccolithovirus*

Type species ***Emiliania huxleyi virus 86***

## Distinguishing features

Viruses assigned to this genus all have large dsDNA genomes (ca. 410–415 kbp) and probably have a common genome structure. The particles have icosohedral symmetry, enveloped by a lipid membrane, are tailless and range from 150 to 200 nm in diameter. The latent period of these viruses is 3–4 h and the burst size is 400–1000 viruses per lysed host cell (mean 620). They infect different isolates of the globally important marine coccolithophorid *Emiliania huxleyi*, a marine alga which has a world-wide distribution and is known for forming vast coastal and mid-oceanic blooms which are easily observed by satellite imagery. The viruses described here were isolated from *E. huxleyi* blooms off the coast of Plymouth, UK, in July 1999 and July/August 2001, from an *E. huxleyi* bloom induced during a mesocosm experiment in a fjord near Bergen, Norway, during June 2000 and from *E. huxleyi* blooms off the coast of Bergen, Norway, in June 1999 and June 2000. The viruses are relatively easy to isolate and susceptible host strains usually lyse 2–7 days after the addition of filtered seawater. Clonal isolates can be obtained by plaque assay.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Coccolithovirus*

| | | |
|---|---|---|
| *Emiliania huxleyi virus 86* | | |
| Emiliania huxleyi virus 86 | [AJ890364] | (EhV-86) |

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Coccolithovirus* but have not been approved as species

| | | |
|---|---|---|
| Emiliania huxleyi virus 84 | | (EhV-84) |
| Emiliania huxleyi virus 88 | | (EhV-88) |
| Emiliania huxleyi virus 163 | | (EhV-163) |
| Emiliania huxleyi virus 201 | | (EhV-201) |
| Emiliania huxleyi virus 202 | | (EhV-202) |
| Emiliania huxleyi virus 203 | | (EhV-203) |
| Emiliania huxleyi virus 205 | | (EhV-205) |
| Emiliania huxleyi virus 207 | | (EhV-207) |
| Emiliania huxleyi virus 208 | | (EhV-208) |
| Emiliania huxleyi virus 99B1 | [FN429076] | (EhV-99B1) |
| Emiliania huxleyi virus 2KB1 | | (EhV-2KB1) |
| Emiliania huxleyi virus 2KB2 | | (EhV-2KB2) |
| Emiliania huxleyi virus Ø28 | | (EhV- Ø28) |
| Emiliania huxleyi virus Ø29 | | (EhV- Ø29) |
| Emiliania huxleyi virus Ø30 | | (EhV- Ø30) |
| Emiliania huxleyi virus Ø42 | | (EhV- Ø42) |
| Emiliania huxleyi virus Ø43 | | (EhV- Ø43) |

# Genus *Prasinovirus*

Type species ***Micromonas pusilla virus SP1***

## Distinguishing features

Viruses assigned to this genus infect marine prasinophytes from the three genera *Bathycoccus*, *Micromonas* and *Ostreococcus*, the latter being the world's smallest free living eukaryote. With a small

host size (less than 1 m in diameter for *Ostreococcus*) and a virus capsid size of around 120 nm, it is estimated there is physically room for no more than 100 virions at any one time. This is reflected in experimental data that suggest a typical burst size of 6–15 viruses per cell. Following viral adsorption, genome replication occurs from 2 hours post infection (p.i.), virions assemble in the cytoplasm from 6 h p.i. until 20 h p.i., after which cellular lysis occurs. The host cell nucleus, mitochondria and chloroplast remain intact through this period. The prasinoviruses have genomes in the size range of 184–198 kbp and are morphologically similar to other phycodnaviruses with icosahedral capsids of 100–130 nm. The viruses are ubiquitous in seawater throughout the world, and have been isolated from the Pacific and Atlantic Oceans, the Gulf of Mexico and the Mediterranean Sea.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Prasinovirus*

*Micromonas pusilla virus SP1*
Micromonas pusilla virus SP1 (MpV-SP1)

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Prasinovirus* but have not been approved as species

| | | |
|---|---|---|
| Bathycoccus prasinos virus 1 | [HM004432] | (BpV-1) |
| Bathycoccus prasinos virus 1 | [HM004430] | (BpV-2) |
| Micromonas pusilla virus 1 | [HM004429] | (MpV-1) |
| Micromonas pusilla virus GM1 | | (MpV-GM1) |
| Micromonas pusilla virus PB6 | | (MpV-PB6) |
| Micromonas pusilla virus PB7 | | (MpV-PB7) |
| Micromonas pusilla virus PB8 | | (MpV-PB8) |
| Micromonas pusilla virus PL1 | | (MpV-PL1) |
| Micromonas pusilla virus SG1 | | (MpV-SG1) |
| Micromonas pusilla virus SP2 | | (MpV-SP2) |
| Ostreococcus luminaris virus 1 | [HM004431] | (OlV-1) |
| Ostreococcus tauri virus 1 | [FN386611] | (OtV-1) |
| Ostreococcus tauri virus 2 | [FN600414] | (OtV-2) |
| Ostreococcus tauri virus 5 | [EU304328] | (OtV-5) |

## GENUS *PRYMNESIOVIRUS*

Type species ***Chrysochromulina brevifilum virus PW1***

### Distinguishing features

Viruses assigned to this genus infect hosts belonging to algal class *Haptophyceae* (also referred to as the *Prymnesiophyceae*). Although viruses in this genus all have dsDNA genomes, there is a wide variety of diameters (100–170 nm) and genome sizes (120–485 kbp). Estimated burst sizes range from 320 to 600 viruses per infected cell. Viruses that infect members of the same marine algae, *Chysochromulina brevifilum* and *C. strobilus*, were isolated from USA (Texas) coastal waters in three locations (Gulf of Mexico, Aransas Pass and Laguna Madre). Viruses that infect *Phaeocystis globosa* were isolated from natural seawater off the coast of the Netherlands during April to July 2000 and 2001, the North Sea in April 2002, from a *P. globosa* bloom induced during a mesocosm experiment during October 2000 and from a *P. globosa* bloom in the English Channel off the coast of Plymouth, UK, in April 2001. These viruses lysed susceptible host strains after 2–7 days after the addition of filtered seawater. Once isolated, susceptible host strains typically lyse within 1–2 days.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Prymnesiovirus*

*Chysochromulina brevifilum virus PW1*
Chysochromulina brevifilum virus PW1 (CbV-PW1)

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Prymnesiovirus* but have not been approved as species

| | |
|---|---|
| Chysochromulina brevifilum virus PW3 | (CbV-PW3) |
| Phaeocystis globosa virus 1 (Texel) | (PgV-01T) |
| Phaeocystis globosa virus 2 (Texel) | (PgV-02T) |
| Phaeocystis globosa virus 3 (Texel) | (PgV-03T) |
| Phaeocystis globosa virus 4 (Texel) | (PgV-04T) |
| Phaeocystis globosa virus 5 (Texel) | (PgV-05T) |
| Phaeocystis globosa virus 6 (Texel) | (PgV-06T) |
| Phaeocystis globosa virus 7 (Texel) | (PgV-07T) |
| Phaeocystis globosa virus 9 (Texel) | (PgV-09T) |
| Phaeocystis globosa virus 10 (Texel) | (PgV-10T) |
| Phaeocystis globosa virus 11 (Texel) | (PgV-11T) |
| Phaeocystis globosa virus 12 (Texel) | (PgV-12T) |
| Phaeocystis globosa virus 13 (Texel) | (PgV-13T) |
| Phaeocystis globosa virus 14 (Texel) | (PgV-14T) |
| Phaeocystis globosa virus 15 (Texel) | (PgV-15T) |
| Phaeocystis globosa virus 16 (Texel) | (PgV-16T) |
| Phaeocystis globosa virus 17 (Texel) | (PgV-17T) |
| Phaeocystis globosa virus 18 (Texel) | (PgV-18T) |
| Phaeocystis globosa virus 102 (Plymouth) | (PgV-102P) |

## GENUS *PHAEOVIRUS*

Type species ***Ectocarpus siliculosus virus 1***

### Distinguishing features

Ectocarpus siliculosus virus 1 isolates infect the free-swimming, zoospore or gamete stages of filamentous brown algal hosts. The virus genome is integrated into the host genome and is inherited in a Mendelian manner. Virus particles are only formed in prospective gametangia or sporangia cells of the host. Phaeoviruses share icosahedral morphologies with internal lipid membranes and large, complex, double stranded DNA genomes. The replication strategy for these virus genomes is yet to be resolved but their genomes occur in both linear and circular forms.

### Species demarcation criteria in the genus

Nine species of viruses are delineated based in part on host specificity. Field isolates of at least seven genera of the *Phaeophycae* contain 120–150 nm diameter polyhedral virus-like particles. The particles contain dsDNA genomes that vary in size from 150 to 350 kb, although the major CP gene and DNA polymerase sequence data indicate that they are closely related. Virus expression is variable; particles are rarely observed in vegetative cells but are common in unilocular sporangia (Feldmannia species virus, FsV) or both unilocular and plurilocular sporangia and gametangia (EsV). Some of the viruses have a narrow host range (FsV), whereas others such as Ectocarpus fasciculatus virus (EfV) and EsV infect members of more than one genus.

### List of species in the genus *Phaeovirus*

| | | |
|---|---|---|
| *Ectocarpus fasciculatus virus a* | | |
| Ectocarpus fasciculatus virus 1 | | (EfV-1) |
| *Ectocarpus siliculosus virus 1* | | |
| Ectocarpus siliculosus virus 1 | [AF204951] | (EsV-1) |
| Ectocarpus siliculosus virus 1-like (pro-virus) | | *(EsV-1)* |
| *Ectocarpus siliculosus virus a* | | |
| Ectocarpus siliculosus virus a | | (EsV-1a) |
| *Feldmannia irregularis virus a* | | |
| Feldmannia irregularis virus 1 | | (FirrV-1) |
| *Feldmannia species virus* | | |
| Feldmannia species virus 158 | [EU916176] | (FsV-158) |
| Feldmannia species virus 178 | | (FsV-178) |
| *Hincksia hinckiae virus a* | | |
| Hincksia hinckiae virus 1 | | (HhV-1) |
| *Myriotrichia clavaeformis virus a* | | |
| Myriotrichia clavaeformis virus 1 | | (McV-1) |
| *Pilayella littoralis virus 1* | | |
| Pilayella littoralis 1 | | (PlV-1) |

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Phaeovirus* but have not been approved as species

None reported.

## GENUS *RAPHIDOVIRUS*

Type species ***Heterosigma akashiwo virus 01***

### Distinguishing features

Viruses assigned to this genus infect the harmful bloom causing raphidophyte, *Heterosigma akashiwo* (*Raphidophyceae*), a marine alga that has a world-wide distribution. Members of the type species *Heterosigma akashiwo virus 01* have a large dsDNA genome (ca. 294 kbp) and virions with icosahedral symmetry that are 202 ± 6 nm in diameter. The viruses were isolated from *H. akashiwo* blooms near the western coast of Japan. The latent period and the burst size of HaV01 is 30–33 h and about 770 at 20 °C, respectively. Although these viruses rapidly degrade to lose infectivity even when kept at 4 °C in the dark, they can be easily cryopreserved. Their infection is considered to be a significant factor influencing the dynamics and termination of *H. akashiwo* blooms. HaV infection has great impacts on *H. akashiwo* populations regarding both the fluctuation of the algal biomass (quantity) and the changes in the clonal composition (quality); the latter is most likely due to the variety in interspecies host specificity of HaV clones. Partial sequencing of the HaV01 genome revealed that several genes resembled other protist-infecting nucleocytoplasmic large DNA viruses. Interestingly, the genome included a 232-amino-acid intein (protein intron) in its DNA polymerase gene.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Raphidovirus*

| | |
|---|---|
| *Heterosigma akashiwo virus 01* | |
| Heterosigma akashiwo virus 01 | (HaV-01) |

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Raphidovirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Phycodnaviridae*

None reported.

## List of other related viruses which may be members of the family *Phycodnaviridae* but have not been approved as species

The following viruses are considered potential candidates for assignment to the family *Phycodnaviridae*. However, this cannot be confirmed without further characterization.

Aureococcus anophagefference virus (AaV) (Brown tide virus): This virus was isolated from Great South Bay New York, USA, in 1992. Viruses are 140–160 nm in diameter and cultures of *Aureococcus anophagefference* usually lyse within 24–48 h.

Chrysochromulina ericina virus 01B (CeV-01B): This virus was isolated from water collected off the coast of Bergen, Norway, in June 1998. Viruses have particle diameters of 160 nm and a genome size of 510 kbp. The lytic cycle is 14–19 h and the burst size is about 1800–4100 viruses per host cell. Although included in the phylogenetic analysis for the *Phycodnaviridae* (Figure 4), the taxonomic position of CeV taxonomic is under debate.

Heterocapsa circularisquama viruses (HcV-01 to HcV-10): These viruses have been isolated from *H. circularisquama* blooms in the coastal waters of Japan. They have a large dsDNA genome (ca. 350 kbp), icosahedral symmetry and are 180–210 nm in diameter (197 ± 8 nm). The latent period and the burst size is 40–56 h and about 1800–2400 infectious units at 20–25 °C, respectively. Although these viruses rapidly degrade to lose infectivity even when kept at 4 °C in the dark, they can be cryopreserved. Classification of HcV is currently under debate since phylogenetic analyses of the HcV DNA polymerase indicates a high similarity with African swine fever virus (genus *Asfarvirus*, family *Asfarviridae*).

Phaeocystis pouchetii virus 01 (PpV-01): This virus was isolated from water collected at the end of a *P. pouchetii* bloom off the coast of Bergen, Norway, in May 1995. Viruses have particle diameters of 130–160 nm and a genome size of 485 kbp. The latent period is 12–18 h, complete lysis of cultures is observed after 48 h and the burst size is about 350–600 viruses per host cell. The taxonomic position of PgV is under debate since it falls in the same clade as the family *Mimiviridae*.

Pyramimonas orientalis virus 01B (PoV-01B): This virus was isolated from water collected off the coast of Bergen, Norway, in June 1998. Viruses have particle sizes of 220 × 180 nm and a genome size of 560 kbp. The lytic cycle is 14–19 h and the burst size is about 800–1000 viruses per host cell. Although included in the phylogenetic analysis for the *Phycodnaviridae* (Figure 4), the taxonomic position of PoV is under debate.

| | |
|---|---|
| Aureococcus anophagefference virus (Brown tide virus) | (AaV) |
| Chrysochromulina ericina virus 01B | (CeV-01B) |
| Heterocapsa circularisquama virus 01 | (HcV-01) |
| Heterocapsa circularisquama virus 02 | (HcV-02) |
| Heterocapsa circularisquama virus 03 | (HcV-03) |
| Heterocapsa circularisquama virus 04 | (HcV-04) |
| Heterocapsa circularisquama virus 05 | (HcV-05) |
| Heterocapsa circularisquama virus 06 | (HcV-06) |
| Heterocapsa circularisquama virus 07 | (HcV-07) |
| Heterocapsa circularisquama virus 08 | (HcV-08) |
| Heterocapsa circularisquama virus 09 | (HcV-09) |
| Heterocapsa circularisquama virus 10 | (HcV-10) |
| Phaeocystis pouchetii virus 01 | (PpV-01) |
| Pyramimonas orientalis virus 01B | (PoV-01B) |

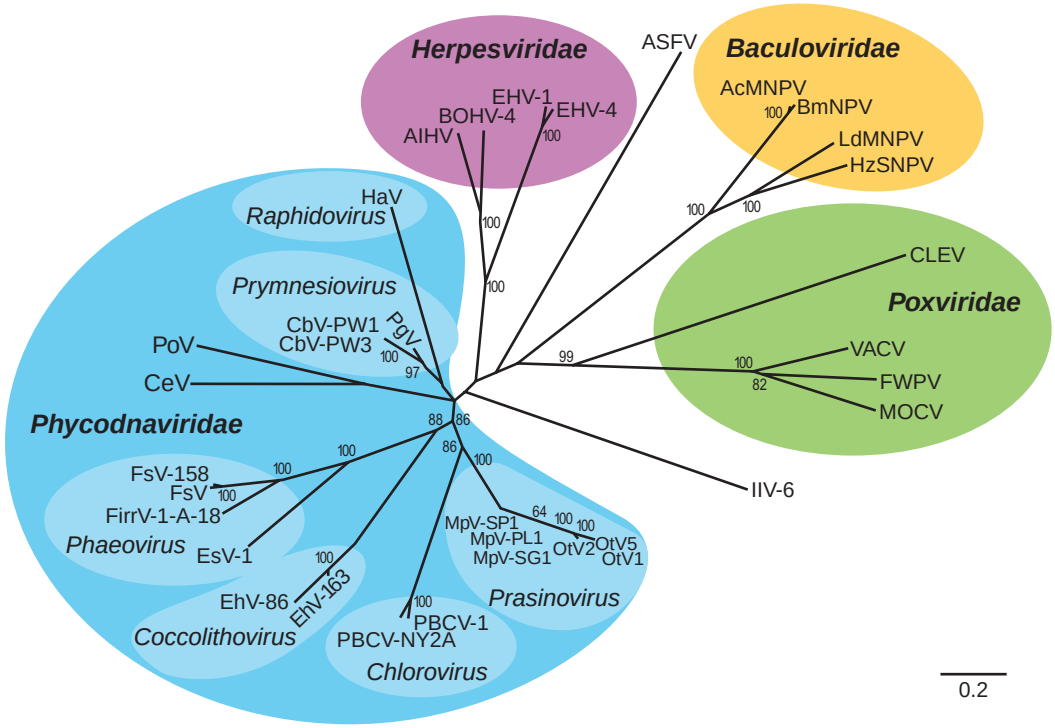


**Figure 4:** Phylogenetic analysis of members of the family *Phycodnaviridae* based on a distance matrix algorithm between the amino acid sequences of DNA pol fragments of phycodnaviruses and other large dsDNA viruses (Neighbor in PHYLIP, version 3.61). The alignment was performed (ClustalW) on the region spanning the highly conserved regions I and IV of the DNA pol genes. Abbreviations: Micromonas pusilla virus (MpV); Ostreococcus tauri virus (OtV); paramecium bursaria chlorella virus (PBCV); Emiliania huxleyi virus (EhV); Ectocarpus siliculosus virus (EsV); Feldmannia irregularis virus (FirrV); Feldmannia species virus (FsV), Chrysochromulina ericina virus (CeV); Pyramimonas orientalis virus (PoV); Chysochromulina brevifilum virus (CbV); Phaeocystis globosa virus (PgV); Heterosigma akashiwo virus (HaV); equid herpesvirus (EHV); alcelaphine herpesvirus (AlHV); bovine herpesvirus (BOHV); African swine fever virus (ASFV); Autographa californica multiple nucleopolyhedrovirus (AcMNPV); Bombyx mori nucleopolyhedrovirus (BmNPV); Lymantria dispar multiple nucleopolyhedrovirus (dMNDV); Helicoverpa zea single nucleopolyhedrovirus (HzSNPV); Chironomus luridus entomopoxvirus; (CLEV); vaccinia virus (VACV); fowlpox virus (FWPV); molluscum contagiosum virus (MOCV); invertebrate iridescent virus (IIV; family *Iridoviridae*). The scale bar indicates a distance of 0.2 fixed mutations per amino acid. (Courtesy of Ilana Gilg.)

## Phylogenetic relationships within the family

At the time of writing, the phylogeny of the *Phycodnaviridae* is in question, owing to the wide variation in DNA sequence that is observed between the six clades of the family *Phycodnaviridae* (ringed in Figure 4; DNA sequence identities range from 29% to 98%). It is possible that the *Phycodnaviridae* may need to be split into separate families as more sequence data become available (e.g. see discussion by Moreau *et al.*, 2010).

## Similarity with other taxa

See comments above about phylogenetic relationships. In addition, many large polyhedral virus-like particles have been observed in electron micrographs of eukaryotic algae. However, for the most part these particles have not been characterized.

## Derivation of names

*Chloro*: from Greek *chloro*, "green".
*Cocco*: derived from Greek *kokkis*, "grain" or "berry" (referring to their shape).
*dna*: for deoxyribonucleic acid.

*Lith*: from Greek *Lithos*, "stone".
*Phaeo*: from Greek *phaeo*, "brown".
*Phyco*: from Greek *phycos*, "plant".
*Prasino*: from Latin *prasino*, "green".
*Prymnesio*: from Greek *prymne*, "stern of a ship".
*Raphido*: from Greek *raphido*, "spine".

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## Contributed by

Wilson, W.H., Van Etten, J.L., Schroeder, D.C., Nagasaki, K., Brussaard, C., Bratbak, G. and Suttle, C.

# Family *Plasmaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Plasmaviridae*** |
| Genus | ***Plasmavirus*** |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Plasmavirus*

Type species ***Acholeplasma phage L2***

## Virion properties

### Morphology

Virions are quasi-spherical, slightly pleomorphic, enveloped and about 80 nm (range 50–125 nm) in diameter (Figure 1). Size varies due to virion heterogeneity: at least three distinct virion forms are produced during infection. Thin-sections show virions with densely stained centers, presumably containing condensed DNA, and particles with lucent centers. The absence of a regular capsid structure suggests the Acholeplasma phage L2 (L2) virion is an asymmetric nucleoprotein condensation bounded by a lipid-protein membrane.

### Physicochemical and physical properties

Virions are extremely heat-sensitive, relatively cold-stable and inactivated by nonionic detergents (Brij-58, Triton X-100 and Nonidet P-40), ether and chloroform. Viral infectivity is resistant to DNAse I and phospholipase A, but sensitive to pronase and trypsin treatment. UV-irradiated virions can be reactivated in host cells by excision and SOS DNA repair systems. Virions are relatively resistant to photodynamic inactivation.

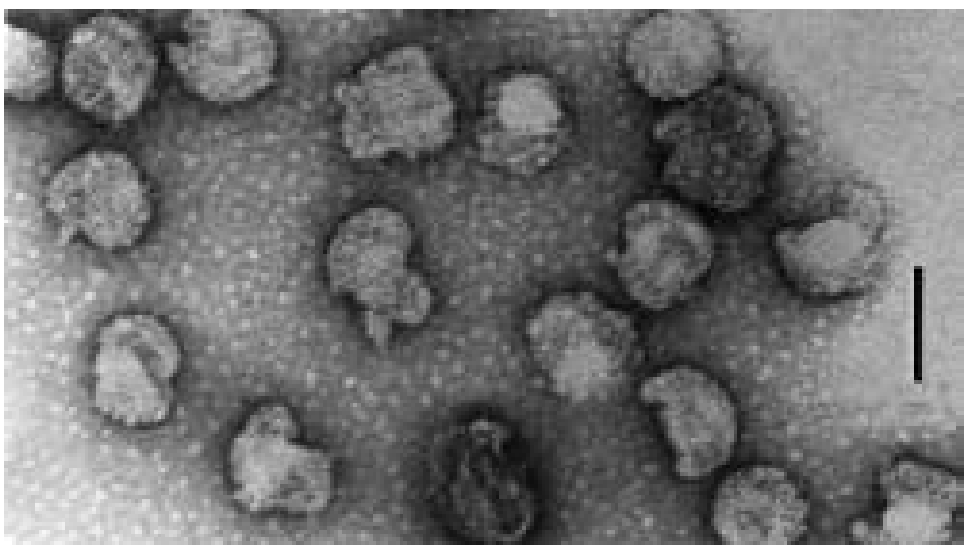

**Figure 1:** Negative contrast electron micrograph of Acholeplasma phage L2 (L2) virions. The pleomorphic virion appears as a core (perhaps a nucleoprotein condensation) within a baggy membrane. The bar represents 100 nm. (From Poddar *et al*. (1985); with permission.)

**Table 1:** Acholeplasma phage L2 ORFs

| Designation | ORF size (codons) | Comments |
|---|---|---|
| ORF 1 | 66,643 | – |
| ORF 2 | 9,620 | – |
| ORF 3 | 37,157 | – |
| ORF 4 | 18,224 | – |
| ORF 5 | 34,868 | putative integrase, gene is upstream from *attP* site |
| ORF 6 | 9,799 | – |
| ORF 7 | 14,047 | – |
| ORF 8 | 7,412 | – |
| ORF 9 | 9,332 | – |
| ORF 10 | 16,143 | – |
| ORF 11 | 25,562 | – |
| ORF 12 | 17,214 | basic protein, putative major virion DNA-binding protein |
| ORF 13 | 81,308 | putative integral membrane protein, has 27 amino acid N-terminal peptidase cleavage signal sequence |
| ORF 13* | 47,699 | translation start site is 295 codons downstream from ORF13 start site and in same reading frame |
| ORF 14 | 26,105 | has 26 aa N-terminal peptidase cleavage signal sequence |

### Nucleic acid

Virions contain one molecule of infectious, circular, superhelical dsDNA. The phage L2 genome is 11,965 bp, with a G+C value of 32%. All ORFs are encoded in one strand. Several genes are translated from overlapping reading frames.

### Proteins

Virions contain at least four major proteins of about 64, 61, 58 and 19 kDa. Several minor protein bands are also observed in virion preparations. DNA sequence analysis indicates 15 ORFs (Table 1).

### Lipids

Virions and host cell membranes have similar fatty acid compositions. Variation of host cell membrane fatty acid composition leads to virions with corresponding fatty acid composition variations. Data indicate viral membrane lipids are in a bilayer structure.

### Carbohydrates

None reported.

## Genome organization and replication

L2 infection involves a noncytocidal productive infectious cycle followed by a lysogenic cycle in each infected cell. At least 11 overlapping mRNAs are transcribed from the DNA coding strand, from at least eight promoters. In non-cytocidal infection, progeny phages are released by budding from the host cell membrane, with the host surviving as a lysogen. Lysogeny involves integration of the phage L2 genome into a unique site in the host cell chromosome. The putative phage L2 attP integration site is CATCTTCAT–7nt-CTGAAGATA. Lysogens are resistant to superinfection by homologous virus but not by heterologous virus (apparently due to a repressor), and are inducible by UV-irradiation and mitomycin C.

## Antigenic properties

None reported.

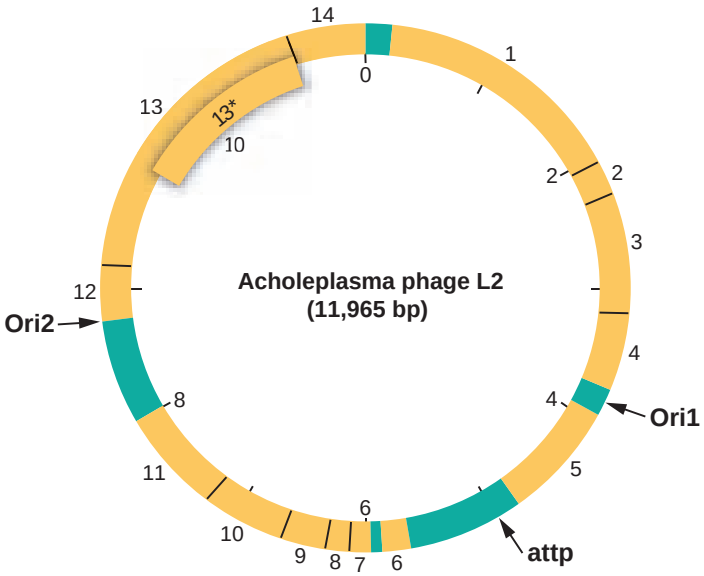


**Figure 2:** Map of genome organization of Acholeplasma phage L2 (L2) showing ORFs as determined from analysis of the 11,965 bp sequence. The base on the 3′-side of the single BstE II cleavage site is taken as the first base of the DNA sequence. The map also shows locations of the phage L2 integration site (attP) and the two phage L2 DNA replication origin sites (ori1 and ori2). (From Maniloff *et al*. (1994); with permission.)

## Biological properties

Host range: the phage L2 infects *Acholeplasma laidlawii* strains. Other putative plasmaviruses have been reported to infect *A. laidlawii* (Acholeplasma phages v1, v2, v4, v5 and v7), *A. modicum* (Acholeplasma phage M1) and *A. oculi* strains (Acholeplasma phage O1).

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Plasmavirus*

*Acholeplasma phage L2*
Acholeplasma phage L2 [L13696] (L2)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Plasmavirus* but have not been approved as species

Acholeplasma phage M1 (M1)
Acholeplasma phage O1 (O1)
Acholeplasma phage v1 (v1)
Acholeplasma phage v2 (v2)
Acholeplasma phage v4 (v4)
Acholeplasma phage v5 (v5)
Acholeplasma phage v7 (v7)

## List of unassigned species in the family *Plasmaviridae*

None reported.

## Phylogenetic relationships within the family

No information available.

## Similarity with other taxa

None reported.

## Derivation of names

*Plasma*: from the Greek *plasma*, "shaped product", referring to the plastic virion shape.

## Further reading

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Maniloff, J., Cadden, S.P. and Putzrath, R.M. (1981). Maturation of an enveloped budding phage: mycoplasma virus L2. In M.S. DuBow (Ed.), *Bacteriophage Assembly* (pp. 503–513). New York: AR Liss Inc..

Maniloff, J., Kampo, G.K. and Dascher, C.C. (1994). Sequence analysis of a unique temperate phage: mycoplasma virus L2. *Gene*, 141, 1–8.

Poddar, S.K., Cadden, S.P., Das, J. and Maniloff, J. (1985). Heterogeneous progeny viruses are produced by a budding enveloped phage. *Intervirology*, **23**, 208–221.

## Contributed by

Maniloff, J.

# Family *Polydnaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Polydnaviridae*** |
| Genus | ***Bracovirus*** |
| Genus | ***Ichnovirus*** |



## Virion properties

### Morphology

Virions have a complex construction, consisting of a nucleocapsid and a single or double layer envelope. Virions consist of one or more enveloped nucleocapsids. Polydnaviruses are divided into two genera, *Bracovirus* and *Ichnovirus*, which share few morphological features (Figure 1). Morphological traits for each genus are described below.

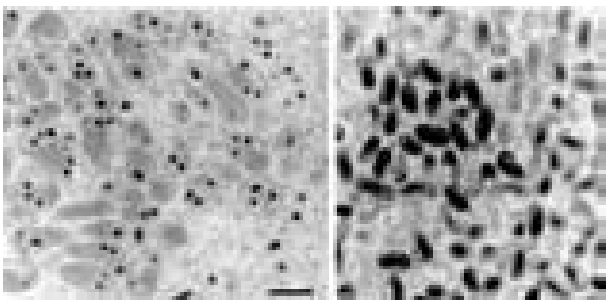

**Figure 1:** Cotesia melanoscela bracovirus (left) and Campoletis sonorensis ichnovirus virions (right). The bars represent 200 nm.

### Physicochemical and physical properties

None reported.

### Nucleic acid

Encapsidated genomes of polydnaviruses consist of multiple dsDNAs of variable size (Figure 2). Genome segments are non-equimolar in abundance. The encapsidated form of polydnavirus genomes is segmented; segments consist of circular supercoiled double stranded DNA. Virions may also contain homologous DNA sequences that are shared among two or more DNA genome segments. Aggregate, non-redundant, genome sizes range from approximately 190 to more than 500 kbp.

### Proteins

Virions are structurally complex and contain at least 20–30 polypeptides, with sizes ranging from 10 to 200 kDa.

### Lipids

Lipids are present, but uncharacterized.

### Carbohydrates

Carbohydrates are present, but uncharacterized.

## Genome organization and replication

Unique among dsDNA viruses, polydnaviruses are specifically associated with parasitoid wasps in the insect order Hymenoptera. Each polydnavirus carried by a given wasp species is genetically unique and exists in two forms. Polydnaviruses persist and are transmitted from adult wasp to

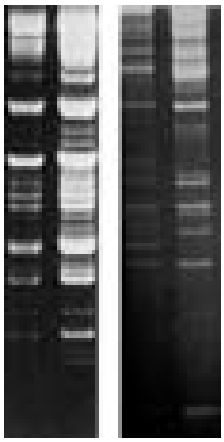

**Figure 2:** Encapsidated DNA genomes from a member of the genus *Ichnovirus* (Campoletis sonorensis ichnovirus, CsIV); left panel, two lanes with two different amounts of DNA); and a *Bracovirus* (Cotesia marginiventris bracovirus, CmaBV; right panel, two lanes with two different amounts of DNA). Genomes were electrophoresed on 1% agarose gels and visualized with ethidium bromide. The different amounts of DNA illustrate the non-equimolarity of individual genomic segments.

offspring as proviruses that are stably integrated into the genome of the wasp. Replication, which results in production of the encapsidated form of the virus, is restricted to specialized calyx cells in the ovaries of female wasps. Replication in calyx cells is nuclear and begins during wasp pupal-adult development. Virus morphogenesis occurs in calyx cells of all female wasps. Viral DNA replication involves amplification of genes plus proviral DNAs corresponding to the viral DNAs packaged into particles. Proviral DNAs are excised via site-specific recombination events and packaged into virus particles, whereas amplified genes required for particle formation are not. Bracovirus particles are released by lysis of calyx cells, while ichnovirus particles bud from calyx cells. Both ichno- and bracoviruses accumulate to high density in the lumen of the oviducts, and wasps inject a quantity of these particles into host insects at oviposition (Figure 3). Shared features of the encapsidated form of polydnavirus genomes include low coding densities and strong A+T biases. A majority of predicted genes in the encapsidated genome consist of related variants, which form multimember gene families. Several genes also contain introns. Transcriptional activity of polydnaviruses is host-specific with some genes expressed only in the wasp, other genes expressed only in the parasitized host of the wasp, and a few genes expressed in both the wasp and parasitized hosts. Bracoviruses and ichnoviruses share few genes or gene families with one another due to their distinct evolutionary origins (see below). Shared genomic and biological features, therefore, reflect convergent evolution. It is also possible that rare genetic exchanges have occurred between polydnaviruses carried by different wasps that parasitize the same host species.

## Antigenic properties

Members of a number of different *Ichnovirus* species share cross-reacting antigenic determinants; in some cases, viral nucleocapsids share at least one major conserved epitope. Campoletis sonorensis ichnovirus (CsIV) and *C. sonorensis* venom proteins display some common epitopes. Although less understood, bracoviruses also share some antigenic determinants with one another.

## Biological properties

All polydnavirus-carrying wasps reside in two families (Braconidae and Ichneumonidae) of the Hymenoptera. All wasp species lay (oviposit) their eggs inside the body of hosts, which are primarily other insects in the order Lepidoptera (Figure 3). Wasp offspring develop by feeding on host tissues. Polydnaviruses are transmitted only vertically from wasp to offspring as proviruses (Figure 3). Reciprocally, wasps infect hosts with only the encapsidated form of polydnaviruses. After parasitism, virus particles infect host tissues and multiple viral genes are thereafter expressed (Figure 3). However, polydnaviruses do not replicate in the wasp's host due to the absence of genes required for

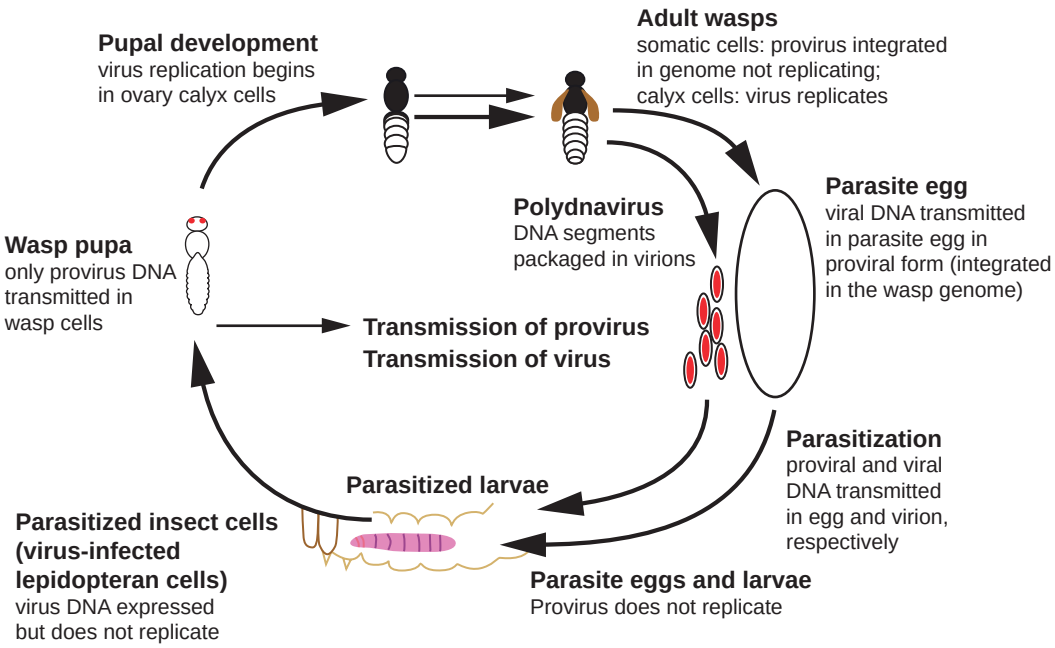


**Figure 3:** Polydnavirus replication and transmission cycles. The replication and transmission of polydnaviruses and the life cycle of an endoparasitic wasp are illustrated. Viral DNA is transmitted as proviral DNA in wasp cells (thin arrows) and as circular episomal DNAs within virions (thick arrows). In replicative wasp cells and in infected lepidopteran cells, viral DNA is present in an unpackaged closed circular form. In non-replicative cells (i.e. all wasp cells except female pupal/adult calyx cells) the virus does not replicate and exists predominantly in the proviral form. Polydnaviruses are vertically transmitted in only the proviral DNA form.

particle formation (see above). Virus-specific gene products cause significant changes in the physiology of the wasp's host, which are required for successful development of offspring. Thus, a mutualism exists because transmission as a provirus depends upon survival of the wasp, and survival of the wasp depends upon infection of its host by the encapsidated, non-replicating form of the virus.

## Genus *Bracovirus*

Type species *Cotesia melanoscela bracovirus*

### Distinguishing features

Bracoviruses are associated with an estimated 18,000 species of wasps in five subfamilies of the Braconidae (Microgastrinae, Cardiochilinae, Miracinae, Khoikholinae and Cheloninae) which together form a monophyletic assemblage called the microgastroid complex. Bracovirus nucleocapsids are cylindrical and surrounded by a single unit membrane envelope. Bracovirus virions contain either single or multiple nucleocapsids in a species-dependent manner.

### Virion properties

#### Morphology

Virions consist of enveloped cylindrical electron-dense nucleocapsids of uniform diameter (34–40 nm) but of variable length (8–150 nm length) and may contain one or more nucleocapsids within a single envelope assembled *de novo* in the nuclei of calyx cells. Bracovirus nucleocapsids also possess long unipolar tail-like appendages (Figure 4).

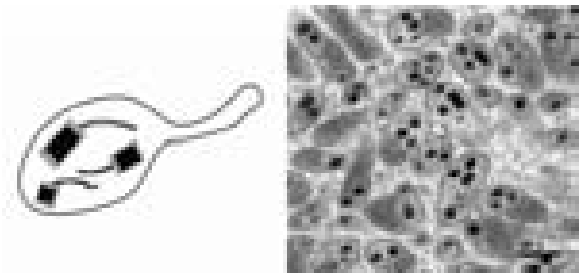

**Figure 4:** (Left) Sectional diagram and (right) negative contrast electron micrograph of particles of Protapanteles paleacritae bracovirus (PpBV). The bar represents 200 nm. (Courtesy of D. Stoltz.)

### Nucleic acid

The encapsidated genomes of bracoviruses consist of multiple, circular dsDNAs ranging in size from approximately 2.0 to more than 30 kbp. Aggregate, non-redundant genome sizes range from 189 kbp for Microplitis demolitor bracovirus (MdBV) to more than 600 kbp for Cotesia congregata bracovirus (CcBV). Genomic segments vary from as few as six for Toxoneuron nigriceps bracovirus (TnBV) to 29 DNAs for Glyptapanteles indiensis bracovirus (GiBV). The corresponding proviral (linear) form of bracovirus genomes reside at multiple macroloci located in specialized regions of the wasp genome. Some of the genes required for replication and the formation of particles (i.e. viral machinery) also cluster in a unique region(s) in the wasp genome.

## Genome organization and replication

The proviral genome segments cluster in tandem arrays in specialized regions of the wasp genome (Figure 5). Proviral DNA is amplified in calyx cells of female wasp ovaries at the onset of replication in the pupal stage. Genomic segments are excised from the amplified cluster at repetitive sequences for packaging into virions. In the case of Glyptapanteles indiensis bracovirus (GiBV), 20 of the 29 segments comprising the encapsidated form of the genome form one macrolocus, while five other proviral loci contain either a single or two proviral segments in tandem array. Small, intersegmental regions (<1kbp), lacking any predicted open reading frames, separate the tandemly arrayed proviral segments. Approximately half of the genes required for particle formation, related to structural genes of nudiviruses, are also clustered in the wasp genome, albeit not in close proximity to proviral loci. At the onset of replication, sequences corresponding to proviral loci are amplified in calyx cells, while genes involved in particle formation are expressed at high levels. Amplified DNAs are then excised and packaged into virus particles, whereas amplified genes required for particle formation are not. Genomic segments are individually packaged into particles and are non-equimolar in abundance. Most genomic segments packaged into particles encode one or more genes but coding densities are overall low. A majority of genes belong to multimember gene families. Gene family members may be clustered on a single viral genomic segment or distributed on multiple segments. Bracoviruses associated with closely related wasps appear to encode similar genes and gene families, whereas the encapsidated genomes of bracoviruses from distantly related wasps share few or no genes. Core genes involved in particle formation in contrast appear conserved among bracoviruses.

## Antigenic properties

Antigenic relationships among the bracoviruses have not been investigated in detail although cross-reacting epitopes have been detected.

## Biological properties

Braconid wasps that carry bracoviruses form a monophyletic lineage. All known bracovirus-carrying wasps inject a quantity of virus particles into hosts during oviposition. For most species, virus

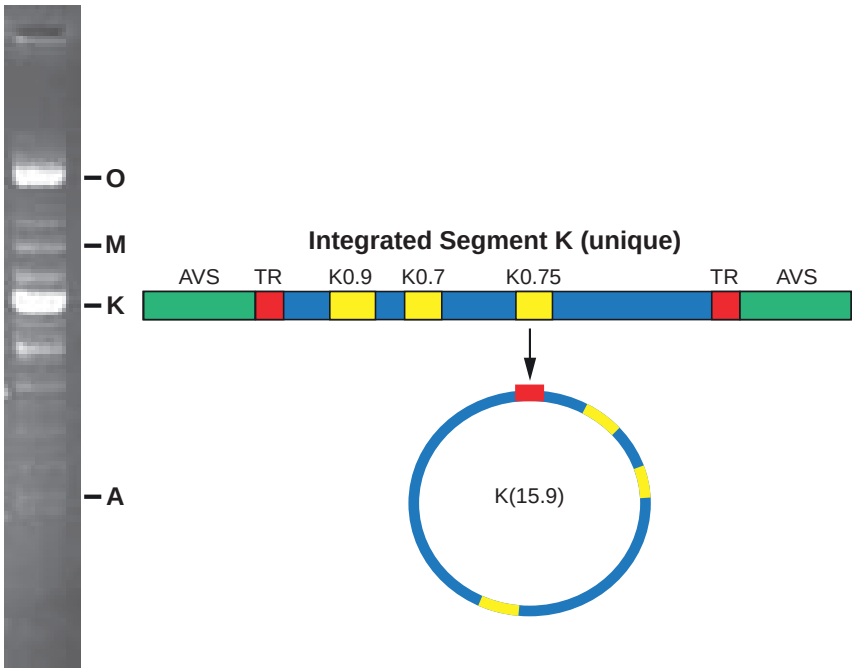


**Figure 5:** Bracovirus genome organization and replication. The Microplitis demolitor bracovirus (MdBV) genome is shown on left with selected viral segments identified. The right panel shows a representative bracovirus segment, MdBV segment K, in the proviral (top) and episomal form (bottom). Proviral segments may be found in tandem arrays with adjacent viral segments (AVS) flanking the integration site of proviral segments. MdBV segments encode genes but are predominantly non-coding sequence.

particles preferentially infect host immune cells (hemocytes) plus selected other tissues including the fat body. Expression of viral gene products in the absence of replication leads to significant changes in host physiology essential for successful parasitism. Bracoviruses from several wasp species prevent the immune system of the host from killing the wasp's offspring. Several immunosuppressive genes encoded by bracovirus have been identified. Bracovirus gene products also alter host development and metabolism.

## Species demarcation criteria in the genus

To demonstrate experimentally that a virus is a bracovirus, the following criteria should be met:

- Virions were isolated from the reproductive tract (calyx region) of adult, female braconid wasps
- Virions have bracovirus morphology, including cylindrical nucleocapsids of variable length, with a diameter of about 30 nm surrounded by a single unit membrane, single or multiple nucleocapsids may be present in the virion
- Nucleic acid isolated from virions is circular dsDNA from multiple molecules (i.e. the DNA genome is segmented)
- Reference specimens of the wasp host are identified by a qualified systematics specialist and deposited in an accessible insect collection.

Criteria that are thought to be of systematic significance:

- Parasitoid wasp species (proviral and replicative host)
- Host(s) of the wasp and its associated bracovirus (the non-replicative host)
- DNA restriction map profiles and/or sequence.

## List of species in the genus *Bracovirus*

| | | |
|---|---|---|
| *Apanteles crassicornis bracovirus* | | |
| Apanteles crassicornis bracovirus | | (AcBV) |
| *Apanteles fumiferanae bracovirus* | | |
| Apanteles fumiferanae bracovirus | | (AfBV) |
| *Ascogaster argentifrons bracovirus* | | |
| Ascogaster argentifrons bracovirus | | (AaBV) |
| *Ascogaster quadridentata bracovirus* | | |
| Ascogaster quadridentata bracovirus | | (AqBV) |
| *Cardiochiles nigriceps bracovirus* | | |
| (Toxoneuron nigriceps bracovirus) | | |
| Toxoneuron nigriceps bracovirus | [Y19010, AJ440973] | (TnBV) |
| *Chelonus altitudinis bracovirus* | | |
| Chelonus altitudinis bracovirus | | (CalBV) |
| *Chelonus blackburni bracovirus* | | |
| Chelonus blackburni bracovirus | | (CbBV) |
| *Chelonus inanitus bracovirus* | | |
| Chelonus inanitus bracovirus | [Z31378, Z58828, AM261417, AM850131, AJ319653, AJ278673, AJ627175] | (CiBV) |
| *Chelonus insularis bracovirus* | | |
| Chelonus insularis bracovirus | | (CinsBV) |
| *Chelonus nr. curvimaculatus bracovirus* | | |
| Chelonus nr. curvimaculatus bracovirus | | (CcvBV) |
| *Chelonus texanus bracovirus* | | |
| Chelonus texanus bracovirus | | (CtBV) |
| *Cotesia congregata bracovirus* | | |
| Cotesia congregata bracovirus | [AF049877, AJ632304, EU493286, D29821, AJ640087, AM180416, AJ583542, AF049876, AF006205] | (CcBV) |
| *Cotesia flavipes bracovirus* | | |
| Cotesia flavipes bracovirus | [EU493300, EU493297] | (CfBV) |
| *Cotesia glomerata bracovirus* | | |
| Cotesia glomerata bracovirus | [FJ713017, AY481559, AY486078, AY466396, EU493327, DQ844603, DQ839630] | (CgBV) |
| *Cotesia hyphantriae bracovirus* | | |
| Cotesia hyphantriae bracovirus | | (ChBV) |
| *Cotesia kariyai bracovirus* | | |
| Cotesia kariyai bracovirus | [AB099714, AB086812, AB074136] | (CkBV) |
| *Cotesia marginiventris bracovirus* | | |
| Cotesia marginiventris bracovirus | | (CmaBV) |
| *Cotesia melanoscela bracovirus* | | |
| Cotesia melanoscela bracovirus | [EU493303] | (CmeBV) |
| *Cotesia rubecula bracovirus* | | |
| Cotesia rubecula bracovirus | [U55279, AY631272, AY234855, AF359344, EU493316] | (CrBV) |
| *Cotesia schaeferi bracovirus* | | |
| Cotesia schaeferi bracovirus | | (CsBV) |
| *Diolcogaster facetosa bracovirus* | | |
| Diolcogaster facetosa bracovirus | | (DfBV) |
| *Glyptapanteles flavicoxis bracovirus* | | |
| Glyptapanteles flavicoxis bracovirus | | (GflBV) |
| *Glyptapanteles indiensis bracovirus* | | |
| Glyptapanteles indiensis bracovirus | [EF051505, AY871265, AY162267, AF414845, EU001243, AF198385] | (GiBV) |
| *Glyptapanteles liparidis bracovirus* | | |
| Glyptapanteles liparidis bracovirus | | (GlBV) |
| *Hypomicrogaster canadensis bracovirus* | | |
| Hypomicrogaster canadensis bracovirus | | (HcBV) |
| *Hypomicrogaster ectdytolophae bracovirus* | | |
| Hypomicrogaster ectdytolophae bracovirus | | (HecBV) |
| *Microplitis croceipes bracovirus* | | |
| Microplitis croceipes bracovirus | | (McBV) |
| *Microplitis demolitor bracovirus* | | |

| | | |
|---|---|---|
| Microplitis demolitor bracovirus | [AF267174, U76033, AY842013, AY848690, AY875680, AY887894, DQ000240] | (MdBV) |
| *Phanerotoma flavitestacea bracovirus* | | |
| Phanerotoma flavitestacea bracovirus | | (PfBV) |
| *Pholetesor ornigis bracovirus* | | |
| Pholetesor ornigis bracovirus | | (PoBV) |
| *Protapanteles paleacritae bracovirus* | | |
| Protapanteles paleacritae bracovirus | | (PpBV) |
| *Tranosema rostrale bracovirus* | | |
| Tranosema rostrale bracovirus | | (TrBV) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Bracovirus* but have not been approved as species

| | | |
|---|---|---|
| Cotesia chilonis bracovirus | [EU493292-6] | (CchBV) |
| Cotesia plutellae bracovirus | [AY651828-30, AY461733, DQ858218-9, EU523381, DQ299488, DI146230, DI145340, DI143592, DI112181, DI111256, DI114377, DI112939, DI110650, EF067331-2, EF067319-30, DQ075354-60] | (CpBV) |
| Cotesia ruficrus bracovirus | [AB099713] | (CrfBV) |
| Cotesia sesamiae bracovirus | [EF710626-35, EF710636-43] | (CseBV) |
| Cotesia vestalis bracovirus | [EU127911, FJ176776-8, EU493308-10, EU081840, EU095951, EF467277-8] | (CvBV) |
| Microplitis bicoloratus bracovirus | [DQ286649] | (MbBV) |
| Toxoneuron nigriceps bracovirus | [Y19010, AJ440973] | (MdBV) |

## Genus *Ichnovirus*

Type species ***Campoletis sonorensis ichnovirus***

### Distinguishing features

Ichnoviruses are associated with an estimated 13,000 species of wasps in two subfamilies of the Ichneumonidae (Campopleginae and Banchinae). Ichnovirus nucleocapsids are fusiform or quasi-cylindrical, often with a short tail-like appendage, and enveloped by two unit membranes. Virions associated with campoplegine ichneumonids contain a single nucleocapsid, whereas virions associated with banchine ichneumonids envelope multiple nucleocapsids.

### Virion properties

#### Morphology

Ichnovirus virions consist of nucleocapsids of uniform size (approximately 85 × 330 nm), having the form of a prolate ellipsoid, surrounded by two unit membrane envelopes (Figure 6). The inner envelope appears to be assembled *de novo* in the nuclei of calyx cells, while the outer envelope is acquired by budding through the plasma membrane of calyx cells.

#### Nucleic acid

The encapsidated genomes of ichnoviruses consist of multiple, circular dsDNAs ranging in size from approximately 2.0 to more than 25 kbp. Ichnoviruses carried by campoplegine ichneumonids like Campoletis sonorensis ichnovirus (CsIV) and Hyposoter fugitivus ichnovirus consist of 20–25 genomic segments with an aggregate size of approximately 250 kbp. In contrast, ichnoviruses associated with banchine ichneumonids, like Glypta fumiferanae virus (GfIV) has an aggregate size of approximately 290 kbp divided into more than 100 segments that range from 1.5 to 5.0 kbp.

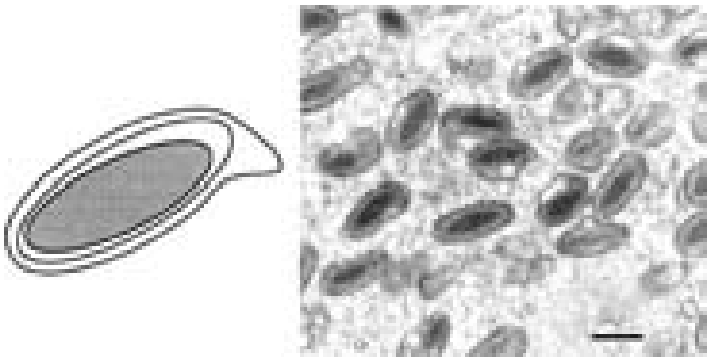

**Figure 6:** (Left) Sectional diagram and (right) negative contrast electron micrograph of particles of Hyposoter exiguae ichnovirus (HeIV). The bar represents 200 nm. (Courtesy of D. Stoltz.)

## Genome organization and replication

Ichnoviruses replicate from proviral DNA. Proviral DNA is amplified in calyx cells of female wasp ovaries at the onset of replication in the pupal stage. Genomic segments are excised from the amplified cluster at repetitive sequences for packaging into virions. Genes required for particle formation are clustered in the wasp genome, forming multiple ichnovirus structural protein-encoding regions. In the wasp *Hyposoter didymator*, two of these structural protein-encoding regions are located in proximity to proviral loci. At the onset of replication, structural protein-encoding regions and proviral loci amplify in calyx cells. Proviral DNAs are then excised and packaged into virus particles, but structural protein-encoding regions are not. Encapsidated ichnovirus genomes are non-equimolar with two recognized viral segment types, nested and unique (Figure 7). Nested segments are often hypermolar and produce from 2 to 5 partially redundant segments from a single proviral locus. Unique segments excise to produce a single segment that is encapsidated. There is evidence that nested segments are preferentially associated with some gene families. Most genomic segments encode one or more genes but coding densities are overall low. A majority of genes belong to multimember gene families. Gene family members may be clustered on a single viral segment or distributed on multiple segments. The encapsidated genomes of ichnoviruses from campoplegine ichneumonids share six gene families, but only one of these gene families is shared with ichnoviruses from banchine ichneumonids. Several genes involved in particle formation are conserved among ichnoviruses, but are unrelated to structural-protein encoding genes of bracoviruses.

## Antigenic properties

Cross-reacting antigenic determinants are shared by a number of different ichnovirus isolates; in some cases, viral nucleocapsids share at least one major conserved epitope. CsIV and *C. sonorensis* venom protein display common epitopes.

## Biological properties

Ichnovirus-carrying wasps inject a quantity of virus particles into host animals during oviposition; virus-specific expression leads to significant changes in host physiology, some of which are responsible for successful parasitism. Ichnoviruses inhibit the immune responses and alter development of infected (parasitized) hosts. Infection impacts translation of some host mRNAs and this impacts the ability of the host to mount effective immune responses (e.g. melanization).

## Species demarcation criteria in the genus

To demonstrate experimentally that a virus is an ichnovirus, the following criteria should be met:

- Virions were isolated from the reproductive tract (calyx region) of adult, female ichneumonid wasps

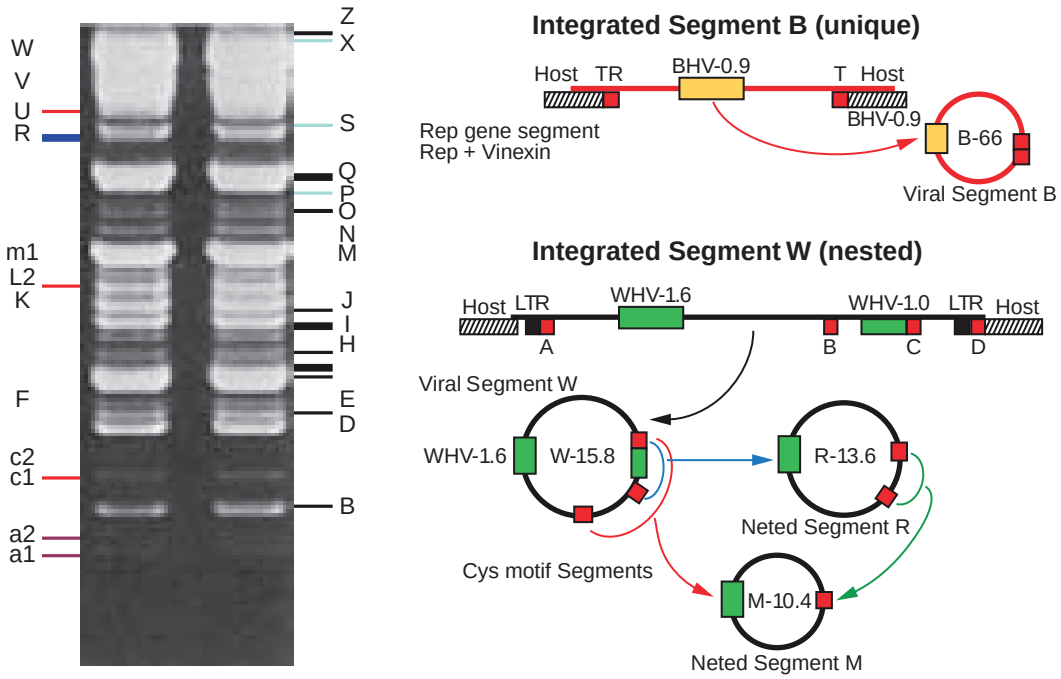


**Figure 7:** Undigested Campoletis sonorensis ichnovirus (CsIV) genome (left panel) with unique segment labels on right and nested segment labels on left. Colors indicate genes known to be associated with each segment. On left all segments encode cys-motif genes. Segments indicated in red are derived from larger segments (nested). Labels on right in this panel indicate unique segments that encode rep genes (black) and "non-rep" genes (e.g. P has similarity to NF-κβ genes). Right panel illustrates unique (top) and nested segments (bottom) in their proviral (integrated-linear) and episomal forms (circular).

- Virions have ichnovirus morphology including fusiform nucleocapsids surrounded by a double unit membrane, single or multiple nucleocapsids may be present per virion
- Nucleic acid isolated from virions is circular dsDNA from multiple molecules (i.e. the DNA genome is segmented)
- Reference specimens of the wasp host identified by a qualified systematics specialist and deposited in an accessible insect collection.

Criteria that are thought to be of systematic significance:

- Parasitoid wasp (proviral and replicative host)
- Host(s) of the wasp and its associated ichnovirus (the non-replicative host)
- DNA restriction map profiles and/or sequence.

## List of species in the genus *Ichnovirus*

| | | |
|---|---|---|
| *Campoletis aprilis ichnovirus* | | |
| Campoletis aprilis ichnovirus | [FJ463032, DQ845287-8, AB100268] | (CaIV) |
| *Campoletis falvincta ichnovirus* | | |
| Campoletis flavicincta ichnovirus | | (CfIV) |
| *Campoletis sonorensis ichnovirus* | | |
| Campoletis sonorensis ichnovirus | [U41655, S47226, AY029394, AY029400, AF361487, AF411011, AF362507, AF361869, AF004367, AY573925, AY197485, AF236017, AF004366, AF004378, L08243, M17405, M23437, M80621-3, AY953130, AH006861, AF004557, M17004, M16999, M17404] | (CsIV) |

| | | |
|---|---|---|
| *Casinaria arjuna ichnovirus* | | |
| Casinaria arjuna ichnovirus | | (CarIV) |
| *Casinaria forcipata ichnovirus* | | |
| Casinaria forcipata ichnovirus | | (CfoIV |
| *Casinaria infesta ichnovirus* | | |
| Casinaria infesta ichnovirus | | (CiIV |
| *Diadegma acronyctae ichnovirus* | | |
| Diadegma acronyctae ichnovirus | | (DaIV) |
| *Diadegma interruptum ichnovirus* | | |
| Diadegma interruptum ichnovirus | | (DiIV) |
| *Diadegma terebrans ichnovirus* | | |
| Diadegma terebrans ichnovirus | | (DtIV) |
| *Enytus montanus ichnovirus* | | |
| Enytus montanus ichnovirus | | (EmIV) |
| *Eriborus terebrans ichnovirus* | | |
| Eriborus terebrans ichnovirus | | (EtIV) |
| *Glypta fumiferanae ichnovirus* | | |
| Glypta fumiferanae ichnovirus | [AB295392, AB289903-99, AB290000-7] | (GfIV) |
| *Hyposoter annulipes ichnovirus* | | |
| Hyposoter annulipes ichnovirus | | (HaIV) |
| *Hyposoter exiguae ichnovirus* | | |
| Hyposoter exiguae ichnovirus | | (HeIV) |
| *Hyposoter fugitivus ichnovirus* | | |
| Hyposoter fugitivus ichnovirus | [AY597814, AY577428-9, AY570798-9, AY563518-9, AY556383-4, AY547319, AB291200-9, AB291165-99, AY935249] | (HfIV) |
| *Hyposoter lymantriae ichnovirus* | | |
| Hyposoter lymantriae ichnovirus | | (HlIV) |
| *Hyposoter pilosulus ichnovirus* | | |
| Hyposoter pilosulus ichnovirus | | (HpIV) |
| *Hyposoter rivalis ichnovirus* | | |
| Hyposoter rivalis ichnovirus | | (HrIV) |
| *Olesicampe benefactor ichnovirus* | | |
| Olesicampe benefactor ichnovirus | | (ObIV) |
| *Olesicampe geniculatae ichnovirus* | | |
| Olesicampe geniculatae ichnovirus | | (OgIV) |
| *Synetaeris tenuifemur ichnovirus* | | |
| Synetaeris tenuifemur ichnovirus | | (StIV) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Ichnovirus* but have not been approved as species

| | | |
|---|---|---|
| Campoletis chlorideae ichnovirus | [FJ463032, DQ845287-8, AB100268] | (CaIV) |
| Campoletis sp. ichnovirus | [AY033945] | (CspIV) |
| Casinaria sp. ichnovirus | | (CaspIV) |
| Dusona sp. ichnovirus | | (DspIV) |
| Glypta sp. ichnovirus | | (GspIV) |
| Hyposoter didymator ichnovirus | [AF132024, DQ295918-20, AF241775, AY519505-6, AY518195-9, AY499565-9, AF479654, AF464931, AF364055-7, AF191723, AF132023, AF131648, AF237946, AF156933, AF464930, AY501381-3, AY486462-4] | (HdIV) |
| Lissonota sp. ichnovirus | | (LspIV) |
| Tranosema rostrale ichnovirus | [DQ790660, DQ790662-3, AF052836-7, AF529168, AF527780, AB291213-5, AB291138-64, AF421353, AY940454] | (TrIV) |

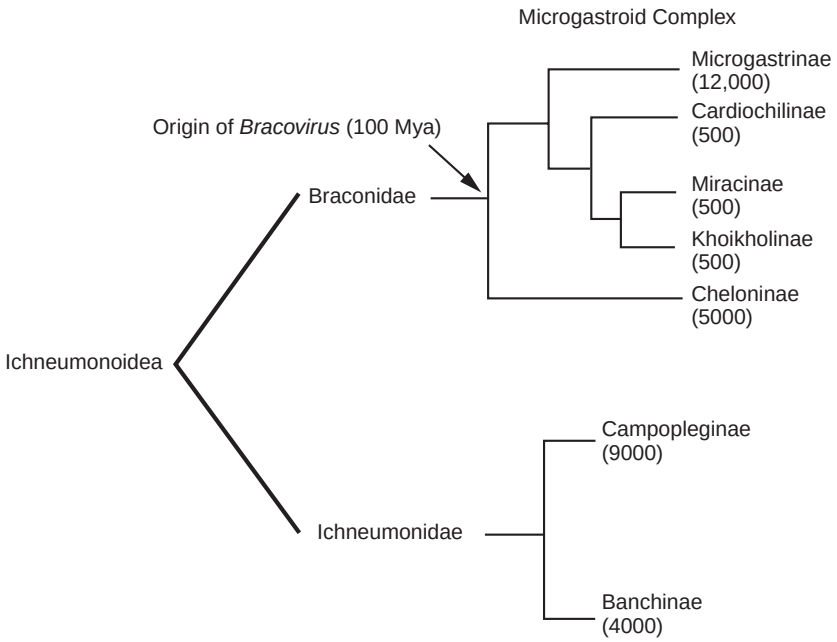


**Figure 8:** Bracovirus and ichnovirus phylogenetic tree illustrating that all members of the *Polydnaviridae* are associated with parasitoid wasps in the superfamily Ichneumonoidea. Subfamilies of the Braconidae that carry bracoviruses and subfamilies of the Ichneumonidae that carry ichnoviruses are shown. Parentheses indicate the approximate number of wasp species in each subfamily.

## Phylogenetic relationships within the family

Phylogenetic data indicate all members of the *Polydnaviridae* are associated with two families of wasps (Braconidae and Ichneumonidae) in the superfamily Ichneumonoidea (Figure 8). However, the members of the genera *Bracovirus* and *Ichnovirus* are unrelated by sequence and serological analyses. All bracoviruses are associated with wasps in specific subfamilies of the family Braconidae. Together, these subfamilies form a monophyletic lineage referred to as the microgastroid complex (Figure 8). Phylogenetic data and fossil calibrations indicate that the microgastroid complex arose approximately 100 million years ago (Mya) and that the bracovirus–braconid wasp association arose from an interaction established between a single ancestral nudivirus and the common ancestor of the microgastroid complex. This interaction then diversified into the thousands of bracovirus-carrying braconid wasp species that exist today. All ichnoviruses are associated with wasps in two subfamilies of the family Ichneumonidae (Figure 8). Phylogenetic analysis indicates that the two families of ichnovirus carrying ichneumonid wasps are separated by groups that do not carry ichnoviruses, which suggest an independent acquisition of viruses in the two groups or a loss of PDVs in some groups. Taken together, dissimilar morphologies and genomes indicate that bracoviruses and ichnoviruses do not share a common ancestor. Instead, ichnoviruses and bracoviruses are currently classified in the family *Polydnaviridae*, because of: (1) similarities in life cycle and (2) both produce replicatively defective encapsidated genomes that are comprised of multiple circular, dsDNAs. These similarities, however, reflect convergent evolution.

## Similarity with other taxa

Sequence analysis of genes required for particle formation indicate that bracoviruses evolved from an ancestral nudivirus. Sequence analysis of genes required for particle formation suggest ichnoviruses also arose from a viral ancestor. Evidence strongly indicates this ancestor was not a nudivirus but a lack of homology with any known taxon of viruses suggests ichnoviruses originated from a virus ancestor now extinct or from a currently undiscovered taxon.

## Derivation of names

*Braco*: from *Braco*nidae, a family of wasps.
*Ichno*: from *Ichn*eumonidae, a family of wasps.
*Polydna*: from Greek *poly*, "several", and *dna*, deoxyribonucleic acid.

## Further reading

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## Contributed by

Strand, M.R. and Drezen, J.-M.

# Family *Polyomaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Polyomaviridae*** |
| Genus | ***Polyomavirus*** |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Polyomavirus*

Type species ***Simian virus 40***

## Virion properties

### Morphology

Virions are non-enveloped and approximately 40–45 nm in diameter. The icosahedral capsid is composed of 72 capsomers in a skewed (T = 7d) lattice arrangement (Figure 1). Right-handed (dextro) skew has been shown for all polyomaviruses examined in cryoelectron-microscopy tilt experiments. Aberrant structures such as empty capsids, microcapsids and tubular forms are regularly observed.

### Physicochemical and physical properties

Virion Mr is $2.5 \times 10^7$. Buoyant density of virions in sucrose and CsCl gradients is 1.20 and 1.34–1.35 g $cm^{-3}$, respectively. Virion $S_{20,w}$ is 240. Virions are resistant to ether, acid and heat treatment (50 °C, 1 h). Virions are unstable at 50 °C for 1 h in the presence of 1 M $MgCl_2$. Greater than 70% of the total virion protein content is Vp1. Recombinant polyomavirus Vp1 (rVp1), expressed from baculovirus or plasmid constructs in eukaryotic cells, self-assembles into virus-like particles (VLPs) under specific chemical and physical conditions (expression of rVp1 in bacteria leads only to capsomeres). These rVp1-VLPs resemble native virions by electron microscopy and are purified by identical procedures (Figure 2). The rVp1 independently forms pentameric assembly units (pentamers) analogous to the capsomeres of virions, minus the centrally located Vp2 or Vp3 proteins. Linking of the carboxy-termini of pentameric rVp1 creates the icosahedral lattice structure of VLPs. Pentamers

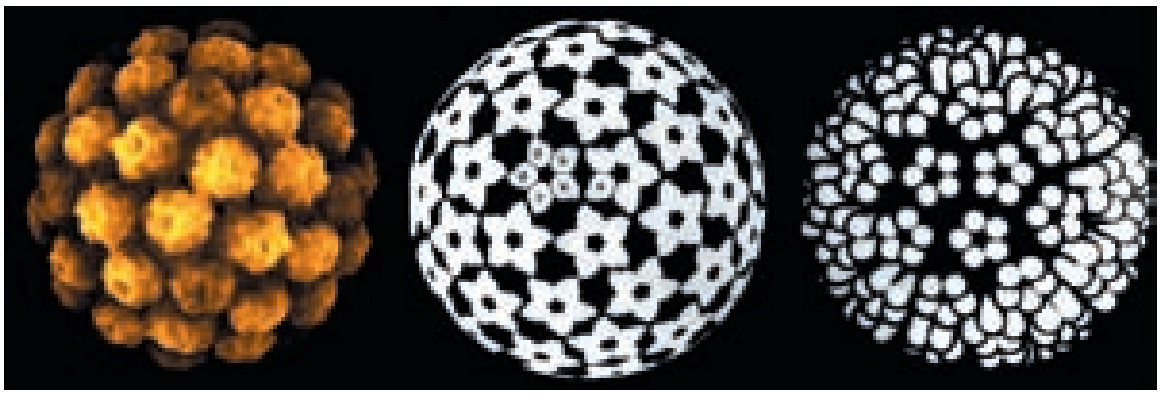

**Figure 1:** (Left) Computer rendering of a particle of murine polyomavirus strain A2. (Center) Capsomer bonding relations. Each icosahedral asymmetric unit comprises six Vp1 subunits, including one (a) from a pentavalent pentamer. The six symmetrically different subunits are designated a, a′, a″, b, b′ and c, corresponding to three different bonding states. (Right) Computer graphics representation of the surface of the capsid of murine polyomavirus strain A2. Five Vp1 subunits form the basis of a polyomavirus capsomer, and 72 capsomers link together in a 12 pentavalent/60 hexavalent arrangement, conveying icosahedral capsid structure. (Left and center, from Salunke, D.M., Casper, D.L.D. and Garcea, R.L. (1986). *Cell* **46**, 895–904; right, from Eckhart W. (1991). In *Fundamental Virology*, 2nd edn (B.N. Fields and D.M. Knipe, Eds.), Raven Press, New York; with permission.)

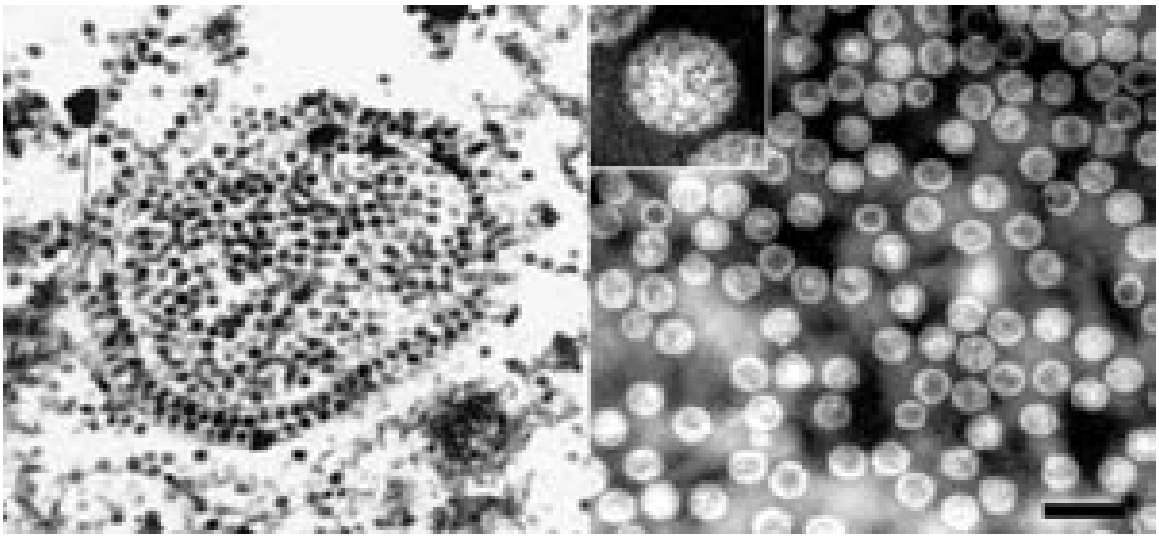

**Figure 2:** Electron micrographs of: (left) brain tissue from a progressive multifocal leukoencephalopathy (PML) patient showing the assembly of JC polyomavirus (JCPyV) particles in the nucleus of an infected oligodendrocyte; and (right) composite of virus-like particles (VLP) self-assembled from recombinant Vp1 of BK polyomavirus (BKPyV) (rBKVp1), purified by CsCl ultracentrifugation from supernatants of Sf9 insect cell cultures infected with recombinant BKVp1-baculovirus. The particles were negatively stained with 2% phosphotungstic acid. The composite includes an insert showing an enlargement of a single VLP. Bar = 100 nm.

that form from rVp1 molecules with truncated carboxy-termini are stable, but do not form VLPs. By modifying the chemical conditions, rVp1-VLPs can be dissociated and subsequently reconstituted. During self-assembly or reconstitution, rVp1-VLPs will non-specifically encapsidate genetic material that is present.

### Nucleic acid

Virions contain a single molecule of circular dsDNA. The genomic size is fairly uniform within the genus, averaging approximately 5 kbp. Simian virus 40 (SV40) strain 776 is 5,243 bp, JC polyomavirus (JCPyV) strain Mad-1 is 5,130 bp, BK polyomavirus (BKPyV) strain Dunlop is 5,153 bp, murine polyomavirus (MPyV) strain A2 is 5,297 bp, and baboon polyomavirus 2 (BPyV) is 4,697 bp. The DNA constitutes about 10–13% of the virion by weight. The G+C content varies between 40 and 50%. In the mature virion, the viral DNA is associated with host cell histone proteins H2a, H2b, H3 and H4 in a supercoiled, chromatin-like complex.

### Proteins

Currently, polyomavirus genomes are known to code for 5–9 proteins, with sizes predicted from the nucleic acid sequences ranging from 7 to 88 kDa (Table 1). Transcription from one side of the viral origin of DNA replication (ORI) results in mRNAs encoding the early proteins. These non-structural proteins are referred to as tumor (T) antigens because they interfere with cell cycle regulation and, in some cases, induce cellular transformation or tumor formation. Alternative splicing appears to be responsible for the 2–5 related, yet distinct, proteins expressed from each polyomavirus T gene. The set of proteins expressed from a single T gene shares amino-terminal sequence. The T antigens initiate bi-directional viral genome replication, as well as transcription of late viral mRNAs. Late mRNA is transcribed from the strand complementary to that used for early transcription and is also initiated from the opposite side of the ORI. Late transcripts code for three structural proteins (Vp1, Vp2 and Vp3) as well as another non-structural protein known as agnoprotein. Of the three structural proteins, Vp1 makes up more than 70% of the total virion protein content and hence is also referred to as the major structural protein. Five Vp1 proteins surround either a Vp2 or Vp3 molecule to form stable assembly units, or capsomers; 72 random capsomers link together in icosahedral symmetry to form the capsid of each virion. The Vp2 and Vp3 molecules may be necessary to ensure specific encapsidation of the replicated polyomavirus genome. Also, VP2 is myristylated (at least in MPyV and SV40) and has a possible role in entry. The agnoprotein may have some role in facilitating capsid assembly, but it is not a component of the mature virion.

**Table 1:** Deduced size of polyomavirus proteins

| Virus | MPyV | SV40 | JCPyV | BKPyV | KPyV | LPyV | BPyV | APyV |
|---|---|---|---|---|---|---|---|---|
| Structural proteins | | | | | | | | |
| VP1 | 42.4 | 39.9 | 39.6 (40) | 40.1 (40) | 41.7 | 40.2 | 40.5 | 37.4 (42) |
| VP2 | 34.8 | 38.5 | 37.4 | 38.3 | 37.4 | 39.3 | 39.1 | 37.3 (39) |
| VP3 | 22.9 | 27.0 | 25.7 | 26.7 | 25.2 | 27.3 | 26.9 | 27.0 (30) |
| Non-structural proteins | | | | | | | | |
| T | 88.0 | 81.6 (94) | 79.3 (94) | 80.5 | 72.3 | 79.9 | 66.9 | 68.3 (80) |
| mT | 48.6 | ND | ND | ND | ND | ND | ND | ND |
| $T'_{135}$ | ND | ND | (17) | * | ND | ND | ND | ND |
| $T'_{136}$ | ND | ND | (17) | * | ND | ND | ND | ND |
| $T'_{165}$ | ND | ND | (22–23) | * | ND | ND | ND | ND |
| 17kT | ND | (17) | ND | 17–20 | ND | ND | ND | ND |
| tT | * | ND | (17) | ND | ND | ND | ND | ND |
| t | 22.8 | 20.4 | 20.2 | 20.5 | 18.8 | 22.2 | 14.0 | 17.0 (24) |
| LP1/agno | ND | 7.3 | 8.1 | 7.4 | ND | ND | 13.1 | ND |

Predicted sizes of polyomavirus proteins in kDa: ( ), observed sizes of the expressed proteins; *, reported proteins that lack both predicted and expressed size; ND, not detected.

The following recently discovered polyomavirus-coded proteins are not listed in Table 1. The SV40 late region, and perhaps that of other mammalian polyomaviruses as well, encodes a 15 kD protein called Vp4 that is not contained in capsids. In the case of SV40, Vp4 appears to be involved in host cell lysis. The recently discovered Merkel cell polyomavirus (MCPyV) encodes a splice variant of the SV40 17kT protein. Except for the recently-discovered canary polyomavirus (CaPyV) (Ref: PMID: 20797969), known avian polyomaviruses have an additional ORF in the late region, at a site corresponding to that of the agnoprotein ORF in mammalian polyomavirus genomes. The protein encoded by this avian polyomavirus ORF is designated Vp4 (also referred to as agnoprotein 1a), but it has no apparent sequence homology to the SV40 Vp4 protein or to the mammalian polyomavirus agnoprotein. Vp4 of avian polyomavirus (APyV; also known as "budgerigar fledgling disease polyomavirus") is a component of the mature virion. It is thought to be involved in packaging the viral genome and inducing apoptosis. Other APyV-coded proteins not listed or designated as such in Table 1 are agnoprotein 1b (same as VP4 delta), agnoprotein 2a and agnoprotein 2b (two poorly characterized hydrophobic proteins). Agnoproteins 1a/1b and 2a/2b could either be seen as functional proteins or alternatively as non-functional splicing variants. The genomes of KI polyomavirus (KIPyV), WU polyomavirus (WUPyV), human polyomavirus-6 (HPyV6), HPyV7 and MCPyV do not appear to contain ORFs that might encode an agnoprotein, nor do they appear to encode a middle T antigen.

### Lipids

None present.

### Carbohydrates

None present

## Genome organization and replication

Virions that attach to cellular receptors are taken up by the cell and transported to the nucleus, where the genome is transcribed and replicated. During a productive infection, transcription of the viral genome is divided into an early and a late stage. Transcription of the early and late coding regions is controlled by separate promoters through the binding of specific transcription factors and cis-acting elements. The sequence that codes for early transcripts is exclusive to one strand of the viral DNA and spans approximately half the genome. Late transcripts are generated in the opposite direction from the other half of the complementary strand (Figure 3).

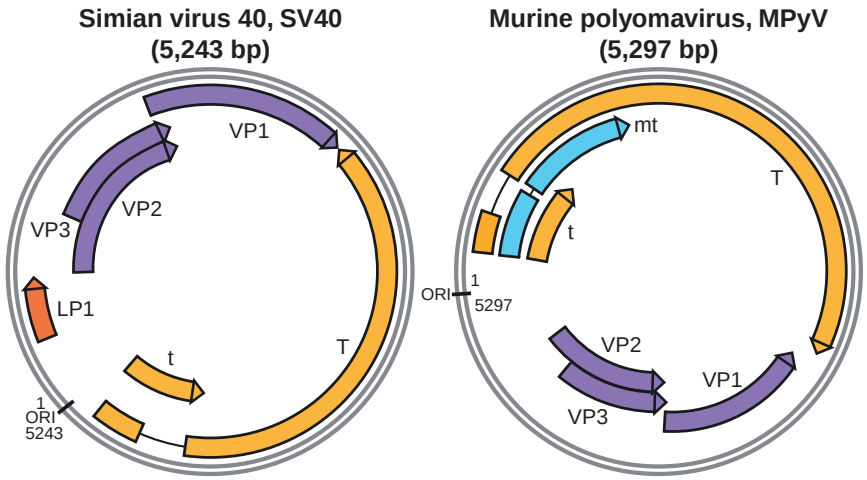


**Figure 3:** Diagram of representative polyomavirus genomes and encoded proteins, SV40 (left) and MPyV (right). The grey circles represent the viral dsDNAs. The origin of DNA replication (ORI) is indicated. Arrows indicate protein-coding regions and their direction of transcription. Introns are denoted by solid lines. Note that the non-coding region in the vicinity of ORI also includes the regulatory region. Alternative splicing is a common characteristic of polyomavirus coding regions. The multiple large T gene products all share identical amino termini.

Precursor mRNAs undergo post-transcriptional processing, which includes capping and polyadenylation of the 5′ and 3′ termini, respectively, as well as splicing. Efficient use of coding information involves differential splicing of the transcripts and use of overlapping ORFs. Early mRNAs encode regulatory, non-structural proteins that may exhibit cis- or trans-activating properties. These include proteins that are required for initiation of viral DNA replication and late protein production. Their expression leads to de-repression of some host cell enzymes and stimulation of cellular DNA synthesis. Prior to the start of the late events, viral DNA replication is initiated in the nucleus. Translation of most of the late transcripts produces structural proteins that are involved in capsid assembly. Post-translational modifications of some early and late viral proteins include phosphorylation, N-acetylation, fatty acid acylation, ADP-ribosylation, methylamination, adenylation, glycosylation and sulphation. Several of the viral proteins contain nuclear localization signals, which facilitate transport of the proteins to the host cell nucleus, where virion maturation occurs. Virions are released by lysis of infected cells.

Identified non-structural proteins include: large T, middle (m) T and small t for mouse and hamster polyomaviruses; large T, 17kT and small t for SV40. JCPyV also encodes small t, and BKPyV makes the equivalent of the SV40 17kT. In addition to large T and small t, three other large T intermediates, termed T Prime ($T'_{135}$, $T'_{136}$ and $T'_{165}$) have been described for JCPyV. Similar T′ antigens expressed from BKPyV have been identified, but the precise size of these proteins has not been reported. No mRNA encoding a protein of size comparable to the small t proteins of other polyomaviruses has been identified in BKPyV (Table 1). T antigens, first named for their involvement in tumorigenicity and transformation, play key roles in the regulation of transcription and DNA replication. The best characterized of these, SV40 large T antigen, exhibits multiple functions that can be mapped to discrete domains.

Replication of the viral genome is initiated by the specific binding of T antigen to ORI and its interaction with host DNA polymerase(s). Due to the limited amount of genetic information encoded by the viral genomes, the polyomaviruses rely heavily upon host cell machinery, including nuclear transcription factors, to replicate their DNA. Replication proceeds bi-directionally via a "Cairns" structure and terminates about 180° from ORI. Late in the replication cycle, rolling circle-type molecules have been identified. The viral proteins involved in initiation may also promote elongation through helicase and ATPase activities.

The non-coding regulatory region of each polyomavirus is positioned between the early and late protein-coding sequences. This sequence contains promoter/enhancer elements. Within

each polyomavirus species, the nucleotide sequence of the regulatory region is hypervariable. Nucleotide sequencing studies have uncovered numerous variations of regulatory region structure. For the human polyomavirus JCPyV, as with other polyomaviruses, the nucleotide sequence of the regulatory region has been shown to control levels of viral transcription and replication. The JCPyV "archetype" regulatory region sequence, which conveys relatively inefficient viral activity, contains a single copy of all sequence sections observed in all other variant forms (Figure 4). From the early side of the archetype, the initial regulatory region sequence section contains ORI followed by sequence sections designated *a*, *b*, *c*, *d*, *e* and *f*. From variant to variant, sequence sections *a* through *e* are the most likely to present deletions, replications and/or unique arrangements; for example, deletion of *b* and *d* leaves *ace*, a 98 bp sequence unit. Although the *ace* sequence unit conveys more activity than archetype, it appears to be the minimal sequence unit required for function. Also, tandem *ace* sequence units, or repeats, constitute the regulatory region of the more robust "prototype" JCPyV sequence, Mad-1. Such modification to the regulatory region structure appears to alter the cellular host range and may also be responsible for switching JCPyV between states of lytic and

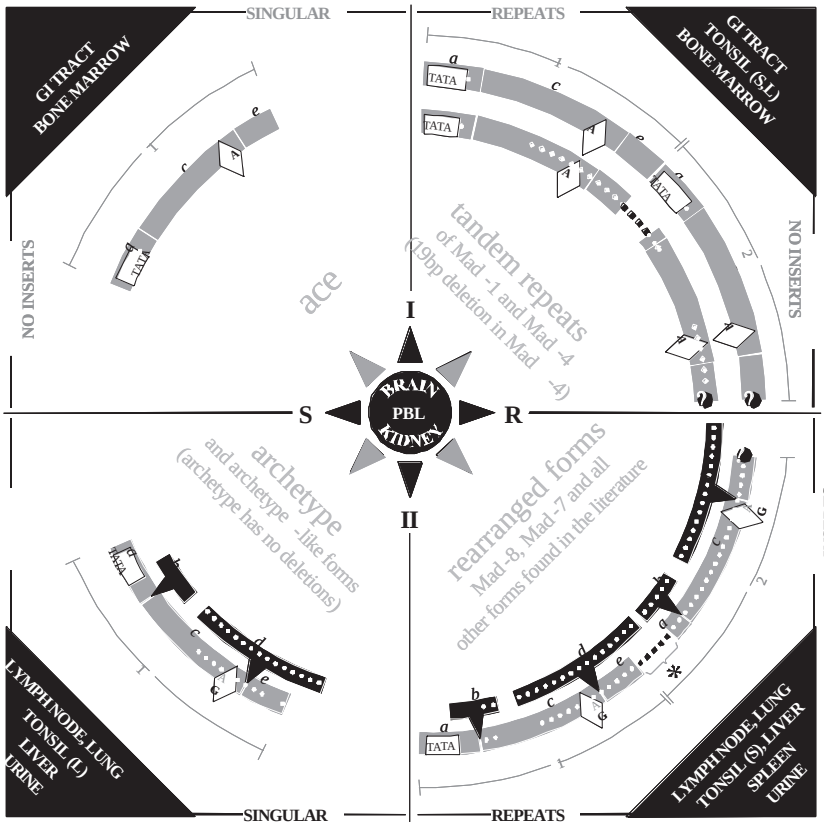


**Figure 4:** The Compass: A schematic diagram of the relationships between JC polyomavirus (JCPyV) regulatory region sequences published worldwide. JCPyV variant regulatory regions are grouped into quadrants (I-S, I-R, II-S and II-R) with ace sequence-units lightly shaded. Upper quadrant variant types (I) have no additional sequence integrated into the ace units (no inserts). Lower quadrant variant types (II) have dark integrated sequence sections (inserts), b (23 bp) and d (66 bp). Both types I and II are divided into singular (S) and repeat (R) forms by the left and right quadrants, respectively. Unshaded boxes are TATA boxes. Dots represent sites of possible deletions. Unshaded diamonds contain the nucleotide that occupies the 49th position in sequence section c (nt 85 of I-S, or 108 of II-S), which is adenine (A) in type I variants, but predominantly guanine (G) in type II variants. Right quadrants (R-forms) have dark dashes where sequence is deleted and ⊕ where additional repeats may occur. The * in the lower right quadrant (II-R) identifies one reported sequence that retains the second TATA box (Ciappi *et al.*, 1999). JCPyV tropism common to all variant regulatory region forms is contained in the dark central circle. Specific JCPyV tropisms are contained in the dark corner triangles. Cells from tonsil are either (L) lymphocytes, or (S) stromal cells (Monaco *et al.*, 1998). Cells in bone marrow that contain JCPyV have been identified as B-lymphocytes (Houff *et al.*, 1988).

latent infection. Arranging all known variant JCPyV regulatory regions into quadrants, according to integration of unique sequence sections and/or repetition of sequence section groups, also links variants by activity. Four distinct structural forms (I-S, I-R, II-S and II-R) are defined along with tissue tropisms. This design, known as the JCV Compass (Figure 4), provides logical connections between variant regulatory regions and may be useful for elucidating crucial steps in JCPyV pathogenesis. Currently, it is not known whether similar arrangements of other polyomavirus species variants also render logical relationships. The consensus sequence of the regulatory region responsible for binding of large T antigen, including that at ORI, is distinctly different in the avian polyomaviruses from that in the mammalian polyomaviruses.

An SV40 DNA sequence, located in the untranslated region 3′ to the polyadenylation cleavage site of the late pre-mRNA, encodes a miRNA that accumulates at late times in infection. This miRNA marks early viral mRNAs for degradation, thereby down-regulating T-antigen expression at late times in infection. This miRNA makes SV40-infected cells less susceptible to attack by cytotoxic T cells *in vitro*. However, a MPyV variant that cannot produce its miRNA shows no difference in pathogenicity. It is not known whether other polyomaviruses encode miRNAs.

## Antigenic properties

The human polyomaviruses JCPyV and BKPyV can be detected by hemagglutination of human type O erythrocytes, while the murine polyomavirus (MPyV) and the goose hemorrhagic polyomavirus (GHPyV) can hemagglutinate sheep and chicken erythrocytes, respectively. The capsids bind to the surface of the erythrocytes, resulting in a three-dimensional lattice-like suspension known as hemagglutination. Using serial dilutions, a titer expressed as hemagglutination units (HA units) can be determined.

Antisera prepared against disrupted virions can also detect antigens shared with other species in the genus. Members of the polyomavirus species can be distinguished antigenically by neutralization, hemagglutination inhibition and immuno-electron microscopy tests. Serum levels of antibodies to JCPyV, BKPyV and SV40 can also be detected by enzyme-linked immunosorbent assay (ELISA) by coating microtiter plates with either whole virion or recombinant VLPs. ELISA tests are also available, and are used routinely for APyV and GHPyV. Polyclonal and monoclonal antibodies can be used to demonstrate cross-reactivity between the T proteins of the primate polyomaviruses. However, there are also specific antibodies currently available that can distinguish among the T antigen epitopes of JCPyV, BKPyV and SV40.

## Biological properties

### Mammalian polyomaviruses

Whereas each of the mammalian polyomaviruses grows most efficiently *in vitro* in cells of its natural host, host species-specificity is not absolute. Cells that fail to support viral replication may be transformed by the action of the viral early gene products.

Mammalian polyomaviruses give rise to primary infections in their natural hosts that are usually not associated with clinical syndromes, although BKPyV infections have sometimes been associated with mild urinary tract and upper respiratory symptoms; the latter consistent with a possible respiratory route of BKPyV transmission. Primary infections in natural hosts then generally lead to clinically uneventful, persistent infections. The murine pneumotropic polyomavirus (MPtV) is a notable exception, being able to cause severe acute disease in newborn mice.

The recognized human polyomaviruses, JCPyV and BKPyV, are distributed worldwide, as demonstrated by detectable levels of circulating antibodies in the majority of the healthy human population. These viruses generally establish persistent infections, usually early in life, after which they can remain latent in several body compartments, including the tonsils, lower urinary tract, lymphoid tissues and bone marrow. Involvement of the kidney is frequently observed, with viruria noted, especially in immunodeficient hosts and patients undergoing renal transplantation.

The exact routes of mammalian polyomavirus transmission are unclear. Since SV40, JCPyV and BKPyV are each known to target the urinary tract, low-level shedding of virus in urine is thought to

play a role in transmission of these polyomaviruses. However, JCPyV and BKPyV have each been found in tonsillar tissue, consistent with respiratory transmission or transmission by hand to mouth contact. Virus spread may also occur when persistent infections are reactivated during periods of immune suppression, including pregnancy. Transmission via tissue transplantation is also thought to play a role in humans. Vectors are not known to play a role in transmission of polyomaviruses.

Whereas BKPyV and JCPyV infections are generally asymptomatic or associated with mild pathologic changes in the respiratory and urinary tracts of immunocompetent individuals, BKPyV infection is an increasingly common complication in transplant recipients, resulting in nephropathy or cystitis; a significant cause of kidney transplant failure. Moreover, there have been reports of disseminated BKPyV infections that gave rise to meningitis, retinitis, pneumonia or vasculopathy. In severely immunocompromised individuals, JCPyV can infect and destroy oligodendrocytes of the central nervous system, thereby giving rise to a fatal demyelinating disease termed progressive multifocal leucencephalopathy (PML). PML is a common complication in HIV-1 infection, eventually affecting 5% of the AIDS population. There is no effective treatment for PML, but the introduction of highly active antiretroviral therapy (HAART) has resulted in a significant decline in the HIV/PML mortality rate. SV40 may cause a PML-like disease in rhesus monkeys, particularly in those infected with HIV.

Two recently discovered putative (insofar as they have not yet been cultivated by inoculating cells in culture) human polyomaviruses, KIPyV and WUPyV, were initially detected in nasopharyngeal aspirates from patients presenting with acute respiratory tract infections, but it is not yet clear whether these viruses are agents of human respiratory tract disease. The association of integrated DNA of the recently discovered MCPyV with human Merkel cell carcinomas, as confirmed by several research groups, may represent the first human malignancy associated with the consistent presence of a particular polyomavirus genome. Interestingly, the MCPyV genomic sequence that encodes the large T antigen was shown to contain a chain-terminating mutation in 9/9 Merkel cell carcinomas. A similar mutation was not seen in MCPyV genomes from non-tumor sources. These experimental findings are consistent with the premise that MCPyV genomes in tumors undergo T-antigen mutations that prevent integrated virus replication (which would lead to cell death), while not effecting oncogenesis. Nevertheless, the role of MCPyV in Merkel cell carcinoma remains uncertain.

Using a rolling circle amplification technique, the genomes of two novel, currently unclassified polyomaviruses, human polyomavirus 6 (HPyV6) and human polyomavirus 7 (HPyV7), were detected in skin swabs from healthy human adults. They are closely related to each other and to WUPyV and KIPyV, but are distantly related to MCPyV. Genomes of the latter virus were also detected in these skin samples, suggesting that at least three polyomaviruses species may be commonly present in human skin.

Beta-lymphotropic polyomavirus (LPyV, or AGMPyV; see list of species in the genus) was isolated from a lymphoblastoid cell line derived from an African green monkey. Nevertheless, LPyV can productively infect some human B cell lymphoma-derived cell lines. This tropism is noteworthy because seroprevalence data suggest that a serologically related counterpart to LPyV circulates in humans, with a prevalence level of 15–20% in adults. Although phylogenetic analyses suggest that LPyV is closely related to MCPyV (in a cluster distinct from that containing WUPyV and KIPyV (Figure 5), the serology of the monkey and putative human viruses is distinct. LPyV transforms hamster embryo cells *in vitro* and induces choroid plexus tumors in transgenic mice that express the LPyV early region. The nature of the supposed human counterpart to LPyV and its possible role in human disease are not yet clear.

At present, there is increasing interest in the medical importance of the human polyomaviruses. The reasons are as follows: (1) polyomaviruses are ubiquitous in humans, (2) polyomaviral pathology is usually seen only in immunocompromised individuals, and (3) the frequency of immmunocompromised individuals has been on the rise because of the increasing numbers of the elderly, tissue transplantation, therapeutic immunomodulators and AIDS.

In November 2009, using a broad-spectrum PCR assay, two new, distinctly different provisional polyomaviruses were reported; one in Bornean orangutans (OranPyV-1) and the other in Sumatran

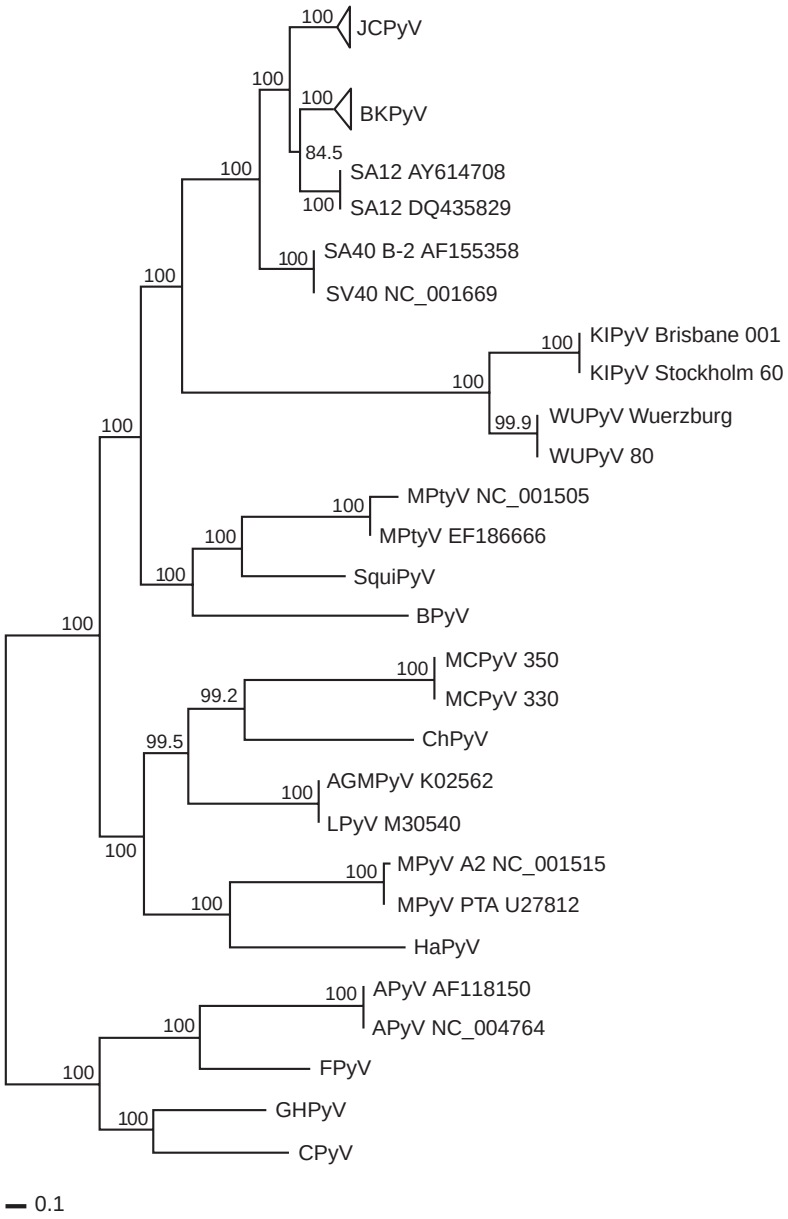


**Figure 5:** Phylogentic relationships of polyomaviruses. The figure shows a concatenated data set of maximum likelihood (ML) trees that were obtained for each of VP1, VP2 and T antigen. Branch lengths are proportional to genetic divergence. The scale bars indicate nuclear substitutions per site. (After Krumbholz, A. *et al.* (2009). *Infect. Genet. Evol.*, **9**, 784–799.)

orangutans (OranPyV-2). Sequencing showed that the genomes of each of these putative polyomaviruses has the characteristic genome architecture, but lacks an obvious agnogene. In addition, using PCR primers designed to identify polyomavirus genomes, putative polyomaviruses were identified in two species of bat (*Myotis lucifugus* and *M. californicus*). These findings may be medically relevant, considering that known instances of emerging diseases in humans are caused by viruses endemic to bats that have been transmitted to humans either directly or through other animal intermediaries.

MPtV is somewhat unique among the mammalian polyomaviruses, with respect to being able to cause severe disease during primary infection. In newborn mice, MPtV causes highly lethal

interstitial pneumonia, perhaps explained by its ability to replicate in vascular endothelial cells of the lung. However, infection generally leads to asymptomatic, persistent infection in immunocompetent adult animals.

Most mammalian polyomaviruses induce neoplastic transformation in cell culture and tumors in rodents and some primates. Transformation *in vitro* and tumorigenesis *in vivo* result from expression of virus-coded early proteins, which interact with specific cellular proteins (p53, pRB and others). Polyomavirus genomes are usually integrated into chromosomes of transformed or tumor cells. *In vivo*, JCPyV can induce brain tumors in owl and squirrel monkeys, and there has been recent interest in the possibility that JCPyV infection might lead to the development of central nervous system tumors in humans. However, this issue remains controversial. As noted above, MCPyV has been associated with Merkel cell carcinomas, although the role of the virus in this neoplasm is as yet uncertain. The inadvertent exposure of millions of human poliovirus vaccine recipients to SV40 (a previously unrecognized contaminant of early vaccines) led to concern that this virus may be a cause of human neoplasms and that it may yet be circulating in the human population. These issues also remain controversial. MPyV produces a wide variety of tumors in its natural host.

#### Avian polyomaviruses

The biology of the recognized avian polyomaviruses (ApyVs) is markedly distinct from that of the mammalian polyomaviruses in several key respects. First, avian viruses display a high degree of pathogenicity, leading to acute and chronic inflammatory diseases, especially in young birds. Second, none of the avian polyomaviruses displays the ability to induce neoplasia. Third, avian polyomavirus displays a broad host range, in comparison to the rather restricted host ranges of the mammalian polyomaviruses. Indeed, whereas the ICTV assigned the species name *Budgerigar fledgling disease virus* in recognition of its typical disease pattern in budgerigars, it is now referred to as APyV in recognition of its broad host range. Moreover, whereas the mammalian polyomaviruses typically display a distinct tissue tropism, APyV is able to replicate in a wide variety of organs. Likewise, GHPyV, the etiological agent of hemorrhagic nephritis and enteritis of geese, displays a broad tissue tropism in geese. A high seroprevalence rate was seen in Germany even in asymptomatic geese. Neither the tissue tropisms nor clinical importance of Finch polyomavirus and Crow polyomavirus have been well characterized.

## Species demarcation criteria in the genus

Species and genus demarcation criteria are currently being developed. In the interim, the list of species is provisional, and all species are assigned to a single genus.

## List of species in the genus *Polyomavirus*

| | | |
|---|---|---|
| *African green monkey polyomavirus* | | |
| African green monkey polyomavirus | [K02562] | (AGMPyV) |
| (Beta-lymphotropic polyomavirus) | | (LPyV) |
| *Baboon polyomavirus 2* | | |
| Baboon polyomavirus 2 | | (BPyV-2) |
| *BK polyomavirus* | | |
| BK polyomavirus | [V01108] | (BKPyV) |
| *Bovine polyomavirus* | | |
| Bovine polyomavirus | [D13942 = NC_001442] | (BPyV) |
| *Budgerigar fledgling disease polyomavirus* | | |
| Avian polyomavirus | [AF241168] | (APyV) |
| *Hamster polyomavirus* | | |
| Hamster polyomavirus | [AJ006015 = NC_001663] | (HaPyV) |
| *Human polyomavirus* | | |
| Human polyomavirus | | |
| *JC polyomavirus* | | |
| JC polyomavirus | [J02226 = NC_001699] | (JCPyV) |
| *Murine pneumotropic virus* | | |
| Murine pneumotropic virus | [M55904] | (MPtV) |
| *Murine polyomavirus* | | |
| Murine polyomavirus | [J02288] | (MPyV) |

| | | |
|---|---|---|
| *Rabbit kidney vacuolating virus* | | |
| Rabbit kidney vacuolating virus | | (RKV) |
| *Simian virus 12* | | |
| Simian virus 12 | [AY614708] | (SV12) |
| (SA12) | | |
| (Baboon polyomavirus type 1) | | |
| *Simian virus 40* | | |
| Simian virus 40 | [J02400 = NC_001669] | (SV40) |

Species names are in italic script; names of isolates are in roman script; synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Polyomavirus* but have not been approved as species

| | | |
|---|---|---|
| **Mammalian viruses** | | |
| KI polyomavirus | [EF127906] | (KIPyV) |
| WU polyomavirus | [EF444549] | (WUPyV) |
| Merkel cell polyomavirus | [EU375803] | (MCPyV) |
| Canary polyomavirus | [GU345044] | (CaPyV) |
| Cynomolgus polyomavirus | | (CyPV) |
| Chimpanzee polyomavirus | [AY691168] | (ChPyV) |
| Athymic rat polyomavirus | | (Rat-PyV) |
| Squirrel monkey polyomavirus | [NC_009951] | (SqPyV) |
| Bornean orangutan polyomavirus | [FN356900] | (OraPyV-1) |
| Sumatran orangutan polyomavirus | [FN356901] | (OraPyV-2) |
| Bat polyomavirus | [NC_011310] | (BatPyV) |
| Human polyomavirus 6 | [NC_014406] | (HPyV6) |
| Human polyomavirus 6 | [NC_014407] | (HPyV7) |
| **Avian viruses** | | |
| Crow polyomavirus | [NC_007922] | (CPyV) |
| Finch polyomavirus | [NC_007923] | (FPyV) |
| Goose hemorrhagic polyomavirus | [NC_004800] | (GHPyV) |

These viruses have not been approved as species because, although distinctive polyomaviral genomes have been detected by PCR-based screening procedures, viruses have not yet been cultivated by inoculating cells in culture.

## List of unassigned species in the family *Polyomaviridae*

None reported.

## Phylogenetic relationships within the family

Phylogenetic relationships among the polyomaviruses, based on the amino acid sequences of VP1, VP2 and large T antigen, are shown in Figure 5.

Genetic distances between species are consistent with separate branching of the mammalian and avian polyomaviruses. Moreover, this separate branching is in accord with the premise that polyomaviruses have co-evolved with their natural hosts, as supported by a 2006 analysis of 72 complete genomes. (However, the putative "human" polyomaviruses, KIPyV and WUPyV, do not cluster with established human polyomaviruses, JCPyV and BKPyV, and MCPyV is even more distant in the phylogenetic tree [Figure 5].) Concerning differences in genomic structures, four of the five avian polyomaviruses contain an additional ORF that is created by alternative splicing in the 5′ end of the late coding region. The encoded protein, designated VP4 in APyV, is incorporated into the APyV capsid. It has no counterpart in the mammalian polyomaviruses and it shares no homology with any mammalian polyomavirus protein. In addition, the DNA-binding domain of the mammalian polyomavirus large T antigens have a different consensus sequence from that of the avian polyomaviruses. Specifically, the mammalian polyomaviruses may all use the pentanucleotide GAGGC as the large T antigen-binding sequence, whereas the avian polyomaviruses may use the palindromic motif CC(A/$T_6$)GG. Concerning biological properties, the mammalian and avian polyomaviruses differ as follows. Whereas mammalian polyomaviruses generally give rise to subclinical

infections in their immunocompetent natural hosts and may be tumorigenic in laboratory animals, avian polyomavirus infections are associated with severe pathologies, including hepatitis, nephritis and feather disorders, and they are not known to induce tumors. Moreover, at least one of the avian polyomaviruses (APyV) has a notably broader host range than any known mammalian polyomavirus.

Based on phylogenetic data (Figure 5), genomic structure and biological properties, the *Polyomaviridae* Study Group is investigating the merits of dividing the family into two genera; one containing the mammalian polyomaviruses and a genus containing the avian polyomaviruses. In addition, the Study Group is considering the merits of creating a separate mammalian genus, comprised of KIPyV, WUPyV, HPyV6 and HPyV7, to reflect their nucleotide sequence divergence from the other mammalian polyomaviruses.

## Similarity with other taxa

Until the VIIth ICTV report, the genus *Polyomavirus* was assigned as one of two genera within the family *Papovaviridae* (the other genus being *Papillomavirus*). However, sequence data established unequivocally that the *Polyomaviridae* and *Papillomaviridae* are not detectably related and constitute distinct virus families.

Bandicoot papillomatosis carcinomatosis virus types 1 and 2 (BPCV1 and BPCV2, respectively) have circular dsDNA genomes that are similar to members of the family *Papillomaviridae* in size (ca. 7.3–7.5 kbp) and possibly in some aspects of gene content in that they encode putative papillomaviral L1 and L2 structural proteins. However, they also encode putative polyomaviral large T and small t antigens. The origins of these viruses is not clear, but they might be explained by recombination between a polyomavirus and a papillomavirus. Although these as yet unclassified viruses appear not to be true polyomaviruses, they have been listed here for the sake of completeness.

## Derivation of name

*Polyoma*: from Greek *poly*, "many", and *-oma*, denoting "tumors".

## Further reading


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## Contributed by

Norkin, L.C., Allander, T., Atwood, W.J., Buck, C.B., Garcea, R.L., Imperiale, M.J., Johne, R., Major, E.O., Pipas, J.M. and Ramqvist, T.

# Family *Poxviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Poxviridae*** |
| Subfamily | ***Chordopoxvirinae*** |
| Genus | ***Avipoxvirus*** |
| Genus | ***Capripoxvirus*** |
| Genus | ***Cervidpoxvirus*** |
| Genus | ***Leporipoxvirus*** |
| Genus | ***Molluscipoxvirus*** |
| Genus | ***Orthopoxvirus*** |
| Genus | ***Parapoxvirus*** |
| Genus | ***Suipoxvirus*** |
| Genus | ***Yatapoxvirus*** |
| Subfamily | ***Entomopoxvirinae*** |
| Genus | ***Alphaentomopoxvirus*** |
| Genus | ***Betaentomopoxvirus*** |
| Genus | ***Gammaentomopoxvirus*** |

## Virion properties

### Morphology

Virions are somewhat pleomorphic, generally brick-shaped (220–450 nm long × 140–260 nm wide × 140–260 nm thick) with a lipoprotein surface membrane displaying tubular or globular units (10–40 nm). They can also be ovoid (250–300 nm long × 160–190 nm diameter) with a surface membrane possessing a regular spiral filament (10–20 nm in diameter) (Figure 1).

Negative contrast EM images show that the surface membrane encloses a biconcave or cylindrical core that contains the genome DNA and proteins organized in a nucleoprotein complex. One or two lateral bodies appear to be present in the concave region between the core wall and a membrane. A recent model suggests that the nucleoprotein complex might be cylindrical, folded at least twice along the long virion axis to form a Z-structure, which presents as three circles, arranged linearly when viewed as a section across the short axis. This virion form is known as the mature virion (MV; also known as intracellular mature virus, IMV). Some MV is wrapped by an additional double layer of intracellular membranes (derived from the trans-Golgi or endosomes) to form wrapped virions (WV; also known as intracellular enveloped virus, IEV). WV can be externalized, losing the outermost of the additional membrane layers via fusion with the cell membrane, to form extracellular virions (EV). EV are antigenically distinct from MV, due to the presence of envelope-specific proteins. They can be bound to the cell surface (in a form specifically known as cell-associated enveloped

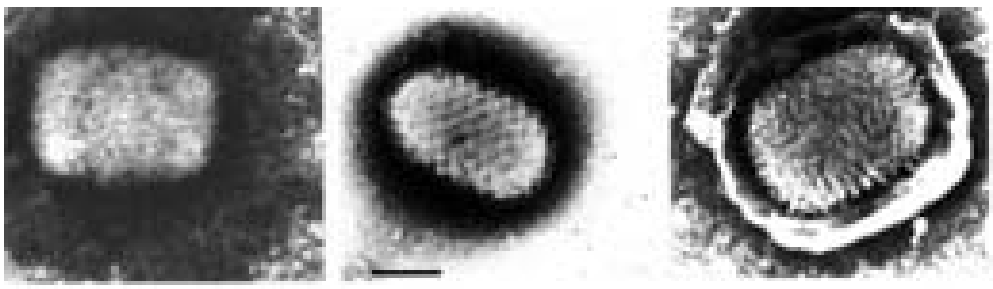

**Figure 1:** Electron micrographs of negatively stained preparations of: (left) an orthopoxvirus mature virion; (center) a parapoxvirus mature virion and (right) a yatapoxvirus enveloped virion. The bar represents 100 nm. (From Esposito, J.J. and Fenner, F. (2001). Poxviruses. In: *Fields Virology*, 4th edn (D.M. Knipe and P.M. Howley, Eds.), Lippincott Williams & Wilkins, Philadelphia, PA, pp. 2885-2921; with permission.)

virus, CEV) or released into the extracellular medium (in a form specifically known as extracellular enveloped virus, EEV). While this pathway commonly predominates in the mammalian poxviruses, the avian viruses (e.g. canarypox virus, fowlpox virus and pigeonpox virus) appear to form EV directly by budding of MV through the cell membrane, rather than by intermediate formation of WV. The avian viruses and some mammalian viruses (e.g. cowpox virus, ectromelia virus and raccoonpox virus, but not vaccinia virus or variola virus, and not all isolates) may also be sequestered within inclusion bodies. Others (e.g. entomopoxviruses) may be occluded into a preformed inclusion body.

#### Physicochemical and physical properties

Particle Mr is $3 \times 10^9$; $S_{20,W}$ is about 5000S. Buoyant density of virions is subject to osmotic influences: in dilute buffers it is about $1.16\,g\,cm^{-3}$, in sucrose about $1.25\,g\,cm^{-3}$, and in CsCl and potassium tartrate about $1.^30\,g\,cm^{-3}$. Virions tend to aggregate in high salt solution. Infectivity of some members is resistant to trypsin. Some members are insensitive to ether. Generally, virion infectivity is sensitive to common detergents, formaldehyde, oxidizing agents and temperatures greater than 40 °C. The virion surface membrane is removed by nonionic detergents and sulfhydryl reducing reagents. Virions are relatively stable in dry conditions at room temperature; they can be lyophilized with little loss of infectivity.

#### Nucleic acid

Nucleic acids constitute about 3% of the particle weight. The genome is a single, linear molecule of covalently-closed, dsDNA, 130–375 kbp in length.

#### Proteins

Proteins constitute about 90% of the particle weight. Genomes encode 150–300 proteins depending on the species; about 100 proteins are present in virions. Virus particles contain many enzymes involved in DNA transcription or modification of proteins or nucleic acids. Enveloped virions have virus-encoded polypeptides in the lipid bilayer, which surrounds the particle. Entomopoxviruses may be occluded by a virus-encoded, major structural protein, spheroidin. Similarly, chordopoxviruses may be within inclusion bodies again consisting of a single protein (the A-type inclusion ATI protein). In general, conserved proteins essential to virus replication in culture (polymerases and other enzymes, and structural proteins) are encoded in the central region of the genome whereas less conserved, non-essential proteins involved in virus–host responses (immunomodulators, anti-apoptotic proteins, etc.) are encoded in the terminal regions of the genome. Several large protein families are encoded within the *Poxviridae*, in some cases with many members. For instance canarypox virus encodes 51 proteins of the ankyrin repeat family.

#### Lipids

Lipids constitute about 4% of the particle weight. Enveloped virions contain lipids, including glycolipids, which may be modified cellular lipids.

#### Carbohydrates

Carbohydrates constitute about 3% of the particle weight. Certain viral proteins, e.g. hemagglutinin in the envelope of orthopoxviruses, have N- and C-linked glycans.

## Genome organization and replication

The poxvirus genome comprises a linear molecule of dsDNA with covalently closed termini; terminal hairpins constitute two isomeric, imperfectly paired, "flip-flop" DNA forms consisting of inverted complementary sequences. Variably sized, tandem repeat sequence arrays may or may not be present near the ends (Figure 2). Replication takes place predominately, if not exclusively, within the cytoplasm (Figure 3).

The entry of poxviruses into mammalian cells is divided into two phases: attachment of the virions to the cell surface and a fusion/entry event that delivers the viral core into the cellular cytoplasm. The attachment phase is mediated largely by electrostatic interactions between the virion and cell surface moieties, particularly glycosaminoglycans and laminin, whereas fusion of the viral membrane with cellular membranes is mediated by a multi-subunit viral entry/fusion complex, comprising at least a dozen highly conserved viral proteins. Although EV differ from MV by possessing an extra membrane (the envelope), it is believed that the EV membrane is disrupted shortly after binding to the cell surface such that the entry/fusion stage is similar for MV and EV.

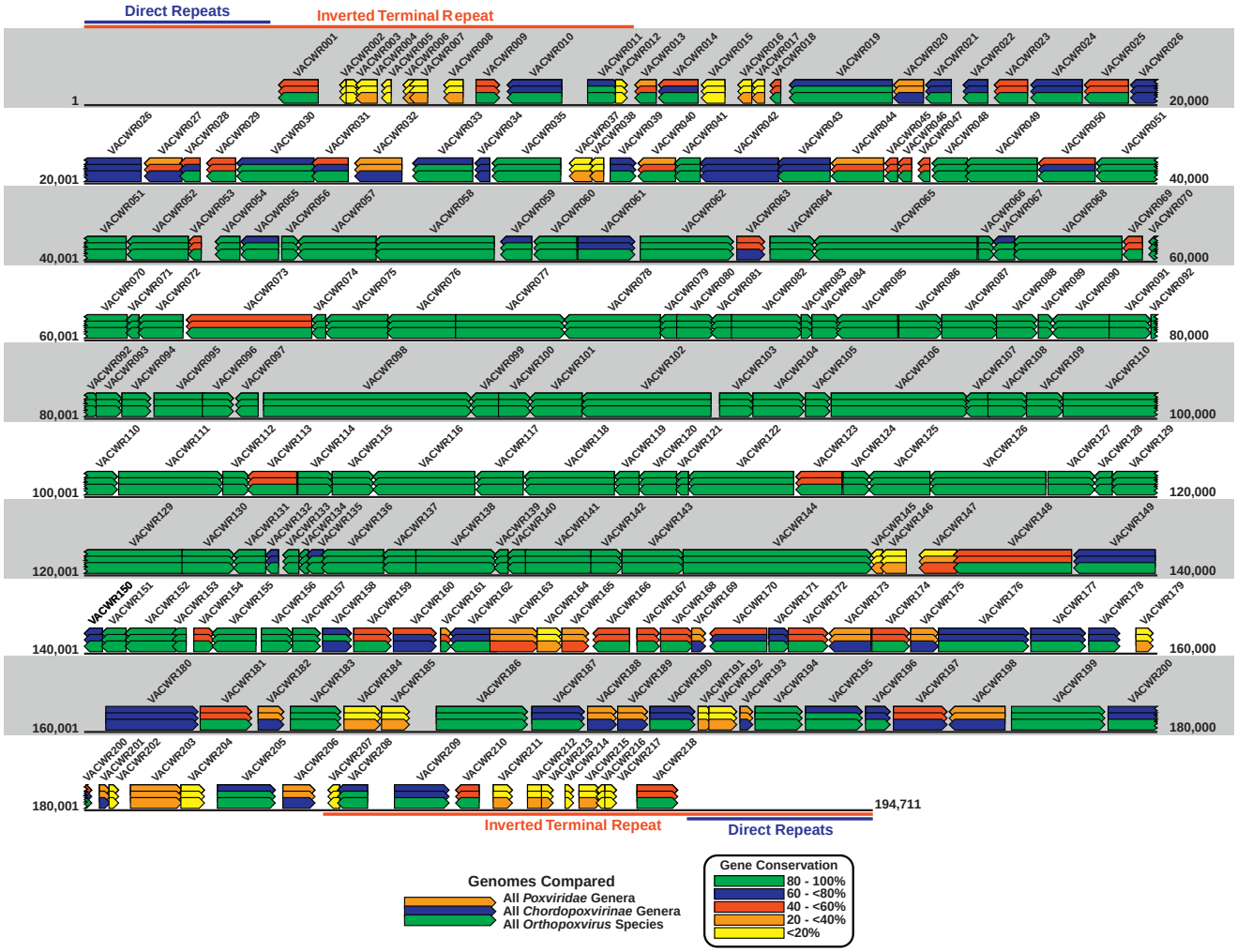


**Figure 2:** Schematic representation of the genome of the WR strain of vaccinia virus (AY243312). The genome is a linear double stranded molecule with terminal hairpins, inverted terminal repeats (ITR) and a series of direct repeats within the ITRs. Coloured rectangles represent each WR gene and the arrow at one end of each rectangle indicates the direction of transcription for that gene from the DNA template. The VACWR name represents the GenBank locus name for each gene. The coloured overlapping gene rectangles indicate the extent to which each gene is conserved (present or absent) in all poxviruses, vertebrate poxviruses (chordopoxviruses) and orthopoviruses. The bars are colour-coded according to the percentage of gene conservation across the indicated taxa.

Polyadenylated, capped primary mRNA transcripts, representing about 50% of the genome, are initially synthesized from both DNA strands by enzymes within the core, including a virus-encoded multisubunit RNA polymerase; transcripts are extruded from the core for translation by host ribosomes. During synthesis of early proteins, host macromolecular synthesis is inhibited. Virus reproduction ensues in the host cell cytoplasm, producing basophilic (B-type) inclusions termed "viroplasms" or "virus factories". The genome contains closely spaced protein-encoding ORFs, lacking introns, some of which may partially overlap. These ORFs are preceded by virus-specific promoters that temporally regulate transcription of three classes of mRNA. One class, the early genes, are expressed from partially uncoated virions prior to DNA replication (these encode many nonstructural proteins, including enzymes involved in replicating the genome and modifying DNA and RNA, and proteins whose role is to neutralize the host response). Early genes also encode intermediate transcription factors. Intermediate genes, which encode late transcription factors, are expressed during the period of DNA replication and are required for subsequent late gene transcription. Finally, late genes are expressed during the post-replicative phase (these mainly encode virion structural proteins but also early transcription factors). Despite a cytoplasmic site of replication, there is

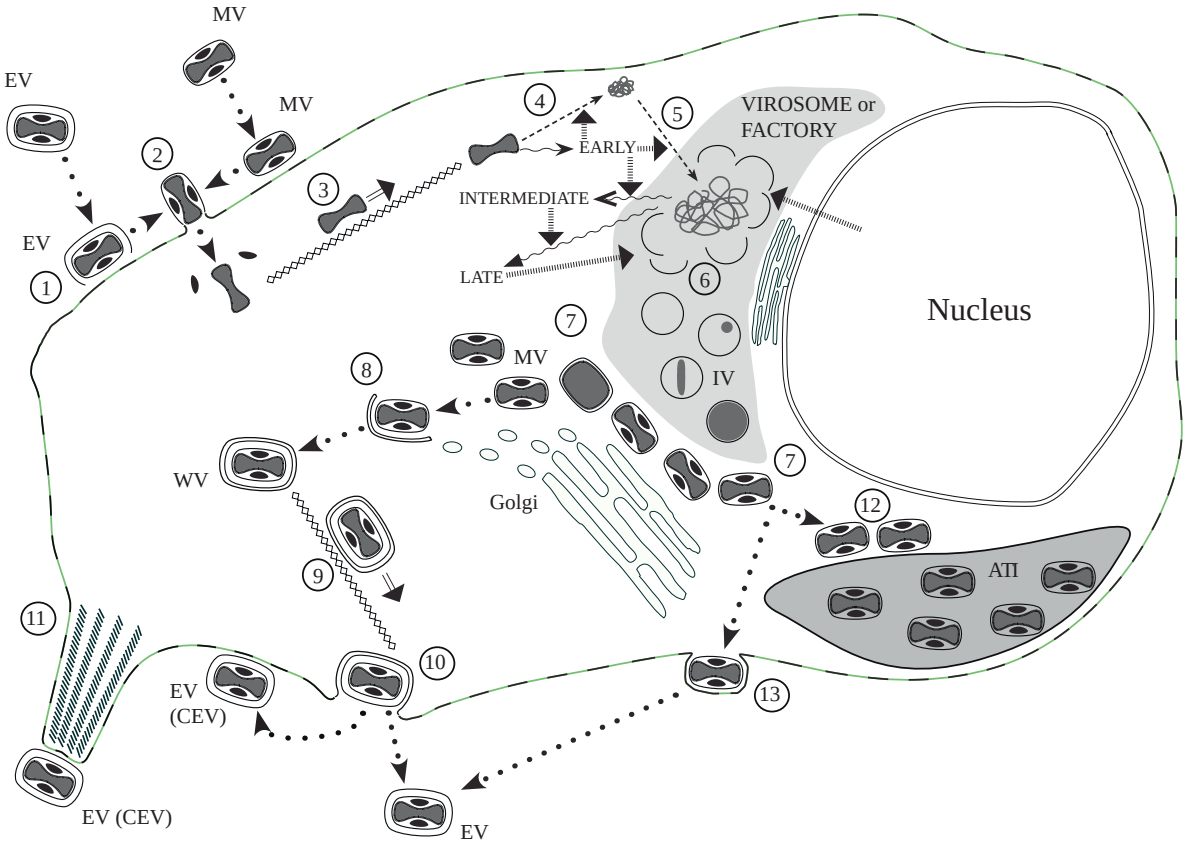


**Figure 3:** The infectious cycle of poxviruses, based primarily on that of vaccinia virus (VACV): ATI, A-type inclusion body; IV, immature virion; MV, mature virion; WV, wrapped virion; EV, enveloped virion; CEV, cell-associated enveloped virion. See text for full details. (1) Disruption of envelope of EV upon binding to cell surface receptors, essentially revealing MV, which like naked MV can (2) fuse directly with the cell membrane (mediated by the fusion complex) to release the naked core (and lateral bodies). The core is (3) transported to the perinuclear region along microtubules. Early genes are expressed (wavy arrows) directly from the intact core; early gene products mediate: (4) uncoating of the core, (5) DNA replication and intermediate gene expression. Intermediate gene products (with involvement of some host proteins derived from the nucleus) mediate late gene expression. Late gene products include structural proteins (including polymerase required for early gene expression) and proteins required for morphogenesis. Single membrane crescents are assembled (6) to enclose viral core proteins and genomic DNA (the latter is cleaved from concatameric intermediates), forming IV. These mature (7) to MV that, in VACV and many other mammalian poxviruses, are transported to the trans-Golgi/endosomal compartment for (8) wrapping with a double membrane to produce WV. These are (9) transported to the cell surface along microtubules, where they (10) exocytose, losing the outer of the two additional membranes, to form EV. The EV can remain on the cell surface as CEV or become free in the medium. CEV can (11) be propelled away from the cell on the tips of actin-driven projections. MV of some poxviruses can (12) alternatively be transported to and incorporated into ATI. The avipoxviruses do not appear to form WV to any significant extent, rather production of EV involves MV transport to the plasma membrane where they undergo budding to exit the cell (13).

evidence for the requirement of host nuclear proteins in post-replicative transcription. The mRNAs are capped, polyadenylated at the 3′ termini, and not spliced. Many intermediate, late and some early mRNAs have 5′-poly(A) tracts, that precede the encoded mRNA. Early protein synthesis is generally decreased during the transition to late gene expression, but some genes are expressed from promoters with both early and late activities. Certain proteins are modified post-translationally (e.g. by proteolytic cleavage, phosphorylation, glycosylation, ribosylation, sulphation, acylation, palmitylation and myristylation). Proteolytic cleavage of late proteins is required for virion morphogenesis.

The replication of the DNA genome appears to be mainly through the action of viral enzymes. DNA replication is initiated with the introduction of a single stranded nick, which serves to prime replication, near one (or both) of the terminal hairpins. The hairpin is unfolded and copied to the

terminus. The two strands are separated at the terminus and hairpins reform, allowing nascent DNA to be extended along the whole length of the genome, through the opposite hairpin and back along the opposite strand, forming a concatemeric product. Resolution of the concatemers for packaging involves at least three virus-encoded functions: a nicking-joining DNase, topoisomerase I and a Holliday junction resolvase.

Genetic recombination within genera has been shown, and may occur between daughter molecules during replication. Non-genetic genome reactivation generating infectious virus has been shown within, and between, members of genera in the subfamily *Chordopoxvirinae*, and forms the basis for the recovery of recombinant vaccinia virus from full-length genomic DNA (in, for instance, bacterial artificial chromosome vectors) by helper fowlpox virus (which can be removed by passage of the progeny through mammalian cells).

Virus morphogenesis begins following DNA replication and expression of early, intermediate and late genes. Particle assembly is initiated with the formation of crescent-shaped membrane structures in the intermediate compartment between the endoplasmic reticulum and the trans-Golgi network. Replicated, concatameric DNA is resolved into unit genomes and packaged, forming virion particles that mature into fully infectious MV (IMV). Some MV acquire an additional double layer of intracellular membrane (derived from the early endosomes or the trans-Golgi network) that contain unique virus proteins, to form WV (IEV). These WV are transported, by association with the cellular microtubule network, to the periphery of the cell where fusion with the plasma membrane ultimately results in release of EV (CEV and EEV). While both MV and EV are infectious, the external antigens on the two virion forms are different, and during infection the two virion types probably bind to different cellular receptors before uptake by mechanisms described above. Virus DNA and several proteins are organized as a nucleoprotein complex within the core of all infectious virions. The MV (IMV) contains an encompassing surface membrane, lateral bodies and the nucleoprotein core complex (see Figure 1). For vaccinia virus, the core wall has a regular subunit structure. Within the vaccinia virion, negative stain indicates that the core assumes a biconcave shape (Figure 1), apparently due to the large lateral bodies. Although the internal structure of vaccinia virions is revealed in thin sections, the detailed internal structure of parapoxvirus particles is less evident (Figure 1). In negatively stained preparations of parapoxviruses, superimposition of dorsal and ventral views of the surface filament sometimes produces a distinctive "criss-cross" surface appearance.

During natural infections, the virus is probably spread within an animal by EV (IEV and CEV) or through the movement of infected cells. A recent *in vitro* study has shown that infected cells, even before they have assembled infectious virions, can repel superinfecting virions and form actin-driven cellular projections ("actin tails" or "actin rockets") that can further propel the superinfecting virion towards uninfected target cells, thereby rapidly spreading the infection.

## Antigenic properties

Within each genus of the subfamily *Chordopoxvirinae* there is considerable serologic cross-protection and cross-reactivity. Neutralizing antibodies are genus-specific. The nucleoprotein antigen, obtained by treatment of virus suspensions with 0.04 M NaOH and 56 °C treatment of virus suspensions, is highly cross-reactive among members. Orthopoxviruses have hemagglutinin antigens, although this is rare in other genera.

## Biological properties

Transmission of various members of the subfamily *Chordopoxvirinae* occurs by (1) aerosol, (2) direct contact, (3) arthropods (via mechanical means), or (4) indirect contact via fomites; transmission of members of the subfamily *Entomopoxvirinae* occurs between arthropods by mechanical means. Host range may be broad in laboratory animals and in tissue culture; however, in nature it is generally narrow. Many poxviruses of vertebrates produce dermal maculopapular, vesicular rashes after systemic or localized infections. Poxviruses infecting humans are zoonotic except for molluscum contagiosum virus (MOCV) and the orthopoxvirus variola virus (VARV) (the etiologic agent of smallpox, now eradicated). Members may or may not be occluded within proteinaceous inclusions (subfamily *Chordopoxvirinae*: acidophilic (A-type) inclusion bodies, or subfamily *Entomopoxvirinae*: occlusions or spheroids). Occlusions may protect such poxviruses in environments where transmission

possibilities are limited. Neutralizing antibodies and cell-mediated immunity play a major role in clearance of vertebrate poxvirus infections. Reinfection rates are generally low and usually less severe. *Molluscum contagiosum* infections may recur, especially by autoinoculation of other areas of the skin with virus derived from the original lesions (e.g., by scratching).

## Taxa demarcation criteria in the family

The following criteria are used as a guideline to establish taxonomic status:

- Natural host range. In some cases, host range may be very narrow, and in others very broad, but in most cases, the delineation of the natural host(s) is a defining characteristic.
- Phylogenetic analysis. Taxonomic groupings can in most cases be readily inferred from the evolutionary clades observed following phylogenetic inference. For new virus isolates, levels of clade separation similar to those of existing taxa are suggestive of the necessity of creating a new taxon.
- Nucleotide sequence identity. Within the conserved, core region of orthopoxvirus species, nucleotide sequence identity of >96% is observed between isolates of all non-North American species. Isolates within a species exhibit >98% nucleotide identity. These levels of identity are sufficiently high that it is frequently difficult to obtain the resolution necessary to use the shared core region of poxvirus genomes as a definitive demarcation criterion. Outside the conserved core region, genome and gene alignments become much more difficult and subjective.
- Amino acid or nucleotide sequence identity between specific, commonly shared genes. Sequence polymorphisms within genes such as the hemagglutinin or A-type inclusion protein can frequently exhibit high levels of variation that provide the resolving power necessary to make demarcation decisions.
- Gene content comparisons. The variability in the content and conservation of gene sequences between poxvirus isolates can serve as a distinguishing characteristic.
- Organization of the genome. Syntenic relationships between genes may in some cases serve to distinguish taxa. But similar to nucleotide sequence identity, conservation of gene synteny can frequently be so high that the resolving power is not available to distinguish between taxa.
- Growth characteristics and host range in cell culture. Characteristics of *in vitro* growth such as the production and morphology of pocks produced on the chorioallantoic membranes of embryonated chicken eggs or plaque characteristics on cell monolayers may distinguish between taxa.
- Disease characteristics. The morbidity, mortality and other distinguishing features of the disease resulting from poxvirus infection can be used to support taxonomic decisions.
- Serological criteria, including plaque neutralization tests and cross-protection in animals, may help to identify new, unique isolates and serve as a criterion for taxonomic demarcation.

## Nomenclature of *Poxvirus* species

Most chordopoxvirus species names consist of two parts:

1. As prefix, a term describing the host from which the Poxvirus is normally isolated and, as a suffix, the term "pox". The prefix should be relevant in nature and scale to the taxonomic entity that best represents the relevant host taxon.
2. The word "virus".

In some cases, intervening terminology describes a clinical feature of the disease caused by the virus.

This nomenclature is normally used for poxviruses that cause characteristic pock-like skin lesions. For those that do not, the following nomenclature would normally be used:

1. A term describing the host from which the poxvirus is normally isolated; it should be relevant in nature and scale to the taxonomic entity that best represents the relevant host taxon.
2. The word "poxvirus" (preferred), though "virus" is also used.

In some case, intervening terminology describes a clinical feature of the disease caused by the virus.

For the entomopoxviruses, the formal taxonomic name (genus, species) of the host precedes the term "entomopoxvirus". "L" refers to lepidopteran, "O" to orthopteran.



# Subfamily *Chordopoxvirinae*

## Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Chordopoxvirinae*** |
| Genus | ***Avipoxvirus*** |
| Genus | ***Capripoxvirus*** |
| Genus | ***Cervidpoxvirus*** |
| Genus | ***Leporipoxvirus*** |
| Genus | ***Molluscipoxvirus*** |
| Genus | ***Orthopoxvirus*** |
| Genus | ***Parapoxvirus*** |
| Genus | ***Suipoxvirus*** |
| Genus | ***Yatapoxvirus*** |

## Distinguishing features

Includes brick-shaped or ovoid poxviruses of vertebrates with a low G+C content (30–40%), except for the parapoxviruses (64%) and MOCV (63%). Extensive serologic cross-reaction and cross-protection is observed within genera, though this is less obvious among the avipoxviruses. A common, conserved co-linear signature core of genes within genera (and in the case of mammalian viruses, spanning genera) is generally maintained with most divergence amongst members occurring at the terminal extremities of the genome. The co-linear signature of core genes appears different for mammalian, avian and insect poxvirus genera. Some viruses produce pocks on the chorioallantoic membranes of embryonated chicken eggs.

# Genus *Avipoxvirus*

Type species ***Fowlpox virus***

## Distinguishing features

Virions are brick-shaped, about 330 × 280 × 200 nm. Infectivity is usually ether-resistant. The genus includes viruses of birds that usually produce proliferative skin lesions (cutaneous form) and/or upper digestive/respiratory tract lesions (diptheritic form), though pneumonic presentation is also seen (e.g. canarypox virus). Cross-protection is variable. Viruses are primarily transmitted mechanically by arthropods, by direct contact or through aerosols. The genomic DNA is about 300 kbp in size. Viruses exhibit extensive serologic cross-reaction. Viruses produce A-type inclusion bodies with considerable amounts of lipid. Viruses grow productively in avian cell cultures, but abortively in mammals and the mammalian cell lines that have been examined. Viruses have been isolated worldwide from more than 250 species of birds but little is known about the total number of species, their host range and their geographic range.

## Species demarcation criteria in the genus

Provisional species demarcation criteria include disease characteristics, nature of the host and ecological niche, growth characteristics on the chicken chorioallantoic membrane, host range in cell culture and cross-neutralization. Restriction enzyme fragment length polymorphisms (RFLP) analysis and cross-hybridization have been used but genomic DNA sequencing studies are becoming more common. The genus comprises highly diverged viruses falling into at least three major clades. Two of these clades, represented by fowlpox-like viruses and canarypox-like viruses (see Figure 4 below), contain fully-sequenced viruses, which show divergence comparable to that observed between some of the genera of mammalian viruses (e.g. in the genera *Suipoxvirus* and

*Capripoxvirus*). Poxviruses of psittacine birds appear to represent a third, similarly diverged clade. Divergence to this extent renders it extremely difficult to identify pan-genus oligonucleotide probes for PCR amplification and sequencing, and consequently existing phylogenetic information across the genus is limited to less than a handful of gene loci. The situation will improve with genome sequencing of a wider range of representative isolates. Until such data provide a better picture of the overall phylogenetic structure of the avian poxviruses, they remain as a single genus.

### List of species in the genus *Avipoxvirus*

| | | |
|---|---|---|
| *Canarypox virus* | | |
| Canarypox virus | [AY318871 = NC_005309] | (CNPV) |
| *Fowlpox virus* | | |
| Fowlpox virus | [AF198100 = NC_002188, AJ581527] | (FWPV) |
| *Juncopox virus* | | |
| Juncopox virus | | (JNPV) |
| *Mynahpox virus* | | |
| Mynahpox virus | | (MYPV) |
| *Pigeonpox virus* | | |
| Pigeonpox virus | | (PGPV) |
| *Psittacinepox virus* | | |
| Psittacinepox virus | | (PSPV) |
| *Quailpox virus* | | |
| Quailpox virus | | (QUPV) |
| *Sparrowpox virus* | | |
| Sparrowpox virus | | (SRPV) |
| *Starlingpox virus* | | |
| Starlingpox virus | | (SLPV) |
| *Turkeypox virus* | | |
| Turkeypox virus | | (TKPV) |

Species names are in italic script; names of isolates are in roman script. Full genome sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Avipoxvirus* but have not been approved as species

| | |
|---|---|
| Crowpox virus | (CRPV) |
| Peacockpox virus | (PKPV) |
| Penguinpox virus | (PEPV) |

## Genus *Capripoxvirus*

Type species ***Sheeppox virus***

### Distinguishing features

Virions are brick-shaped, about 300 × 270 × 200 nm. Infectivity is sensitive to trypsin and ether. The genus includes viruses of sheep, goats and cattle. Viruses can be mechanically transmitted by arthropods and by direct contact or fomites. The genomic DNA is about 154 kbp in size. There is extensive DNA cross-hybridization between species. In addition, extensive serologic cross-reaction and cross-protection is observed among members.

### Species demarcation criteria in the genus

Provisional species demarcation criteria have included RFLP analysis while more recently genomic DNA sequence analysis has become more important.

### List of species in the genus *Capripoxvirus*

| | | |
|---|---|---|
| *Goatpox virus* | | |
| Goatpox virus G20-LKV | [AY077836] | (GTPV-G20) |
| Goatpox virus Pellor | [AY077835 = NC_004003] | (GTPV-Pell) |
| *Lumpy skin disease virus* | | |
| Lumpy skin disease virus NI-2490 | [AF325528 = NC_003027] | (LSDV-NI) |
| Lumpy skin disease virus NW-LW | [AF409137] | (LSDV-NW) |
| *Sheeppox virus* | | |
| Sheeppox virus A | [AY077833] | (SPPV-A) |
| Sheeppox virus NISKHI | [AY077834] | (SPPV-NIS) |
| Sheeppox virus 17077-99 | [AY077832 = NC_004002] | (SPPV-17077-99) |

Species names are in italic script; names of isolates are in roman script. Full genome sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## GENUS *CERVIDPOXVIRUS*

Type species ***Deerpox virus W-848-83***

### Distinguishing features

Deerpox viruses (DPVs) are poorly characterized viruses responsible for non-parapoxvirus-like infections in members of two subfamilies of cervids, American deer (Odocoileinae) and reindeer (Rangiferinae). Until recently, there has been insufficient information available to classify these chordopoxviruses but the protein coding regions of DPV isolate W-848-83 (W83) and W-1170-84 (W84) have now been determined. DNA sequence comparisons of DPV-W83 with available genomic sequences of members of the subfamily *Chordopoxvirinae* indicate that DPV-W83 is most similar to members of the genera *Capripoxvirus*, *Suipoxvirus*, *Leporipoxvirus* and *Yatapoxvirus*, yet the phylogenetic distance estimate between DPV-W83 and members of these genera is of a similar order of magnitude as the distance between these genera. DPVs are also distinguished from members of these genera by the presence of five DPV-specific genes and an ortholog of VACV A31R that has so far been observed only in orthopoxviruses and avipoxviruses. The sum total of the data support the continued status of *Deerpox virus W-848-83* as the type species in the new *Cervidpoxvirus* genus in the subfamily *Chordopoxvirinae*. It should be noted, however, that a proposal to change the name of this species is currently under consideration.

### List of species in the genus *Cervidpoxvirus*

| | | |
|---|---|---|
| *Deerpox virus W-848-83* | | |
| Deerpox virus W-848-83 | [AY689436 = NC_006966] | (DPV W-848-83) |
| Deerpox virus W-1170-84 | [AY689437 = NC_006967] | (DPV W-1170-84) |

Species names are in italic script; names of isolates are in roman script. Full genome sequence accessions [ ] and assigned abbreviations ( ) are also listed.

## GENUS *LEPORIPOXVIRUS*

Type species ***Myxoma virus***

### Distinguishing features

Virions are brick-shaped, about 300250200 nm. Infectivity is ether-sensitive. The genus includes viruses of lagomorphs and squirrels with extended cell culture host range. Usually, viruses are mechanically transmitted by arthropods; but they are also transmitted by direct contact and fomites. Myxoma and fibroma viruses cause localized benign tumor-like lesions in their natural hosts. Myxoma viruses cause severe generalized disease in European rabbits. The genomic DNA is about

160 kbp, and the G+C content about 40%. Extensive DNA cross-hybridization is observed between member viruses. Serologic cross-reaction and cross-protection have been demonstrated between different species.

### Species demarcation criteria in the genus

Provisional species demarcation criteria include various serological criteria, including plaque neutralization tests, cross-protection in animals and agar diffusion methods. Distribution, ecological niche, host range and disease, plaque characteristics, host range in cell culture, and RFLP analysis have been useful and genomic DNA sequence analysis is now expected.

### List of species in the genus *Leporipoxvirus*

| | | |
|---|---|---|
| *Hare fibroma virus* | | |
| Hare fibroma virus | | (FIBV) |
| *Myxoma virus* | | |
| Myxoma virus Lausanne | [AF170726 = NC_001132] | (MYXV-LAU) |
| *Rabbit fibroma virus* | | |
| Rabbit fibroma virus | [AF170722 = NC_001266] | (RFV) |
| (Shope fibroma virus) | | (SFV) |
| *Squirrel fibroma virus* | | |
| Squirrel fibroma virus | | (SQFV) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## Genus *Molluscipoxvirus*

Type species ***Molluscum contagiosum virus***

### Distinguishing features

Virions are brick-shaped, about 320 × 250 × 200 nm. Their buoyant density in CsCl is about 1.288 g cm$^{-3}$. The genomic DNA is about 190 kbp in size and G+C content is >60%. DNAs cross-hybridize extensively. RFLP maps suggest four sequence divergences among the isolates examined. Molluscum contagiosum virus (MOCV) has not been propagated in tissue cultures. It is transmitted mechanically by direct contact between children, or between young adults. It is often sexually transmitted. Sometimes the virus causes opportunistic infections of persons with eczema or AIDS. Virus produces localized lesions containing enlarged cells with cytoplasmic inclusions known as molluscum bodies. Infections can recur and lesions may be disfiguring when combined with bacterial infections. Unnamed viruses of horses, donkeys, and chimpanzees have also been identified.

### List of species in the genus *Molluscipoxvirus*

| | | |
|---|---|---|
| *Molluscum contagiosum virus* | | |
| Molluscum contagiosum virus | [U60315 = NC_001731] | (MOCV) |

Species names are in italic script; names of isolates are in roman script. Full genome sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## Genus *Orthopoxvirus*

Type species ***Vaccinia virus***

### Distinguishing features

Virions are brick-shaped, about 200 × 200 × 250 nm. Infectivity is ether-resistant. Extensive serologic cross-reactivity exists between the viruses. Virus-infected cells synthesize a hemagglutinin (HA)

glycoprotein that contributes to the modification of cell membranes and enables hemadsorption and hemagglutination of certain avian erythrocytes and alteration of the envelope of extracellular enveloped viruses. Neutralization sites on enveloped viruses are distinct from those on IMVs. The host range is broad in laboratory animals and tissue culture; in nature it may be relatively narrow. Most infections are generalized and disseminated. The genomic DNA is 170–250 kbp, and the G+C content is about 36%. The DNAs cross-hybridize extensively between members of the genus and sometimes with DNA of members of other genera. By comparison to the American species, DNA restriction maps suggest independent evolution of the Eurasian-African species.

## Species demarcation criteria in the genus

The criteria are provisional and reflect the fact that species definitions can be rather arbitrary and reflective of attempts to define natural transmission lineages. Most orthopoxviruses contain a hemagglutinin (HA) and many contain an A-type inclusion protein; polymorphisms within these genes distinguish species. Species can be classified by pock morphologies and by ceiling temperature for growth on the chorioallantoic membrane of embryonated chicken eggs. Ecological niche and host range are useful in some cases, but in others (rabbitpox virus and buffalopox virus) these can be misleading. RFLP analysis of the terminal regions of viral DNA outside of the core of common genes has aided the classification process. Detailed polymerase chain reaction (PCR) polymorphism analysis throughout the entire genome and subsequent genomic DNA sequencing studies have shown all orthopoxviruses to be unique. With genomic sequence analysis, it has become apparent that members of the species *Cowpox virus* are not monophyletic, as indicated by the different positions of cowpox virus GRI-90 and cowpox virus Brighton Red in the phylogenetic structure of the genus *Orthopoxvirus* (see Figure 4 below). This is still not reflected in the current taxonomy because the results of a wide-scale genome sequence study to clarify the issue are pending.

## List of species in the genus *Orthopoxvirus*

| | | |
|---|---|---|
| *Camelpox virus* | | |
| Camelpox virus CMS | [AY009089] | (CMLV-CMS) |
| Camelpox virus M-96 | [AF438165 = NC_003391] | (CMLV-M-96) |
| *Cowpox virus* | | |
| Cowpox virus Brighton Red | [AF482758 = NC_003663] | (CPXV-BR) |
| Cowpox virus GRI-90 | [X94355] | (CPXV-GRI) |
| *Ectromelia virus* | | |
| Ectromelia virus Moscow | [AF012825 = NC_004105] | (ECTV-MOS) |
| *Monkeypox virus* | | |
| Monkeypox virus Zaire-96-I-16 | [AF380138 = NC_003310] | (MPXV-ZAI) |
| *Raccoonpox virus* | | |
| Raccoonpox virus | | (RCNV) |
| *Taterapox virus* | | |
| Taterapox virus | [DQ437594 = NC_008291] | (GBLV) |
| *Vaccinia virus* | | |
| Buffalopox virus | | (BPXV) |
| Cantagalo virus | | (CTGV) |
| Rabbitpox virus Utrecht | | (RPXV-UTR) |
| Vaccinia virus Ankara | [AM501482] | (VACV-ANK) |
| Vaccinia virus Copenhagen | [M35027] | (VACV-COP) |
| Vaccinia virus WR | [AY243312 = NC_006998] | (VACV-WR) |
| *Variola virus* | | |
| Variola major virus Bangladesh-1975 | [L22579] | (VARV-BSH) |
| Variola major virus India-1967 | [X69198 = NC_001611] | (VARV-IND) |
| Variola virus minor Garcia-1966 | [Y16780] | (VARV-GAR) |
| *Volepox virus* | | |
| Volepox virus | | (VPXV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Orthopoxvirus* but have not been approved as species

| | |
|---|---|
| Skunkpox virus | (SKPV) |
| Uasin Gishu disease virus | (UGDV) |

## GENUS *PARAPOXVIRUS*

Type species **Orf virus**

### Distinguishing features

Virions are ovoid, 220–300 × 140–170 nm in size, with a surface filament that may appear as a regular cross-hatched, spiral coil involving a continuous thread. Infectivity is ether-sensitive. DNA is 130–150 kbp in size; G+C content is about 64%. Most species show extensive DNA cross-hybridization and serological cross-reactivity. Cross-hybridizations and DNA maps suggest extensive sequence divergence among members, higher than seen for members of the genus *Orthopoxvirus*. Generally the member viruses come from ungulates and domesticated livestock. They exhibit a narrow cell culture host range.

### Species demarcation criteria in the genus

Originally the major provisional species demarcation criterion was host range, coupled with RFLP and cross-hybridization analyses at the terminal regions of the genome, external to the core of conserved genes. With expansion of the genus, the host-range distinctions became less relevant and genomic sequence data are now more important.

### List of species in the genus *Parapoxvirus*

| | | |
|---|---|---|
| *Bovine papular stomatitis virus* | | |
| Bovine papular stomatitis virus | [AY386265 = NC_005337] | (BPSV) |
| *Orf virus* | | |
| Orf virus | [AY386264 = NC_005336] | (ORFV) |
| (Contagious pustular dermatitis virus) | | |
| (Contagious ecthyma virus) | | |
| *Parapoxvirus of red deer in New Zealand* | | |
| Parapoxvirus of red deer in New Zealand | | (PVNZ) |
| *Pseudocowpox virus* | | |
| Pseudocowpox virus | | (PCPV) |
| (Milker's nodule virus) | | |
| (Paravaccinia virus) | | |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Parapoxvirus* but have not been approved as species

Auzduk disease virus
Camel contagious ecthyma virus
Chamois contagious ecthyma virus
Sealpox virus

# GENUS *SUIPOXVIRUS*

Type species ***Swinepox virus***

## Distinguishing features

Virions are brick-shaped, about 300 × 250 × 200 nm. The genomic DNA is about 175 kbp in size with inverted terminal repeats of about 5 kbp. Virus forms foci or plaques in pig kidney cell culture (one-step growth is about 3 days at 37 °C) and plaques in swine testes cell cultures. Virus causes asymptomatic generalized skin disease in swine that appears to be localized to epithelial cells and draining lymph nodes. Virus neutralizing antibodies are not usually detected. Mechanical transmission by arthropods (probably lice) is suspected. Viruses have a worldwide distribution. Rabbits can be infected experimentally; however serial transmission in rabbits is unsuccessful.

## List of species in the genus *Suipoxvirus*

| | | |
|---|---|---|
| *Swinepox virus* | | |
| Swinepox virus | [AF410153 = NC_003389] | (SWPV) |

Species names are in italic script; names of isolates are in roman script. Full genome sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

# GENUS *YATAPOXVIRUS*

Type species ***Yaba monkey tumor virus***

## Distinguishing features

Virions are brick-shaped, about 300 × 250 × 200 nm. The genomic DNA is about 145 kbp in size, and the G+C content is about 33%. Although DNAs cross-hybridize extensively, DNA RFLP maps suggested major sequence divergences between Tanapox virus (TANV) and Yaba monkey tumor virus (YMTV); this was confirmed by complete genome sequences of both viruses. YMTV in primates causes histiocytomas, which are tumour-like masses of mononuclear cells. Viruses have been isolated from captive monkeys, baboons and experimentally infected rabbits. Accidental human infection in the laboratory has been reported. Human infection due to TANV has been observed in equatorial Africa and in laboratory personnel handling infected primates. In primates, TANV produces localized lesions that likely result from mechanical transmission by insects generally during the rainy season in African rain forests. Lesions commonly contain virions with a double-layer envelope surrounding the viral surface membrane. Yaba-like disease virus should be regarded as a strain of tanapox virus (strain Davis) isolated directly from primates.

## Species demarcation criteria in the genus

Species demarcation criteria include RFLP analysis, genomic DNA sequencing studies, serological criteria including cross-protection in animals and plaque neutralization tests, geographical distribution, ecological niche and nature of the disease.

## List of species in the genus *Yatapoxvirus*

| | | |
|---|---|---|
| *Tanapox virus* | | |
| (Yaba-like Disease virus) | | |
| Tanapox virus | [EF420156 = NC_009888] | (TANV) |
| Yaba-like Disease virus | [AJ293568 = NC_002642] | (YLDV) |

| | | |
|---|---|---|
| *Yaba monkey tumor virus* | | |
| Yaba monkey tumor virus | [AY386371 = NC_005179] | (YMTV) |

Species names are in italic script; names of isolates are in roman script. Full genome sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of unassigned species in the subfamily *Chordopoxvirinae*

| | |
|---|---|
| *Squirrel poxvirus* | |
| Squirrel poxvirus | SQPV |

Species names are in italic script; names of isolates are in roman script. Full genome sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

Although previously classified as a parapoxvirus due to morphological similarities when viewed by electron microscopy, careful examination suggests that squirrel parapoxvirus (SPPV) virions possess a longitudinal regular cross-hatched spiral coil surface element rather than the transverse surface element seen in the other parapoxviruses. Again, although similar in length to the parapoxviruses (280–320 nm), the virion is more brick shaped with a width estimated at approximately 180–220 nm (compared with 140–170 nm). The virus is also antigenically distinct from the type species of the *Parapoxvirus* genus, *Orf virus*, with only 2 out of 27 monoclonal antibodies raised against orf virus (ORFV) cross-reacting with SPPV. Sequence analysis of the genomic termini of the SPPV genome also failed to find genes characteristic of the parapoxviruses and instead found numerous putative genes that, so far, do not have counterparts in any of the other sequenced poxviruses. Phylogenetic analysis (see Figure 4 below) also supports a classification separate from the parapoxviruses and indeed from all the other currently recognized genera. Taken together these data indicated that SPPV did not belong in the genus *Parapoxvirus*. It was therefore renamed *Squirrel poxvirus* (SQPV) as an unassigned species in the subfamily *Chordopoxvirinae*, though a proposal to rationalize the nomenclature by changing the name to *Squirrelpox virus* is under consideration.

## List of other related viruses which may be members of the subfamily *Chordopoxvirinae* but have not been approved as species

| | | |
|---|---|---|
| California harbor seal poxvirus | | |
| Cotia virus | | |
| Dolphin poxvirus | | |
| Embu virus | | |
| Grey kangaroo poxvirus | | |
| Marmosetpox virus | | |
| Molluscum-like poxvirus | | |
| Nile crocodile poxvirus | [DQ356948 = NC_008030] | (CRV) |
| Quokka poxvirus | | |
| Red kangaroo poxvirus | | |
| Salanga poxvirus | | |
| Spectacled caiman poxvirus | | |
| Yoka poxvirus | | |

With the assignment of *Deerpox virus W-848-83* as type species of the new genus *Cervidpoxvirus*, mule deer poxvirus (a synonym for deerpox virus) has been removed from the current list of unassigned viruses in the subfamily *Chordopoxvirinae*.

The genome sequence of an isolate of Nile crocodile poxvirus (CRV) has been reported, indicating it to be diverged from the mammalian poxvirus genera and from the *Avipoxvirus* genus. It is likely that CRV will become the type species of a new genus representing poxviruses of the *Crocodylidae*. It remains to be seen whether genome sequencing in the longer term will indicate that other poxviruses of *Crocodilia* (or even *Reptilia*) in general could be encompassed in the same genus.

# SUBFAMILY *ENTOMOPOXVIRINAE*

Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Entomopoxvirinae*** |
| Genus | ***Alphaentomopoxvirus*** |
| Genus | ***Betaentomopoxvirus*** |
| Genus | ***Gammaentomopoxvirus*** |

## Distinguishing features

Entomopoxviruses infect insects. The viruses include different morphologic forms, e.g., brick-shaped, or ovoid. They are about 70–250 × 350 nm in size and are chemically similar to other family members. Virions contain at least four enzymes equivalent to those found in vaccinia virus. Virions of several morphological types have globular surface units that give a mulberry-like appearance; some have one lateral body, others have two. The DNA G+C content is about 20%. A common co-linear signature of core genes, different from those of members of the subfamily *Chordopoxvirinae*, is beginning to emerge, and is characteristic of the subfamily. No serologic relationships have been demonstrated between entomopoxviruses and chordopoxviruses. Entomopoxviruses replicate in the cytoplasm of insect cells (hemocytes and adipose tissue cells). Mature virions are usually occluded in spheroids comprised of a major crystalline occlusion body protein (termed "spheroidin"). The subdivision into genera is based on virion morphology, host range and the genome sizes of a few isolates. The genetic basis for these different traits is unknown.

# GENUS *ALPHAENTOMOPOXVIRUS*

Type species ***Melolontha melolontha entomopoxvirus***

## Distinguishing features

The genus includes poxviruses of *Coleoptera*. Virions are ovoid, about 450 × 250 nm in size, with one lateral body and a unilateral concave core. Surface globular units are 22 nm in diameter. The genomic DNA is about 260–370 kbp in size.

## Species demarcation criteria in the genus

The primary species demarcation criterion is currently host range recognizing that adequate molecular information is limited and available for only two members. In the future, genetic content, gene order and RFLP analysis between members within a defined region of the genome and cross-hybridization analysis are likely to be useful. Serological criteria including plaque and virus neutralization tests are also used.

## List of species in the genus *Alphaentomopoxvirus*

| | |
|---|---|
| *Anomala cuprea entomopoxvirus* | |
| Anomala cuprea entomopoxvirus | (ACEV) |
| *Aphodius tasmaniae entomopoxvirus* | |
| Aphodius tasmaniae entomopoxvirus | (ATEV) |
| *Demodema boranensis entomopoxvirus* | |
| Demodema boranensis entomopoxvirus | (DBEV) |
| *Dermolepida albohirtum entomopoxvirus* | |
| Dermolepida albohirtum entomopoxvirus | (DAEV) |
| *Figulus subleavis entomopoxvirus* | |
| Figulus subleavis entomopoxvirus | (FSEV) |

*Geotrupes sylvaticus entomopoxvirus*
Geotrupes sylvaticus entomopoxvirus (GSEV)
*Melolontha melolontha entomopoxvirus*
Melolontha melolontha entomopoxvirus (MMEV)

Species names are in italic script; names of isolates are in roman script. Assigned abbreviations ( ) are also listed.

# GENUS *BETAENTOMOPOXVIRUS*

Type species ***Amsacta moorei entomopoxvirus "L"***

## Distinguishing features

The genus includes poxviruses of Lepidoptera and Orthoptera. Virions are ovoid, about 350 × 250 nm in size, with a sleeve-shaped lateral body and cylindrical core. Surface globular units are 40 nm in diameter. The genomic DNA is about 225 kbp in size with covalently closed termini and inverted terminal repetitions. The G+C content is about 18.5%. Viruses produce a 115 kDa occlusion body protein encoded by the spheroidin gene.

## Species demarcation criteria in the genus

The main species demarcation criteria are currently host range and virion morphology. Serological criteria based on plaque neutralization may also be used. Genetic content, gene order, RFLPs within specific genes or within larger selected regions of the genome and nucleic acid cross-hybridization analysis are likely to become increasingly important. The species *Melanoplus sanguinipes entomopoxvirus "O"* was previously removed from the genus *Betaentomopoxvirus* (and is now unclassified within the subfamily) based on genomic DNA sequence comparisons with the type species. This suggests that other betaentomopoxviruses, so classified based on morphological and host range criteria, may eventually need reclassification once sequence information becomes available.

## List of species in the genus *Betaentomopoxvirus*

| | | |
|---|---|---|
| *Acrobasis zelleri entomopoxvirus "L"* | | |
| Acrobasis zelleri entomopoxvirus "L" | | (AZEV) |
| *Amsacta moorei entomopoxvirus "L"* | | |
| Amsacta moorei entomopoxvirus "L" | [AF250284 = NC_002520] | (AMEV) |
| *Arphia conspersa entomopoxvirus "O"* | | |
| Arphia conspersa entomopoxvirus "O" | | (ACOEV) |
| *Choristoneura biennis entomopoxvirus "L"* | | |
| Choristoneura biennis entomopoxvirus "L" | | (CBEV) |
| *Choristoneura conflicta entomopoxvirus "L"* | | |
| Choristoneura conflicta entomopoxvirus "L" | | (CCEV) |
| *Choristoneura diversuma entomopoxvirus "L"* | | |
| Choristoneura diversuma entomopoxvirus "L" | | (CDEV) |
| *Choristoneura fumiferana entomopoxvirus "L"* | | |
| Choristoneura fumiferana entomopoxvirus "L" | | (CFEV) |
| *Chorizagrotis auxiliars entomopoxvirus "L"* | | |
| Chorizagrotis auxiliars entomopoxvirus "L" | | (CXEV) |
| *Heliothis armigera entomopoxvirus "L"* | | |
| Heliothis armigera entomopoxvirus "L" | | (HAVE) |
| *Locusta migratoria entomopoxvirus "O"* | | |
| Locusta migratoria entomopoxvirus "O" | | (LMEV) |
| *Oedaleus senigalensis entomopoxvirus "O"* | | |
| Oedaleus senigalensis entomopoxvirus "O" | | (OSEV) |
| *Operophtera brumata entomopoxvirus "L"* | | |
| Operophtera brumata entomopoxvirus "L" | | (OBEV) |
| *Schistocera gregaria entomopoxvirus "O"* | | |
| Schistocera gregaria entomopoxvirus "O" | | (SGEV) |

Species names are in italic script; names of isolates are in roman script. "L" represents lepidopteran, "O" represents orthopteran. Full genome sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

# GENUS *GAMMAENTOMOPOXVIRUS*

Type species ***Chironomus luridus entomopoxvirus***

## Distinguishing features

This genus includes poxviruses of Diptera. Virions are brick-shaped, about 320 × 230 × 110 nm in size, with two lateral bodies and a biconcave core. The genomic DNA is about 250–380 kbp in size.

## Species demarcation criteria in the genus

The major species demarcation criterion is currently host range. However, as molecular information becomes available, genetic content, gene order and RFLPs within specific genes or within larger selected regions of the genome and cross-hybridization studies are likely to become increasingly important. Again, serological criteria, such as plaque neutralization, can be considered.

## List of species in the genus *Gammaentomopoxvirus*

| | | |
|---|---|---|
| *Aedes aegypti entomopoxvirus* | | |
| Aedes aegypti entomopoxvirus | | (AAEV) |
| *Camptochironomus tentans entomopoxvirus* | | |
| Camptochironomus tentans entomopoxvirus | | (CTEV) |
| *Chironomus attenuatus entomopoxvirus* | | |
| Chironomus attenuatus entomopoxvirus | | (CAEV) |
| *Chironomus luridus entomopoxvirus* | | |
| Chironomus luridus entomopoxvirus | | (CLEV) |
| *Chironomus plumosus entomopoxvirus* | | |
| Chironomus plumosus entomopoxvirus | | (CPEV) |
| *Goeldichironomus haloprasimus entomopoxvirus* | | |
| Goeldichironomus haloprasimus entomopoxvirus | | (GHEV) |

Species names are in italic script; names of isolates are in roman script. Assigned abbreviations ( ) are also listed.

## List of unassigned species in the subfamily *Entomopoxvirinae*

| | | |
|---|---|---|
| *Diachasmimorpha entomopoxvirus* | | |
| Diachasmimorpha entomopoxvirus | | (DIEV) |
| *Melanoplus sanguinipes entomopoxvirus* | | |
| Melanoplus sanguinipes entomopoxvirus | [AF063866 = NC_001993] | (MSEV) |

Species names are in italic script; names of isolates are in roman script. Full genome sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## Phylogenetic relationships within the family *Poxviridae* (Figure 4)

Members of the family *Poxviridae* share a characteristic virion morphology, with subtle differences between some genera. Use of serology, nucleic acid hybridization and restriction enzyme fragment polymorphism are generally limited to within-genus studies. The two subfamilies represent the fundamental difference in hosts, based on whether or not they are chordate. The subfamilies were divided into genera based on host, disease (and *in vitro* biological characteristics) and lack of intergenus antigenic cross-reactivity, later using criteria reflecting the nature of the genome. Modern sequence analysis essentially supported this classification, though it is apparent that the divergence between the avian viruses is probably greater than would be accommodated within a single genus.

## Similarity with other taxa

See Figure 2 in family *Asfarviridae*.

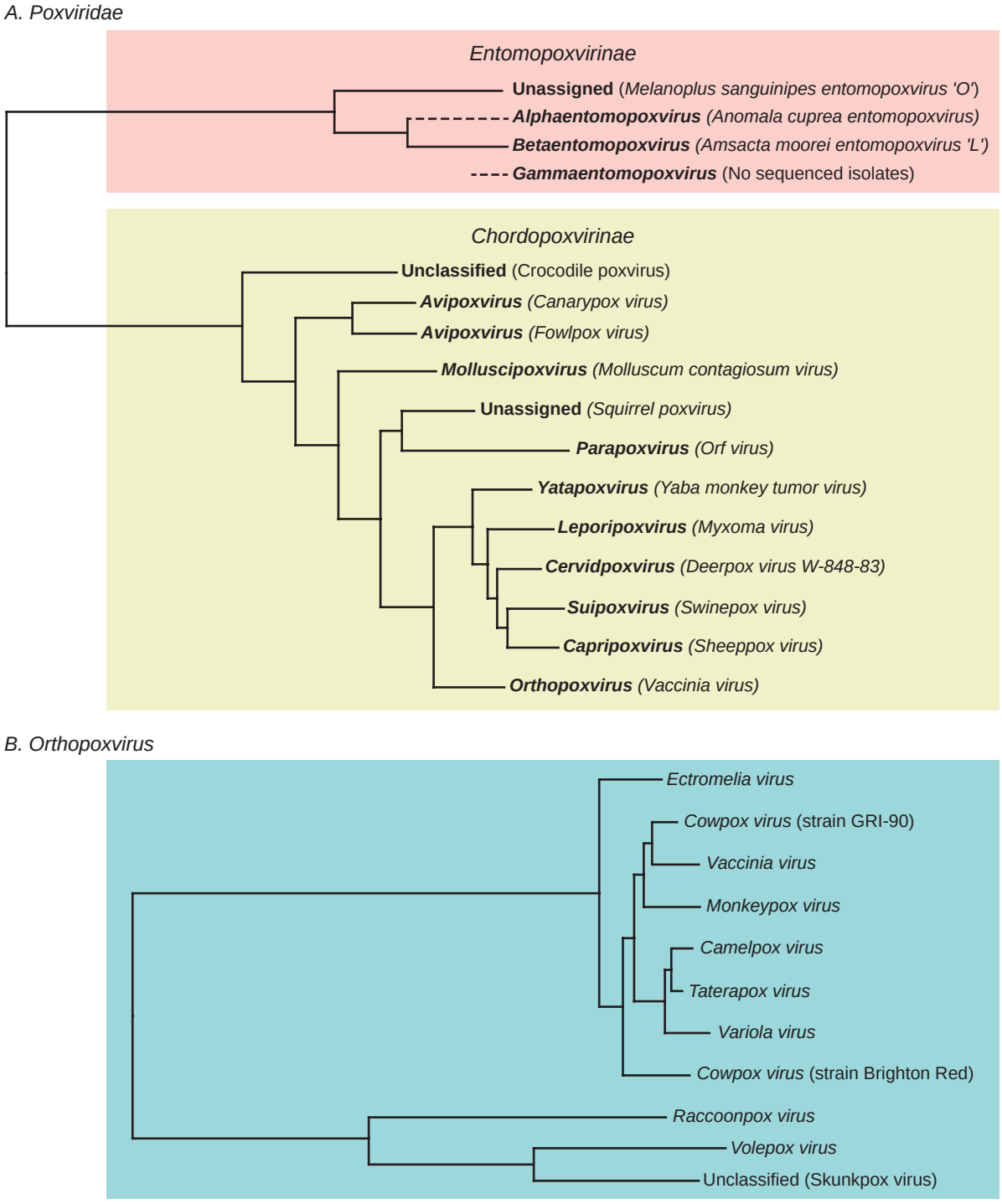


**Figure 4:** Panel A: Phylogenetic relationships in the family *Poxviridae*. Phylogenetic predictions are based upon aligned amino acid sequences from 19 conserved genes of virus isolates from representative species of each genus. Genera are indicated by bold, italic text, while species are represented in italic text. Branches with dotted lines indicate virus isolates for which limited sequence information is available and therefore their placement on the tree is not definitive. The species *Squirrel poxvirus* has not yet been assigned to a genus. Unclassified viruses have not yet been assigned to a taxon. There are no sequenced isolates within the genus *Gammaentomopoxvirus*. Panel B: Phylogenetic relationships in the genus *Orthopoxvirus*. Phylogenetic predictions are based upon codon-aligned nucleic acid sequences from nine conserved genes of isolates from each species. Two strains of cowpox virus were included in the analysis to demonstrate the discordant placement of different isolates of this species on the genus tree. Tree topologies for both analyses were inferred using Bayesian analysis as implemented by the program MrBayes.

## Derivation of names

*Avi*: from Latin *avis*, "bird".
*Capri*: from Latin *caper*, "goat".
*Entomo*: from Greek *entomon*, "insect".
*Lepori*: from Latin *lepus*, "hare".
*Mollusci*: from Latin *molluscum*, "clam", "snail"; related to appearance of lesion.
*Orf*: Scottish word based on Icelandic *hrufa*, "scab", "boil".
*Ortho*: from Greek *orthos*, "straight".
*Para*: from Greek *para*, "by side of".
*Pox*: from poc, pocc, "pustule".
*Sui*: from Latin *sus*, "swine".
*Yata*: sigla from *Ya*ba and *ta*napox viruses.

## Further reading

### Journals and books

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Roberts, K.L. and Smith, G.L. (2008). Vaccinia virus morphogenesis and dissemination. *Trends Microbiol.*, **16**, 472–479.

### Websites

The Vaccinia Virion 3D Tour: http://vacciniamodel.com
Poxvirus Bioinformatics Resource Center: http://www.poxvirus.org

## Contributed by

Skinner, M.A., Buller, R.M., Damon, I.K., Lefkowitz, E.J., McFadden, G., McInnes, C.J., Mercer, A.A., Moyer, R.W. and Upton, C.

# Family *Rudiviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Rudiviridae*** |
| Genus | ***Rudivirus*** |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Rudivirus*

Type species ***Sulfolobus islandicus rod-shaped virus 2***

## Virion properties

### Morphology

Virion has a stiff rod shape and measures about 600–900 × 23 nm (Figure 1). It is not enveloped and consists of a tube-like superhelix formed by dsDNA and multiple copies of a major structural protein. At each end, the tube carries plugs, about 50 × 6 nm, to which three tail fibers are anchored (Figure 1). These tail fibers appear to be involved in adsorption onto the host cell surface. The length of the virions is proportional to the size of the packaged viral DNA (Table 1).

### Physicochemical and physical properties

The buoyant density of virions is 1.36 g cm$^{-3}$. Virions are highly thermostable; autoclaving at 120 °C for at least 50 min is required for their inactivation. Virions are not inactivated by treatment with 6M urea, 0.1% Triton X-100, absolute ethanol and 2-octanol, but can be degraded by prolonged treatment with 0.1% SDS at 50 °C. Virions can act as a template for site-selective and spatially controlled chemical modification. Both the ends and the body of the virus, or the ends only, can be chemically addressed.

### Nucleic acid

Genome is a single molecule of linear dsDNA, which ranges from 24,655 to 35,482 bp (Table 1).

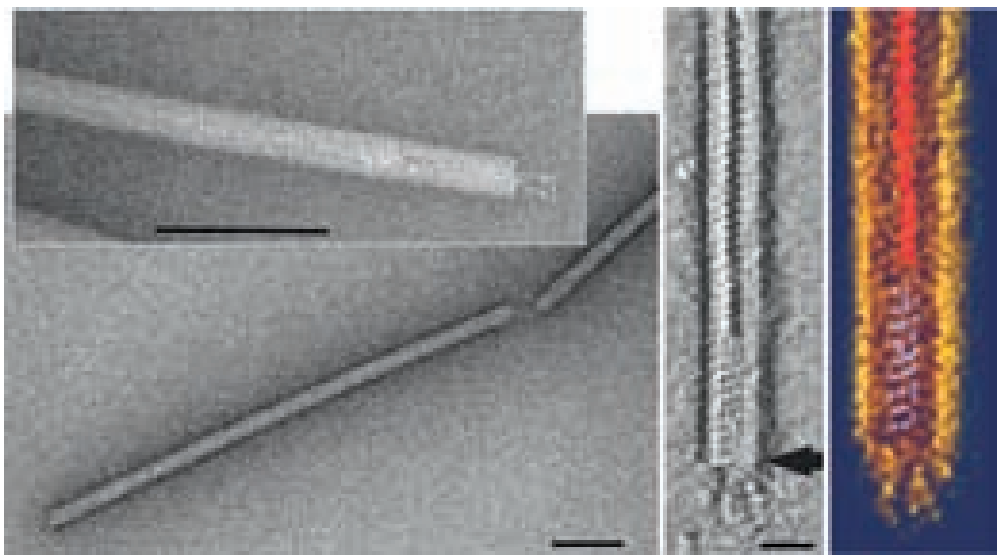

**Figure 1:** (Left panel) Negative contrast electron micrographs of virions of Stygiolobus rod-shaped virus. The bars correspond to 200 nm. (Right panels) Electron tomography image of the virion: horizontal slice (0.7) showing the accumulated stain in the central part, and visualization of the data using Amira software. With an arrow is indicated a point of attachment of the three tail fibers. The scale bar represents 50 nm. (Modified from Vestergaard *et al.* (2008). *J. Bacteriol.*, **190**, 6837-6845.)

**Table 1:** Properties of the rudiviruses

| Name | Origin | Virion length (nm) | Genome size (bp) | Total ORFs | G+C (%) | ITR length (bp) |
|---|---|---|---|---|---|---|
| SRV | Portugal | 702 | 28097 | 37 | 29.3 | 1030 |
| ARV1 | Italy | 610 | 24655 | 41 | 39.1 | 1365 |
| SIRV1 | Iceland | 830 | 32308 | 45 | 25.3 | 2032 |
| SIRV2 | Iceland | 900 | 35498 | 54 | 25.2 | 1626 |

### Proteins

Virions contain one major highly glycosylated protein of about 14.5 kDa, and three minor proteins with molecular masses of about 50, 58 and 110 kDa, the largest of which is involved in formation of the terminal filaments. The major structural protein is shown to generate long tubular structures *in vitro*. The protein structure adopts a four-helix bundle fold that is stabilized by an extensive hydrophobic core, with helices ranging from 11 to 19 amino acids in length, and is identical to the four-helix bundle fold of the two major structural proteins of the Acidianus filamentous virus 1, a member of the family *Lipothrixviridae*.

### Lipids

No lipid was detected.

### Carbohydrates

None reported.

## Genome organization and replication

The two strands of the linear DNA are covalently linked at both ends of the genome. The genomes carry long inverted terminal repeats, ranging from 1365 bp to 2032 bp (Table 1), which include multiple direct repeats. Although the sequences of the inverted terminal repeats are different for different rudiviruses, they all carry the 21 bp sequence AATTTAGGAATTTAGGAATTT near the genome ends, which may be an important signal for DNA replication. The finding of head-to-head and tail-to-tail linked replicative intermediates suggests a self-priming replication model.

The genome sequence and composition of the *Acidianus* and *Stygiolobus* rudiviruses differ significantly from those of the two *Sulfolobus* rudiviruses (Figure 2). A dUTPase and a Holliday junction resolvase are encoded in the virus genomes, and those of the Sulfolobus rod-shaped virus 2 have been functionally characterized as recombinant proteins *in vitro*. At least 10% of the encoded proteins are predicted to have different DNA-binding motifs and are presumed to be transcriptional regulators. One of these, protein SvtR of Sufolobus rod-shaped virus 2, with the structure similar to that of bacterial RHH proteins, was characterized in detail and shown to strongly repress the transcription of the minor structural protein. The transcriptional patterns of the rudiviruses are relatively simple, with few temporal expression differences.

## Antigenic properties

No information is available.

## Biological properties

The viruses were isolated from extreme acidic geothermal environments, with temperatures above 80 °C and pH values below 3, of Iceland, Portugal and Italy. The hosts are members of the hyperthermophilic archaeal genera *Sulfolobus*, *Acidianus* and *Stygiolobus.* The viral genome does not integrate into the host chromosome. Sulfolobus islandicus rod-shaped virus 2 kills the host cell as a consequence of elaborated, well-orchestrated mechanism. Following virus infection, massive degradation of the host chromosomes occurs accompanied by formation of pyramidal structures on the host cell

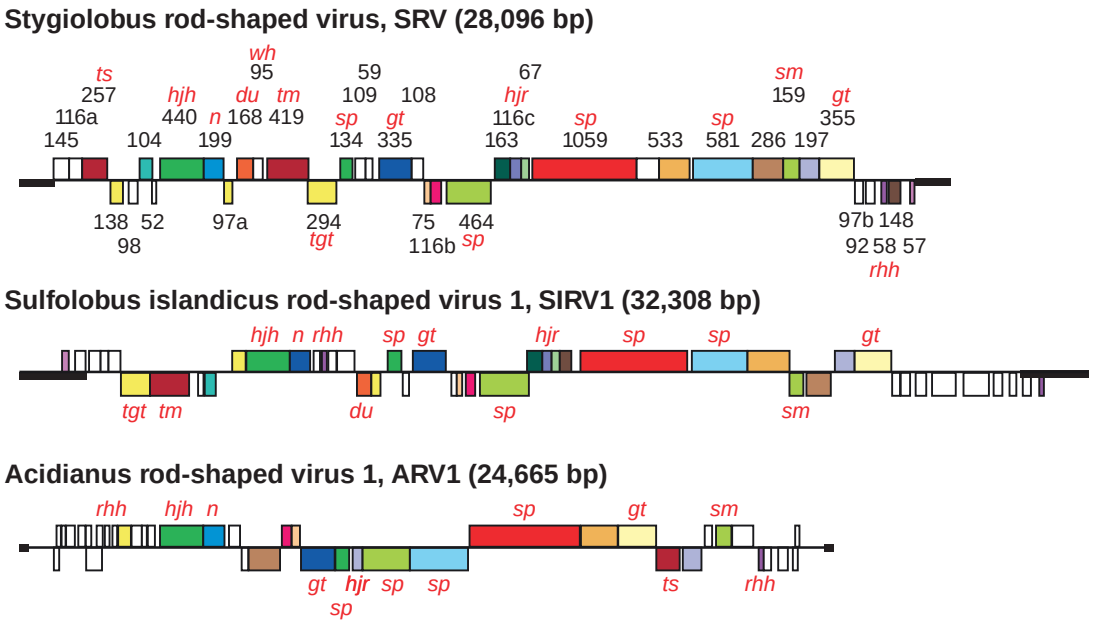


**Figure 2:** Genome organization of Stygiolobus rod-shaped virus (SRV), Sulfolopbus islandicus rod-shaped virus 1 (SIRV1) and Acidianus rod-shaped virus 1 (ARV1), showing the predicted ORFs and the ITRs (bold lines). SRV ORFs are identified by their amino acid lengths. Homologous genes shared between the rudiviruses are color-coded. Genes above the horizontal line are transcribed from left to right, and those below the line are transcribed in the opposite direction. Predicted functions or structural characteristics of the gene products are indicated as follows: sp, structural protein; rhh, ribbon-helix-helix protein; wh, winged helix protein; tm, transmembrane; tgt, tRNA guanine transglycosylase; hjh; Holliday junction helicase; hjr, Holliday junction resolvase; n, nuclease; du, dUTPase; ts, thymidylate synthase; sm, *S*-adenosylmethionine-dependent methyltransferase; gt, glycosyl transferase. (From Vestergaard *et al.* (2008). *J. Bacteriol.* **190**, 6837-6845.)

surface that rupture the S-layer (Figure 3). Close to the end of eclipse phase, the pyramidal structures open outwards, and create apertures through which mature virions escape the cell (Figure 3). SIRV2-encoded protein P98 is the major constituent of these exceptional cellular ultrastructures. This mechanism of virus release is unique and differs from lysis and egress systems of known bacterial and eukaryotic viruses.

## Species demarcation criteria in the genus

Species in the genus differ in virion size, host range, size and nucleotide sequence of the genome.

## List of species in the genus *Rudivirus*

| | | |
|---|---|---|
| *Sulfolobus islandicus rod-shaped virus 1* | | |
| Sulfolobus islandicus rod-shaped virus 1 | [AJ414696] | (SIRV1) |
| *Sulfolobus islandicus rod-shaped virus 2* | | |
| Sulfolobus islandicus rod-shaped virus 2 | [AJ344259] | (SIRV2) |
| *Acidianus rod-shaped virus 1* | | |
| Acidianus rod-shaped virus 1 | [AJ875026] | (ARV1) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Rudivirus* but have not been approved as species

| | | |
|---|---|---|
| Stygiolobus rod-shaped virus | [FM164764] | (SRV) |

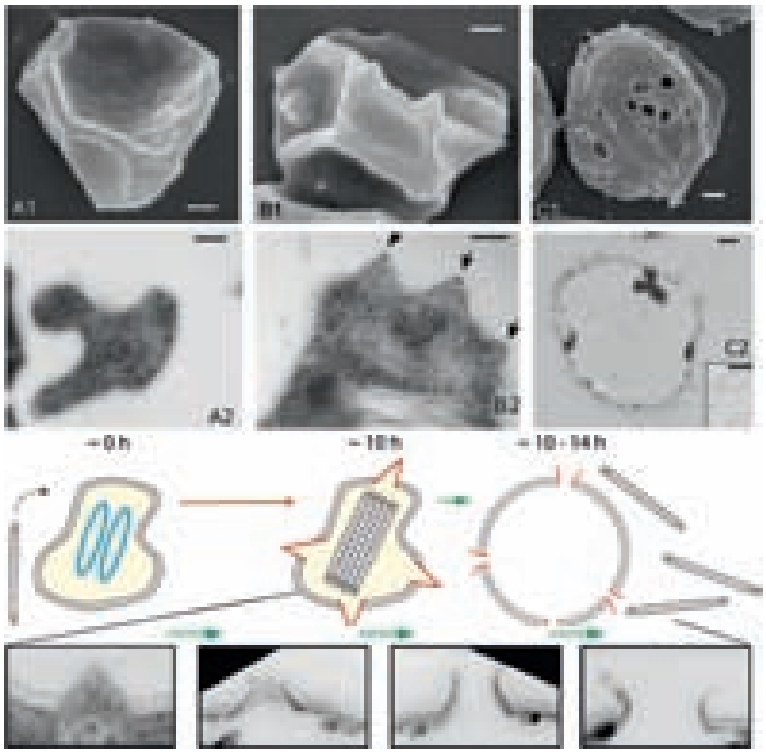


**Figure 3:** Transformation of *Sulfolobus* host cells following infection with Sulfolobus islandicus rod-shaped virus 2: (A1, A2), uninfected cells; (B1, B2), cells 10 h post-infection; (C1,C2), cells 10–14 h post-infection. (A1, B1, C1) scanning electron micrographs; (A2, B2, C2) transmission electron micrographs of thin sections of negatively stained cells. Scale bars: 200 nm. The electron micrographs are accompanied by the schematic representation of the cell at different stages of the virus life cycle. Times after infection are indicated in hours. At 0 h, two chromosomes of the *Sulfolobus* host are shown in blue. Later they degrade concomitantly with formation of virus-induced pyramids (shown in red) and the intracellular clusters of assembled virions. Finally, at time points between 10 and 14 h, the virus-induced pyramids open (their remains are shown in red), the cell lyses and the virions are extruded. The gradual opening out of the virus-induced pyramids (at time points between 10 and 14 h) is illustrated in more details with fragments from electron micrographs of thin sections of the infected cell. (Modified from Bize *et al.*, 2009.)

## List of unassigned species in the family *Rudiviridae*

None reported.

## Phylogenetic relationships within the family

Not available.

## Similarity with other taxa

Originally, the two families of dsDNA viruses with linear genomes, the *Rudiviridae* and the *Lipothrixviridae*, were distinguished by differences in virion structure and this was later supported by comparative genomics. Nevertheless, a substantial fraction of orthologous genes, including some encoding glycosyl transferases and transcriptional regulators, are shared by the rudiviruses and lipothrixviruses. For example, of the 45 predicted genes of the rudivirus Sulfolobus islandicus rod-shaped virus 1, nine share orthologs with the lipothrixvirus Sulfolobus islandicus filamentous virus. Moreover, the structure of the major virion protein of the rudiviruses turned out to be identical to the structures of the two major virion proteins of the lipothrixvirus Acidianus filamentous virus 1. These observations indicate that the known linear dsDNA viruses, all of which infect hyperthermopilic members of the domain Archaea, share a common ancestor. One can suggest a sequence of evolutionary events in which the major virion protein of a "simpler" non-enveloped virion of the

*Rudiviridae* has been duplicated and evolved so as to facilitate interactions with a hydrophobic envelope, producing the more complex virion of the *Lipothrixviridae*. Therefore it has been suggested that the families *Rudiviridae* and *Lipothrixviridae* could become members of a new viral order.

## Derivation of name

*Rudi*: from Latin *rudis*, "small rod".



## Further reading

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## Contributed by

Prangishvili, D.

# Family *Tectiviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Tectiviridae* |
| Genus | *Tectivirus* |

Since only one genus is currently recognized, the family description corresponds to the genus description.



# Genus *Tectivirus*

Type species *Enterobacteria phage PRD1*

## Distinguishing features

Characteristic features of the tectiviruses are a linear dsDNA genome and a protein-rich internal membrane, which is enclosed in an icosahedral protein capsid. The genome has around 100 bp long inverted terminal repeats (ITR) and covalently linked 5′-terminal proteins used in replication as primers. Upon infection the viral membrane is transformed to a tubular structure used in genome delivery.

## Virion properties

### Morphology

Virions are icosahedra, have no external envelope, and measure 66 nm from facet to facet (Figure 1). Capsids have flexible spikes extending about 20 nm from the virion vertices. The capsid of Enterobacteria phage PRD1 (PRD1) is constructed of 240 major capsid protein (P3) trimers that form a pseudo T = 25 lattice. Protein P3 contains two beta-barrels and forms very tight trimers. Underneath the capsomers the minor coat protein P30 dimers stretch out from one vertex to another cementing the P3 facets together. The spikes are formed by two proteins (P2 and P5) extending from the penton protein (P31) and are used for receptor recognition. One virion vertex is different and used for the phage DNA packaging (Figure 1). The packaging vertex contains packaging ATPase P9 and three other proteins (P6, P20 and P22). The capsid encloses an inner membrane formed of approximately equal amounts of virus-encoded proteins and lipids derived from host cell plasma membrane. The DNA is coiled within this membrane. Virions are normally tailless, but produce tail-like tubes of about 60 × 10 nm upon DNA release or after chloroform treatment (Figure 1).

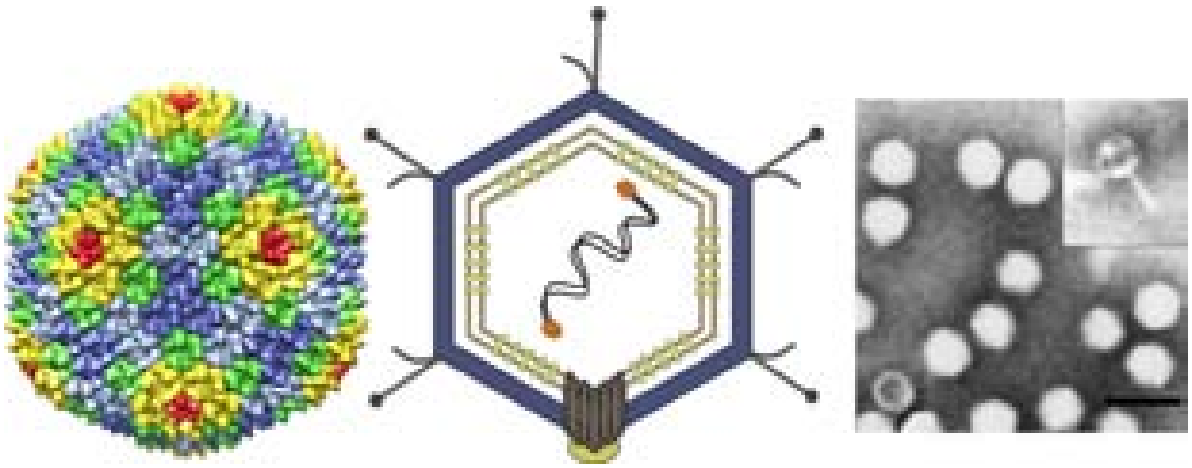

**Figure 1:** (Left) X-ray crystallography-based structure of the virion of Enterobacteria phage PRD1 (pseudo T = 25) viewed down two-fold axis of symmetry. (Middle) Schematic picture of the virion. (Right) Negative stain electron micrograph of phage PRD1 particles. The inset shows a particle with a protruding membranous tail-like structure. The bar represents 100 nm.

**Table 1:** Enterobacteria phage PRD1 (PRD1) proteins and their functions

| Phage protein | Gene | Mutant | Mass (kDa) | Function | Location |
|---|---|---|---|---|---|
| P1 | *I* | + | 63.3 | DNA polymerase | Cytoplasm |
| P2 | *II* | + | 63.7 | Receptor binding | Vertices |
| P3 | *III* | + | 43.1 | Major capsid protein | Capsid |
| P5 | *V* | + | 34.2 | Spike | Vertices |
| P6 | *VI* | + | 17.6 | Packaging | Unique vertex |
| P7 | *VII* | + | 27.1 | DNA entry, transglycosylase | Viral membrane |
| P8 | *VIII* | + | 29.6 | Genome terminal protein | Viral DNA |
| P9 | *IX* | + | 25.8 | DNA packaging | Unique vertex |
| P10 | *X* | + | 20.6 | Assembly | Host plasma membrane |
| P11 | *XI* | + | 22.2 | DNA entry | Membrane surface |
| P12 | *XII* | + | 16.6 | ssDNA binding | Cytoplasm |
| P14 | *XIV* | + | 15.0 | DNA entry | Vertices |
| P15 | *XV* | + | 17.3 | Lytic muramidase | Cytoplasm? |
| P16 | *XVI* | + | 12.6 | Infectivity | Viral membrane |
| P17 | *XVII* | + | 9.5 | Assembly | Cytoplasm |
| P18 | *XVIII* | + | 9.8 | DNA entry | Viral membrane |
| P19 | *XIX* | + | 10.5 | ssDNA binding | Cytoplasm |
| P20 | *XX* | + | 4.7 | DNA packaging | Unique vertex |
| P22 | *XXII* | + | 5.5 | DNA packaging | Unique vertex |
| P30 | *XXX* | + | 9.1 | Capsid stability | Capsid |
| P31 | *XXXI* | + | 13.7 | Penton | Capsid |
| P32 | *XXXII* | + | 5.4 | DNA entry | Viral membrane |
| P33 | *XXXIII* | − | 7.5 | Assembly | Cytoplasm |
| P34 | *XXXIV* | − | 6.7 | ? | Viral membrane |
| P35 | *XXXV* | + | 12.8 | Holin | Host plasma membrane |
| P36 | *XXXVI* | + | 12.6 | Lysis | Host plasma membrane |
| P37 | *XXXVII* | + | 10.1 | Lysis | Host plasma membrane |

### Physicochemical and physical properties

The virion Mr is about $66 \times 10^6$, the $S_{20,w}$ is 357–416S, and the buoyant density in CsCl is about $1.29\,g\,cm^{-3}$. Virions are usually stable at pH 5–8. Infectivity is sensitive to organic solvents and detergents.

### Nucleic acid

Virion contains a single molecule of linear dsDNA of about 15 kb. The DNA corresponds to 14–15% of particle weight and the G+C content is 48%. The complete DNA sequences of phage PRD1 and five closely related phages (Enterobacteria phages PR3, PR4, PR5, PR772 and L17) as well as Bacillus phages Bam35, GIL16, GIL01 and AP50 have been determined. The 100 bp long ITRs of PRD1 are 100% identical.

### Proteins

PRD1 proteins and their functions are listed in Table 1. The proteins of other enterobacterial tectiviruses are closely related to PRD1. Tectiviruses infecting members of the genus *Bacillus* (Bam35, GIL16, GIL01, and AP50) contain approximately the same number of proteins as PRD1.

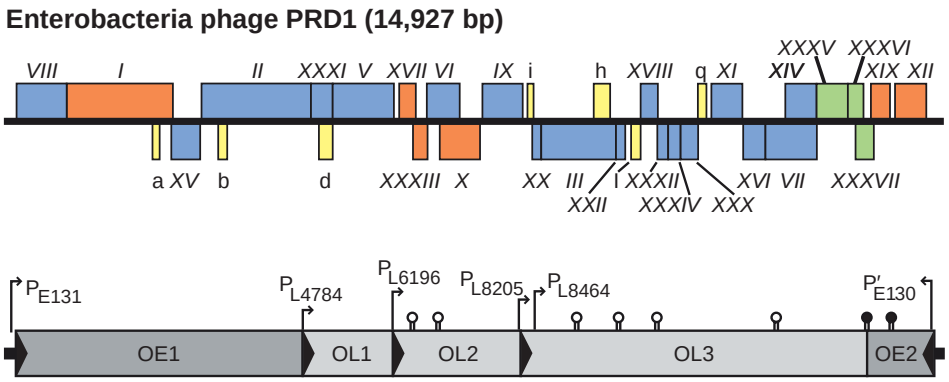


**Figure 2:** The genome of Enterobacteria phage PRD1 (PRD1) indicating the organization of genes and promoters (P) and their reading direction. Gene and protein nomenclature are correlated so that each protein has the same number (Arabic numeral) as the gene (Roman numeral). ORFs with no confirmed protein product are indicated by lower case letters. (Top) Order of genes; genes *XIX* and *XII* are on one strand and all other genes are on the other strand. The different colors indicate the ORFs (yellow) and the genes coding structural proteins (blue), nonstructural proteins (orange) and proteins involved in lysis (green). (Bottom) Promoters (P), terminators (lollipops) and operons (O) and their reading directions; E = early, L = late.

### Lipids

Virions contain about 15% lipids by weight. Lipids form an internal membrane with virus-specific proteins. Lipids constitute about 60% of the inner membrane. In PRD1, lipids form a bilayer and seem to be in a liquid crystalline phase. Phospholipid content (52% phosphatidylethanolamine and 43% phosphatidylglycerol, 5% cardiolipin) deviates from the host plasma membrane such that the phosphatidylglycerol is enriched. The fatty acid composition is close to that of the host.

### Carbohydrates

Not detected.

## Genome organization and replication

The PRD1 genome is a linear dsDNA molecule of 14,927 bp with proteins covalently linked to both 5′-termini. Replication is protein-primed, proceeds by strand displacement and can start at both ends of the genome as the ITRs contain sites for the replication initiation. A total of 27 identified genes are distributed in both strands and organized into five operons. Both ends of the genome contain early genes involved in replication and regulation of gene expression (Figure 2). The genes located in the three late operons encode structural proteins and proteins involved in assembly and lysis.

After DNA entry, replication and transcription, capsid proteins polymerize in the cytoplasm, whereas membrane-associated proteins are inserted into the host plasma membrane (Figure 3). With the help of nonstructural virion-encoded assembly factors and the coat-forming proteins, a virus-specific lipoprotein membrane obtains an outer protein shell leading to the formation of a procapsid. This is translocated to the interior of the cell. The linear genome is packaged into the procapsid through a unique packaging vertex. Mature virions are released by lysis of the host cell (Figure 3).

## Antigenic properties

No information available.

## Biological properties

Enterobacterial phages are virulent. The tail-like membranous tube probably acts as a DNA injection device. Phages Bam35 and AP50 and a number of additional isolates are specific for *Bacillus*

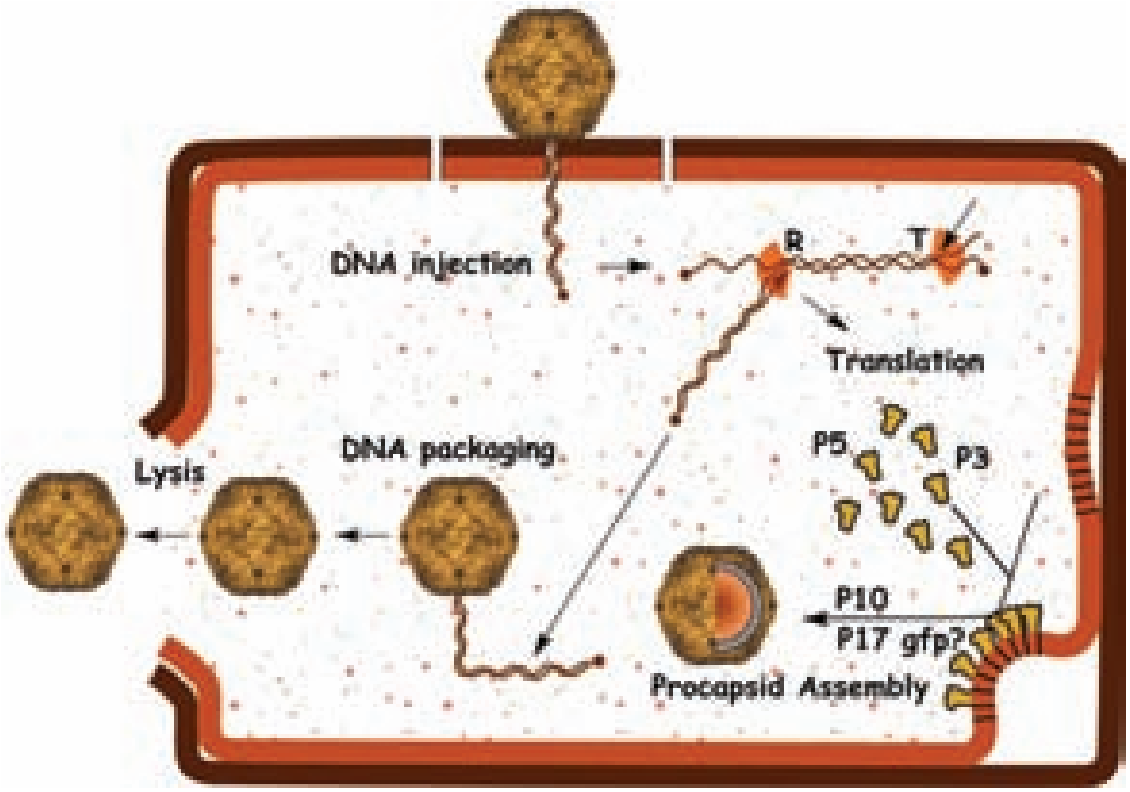


**Figure 3:** Diagram of the life cycle of Enterobacteria phage PRD1; for details see the text.

species and are temperate. Thermus phage P37-14 and a number of similar isolates are specific for *Thermus* and are found in volcanic hot springs.

## Species demarcation criteria in the genus

The sequence comparison of tectiviruses infecting *Enterobacteria* (phage PRD1) and *Bacillus* (phage Bam35) reveals very little identity, but genome organization and location of key genes are similar. The *Thermus* phage hosts are thermophilic and the other tectivirus hosts are mesophilic.

## List of species in the genus *Tectivirus*

| | | |
|---|---|---|
| *Bacillus phage AP50* | | |
| Bacillus phage AP50 | [EU408779] | (AP50) |
| *Bacillus phage Bam35* | | |
| Bacillus phage Bam35 | [AY257527] | (Bam35) |
| *Enterobacteria phage PRD1* | | |
| Enterobacteria phage PRD1 | [AY848689] | (PRD1) |
| *Thermus phage P37-14* | | |
| Thermus phage P37-14 | | (P37-14) |

Species names are in italic script; names of strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Tectivirus* but have not been approved as species

| | | |
|---|---|---|
| Enterobacteria phage L17 | [AY848684] | (L17) |
| Enterobacteria phage PR3 | [AY848685] | (PR3) |

| | | |
|---|---|---|
| Enterobacteria phage PR4 | [AY848686] | (PR4) |
| Enterobacteria phage PR5 | [AY848687] | (PR5) |
| Enterobacteria phage PR772 | [AY848688] | (PR772) |
| Bacillus phage GIL16 | [AY701338] | (GIL16) |
| Bacillus phage GIL01 | [AJ536073] | (GIL01) |



## List of unassigned species in the family *Tectiviridae*

*Bacillus phage phiNS11*
Bacillus phage phiNS11 (phiNS11)

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## Phylogenetic relationships within the family

The Enterobacteria phages (PRD1-types) show nucleotide sequence similarity between 91.9% and 99.8%. The Bacillus phages (Bam35-types) are more diverse in sequence (59–99% identity). Bam35- and PRD1-type phages most probably share a common ancestor.

## Similarity with other taxa

PRD1, and presumably other tectiviruses, have many features in common with adenoviruses. These include: (1) a linear dsDNA genome, (2) genomic inverted terminal repeats, (3) protein-primed initiation of replication, (4) type B (*E. coli* Pol II) DNA polymerase, (5) trimeric capsid proteins that contains two beta-barrels and form hexagonal structures, (6) arrangement of the capsid protein in a pseudo T = 25 lattice, (7) five-fold protein (penton) fold and (8) receptor-binding spikes at capsid vertices. The same principal capsid architecture and capsid protein fold have also been described in eukaryotic Paramecium bursaria Chlorella virus 1 (family *Phycodnaviridae*), Chilo iridescent virus (family *Iridoviridae*), archaeal Sulfolobus turreted icosahedral virus (unassigned family) and bacterial Pseudoalteromonas phage PM2 (family *Corticoviridae*).

## Derivation of name

*Tecti*: from Latin *tectus*, "covered".

## Further reading

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## Contributed by

Oksanen, H.M and Bamford, D.H.

# Genus *Rhizidiovirus*

Type species ***Rhizidiomyces virus***

## Virion properties

### Morphology
Virions are isometric, 60 nm in diameter (Figure 1).

### Physicochemical and physical properties
The buoyant density of virions in CsCl is 1.31 g cm$^{-3}$; $S_{20,w}$ is 625. Virions contain 10% nucleic acid.

### Nucleic acid
Virions contain a single molecule of dsDNA with a Mr of 16.8 $10^6$ and a G+C ratio of 42%.

### Proteins
Virions contain at least 14 polypeptides with sizes in the range of 26−84.5 kDa.

### Lipids
None reported.

### Carbohydrates
None reported.

## Genome organization and replication
Particles appear first in the nucleus.

## Biological properties
The virus appears to be transmitted in a latent form in the zoospores of its host *Rhizidiomyces* sp. Activation of the virus, which occurs under stress conditions such as heat, poor nutrition, or aging, results in cell lysis. *Rhizidiomyces* sp. is a water mold belonging to the kingdom Protista (Chromalveolata); it is no longer classified in the kingdom Fungi. Therefore, there are currently no recognized dsDNA viruses that naturally replicate in fungi.

No research has been done on this dsDNA virus system since 1983.

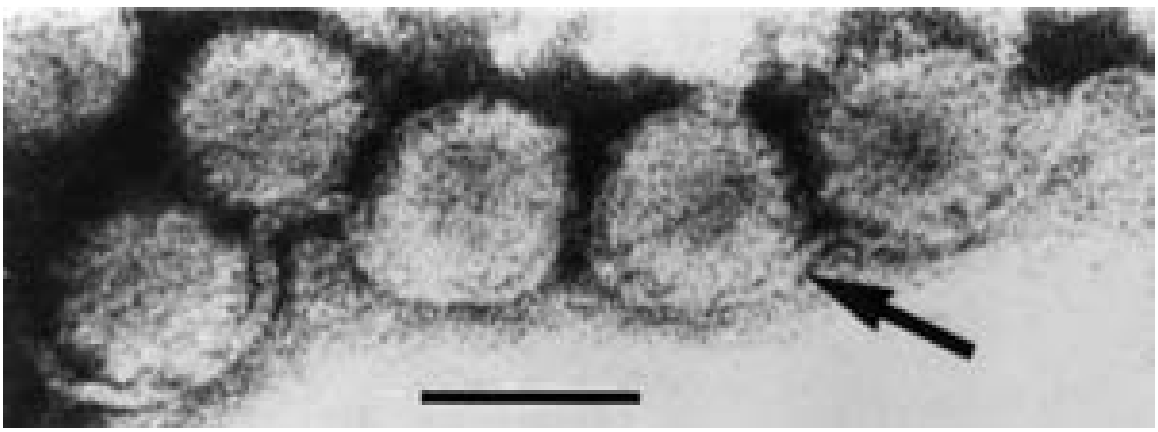

**Figure 1:** Negative contrast electron micrograph of particles of an isolate of Rhizidiomyces virus, which have been isolated from mechanically broken sporangia, are observed attached on a membrane-like structure (arrow). The bar represents 50 nm. (From Dawe and Kuhn (1983). *Virology*, **130**, 10−20; with permission.)

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Rhizidiovirus*

*Rhizidiomyces virus*

Rhizidiomyces virus (RZV)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Rhizidiovirus* but have not been approved as species

None reported.

## Similarity with other taxa

None reported.

## Derivation of name

*Rhizidio*: from name of the host *Rhizidiomyces* sp.

## Further reading

Dawe, V.H. and Kuhn, C.W. (1983). Virus-like particles in the aquatic fungus, *Rhizidiomyces. Virology*, **130**, 10–20.
Dawe, V.H. and Kuhn, C.W. (1983). Isolation and characterization of a double-stranded DNA mycovirus infecting the aquatic fungus, *Rhizidiomyces. Virology*, **130**, 21–28.

## Contributed by

Ghabrial, S.A.

# GENUS *SALTERPROVIRUS*

Type species ***His1 virus***



## Distinguishing features

Salterproviruses possess a spindle-shaped capsid surrounding a dsDNA linear genome of about 15 kb with inverted terminal repeat sequences and terminal proteins attached at 5′ ends. They replicate via protein-priming and an encoded DNA polymerase. They infect extremely halophilic Archaea.

## Virion properties

### MORPHOLOGY

Virus particles are spindle-shaped (44 × 77 nm) with a short, 7 nm long tail at one end. In Figure 1, many of the particles can be seen attached to host flagella via the tail. Virions can vary somewhat in shape in negative stain preparations, indicating that the capsid is flexible. A small proportion of particles are much larger or more elongated (up to about 204 nm in length). Virions of his2 virus (His2) are even more fragile than those of his1 virus (His1), and negatively-stained preparations usually show severely distorted or disintegrated capsids. However, a few particles can be found that retain the typical spindle shape (44 × 67 nm).

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

The buoyant density of His1 in CsCl is 1.28 g cm$^{-3}$. The virus is sensitive to chloroform, ethanol and detergents such as TritonX-100, but resistant to trichlorotrifluoroethane. Particles are stable if maintained in high salt solutions. Stable over pH range 3–9, and up to 60 °C.

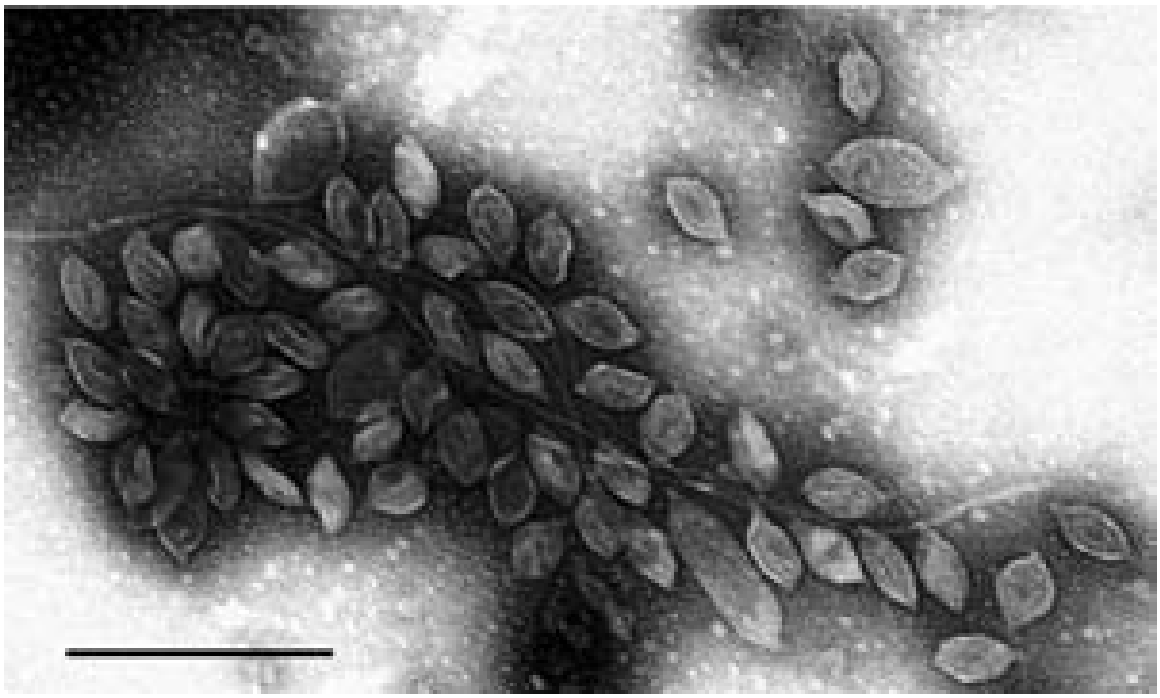

**Figure 1:** Negative-stain electron-micrograph (2% uranyl acetate) of an isolate of His1 virus. Many particles are seen binding to contaminating flagella derived from the host, *Haloarcula hispanica*. The bar represents 200 nm.

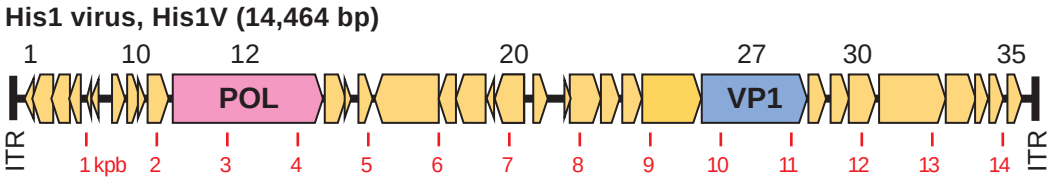


**Figure 2:** His1 genome, showing predicted ORFs (numbers above), inverted terminal repeats (ITR), the DNA polymerase (POL) and probable major capsid protein gene (VP1). Scale shown underneath is in kbp.

### Nucleic acid

The genome of His1 is linear dsDNA, and is 14,464 bp in length. It has 39% G+C, which is significantly lower than that of its host (62.7% G+C). The 5′ ends have protein attached. The related His2 has a genome of similar length, 16,067 bp.

### Proteins

The CP(s) or the terminal protein have, as yet, not been determined.

### Lipids

Not known.

### Carbohydrates

Not known.

## Genome organization and replication

His1 has a linear, double stranded DNA genome of 14.5 kb with covalently bound proteins attached to the 5′ termini. The termini contain inverted terminal repeat sequences of 105 bp (Figure 2). The genome is predicted to encode 35 proteins, one of which is a member of the type B group of DNA polymerases, and which uses protein-priming. Replication most likely occurs from both ends of the genome via protein-priming. The His1 genome, with intact terminal proteins, can be transfected into competent cells of *Har. hispanica*, resulting in the production of infectious virus.

In single-step growth curves, virus release is seen to occur well before significant cell lysis, indicating that virus exit is not linked to lysis. Given the nature of the host cell wall, a thin layer of protein immediately outside of the cell membrane (S-layer), virus particles may bud from the cell, as observed for the morphologically similar Sulfolobus spindle-shaped virus (SSV-1).

## Antigenic properties

Not known.

## Biological properties

His1 was isolated from a saltern crystallizer pond (Avalon, Victoria, Australia), in which the water was at salt saturation. Not surprisingly, virus particles are most stable at high salt concentration. The isolating host was *Haloarcula hispanica*, a member of the extremely halophilic Archaea (family Halobacteriaceae). The virus name comes from the first three letters of the host species name. Infection is lytic, but the virus is also capable of existing in an unstable carrier state. Virus particles are able to exit the cell without causing cell lysis. The entire virus genome does not integrate into that of the host, but homologs of the His2 VP1 gene (and 2–3 adjacent genes, probably also membrane proteins) are found in a wide variety of haloarchaeal genome sequences, usually clustered together as a block and keeping the same order and relative orientation. These gene blocks are often nearby genes related to other haloviruses (e.g. HRPV-1, HHPV-1) or haloarchaeal plasmids (e.g. pHK2). Their distribution and significance are currently being studied.

## Species demarcation criteria in the genus

Not yet determined.

## List of species in the genus *Salterprovirus*

*His1 virus*

| | | |
|---|---|---|
| His1 virus - DSM22484 | [AF191796] | (His1V-DSM22484)[a] |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
[a]Deposited with the German culture collection (DSMZ).

## List of other related viruses which may be members of the genus *Salterprovirus* but have not been approved as species

| | | |
|---|---|---|
| His2 virus | [AF191797] | (His2V) |

## Phylogenetic relationships within the genus

The genomes sequences of His1 and His2 share little nucleotide similarity but the predicted protein sequences of their DNA polymerase genes share 42% amino acid identity, confirming their specific, phylogenetic relationship.

## Similarity with other taxa

Morphologically, virus capsids resemble spindle-shaped archaeal viruses of *Sulfolobus* (SSV1) but on all other available criteria – sequence similarity, genome structure and replication – they appear to be very different.

## Derivation of name

*Salterpro*: acronym of *salt* *ter*minal proteins, referring to their characteristic genome structure of linear dsDNA with 5′-attached terminal proteins.

## Further reading

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## Contributed by

Dyall-Smith, M.L.

# FAMILY *ANELLOVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Anelloviridae* |
| Genus | *Alphatorquevirus* |
| Genus | *Betatorquevirus* |
| Genus | *Gammatorquevirus* |
| Genus | *Deltatorquevirus* |
| Genus | *Epsilontorquevirus* |
| Genus | *Zetatorquevirus* |
| Genus | *Etatorquevirus* |
| Genus | *Thetatorquevirus* |
| Genus | *Iotatorquevirus* |



## Virion properties

### MORPHOLOGY

Virions are non-enveloped, with reported diameters of about 30 nm for torque teno viruses (TTVs, genus *Alphatorquevirus*) and torque teno mini viruses (TTMVs, genus *Betatorquevirus*) (Figure 1).

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

The buoyant density of virions in CsCl is 1.31–1.33 g cm$^{-3}$ for TTVs and 1.27–1.28 g cm$^{-3}$ for TTMVs, both estimated using virus purified from serum.

### NUCLEIC ACID

Virions contain a single molecule of circular ssDNA, which ranges from about 2 to about 3.9 kb in size. Genomes are of negative sense. The putative non-coding region generally contains one or two sequences of about 80–110 nt with high G+C content (ca. 90%), which is postulated to form a secondary structure composed of stems and loops. A region of about 130 nt, located in the untranslated part of the genome, is relatively well conserved between members of the family.

### PROTEINS

Two main open reading frames, ORF1 and ORF2, and additional ORFs, may be deduced directly from the nucleotide sequence. These ORFs overlap partially, and their estimated sizes differ widely among isolates. Transfection approaches, restricted to the study of some TTV isolates, have demonstrated that at least 5–7 proteins ranging from about 12 to 80 kDa may be expressed via alternative translational initiation. The ORF1 proteins of human and animal anelloviruses

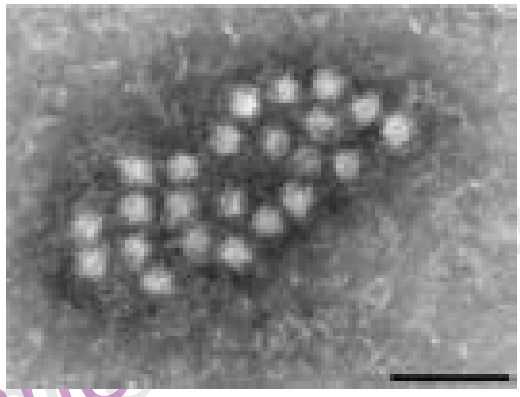

**Figure 1:** Negative contrast electron microscopy of particles of an isolate of *Torque teno virus*, stained with uranyl acetate. The bar represents 100 nm. (From Itoh *et al.* (2000.) *Biochem. Biophys. Res. Commun.*, **279**, 718–724.)

possess arginine-rich, hydrophilic N-terminal sequences, and at least one amino acid sequence motif with which rolling circle replication (RCR) of the virus DNA may be associated. On this basis, ORF1 is believed to encode the putative capsid protein and replication-associated protein of anelloviruses. Hypotheses regarding functions of the other proteins are based on studies involving specific isolates. ORF2, which presents a highly conserved motif, $WX_7HX_3CXCX_5H$, identifiable in its N-terminal part, may encode a protein with phosphatase activity (TTMVs), or a peptide able to suppress NF-κB pathways (TTVs). ORF3 of TTVs has a serine-rich domain at the C-terminus capable of generating different phosphorylation sites, and might play some role in maintaining persistent viral infection. It was also demonstrated that a short TTV peptide, encoded by the N-terminus of a putative ORF, is able to induce p53-independent apoptosis in human hepatocellular carcinoma cell lines.

### Lipids
Unknown.

### Carbohydrates
Unknown.

## Genome organization and replication

Anelloviruses harbour a relatively well conserved genetic organization with a coding region containing a major ORF, ORF1, an overlapping ORF2 and several additional ORFs, and an untranslated region (Figure 2). Knowledge of the genome expression and replication mechanisms remains limited, mainly owing to the lack of an efficient cell culture propagation system. TTV-specific mRNAs have been detected in various tissues and organs in humans, and following transfection studies. At least theree mRNAs of different sizes (ca. 2.9, 1.2 and 1.0 kb) are transcribed from the negative strand of the putative circular ds replicative form TTV DNA. The existence of these mRNAs supports the view that both ORF1 and ORF2 are functional, and also suggests that additional transcripts are generated by complex splicing. The transcription profile of other members of the family is not known, but the fact that they share similar genome organizations is highly suggestive that several mRNAs may be expressed as for TTVs. The presence within ORF1 of conserved amino acid sequence motifs, which occur in the Rep proteins of other animal and plant viruses with circular ssDNA genomes (within *Circoviridae* and *Nanoviridae*), suggests that replication of anellovirus DNA may use a rolling circle mechanism of replication.

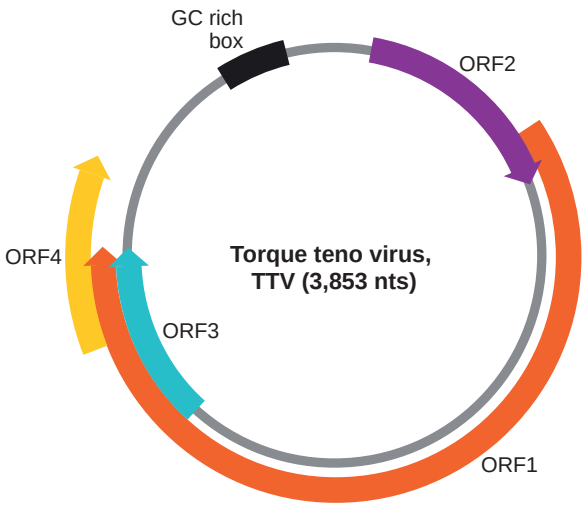


**Figure 2:** Genome organization of an isolate of Torque teno virus.

### Antigenic properties

It has been demonstrated that TTV particles in the blood are bound to immunoglobulins G (IgG) and M (IgM), forming immune complexes; they exist as free virions in feces.

### Biological properties

Epidemiological studies have demonstrated the global distribution of anelloviruses in rural and urban populations. Their overall prevalence in the general population is high (>90%). Although they were initially suspected of being transmitted only by blood transfusion, the global dispersion of the viruses in populations and their detection in various biological samples (e.g. plasma, saliva and feces) suggests combined modes of diffusion, and in particular the spread by saliva droplets. Other modes of transmission, such as those involving maternal or sexual routes, have also been suggested. The link between anellovirus infection and a specific pathology remains unproven, although some studies suggested possible associations with liver or respiratory diseases, hematological disorders or cancer. The effects of anelloviruses on the immune system are also generally unknown.

Infection with anelloviruses is not restricted to human hosts. Viruses have been detected in non-human primates (chimpanzee, macaque, tamarin and douroucouli), tupaia, pets (cat and dog) and farm animals (pig and cow). Such identifications were recently extended to a marine mammal (sea lion). The analysis of complete viral sequences from different animal sources reveals a high heterogeneity in the size of the viral genome (ca. 2 to 3.9 kb), along with a high genetic divergence when compared with human isolates. However, genomic organization and predicted transcription profiles correspond to those found in human isolates.



## GENUS *ALPHATORQUEVIRUS*

Type species ***Torque teno virus 1***

### Distinguishing features

The genus contains viruses identified in humans and non-human primates, with genomes ranging from about 3.6 to 3.9 kb.

### Species demarcation criteria in the genus

Based on analysis of ORF1 in its entirety, a cut-off value of 35% nucleotide sequence identity is applied as a demarcation criterion.

### List of species in the genus *Alphatorquevirus*

| | | |
|---|---|---|
| *Torque teno virus 1* | | |
| Torque teno virus 1-TA278 | [AB008394] | (TTV1-TA278) |
| *Torque teno virus 2* | | |
| Torque teno virus 2-CH71 | [AB049608] | (TTV2-CH71) |
| *Torque teno virus 3* | | |
| Torque teno virus 3-HEL32 | [AY666122] | (TTV3-HEL32) |
| *Torque teno virus 4* | | |
| Torque teno virus 4-Pt-TTV6 | [AB041957] | (TTV4-Pt-TTV6) |
| *Torque teno virus 5* | | |
| Torque teno virus 5-TCHN-C1 | [AF345523] | (TTV5-TCHN-C1) |
| *Torque teno virus 6* | | |
| Torque teno virus 6-KAV | [AF435014] | (TTV6-KAV) |
| *Torque teno virus 7* | | |
| Torque teno virus 7-PMV | [AF261761] | (TTV7-PMV) |

| | | |
|---|---|---|
| *Torque teno virus 8* | | |
| Torque teno virus 8-Kt-08F | [AB054647] | (TTV8-Kt-08F) |
| *Torque teno virus 9* | | |
| Torque teno virus 9-BM1C-18 | [DQ187006] | (TTV9-BM1C-18) |
| *Torque teno virus 10* | | |
| Torque teno virus 10-JT34F | [AB064607] | (TTV10-JT34F) |
| *Torque teno virus 11* | | |
| Torque teno virus 11-TCHN-D1 | [AF345524] | (TTV11-TCHN-D1) |
| *Torque teno virus 12* | | |
| Torque teno virus 12-CT44F | [AB064605] | (TTV12-CT44F) |
| *Torque teno virus 13* | | |
| Torque teno virus 13-TCHN-A | [AF345526] | (TTV13-TCHN-A) |
| *Torque teno virus 14* | | |
| Torque teno virus 14-CH65-1 | [AB037926] | (TTV14-CH65-1) |
| *Torque teno virus 15* | | |
| Torque teno virus 15-TJN01 | [AB028668] | (TTV15-TJN01) |
| *Torque teno virus 16* | | |
| Torque teno virus 16-TUS01 | [AB017613] | (TTV16-TUS01) |
| *Torque teno virus 17* | | |
| Torque teno virus 17-SENV-G | [AX025830] | (TTV17-SENV-G) |
| *Torque teno virus 18* | | |
| Torque teno virus 18-SENV-C | [AX025718] | (TTV18-SENV-C) |
| *Torque teno virus 19* | | |
| Torque teno virus 19-SANBAN | [AB025946] | (TTV19-SANBAN) |
| *Torque teno virus 20* | | |
| Torque teno virus 20-SAa-10 | [AB060594] | (TTV20-SAa-10) |
| *Torque teno virus 21* | | |
| Torque teno virus 21-TCHN-B | [AF348409] | (TTV21-TCHN-B) |
| *Torque teno virus 22* | | |
| Torque teno virus 22-svi-1 | [AX174942] | (TTV22-svi-1) |
| *Torque teno virus 23* | | |
| Torque teno virus 23-CH65-2 | [AB049607] | (TTV23-CH65-2) |
| *Torque teno virus 24* | | |
| Torque teno virus 24-SAa-01 | [AB060597] | (TTV24-SAa-01) |
| *Torque teno virus 25* | | |
| Torque teno virus 25-Mf-TTV9 | [AB041959] | (TTV25-Mf-TTV9) |
| *Torque teno virus 26* | | |
| Torque teno virus 26-Mf-TTV3 | [AB041958] | (TTV26-Mf-TTV3) |
| *Torque teno virus 27* | | |
| Torque teno virus 27-CT23F | [AB064595] | (TTV27-CT23F) |
| *Torque teno virus 28* | | |
| Torque teno virus 28-CT43F | [AB064598] | (TTV28-CT43F) |
| *Torque teno virus 29* | | |
| Torque teno virus 29-yonKC009 | [AB038621] | (TTV29-yonKC009) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Alphatorquevirus* but have not been approved as species

None reported.

## Genus *Betatorquevirus*

Type species ***Torque teno mini virus 1***

### Distinguishing features

The genus contains viruses identified in humans and non-human primates, with genomes ranging from about 2.8 to 2.9 kb.

### Species demarcation criteria in the genus

Based on analysis of ORF1 in its entirety, a cut-off value of 35% nucleotide sequence identity is applied as a demarcation criterion.

### List of species in the genus *Betatorquevirus*

| | | |
|---|---|---|
| *Torque teno mini virus 1* | | |
| Torque teno mini virus 1-CBD279 | [AB026931] | (TTMV1-CBD279) |
| *Torque teno mini virus 2* | | |
| Torque teno mini virus 2-NLC023 | [AB038629] | (TTMV2-NLC023) |
| *Torque teno mini virus 3* | | |
| Torque teno mini virus 3-NLC026 | [AB038630] | (TTMV3-NLC026) |
| *Torque teno mini virus 4* | | |
| Torque teno mini virus 4-Pt-TTV8-II | [AB041963] | (TTMV4-Pt-TTV8-II) |
| *Torque teno mini virus 5* | | |
| Torque teno mini virus 5-TGP96 | [AB041962] | (TTMV5-TGP96) |
| *Torque teno mini virus 6* | | |
| Torque teno mini virus 6-CBD203 | [AB026929] | (TTMV6-CBD203) |
| *Torque teno mini virus 7* | | |
| Torque teno mini virus 7-CLC156 | [AB038627] | (TTMV7-CLC156) |
| *Torque teno mini virus 8* | | |
| Torque teno mini virus 8-PB4TL | [AF291073] | (TTMV8-PB4TL) |
| *Torque teno mini virus 9* | | |
| Torque teno mini virus 9-NLC030 | [AB038631] | (TTMV9-NLC030) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Betatorquevirus* but have not been approved as species

| | | |
|---|---|---|
| Torque teno mini virus-LIL-y1 | [EF538880] | (TTMV-LIL-y1) |
| Torque teno mini virus-LIL-y2 | [EF538881] | (TTMV-LIL-y2) |
| Torque teno mini virus-LIL-y3 | [EF538882] | (TTMV-LIL-y3) |

## GENUS *GAMMATORQUEVIRUS*

Type species ***Torque teno midi virus 1***

### Distinguishing features

The genus contains viruses identified in humans and non-human primates, with genomes of about about 3.2 kb. Some isolates harbouring shorter genomes (ca. 2–2.6 kb) have been identified.

### Species demarcation criteria in the genus

Based on analysis of ORF1 in its entirety, a cut-off value of 35% nucleotide sequence identity is applied as a demarcation criterion.

### List of species in the genus *Gammatorquevirus*

| | | |
|---|---|---|
| *Torque teno midi virus 1* | | |
| Torque teno midi virus 1-MD1-073 | [AB290918] | (TTMDV1-MD1-073) |
| *Torque teno midi virus 2* | | |
| Torque teno midi virus 2-MD2-013 | [AB290919] | (TTMDV2-MD2-013) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Gammatorquevirus* but have not been approved as species

| | | |
|---|---|---|
| Torque teno midi virus-2PoSMA | [EF538875] | (TTMDV-2PoSMA) |
| Torque teno midi virus-6PoSMA | [EF538876] | (TTMDV-6PoSMA) |
| Torque teno midi virus-MDJHem2 | [AB303552] | (TTMDV-MDJHem2) |
| Torque teno midi virus-MDJHem3-1 | [AB303553] | (TTMDV-MDJHem3-1) |
| Torque teno midi virus-MDJHem3-2 | [AB303554] | (TTMDV-MDJHem3-2) |
| Torque teno midi virus-MDJHem5 | [AB303555] | (TTMDV-MDJHem5) |
| Torque teno midi virus-MDJN2 | [AB303559] | (TTMDV-MDJN2) |
| Torque teno midi virus-MDJN14 | [AB303560] | (TTMDV-MDJN14) |
| Torque teno midi virus-MDJN47 | [AB303561] | (TTMDV-MDJN47) |
| Torque teno midi virus-MDJN51 | [AB303562] | (TTMDV-MDJN51) |
| Torque teno midi virus-MDJN69 | [AB303564] | (TTMDV-MDJN69) |
| Torque teno midi virus-MDJN97 | [AB303566] | (TTMDV-MDJN97) |
| Torque teno midi virus-Pt-TTMDV210 | [AB449062] | (TTMDV-Pt-TTMDV210) |

## GENUS *DELTATORQUEVIRUS*

Type species ***Torque teno tupaia virus***

### Distinguishing features

The genus contains virus identified in tupaia.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Deltatorquevirus*

*Torque teno tupaia virus*

| | | |
|---|---|---|
| Torque teno tupaia virus-Tbc-TTV14 | [AB057358] | (TTTuV-Tbc-TTV14) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Deltatorquevirus* but have not been approved as species

None reported.

## GENUS *EPSILONTORQUEVIRUS*

Type species ***Torque teno tamarin virus***

### Distinguishing features

The genus contains a virus identified in the cotton-top tamarin.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Epsilontorquevirus*

*Torque teno tamarin virus*
Torque teno tamarin virus-So-TTV2 [AB041960] (TTTaV-So-TTV2)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Epsilontorquevirus* but have not been approved as species

None reported.

## GENUS *ZETATORQUEVIRUS*

Type species ***Torque teno douroucouli virus***

### Distinguishing features

The genus contains virus identified in the douroucouli (owl monkey or night monkey).

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Zetatorquevirus*

*Torque teno douroucouli virus*
Torque teno douroucouli virus-At-TTV3 [AB041961] (TTDoV-At-TTV3)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Zetatorquevirus* but have not been approved as species

None reported.

## GENUS *ETATORQUEVIRUS*

Type species ***Torque teno felis virus***

### Distinguishing features

The genus contains viruses identified in the domestic cat.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Etatorquevirus*

*Torque teno felis virus*
Torque teno felis virus-Fc-TTV4 [AB076003] (TTFeV-Fc-TTV4)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Etatorquevirus* but have not been approved as species

| | | |
|---|---|---|
| Torque teno felis virus-PRA1 | [EF538877] | (TTFeV-Fc-PRA1) |

## GENUS *THETATORQUEVIRUS*

Type species ***Torque teno canis virus***

### Distinguishing features

The genus contains virus identified in the domestic dog.

### List of species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Thetatorquevirus*

*Torque teno canis virus*

| | | |
|---|---|---|
| Torque teno canis virus-Cf-TTV10 | [AB076002] | (TTCaV-Cf-TTV10) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Thetatorquevirus* but have not been approved as species

None reported.

## GENUS *IOTATORQUEVIRUS*

Type species ***Torque teno sus virus 1***

### Distinguishing features

The genus contains viruses identified in the pig.

### Species demarcation criteria in the genus

Based on analysis of ORF1 in its entirety, a cut-off value of 35% nucleotide sequence identity is applied as a demarcation criterion.

### List of species in the genus *Iotatorquevirus*

*Torque teno sus virus 1*

| | | |
|---|---|---|
| Torque teno sus virus 1-Sd-TTV31 | [AB076001] | (TTSuV1-Sd-TTV31) |

*Torque teno sus virus 2*

| | | |
|---|---|---|
| Torque teno sus virus 2-1p | [AY823990] | (TTSuV2-1p) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Iotatorquevirus* but have not been approved as species

Torque teno sus virus-2p [AY823991] (TTSuV-2p)

Note: The nucleotide sequence of this virus identified in swine presents a degree of sequence divergence compatible with the creation of a distinct genus. However, it has been proposed to classify this virus in the genus *Iotatoquevirus* until further data have been collected in swine species.

## List of other related viruses which may be members of the family *Anelloviridae* but have not been approved as species

Torque teno zalophus virus – ZcAV [FJ459582] (TTZaV-ZcAV)

Note: virus identified in California sea lion.



## Phylogenetic relationships within the family

The progressive discovery of highly divergent, complete genomes ranging from about 2 to 4 kb in humans and other animals impairs a reliable phylogenetic and taxonomic analysis of full-length sequences. Based on these considerations, the analysis of the entire ORF1 at the nucleotide level (ORF1-nt) is the most convenient approach. Analysis of the distribution of pairwise comparisons and the corresponding phylogenetic tree (see Figure 3) facilitates identification of the levels of genera and species. Based on the currently available data, a taxonomic classification is proposed with the following cut-off values for sequence divergence: genera >56%, species >35%.

## Similarity with other taxa

Members of the family *Anelloviridae* have features in common with *Chicken anaemia virus*, the type species of genus *Gyrovirus*, family *Circoviridae*. Namely:

- All viruses possess negative sense, circular, ssDNA genomes.
- The genome organizations are similar.
- The CP of CAV and the putative CPs of anelloviruses both possess amino acid sequence motifs that are characteristic of RCR Rep proteins. The proteins encoded by ORF2 in CAV, TTVs and TTMVs contain amino acid sequences that are characteristic of protein tyrosine phosphatases (PTPase). ORF2 in anelloviruses and CAV share a common motif, $WX_7HX_3CXCX_5H$.
- The non-coding region of the CAV genome and those of most anellovirus genomes contain G+C-rich sequences.
- Spliced transcripts have been detected for CAV and TTV.
- Peptides identified in CAV and TTV are able to induce apoptosis in human hepatocellular carcinoma cell lines.

## Derivation of names

*Anello*: from Latin *anello*, "ring".
*Torque*: from Latin *torques*, "necklace".
*Teno*: from Latin *tenuis*, "thin".
*Mini*: from Latin *minimus*, "small".
*Midi*: from Latin *medius*, "intermediate".

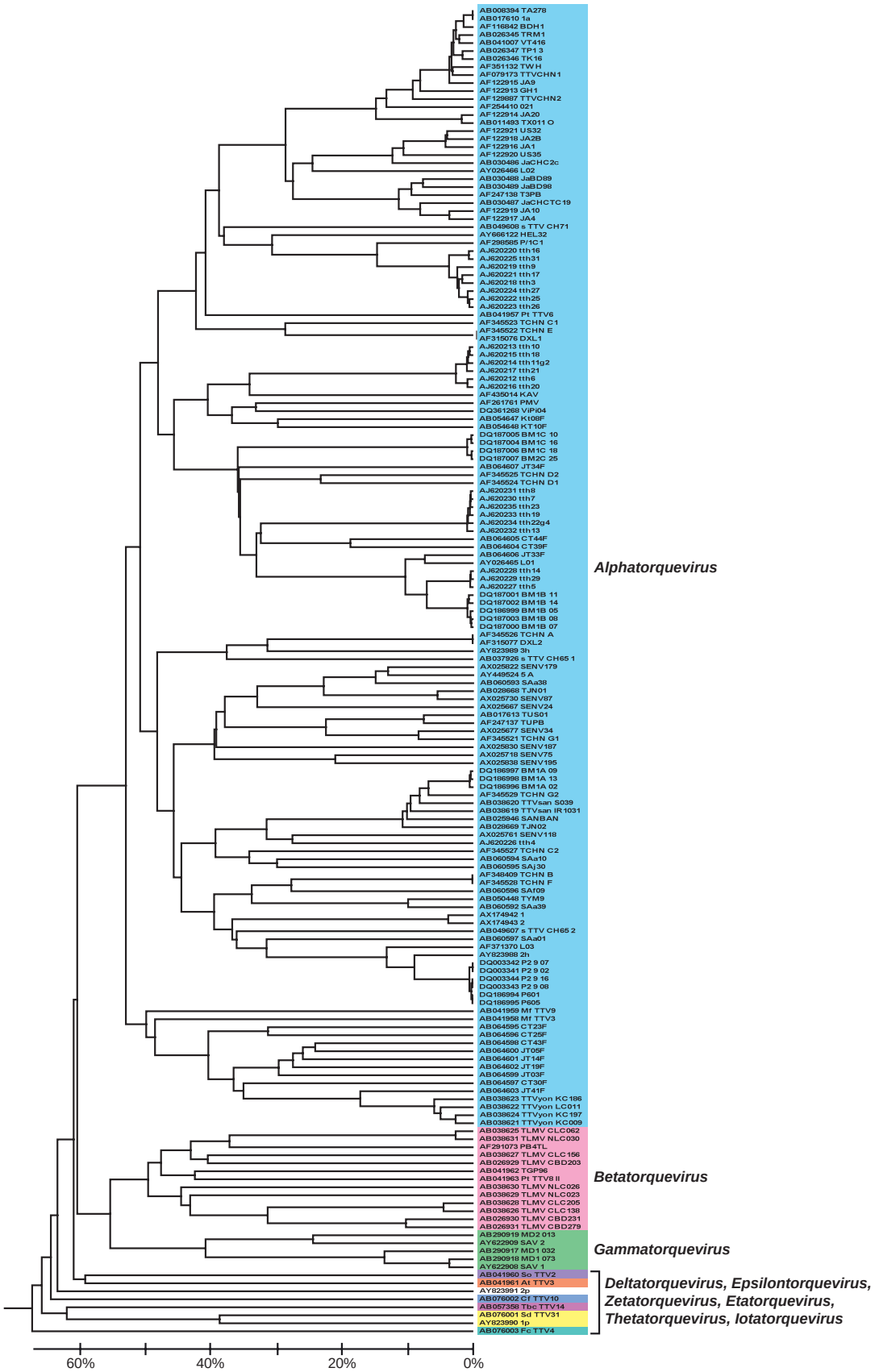

**Figure 3 (*opposite*):** UPGMA phylogenetic tree built for members of the family *Anelloviridae*, using ORF1-nt sequences (virus strains are identified on the figure by their GenBank accession numbers).

## Further reading

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# Family *Circoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Circoviridae*** |
| Genus | ***Circovirus*** |
| Genus | ***Gyrovirus*** |

## Virion properties

### Morphology

Virions are not enveloped and exhibit icosahedral symmetry (Figure 1). Ranges reported for virion diameters of chicken anemia virus (CAV), porcine circovirus 1 (PCV1) and beak and feather disease virus (BFDV) are 19.1–26.5 nm, 17–20.7 nm and 12–20.7 nm, respectively. Comparative analysis indicated that the diameters of CAV virions are about 20% greater than those of PCV1 and BFDV, and that CAV exhibits a distinctive surface structure, which is not exhibited by PCV1 and BFDV.

### Physicochemical and physical properties

The buoyant densities of virions in CsCl range from 1.33 to 1.37 g cm$^{-3}$. CAV and PCV1 are resistant to inactivation by treatment at pH 3, and both can withstand incubation at 70°C for 15 min. Both viruses resist treatment with organic solvents such as chloroform, and both show at least partial resistance to sodium dodecyl sulphate.

### Nucleic acid

Virions contain a single molecule of circular ssDNA, which ranges from about 1.7 to 2.3 kb in size.

### Proteins

The virions of CAV, PCV1 and porcine circovirus 2 (PCV2) are each comprised of one structural protein (capsid protein), for which approximate sizes of 50 kDa (CAV), 30 kDa (PCV1) and 30 kDa (PCV2) have been estimated, respectively. BFDV is reported to contain three proteins, 26.3, 23.7 and 15.9 kDa. The protein composition of virions of the other members of the family *Circoviridae* is not known, but putative structural proteins have been identified by amino acid similarity searches.

### Lipids

Unknown.

### Carbohydrates

Unknown.

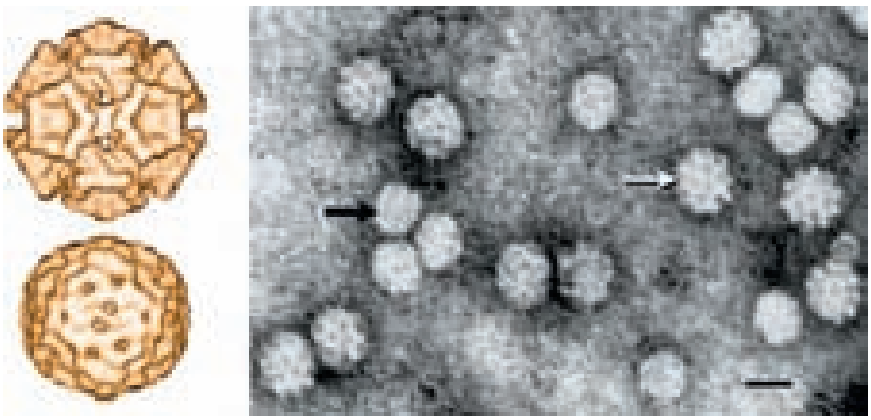

**Figure 1:** (Left upper) Cryo-electron microscopy image of a particle of an isolate of chicken anemia virus. A structural model comprising 60 subunits (T = 1) arranged in 12 trumpet-shaped pentameric rings has been proposed. (Left lower) Cryo-electron microscopy image of a particle of an isolate of porcine circovirus-2. A structural model comprising 60 subunits (T = 1) arranged in 12 flat pentameric morphological units has been proposed. (Right) Negative contrast electron microscopy of particles of an isolate of chicken anemia virus (black arrow) and beak and feather disease virus (white arrow), stained with uranyl acetate. Bar = 20 nm.

### Genome organization and replication

CAV has a negative sense genome organization, whereas those of the other circoviruses are ambisense. All viruses of the family replicate their genomes using a circular, double strand (ds) replicative form (RF) DNA intermediate, which is produced using host cell DNA polymerases during the S phase of cell division. The RF serves as template for generation of viral ssDNA, probably using the rolling circle replication (RCR) mechanism. Since complex transcription patterns (up to 12 transcripts) have been identified for PCV1 and PCV2, it is also probable that some, if not all, members of the genus *Circovirus* employ similar transcription strategies. The existence of spliced mRNAs was recently demonstrated for CAV.

### Antigenic properties

CAV is antigenically distinct from other circoviruses. PCV1 and BFDV are antigenically different, but PCV1 and PCV2 share common epitopes. The antigenic relationships of avian members of the genus *Circovirus* are not known.

### Biological properties

Circoviruses are host-specific or exhibit a narrow host range, and the majority of those reported infect avian species. Most infections are thought to spread by the fecal–oral route, but vertical transmission has been demonstrated. Circovirus infections are highly prevalent and have widespread geographic distributions. Although subclinical infections are common, circovirus infections are associated with a range of clinical diseases, including infectious chicken anemia, psittacine beak and feather disease, circovirus disease of pigeons, and the postweaning multisystemic wasting syndrome of pigs (PMWS), for which PCV2 has been recognized as an essential component of the disease process. Circovirus infections cause lymphoid depletion and are immunosuppressive.

## Genus *Circovirus*

Type species ***Porcine circovirus-1***

### Distinguishing features

Viruses have very similar ambisense genome organizations in which the genes encoding the Rep and CP are divergently arranged on different strands of the ds RF. The intergenic region between the start sites of these genes contains the conserved nonanucleotide motif (TAGTATTAC), located at the apex of a potential stem-loop, at which rolling circle replication (RCR) of the virus DNA initiates.

### Virion properties

#### Morphology

Virions are non-enveloped and show spherical morphology. Electron microscopy studies showed that BFDV and PCV2 have a diameter of about 20.5 nm, and an icosahedral T = 1 structure containing 60 CP molecules with dodecahedral symmetry. The virion sizes reported for other circoviruses are similar and appear to depend on the type of negative stain used and on whether measurement is made using negative contrast or thin section electron microscopy.

#### Physicochemical and physical properties

PCV1 virions have a sedimentation coefficient of 57S and a buoyant density in CsCl of 1.35–1.37 g cm$^{-3}$.

#### Nucleic acid

Virions contain covalently closed circular ssDNA. The genomes of PCV1 and PCV2 are the smallest viruses replicating autonomously in mammalian cells (1759 and 1768 nt), and the genomes of the other circoviruses are slightly larger (about 1.7–2 kb). All virus genomes carry a putative stem-loop element with the conserved nonamer (TAGTATTAC) in its apex.

## Genome organization and replication

Genomes consist of one ssDNA component approximately 1.7–2 kb in size. Circoviruses possess ambisense genomes with the two major ORFs, from the *rep* and *cap* genes, encoded on opposite strands of the replicative dsDNA intermediate (Figure 2). This arrangement creates two intergenic regions, a larger one between the 5′ ends of the two major ORF genes and a shorter one between their 3′ ends. In the case of PCV1 and PCV2, the non-coding region between the initiation codons of the *rep* and the *cap* genes comprises the origin of replication. After infection, the ssDNA genome is converted into dsDNA RF, presumably by host enzymes. By analogy to geminiviruses, replication is thought to follow the RCR model and is initiated at the conserved TAGTAT$^{T}/_{A}$C sequence. The *rep* gene of PCV1 and PCV2 expresses two proteins, Rep and Rep′, which are derived from differentially spliced transcripts. Rep is translated from the full-length transcript (PCV1: 312 amino acid residues (aa); PCV2: 314 aa), whereas a spliced transcript encodes truncated and C-terminal frameshifted Rep′ (PCV1: 168 aa; PCV2: 178 aa). Rep and Rep′ of PCV1 bind to two genomic hexameric repeats, which are located close to the potential stem loop at the origin of replication. Both proteins and binding to the hexamers are essential for initiation of replication. The *cap* gene of PCV1 and PCV2 encodes the major structural protein Cap (PCV1: 232 aa; PCV2: 233 aa). Cap possesses an arginine-rich N-terminus sequence that is possibly involved in binding to viral DNA, as suggested for PCV1 and PCV2, and BFDV, too. Several smaller ORFs have been identified, but only ORF3 of PCV2 (also identifiable in PCV1) has been recognized as functional, exhibiting an apoptotic activity. The porcine kidney PK-15 cell line can be used successfully for propagation of PCV1 and PCV2. Information regarding other members of the genus *Circovirus* remains limited.

## Antigenic properties

Cap is the main antigenic determinant of PCV1 and PCV2. Both share antigenic epitopes and show cross-reaction. A detailed serological analysis for the avian circoviruses has not yet been performed.

## Biological properties

Natural infections with PCV1 and PCV2 appear to be restricted to pigs, while BFDV infections are detected in over 40 species of psittacine birds. Available evidence suggests that viruses are host-specific or have narrow host ranges. The fecal–oral route of transmission is likely, although vertical transmission has been reported in cases of PCV2, BFDV and pigeon circovirus (PiCV).

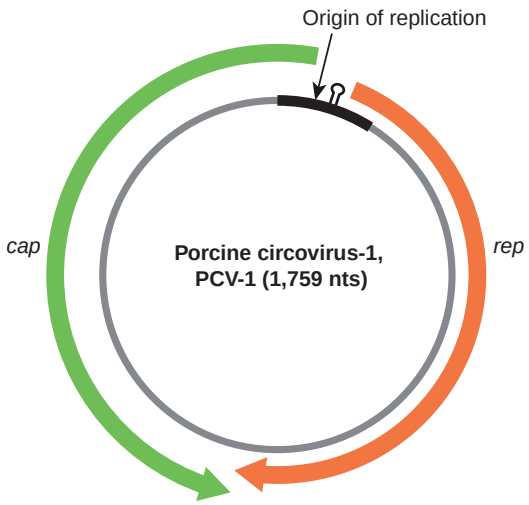


**Figure 2:** Genome organization of an isolate of porcine circovirus-1.

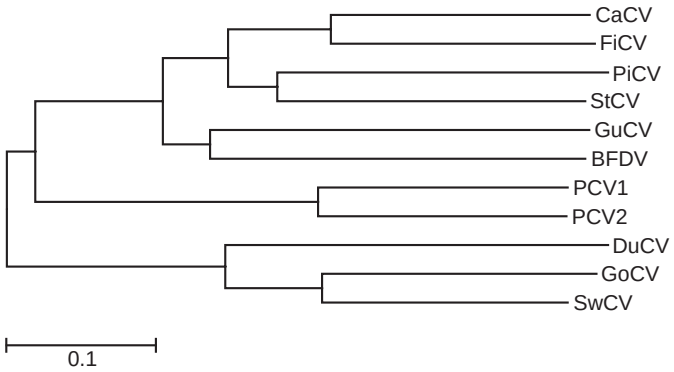


**Figure 3:** Genus *Circovirus*: neighbour-joining phylogenetic tree built with CP amino acid sequences.

## Species demarcation criteria in the genus

Since the *rep* genes are highly conserved, species demarcation can be best resolved by phylogenetic comparison of the CP coding sequence (Figure 3). The current suggested criteria demarcating species in the genus are (i) complete genome nt sequence identity <75%, and (ii) CP sequence identity <70%.

## List of species in the genus *Circovirus*

| | | |
|---|---|---|
| *Beak and feather disease virus* | | |
| Beak and feather disease virus | [AF080560] | (BFDV) |
| *Canary circovirus* | | |
| Canary circovirus | [AJ301633] | (CaCV) |
| *Duck circovirus* | | |
| Duck circovirus | [AY228555] | (DuCV) |
| *Finch circovirus* | | |
| Finch circovirus | [DQ845075] | (FiCV) |
| *Goose circovirus* | | |
| Goose circovirus | [AJ304456] | (GoCV) |
| *Gull circovirus* | | |
| Gull circovirus | [DQ845074] | (GuCV) |
| *Pigeon circovirus* | | |
| Pigeon circovirus | [AF252610] | (PiCV) |
| *Porcine circovirus-1* | | |
| Porcine circovirus-1 | [Y09921] | (PCV1) |
| *Porcine circovirus-2* | | |
| Porcine circovirus-2-pws-PCV | [AF027217] | (PCV2-pws-PCV) |
| *Starling circovirus* | | |
| Starling circovirus | [DQ172906] | (StCV) |
| *Swan circovirus* | | |
| Swan circovirus | [EU056309] | (SwCV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Circovirus* but have not been approved as species

| | | |
|---|---|---|
| Raven circovirus-4-1131 | [DQ146997] | (RaCV-4-1131) |

# Genus *Gyrovirus*

Type species *Chicken anemia virus*

## Distinguishing features

Chicken anemia virus (CAV), the only member of the genus, can be distinguished from viruses belonging to the genus *Circovirus* on the basis of its negative sense genome organization. Also, CAV virions are larger than those of other circoviruses, and surface structure is more evident.

## Virion properties

### Morphology

Virions are non-enveloped and show icosahedral morphology. Electron microscopy studies showed that CAV have a diameter of about 25 nm, with a capsid consisting of 60 subunits (T = 1) arranged in 12 pentameric rings.

### Physicochemical and physical properties

CAV virions have a sedimentation coefficient of 91S and a buoyant density in CsCl of 1.33–1.35 g cm$^{-3}$.

### Nucleic acid

The genomes of CAV isolates are about 2.3 kb in size and share high sequence identity. Part of the non-transcribed region (NTR) of the genome is G+C-rich and capable of forming stem-loop structures.

## Genome organization and replication

Gyroviruses have a negative sense genome organization, with a coding region containing three partially overlapping ORFs (Figure 4). CAV was initially thought to transcribe a single, unspliced mRNA, but recent studies have demonstrated that several spliced transcripts (ca. 2, 1.6, 1.3 and 1.2 kb) are detectable during the life cycle of the virus. NTR contains transcription initiation and termination signals, and a tandemly arranged array of either four or five 19 nt repeats with which promoter–enhancer activity is associated. The circular ssDNA genome is thought to replicate using

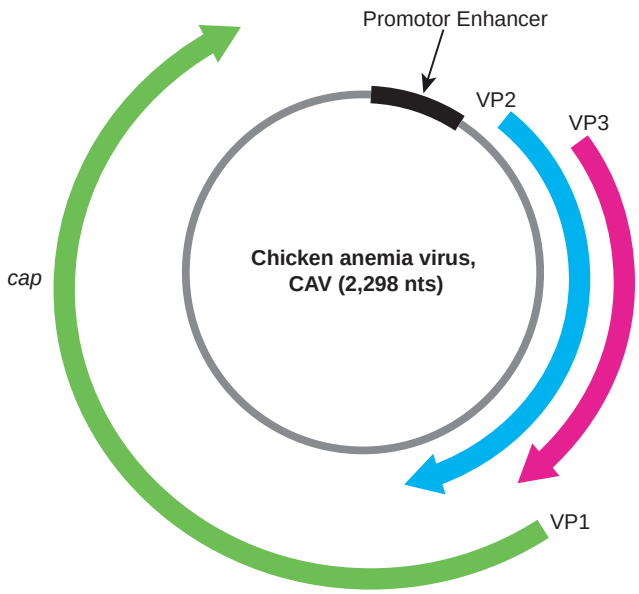


**Figure 4:** Genome organization of an isolate of chicken anemia virus.

the RCR mechanism. However, although a semi-conserved nonanucleotide motif, at which RCR initiates in other circular ssDNA replicons, is found within NTR, it is not located at the apex of a potential stem-loop, as is the case with the replicons of some other families of viruses. Three virus proteins are synthesized. VP1 (ca. 52 kDa), encoded by ORF1, is the only structural protein. VP2 (ca. 26 kDa), encoded by ORF2, is a protein phosphatase. VP3 (ca. 14 kDa) encoded by ORF3 is capable of causing apoptosis and has been called "apoptin". The occurrence within VP1 of two amino acid motifs, which have putative roles in RCR, suggests that this structural protein possesses DNA replication function. Marek's disease virus-transformed chicken lymphoblastoid MDCC-MSB1 and MDCC-CU147 cell lines can be used successfully for the propagation of CAV isolates.

## Antigenic properties

Cell culture isolates of CAV, collected from throughout the world, belong to a single serotype, but isolates can be differentiated by type-specific virus neutralizing monoclonal antibodies.

## Biological properties

Although the chicken is the major host, CAV has also been isolated from turkeys, and the detection of virus-specific antibody suggests that Japanese quail may also be susceptible to infection. Vertical (egg) transmission from infected parent chickens results in clinical disease in progeny chicks at 1–2 weeks post-hatching. Clinical signs include anemia and lymphoid depletion. Sub-clinical infections with horizontally acquired virus, probably spread by the fecal–oral route, occur when maternally derived antibody is no longer protective.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Gyrovirus*

*Chicken anemia virus*
Chicken anemia virus-Cuxhaven-1 [M55918] (CAV-Cuxhaven-1)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Gyrovirus* but have not been approved as species

None reported.

## List of other related viruses which may be members of the family *Circoviridae* but have not been approved as species

Cyclovirus-PK5006 [GQ404844] (CyCV1-PK5006)

Note: isolate representative of a group of highly divergent viral genomes identified in humans and chimpanzees.

## Phylogenetic relationships within the family *Circoviridae*

Comparison of the Rep proteins of all members of the family *Circoviridae* reveals that CAV has no close phylogenetic relationship to the species in the genus *Circovirus* (Figure 5). Similar observations are made when the complete viral genomes are compared.

## Similarity with other taxa

Analysis of the genome structure, the origin of replication and the Rep proteins of viruses of the genus *Circovirus* show similarity to members of the plant virus family *Nanoviridae* and less pronounced similarity to members of the plant virus family *Geminiviridae*.

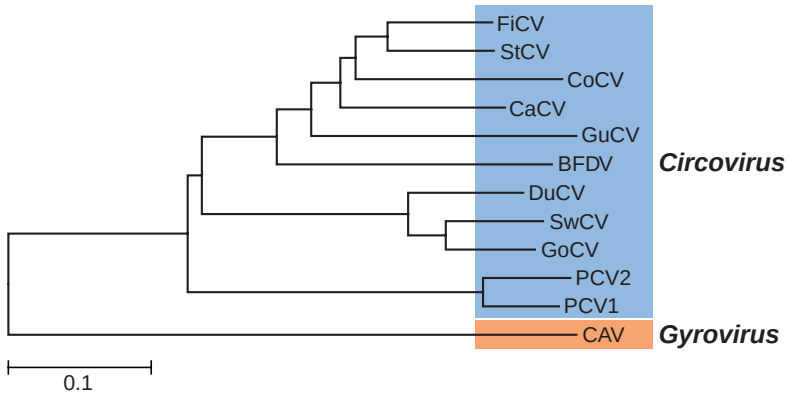


**Figure 5:** Family *Circoviridae*: neighbour-joining phylogenetic tree built with Rep amino acid sequences.



Gyroviruses have features in common with members of the familly *Anelloviridae*, since they possess circular ssDNA genomes with similar, but not identical, negative sense genome organizations. The non-coding regions of CAV and those of most of the anelloviruses genomes are G+C-rich. The CP of CAV and the putative CPs of anelloviruses possess amino acid sequence motifs that are characteristic of RCR Rep proteins. The proteins encoded by ORF2 in CAV and some anelloviruses contain amino acid sequences that are characteristic of protein tyrosine phosphatases (PTPase), including the motif $WX_7HX_3CXCX_5H$. Spliced transcripts have been detected for CAV and some anelloviruses, along with peptides able to induce apoptosis in human hepatocellular carcinoma cell lines.

## Derivation of names

*Circo*: from *cir*cular *co*nformation.
*Gyro*: from Latin *gyrus*, "ring" or "circuit".

## Further reading

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# FAMILY *GEMINIVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Geminiviridae*** |
| Genus | ***Mastrevirus*** |
| Genus | ***Curtovirus*** |
| Genus | ***Topocuvirus*** |
| Genus | ***Begomovirus*** |

## Virion properties

### MORPHOLOGY

Virions are typically twinned (so-called "geminate"). For maize streak virus (MSV) particles, cryo-electron microscopy has shown that virions are about 22 × 38 nm, consisting of two incomplete icosahedra (T = 1) containing a total of 110 coat protein subunits organized as 22 pentameric capsomers (Figure 1).

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virion $S_{20,w}$ is approximately 70S.

### NUCLEIC ACID

Twinned virions are presumed to contain a single copy of circular ssDNA, ranging in size from 2.5 to 3.0 kb. Hence, for viruses with bipartite genomes, two virions containing different genomic components will be required for infection. Half-size defective components and ssDNA satellites are also encapsidated, possibly in isometric virions.

### PROTEINS

Virions contain a single structural protein (CP; Mr about 28–34×$10^3$). No other proteins have been found associated with virions.

### LIPIDS

None reported.

### CARBOHYDRATES

None reported.

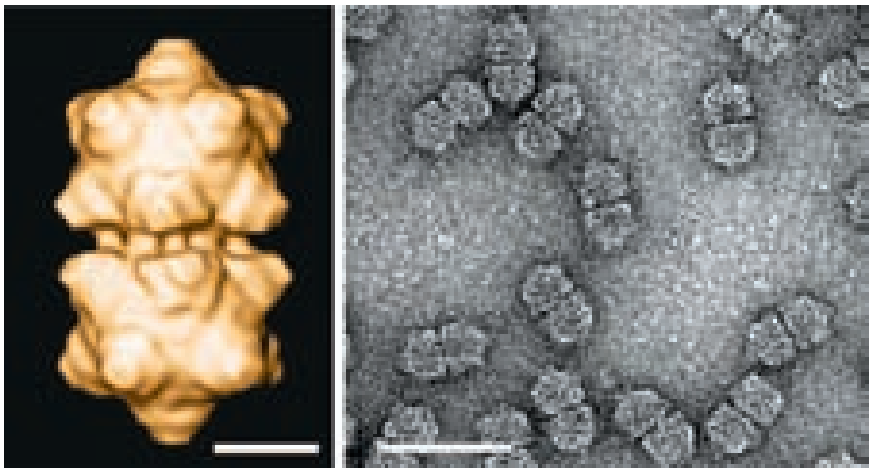

**Figure 1:** (Left) Cryo-electron microscopic reconstruction of maize streak virus (MSV) viewed along a two-fold axis of symmetry. The bar represents 10 nm. (Right) Purified particles of MSV stained with uranyl acetate showing typical twinned quasi-isometric subunits. The bar represents 50 nm. (From Zhang *et al.* (2001). *Virology*, **279**, 471–477 and courtesy of R. McKenna).

### Genome organization and replication

Viruses in the genera *Mastrevirus*, *Curtovirus* and *Topocuvirus* have a single genomic component, whereas those in the genus *Begomovirus* have either one or two components. Replication occurs through double stranded replicative intermediates by a rolling circle mechanism. Complementary-sense DNA synthesis on the virion-sense (encapsidated) strand to produce dsDNA depends solely on host factors. Virus ssDNA synthesis is initiated by cleavage of the virion-sense strand by the virus-encoded replication-associated protein (Rep) immediately downstream of the 3′ thymidine residue in an absolutely conserved TAATATT/AC sequence located in the loop of a potential stem-loop structure within the intergenic region. Geminiviruses do not encode a DNA polymerase, and consequently are heavily dependent on host factors that must be recruited during early stages of replication. In all cases, coding regions in both virion-sense and complementary-sense strands diverge from an intergenic region, and transcription is bi-directional, with independently controlled transcripts initiating within the intergenic region. Viruses in the genus *Mastrevirus* use transcript splicing for gene expression, those in other genera use multiple overlapping transcripts.

## Genus *Mastrevirus*

Type species ***Maize streak virus***

### Distinguishing features

Mastreviruses are monopartite, leafhopper-transmitted geminiviruses that are found only in the Eastern Hemisphere. They infect tropical and temperate cereals and vegetable crops. Many cereal-infecting mastreviruses also infect wild grasses, in which they are considered to be native. It is the only genus to employ two viral-encoded proteins for replication, RepA (C1 transcript) and Rep (C1:C2 transcripts). This is also the only group of geminiviruses thus far known to utilize transcript splicing, and therefore their sequences contain introns. The proteins translated from the spliced mRNAs encoded by C1 and C2 are involved in replication and encode the replication initiator protein. In addition, regulation of virion-sense gene expression in some grass-infecting mastreviruses occurs by differential splicing of two virion sense transcripts, V1 and V2. The working cut-off for species in this genus has been established at less than 75% shared nt identity, in contrast to the other three genera for which isolates sharing less than 89% nt identity are usually considered separate species. Recent analysis of additional mastrevirus sequences provides evidence that cut-off is greater than 75%, however, detailed analyses are in progress (unpublished data).

### Genome organization and replication

The mastrevirus genome consists of a single component of circular ssDNA, 2.6–2.8 kb in size. A small complementary-sense DNA containing 5′-ribonucleotides, annealed to the genomic DNA within the small intergenic region of Chloris striate mosaic virus (CSMV), Digitaria streak virus (DSV), MSV, tobacco yellow dwarf virus (TYDV) and wheat dwarf virus (WDV), may be involved in priming complementary-sense DNA synthesis. The small, annealed DNA is subsequently encapsidated with genomic ssDNA. The nucleotide sequences of infectious genomic clones of bean yellow dwarf virus (BeYDV), CSMV, DSV, MSV, Miscanthus streak virus (MiSV), Panicum streak virus (PanSV), sugarcane streak virus (SSV) sugarcane streak Egypt virus (SSEV), sugarcane streak Reunion virus (SSRV), TYDV and WDV have been determined. Their genomes encode four proteins (Figure 2). The two encoded on the virion-sense strand are the coat protein (CP, ORF V1) that encapsidates the virion-sense ssDNA and acts as a nuclear shuttle protein (NSP) for viral DNA, and the movement protein (MP, ORF V2), that functions in cell-to-cell movement. The CP appears to regulate the balance of ssDNA and dsDNA accumulation. Regulation of virion-sense gene expression in MSV and grass-infecting relatives occurs by differential transcript splicing. The complementary-sense strand encodes the replication-associated protein (Rep gene, ORFs C1/C2), expressed from ORFs C1 and C2 by transcript splicing. The Rep initiates rolling circle replication by introducing a nick into the conserved TAATATTAC sequence in the virion-sense strand. Rep binds to the large subunit of the replication factor C clamp loader complex, suggesting a role in the recruitment of host replication factors to the origin of replication. Rep-A protein (ORF C1) also encoded on the

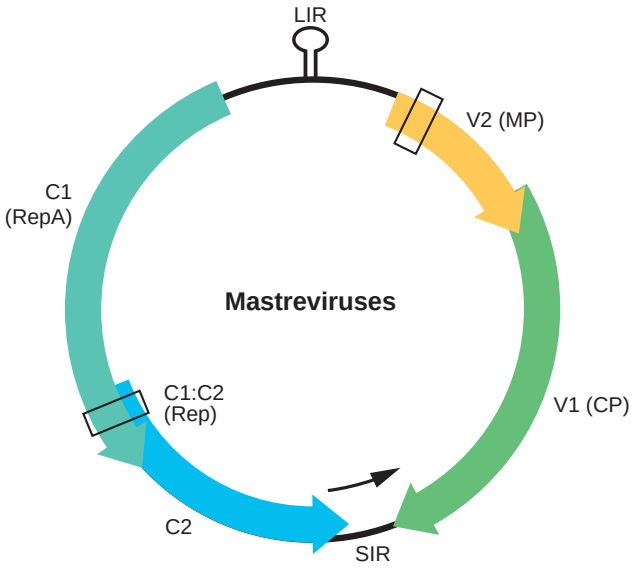


**Figure 2:** Typical genomic organization of mastreviruses. ORFs are denoted as either being encoded on the virion-sense (V) or complementary-sense (C) strand, and corresponding genes are indicated. The positions of the stem-loop motif containing the conserved TAATATTAC sequence in the large intergenic region (LIR) and the encapsidated complementary-sense primer-like molecule (small arrow) in the small intergenic region (SIR) are shown. Introns (open boxes) occur in ORF V2 and at the overlap between ORFs C1 and C2. MP; movement protein, CP; coat protein, REP; replication-associated protein.

complementary-sense strand, binds to the plant homologue of retinoblastoma protein (Rb) to regulate cell-cycle progression, altering the environment of terminally differentiated cells to provide host factors that support viral DNA replication. Both Rep and Rep-A bind to the origin of replication as multimeric proteins.

## Antigenic properties

Serological analyses indicate that grass-infecting geminiviruses from the same continent constitute distinct groupings: there is an African streak virus group (MSV, PanSV, SSV, SSEV and SSREV), an Australasian striate mosaic virus group (CSMV, Bromus striate mosic virus [BrSMV], DiSMV, Paspalum striate mosaic virus [PSMV]), and the very distinct Asian MiSV and European WDV. Although DSV originates from Vanuatu, it is most closely related to the African mastreviruses. Grass geminiviruses originating from different continents are either unrelated or distantly related. Mastreviruses that infect dicotyledonous plants (TYDV and BeYDV) are not antigenically related to those that infect monocotyledonous plants.

## Biological properties

### Host range

The host range of Mastreviruses is relatively narrow. With the exception of TYDV, BeYDV and the candidate member chickpea chlorotic dwarf virus (CpCDV), which infect certain *Solanaceae* and *Fabaceae*, among others, the host range of mastreviruses is limited to members of the *Poaceae* (*Gramineae*).

### Transmission

Mastreviruses are transmitted in nature by leafhoppers (suborder Homoptera / order Hemiptera, family Cicadellidae), in most cases by a single species. Transmission is persistent and circulative, and viruses are non-propagative. Mastreviruses are normally not transmissible by mechanical inoculation, although MSV has been transmitted experimentally using a vascular puncture technique using maize seeds. Most members are transmitted experimentally to plants by *Agrobacterium*-mediated transfer (agroinoculation) from partially or tandemly repeated cloned genomic DNA.

## Species demarcation criteria in the genus

The following criteria are used as a guideline to establish taxonomic status:

- Nucleotide sequence identity: full-length nucleotide sequence identity <75% has in the past been considered indicative of a distinct species. Recent analysis of additional mastreviruses sequences provides evidence for a cut-off closer to 89% (unpublished data). However, decisions based on nucleotide sequence comparisons, particularly when approaching the cut-off value, must take into account the biological properties of the virus.
- *Trans*-replication of genomic components: the inability of Rep protein to *trans*-replicate a genomic component suggests a distinct species.
- Coat protein characteristics: serological differences may be indicative of a distinct species.
- Different vector species.
- Natural host range and symptom phenotype: these characteristics may relate to a particular species but their commonest use will be to distinguish strains.

## List of species in the genus *Mastrevirus*

| | | |
|---|---|---|
| *Bean yellow dwarf virus* | | |
| Bean yellow dwarf virus - [Pakistan:Faisalabad 14:2006] | [AM849096] | (BeYDV-[PK:Fai14:06]) |
| *Chloris striate mosaic virus* | | |
| Chloris striate mosaic virus - [Australia] | [M20021] | (CSMV-[AU]) |
| *Digitaria streak virus* | | |
| Digitaria streak virus - [Vanuatu] | [M23022] | (DSV-[VU]) |
| *Eragrostis streak virus* | | |
| Eragrostis streak virus - [Zimbabwe:Guruwe 186:2007] | [EU244915] | (ESV-[ZM:Gur186:07]) |
| *Maize streak virus* | | |
| Maize streak virus - A [Kenya:Amagoro:1998] | [AF329878] | (MSV-A[KE:Ama:98]) |
| Maize streak virus - B [Kenya:Jamaica:1999] | [AF329887] | (MSV-B[KE:Jam:99]) |
| Maize streak virus - C1 [Burundi:Rob3:1990] | [EU628627] | (MSV-C1[BI:Rob3:90]) |
| Maize streak virus - D [JIC16:Paspalum:2002] | [EU628637] | (MSV-D[JIC16:Pas:02]) |
| Maize streak virus - E [Nigeria:g79:2007] | [EU628638] | (MSV-E[NG:g79:07]) |
| *Miscanthus streak virus* | | |
| Miscanthus streak virus - [Japan:1991] | [D01030] | (MiSV-[JP91]) |
| *Panicum streak virus* | | |
| Panicum streak virus - A [Kenya:1990] | [X60168] | (PanSV-A[KE:90]) |
| Panicum streak virus - B [Zimbabwe:Guruwe 169:Urochloa:2006] | [EU224264] | (PanSV-B1[ZM:Gur169:Uro:06]) |
| Panicum streak virus - C [South Africa:1989] | [L39638] | (PanSV-C[ZA:89]) |
| Panicum streak virus - D [Nigeria:Ifon 91:Urochloa:2007] | [EU224265] | (PanSV-D1[NG:Ifo91:Uro:07]) |
| Panicum streak virus - E [Kenya:Jic10:1997] | [GQ415391] | (PanSV-E[KE:Jic10:97]) |
| Panicum streak virus - F [Kenya:Nye2:g364:2008] | [GQ415392] | (PanSV-F[KE:Nye2:08]) |
| Panicum streak virus - G [Mayotte:Cac:g385:2008] | [GQ415395] | (PanSV-G[YT:Cac:g385:08]) |
| Panicum streak virus - H [Central African Republic:Bai2:Car11:Brachiaria:2008] | [GQ415397] | (PanSV-H[CF:Bai2:Car11:Bd:08]) |
| Panicum streak virus - I [Kenya:Nra1:g374:2008] | [GQ415399] | (PanSV-I[KE:Nra1:g374:08]) |
| *Setaria streak virus* | | |
| Setaria streak virus - A [South Africa:Setaria:1988] | [AF007881] | (SetSV-A[ZA:Set:88]) |
| Setaria streak virus - B [South Africa:g217:2007] | [EU628639] | (SetSV-B[ZA:g217:07]) |
| Setaria streak virus - C [Zimbabwe:MIC24K:Pennisetum:1987] | [EU628641] | (SetSV-C[ZM:MIC24K:Pen:87]) |
| Setaria streak virus - D [KSV-JIC2:2002] | [EU628642] | (SetSV-D[KSV-JIC2:02]) |
| Setaria streak virus - E [Uganda:Luwero 1:2007] | [EU628622] | (SetSV-E2[UG:Luw1:07]) |
| *Sugarcane streak Egypt virus* | | |
| Sugarcane streak Egypt virus - [Egypt:Aswan] | [AF039528] | (SSEV-[EG:Asw]) |
| *Sugarcane streak Reunion virus* | | |
| Sugarcane streak Reunion virus - A [Reunion:R574] | [AF072672] | (SSREV-A[RE:574]) |

| | | |
|---|---|---|
| Sugarcane streak Reunion virus - B [Zimbabwe:Nyangombe 177:Paspalum:2006] | [EU244916] | (SSREV-B[ZM:Nya177:Pas:06]) |
| *Sugarcane streak virus* | | |
| Sugarcane streak virus - [South Africa:Natal] | [M82918] | (SSV-[ZA:Nat]) |
| *Tobacco yellow dwarf virus* | | |
| Tobacco yellow dwarf virus - [Australia] | [M81103] | (TbYDV-[AU]) |
| *Urochloa streak virus* | | |
| Urochloa streak virus - [Nigeria:g226:2007] | [EU445697] | (UroSV-[NG:g226:07]) |
| *Wheat dwarf virus* | | |
| Wheat dwarf virus - [China:Gansu Gangu 01:05] | [EF536859] | (WDV-[CN:GsGg01:05]) |

Species names are in italic script; names of isolates are in roman script. Genome sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.



### List of other related viruses which may be members of the genus *Mastrevirus* but have not been approved as species

| | | |
|---|---|---|
| Barley dwarf virus | [AM989927] | (BDV) |
| Cenchrus streak virus | [EU244914] | (CSV) |
| Chickpea chlorotic dwarf Pakistan virus | [AM849097] | (CpCDPKV) |
| Chickpea chlorotic dwarf Sudan virus | [AM933134] | (CpCDSDV) |
| Eragrostis curvula streak virus | [FJ665629] | (ECSV) |
| Oat dwarf virus | [AM296025] | (ODV) |
| Saccharum streak virus | [GQ273988] | (SacSV) |

Full table available online on Science Direct®, www.sciencedirect.com.

## GENUS *CURTOVIRUS*

Type species ***Beet curly top virus***

### Distinguishing features

Curtoviruses are monopartite geminiviruses that are vectored by leafhoppers, often in a species-specific manner. Like the genus *Begomovirus*, curtoviruses are found in both the Eastern and Western Hemisphere. They infect a wide variety of vegetable crops and are thought to be widely prevalent in uncultivated, endemic eudicot species. The genomes most often have seven open-reading frames, but recently isolates have been discovered that encode only five or six ORFs. Curtovirus genomes have been associated with defective-interfering DNAs (DI particles) that function in some instances to reduce symptom severity. The species working cut-off in this genus has been established at less than 89% shared nt identity.

### Genome organization and replication

The genomes of curtoviruses consist of a single circular ssDNA component, 2.9–3.0 kb in size. The nucleotide sequences of infectious genomic clones of beet curly top virus (BCTV), beet mild curly top virus (BMCTV), beet severe curly top virus (BSCTV) and horseradish curly top virus (HrCTV) have been determined. Their genomes encode six to seven proteins (Figure 3). Three encoded on the virion-sense strand are the coat protein (CP, ORF V1), that encapsidates the virion-sense ssDNA and is involved in virus movement and insect vector transmission, V2 a movement protein (MP) and V3, involved in the regulation of the relative levels of ssDNA and dsDNA. The complementary-sense strand encodes the replication-associated protein (Rep, ORF C1), required for the initiation of viral DNA replication, C2 protein that acts as a pathogenicity factor in some hosts, a replication enhancer protein (REn, ORF C3) and C4 protein (ORF C4), an important symptom determinant that is implicated in cell-cycle control. Nucleotide sequence comparisons suggest that curtoviruses and begomoviruses diverged after a recombination event altered insect vector specificity.

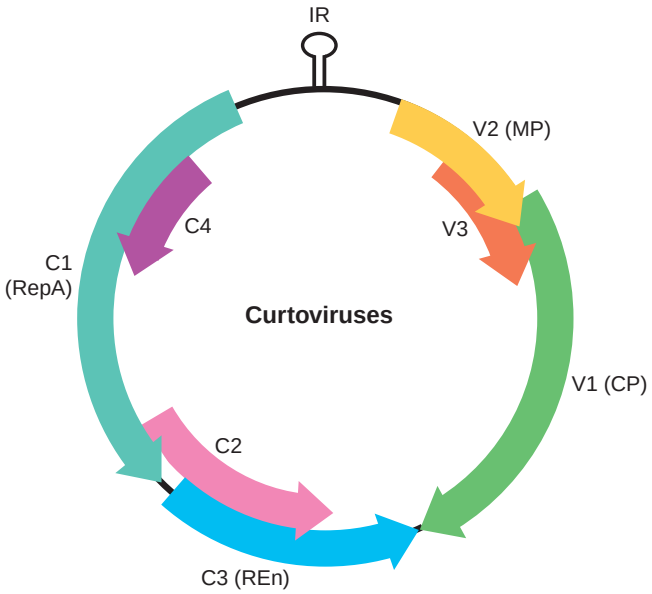


**Figure 3:** Typical genomic organization of curtoviruses. ORFs are denoted as either being encoded on the virion-sense (V) or complementary-sense (C) strand. Gene designations are shown where these are known. ORF C3 is not present in horseradish curly top virus (HrCTV). The position of the stem-loop containing the conserved TAATATTAC sequence located in the intergenic region (IR) is shown. CP; coat protein, MP; movement protein, Rep; replication-associated protein, REn; replication enhancer.

## Antigenic properties

Serological tests show BCTV, tomato leaf roll virus (TLRV) and tomato pseudo-curly top virus (TPCTV, genus *Topocuvirus*) to be relatively closely related. Distant relationships between curtoviruses and begomoviruses have been shown in serological tests.

## Biological properties

### Host range

The type member BCTV has a very wide host range within dicotyledonous plants, including over 300 species in 44 plant families.

### Transmission

Curtoviruses are transmitted in nature by leafhoppers (suborder Homoptera / order Hemiptera, family Cicadellidae) in a persistent (circulative, non-propagative) manner. BCTV may be transmitted with difficulty by mechanical inoculation. Most members are transmitted experimentally to plants by *Agrobacterium*-mediated transfer (agroinoculation) from partially or tandemly repeated cloned genomic DNA.

## Species demarcation criteria in the genus

The following criteria should be used as a guideline to establish taxonomic status:

- Nucleotide sequence identity: full-length nucleotide sequence identity <89% is generally indicative of a distinct species; however, decisions based on nucleotide sequence comparisons, particularly when approaching this value, must take into account the biological properties of the virus.
- *Trans*-replication of genomic components: the inability of Rep protein to *trans*-replicate a genomic component suggests a distinct species.

- Coat protein characteristics: serological differences may be indicative of a distinct species although the coat protein is highly conserved, suggesting that this criterion may be of limited use.
- Natural host range and symptom phenotype: these characteristics may relate to a particular species but their most common use will be to distinguish strains, when the information is available.

### List of species in the genus *Curtovirus*

| | | |
|---|---|---|
| *Beet curly top Iran virus* | | |
| Beet curly top Iran virus - [Iran:Kerman:2007] | [EU273818] | (BCTIRV-[IR:Ker:07]) |
| *Beet curly top virus* | | |
| Beet curly top virus - A [US:California:1985] | [M24597] | (BCTV-A[US:Cal:85]) |
| *Beet mild curly top virus* | | |
| Beet mild curly top virus - [Mexico:2006] | [EU193175] | (BMCTV-[MX:06]) |
| *Beet severe curly top virus* | | |
| Beet severe curly top virus - [Iran:1986] | [X97203] | (BSCTV-[IR:86]) |
| *Horseradish curly top virus* | | |
| Horseradish curly top virus - [US:Salinas:1988] | [U49907] | (HrCTV-[US:Sal:88]) |
| *Pepper curly top virus* | | |
| Pepper curly top virus - [US:New Mexico:2005] | [EF501977] | (PepCTV-[US:NM:05]) |
| *Spinach curly top virus* | | |
| Spinach curly top virus - [US:Spinach 3:1996] | [AY548948] | (SpCTV-[US:Sp3:96]) |

Species names are in italic script; names of isolates are in roman script. Genome sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Curtovirus* but have not been approved as species

| | | |
|---|---|---|
| Pepper yellow dwarf virus | [EU921828] | (PepYDV) |

## GENUS *TOPOCUVIRUS*

Type species ***Tomato pseudo-curly top virus***

### Distinguishing features

*Tomato pseudo-curly top virus* is the only member of the genus. The monopartite virus encodes six ORFs that are organized similarly to monopartite begomoviral genomes. It is the only geminivirus to be transmitted by the treehopper *Micrutalis malleifera*, a specificity conferred by unique amino acids in the coat protein. It infects only eudicots (dicotyledonous plants), and has been found only in the southeastern United States.

### Genome organization and replication

The genome of tomato pseudo-curly top virus (TPCTV) consists of a single component of circular ssDNA, 2.8 kb in size. The genome, encoding six proteins, resembles that of monopartite members of the genus *Begomovirus* (Figure 4). Nucleotide sequence comparisons suggest that TPCTV and begomoviruses diverged after a recombination event altered insect vector specificity. The coat protein is more closely related to those of the leafhopper-transmitted curtoviruses than the whitefly-transmitted begomoviruses. The V2 protein is distantly related to the curtovirus V2 protein.

### Antigenic properties

Serological tests show TPCTV to be relatively closely related to BCTV and TLRV in the genus *Curtovirus*.

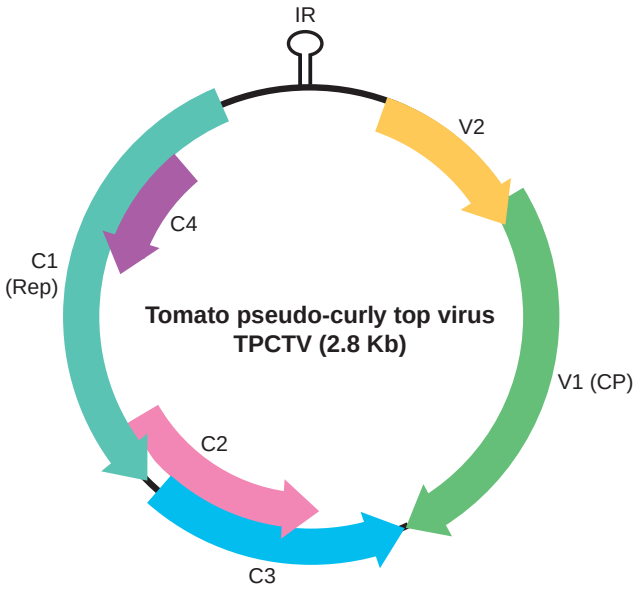


**Figure 4:** Genomic organization of TPCTV. ORFs are denoted as either being encoded on the virion-sense (V) or complementary-sense (C) strand. Gene designations are shown where these are known. The position of the stem-loop containing the conserved TAATATTAC sequence, located within the intergenic region, is shown. CP, coat protein; Rep, replication-associated protein.

## Biological properties

### Host range

The host range of TPCTV is restricted to dicotyledonous plants, and includes weed species such as nightshade (*Solanum nigrum*), *Datura stramonium* and common chickweed (*Stellaria media*), crops such as tomato (*Lycopersicon esculentum*) and bean (*Phaseolus vulgaris*) and the experimental host *Nicotiana benthamiana*. TPCTV induces symptoms resembling those associated with BCTV infection in many hosts.

### Transmission

TPCTV is transmitted in nature by the treehopper *Micrutalis malleifera* Fowler (suborder Hemiptera / order Homoptera, family Membracidae), and has been transmitted experimentally to plants by *Agrobacterium*-mediated transfer (agroinoculation) from a tandemly repeated cloned genomic DNA.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Topocuvirus*

Tomato pseudo-curly top virus
Tomato pseudo-curly top virus - [US:Florida:1994] [X84735] (TPCTV-[US:FL:94])

Species names are in italic script; names of isolates are in roman script. Genome sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Topocuvirus* but have not been approved as species

None reported.

# Genus *Begomovirus*

Type species ***Bean golden yellow mosaic virus***

## Distinguishing features

Begomoviruses are monopartite or bipartite, whitefly-transmitted geminiviruses that are found in the Eastern (both genome types) and Western Hemisphere (only bipartite are thought to be endemic). The whitefly vector *Bemisia tabaci* (Genn.) is considered to be a sibling species group owing to the considerable genetic and behavioral variation between different haplotypes. Co-adaptation has been shown to influence transmission efficieny for co-evolved *B. tabaci*-begomoviral complexes, though at least one introduced haplotype from the Eastern Hemisphere has been shown to readily transmit viruses endemic to the Western Hemisphere and so this relationship may range from relaxed to strict. Nonetheless, whitefly vector specificity is associated with specific amino acids that reside on the viral coat protein. Bipartite begomoviruses are extant in both hemispheres, but monopartite genomes appear to have evolved only in the Eastern Hemisphere. Some monopartite begomoviral genomes have one or more types of satellite molecules that contain no viral sequences except non-coding intergenic region sequences (probably of viral origin) that are required for replication. Their contributions to the begomoviral infection cycle are not entirely clear but studies thus far point to modulation of virulence, mainly owing to suppression of host gene silencing (innate defense system), and therefore they appear to rescue viral species from extinction that can no longer outcompete the host defense system using viral encoded suppressors of host gene silencing. All types of begomoviruses infect only eudicot hosts (dicots) that are primarily endemic to tropical, subtropical and mild temperate climes. Bipartite begomoviruses encode 6–8 ORFs, and monopartite begomoviruses encode six ORFs. The working cut-off for species in this genus has been established at less than 89% shared nt identity, as for curtoviruses.

## Genome organization and replication

The genomes of bipartite begomoviruses consist of two components, referred to as DNA A and DNA B, each 2.5–2.6 kb in size. The DNA A component of the bipartite begomoviruses can replicate autonomously and produce virions but requires the DNA B component for nuclear localization (BV1) and for systemic infection (BC1). DNA A and DNA B components share approximately 200 bp of sequence within the intergenic region, encompassing the conserved stem-loop and TAATATTAC sequence that is termed the common region (Figure 5). Some begomoviruses have a monopartite genome, and this type is found only the Old World. The organization of the ORFs resembles that seen in the bipartite viral DNA-A component (for example Ageratum yellow vein virus (AYVV), tomato yellow leaf curl virus (TYLCV) and tomato leaf curl virus (ToLCV)).

The nucleotide sequences of full-length genomic clones of more than 100 distinct species have been established. The DNA A virion-sense strand encodes the coat protein (CP, ORF AV1/V1) that encapsidates the virion-sense ssDNA and may be involved in virus movement, and ORF AV2/V2, which has also been implicated in virus movement. The New World bipartite viruses lack an AV2 ORF. The DNA A complementary-sense strand encodes the replication-associated protein (Rep, ORF AC1/C1), a transcriptional activator protein (TrAP, ORF AC2/C2), a replication enhancer protein (REn, ORF AC3/C3) and C4 protein (ORF AC4/C4). Rep initiates viral DNA replication by binding to reiterated motifs (iterons) within the intergenic region and introducing a nick into the conserved TAATATT/AC sequence. Rep also binds to the plant homologue of retinoblastoma protein (Rb) to regulate cell-cycle progression, altering the environment of terminally differentiated cells to provide host factors that support viral DNA replication. TrAP transactivates expression of virion-sense gene expression from both DNA A and DNA B, and also functions in the suppression of post-transcriptional gene silencing. REn is required for efficient viral DNA replication. C4 protein is an important symptom determinant implicated in cell-cycle control, and AC4 protein may counter a host response to Rep expression. DNA B encodes a nuclear shuttle protein (NSP, ORF BV1) on the virion-sense strand and a movement protein (MP, ORF BC1) on the complementary-sense strand.

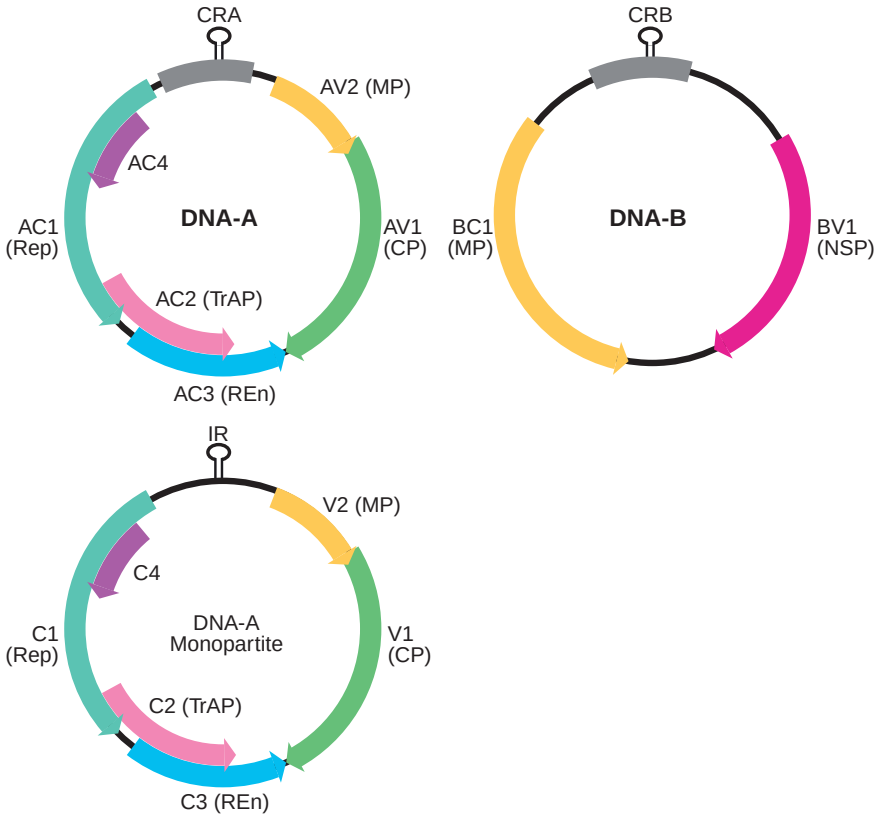


**Figure 5:** Genomic organization of begomoviruses. ORFs are denoted as either being encoded on the virion-sense (V) or complementary-sense (C) strand, preceded by component designation (A or B) if bipartite (top). The "common region" (CRA and CRB; top), representing largely intergenic sequences that are shared between the two genomic components of bipartite viruses are shown as grey boxes. The position of the stem-loop containing the conserved TAATATTAC sequence, located within the intergenic regions (CRA and CRB, top; IR, bottom) is shown. CP, coat protein; Rep, replication-associated protein; TrAP, transcriptional activator protein; REn, replication enhancer; MP, movement protein; NSP, nuclear shuttle protein.

## Satellites

Small circular ssDNA satellites, approximately 1.3 kb in size (smaller, unit length molecules also are found), are associated with some (but not all) Eastern Hemisphere monopartite begomoviruses. One type of satellite, termed betasatellite (DNA β), contains a single coding region termed C1 (ORF βC1) whose gene product has been shown to function as a suppressor of host gene silencing. Additional functions may be found. In any case this can have the effect of making the begomovirus capable of systemically infecting the plant when it was not capable of doing so in the absence of the betasatellite, and/or of increasing virus virulence for begomoviral genomes already capable of some degree of systemic infection. The only recognizable begomoviral sequence in the betasatellite is the stem-loop and TAATATTAC sequences. The remainder of the satellite sequence is otherwise unrelated to the so-called "helper" begomovirus. The betasatellites depend on the associated begomoviruses for their replication and encapsidation, and are considered essential for maintenance of the disease in the field. They are thought to be promiscuous in that they may associate and function with more than a single helper begomovirus. Autonomously replicating nanovirus-like (master Rep gene) components of unknown biological function are frequently associated with the begomovirus/DNA β complexes, and are referred to as alphasatellites. At least three types of alphasatellites are known. One type has recently been shown to ameliorate symptoms when co-infecting plants with the helper virus and its associated betasatellite, suggesting that it "down-regulates virulence" to some degree, possibly by reducing the accumulation of betasatellite. The taxonomic classification of alpha- and betasatellites is in progress.

## Antigenic properties

Serological tests show all begomoviruses to be relatively closely related. The use of monoclonal antibodies has shown that begomoviruses may be grouped geographically based on shared epitopes.

## Biological properties

### Host range

Collectively, begomoviruses infect a wide range of dicotyledonous plants although, individually, most have limited host ranges.

### Transmission

Transmitted in nature by the whitefly *Bemisia tabaci* (Genn.) sibling species group. Some begomoviruses are known to be differentially adapted for efficient transmission by their local *B. tabaci* biotype but for the most part this proposed co-evolution is poorly studied. Some begomoviruses are transmissible by mechanical inoculation, although many require either *Agrobacterium*-mediated transfer (agroinoculation) from partially or tandemly repeated cloned genomic DNA or biolistic delivery of cloned genomic DNA for their experimental transmission.



## Species demarcation criteria in the genus

The following criteria should be used as a guideline to establish taxonomic status:

- Number of genomic components: presence or absence of a DNA B component.
- Organization of the genome: presence or absence of ORF AV2.
- Nucleotide sequence identity: because of the growing number of recognized species, derivation of the complete nucleotide sequence will be necessary to distinguish species. Nucleotide sequence identity <89% is generally indicative of a distinct species. However, decisions based on nucleotide sequence comparisons, particularly when approaching this value, must take into account the biological properties of the virus. The taxonomic status of a recombinant will depend on relatedness to the parental viruses, the frequency and extent of recombination events, and its biological properties compared with the parental viruses. Information concerning the diversity of related recombinants may be helpful to determine status.
- *Trans*-replication of genomic components: the inability of Rep protein to *trans*-replicate a genomic component suggests a distinct species. However, when considering this criterion, it should be kept in mind that small changes in the Rep binding site of otherwise identical viruses might prevent functional interaction and recombination involving a small part of the genome may confer replication competence on a distinct species.
- Production of viable pseudorecombinants: account should be taken of the fitness of the pseudorecombinant in the natural host(s) of the parental viruses. It should be ensured that pseudorecombinant viability is not the result of inter-component recombination.
- Coat protein characteristics: amino acid sequence identity <90% and substantial serological differences may be indicative of a distinct species in the first instance, but derivation of the complete sequence will be necessary to confirm taxonomic status.
- Natural host range and symptom phenotype. These characteristics may relate to a particular species but their most common use will be to distinguish strains, when such information is available.

## List of species in the genus *Begomovirus*

| | DNA A | DNA B | |
|---|---|---|---|
| *Abutilon mosaic virus* | | | |
| Abutilon mosaic virus - [Germany] | [X15983] | [X15984] | (AbMV-[DE]) |
| *African cassava mosaic virus* | | | |
| African cassava mosaic virus - [Cameroon:1998] | [AF112352] | [AF112353] | (ACMV-[CM:98]) |
| *Ageratum enation virus* | | | |
| Ageratum enation virus - India [India:Kangra:2008] | [FN543099] | | (AEV-IN[IN:Kan:08]) |
| *Ageratum leaf curl virus* | | | |
| Ageratum leaf curl virus - [China:Guangxi 52:2003] | [AJ851005] | | (ALCuV-[CN:Gx52:03]) |

| | | | |
|---|---|---|---|
| *Ageratum yellow vein Hualian virus* | | | |
| Ageratum yellow vein Hualian virus - Hsinchu [Taiwan:Hsinchu:Tom:2003] | [DQ866124] | | (AYVHuV-Hsi[TW:Hsi:Tom:03]) |
| *Ageratum yellow vein Sri Lanka virus* | | | |
| Ageratum yellow vein Sri Lanka virus - [Sri Lanka:1999] | [AF314144] | | (AYVSLV-[LK:99]) |
| *Ageratum yellow vein virus* | | | |
| Ageratum yellow vein virus - Guangxi [China:Guangxi 129:2005] | [AM940137] | | (AYVV-Gx[CN:Gx129:05]) |
| *Alternanthera yellow vein virus* | | | |
| Alternanthera yellow vein virus - A [China:Fujian 22:2006] | [EF544604] | | (AlYVV-A[CN:Fuj22:06]) |
| *Bean calico mosaic virus* | | | |
| Bean calico mosaic virus - [Mexico:Sonora:1986] | [AF110189] | [AF110190] | (BCaMV-[MX:Son:86]) |
| *Bean dwarf mosaic virus* | | | |
| Bean dwarf mosaic virus - [Colombia:1987] | [M88179] | [M88180] | (BDMV-[CO:87]) |
| *Bean golden mosaic virus* | | | |
| Bean golden mosaic virus - [Brazil:Campinas 1:1978] | [M88686] | [M88687] | (BGMV-[BR:Cam1:78]) |
| *Bean golden yellow mosaic virus* | | | |
| Bean golden yellow mosaic virus - [Dominican Republic:1987] | [L01635] | [L01636] | (BGYMV-[DO:87]) |
| *Bhendi yellow vein mosaic virus* | | | |
| Bhendi yellow vein mosaic virus - India [India:Madurai] | [AF241479] | | (BYVMV-IN[IN:Mad]) |
| *Bitter gourd yellow vein virus* | | | |
| Bitter gourd yellow vein virus - [Pakistan:Lahore:2004] | [AM491590] | [AM709505] | (BGYVV-[PK:Lah:04]) |
| *Boerhavia yellow spot virus* | | | |
| Boerhavia yellow spot virus - [Mexico:Yucatan:2005] | [EF121755] | | (BoYSV-[MX:Yuc:05]) |
| *Cabbage leaf curl Jamaica virus* | | | |
| Cabbage leaf curl Jamaica virus - [Jamaica:CUc3:2005] | [DQ178608] | [DQ178609] | (CabLCJV-[JM:CUc3:05]) |
| *Cabbage leaf curl virus* | | | |
| Cabbage leaf curl virus - [United States:Florida:1996] | [U65529] | [U65530] | (CabLCV-[US:Flo:96]) |
| *Chayote yellow mosaic virus* | | | |
| Chayote yellow mosaic virus - [Nigeria:Ibadan] | [AJ223191] | | (ChaYMV-[NG:Iba]) |
| *Chilli leaf curl virus* | | | |
| (*Pepper leaf curl Pakistan virus**) | | | |
| Chilli leaf curl virus - India [India:Amritsar:Papaya:2009] | [GU136803] | | (ChiLCV-IN[IN:Amr:Pap:09]) |
| *Chino del tomate virus* | | | |
| Chino del tomate virus - Tomato [Mexico:Sinaloa IC:1983] | [AF101476] | [AF101478] | (CdTV-To[MX:SinIC:83]) |
| *Clerodendron golden mosaic virus* | | | |
| Clerodendron golden mosaic virus - [Vietnam:Sonla:2005] | [DQ641692] | [DQ641693] | (ClGMV-[VN:Son:05]) |
| *Corchorus golden mosaic virus* | | | |
| Corchorus golden mosaic virus - [India:Bahraich:2008] | [FJ463902] | [FJ463901] | (CoGMV-[IN:Bah:08]) |
| *Corchorus yellow spot virus* | | | |
| Corchorus yellow spot virus - [Mexico:Yucatan:2005] | [DQ875868] | [DQ875869] | (CoYSV-[MX:Yuc:05]) |
| *Corchorus yellow vein Vietnam virus* | | | |
| (Corchorus yellow vein virus**) | | | |
| Corchorus yellaow vein Vietnam virus - [Vietnam:Hoa Binh:2000] | [AY727903] | [AY727904] | (CoYVV-[VN:Hoa:00]) |
| *Cotton leaf crumple virus* | | | |
| Cotton leaf crumple virus - Arizona [Mexico:Sonora:1991] | [AF480940] | [AF480941] | (CLCrV-AZ[MX:Son:91]) |
| *Cotton leaf curl Alabad virus* | | | |
| Cotton leaf curl Alabad virus - [Pakistan:Alabad 804a:1996] | [AJ002452] | | (CLCuAlV-[PK:Ala804a:96]) |
| *Cotton leaf curl Bangalore virus* | | | |
| Cotton leaf curl Bangalore virus - [India:Bangalore:2004] | [AY705380] | | (CLCuBaV-[IN:Ban:04]) |

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| *Cotton leaf curl Gezira virus* | | | |
| Cotton leaf curl Gezira virus - Burkina Faso [Burkina Faso:Bazega:Okra:2009] | [FN554540] | | (CLCuGeV-BF[BF:Baz:Ok:09]) |
| *Cotton leaf curl Kokhran virus* | | | |
| Cotton leaf curl Kokhran virus - Faisalabad [Pakistan:Faisalabad 1] | [AJ496286] | | (CLCuKoV-Fai[PK:Fai1]) |
| *Cotton leaf curl Multan virus* | | | |
| Cotton leaf curl Multan virus - Darwinii [Pakistan:Multan:Darwinii 1:2006] | [EU365613] | | (CLCuMuV-Dar[PK:Mul:Dar1:06]) |
| *Cowpea golden mosaic virus* | | | |
| Cowpea golden mosaic virus - [Nigeria:Nsukka:1990] | [AF029217] | | (CPGMV-[NG:Nsu:90]) |
| *Croton yellow vein mosaic virus* | | | |
| Croton yellow vein mosaic virus - [India:Lucknow:2007] | [EU727086] | | (CYVMV-[IN:Luc:07]) |
| *Cucurbit leaf curl virus* (Cucurbit leaf crumple virus**) | | | |
| Cucurbit leaf crumple virus - [United States:Arizona:1991] | [AF256200] | [AF327559] | (CuLCrV-[US:Ari:91]) |
| *Desmodium leaf distortion virus* | | | |
| Desmodium leaf distortion virus - [Mexico:Yucatan:2005] | [DQ875870] | [DQ875871] | (DesLDV-[MX:Yuc:05]) |
| *Dicliptera yellow mottle Cuba virus* | | | |
| Dicliptera yellow mottle Cuba virus - [Cuba] | [AJ549960] | | (DiYMoCUV-[CU]) |
| *Dicliptera yellow mottle virus* | | | |
| Dicliptera yellow mottle virus - [United States:Florida:1998] | [AF139168] | [AF170101] | (DiYMoV-[US:Flo:98]) |
| *Dolichos yellow mosaic virus* | | | |
| Dolichos yellow mosaic virus - [Bangladesh:Gazipur] | [AY271891] | | (DoYMV-[BD:Gaz]) |
| *East African cassava mosaic Cameroon virus* | | | |
| East African cassava mosaic Cameroon virus - Cameroon [Cameroon:1998] | [AF112354] | [AF112355] | (EACMCV-CM[CM:98]) |
| *East African cassava mosaic Kenya virus* | | | |
| East African cassava mosaic Kenya virus - [Kenya:Kathiana:K300:2002] | [AJ717580] | [AJ704965] | (EACMKV-[KE:Kat:K300:02]) |
| *East African cassava mosaic Malawi virus* | | | |
| East African cassava mosaic Malawi virus - [Malawi:K:1996] | [AJ006460] | | (EACMMV-[MW:K:96]) |
| *East African cassava mosaic virus* | | | |
| East African cassava mosaic virus - Kenya [Kenya:Boa:K48:2001] | [AJ717542] | [AJ704949] | (EACMV-KE[KE:Boa:K48:01]) |
| *East African cassava mosaic Zanzibar virus* | | | |
| East African cassava mosaic Zanzibar virus - [Kenya:Felunzi:K19:2001] | [AJ717562] | [AJ704942] | (EACMZV-[KE:Fel:K19:01]) |
| *Erectites yellow mosaic virus* | | | |
| Erectites yellow mosaic virus - [Vietnam:Hoabinh:2005] | [DQ641698] | | (ErYMV-[VN:Hoa:05]) |
| *Eupatorium yellow vein mosaic virus* | | | |
| Eupatorium yellow vein mosaic virus - [Japan:SOJ3:2000] | [AJ438937] | | (EpYVMV-[JR:SOJ3:00]) |
| *Eupatorium yellow vein virus* | | | |
| Eupatorium yellow vein virus - A [Japan:Kumamoto] | [AB007990] | | (EpYVV-A[JR:Kum]) |
| *Euphorbia leaf curl Guangxi virus* | | | |
| Euphorbia leaf curl Guangxi virus - A [China:Guangxi 35-1:2002] | [AM411424] | | (EuLCGxV-A[CN:Gx35-1:02]) |
| *Euphorbia leaf curl virus* | | | |
| Euphorbia leaf curl virus - [China:Fujian:2006] | [FJ487911] | | (EuLCuV-[CN:Fuj:06]) |
| *Euphorbia mosaic virus* | | | |
| Euphorbia mosaic virus - [Cuba:Tobacco:2008] | [FJ807782] | [FJ807783] | (EuMV-[CU:Tb:08]) |
| *Hollyhock leaf crumple virus* | | | |
| Hollyhock leaf crumple virus - [Egypt:Cairo:1997] | [AY036009] | | (HoLCrV-[EG:Cai:97]) |
| *Honeysuckle yellow vein Kagoshima virus* | | | |
| Honeysuckle yellow vein Kagoshima virus - A [Japan:Kagoshima 5:Tobacco] | [AB178949] | | (HYVKgV-A[JR:Kag5:Tob]) |

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| *Honeysuckle yellow vein mosaic virus* | | | |
| Honeysuckle yellow vein mosaic virus - A [Japan:Fukuoka 1] | [AB178945] | | (HYVMV-A[JR:Fuk1]) |
| *Honeysuckle yellow vein virus* | | | |
| Honeysuckle yellow vein virus - Japan [Japan: Sapporo 1:2000] | [AB182261] | | (HYVV-JR[JR:SP1:00]) |
| *Horsegram yellow mosaic virus* | | | |
| Horsegram yellow mosaic virus - [India:Bangalore:2004] | [AM932427] | [AM932428] | (HgYMV-[IN:Ban:04]) |
| *Indian cassava mosaic virus* | | | |
| Indian cassava mosaic virus - India [India:Maharashstra 2:1988] | [AY730035] | [AY730036] | (ICMV-IN[IN:Mah2:88]) |
| *Ipomoea yellow vein virus* | | | |
| Ipomoea yellow vein virus - [Spain:1998] | [AJ132548] | | (IYVV-[ES:98]) |
| *Kudzu mosaic virus* | | | |
| Kudzu mosaic virus - [China:Fujian:2008] | [FJ539014] | [FJ539015] | (KuMV-[CN;Fuj;08]) |
| *Lindernia anagallis yellow vein virus* | | | |
| Lindernia anagallis yellow vein virus - [China:Hainan:2004] | [AY795900] | | (LaYVV-[CN:Hn:04]) |
| *Ludwigia yellow vein Vietnam virus* | | | |
| Ludwigia yellow vein Vietnam virus - [Vietnam:Hochiminh:2005] | [DQ641699] | | (LuYVVNV-[VN:Hoc:05]) |
| *Ludwigia yellow vein virus* | | | |
| Ludwigia yellow vein virus - [China:Guangxi 37:2003] | [AJ965539] | | (LuYVV-[CN:Gx37:03]) |
| *Luffa yellow mosaic virus* | | | |
| Luffa yellow mosaic virus - [Vietnam] | [AF509739] | [AF509740] | (LYMV-[VN]) |
| *Macroptilium mosaic Puerto Rico virus* | | | |
| Macroptilium mosaic Puerto Rico virus - [Puerto Rico:1990] | [AY044133] | [AY044134] | (MacMPRV-[PR:90]) |
| *Macroptilium yellow mosaic Florida virus* | | | |
| Macroptilium yellow mosaic Florida virus - [United States:Florida:1985] | [AY044135] | [AY044136] | (MacYMFV-[US:Flo:85]) |
| *Macroptilium yellow mosaic virus* | | | |
| Macroptilium yellow mosaic virus - Cuba [Cuba] | [AJ344452] | | (MacYMV-CU[CU]) |
| *Malvastrum leaf curl Guangdong virus* | | | |
| Malvastrum leaf curl Guangdong virus - [China:Guangdong 6:2004] | [AM236779] | | (MaLCGdV-[CN:Gd6:04]) |
| *Malvastrum leaf curl virus* | | | |
| Malvastrum leaf curl virus - [China:Guangxi 100 :Papaya:2005] | [AM260699] | | (MaLCuV-[CN:Gx100:Pap:05]) |
| *Malvastrum yellow leaf curl virus* | | | |
| Malvastrum yellow leaf curl virus - [China:Yunnan 193:2003] | [AJ971524] | | (MaYLCV-[CN:Yn193:03]) |
| *Malvastrum yellow mosaic virus* | | | |
| Malvastrum yellow mosaic virus - [China:Hainan 36:2004] | [AM236755] | | (MaYMV-[CN:Hn36:04]) |
| *Malvastrum yellow vein virus* | | | |
| Malvastrum yellow vein virus - [China:Yunnan 206:Ageratum:2003] | [AJ744881] | | (MaYVV-[CN:Yn206:Age:03]) |
| *Malvastrum yellow vein Yunnan virus* | | | |
| Malvastrum yellow vein Yunnan virus - [China:Yunnan 160:2003] | [AJ786711] | | (MaYVYnV-[CN:Yn160:03]) |
| *Melon chlorotic leaf curl virus* (*Squash yellow mild mottle virus**) | | | |
| Melon chlorotic leaf curl virus - Costa Rica [Costa Rica:Guanacaste:1998] | [AY064391] | [AF440790] | (MCLCuV-CR[CR:Gua:98]) |
| *Mesta yellow vein mosaic virus* | | | |
| Mesta yellow vein mosaic virus - Andhra Pradesh [India:Amadalavalasa 27:2008] | [FJ159269] | | (MeYVMV-And[IN:Ama27:08]) |
| *Mimosa yellow leaf curl virus* | | | |
| Mimosa yellow leaf curl virus - [Vietnam:Binhduong:2005] | [DQ641695] | | (MiYLCV-[VN:Bin:05]) |

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| *Mungbean yellow mosaic India virus* | | | |
| Mungbean yellow mosaic India virus - [India: Sriganganagar:Mungbean 1:1996] | [AF416742] | [AF416741] | (MYMIV-[IN:Sri:Mg1:96]) |
| *Mungbean yellow mosaic virus* | | | |
| Mungbean yellow mosaic virus - [India:Madurai:Soybean] | [AJ421642] | [AJ867554] | (MYMV-[IN:Mad:Sb]) |
| *Okra yellow crinkle virus* | | | |
| Okra Yellow crinkle virus - [Cameroon:Muea:2008] | [FM210275] | | (OYCrV-[CM:Mue:08]) |
| *Okra yellow mosaic Mexico virus* | | | |
| Okra yellow mosaic Mexico virus - [Mexico:Mazatepec 3:2004] | [DQ022611] | | (OYMMV-[MX:Maz3:04]) |
| *Okra yellow mottle Iguala virus* | | | |
| Okra yellow mottle Iguala virus - [Mexico:Iguala] | [AY751753] | | (OYMoIgV-[MX:Igu]) |
| *Okra yellow vein mosaic virus* | | | |
| Okra yellow vein mosaic virus - Pakistan [India:Chandigarh 177:2007] | [FJ179371] | | (OYVMV-PK[IN:Cha177:07]) |
| *Papaya leaf curl China virus* | | | |
| Papaya leaf curl China virus - Ageratum [China:Guangxi 10:Ageratum:2002] | [AJ558125] | | (PaLCuCNV-Age[CN:Gx10:02]) |
| *Papaya leaf curl Guandong virus* | | | |
| Papaya leaf curl Guangdong virus - [China:Fujian:Euphorbia:2006] | [FJ495184] | | (PaLCuGdV-[CN:Fj:Eu:06]) |
| *Papaya leaf curl virus* | | | |
| Papaya leaf curl virus - India [India:Lucknow] | [Y15934] | | (PaLCuV-IN[IN:Luc]) |
| *Pedilenthus leaf curl virus* | | | |
| (*Tomato leaf curl Pakistan virus**) | | | |
| Pedilenthus leaf curl virus - Pedilenthus [Pakistan:Multan:2006] | [AM712436] | | (PeLCuV-Pe[PK:Mul:06]) |
| *Pepper golden mosaic virus* | | | |
| Pepper golden mosaic virus - Costa Rica [Costa Rica] | [AF149227] | | (PepGMV-CR[CR]) |
| *Pepper huasteco yellow vein virus* | | | |
| Pepper huasteco yellow vein virus - [Mexico:Guanajuato 1; 2008] | [GU128150] | [GU128146] | (PHYVV-[MX:Gua1:08]) |
| *Pepper leaf curl Bangladesh virus* | | | |
| Pepper leaf curl Bangladesh virus - Bangladesh [Bangladesh:Bogra:1999] | [AF314531] | | (PepLCBV-BD[BD:Bog:99]) |
| *Pepper leaf curl Lahore virus* | | | |
| Pepper leaf curl Lahore virus - [Pakistan:Lahore:2004] | [AM404179] | | (PepLCLaV-[PK:Lah:04]) |
| *Pepper leaf curl virus* | | | |
| Pepper leaf curl virus - Malaysia [Malaysia:Klang:1997] | [AF414287] | | (PepLCV-MY[MY:Kla:97]) |
| *Pepper yellow leaf curl Indonesia virus* | | | |
| Pepper yellow leaf curl Indonesia virus - [Indonesia:2005] | [AB267834] | [AB267835] | (PepYLCIV-[ID:05]) |
| *Pepper yellow vein Mali virus* | | | |
| Pepper yellow vein Mali virus - [Burkina Faso:Banfora:hot pepper1:2009] | [FN555172] | | (PepYVMLV-[BF:Ban:Hpe1:09]) |
| *Potato yellow mosaic Panama virus* | | | |
| Potato yellow mosaic Panama virus - [Panama:Divisa:Tomato:1996] | [Y15034] | [Y15033] | (PYMPV-[PA:Div:Tom:96]) |
| *Potato yellow mosaic virus* | | | |
| Potato yellow mosaic virus - Potato [Venezuela:1991] | [D00940] | [D00941] | (PYMV-Po[VE:91]) |
| *Pumpkin yellow mosaic virus* | | | |
| Pumpkin yellow mosaic virus - [Malaysia:Negeri Sambilan:2001] | [EF197941] | | (PuYMV-[MY:Neg:01]) |
| *Radish leaf curl virus* | | | |
| Radish leaf curl virus - [India:Varanasi:2005] | [EF175733] | | (RaLCuV-[IN:Var:03]) |
| *Rhynchosia golden mosaic Sinaloa virus* | | | |
| Rhynchosia golden mosaic Sinaloa virus - [Mexico:Sinaloa:2005] | [DQ406672] | [DQ406673] | (RhGMSiV-[MX:Sin:05]) |
| *Rhynchosia golden mosaic virus* | | | |
| Rhynchosia golden mosaic virus - Mexico [Mexico:Sinaloa:2005] | [DQ347950] | [DQ356429] | (RhGMV-MX[MX:Sin:05]) |

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| *Senecio yellow mosaic virus* | | | |
| Senecio yellow mosaic virus - [China:Guangxi 46:2003] | [AJ876550] | | (SeYMV-[CN:Gx46:03]) |
| *Sida golden mosaic Costa Rica virus* | | | |
| Sida golden mosaic Costa Rica virus - [Costa Rica] | [X99550] | [X99551] | (SiGMCRV-[CR]) |
| *Sida golden mosaic Florida virus* | | | |
| Sida golden mosaic Florida virus – [Cuba:Havana:Malvastrum:111:2009] | [HM003779] | [HM003778] | (SiGMFlV-[CU:Hav:Mal:111:09]) |
| *Sida golden mosaic Honduras virus* | | | |
| Sida golden mosaic Honduras virus - [Honduras] | [Y11097] | [Y11098] | (SiGMHV-[HN]) |
| *Sida golden mosaic virus* | | | |
| Sida golden mosaic virus - [United States:Florida] | [AF049336] | [AF039841] | (SiGMV-[US:Flo]) |
| *Sida golden yellow vein virus* | | | |
| Sida golden yellow vein virus - [Cuba:Havana] | [AJ577395] | | (SiGYVV-[CU:Hav]) |
| *Sida leaf curl virus* | | | |
| Sida leaf curl virus - [China:Hainan 57:2004] | [AM050730] | | (SiLCuV-[CN:Hn57:04]) |
| *Sida micrantha mosaic virus* | | | |
| Sida micrantha mosaic virus - [Brazil:A2B2] | [AJ557451] | [AJ557453] | (SiMMV-[BR:A2B2]) |
| *Sida mottle virus* | | | |
| Sida mottle virus - Micrantha [Brazil:A1B3] | [AJ557450] | [AJ557454] | (SiMoV-Mic[BR:A1B3]) |
| *Sida yellow mosaic China virus* | | | |
| Sida yellow mosaic China virus - [China:Hainan 7:Ageratum:2003] | [AM048837] | | (SiYMCNV-[CN:Hn7:Age:03]) |
| *Sida yellow mosaic virus* | | | |
| Sida yellow mosaic virus - [Brazil:Vicosa 2:1999] | [AY090558] | | (SiYMV-[BR:Vic2:99]) |
| *Sida yellow mosaic Yucatan virus* | | | |
| Sida yellow mosaic Yucatan virus - [Mexico:Yucatan:2005] | [DQ875872] | [DQ875873] | (SiYMYuV-[MX:Yuc:05]) |
| *Sida yellow vein Madurai virus* | | | |
| Sida yellow vein Madurai virus - [India:Madurai:2005] | [AM259382] | | (SiYVMaV-[IN:Mad:05]) |
| *Sida yellow vein Vietnam virus* | | | |
| Sida yellow vein Vietnam virus - [Vietnam:Hanoi:2005] | [DQ641696] | | (SiYVVV-[VN:Han:05]) |
| *Sida yellow vein virus* | | | |
| Sida yellow vein virus - [Honduras] | [Y11099] | [Y11100] | (SiYVV-[HN]) |
| *Siegesbeckia yellow vein Guangxi virus* | | | |
| Siegesbeckia yellow vein Guangxi virus - [China:Guangxi 111:2005] | [AM238692] | | (SgYVGxV-[CN:Gx111:05]) |
| *Siegesbeckia yellow vein virus* | | | |
| Siegesbeckia yellow vein virus - [China: Guangdong 13:2004] | [AM183224] | | (SgYVV-[CN:Gd13:04]) |
| *South African cassava mosaic virus* | | | |
| South African cassava mosaic virus - [Madagascar:12] | [AJ422132] | | (SACMV-[MG:12]) |
| *Soybean blistering mosaic virus* | | | |
| Soybean blistering mosaic virus - [Argentina:NOA:2005] | [EF016486] | | (SbBMV-[AR:NOA:05]) |
| *Soybean crinkle leaf virus* | | | |
| Soybean crinkle leaf virus - [Japan] | [AB050781] | | (SbCrLV-[JR]) |
| *Spilanthes yellow vein virus* | | | |
| Spilanthes yellow vein virus - [Vietnam:Dalat:2005] | [DQ641694] | | (SpYVV-[VN:Dal:05]) |
| *Squash leaf curl China virus* | | | |
| Squash leaf curl China virus - China [China:Guangxi25:2005] | [AM260206] | [AM260208] | (SLCCNV-CN[CN:Gx25:05]) |
| *Squash leaf curl Philippines virus* | | | |
| Squash leaf curl Philippines virus - [Philippines:Batangas P133:Pumpkin:2007] | [EU487041] | | (SLCuPV-[PH:BatP133:Pum:07]) |
| *Squash leaf curl virus* | | | |
| Squash leaf curl virus - [Jordan:Malva:2006] | [EF532620] | [EF532621] | (SLCuV-[JD:Mal:06]) |
| *Squash leaf curl Yunnan virus* | | | |
| Squash leaf curl Yunnan virus - [China:Yunnan 23:2000] | [AJ420319] | | (SLCuYnV-[CN:Y23:00]) |

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| *Squash mild leaf curl virus* | | | |
| Squash mild leaf curl virus - [United States: Imperial Valley:1979] | [AF421552] | [AF421553] | (SMLCuV-[US:IV:79]) |
| *Sri Lankan cassava mosaic virus* | | | |
| Sri Lankan cassava mosaic virus - India [India:Adivaram:2003] | [AJ579307] | [AJ579308] | (SLCMV-IN[IN:Adi:03]) |
| *Stachytarpheta leaf curl virus* | | | |
| Stachytarpheta leaf curl virus - [China:Hainan 30:2004] | [AJ810156] | | (StaLCuV-[CN:Hn30:04]) |
| *Sweet potato leaf curl Canary virus* | | | |
| Sweet potato leaf curl Canary virus - [Spain: Canary Islands:BG21:2002] | [EU856365] | | (SPLCCaV-[ES:CI:BG21:02]) |
| *Sweet potato leaf curl China virus* | | | |
| Sweet potato leaf curl China virus - [China:2005] | [DQ512731] | | (SPLCCNV-[CN::05]) |
| *Sweet potato leaf curl Georgia virus* | | | |
| Sweet potato leaf curl Georgia virus - [United States:Georgia 16] | [AF326775] | | (SPLCGoV-[US:Geo16]) |
| *Sweet potato leaf curl Lanzarote virus* | | | |
| Sweet potato leaf curl Lanzarote virus - [Spain:Canary Islands:BG27:2002] | [EF456746] | | (SPLCLaV-[ES:CI:BG27:02]) |
| *Sweet potato leaf curl Spain virus* | | | |
| Sweet potato leaf curl Spain virus - [Spain:Canary Islands:BG1:2002] | [EF456741] | | (SPLCESV-[ES:CI:BG1:02]) |
| *Sweet potato leaf curl virus* | | | |
| Sweet potato leaf curl virus - China [China:Yunnan:RL31:2006] | [EU253456] | | (SPLCV-CN[CN:Yn:RL31:07]) |
| *Tobacco curly shoot virus* | | | |
| Tobacco curly shoot virus - [China:Alternanthera:2008] | [GU199583] | | (TbCSV-[CN:Alt:08]) |
| *Tobacco leaf curl Cuba virus* | | | |
| Tobacco leaf curl Cuba virus - [Cuba:Taguasco:2005] | [AM050143] | | (TbLCuCV-[CU:Tag:05]) |
| *Tobacco leaf curl Japan virus* | | | |
| Tobacco leaf curl Japan virus - Ibaraki [Japan:Ibaraki:Honeysucke:2006] | [AB287439] | | (TbLCJV-Iba[JR:Iba:06]) |
| *Tobacco leaf curl Yunnan virus* | | | |
| Tobacco leaf curl Yunnan virus - China [China:Yunnan 136:2002] | [AJ512761] | | (TbLCYnV-CN[CN:Yn136:02]) |
| *Tobacco leaf curl Zimbabwe virus* | | | |
| Tobacco leaf curl Zimbabwe virus - [Comoros:Foumboudziouni:2005] | [AM701756] | | (TbLCZV-[KM:Fou:05]) |
| *Tomato chino La Paz virus* | | | |
| Tomato chino La Paz virus - A [Mexico:Baja La Paz:2002] | [AY339618] | | (ToChLPV-A[MX:BLP:02) |
| *Tomato chlorotic mottle virus* | | | |
| Tomato chlorotic mottle virus - Bahia [Brazil:Seabra 1:1996] | [AF490004] | [AF491306] | (ToCMoV-BA[BR:Sea1:96]) |
| *Tomato curly stunt virus* | | | |
| Tomato curly stunt virus - [South Africa:Onderberg:1998] | [AF261885] | | (ToCSV-[ZA:Ond:98]) |
| *Tomato golden mosaic virus* | | | |
| Tomato golden mosaic virus - [Brazil:Common:1984] | [K02029] | [K02030] | (TGMV-[BR:Com:84]) |
| *Tomato golden mottle virus* | | | |
| Tomato golden mottle virus - [Mexico:San Luiz Potosi:2005] | [DQ520943] | [DQ406674] | (ToGMoV-[MX:SLP:05]) |
| *Tomato leaf curl Arusha virus* | | | |
| Tomato leaf curl Arusha virus - [Tanzania:Kilimandjaro:2005] | [EF194760] | | (ToLCArV-[TZ:Kil:05]) |
| *Tomato leaf curl Bangalore virus* | | | |
| Tomato leaf curl Bangalore virus - A [India:Bangalore 1] | [Z48182] | | (ToLCBaV-A[IN:Ban1]) |
| *Tomato leaf curl Bangladesh virus* | | | |
| Tomato leaf curl Bangladesh virus - [Bangladesh:2] | [AF188481] | | (ToLCBV-[BD:2]) |

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| *Tomato leaf curl China virus* | | | |
| Tomato leaf curl China virus - Baise [China:Guangxi 32:2002] | [AJ558118] | | (ToLCCNV-BS[CN:Gx32:02]) |
| *Tomato leaf curl Comoros virus* | | | |
| Tomato leaf curl Comoros virus - Comoros [Comoros:Bambas:2004] | [AM701759] | | (ToLCKMV-KM[KM:Bam:04]) |
| *Tomato leaf curl Guangdong virus* | | | |
| Tomato leaf curl Guangdong virus - [China:Guangzhou 2:2003] | [AY602165] | | (ToLCGdV-[CN:Gz2:03]) |
| *Tomato leaf curl Guangxi virus* | | | |
| Tomato leaf curl Guangxi virus - [China:Guangxi 1:2003] | [AM236784] | | (ToLCGxV-[CN:Gx1:03]) |
| *Tomato leaf curl Gujarat virus* | | | |
| Tomato leaf curl Gujarat virus - [India:Varanasi:2001] | [AY190290] | [AY190291] | (ToLCGuV-[IN:Var:01]) |
| *Tomato leaf curl Hsinchu virus* | | | |
| Tomato leaf curl Hsinchu virus - [China:Hainan:Ramie:2007] | [EU596959] | [EU596960] | (ToLCHsV-[CN:Hn:Ram:07]) |
| *Tomato leaf curl Java virus* (*Tomato leaf curl Indonesia virus**) | | | |
| Tomato leaf curl Java virus - A [Indonesia] | [AB100304] | | (ToLCJaV-A[ID]) |
| *Tomato leaf curl Joydebpur virus* | | | |
| Tomato leaf curl Joydebpur virus - Bangladesh [Bangladesh] | [AJ875159] | | (ToLCJoV-BD[BD] ) |
| *Tomato leaf curl Karnataka virus* | | | |
| Tomato leaf curl Karnataka virus - Bangalore [India:Bangalore:1993] | [U38239] | | (ToLCKaV-Ban[IN:Ban:93]) |
| *Tomato leaf curl Kerala virus* | | | |
| Tomato leaf curl Kerala virus - [India:Kerala 3:2007] | [EU910141] | | (ToLCKeV-[IN:Ker3:07]) |
| *Tomato leaf curl Laos virus* | | | |
| Tomato leaf curl Laos virus - [Laos] | [AF195782] | | (ToLCLV-[LA]) |
| *Tomato leaf curl Madagascar virus* | | | |
| Tomato leaf curl Madagascar virus - Atsimo [Madagascar:Toliary:2001] | [AJ865339] | | (ToLCMGV-Ats[MG:Tol:01]) |
| *Tomato leaf curl Malaysia virus* | | | |
| Tomato leaf curl Malaysia virus - Malaysia [Malaysia:Klang:1997] | [AF327436] | | (ToLCMYV-MY[MY:Kla:97]) |
| *Tomato leaf curl Mali virus* | | | |
| Tomato leaf curl Mali virus - [Mali] | [AY502936] | | (ToLCMLV-[ML]) |
| *Tomato leaf curl Mayotte virus* | | | |
| Tomato leaf curl Mayotte virus - [Mayotte: Kahani:2003] | [AJ865340] | | (ToLCYTV-[YT:Kah:03]) |
| *Tomato leaf curl New Delhi virus* | | | |
| Tomato leaf curl New Delhi virus - [Bangladesh:Jessore: Severe:2005] | [AJ875157] | [AJ875158] | (ToLCNDV-[BG:Jes:Svr :05]) |
| *Tomato leaf curl Philippines virus* | | | |
| Tomato leaf curl Philippines virus - A [Philippines:Laguna:2006] | [AB377113] | | (ToLCPV-A[PH:Lag:06]) |
| *Tomato leaf curl Pune virus* | | | |
| Tomato leaf curl Pune virus - [India:Pune:2005] | [AY754814] | | (ToLCPuV-[IN:Pun:05]) |
| *Tomato leaf curl Seychelles virus* | | | |
| Tomato leaf curl Seychelles virus - [Seychelles:Val d'Endor:2004] | [AM491778] | | (ToLCSCV-[SC:VE:04]) |
| *Tomato leaf curl Sinaloa virus* | | | |
| Tomato leaf curl Sinaloa virus - [Nicaragua:Santa Lucia] | [AJ608286] | [AJ508783] | (ToLCSiV-[NI:SL]) |
| *Tomato leaf curl Sri Lanka virus* | | | |
| Tomato leaf curl Sri Lanka virus - [Sri Lanka:Bandarawela:1997] | [AF274349] | | (ToLCLKV-[LK:Ban:97]) |
| *Tomato leaf curl Sudan virus* | | | |
| Tomato leaf curl Sudan virus - Gezira [Sudan:Gezira:1996] | [AY044137] | | (ToLCSDV-Gez[SD:Gez:96]) |
| *Tomato leaf curl Taiwan virus* | | | |
| Tomato leaf curl Taiwan virus - A [China:Hong Kong T1:2007] | [EU624503] | | (ToLCTV-A[CN:HKT1:07]) |

| | | | |
|---|---|---|---|
| *Tomato leaf curl Uganda virus* | | | |
| Tomato leaf curl Uganda virus - [Uganda:Iganga:2005] | [DQ127170] | | (ToLCUV-[UG:Iga:05]) |
| *Tomato leaf curl Vietnam virus* | | | |
| Tomato leaf curl Vietnam virus - [Vietnam:Dan Xa 2:2007] | [EU189149] | | (ToLCVV-[VN:DX1:07]) |
| *Tomato leaf curl virus* | | | |
| Tomato leaf curl virus - Solanum [Australia:Solanum:D1] | [AF084006] | | (ToLCV-Sol[AU:Sol:D1]) |
| *Tomato mild yellow leaf curl Aragua virus* | | | |
| Tomato mild yellow leaf curl Aragua virus - [Venezuela:10:2003] | [AY927277] | [EF547938] | (ToMYLCV-[VE:10:03]) |
| *Tomato mosaic Havana virus* | | | |
| Tomato mosaic Havana virus - [Cuba:Quivican] | [Y14874] | [Y14875] | (ToMHaV-[CU:Qui]) |
| *Tomato mosaic leaf curl virus* | | | |
| (Merremia mosaic virus**) | | | |
| Meraremia mosaic virus - Puerto Rico [Puerto Rico:] | [AF068636] | [AY965899] | (MerMV-PR[PR:]) |
| *Tomato mottle Taino virus* | | | |
| Tomato mottle Taino virus - [Cuba] | [AF012300] | [AF012301] | (ToMoTaV-[CU]) |
| *Tomato mottle virus* | | | |
| Tomato mottle virus - [PR:2004] | [AY965900] | [AY965901] | (ToMoV-[PR:04]) |
| *Tomato rugose mosaic virus* | | | |
| Tomato rugose mosaic virus - [Brazil:Uberlandia 1:1996] | [AF291705] | [AF291706] | (ToRMV-[BR:Ube1:96]) |
| *Tomato severe leaf curl virus* | | | |
| Tomato severe leaf curl virus - Guatemala [Guatemala:Sansirisay:1996] | [AF130415] | | (ToSLCV-GT[GT:San:96]) |
| *Tomato severe rugose virus* | | | |
| Tomato severe rugose virus - [Brazil:Petrolina de Goias 1:Capsicum:2003] | [DQ207749] | | (ToSRV-[BR:PG1:Cap:03]) |
| *Tomato yellow leaf curl Axarquia virus* | | | |
| Tomato yellow leaf curl Axarquia virus - Sicily1 [Italy:Sicily2/2:2007] | [EU734831] | | (TYLCAxV-Sic1[IT:Sic2/2:07]) |
| *Tomato yellow leaf curl China virus* | | | |
| Tomato yellow leaf curl China virus - Baoshan1 [China:Yunnan 10:Tobacco:2000] | [AJ319675] | | (TYLCCNV-Bao1[CN:Yn10:Tob :00]) |
| *Tomato yellow leaf curl Guangdong virus* | | | |
| Tomato yellow leaf curl Guangdong virus - [China:Guangdong 32:2007] | [GQ169042] | | (TYLCGdV-[CN:Gd32:07]) |
| *Tomato yellow leaf curl Indonesia virus* | | | |
| Tomato yellow leaf curl Indonesia virus - [Indonesia:Lembang:2005] | [AF189018] | | (TYLCIDV-[ID:Lem:05]) |
| *Tomato yellow leaf curl Kanchanaburi virus* | | | |
| Tomato yellow leaf curl Kanchanaburi virus - [Thailand:Kanchanaburi 1:2001] | [AF511529] | [AF511528] | (TYLCKaV-[TH:Kan1:01]) |
| *Tomato yellow leaf curl Malaga virus* | | | |
| Tomato yellow leaf curl Malaga virus - [Spain:421:1999] | [AF271234] | | (TYLCMaV-[ES:421:99]) |
| *Tomato yellow leaf curl Mali virus* | | | |
| Tomato yellow leaf curl Mali virus - Ethiopia [Ethiopia:Melkassa:2005] | [DQ358913] | | (TYLCMLV-ET[ET:Mel:05]) |
| *Tomato yellow leaf curl Sardinia virus* | | | |
| Tomato yellow leaf curl Sardinia virus - Sardinia [Italy:Sardinia:1988] | [X61153] | | (TYLCSV-Sar[IT:Sar:88]) |
| *Tomato yellow leaf curl Thailand virus* | | | |
| Tomato yellow leaf curl Thailand virus - A [Thailand:1] | [X63015] | [X63016] | (TYLCTHV-A[TH:1]) |
| *Tomato yellow leaf curl Vietnam virus* | | | |
| Tomato yellow leaf curl Vietnam virus - [Vietnam:Hanoi:2005] | [DQ641697] | | (TYLCVV-[VN:Han:05]) |
| *Tomato yellow leaf curl virus* | | | |
| Tomato yellow leaf curl virus - Gezira [Iran:Bandar Abbas:2007] | [EU085423] | | (TYLCV-Gez[IR:BA:07]) |

| | | | |
|---|---|---|---|
| *Tomato yellow margin leaf curl virus* | | | |
| Tomato yellow margin leaf curl virus - [Venezuela:Merida 57] | [AY508993] | [AY508994] | (TYMLCV-[VE:Mer57]) |
| *Tomato yellow spot virus* | | | |
| Tomato yellow spot virus - [Brazil:Bicas 2:1999] | [DQ336350] | [DQ336351] | (ToYSV-[BR:Bic2:99]) |
| *Tomato yellow vein streak virus* | | | |
| Tomato yellow vein streak virus - Potato[Brazil:Potato:1983] | [EF417915] | [EF417916] | (ToYVSV-Po[BR:Pot:83]) |
| *Vernonia yellow vein virus* | | | |
| Vernonia yellow vein virus - [India:Madurai:2005] | [AM182232] | | (VeYVV-[IN:Mad:05]) |
| *Watermelon chlorotic stunt virus* | | | |
| Watermelon chlorotic stunt virus - [Iran:1997] | [AJ245652] | [AJ245653] | (WmCSV-[IR:97]) |

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers are provided for the complete A and (where appropriate) B components. Assigned abbreviations are also listed.
*Species created in error. The species *Pepper leaf curl Pakistan virus*, *Squash yellow mild mottle virus*, *Tomato leaf curl Indonesia virus* and *Tomato leaf curl Pakistan virus* appear in earlier publications and lists of the ICTV. Analysis shows that these are clearly synonyms of the species under which they are listed and proposals to remove them as recognized species will be made at the earliest opportunity.
**Preferred name for species. Proposals to change the names of *Corchorus yellow vein Vietnam virus*, *Cucurbit leaf curl virus* and *Tomato mosaic leaf curl virus* to those of the synonyms listed will be made at the earliest opportunity.
Full table available online on Science Direct ®, www.sciencedirect.com.

## List of other related viruses which may be members of the genus *Begomovirus* but have not been approved as species

The following table lists viruses for which complete genome (or DNA A) sequences are available and which from sequence comparisons appear to be candidates for further species in the genus *Begomovirus*.

| | DNA A | DNA B | |
|---|---|---|---|
| Ageratum yellow vein China virus | [AJ564744] | | (AYVCNV) |
| Allamanda leaf curl virus | [EF602306] | | (AllLCV) |
| Bean leaf curl Madagascar virus | [AM701757] | | (BLCMGV) |
| Bhendi yellow vein Bhubhaneswar virus | [FJ589571] | | (BYVBhV) |
| Bhendi yellow vein Delhi virus | [FJ515747] | | (BYVDeV) |
| Bhendi yellow vein Haryana virus | [FJ561298] | | (BYVHaV) |
| Bhendi yellow vein Maharashtra virus | [EU482411] | | (BYVMaV) |
| Bhendi yellow vein virus | [GU181356] | | (BYVV) |
| Blainvillea yellow spot virus | [EU710756] | [EU710757] | (BlYSV) |
| Cherry tomato leaf curl virus | [DQ629102] | | (CtoLCV) |
| Chilli leaf curl Pakistan virus | [DQ116879] | | (ChiLCPKV) |
| Clerodendron golden mosaic China virus | [FJ011668] | [FJ011669] | (ClGMCNV) |
| Clerodendron golden mosaic Jiangsu virus | [FN396966] | | (ClGMJgV) |
| Clerodendron yellow mosaic virus | [EF408037] | | (ClYMV) |
| Cotton leaf curl Burewala virus | [AM421522] | | (CLCuBuV) |
| Cotton leaf curl Rajasthan virus | [AY795606] | | (CLCuRaV) |
| Crassocephalum yellow vein virus | [EF165536] | | (CraYVV) |
| Cucumber leaf curl virus | [EF450316] | | (CuLCuV) |
| Emilia yellow vein virus | [EU377539] | | (EmYVV) |
| Euphorbia mosaic Peru virus | [AM886131] | | (EuMPV) |
| Euphorbia yellow mosaic virus | [FJ619507] | [FJ619508] | (EuYMV) |
| Gossypium punctatum mild leaf curl virus | [EU384575] | [EU384578] | (GPMLCuV) |
| Hollyhock leaf curl virus | [AJ542539] | | (HoLCuV) |
| Ipomoea yellow vein Malaga virus | [EU839576] | | (IYVMaV) |
| Jatropha leaf curl virus | [EU798996] | | (JLCuV) |
| Jatropha yellow mosaic virus | [FJ177030] | | (JYMV) |
| Kenaf leaf curl virus | [EU366903] | | (KLCuV) |
| Macroptilium golden mosaic virus | [EU158096] | [EU158097] | (MacGMV) |
| Malvastrum leaf curl Fujian virus | [FJ712189] | | (MaLCFuV) |
| Malvastrum yellow mosaic Helshire virus | [FJ600483] | | (MaYMHeV) |
| Malvastrum yellow mosaic Jamaica virus | [FJ601917] | [FJ600485] | (MaYMJV) |
| Malvastrum yellow vein Baoshan virus | [FN386459] | | (MaYVBaV) |

| | | | |
|---|---|---|---|
| Malvastrum yellow vein Honghe virus | [FN552749] | | (MaYVHoV) |
| Merremia leaf curl virus | [DQ644561] | | (MerLCuV) |
| Mesta yellow vein mosaic Bahraich virus | [EU360303] | | (MeYVMBaV) |
| Okra leaf curl virus | [FM164726] | | (OLCuV) |
| Okra mottle virus | [EU914817] | [EU914818] | (OMoV) |
| Papaya leaf curl New Delhi virus | [DQ989325] | | (PaLCuNDV) |
| Passionfruit severe leaf distortion virus | [FJ972767] | [FJ972768] | (PSLDV) |
| Pepper leaf curl Yunnan virus | [EU585781] | | (PepLCYnV) |
| Potato yellow mosaic Trinidad virus | [AF039031] | [AF039032] | PYMTTV |
| Rhynchosia golden mosaic Yucatan virus | [EU021216] | [FJ792608] | (RhGMYuV) |
| Rhynchosia yellow mosaic virus | [AM999981] | [AM999982] | (RhYMV) |
| Sida common mosaic virus | [EU710751] | | (SiCMV) |
| Sida mosaic Sinaloa virus | [DQ520944] | [DQ356428] | (SiMSiV) |
| Sida yellow leaf curl virus | [EU710750] | | (SiYLCV) |
| Sun hemp leaf distortion virus | [FJ455449] | | (SHLDV) |
| Sweet potato leaf curl Bengal virus | [FN432356] | | (SPLCBeV) |
| Sweet potato leaf curl Italy virus | [AJ586885] | | (SPLCITV) |
| Sweet potato leaf curl Japan virus | [AB433787] | | (SPLCJV) |
| Sweet potato leaf curl Shangai virus | [EU309693] | | (SPLCShV) |
| Tobacco curly shoot India virus | [EU194914] | | (TbCSIV) |
| Tobacco leaf curl Comoros virus | [AM701762] | | (TbLCKMV) |
| Tobacco leaf curl Thailand virus | [DQ871221] | | (TbLCTHV) |
| Tobacco leaf rugose virus | [AJ488768] | | (TbLRV) |
| Tobacco mottle leaf curl virus | [FM160943] | | (TbMoLCV) |
| Tobacco yellow crinkle virus | [FJ213931] | | (TbYCV) |
| Tomato common mosaic virus | [EU710754] | [EU710755] | (ToCMV) |
| Tomato leaf curl Antsiranana virus | [AM701766] | | (ToLCAnV) |
| Tomato leaf curl Cameroon virus | [FM210277] | | (ToLCCMV) |
| Tomato leaf curl Cebu virus | [EU295549] | | (ToLCCeV) |
| Tomato leaf curl Cotabato virus | [EU487047] | | (ToLCCoV) |
| Tomato leaf curl Diana virus | [AM701765] | | (ToLCDiV) |
| Tomato leaf curl Ghana virus | [EU350585] | | (ToLCGV) |
| Tomato leaf curl Hainan virus | [FN256261] | | (ToLCHaV) |
| Tomato leaf curl Ilocos virus | [EU295551] | | (ToLCIlV) |
| Tomato leaf curl Laguna virus | [AB307731] | | (ToLCLaV) |
| Tomato leaf curl Mindanao virus | [EU487046] | | (ToLCMiV) |
| Tomato leaf curl Mohely virus | [AM701763] | | (ToLCMoV) |
| Tomato leaf curl Namakely virus | [AM701761] | | (ToLCNaV) |
| Tomato leaf curl Nigeria virus | [FJ685621] | | (ToLCNGV) |
| Tomato leaf curl Ouani virus | [AM701758] | | (ToLCOuV) |
| Tomato leaf curl Palampur virus | [AM884015] | [AM992534] | (ToLCPalV) |
| Tomato leaf curl Patna virus | [EU862323] | | (ToLCPaV) |
| Tomato leaf curl Rajasthan virus | [DQ339117] | | (ToLCRaV) |
| Tomato leaf curl Sulawesi virus | [FJ237614] | | (ToLCSuV) |
| Tomato leaf curl Togo virus | [EU847739] | | (ToLCTOV) |
| Tomato leaf curl Toliara virus | [AM701768] | | (ToLCToV) |
| Tomato leaf distortion virus | [EU710749] | | (ToLDV) |
| Tomato mild mosaic virus | [EU710752] | [EU710753] | (ToMMV) |
| Tomato yellow distortion leaf virus | [FJ174698] | [FJ999999] | (ToYDLV) |
| Tomato yellow leaf curl Chuxiong virus | [AJ457985] | | (TYLCChuV) |
| Tomato yellow leaf curl Dan Xa virus | [EU189150] | | (TYLCDXV) |
| Tomato yellow leaf curl Iran virus | [AJ132711] | | (TYLCIRV) |
| Velvet bean severe mosaic virus | [FN543425] | [FN543426] | (VBSMV) |
| Wissadula golden mosaic virus | [GQ355488] | [GQ355487] | (WGMV) |

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The following table lists begomoviruses for which the data are less complete. More information is needed to determine whether they are candidates for new species.

| | DNA A | DNA B | |
|---|---|---|---|
| Tomato crinkle yellow leaf virus | | [AY090556] | (ToCYLV) |
| Tomato mild leaf curl virus | | [DQ336352] | (ToMLCV) |

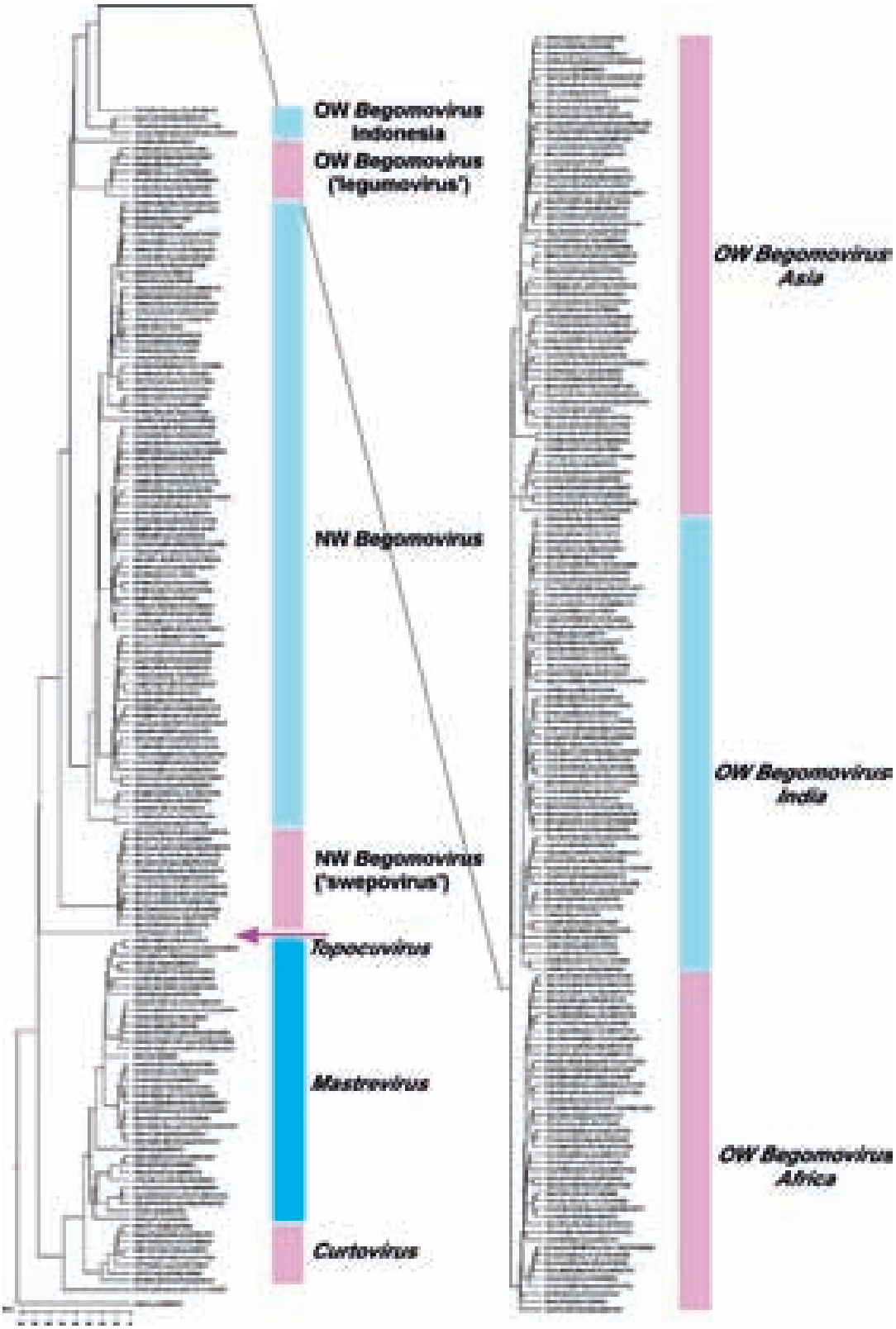


**Figure 6:** Dendrogram based on the complete DNA (or DNA A) component nucleotide sequences representing 215 approved and 103 candidate species of geminiviruses. Accession numbers and nucleotide sequences were obtained from GenBank. Sequences were aligned using the clustal algorithm (MegAlign 3.11, DNASTAR) and the bootstrap analysis was done with PAUP 4.0. The vertical axis is arbitrary and the horizontal axis represents a distance expressed in percentage of nucleotide substitution × 100. NW, New World; OW, Old World.

## List of unassigned species in the family *Geminiviridae*

None reported.

## Phylogenetic relationships within the family

Phylogenetic analysis of 318 complete genomic sequences (DNA A sequences in the case of bipartite begomoviruses) of 215 approved and 103 candidate species shows that geminiviruses cluster according to current taxonomic classification into four genera. In addition, they cluster according to geographic distribution, at least within the begomoviruses, probably reflecting their evolutionary divergence as a consequence of isolation due to the inability of their insect vectors to fly over long distances. Despite frequent inter-species recombination events and the increasing worldwide movement of infected plants, it is remarkable that this geographical distribution is still apparent.

## Similarity with other taxa

Members of the plant virus families *Geminiviridae* and *Nanoviridae* have circular ssDNA genomes and replicate by a rolling circle mechanism. All these viruses have highly conserved sequences, either TAATATTAC (geminiviruses) or predominantly TAGTATTAC (nanoviruses) in the loop of a putative stem-loop structure within the intergenic region, in which a nick is introduced during the initiation of replication. Similar structures are found in members of the animal virus family *Circoviridae* as well as the genus *Anellovirus* that also have small circular ssDNA genomes. It is speculated that geminiviruses derive from prokaryotic episomal replicons based on conservation of motifs in proteins that function in rolling circle replication initiation.

## Derivation of names

*Begomo*: from the type species *be*an *go*lden yellow *mo*saic virus (previously bean golden mosaic virus).
*Curto*: from the type species beet *cu*rly *to*p virus.
*Gemini*: from Latin meaning "twin", describing the characteristic twinned (geminate) particle morphology.
*Mastre*: from the type species *ma*ize *stre*ak virus.
*Topocu*: from the type species *to*mato *p*seud*o*-*cu*rly top virus.

## Further reading


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## Contributed by

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# Family *Inoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Inoviridae*** |
| Genus | ***Inovirus*** |
| Genus | ***Plectrovirus*** |

## Virion properties

### Morphology

Virions in this family contain a circular, positive sense, single stranded DNA genome within a helical array of thousands of copies of a major capsid protein. Inoviruses are flexible filaments about 7 nm in diameter that infect gram-negative and gram-positive bacteria, while plectroviruses are short rods about 15 nm in diameter that infect mycoplasmas (Figure 1). The packaged loops of DNA have anti-parallel strands extending between the virion ends, with fold-backs at the ends. The fold-back at one end is a specific DNA sequence that initiates packaging, and that at the other end is a random sequence that triggers the addition of a multi-domain adsorption protein. A crystal structure has been determined for the adsorption protein of one inovirus, but otherwise little is known about the structures of end proteins. In micrographs, the initiating ends of inoviruses are blunt, while the adsorption ends are tapered with extensions, as shown in Figure 1b. The virions are extruded, as shown in Figure 1c for plectrovirus progeny emerging from innumerable sites on the membrane of the wall-less mycoplasma host. Inoviruses of gram-negative hosts assemble and extrude from a more limited number of sites at adhesions between outer and inner membranes.

Virion contour lengths depend on DNA size and on the average nucleotide rise, h, characterizing the DNA conformation. Lengths of inoviruses range from 700 nm for Pseudomonas phage Pf3 (5833 nt, h = 0.24 nm), to 900 nm for *E. coli* phages in the Ff group (f1, fd, and M13; 6407 nt, h = 0.28 nm) and up to 3700 nm projected for Pseudomonas phage Pf4 (12437 nt, h = 0.61 nm). Major capsid subunits (gp8 or equivalent) have from 42 to 57 amino acids, the N-terminal domains of which maintain virion solubility, while hydrophobic central domains stabilize subunit–subunit interactions, and C-terminal domains interact with the DNA. The gene 8 subunits are highly $\alpha$-helical. They overlap each other in arrays in either Class I symmetry or Class II symmetry. Class I capsids have subunits arranged in steps of two interdigitated pentamers, and Class II capsids have subunits arranged in helices of approximately 5.4 interdigitated monomers per turn. The DNA helices within many of these capsids have bases stacked at the center and H-bonded, albeit with only about 25% Watson–Crick H-bonding possible, but otherwise similar to classical right-handed A- and B-form DNA helices. The DNA helix in Escherichia coli phage fd has a nucleotide rotation of 36° and a rise of 0.28 nm. However, the Pseudomonas phage Pf1 has an everted (inside-out), highly twisted and stretched DNA helix with a nucleotide rotation of 131.8° and a rise of 0.61 nm; the sugar-phosphate backbones are in the center and the bases are on the outside. Coat protein sequence similarities indicate Pf1-like DNA conformations in Psuedomonas phage Pf4 and Vibrio phage f237 (VfO3K6). Atomic models of inovirus structures are available in the structure databases, and refined models based on X-ray and spectroscopic date are continually being developed.

Plectrovirus virions are nearly straight rods with one end rounded and the other more variable. *Acholeplasma* phages are 70–90 nm long and 15 nm in diameter, whereas *Spiroplasma* phages are 230–280 nm long and 10–15 nm in diameter (Figure 1, lower). Negative stained images suggest 4 ± 2 nm hollow cores. Optical diffraction of images of Acholeplasma phage MV-L1 suggest morphological units arranged with two-fold rotational and 5.6-fold screw symmetry. DNA conformations in this genus have apparent nucleotide rise values, h, near 0.07 nm.

### Physicochemical and physical properties

Members of the family *Inoviridae* are sensitive to chloroform and ether, but have differing sensitivities to detergents. They are resistant to wide ranges of pH, and to extremes of cold and heat. The inovirus Pf1 has a unique thermally induced virion symmetry transition near 10 °C that is of considerable value to structural studies. Inoviruses have DNA contents from 6% to 14% and buoyant densities in CsCl in the narrow range 1.28 ± 0.02 g cm$^{-3}$. Virions range in total mass from 10 MDa

to over 60 MDa, but $S_{20,w}$ values are in a narrow range 40–45S because mass-per-length values are in a narrow range near 18,000 Da nm$^{-1}$. Translational and rotational diffusion constants are consistent with persistence lengths on the order of their contour lengths. Plectrovirus buoyant densities are 1.39 g cm$^{-3}$ in CsCl and 1.21 g cm$^{-3}$ in metrizamide, as reported for Spiroplasma phage SpV1-KC3. Virions in the family are easily precipitated from dilute solutions by low concentrations of polyethylene glycol.

In addition to random flexing, most inoviruses exhibit large-length-scale coiling. Strong coiling is evident for *E. coli* phages X and C-2 in electron micrographs, and gentle coiling is evident in liquid crystal behaviour for phage fd and several others. However, Pseudomonas phage Pf1 shows no evidence of the phenomenon. The varying degrees of coiling depend on the varying characteristics of capsid–DNA interactions, including the stoichiometric ratio of nucleotides per subunit, the value of which lies between 2 and 2.5 for many species, but which is uniquely 1 (unity) for Pf1.

### Nucleic acid

The genomes in the family range in size from 4.5 kb to as much as 12.4 kb and encode from 4 to 17 or more genes. Virions, as well as assembly precursor complexes in the cytoplasm, contain one molecule of positive sense ssDNA. In some cases genes are expressed from complementary strands, in particular the repressor genes when the viral genome is a latent prophage integrated in the cellular genome.

### Proteins

In the genus *Inovirus*, the type virus M13 has five different proteins in its virion (Figures 1 and 2). The cylindrical shells are composed of 2700 copies of gp8 (5.2 kDa), the adsorption end has probably

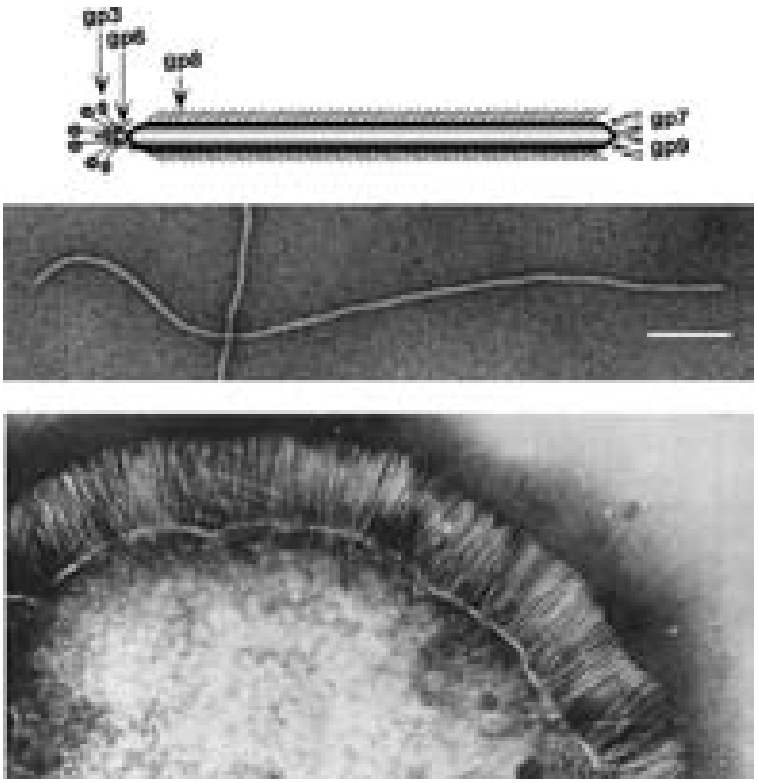


**Figure 1:** (Upper) A fanciful diagram to represent all members of the *Inoviridae* family (from A. Kornberg and T.A. Baker (1991). *DNA Replication*, 2nd edn. W.H. Freeman, New York; with permission); the gene numbering is for the closely related inoviruses f1, fd and M13 (often referred to, collectively or individually, as Ff phage). (Center) Negative contrast electron micrograph E. coli phage fd showing the differing end morphologies. Bar represents 100 nm; magnification about 180,000× (from C. Gray *et al.* (1981). *J. Mol. Biol.*, **146**, 621–627). (Lower) Electron micrograph of innumerable copies of the plectrovirus species *Spiroplasma phage SpV1* extruding from the membrane of *S. citri*; magnification 60,000× (reproduced from Renaudin, J. and Bove, J.M. (1994). *Adv. Virus Res.*, **44**, 429–463; with permission of Elsevier).

five each of gp3 (43 kDa) and gp6 (12 kDa), and the assembly nucleation end has probably five each of gp7 (3.5 kDa) and gp9 (3.3 kDa).

In the genus *Plectrovirus*, the Spiroplasma phages SpV1-R8A2B and SpV1-T78 have major capsid proteins of 7.5 kDa, while the Acholeplasma phages MVL1 and MVL51 have major coat proteins apparently of 19 kDa, according to gel data, although there is a strong tendency to aggregate.

### Lipids
None reported.

### Carbohydrates
None reported.

## Genome organization and replication

Inovirus genomes all have core regions similar to the genome of type virus M13 which is comprised of a DNA replication module (g2, g5 and g10), a capsid protein module (g7, g9, g8, g3 and g6), a morphogenesis module (g1,g4 and g11), and intergenic module with control sequences (Figure 2). Genes are closely spaced, even overlapping. Many inoviruses are integrative phages present in the host chromosome as latent prophages with their core regions flanked by genes for integrases, repressors, transposases and virulence factors such as toxin genes, as well as additional control sequences, such as operators and insertion sequences. An example is the well-characterized genome of vibriophage CTXφ, in which the genes for the subunits of cholera toxin are in the locus of a key morphogenesis gene of M13. Extensive sequence homologies between non-integrative and integrative phages, as between Pseudomonas phages Pf1 and Pf4, and many other pairs, indicate close evolutionary relationships. Members of the family *Inoviridae* do not lyse their hosts, so the established terms "lytic" and "lysogenic" are not fully suitable, but they are commonly used and not likely to be misunderstood.

Non-integrative, productive infections invariably involve the following steps: (1) phage adsorption to specific cell surface receptors, usually plasmid encoded conjugative pili, then binding to periplasmic protein co-receptors that couple adsorption with DNA uptake into the cytoplasm; (2) host enzyme conversion of the released ssDNA to an initial dsDNA circle from which viral proteins are expressed; (3) semi-conservative DNA replication initiated by viral endonuclease at a specific origin sequence, concomitant with continued expression of structural and assembly proteins; (4) rolling circle synthesis of progeny ssDNA that become sequestered by viral ssDNA binding protein; (5) and finally membrane-based assembly and extrusion of virions without cell lysis. Once infection is established virus extrusion continues indefinitely as cells divide. Primary control of expression

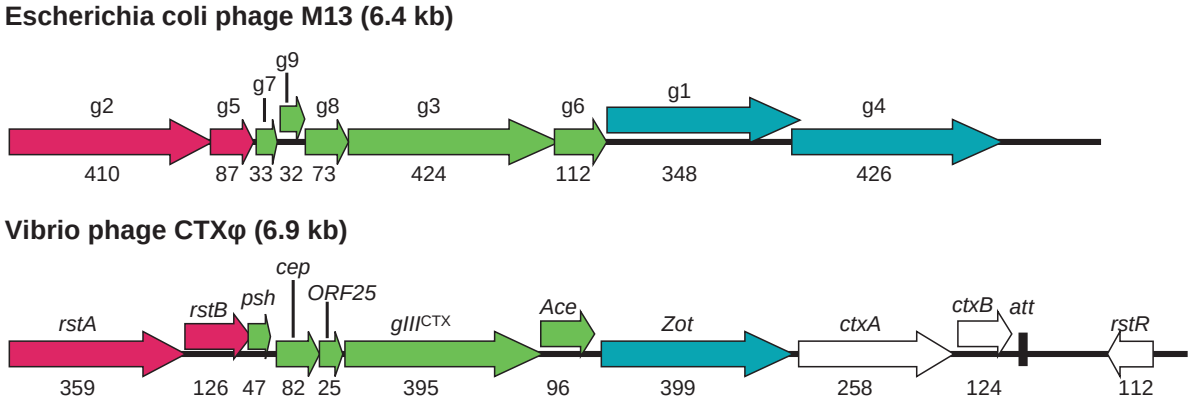


**Figure 2:** Linear presentations of circular genomes of non-integrative *E. coli phage* M13 (Upper, 6.4 kb), and integrative Vibrio phage CTXφ (Lower; 6.9 kb). They are organized in modules of replication genes (red), structural genes (green) and morphogenesis genes (blue). Gene numbers and names are from classical genetic mapping studies Gene 10 (DNA replication) and gene 11 (morphogenesis) are derived translationally from the C-terminal thirds of genes 2 and 1, respectively. Note the loci of the cholera toxin subunits A and B correspond to the locus of g4 in M13 and that the repressor RstR is expressed from the complementary strand. (Adapted from Faruque, S.M. and Mekalanos, J.J. (2005). *J. Bacteriol.*, **187**, 4095–4103; with permission).

is transcriptional from promoters of various strengths around the genome. Translational controls use overlapping reading frames and alternate starts in the same frame. Intergenic regions contain the complementary-strand and viral-strand replication origins and DNA packaging signals. Cell growth rates are usually slowed enough on lawns to generate plaques, but phage production on lawns without plaque formation can occur.

Amongst members of the family *Inoviridae*, the integration of genomes into host chromosomes was first reported for Xanthomonas phages Cf1t and Cf16 and for the SpV1-type Spiroplasma phages. The Cf1 insertions were at one bacterial site, whereas the plectrovirus insertions were at 17 or more sites in their plant pathogenic mycoplasmas. These insertions are mediated by viral integrases and transposases. In contrast, insertions mediated by bacterial host XerCD recombinases were later observed for vibriophage/prophage CTX$\phi$, the pathogenicity island of *V. cholera* carrying the cholera toxin genes, and for prophages CUS1 and CUS2, which are virtually identical elements bearing an apparent virulence factor in the genomes of the human pathogens *E. coli* O18:K1:H7 and *Y. pestis.* Thus, the mechanisms for conversions into prophage states, as well as mechanisms of maintenance and induction vary considerably within the family *Inoviridae*. Vibriophage CTX$\varphi$ infections have been the most extensively studied. They start with virus binding to TCP pili receptors followed by ssDNA release and formation of dsDNA, as described above for non-integrative infections. Holliday junctions can then form between att sites on phage dsDNA and the bacterial dif sites. Repeated formations of junctions produce tandem, or multiple, insertions which allow the replication of circular viral DNA from the linear chromosome site. Maintenance of the prophage state is through the combined actions of a phage-encoded repressor, RstR, and a host-encoded repressor, LexA. Induction of the prophage state to phage and toxin production occurs when the pathogenic bacterium colonizes the gut.

Known plectroviruses infect members of the genera *Acholeplasma* and *Spiroplasma*. Their surface receptors are not well characterized, but may have both polysaccharide as well as protein components. The replication pathways of Acholeplasma phage MV-L51 and the SpV1 group of Spiroplasma phages are similar to each other and to inoviruses with respect to freely replicating circular dsDNA and to the assembly and release of progeny virions at the membrane while the cells continue to divide. Genomes of SpV1-type phages contain insertion sequences and transposase genes, as do chromosomes of mycoplasmas infected by them. Certain high passage mycoplasma strains contain the latent prophages without producing virus, and these strains produce turbid plaques when superinfected with isolated virus. Mechanisms of insertion, repression, immunity and induction amongst the systems used by members of the family *Inoviridae* present many open questions.

## Biological properties

Members of the family *Inoviridae* mobilize DNA in the microbial world, and thus play a role in the evolution of microorganisms. They do not lyse their hosts, so that progeny cells remain infected, whether in a virulent productive state or in a latent prophage state. Thus vertical transfers of genomes continually occur, and horizontal transfers occur through the extruded virions. Members of the family are found in most, if not all, ecological niches of the planet, being present in all manner of commensal and pathogenic species in plants and animals, and surviving free under a range of environmental conditions. Most are either themselves integrative phages, or they are phylogenetically related to integrative phages. They carry virulence factors and are implicated in the pathogenesis of plant diseases including wilts and cankers, and of animal diseases including cholera, cystic fibrosis, acute gastroenteritis, neonatal meningitis, gonorrhea and plague. In many cases, the pathogenicity depends on the induction of a latent prophage.

The structures and lifestyles of these phages make them particularly well suited for gene transfer functions. Their virions can lengthen to carry extra genes and can adapt adsorption specificities to new hosts. In some cases their genomes can be mobilized in alternate capsids, and the physicochemical nature of DNA packaging can evolve to adapt to new environments. With regard to the latter, the types of DNA-protein interaction stabilizing virions vary from predominantly electrostatic, as in phages of the Ff group and many others, to predominantly hydrophobic, as in KSF-1$\varphi$, which has no basic amino acid residues in its major capsid protein. The properties are exemplified in several

systems, such as by the presence of Pseudomonas prophage Pf4 (12.4 kb) in the genome of pathogenic *Ps. aeruginosa* strain O1 (PAO1), carrying 5.1 kb of control functions and virulence factors, on a virtually unmodified core genome of Pseudomonas phage Pf1 (7.3 kb) specific for *Ps. aeruginosa* strain K (PAK). Further, the existence of virtually identical prophages CUS1 and CUS2 in the genomes of the pathogens *E. coli* O18:K1:H7 and *Y. pestis*, mentioned above, points to significant roles for inoviruses in pathogen evolution. Contributing to these roles are cellular receptors that are conjugative type IV pili encoded by transmissible plasmids so that lateral transfer of these plasmids concomitantly transfers phage sensitivity to new hosts. Of special importance in regard to host range among bacteria of the genus *Vibrio*, is that vibriophages fs1, KSF-1φ, VEJφ, VGJφ, and VSK all use as receptors the mannose-sensitive hemagglutinin (MSHA) pili present on many vibrio strains, and all of them are *xer*-recombinase intergrative phages capable of transducing genetic elements. All of them contribute to the diversity of the *Vibrionacea*. And finally, as a plectrovirus example, many pathogenic *Spiroplasma citri* strains from plants bear freely replicating circular DNA and extrude *SpV1-like* virions while also bearing prophages in the chromosomes of continually dividing mycoplasma cells (Figure 1).



# GENUS *INOVIRUS*

Type species ***Enterobacteria phage M13***

## Distinguishing features

Virions are slender and flexible 7 ± 1 nm filaments having a range of lengths, but narrow range of mass-per-length values near 18,000 Da nm$^{-1}$, hence a narrow range of sedimentation coefficients near 42 $S_{20,w}$. Nucleic acid contents are low, 6–14% by weight, keeping buoyant densities in CsCl in the range of 1.28 ± 0.02 g cm$^{-3}$. Hosts are gram-negative and gram-positive bacteria.

## Species demarcation criteria within the genus

- Host range
- Non-exchangeability of structural genes

Species demarcations are not definitive because of mosaicism generated by widespread lateral gene transfers and host chromosome integration as described above. Inovirus species are listed under the bacterial host species for the initial isolate together with incompatibility group plasmid that defines host ranges.

## List of species in the genus *Inovirus*

**1. Phages of *Enterobacteriaceae***

| | | | |
|---|---|---|---|
| *Enterobacteria phage AE2* | *{IncF}* | | |
| Enterobacteria phage AE2 | | | (AE2) |
| *Enterobacteria phage C-2* | *{IncC}* | | |
| Escherichia coli phage C-2 | | | (C-2) |
| *Enterobacteria phage dA* | *{IncF}* | | |
| Enterobacteria phage dA | | | (dA) |
| *Enterobacteria phage Ec9* | *{IncF}* | | |
| Enterobacteria phage Ec9 | | | (Ec9) |
| *Enterobacteria phage f1* | *{IncF}* | | |
| Escherichia coli phage f1* | | [J02448] | (f1) |
| *Enterobacteria phage fd* | *{IncF}* | | |
| Escherichia coli phage fd* | | [J02451] | (fd) |
| *Enterobacteria phage HR* | *{IncF}* | | |
| Enterobacteria phage HR | | | (HR) |
| *Enterobacteria phage I2-2* | *{IncI$_2$}* | | |
| Escherichia coli phage $I_2$-2 | | [X14336] | ($I_2$-2) |
| *Enterobacteria phage If1* | *{IncI}* | | |
| Escherichia coli phage If1 | | [ U02303] | (If1) |

| | | | |
|---|---|---|---|
| *Enterobacteria phage IKe* | *{IncI$_2$, IncN, IncP-1}* | | |
| Escherichia coli phage IKe | | [X02139] | (IKe) |
| *Enterobacteria phage M13* | *{IncF}* | | |
| Escherichia coli phage M13* | | [V00604] | (M13) |
| *Enterobacteria phage PR64FS* | *{IncI}* | | |
| Enterobacteria phage PR64FS | | | (PR64FS) |
| *Enterobacteria phage SF* | *{IncS}* | | |
| Enterobacteria phage SF | | | (SF) |
| *Enterobacteria phage tf-1* | *{IncT}* | | |
| Escherichia coli phage tf-1 | | | (tf-1) |
| *Enterobacteria phage X* | *{IncX, IncI$_2$, IncN, IncP-1}* | | |
| Escherichia coli phage X | | | (X) |
| *Enterobacteria phage X-2* | *{IncX}* | | |
| Escherichia coli phage X-2 | | | (X-2) |
| *Enterobacteria phage ZJ/2* | *{IncF}* | | |
| Escherichia coli phage ZJ/2 | | | (ZJ/2) |
| **2. Phages of *Pseudomonadaceae*** | | | |
| *Pseudomonas phage Pf1* | *{PAK}* | | |
| Pseudomonas phage Pf1 | | [X52107] | (Pf1) |
| *Pseudomonas phage Pf2* | | | |
| Pseudomonas phage Pf2 | | | (Pf2) |
| *Pseudomonas phage Pf3* | *{PA01}* | | |
| Pseudomonas phage Pf3 | | [M11912] | (Pf3) |
| **3. Phages of *Vibrionaceae*** | | | |
| *Vibrio phage 493* | | | |
| V. cholera phage/prophage 493 | | | (493) |
| *Vibrio phage CTX* | | | |
| V. cholera phage/prophage CTXϕ | | [GU942563] | (CTX) |
| *Vibrio phage fs1* | | | |
| V. cholera phage/prophage fs1 | | [D89074] | (fs1) |
| *Vibrio phage fs2* | | | |
| V. cholera phage fs2 | | [AB002632] | (fs2) |
| *Vibrio phage v6* | | | |
| Vibrio phage v6 | | | (v6) |
| *Vibrio phage Vf12* | | | |
| V. parahaemolyticus phage Vf12 | | [AB012574] | (Vf12) |
| *Vibrio phage Vf33* | | | |
| V. parahaemolyticus phage Vf33 | | [AB012573] | (Vf33) |
| *Vibrio phage VSK* | | | |
| V. cholera phage VSK | | [AF453500] | (VSK) |
| **4. Phages of *Xanthomonadaceae*** | | | |
| *Xanthomonas phage Cf16* | | | |
| Xanthomonas phage/prophage Cf16 | | | (Cf16) |
| *Xanthomonas phage Cf1c* | | | |
| Xanthomonas phage/prophage Cf1c | | [M57538, U41819] | (Cf1c) |
| *Xanthomonas phage Cf1t* | | | |
| Xanthomonas phage/prophage Cf1t | | [U08370] | (Cf1t) |
| *Xanthomonas phage Cf1tv* | | | |
| Xanthomonas phage/prophage Cf1tv | | | (Cf1tv) |
| *Xanthomonas phage Lf* | | | |
| Xanthomonas phage/prophage Lf | | [U10884, U38235, X70327-31, AF018286] | (Lf) |
| *Xanthomonas phage Xf* | | | |
| Xanthomonas phage Xf | | | (Xf) |
| *Xanthomonas phage Xfo* | | | |
| Xanthomonas phage Xfo | | | (Xfo) |
| *Xanthomonas phage Xfv* | | | |
| Xanthomonas phage Xfv | | | (Xfv) |

Species names are in italic script; names of isolates are in roman script. Sequence accession n umbers [ ] and assigned abbreviations ( ) are also listed. For the many phages of *Enterobacteriaceae* (Group 1) the transmissible plasmids that largely define host ranges are listed in the second column.
*Phages f1, fd and M13 have virtually identical structures and their 6.4 kb genomes differ at only about 1% of the nucleotide positions. They are often referred to, collectively or individually, as Ff phage. It is expected that they will be grouped into a single species in a future taxonomic revision.

### List of other related viruses which may be members of the genus *Inovirus* but which have not yet been approved as species

| | | |
|---|---|---|
| **Phages of Enterobacteriaceae** | | |
| E. coli O18:K1:H7 phage/prophage CUS1 | | (CUS1) |
| **Phages of Neissericeae** | | |
| N. menigitidis prophage MDA | [NC_003116] | (MDAφ) |
| N. menigitidis prophage Nf1-A | [NC_003116] | (Nf1A) |
| N. menigitidis prophage Nf3-A | [NC_003116] | (Nf3A) |
| N. menigitidis prophages Nf1-B1,B2 | [NC_003112] | (Nf1B) |
| N. menigitidis prophages Nf1-C1,C2,C3,C4 | [NC_008767] | (Nf1C) |
| N. gonorrhea prophages Nf4-G2,G3,G5 | [NC_002946] | (Nf4G) |
| **Phages of Pseudomonadaceae** | | |
| Ps. aeruginosa phage/prophage Pf4 | [AE004091] | Pf4 |
| Ps. aeruginosa phage/prophage Pf5 | [NC_008463] | Pf5 |
| Ps. aeruginosa phage/prophage Pf7 | [CP000744] | Pf7 |
| Ps. aeruginosa prophage Pf-LESB58 | [FM209186] | (Pf-LES) |
| **Phages of Ralstonaceae** | | |
| R. solanacearum phage/prophage φRSM1 | [AB259123] | (φRSM1) |
| R. solanacearum phage/prophage φRSS1 | [AB259124] | (φRSS1) |
| R. pickettii phage/prophage p12J | [AY374414] | (p12J) |
| **Phages of Shewanellaceae** | | |
| S. piezotolerans phage/prophage SW1 | [CP000472] | (SW1) |
| **Phages of Stenotrophomonaceae** | | |
| S. maltophilia phage φSMA9 | [AM040673] | (φSMA9) |
| **Phages of Thermusacea** | | |
| T. thermophilus phage PH75 | | (PH75) |
| **Phages of Vibrionaceae** | | |
| V. parahaemolyticus phage VfO4K68 | [AB043679] | (VfO4K68) |
| V. parahaemolyticus phage/prophage VfO3K6 (f237) | [AB043678] [BA000031] | (VfO3K6) (f237) |
| V. cholera phage/prophage KSF-1φ | [AY714348] | (KSF-1φ) |
| V. cholera phage/prophage VEJφ | [FJ904927] | (VEJφ) |
| V. cholera phage/prophage VGJφ | [AY242528] | (VGJφ) |
| V. cholera phage VSKK | [AF452449] | (VSKK) |
| **Phages of Xyllelaceae** | | |
| X. fastidiosa phage Xfφf1 | | (Xfφf1) |
| **Phages of Yersiniaceae** | | |
| Y. pestis phage/prophage CUS2 | [NC_003143] | (CUS2) (φYp01) (Ypfφ) |
| **Phages of Gram positive bacteria** | | |
| Clostridium beijerinickii phage CAK1 | | (CAK1) |
| Propionibacterium freudenreichii phage B5 | [AF428260] | (B5) |

## GENUS *PLECTROVIRUS*

Type species ***Acholeplasma phage MV-L51***

### Distinguishing features

Virions are rods with lengths of about 300 nm or less and diameters of about 15 nm. Virions are resistant to non-ionic detergents (Nonidet P-40 and Triton X-100) and sensitive to chloroform and ether. Adsorption is to cell membranes of wall-less mycoplasma hosts. Buoyant densities are 1.39 g cm$^{-3}$ in CsCl and 1.21 g cm$^{-3}$ in metrizamide, as reported for Spiroplasma phage SpV1-KC3.

### Species demarcation criteria in the genus

- Host range

Acholeplasma viruses infect some *Acholeplasma laidlawii* strains. SpV1-like viruses have broad host ranges among *Spiroplasma* species *S. citri*, *S. melliferum* and *S. kunkelii*.

## List of species in the genus *Plectrovirus*

| | | |
|---|---|---|
| **1. Phages of *Acholeplasma* spp.** | | |
| *Acholeplasma phage MV-L51* | | |
| Acholeplasma phage MV-L51 | | (MVL51) |
| Acholeplasma phage/prophage MV-L1 | [NC_001341] | (MVL1) |
| **2. Phages of *Spiroplasma* spp.** | | |
| *Spiroplasma phage 1-aa* | | |
| Spiroplasma phage SpV1-aa | | (SpV1-aa) |
| *Spiroplasma phage 1-C74* | | |
| Spiroplasma phage/prophage SpV1-C74 | [U28974] | (SpV1-C74) |
| *Spiroplasma phage 1-KC3* | | |
| Spiroplasma phage/prophage SpV1-KC3 | | (SpV1-KC3) |
| *Spiroplasma phage 1-R8A2B* | | |
| Spiroplasma phage/prophage SpV1-R8A2B | [X51344] | (SpV1-R8A2B) |
| *Spiroplasma phage 1-S102* | | |
| Spiroplasma phage/prophage SpV1-S102 | | (SpV1-S102) |
| *Spiroplasma phage 1-T78* | | |
| Spiroplasma phage/prophage SpV1-T78 | | (SpV1-T78) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations () are also listed.

## List of other related viruses which may be members of the genus *Plectrovirus* but which have not yet been approved as species

| | | |
|---|---|---|
| Spiroplasma phage SkV1-CR2-3x | [EF506570] | (SkV1-S2) |
| Spiroplasma phage/prophage SVGII3 | [AJ969242] | (SVGII3) |
| Spiroplasma phage/prophage SVTS2 | [AF133242] | (SVTS2) |

## List of unassigned species in the family *Inoviridae*

None reported.

## Phylogenetic relationships within the family

Phylogenetic distances between KSF-1φ and nine other filamentous vibriophages showed distinctly different cluster patterns depending upon which of three genes were used in the analysis, namely any of a gene for replication, one for phage morphogenesis, or one for receptor binding. The results showed the evolutionary effects of horizontal gene transfers resulting in mosaicism amongst the vibriophages. Some phages currently classified as different species are very closely related in all their genes (e.g. E. coli phages f1, fd and M13) and should be grouped into a single species in a future taxonomic revision.

## Similarity with other taxa

The repressed latent prophage states of many members of the family *Inoviridae* are similar to those of temperate phages in other taxa in being inducible to virulent states that produce copius progeny, but they are distinctly different in not causing cell lysis. Hence the terms like "lysogenic" and "lytic" states are not strictly appropriate despite their wide usage.

## Derivation of names

*Ino*: from Greek *nos*, "muscle filament".
*Plectro*: from Greek *plektron*, "small stick".

## Further reading

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## Contributed by

Day, L.A.

# Family *Microviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Microviridae*** |
| Genus | ***Microvirus*** |
| Subfamily | ***Gokushovirinae*** |
| Genus | ***Chlamydiamicrovirus*** |
| Genus | ***Bdellomicrovirus*** |
| Genus | ***Spiromicrovirus*** |

## Virion properties

### Morphology

Members of the family *Microviridae* are non-enveloped, ssDNA, prokaryotic viruses with T = 1 icosahedral symmetry. There are two morphologies represented within the family *Microviridae* (Figure 1), which along with genome organization, sequence homologies and host lifestyle, separate the family into two distinct parts, the genus *Microvirus* and the three genera of subfamily *Gokushovirinae*. Members of the genus *Microvirus* infect enterobacteria, and share a morphology and genome organization typified by Enterobacteria phage ϕX174 (ϕX174). Members of the *Gokushovirinae* infect obligate intracellular parasitic bacteria (*Bdellovibrio* and *Chlamydia*) and mollicutes (*Spiroplasma*), and share the morphology, typified by Spiroplasma phage 4 (SpV4).

Phage ϕX174 typifies microvirus morphology, in which pentamers of a major spike protein decorate the five-fold axes of symmetry of the T = 1 lattice. The structures of ϕX174, Enterobacteria phage

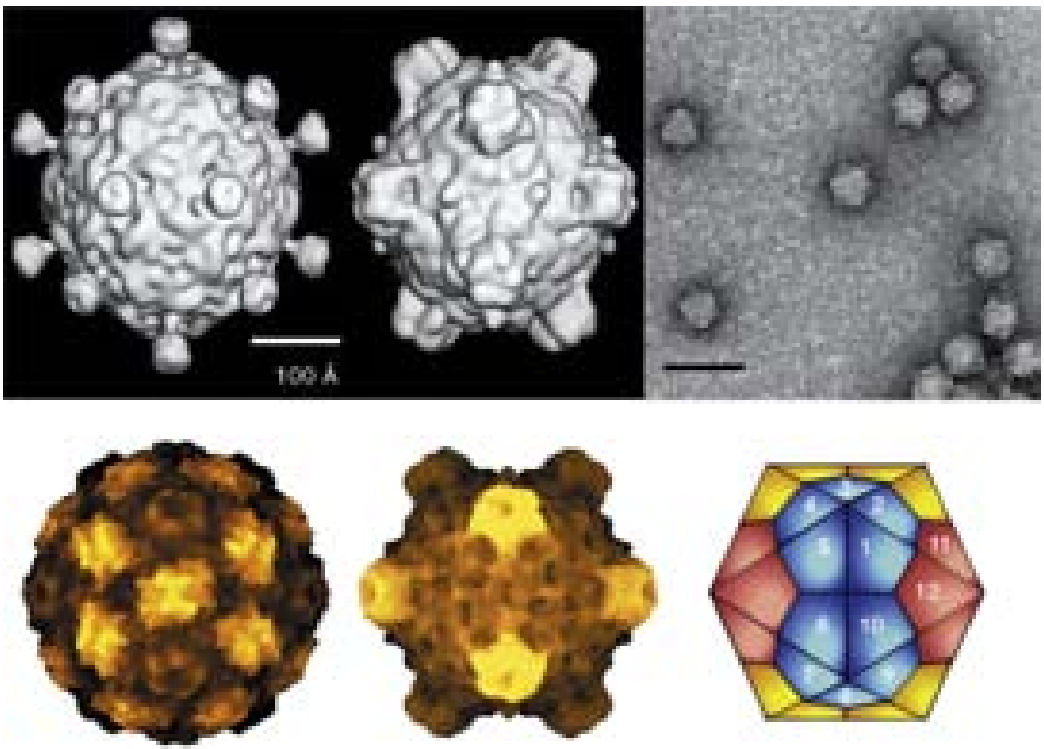


**Figure 1:** (Top left) Cryo-image reconstructions of the two morphologies represented within the family *Microviridae*: (left) Spiroplasma phage 4 (SpV4) (genus *Spiromicrovirus*); (right) Enterobacteria phage ϕX174 (ϕX174) (genus *Microvirus*) (courtesy T. Baker, R. McKenna and M.G. Rossmann). (Top right) Negative contrast electron micrograph of ϕX174 particles. The bar represents 50 nm. (Bottom) Electronic rendering surface of ϕX174 particles (left, scaffold; center, procapsid) and diagram representing the T = 1 lattice. (Dokland *et al.* (1997). *Nature*, **389**, 308–313)

**Table 1:** Proteins found in members of the family *Microviridae*

| *Microvirus* proteins | *Chlamydiamicrovirus* and *Bdellomicrovirus* proteins[1] | *Spiromicrovirus* proteins | Protein function |
|---|---|---|---|
| A | Vp4 | Vp2 | DNA replication protein |
| A* | | | Function unknown, non-essential |
| B | Vp3 | | Internal scaffolding protein |
| C | *Vp5* | | ssDNA synthesis, inhibitor of dsDNA synthesis |
| D | Absent | Absent | External scaffolding protein |
| E | *OrfN product* in ϕMH2K | | Lysis protein |
| F | Vp1 | Vp1 | Major capsid protein |
| G | Absent | Absent | Major spike protein |
| H | Vp2 | *Vp4* | Minor spike protein, DNA pilot protein. *De novo* H protein synthesis is required for efficient viral protein synthesis |
| J | *Orf8 product* | *Orf8 product* | DNA binding protein |
| K | | | Burst size modulation (host specific), non-essential |
| L, M | 6, 7 (Chp2) | 3,5,6,7,8,9 | Orfs of unknown coding capacity and/or proteins of unknown function |
| | W, X, Y, Z (ϕMH2K) | | |

[1]Italics indicate a hypothesized homolog. Blank entries indicate that the identity of the homolog, if it exists, is not readily apparent. Absent indicates that no homolog exists.

α3 (α3) and Enterobacteria phage G4 (G4) capsids (the G4 capsids were empty particles) have been determined to at least 3.5 Å resolution. CPs can be superimposed with root mean square deviations less than or equal to 0.8 Å and exhibit the common β-barrel motif. Capsids have a diameter of 250 Å. The 70 Å-diameter spike protein pentamers rise 30 Å from the surface of the capsid. Virions of the other three genera lack major spike proteins and, hence, their five-fold axes of symmetry are not decorated. Cryo-EM image reconstruction of SpV4 reveals mushroom-shaped protrusions at the three-fold axes of symmetry, which rise 54 Å above the surface of the 270 Å diameter capsids. These three-fold related structures appear to be composed of three interacting CPs and are formed by a distinct insertion loop unique to the gokushovirus CPs.

### Physicochemical and physical properties

The buoyant densities of family members range from 1.38–1.41 g cm$^{-3}$ for microviruses and 1.30–1.31 g cm$^{-3}$ for bdellomicroviruses and chlamydiamicroviruses. The only known spiromicrovirus, SpV4, has a reported buoyant density of 1.40 g cm$^{-3}$. Particles of both morphologies are very stable, resistant to detergents, ether, chloroform, pH 6.0–9.0 and freezing. Microviruses have S values of about 115S, while phages in the other genera sediment with S values near 90S.

### Nucleic acid

Genomes are circular positive sense ssDNA molecules. As with morphological, biochemical and biophysical properties, genome sizes appear to fall into two size ranges. Microvirus genomes are 5.3–6.1 kb, while gokushovirus genomes are considerably smaller, 4.4–4.9 kb. The smaller genomes reflect the absence of genes encoding major spike and external scaffolding proteins.

For those genera in which multiple species have been sequenced (*Microvirus* and *Chlamydiamicrovirus*), genome arrangement is close to identical within the genus. The genome arrangement of the only sequenced member of bdellomicroviruses, Bdellovibrio phage ϕMH2K (ϕMH2K), is extremely similar to that found in chlamydiamicroviruses, with the exception of the location of gene 5.

### Proteins

Although only the ϕX174-like phages have been studied in detail, sequence similarities and structurally based computational analyses have led to reasonable hypotheses regarding some of the viral proteins in the three less-studied gokushoviruses (Table 1).

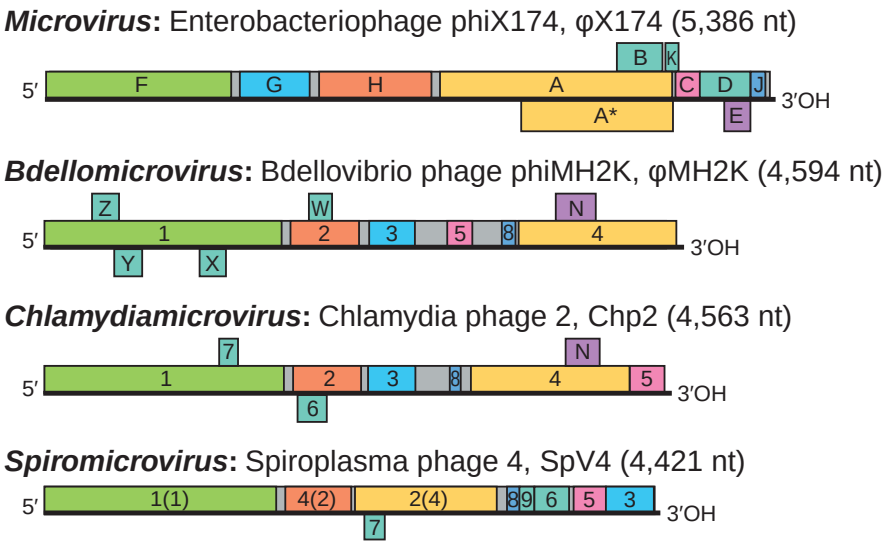


**Figure 2:** Genome organization of members of the family *Microviridae*. The circular genomes are presented linearly. Due to pronounced protein homologies and genome arrangements, the same gene number scheme is used for both the bdellomicroviruses (Bdellovibrio phage ϕMH2K; ϕMH2K) and chlamydiamicroviruses (Chlamydia phage 2; Chp2). Four proteins in Spiroplasma phage 4 (SpV4), (the products of genes 1, 2, 4, and 8) have homologs in the chlamydia- and bdellomicroviruses, the number in parentheses indicates the homologous gene.

ssDNA

### Lipids
None reported.

### Carbohydrates
None reported.

## Genome organization and replication

Genome organization is summarized in Figure 2. DNA replication and capsid assembly has been studied only for microviruses. Considering the distant relationship with the other genera, generalizations regarding common mechanisms within the family are not appropriate. Therefore, replication is discussed at the genus level.

## Antigenic properties

Both neutralizing and non-neutralizing monoclonal antibodies have been produced against microviruses. These antibodies often cross-react with other genus members. Non-neutralizing monoclonal antibodies have been produced against chlamydiamicrovirus proteins. Antibodies against the Chp2 CP recognize the ϕMH2K CP, further demonstrating the close relationship between these two genera.

# Genus *Microvirus*

Type species ***Enterobacteriophage phiX174***

## Distinguishing features

All current members of the genus *Microvirus* were isolated from *Enterobacteriaceae*. However, it should be noted that rigorous searches for members of the family *Microviridae* have not been conducted in other hosts. Since most isolation procedures are optimized for large dsDNA viruses, which are considerably denser than microviruses, easier to visualize by electron microscopy, and have much larger S values, special techniques must be employed for the isolation of members of the family *Microviridae*.

Although the viruses in this group are dependent on the same host cell proteins for replication, genetic studies suggest that individual phages may be particularly sensitive to host cell alleles of *rep*, *slyD* and *mraY*. Even though the primary sequences of the microvirus proteins have diverged, some of these proteins can cross-function (e.g., DNA binding proteins and internal scaffolding proteins), indicating productive use; while other proteins cross-inhibit (e.g., external scaffolding proteins), indicating the ability to interact with other proteins across species lines. However, after interaction, other functions required for productive morphogenesis are hindered. Cross-species inhibitory protein domains have been identified with the use of chimeric proteins.

## Genome organization and replication

Microvirus genome organization is strictly conserved in the 47 known genome sequences. The genomes most distinguishing feature is the presence of overlapping reading frames. Seven of the 11 genes (genes A through E and gene K) reside in such regions (Figure 2). Most of these genes code for non-structural proteins (Table 1). Genes found within the coding sequences of other genes (A*, B, K and E) encode non-essential proteins (A* and K), proteins that do not affect particle formation, such as lysis proteins (E); or highly flexible proteins that tolerate substitutions, like the internal scaffolding protein (B). Moreover, multiple mutant strains that no longer require B protein function have been isolated. Packaged microvirus genomes do not form the densely condensed cores seen in most dsDNA bacteriophages. Instead, genomes are intimately associated with the capsid's inner surface, which contributes to late stages of morphogenesis and virion stability. This association, as opposed to fixed capsid dimensions, may hinder the acquisition of new genes (or morons), but has not appeared to hinder horizontal gene exchanges between different microvirus species.

Procapsid morphogenesis and DNA synthesis proceed independently of each other during microvirus replication. After phage ssDNA enters the cell, stage I DNA replication commences. This process converts the infecting (+) single stranded circular genome into a covalently closed double stranded molecule called replicative form I DNA (RFI). No viral proteins are involved. With the synthesis of the (−) strand, transcription can begin. Although microvirus gene expression is not dependent on elaborate *trans*-acting mechanisms to ensure temporal regulation, the relative timing and amounts of viral proteins synthesized is controlled by highly sophisticated sets of *cis*-acting promoters, transcription terminators and ribosome binding sites. Stage II DNA synthesis is dependent on the viral A protein, which cleaves the RFI DNA (+) strand at the origin of replication and covalently attaches itself to the DNA. This generates RF II molecules. *De novo* (+) strand replication involves a rolling circle mechanism, while *de novo* (−) strand synthesis occurs via a mechanism similar to stage I replication. Stage II DNA synthesis continues until one copy of viral protein C binds displaced ssDNA at the initiation of another round of stage II DNA synthesis, which terminates further dsDNA replication. With the binding of the C protein, the stage III DNA pre-initiation complex forms, consisting of one copy each of protein A, protein C and the host cell rep protein, a DNA helicase which associates with protein A during stage II DNA synthesis.

The packaging mechanisms involved in members of the family *Microviridae* differ substantially from those found in other bacteriophages. The pre-initiation complex associates with the viral procapsid (see below), forming the 50S complex in which ssDNA is concurrently synthesized and packaged. Genome length is strictly governed by a single origin of replication, which determines both the initiation and termination of biosynthesis and packaging. DNA concatamers, unique translocating vertices, and head-full mechanisms are not involved. The viral A protein bound to the origin of replication in the RF II DNA is both necessary and sufficient for packaging specificity. Although any circular DNA molecule with a microvirus origin of replication can serve as a template, the secondary structure formed by the packaged genome can affect the biophysical properties of the resulting virion. After one round of rolling circle synthesis, protein A cuts the newly generated origin and acts as a ligase, generating a covalently closed circular molecule.

The first assembly intermediates in procapsid morphogenesis are 9S and 6S particles, pentamers of viral coat and spike proteins, respectively (Figure 3). Five internal scaffolding proteins (protein B) bind to the underside of a 9S particle, which produces the 9S* particle. The upper surface of the viral coat protein can now interact with spike and external scaffolding proteins. 9S* particles are also less prone to aggregate prematurely than 9S particles and the presence of protein B may

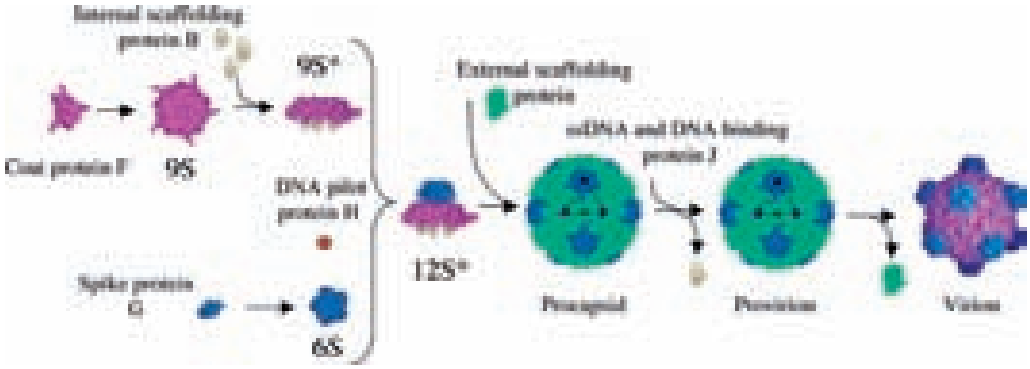


**Figure 3:** Microvirus capsid morphogenesis.



facilitate the incorporation of the minor vertex protein, or DNA pilot protein, protein H. Twelve 12S* particles then associate with external scaffolding proteins (protein D) to form the procapsids. In the ϕX174 procapsid crystal structure, four D proteins, as dimers of dimers, are associated with one CP. After particle formation, two-fold related B-B and D-D scaffolding contacts keep the CP pentamers from dissociating during DNA packaging.

The genome replication/packaging machinery binds to a depression along the two-fold axis of symmetry and an ssDNA genome is then concurrently synthesized and packaged, along with 60 copies of the DNA binding protein, protein J. Procapsids are probably filled through one of the 30 Å diameter pores at the three-fold axes of symmetry. During packaging, B proteins are extruded, probably displaced by the DNA binding protein J, which shares a binding cleft in the viral CP. Finally, D proteins dissociate, yielding the virion. This is accompanied by an 8.5 Å radial collapse of CPs around the packaged genome, which is tethered to the underside of the CP pentamers by F-DNA and J-DNA interactions. Genetic and biochemical data indicate that capsid-ssDNA interactions may mediate the integrity of this final stage of morphogenesis.

## Biological properties

The nature of the interaction of ϕX174-like phages with their hosts is poorly understood. Initial attachment to host cells occurs via a sugar residue, most likely glucose, in the lipopolysaccharide (LPS). A site on the surface of the capsid, near the three-fold axes of symmetry, has been shown to bind glucose reversibly. However, host range mutations change amino acids in spike proteins G and H, suggesting that a second host cell receptor may be required for DNA ejection. The identity of this second factor is unknown. Although the members of the family *Microviridae* are tailless, the virus may follow a pathway similar to that of the large-tailed Enterobacteria phage T4 (T4). T4 reversibly interacts with LPS via its long tail fibers. The phage then "walks" along the surface of the cell until it finds a second receptor, which triggers ejection. Instead of walking, microviruses may "rock and roll" along the cell surface, until this second receptor is found.

Intracellular localization of ϕX174-like phage maturation is strongly dependent on the host cell *rep* allele, which must physically interact with the viral A protein and the procapsid during ssDNA synthesis and packaging. Proper interactions between the viral E protein and the gene products of host cell *slyD* and *mraY* alleles are probably required for lysis.

## Species demarcation criteria in the genus

Currently, species demarcation criteria are temperature and host range. However, both phenotypes can be changed by single point mutations. Therefore, these criteria may not be rigorous for distinguishing between species. The results of phylogenetic analyses of 42 novel isolates suggest that the microviruses fall into three clades represented by bacteriophage ϕX174, α3 and G4. Thus, it may be more appropriate to consider most isolates as varieties of these three phages.

### List of species in the genus *Microvirus*

| | | |
|---|---|---|
| *Enterobacteria phage alpha3* | | |
| Enterobacteria phage α3 | [X60322] | (α3) |
| *Enterobacteria phage G4* | | |
| Enterobacteria phage G4 | [J02454] | (G4) |
| *Enterobacteria phage phiX174* | | |
| Enterobacteria phage ϕX174 | [J02482] | (ϕX174) |
| Enterobacteria phage S13 | [M14428] | (S13) |
| *Enterobacteria phage phiK* | | |
| Enterobacteria phage ϕK | [X60323] | (ϕK) |
| *Enterobacteria phage St-1* | | |
| Enterobacteria phage St-1 | [GQ149088] | (St-1) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Microvirus* but which have not yet been approved as species

None reported.

## SUBFAMILY *GOKUSHOVIRINAE*

### Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Gokushovirinae*** |
| Genus | ***Chlamydiamicrovirus*** |
| Genus | ***Bdellomicrovirus*** |
| Genus | ***Spiromicrovirus*** |

### Distinguishing features

The members of the subfamily *Gokushovirinae* differ from those in the genus *Microvirus* in virion morphology (see Figure 1), genome organization (see Figure 2), sequence homologies and host lifestyle. They infect obligate intracellular parasitic bacteria and mollicutes whereas microviruses infect enterobacteria. The three genera are distinguished by host organism.

## GENUS *CHLAMYDIAMICROVIRUS*

Type species ***Chlamydia phage 1***

### Distinguishing features

These phages infect various species of *Chlamydia and Chlamydophila*. Stocks of the type species phage no longer exist. Computational analyses indicate that chlamydiamicrovirus capsids will resemble those of SpV4.

### Genome organization and replication

The genome organization of the *Chlamydiamicrovirus* Chp-2 is depicted in Figure 2. Since double stranded replicative form DNA has been isolated and homologs of microvirus proteins A and C are present, DNA replication is thought to occur via a similar mechanism. The mechanisms involved in capsid formation in the genus *Chlamydiamicrovirus* are not known, but will probably not resemble microviruses morphogenesis because chlamydiamicroviruses lack external scaffolding and major spike proteins. However, Vp3 has been demonstrated to be an internal scaffold protein. The Chp-2 viral lifecycle has been characterized and is tightly regulated with the developmental cycle of its host.

### Biological properties

Unlike the microviruses, the chlamydiamicroviruses probably recognize a protein receptor.

### Species demarcation criteria in the genus

There are no formal criteria for species demarcation.

### List of species in the genus *Chlamydiamicrovirus*

| | | |
|---|---|---|
| *Chlamydia phage 1* | | |
| Chlamydia phage 1 | [D00624] | (Chp-1) |
| *Chlamydia phage 2* | | |
| Chlamydia phage 2 | [AJ270057] | (Chp-2) |
| *Chlamydia pneumoniae phage CPAR39* | | |
| Chlamydia pneumoniae phage CPAR39 | [AE002163] | (ϕCPAR39) |
| *Guinea pig Chlamydia phage* | | |
| Guinea pig Chlamydia phage | [U41758] | (ϕCPG1) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Chlamydiamicrovirus* but which have not yet been approved as species

None reported.

## GENUS *BDELLOMICROVIRUS*

Type species ***Bdellovibrio phage MAC 1***

### Distinguishing features

These phages infect *Bdellovibrio* strains. DNA of isolates of the type species, *Bdellovibrio phage MAC 1*, has not been sequenced and stocks of this phage no longer exist. All bdellovibriovirus genome and biophysical data come from the study of ϕMH2K. Computational analyses suggest that the structure of ϕMH2K resembles SpV4.

### Genome organization and replication

The genome organization of the ϕMH2K is depicted in Figure 2. Replication is most likely similar to Chp-2.

### Species demarcation criteria in the genus

There are no formal criteria for species demarcation.

### List of species in the genus *Bdellomicrovirus*

| | | |
|---|---|---|
| *Bdellovibrio phage phiMH2K* | | |
| Bdellovibrio phage ϕMH2K | [AF306496] | (ϕMH2K) |
| *Bdellovibrio phage MAC 1* | | |
| Bdellovibrio phage MAC 1 | | (MAC-1) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Bdellomicrovirus* but which have not yet been approved as species

None reported.

# GENUS *SPIROMICROVIRUS*

Type species ***Spiroplasma phage 4***

## Distinguishing features

The one isolated phage of this group infects *Spiroplasma melliferum*.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Spiromicrovirus*

*Spiroplasma phage 4*
Spiroplasma phage 4 [M17988] (SpV4)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Spiromicrovirus* but which have not yet been approved as species

None reported.

## Phylogenetic relationships within the family (Figure 4)

Phylogenetic trees built with four different proteins (CP, Rep protein, DNA pilot protein and the internal scaffolding protein) clearly illustrate the existence of the two major groups of the family.

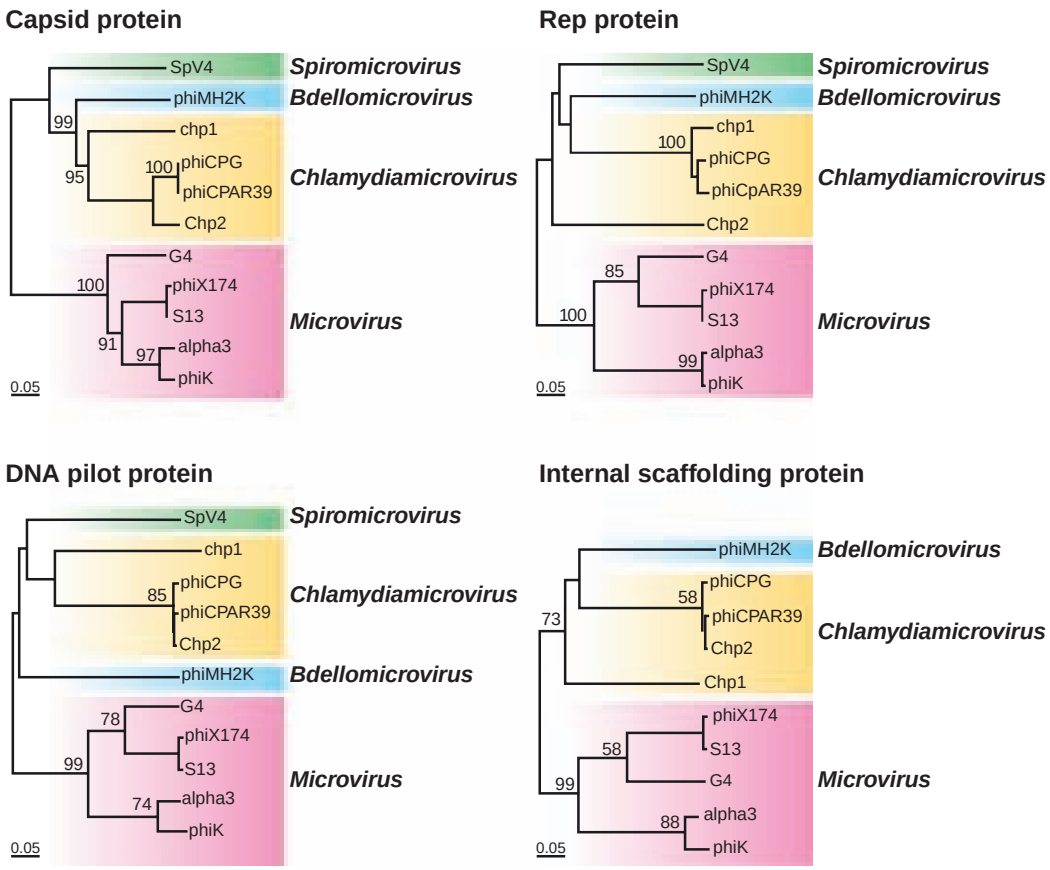

**Figure 4 (opposite):** Phylogeny of the family *Microviridae*. The aa sequences of the capsid protein (tTop left), the Rep protein (top right), the DNA pilot protein (bottom left) and the internal scaffolding protein (bottom right) were used to make alignments with the CLUSTAL X software. The trees were designed with PAUP and the bootstrap values are indicated above 50%. The abbreviations of the viruses used and their GenBank accession numbers are listed in the List of Species of the description.

## Similarity with other taxa

In some instances ssDNA genomes of viruses in the family *Microviridae* are similar in organization to those of members of the family *Inoviridae*. Structurally, viruses in the family *Microviridae* resemble those in the family *Parvoviridae*. Atomic structures of the CPs are rich in insertion loops coming off the β-barrel core. Similarities between the capsids of spiromicroviruses, bdellomicroviruses, chlamydiamicroviruses and parvoviruses are more pronounced due to the complex interactions of CPs at the three-fold axes of symmetry.

## Derivation of names

*Micro*: from the Greek *micros*, "small".
*Gokusho*: from the Japanese *Gokusho*, "very small" or "very holy".

## Further reading

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## Contributed by

Cherwa, J.E. Jr. and Fane, B.A.

# Family *Nanoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Nanoviridae*** |
| Genus | ***Nanovirus*** |
| Genus | ***Babuvirus*** |

## Distinguishing features

The family *Nanoviridae* comprises plant viruses possessing very small virions containing a multipartite (6–8), circular, single stranded DNA genome and being transmitted by aphids in a circulative (non-propagative) persistent manner. Each virion contains one component of the multipartite genome and 60 subunits of a capsid protein (CP) of about 19 kDa.



## Virion properties

### Morphology

Virions are 17–20 nm in diameter, and presumably of an icosahedral T = 1 symmetry structure containing 60 subunits. They are not enveloped. Capsomeres may be evident, producing an angular or hexagonal outline (Figure 1).

### Physicochemical and physical properties

Virions are stable in $Cs_2SO_4$ but may not be stable in CsCl. The buoyant density of virions is about 1.24 to 1.30 g cm$^{-3}$ in $Cs_2SO_4$, and 1.34 g cm$^{-3}$ in CsCl. They sediment as a single component in sucrose rate-zonal and $Cs_2SO_4$ isopycnic density gradients. The sedimentation coefficient of banana bunchy top virus (BBTV) virions is 46S. The particle weight of subterranean clover stunt virus (SCSV) is approximately $1.6 \times 10^6$. The extinction coefficient of SCSV is 3.6 at $A_{260}$ (corrected for light scattering).

### Nucleic acid

Six or eight circular single stranded (ss)DNAs ranging in size from 923 to 1111 nucleotides (nt) are consistently found in virion preparations of different babu- and nanovirus isolates. They are encapsidated as individual positive sense strands in separate particles. In addition, up to four different satellite-like ssDNAs of about 1000–1100 nt, also referred to as alphasatellites, are found associated with the majority of the nanovirid isolates.

### Proteins

Virions have a single CP of about 19 kDa. No other proteins have been found associated with virions. In addition, at least 5–7 non-structural proteins are encoded by the mRNA(s) transcribed from the nanovirid ssDNAs (Table 1). All but one of the nanovirid DNAs encode only a single protein.

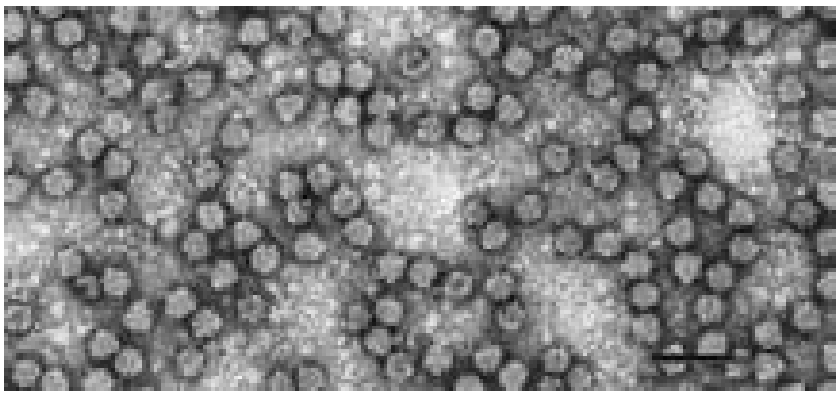

**Figure 1:** Negative contrast electron micrograph of particles of Faba bean necrotic yellows virus (FBNYV). The bar represents 50 nm. (Courtesy of L. Katul and D.-E. Lesemann.)

**Table 1:** Designation, size and functions of the proteins encoded by the various DNA components of the members of the family *Nanoviridae*

| Protein[a] | Protein size (in kDa) | Encoded by DNA component | Identified from[b] | | | | Protein function(s)[c] |
|---|---|---|---|---|---|---|---|
| | | | Nanoviruses | | Babuviruses | | |
| | | | FBNYV | SCSV | BBTV | ABTV | |
| M-Rep | 33.1–33.7 | DNA-R | + | + | + | + | *R*eplication initiator protein for all genomic DNAs |
| CP | 18.7–19.6 | DNA-S | + | + | + | + | Structural protein, virion formation (encapsidation) |
| Clink | 18.5–19.9 | DNA-C | + | + | + | + | Cell-cycle regulation |
| MP | 12.7–13.7 | DNA-M | + | + | + | + | Cell-to-cell *m*ovement |
| NSP | 17.3–17.7 | DNA-N | + | + | + | + | Presumed *N*SP (by analogy to geminiviruses) |
| U1 | 16.9–18.0 | DNA-U1 | + | + | − | − | *U*nknown |
| U2 | 14.2–15.4 | DNA-U2 | + | − | − | − | *U*nknown |
| U3 | 10.3 | DNA-U3 | − | − | + | − | *U*nknown |
| U4 | 10 or 12.5 | DNA-U4 | + | − | − | − | *U*nknown |

[a]Master replication initiator protein (M-Rep), coat protein (CP), cell-cycle link protein (Clink), movement protein (MP) and nuclear shuttle protein (NSP). U1 to U4 are temporary designations until the protein function is determined.
[b] + or − indicates whether a protein has been described from a virus species or not. Note that the genome organization of all other nanoviruses is identical to that of FBNYV.
[c]The italicized letters indicate how the DNA component designation has been derived.

A second virion-sense ORF, completely nested within the M-Rep-encoding ORF and encoding a putative 5 kDa protein of unknown function (U5), was identified only from banana bunchy top virus (BBTV) DNA-R but not from any other nano- and babuvirus DNA-R.

**Lipids**
Not known.

**Carbohydrates**
Not known.

## Genome organization and replication

Based on a range of geographical isolates, there is compelling evidence that 6 and 8 DNAs are essential and form the genome of a babu- and nanovirus, respectively (Figures 2 and 3). All nanovirid DNAs have a similar structural organization, containing conserved inverted repeat sequences potentially forming a stem-loop structure that is part of the common region-stem loop [CR-SL], and a second common region named CR-M (for babuviruses) or CR-II (for nanoviruses) (Figures 2 and 3). The additional satellite-like DNAs contain different inverted repeat sequences (stem loops).

Babu- and nanoviruses share a set of five homologous DNA components, referred to as DNA-R, -S, -C, -M and -N (Figures 2 and 3). Three other DNAs (DNA-U1, -U2 and -U4) encoding proteins of as yet unknown functions have been identified from nanoviruses only and one further DNA (DNA-U3) only from babuviruses. It should be noted, however, that no ORF has been identified from the DNA-U3 of the two Abaca bunchy top virus (ABTV) isolates and several Asian isolates of BBTV. Whereas the difference in the number and types of DNAs between babu- and nanoviruses reflects a fundamental difference between viruses of these two genera, the apparent disparity in genomic organizations between some viruses of a genus may indicate that certain genome components have not yet been identified. This might be true for DNA-U2 and -U4 of SCSV and seems definitely to be the case for cardamom bushy dwarf virus (CBDV) DNAs other than DNA-R.

All nanovirid DNAs contain a major virion sense ORF and are transcribed unidirectionally. However, two mRNAs (encoding M-Rep and U5 protein) are transcribed from the BBTV DNA-R, whereas all the other babu- and nanovirus DNAs (incl. the other babu- and nanovirus DNA-R) seem to encode a single protein only. Each coding region is preceded by a promoter sequence with a TATA box and followed by a polyadenylation signal (Figures 2 and 3). For the nanovirus DNA-R, however, the position of the polydenylation signal between the TATA box and the ORF leads to transcription of the replication origin and synthesis of a terminally redundant mRNA that is capable of folding into extended secondary structures. This may be a way to regulate the expression of the encoded master replication initiator (M-Rep) protein.

Since the nanovirid DNAs and some of the biochemical events determined for nanovirid replication resemble those of the geminiviruses, their replication is also thought to occur in the nucleus through transcriptionally and replicationally active dsDNA intermediates by a rolling circle type of replication mechanism. Upon decapsidation of viral ssDNA, one of the first events is the synthesis of viral dsDNA with the aid of host DNA polymerase. As the virus DNAs have the ability to self-prime during dsDNA synthesis, it is likely that pre-existing primers are used for dsDNA replicative form (RF) synthesis, as has been shown for BBTV. From these dsDNA forms, host RNA polymerase then transcribes mRNAs encoding the M-Rep and other viral proteins. Viral DNA replication is initiated by the M-Rep protein. There is experimental evidence for faba bean necrotic yellows virus (FBNYV) and BBTV that M-Rep has DNA cleavage and nucleotidyl transfer activity *in vitro* and initiates the replication of all genomic DNAs. These biochemical reactions involve a conserved nonanucleotide sequence flanked by inverted repeat sequences that potentially form a stem-loop structure and are a part of the viral origin of replication (*ori*). This sequence arrangement including the loop-forming sequence containing the nonanucleotide TATTATTAC or TAGTATTAC is perfectly conserved in babuviruses and nanoviruses, respectively. The non-coding regions (NCRs) of all genomic DNAs of a given nanovirid share this highly conserved stem-loop region (CR-SL) that also encompasses short repeated sequences (iterons) that are presumed to be binding sites for the M-Rep protein, the only viral protein essential for nanovirus replication. All other replication proteins including DNA

polymerases are provided by the host cell. Viral DNA replication is enhanced by the action of Clink, a nanovirid-encoded cell cycle modulator protein.

In addition to the genomic DNAs, a number of additional DNAs encoding Rep proteins have been found associated with some, but not all nanovirid infections. These additional DNAs, referred to as "alphasatellites" (see chapter on "Satellites"), are genetically very diverse and phylogenetically distinct from the DNA-R of nano- and babuviruses. Tentative evidence suggests that these DNAs interfere with the establishment and expression of nanovirid disease symptoms.

## Antigenic properties

Virions are strong immunogens. Most viruses belonging to the same genus are serologically related to, but distinct from, one another. No serological relationship between babu- and nanoviruses has been observed.

## Biological properties

### Host range

Viruses of the individual species have narrow host ranges. Nanoviruses naturally infect only a limited range of leguminous species (*Fabaceae*), whereas babuviruses have been reported only from few monocots, such as the *Musaceae* and *Zingiberaceae*. All viruses are associated with stunting of infected plants, and infected hosts may also show leaf roll, chlorosis and premature death. Nanovirids are restricted to the phloem tissue of their host plants and are not transmitted mechanically or through seeds. Until recently, plants could only be experimentally infected by graft or vector transmission. However, infectivity of purified FBNYV virions by biolistic bombardment has now been demonstrated and aphid-transmissible FBNYV and faba bean necrotic stunt virus (FBNSV) virions have been reconstituted using eight cloned DNAs.

### Transmission

Under natural conditions, all viruses are transmitted by certain aphid species in a persistent manner and do not replicate in their vectors. Together with results from complementation experiments, there is evidence that vector transmission of FBNYV (and probably also other nanovirids) require a virus-encoded helper factor that is either dysfunctional or absent in purified virion preparations.

### Geographical distribution

While BBTV is widely distributed in banana growing countries in the Asia-Pacific region and Africa, ABTV has been reported only from Sarawak (Malaysia) and the Philippines, and CBDV only from India. SCSV occurs in Australia, milk vetch dwarf virus (MDV) in China and Japan, pea necrotic yellow dwarf virus (PNYDV) in Europe and FBNSV in Ethiopia and Morocco. In contrast, the reported geographic distribution of FBNYV is much wider, occurring in several countries of West Asia and North and East Africa as well as in a European country (Spain). No nanovirus has been recorded from the New World.

## Genus and species demarcation criteria in the family

Features demarcating the two genera in the family, i.e., criteria to be used for distinguishing babuviruses from nanoviruses, are shown in Table 2.

Criteria to be used as guidelines for species demarcation are:

- Differences in natural host range
- Differences in the number and types of vector aphid species
- Different reactions to antibodies to individual species
- Differences in CP aa sequences of $>15\%$, and/or
- Overall nt sequence identity of $<75\%$ is generally indicative of a distinct species.

Since several nanovirids are now known to have overlapping host ranges and to be transmitted by a similar range of aphid species, biological criteria appear no longer useful for species

**Table 2:** Features distinguishing viruses of the genera *Nanovirus* and *Babuvirus*

| Features | Genus *Nanovirus* | Genus *Babuvirus* |
|---|---|---|
| Major hosts | Dicots (legumes) | Monocots |
| Major aphid vectors | *Aphis craccivora* and a few other legume-colonizing aphid species | *Pentalonia* and *Micromyzus* spp. |
| No. of genomic DNAs | 8 | 6 |
| Presence of specific DNAs | DNA-U1, -U2, -U4 | DNA-U3 |
| Size of genomic DNAs | 923–1020 nt | 1013–1111 nt |
| Overall nucleotide sequence relationship in DNA-R, -N, -S, -C and -M | >50% (between species) | >50% (between species) |
| | <45% (between babu- and nanoviruses) | |



discrimination within a genus. Although species-specific monoclonal antibodies (where available) can be used for species discrimination, preference should nowadays be given to the molecular criteria specified above.

## GENUS *NANOVIRUS*

Type species ***Subterranean clover stunt virus***

### Distinguishing features

Experimental and circumstantial evidence suggests that the nanovirus genome consists of eight different ssDNA components, referred to as DNA-R, -S, -M, -C, -N, -U1, -U2 and -U4 (Figure 2). Thus, the latter three DNAs are characteristic for viruses of the genus *Nanovirus*. The other major differences between babu- and nanoviruses are that the latter naturally infect legumes (dicots), are vectored by several aphid species colonizing legumes, and share low levels of aa sequence identities ranging from 18 to 56% in individual genes with babuviruses. Moreover, the nanovirus DNA components ranging in size from 923 to 1020 nt are slightly smaller (by ca. 100 nt) than those of babuviruses (1013–1111 nt) and, in contrast to babuviruses, the nanovirus DNA-R transcripts are terminally redundant.

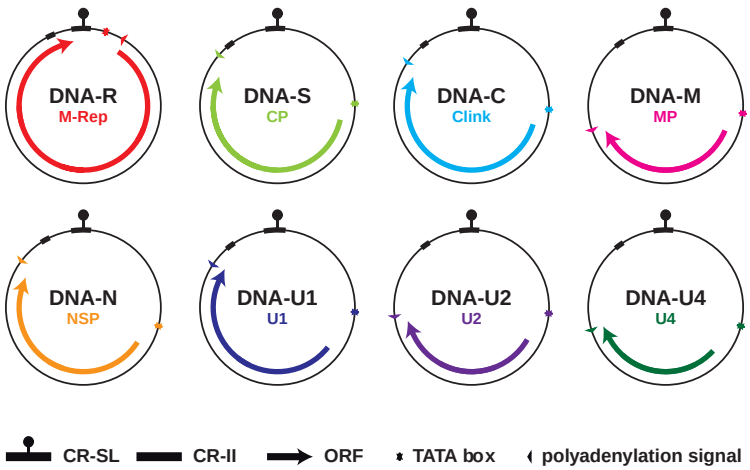


**Figure 2:** Diagram illustrating the genomic organization of viruses of the genus *Nanovirus* and depicting the structure of the eight identified viral DNA components (see also Table 1). Each DNA circle (1000 ± 20 nt) contains its designated name and the name of the encoded protein. Arrows refer to the location and approximate size of the ORFs and the direction of transcription. Note that DNA-U2 and -U4 have not been identified from SCSV. The position of the common stem-loop region (CR-SL) and the second common region (CR-II) are indicated.

Antisera to FBNYV and SCSV cross-react weakly with SCSV and FBNYV respectively in Western blots and immunoelectron microscopy, but not at all in DAS-ELISA, suggesting that the serological relationship between these two viruses is distant (CP aa sequence identity ca. 57%). However, MDV and FBNSV react strongly not only with FBNYV antisera but also with the majority of monoclonal antibodies to FBNYV. Therefore, species-specific MAbs are required for the differentiation and specific detection of these three closely related species, which share CP aa sequence identities of 83–85%.

Nanoviruses have largely overlapping but relatively narrow host ranges. They infect over 50 legume species and only a few non-legume species under experimental and natural conditions. They are transmitted by several aphid species. *Aphis craccivora* appears to be the major natural vector of these viruses as it is the most abundant aphid species on legume crops in the afflicted areas and was among the most efficient vectors under experimental conditions. Other aphid vectors are *Acyrthosiphon pisum* and *Aphis fabae* but for MDV also *A. gossypii* and *Megoura viciae*.

### List of species in the genus *Nanovirus*

| | | |
|---|---|---|
| *Faba bean necrotic yellows virus* | | |
| Faba bean necrotic yellows virus-[Egypt] | [AJ132179 to -84, AJ132186, AJ749902] | (FBNYV-[EG]) |
| Faba bean necrotic yellows virus-[Morocco] | [GQ274023 to -30] | (FBNYV-[MA]) |
| *Milk vetch dwarf virus* | | |
| Milk vetch dwarf virus-[Japan] | [AB000923 to -7; AB009046, AB027511, AB044387, AB255373] | (MDV-[JP]) |
| *Subterranean clover stunt virus* | | |
| Subterranean clover stunt virus-[Australia] | [U16730, U16732–4, U16736, AJ290434] | (SCSV-[AU]) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Nanovirus* but have not been approved as species

| | | |
|---|---|---|
| *Faba bean necrotic stunt virus* | | |
| Faba bean necrotic stunt virus-[Ethiopia] | [GQ150778 to -85] | (FBNSV-[ET]) |
| Faba bean necrotic stunt virus-[Morocco] | [GQ274031 to -8] | (FBNSV-[MA]) |
| *Pea necrotic yellow dwarf virus* | | |
| Pea necrotic yellow dwarf virus-[Germany] | [GU553134*] | (PNYDV-[DE]) |

* Although only a DNA-R sequence has been described for PNYDV-[DE] (Grigoras et al., *Plant Dis.* **94**, 643, 2010), the seven other genome segments have been sequenced and aphid transmissible virus has been reconstituted using cloned DNAs.

## Genus *Babuvirus*

Type species ***Banana bunchy top virus***

### Distinguishing features

Babuviruses naturally infect monocots (*Musa* and *Amomum* spp.) and are vectored by the banana aphid *Pentalonia nigronervosa* (CBDV also by *Micromyzus kalimpongensis* Basu). BBTV and ABTV infect bananas (*Musa* spp.) and closely related species within the *Musaceae*, such as *M. textilis* Née and *Ensete ventricosum* Cheesem. There are no confirmed non-*Musa* hosts of BBTV and ABTV. Symptoms of BBTV include plant stunting, foliar yellowing and most characteristic dark green streaks on the pseudostem, petioles and leaves. There is not much information on CBDV, which has recently been found associated with a serious disease ("Foorkey") of large cardamom (*Amomum subulatum* Roxb.) in India.

BBTV and ABTV have a genome consisting of six different ssDNA components, referred to as DNA-R, -S, -C, -M, -N, and -U3 (Figure 3). Since they have been identified from all babuvirus isolates studied in greater detail, they are considered integral components of the babuvirus genome. Although there are several BBTV isolates from which a DNA-U3 has not been identified, most babuviruses seem to possess a specific DNA component (DNA-U3) of unknown function. Only for DNA-U3 of BBTV-[AU] (L41576) has an RNA transcript been reported.

Unlike the relative conservation among the genes within nanovirus species, there is considerable variation in certain genes among individual isolates of a babuvirus species. Striking genetic differences ranging from 7 to 20% have been observed for the individual gene products of two ABTV isolates, one from the Philippines and the other from Sarawak (Malaysia). Similarly, two groups of BBTV isolates, designated the Asian and South Pacific groups, are distinguished based on DNA-R, -N and -S sequences. The nt sequences of the major gene of DNA-R, -N and -S differ by 7.5, 8.6 and 6.3% which translate to mean differences of 5.6, 6.7 and 1.4%, respectively, in aa sequences between the two geographic groups. Particularly striking are the differences between the two groups of BBTV isolates in the CR-M of DNA-R (32%), -N (27%) and -S (39%), whereas the intragroup CR-M variation does not exceed 6%. Whereas the DNA-R, -N and -S genes appear to be well conserved among BBTV isolates, striking differences (up to 19%) between BBTV isolates in less conserved gene products (e.g., movement protein) have been observed (Figure 4). If these genetic differences can be substantiated by completely sequencing a number of Asian isolates, this might form the basis for a future subdivision of BBTV into two separate species.

BBTV is serologically unrelated to members of the genus *Nanovirus*. However, BBTV antibodies have been used for the detection of ABTV and CBDV, the two other babuviruses. Of 10 monoclonal antibodies raised to BBTV, only two reacted with ABTV. This is consistent with a CP amino acid sequence difference of about 20% between ABTV and BBTV.

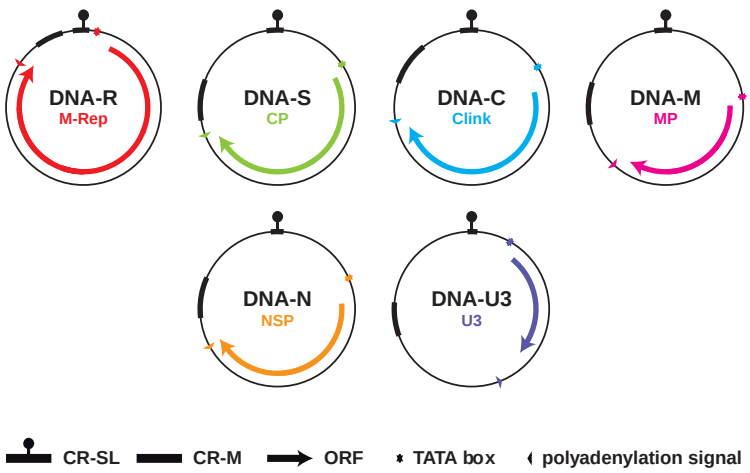


**Figure 3:** Diagram illustrating the genomic organization of viruses of the genus *Babuvirus* and depicting the structure of the six identified viral DNA components (see also Table 1). Each DNA circle (1060 ± 50 nt) contains its designated name and the name of the encoded protein. The position of the common stem-loop region (CR-SL) and the major common region (CR-M) are indicated. Arrows refer to the location and approximate size of the ORFs and the direction of transcription. Note that (i) no ORF has been identified from DNA-U3 of the two ABTV isolates and several Asian isolates of BBTV and (ii) DNA-R of BBTV has a small internal ORF which potentially encodes a 5 kDa protein (U5) and has been shown to be transcribed from DNA-R of BBTV-[AU].

## List of species in the genus *Babuvirus*

| | | |
|---|---|---|
| *Abaca bunchy top virus* | | |
| Abaca bunchy top virus-[Malaysia] | [EF546808 to -13] | (ABTV-[MY]) |
| Abaca bunchy top virus-[Philippines] | [EF546802 to -7] | (ABTV-[PH]) |
| *Banana bunchy top virus* | | |
| Banana bunchy top virus-[Australia] | [S56276, L41574 to -78] | (BBTV-[AU]) |
| Banana bunchy top virus-[Taiwan] | [DQ826390 to -1; DQ826393 to -6] | (BBTV-[TW]) |
| *Cardamom bushy dwarf virus* | | |
| Cardamom bushy dwarf virus-[India] | [AY485960*] | (CdBDV-[IN]) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Although the DNA-R sequence available from GenBank is defective, an apparently complete DNA-R sequence has been determined.

## List of other related viruses which may be members of the genus *Babuvirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Nanoviridae*

| | | |
|---|---|---|
| *Coconut foliar decay virus* | | |
| Coconut foliar decay virus-[Vanuatu] | [M29963*] | (CFDV-[VU]) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
* Since this appears to be an alphasatellite sequence, it should no longer be assigned to the family *Nanoviridae*.

## Phylogenetic relationships within the family

Analysis of the five proteins identified from the majority of the assigned nanovirid species (Figure 4) suggests that the most conserved nanovirid proteins are the M-Rep protein (54–97% identity) and the NSP (41–91%), followed by the CP (20–84%), the MP (14–76%) and the Clink protein (18–72%). Babuviruses share significant levels of aa sequence similarity with nanoviruses only in the M-Rep (54–56%) and NSP (41–45%), whereas the aa sequence similarities between the two genera are negligible in the CP (20–27%), the MP (20–23%) and the Clink protein (18–23%).

## Similarity with other taxa

All Rep proteins of the assigned species have most of the aa domains characteristic of Rep proteins of geminiviruses and other ssDNA viruses. The nanovirus Rep proteins differ from those of members of the family *Geminiviridae* in being smaller (about 33 kDa), having a slightly different dNTP-binding motif (GPQ/NGGEGKT), lacking the retinoblastoma-like protein (Rb)-binding motif (LxCxE) and sharing aa sequence identities of only 17 to 22% with them. Moreover, the assigned species are clearly distinct from geminiviruses in particle morphology, genome size, number and size of DNA components, mode of transcription, and in vector species. Although circo- and nanovirids possess closely related Rep proteins and morphologically similar virions, circovirids infect vertebrates and have a much smaller genome (1.8–2.3 kb) that is not only monopartite but also bidirectionally transcribed. All these ssDNA viruses have a conserved nonanucleotide motif at the apex of the stem-loop sequence which is consistent with the operation of a rolling circle model for DNA replication.

## Derivation of names

*Babu*: from *ba*nana *bu*nchy top virus.
*Nano*: from the Greek *nanos*, meaning "dwarf", referring to the observations that these plant viruses have the smallest known virions and genome segment sizes, and dwarf their hosts.

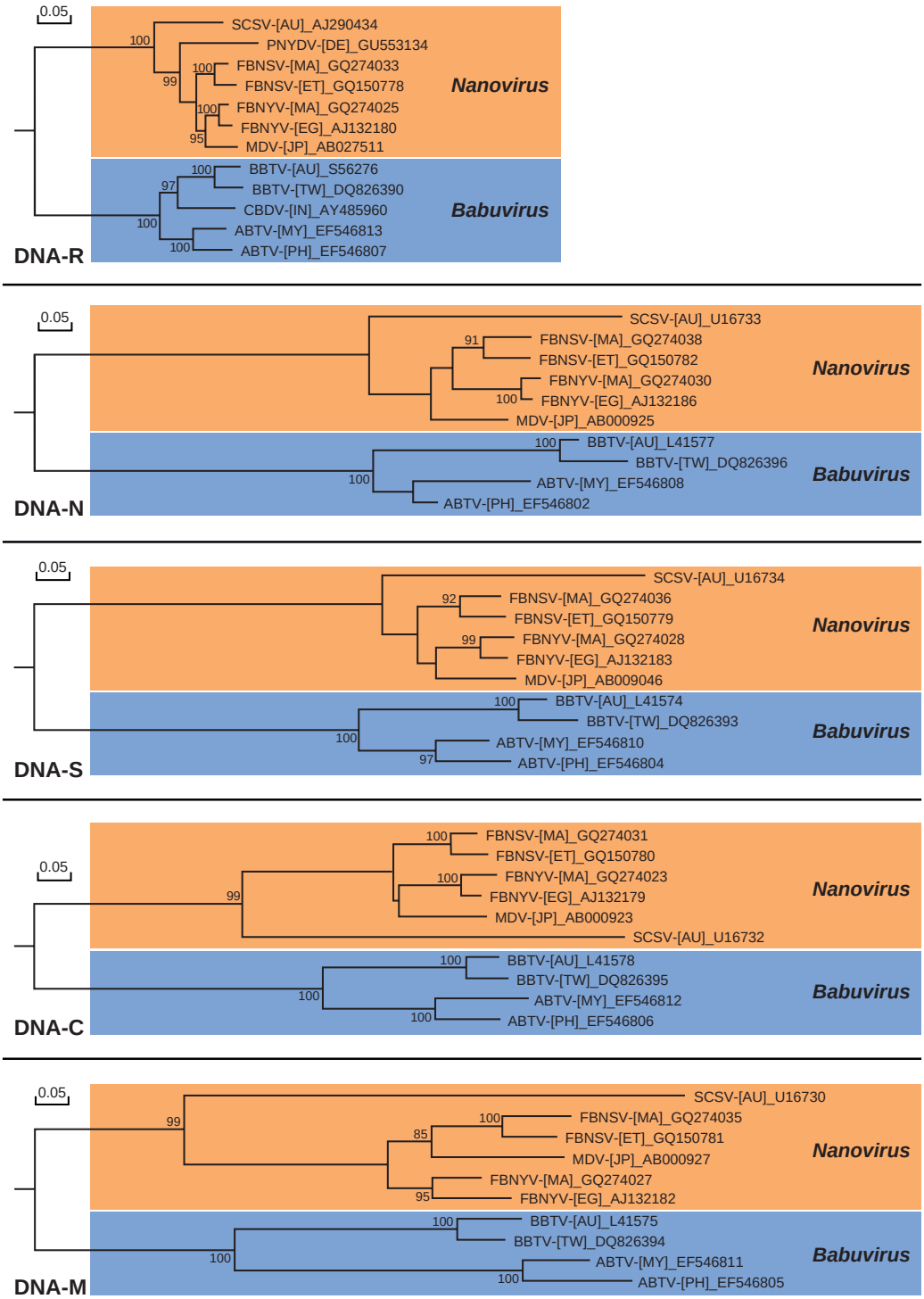


**Figure 4:** Neighbor-joining dendrograms illustrating nucleotide sequence relationships in the five DNAs (DNA-R, -N, -S, -C and -M) identified from all assigned and (still) putative members of the genera *Nanovirus* and *Babuvirus* (family *Nanoviridae*): abaca bunchy top virus (ABTV), banana bunchy top virus (BBTV), cardamom bushy dwarf virus (CBDV), faba bean necrotic yellows virus (FBNYV), faba bean necrotic stunt virus (FBNSV), milk vetch dwarf virus (MDV), pea necrotic yellow dwarf virus (PNYDV), and subterranean clover stunt virus (SCSV). Since sequence information is available for genetically distinct isolates of some nano- and babuviruses and this may have future taxonomic implications, the sequences of ABTV isolates from Malaysia (MY) and the Philippines (PH), BBTV isolates from Australia (AU) and Taiwan (TW), FBNYV isolates from Egypt (EG) and Morocco (MA), and FBNSV isolates from Ethiopia (ET) and Morocco (MA) were included in the comparison. Vertical branch lengths are arbitrary and horizontal distances are proportional to the number of base substitutions per site (see scale bar). Sequence alignments and dendrograms were produced using DNAMAN (version 6, Lynnon Biosoft, Quebec, Canada) which uses a CLUSTAL-type algorithm. The dendrograms were bootstrapped 1000 times (scores are shown at nodes).

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Vetten, H.J., Dale, J.L., Grigoras, I., Gronenborn, B., Harding, R., Randles, J.W., Sano, Y., Thomas, J.E., Timchenko, T. and Yeh, H.H.

# Family *Parvoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Parvoviridae* |
| Subfamily | *Parvovirinae* |
| Genus | *Parvovirus* |
| Genus | *Erythrovirus* |
| Genus | *Dependovirus* |
| Genus | *Amdovirus* |
| Genus | *Bocavirus* |
| Subfamily | *Densovirinae* |
| Genus | *Iteravirus* |
| Genus | *Brevidensovirus* |
| Genus | *Densovirus* |
| Genus | *Pefudensovirus* |



## Virion properties

### Morphology

Parvoviruses are among the smallest of isometric viruses, with diameters ranging from 215 Å (Penaeus stylirostris densovirus, PstDNV) to 255 Å (canine parvovirus, CPV). X-ray crystallography studies have unequivocally established the icosahedral nature of parvoviruses, with 31 rotational elements (six 5-folds, ten 3-folds and fifteen 2-folds). The final resolution obtained ranges from 3.7 (Galleria mellonella densovirus; GmDNV) to 2.5 (PstDNV) Å, but the N-termini of the capsid proteins have not yet been solved. Some structures have a smooth appearance (GmDNV), whereas others (adeno-associated virus-2, AAV-2, and PstDNV) are spiky at the 3- or 5-fold symmetry axes (Figure 1). Parvoviruses lack envelopes.

### Physicochemical and physical properties

Infectious particles are composed of about 75% protein and about 25% DNA, and their Mr is about $5.5–6.2 \times 10^6$. Virion buoyant density is 1.39–1.43 g cm$^{-3}$ in CsCl and the $S_{20,w}$ is 110–122S. Infectious particles with buoyant densities of about 1.45 g cm$^{-3}$ may represent conformational or other variants, or precursors to the mature particles. Defective particles with deletions in the genome occur and exhibit lower densities (about 1.34 g cm$^{-3}$). Mature virions are stable in the presence of lipid solvents, or on exposure to pH 3–9 or, for most species, incubation at 56 °C for 60 min. Viruses can be inactivated by treatment with formalin, β-propriolactone, hydroxylamine, ultraviolet light and oxidizing agents such as sodium hypochlorite, although virus aggregates are somewhat more resistant.

### Nucleic acid

The genome is a linear, nonsegmented molecule of ssDNA, 4–6.3 kb in size (Mr $1.5–2.0 \times 10^6$), with a G+C content of about 40–55%. The 5′- and 3′-ends of the genome contain palindromic sequences, 120 to ~550 nt in length, which can be folded into hairpin structures essential for viral DNA replication. These terminal hairpins may be part of a terminal repeat, and therefore related in sequence (e.g. dependoviruses), whereas some genomes have terminal hairpins that are unrelated in sequence to one another (e.g. members of the genus *Parvovirus*). Some parvoviruses preferentially encapsidate ssDNA of negative polarity (i.e. complementary to the viral mRNA species; e.g. minute virus of mice, MVM), whereas others may encapsidate ssDNA species of either polarity in equivalent (e.g. adeno-associated viruses, AAVs) or different proportions (e.g. bovine parvovirus, BPV). The percentage of particles encapsidating the positive strand can vary from 1 to 50% and may be influenced by the host cell in which the virus is produced (e.g. LuIII virus, LuIIIV). Some insect parvoviruses (densoviruses) have genes on both strands.

### Proteins

Most parvoviruses have 2–4 virion proteins, and up to five in the case of brevidensoviruses and pefudensoviruses (VP1-VP5). Depending on the species, protein sizes are: VP1 75–96 kDa, VP2 65–85 kDa, VP3 55–75 kDa and VP4 45–52 kDa (for brevidensoviruses somewhat smaller). The viral proteins represent different N-extended forms of the same gene product, except for the

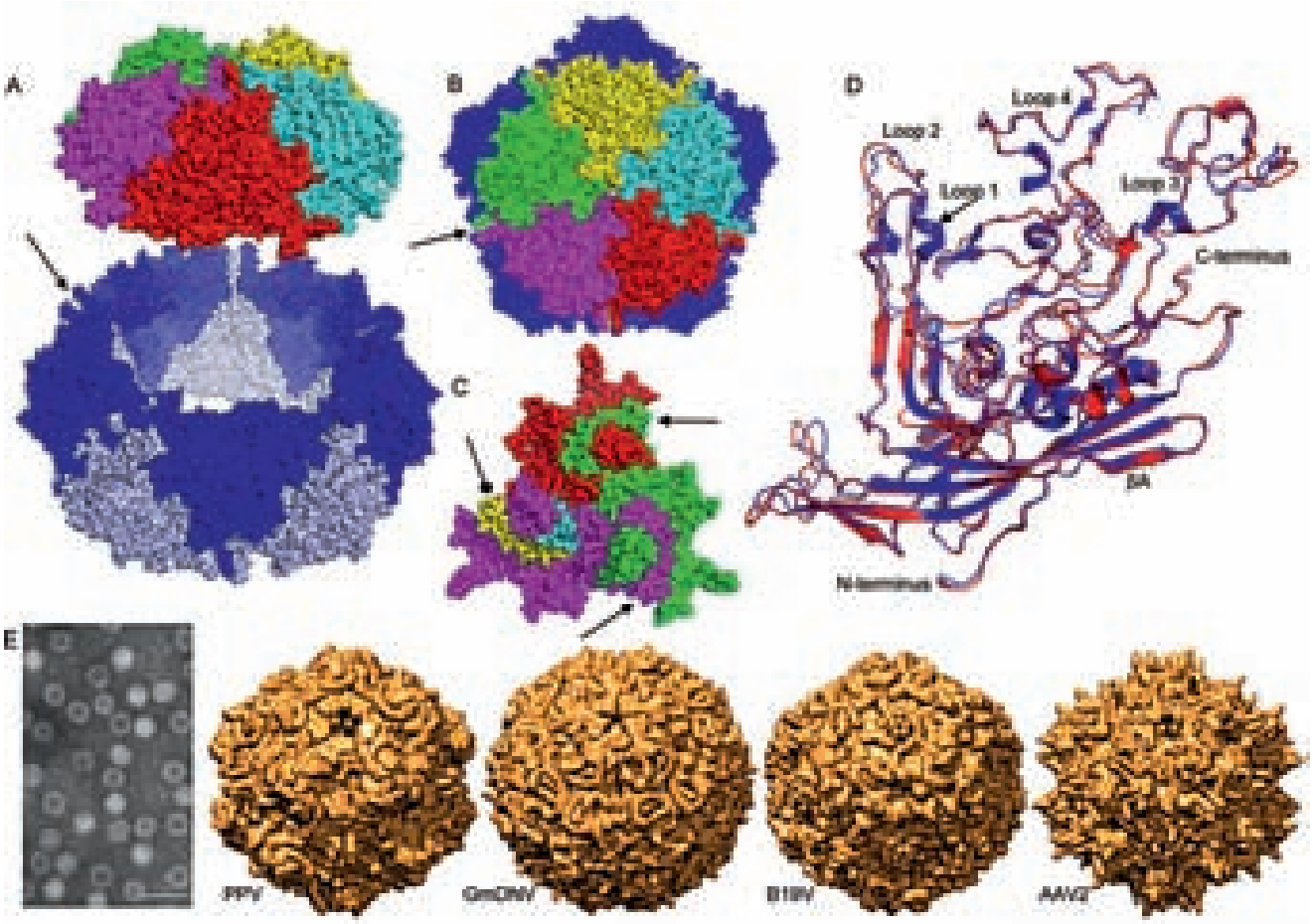


**Figure 1:** Morphology of parvoviruses. (A) Side-view, at a resolution of 3.5 Å, of a tilted model of a porcine parvovirus (PPV) capsid with the top five trimers translated 120 Å along its 5-fold axis from the middle body of 10 trimers (five shown in dark blue and five in light blue); the bottom five trimers are not shown. The 5-fold axes are located at the intersection of five trimers (e.g. at arrow: green, magenta, two dark blue and one light blue colored trimer). (B) Top view of model shown in (A), without light-blue trimers, along the 5-fold axis. The channels at the 5-fold axes are clearly visible (arrow indicates same 5-fold axis as shown by arrow in (A)). (C) Structure of a PPV trimer. The arrows indicate the intertwining of the GH-loop (between β strands G and H) in the counter-clockwise located proteins from near the 3-fold axis towards the 2-fold axis (arrows). The GH-loop actually consists of two loops of which one (loop 3, yellow), running to the 2-fold axis, is partly covered by loop 4 (cyan) near the 3-fold axis (oval arrow). (D) Parvoviruses may have remarkably similar structures despite low sequence identities. This figure shows the alignment of minute virus of mice (MVM, in red; 549 amino acid residues, PDB 1mvm) and PPV (in blue; 542 amino acid residues, PDB 1k3v) structural proteins that have only 52% sequence identity. Nevertheless, 528 $C_\alpha$s (97%) of the residue pairs occupy the same position in the capsid (root-mean-square error of 1.0 Å). (E) Negative contrast electron micrograph of empty and full PPV particles (bar = 50 nm) and space-filling models of the capsid structures of PPV, Galleria mellonella densovirus (GmDNV; PDB: 1DNV), human B19 virus (B19V; PDB: 1s58), and adeno-associated virus - 2 (AAV-2; PDB: 1lp3) shown at a resolution of 4 Å. In each case, the view is down a 2-fold axis at the centre of the virus, with 3-fold axes left and right of centre, and 5-fold axes above and below. Models (A-D) have been rendered by PyMOL and the space-filling models by CHIMERA (Multiscale Models).

pefudensoviruses that generate two different N-terminal extensions. Some shrimp densoviruses have a single 54 kDa capsid protein. The VP1-specific regions of viruses of all genera, except members of the genera *Brevidensovirus* and *Amdovirus* and some unclassified parvoviruses, contain the enzymatic core of a phospholipase A2 (PLA2). The two smallest proteins are usually the principal proteins. Spermidine, spermine and putrescine have been identified as components of some densoviruses and iteraviruses.

### Lipids

Virions lack essential lipids.

### Carbohydrates

None of the viral proteins are known to be glycosylated.

## Genome organization and replication

Parvoviruses usually possess two major gene cassettes (Figure 2). The REP ORF encodes the non-structural proteins (NS), which are required for transcription and DNA replication, and the CP ORF encodes the structural proteins of the capsid (the CAP, VP or S proteins). Both gene cassettes are present on the same DNA strand of all parvoviruses except for members of the genera *Densovirus* and *Pefudensovirus* and some as yet unclassified ambisense densoviruses. The REP functions and the CPs of the latter genera are encoded in the 5′-halves of the complementary strands and represented, as for monosense parvoviruses, with the REP cassette to the left (Figure 2). In some viruses, other minor ORFs have been detected. For some of these, a protein product has been identified (the ORF for the N terminus of VP1). Mutations within the REP ORF block virus replication and gene expression. Mutants in REP or CP can be complemented in *trans*. The palindromic sequences (at both termini) are required in *cis* for DNA replication to occur.

Some viruses use alternative splicing, leaky scanning, or a combination thereof, to obtain different forms of the REP and CAP gene products. The MVM REP ORF produces two major non-structural proteins NS1 and NS2P, and two minor ones (NS2Y and NS2L). A subset of the same alternative splicing strategy allows translation of the CP ORF to produce two proteins, VP1 and VP2. MVM VP3 is generated in the intact virion by proteolytic cleavage of VP2. VP1 and VP2 are identical except for their N termini. Synthesis of VP1 derives from a minor spliced mRNA containing a methionine initiation codon that allows translation of a small ORF, which encodes basic amino acid sequence motifs, upstream (5′) of the VP2-coding sequence. This expression strategy varies among viruses in the different genera and even, to a minor extent, within a genus. Parvoviruses use an alternative splice donor, while dependoviruses use an alternative splice acceptor for this purpose. The use of a leaky scanning mechanism for the generation of NS and VP products is common among densoviruses.

Members of the genera *Erythrovirus*, *Amdovirus* and *Bocavirus* use only a single promoter in their left genome end, and so regulation of their expression must be exclusively post-transcriptional. All of the parvoviruses make extensive use of alternative splicing strategies, and all members of the genera *Erythroviruses*, *Dependovirus*, *Amdovirus* and *Bocavirus* examined also use alternative polyadenylation at a site within the centre of the genome. In addition, the parvoviruses make use of alternative translation strategies for the expression of both their structural and nonstructural proteins:

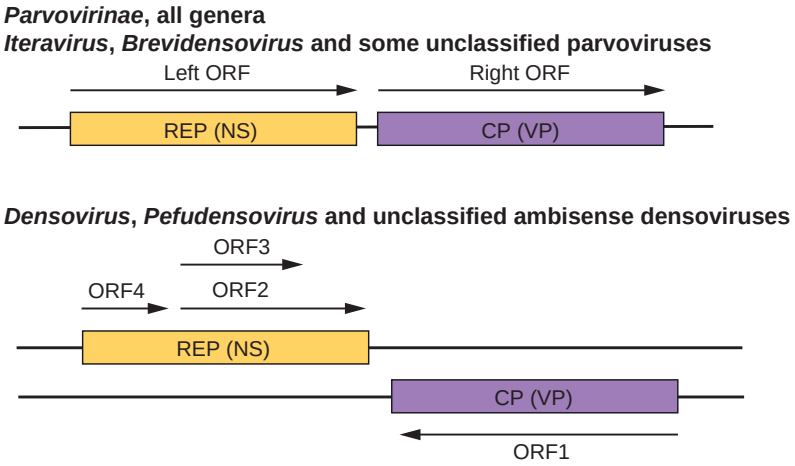


**Figure 2:** Gene organization for members of the monosense subfamily *Parvovirinae*, the genera *Iteravirus* and *Brevidensovirus*, and some unassigned parvoviruses (top). Gene organization for the ambisense densoviruses and some unassigned densoviruses (bottom).

regulation of translation is known to govern the expression of the VP2 protein of AAV-2, the capsid proteins generated by members of the genera *Amdovirus* and *Bocavirus*, and the nonstructural proteins of the goose parvovirus (GPV) subgroup of the genus *Dependovirus*.

Other parvoviruses contain two (genera *Parvovirus* and *Densovirus*) or three (genera *Dependovirus* and *Brevidensovirus*) promoters for mRNA transcription. Some of the mRNAs are spliced, thus allowing alternative forms of the protein products to be produced. The mRNA species are capped and polyadenylated either at a common 3′ site near the end of the genome (MVM and AAVs of primates), or at an alternative polyadenylation site in the centre of the genome as well as at a site near the end of the genome (all known members of the genus *Dependovirus* other than those that infect primates). Depending on the species, viruses may benefit from co-infection with other viruses, such as adenoviruses or herpesviruses, or from the effects of chemical or other treatments of the host cells. Viral proteins accumulate in the nucleus in the form of empty capsid structures. Progeny infectious virions accumulate in the cell nucleus.

Viral entry into the cell is usually by receptor-mediated endocytosis and is blocked by antagonists of vacuolar ATPase. The trafficking of virus within the cell appears to vary among members of the family, and even among species within individual genera. The PLA2 domain plays a role in the trafficking or release of particles from endosomal compartments. The process of uncoating is not well understood. Virus replication takes place in the cell nucleus and appears to require the cell to go through S-phase, indicating a close association between the host and virus replication processes. Autonomously replicating parvoviruses probably do not initiate gene expression until the host cell enters S-phase to produce viral dsDNA, whereas this transition is effected by a positive process under the control of the helper virus in the case of helper-dependent parvoviruses.

Replication proceeds through a series of duplex, concatemeric intermediates by the rolling hairpin mechanism (Figure 3), and probably involves a processive host DNA polymerase(s) (probably pol δ, possibly pol ϵ or others). In step (a) the base-paired 3′ nucleotide of the left-end hairpin is used by a host polymerase to prime conversion of virion DNA to the first duplex intermediate. This generates a monomer length duplex molecule in which the two strands are covalently continuous at the viral left-end telomere. Synthesis of this intermediate precedes viral gene expression. The 3′ end of the new DNA strand is ligated to the 5′ end of the hairpin by a host ligase, creating a covalently continuous duplex molecule (step b). Replication beyond this point requires expression of NS1, which carries out a "hairpin transfer" reaction, in which it nicks the ligated strand (step c). The replication

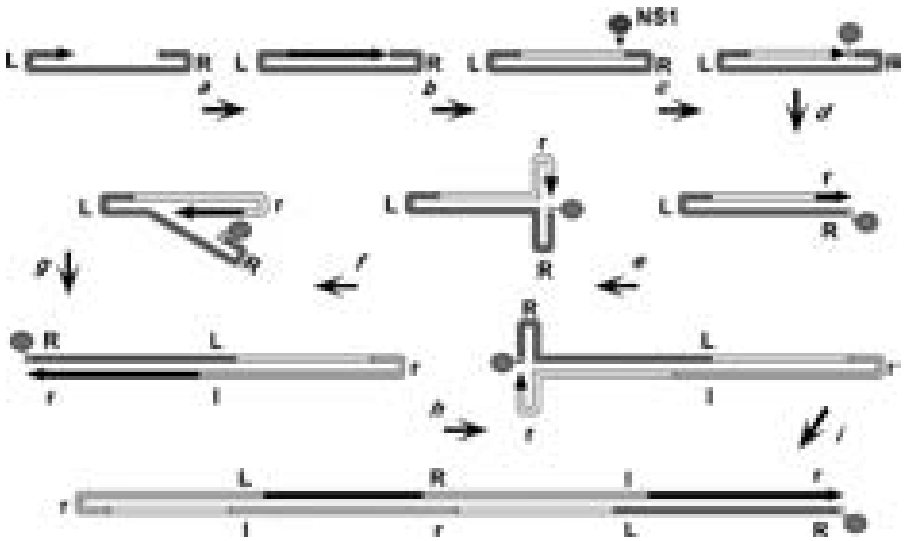


**Figure 3:** DNA replication model as determined for minute virus of mice (MVM). The newly synthesized DNA of the growing 3′ end is represented by a black arrowed line, with the original genome in dark grey. Light grey denotes progeny genomes embedded within the oligomeric intermediates. Upper and lower cases of R and L represent flip and flop forms of the right and left ends, respectively. For details see the text.

fork now unfolds and copies the hairpin, thus replacing the original sequence of the terminus with its inverted complement (step d). The terminal sequences are imperfect palindromes, and since this inversion occurs with every round of replication, progeny genomes comprise equal numbers of each terminal orientation, dubbed "flip" and "flop". In MVM replication, this hairpin transfer reaction occurs only at the right-end, because of different structural and co-factor requirements needed to activate the NS1 nickase at each terminus.

When inversion occurs on the first monomer formed after uncoating, it regenerates the "tether" sequence, lost during entry, and now attached to a newly synthesized NS1 molecule. Extended-form right-end termini are melted out and reformed into hairpin "rabbit ear" structures in a process facilitated by direct binding of NS1 to sequences in the terminus (step e). This allows the newly synthesized DNA to create the base-paired hairpin structures needed to prime synthesis of additional linear sequences (step f). This gives rise to a palindromic duplex dimeric (step g), which can undergo the same right-end rearrangement (step h), leading to the synthesis of tetrameric concatemers (step h), in which alternating unit length genomes are fused in left-end:left-end and right-end:right-end orientations. Individual genomic monomer duplexes are then excised from these concatemers by a process called junction resolution.



### Antigenic properties

Parvoviruses appear to have very stable virions that are quite simple antigenically. This has led to the use of individual serotype as a major criterion for species demarcation. Serotype has been defined by neutralization of infectivity in cell culture, hemagglutination-inhibition or specific ELISA using a capture format. Two antigenic sites, defined by mutations that confer resistance to neutralization by monoclonal antibodies, have been determined for CPV. Some, but not all, viruses representing species in a genus may be antigenically related by epitopes in the NS proteins.

### Biological properties

Autonomous parvoviruses require host cell passage through S-phase. Certain parvoviruses replicate efficiently only in the presence of helper viruses (e.g. adenoviruses or herpesviruses). These helper functions involve the adenovirus or herpesvirus early gene products and trans-activation of parvovirus replication. The helper functions appear to relate to effects of the helper virus upon the host cell rather than direct involvement of helper virus gene products in parvovirus replication. Association of parvoviruses with tumor cell lines appears to relate to increased DNA replication and/or the state of differentiation in such cells, rather than previous involvement as an etiologic agent of oncogenesis. Co-infection involving certain parvoviruses and selected oncogenic adenoviruses (or other viruses) may reduce the oncogenic effect of those viruses, possibly by promoting cell death. In certain circumstances, parvovirus DNA may integrate into the host genome, from which it may be activated by subsequent helper virus infection. The site of integration may be specific in certain hosts (e.g. the q arm of human chromosome 19 for AAV-2).

## SUBFAMILY *PARVOVIRINAE*

### Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Parvovirinae*** |
| Genus | ***Parvovirus*** |
| Genus | ***Erythrovirus*** |
| Genus | ***Dependovirus*** |
| Genus | ***Amdovirus*** |
| Genus | ***Bocavirus*** |

### Distinguishing features

Viruses assigned to the subfamily *Parvovirinae* infect vertebrates and vertebrate cell cultures, sometimes in association with other viruses.

# GENUS *PARVOVIRUS*

Type species *Minute virus of mice*

## Distinguishing features

For some members of the genus, mature virions contain virtually only negative strand DNA of 5 kb. In other members, positive strand DNA occurs also in variable proportions (1–50%). The linear molecule of ssDNA has hairpin structures at both the 5′ and 3′ ends. The 3′ terminal hairpin (left end, "–" strand) is 115–116 nt in length, the 5′ structure is 200–242 nt long. There are two mRNA promoters (map units 4 and 39) and a single polyadenylation site at the 3′ end. Characteristic cytopathic effects are induced by the viruses during replication in cell culture. Many species exhibit hemagglutination with red blood cells of one or more species. Under experimental conditions, the host range may be extended to a large number of vertebrate species (e.g. rodent viruses and LuIII virus (LuIIIV) replicate in Syrian hamsters). Transplacental transmission has been detected for a number of species.

## Genome organization and replication

Alternate splicing controls viral gene expression (Figure 4). For MVM, transcripts 1–6 are made from the promoter at 4 map units. Transcripts 1–3 encode only NS1, while transcripts 4–6, which are spliced into another reading frame by the major intron, encode NS2P, NS2Y and NS2L, respectively, the C-termini of which are different due to the use of alternative donor and acceptor splice sites bordering the small intron. A promoter at 38 map units is transactivated by NS1 to drive the synthesis of transcripts 7–9. The use of the same alternative donor splice sites that are present in the P4 transcripts controls the synthesis of the CPs, such that transcripts 7 and 9 encode VP2, while transcript 8 encodes VP1.

## Species demarcation criteria in the genus

Members of each species are antigenically distinct, as assessed by neutralization using polyclonal antisera, and natural infection is usually confined to a single host species. Generally, species are <95% related by non-structural gene DNA sequence.

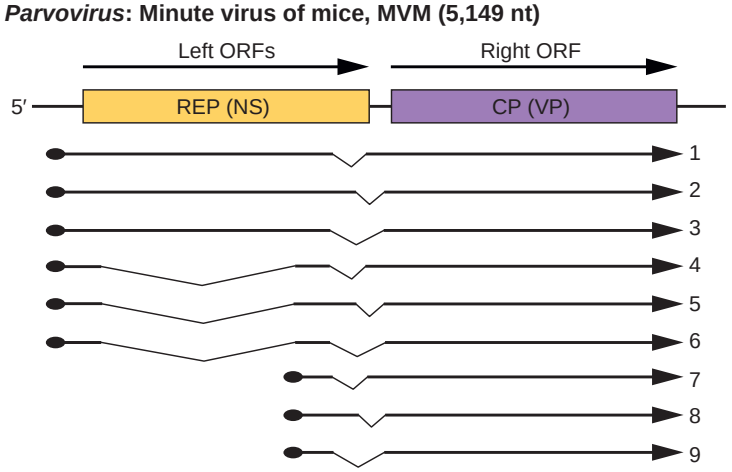


**Figure 4:** Gene organization and transcription scheme for members of the genus *Parvovirus*, as shown for minute virus of mice (MVM). Genes are shown as boxes. The left ends of the mRNAs (thick lines) are the sites of the mRNA caps (filled circles), the right ends are the polyadenylation sites (arrows); introns are indicated by thin lines.

## List of species in the genus *Parvovirus*

| | | |
|---|---|---|
| *Chicken parvovirus*[1] | | |
| Chicken parvovirus | [GU214704] | (ChPV) |
| *Feline panleukopenia virus* | | |
| Canine parvovirus strain CPV-N | [M19296] | (CPV) |
| Feline panleukopenia virus-b | [M75728] | (FPV-b) |
| Mink enteritis virus Abashiri | [D00765] | (MEV) |
| Raccoon parvovirus Georgia | [M24005] | (RPV) |
| *H-1 parvovirus* | | |
| H-1 parvovirus | [X01457] | (H-1PV) |
| *HB parvovirus* | | |
| HB parvovirus | | (HBPV) |
| *Kilham rat virus* | | |
| Kilham rat virus | [AF321230] | (KRV) |
| *Lapine parvovirus* | | |
| Lapine parvovirus | | (LPV) |
| *LuIII virus* | | |
| LuIII virus | [M81888] | (LuIIIV) |
| *Minute virus of mice* | | |
| Minute virus of mice (Cutter) | [U34256] | (MVMc) |
| Minute virus of mice (immunosuppressive) | [M12032] | (MVMi) |
| Minute virus of mice (prototype) | [J02275] | (MVMp) |
| *Mouse parvovirus 1* | | |
| Mouse parvovirus 1 | [U12469] | (MPV-1) |
| *Porcine parvovirus* | | |
| Porcine parvovirus Kresse | [U44978] | (PPV-Kr) |
| Porcine parvovirus NADL-2 | [L23427] | (PPV-NADL2) |
| *RT parvovirus* | | |
| Rat parvovirus 1 | [AF036710] | (RTPV-1) |
| *Tumor virus X* | | |
| Tumor virus X | | (TVX) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
[1]A proposal to reclassify the species *Chicken parvovirus* is currently under consideration by the ICTV.

## List of other related viruses which may be members of the genus *Parvovirus* but have not been approved as species

| | | |
|---|---|---|
| Hamster parvovirus | [U34255] | (HaPV) |
| Mouse parvovirus 2 | | (MPV-2) |
| Rat minute virus 1 | [AF332882] | (RMV-1) |
| Tumor virus X | | (TVX) |

# GENUS *ERYTHROVIRUS*

Type species ***Human parvovirus B19***

## Distinguishing features

Populations of mature virions contain equivalent numbers of positive and negative sense ssDNA, 5.5 kb in size. The DNA molecules contain inverted terminal repeats of 383 nt, the first 365 nt of which form a palindromic sequence. Upon extraction, the complementary DNA strands usually self-anneal to form dsDNA. There is a single mRNA promoter (map unit 6) and two polyadenylation signals: one near the middle of the genome, the other near the 3′ end. Efficient replication occurs in primary erythrocyte precursors. There have also been reports of productive infection in cell lines of megakaryoblastoid erythroleukemic origin.

## Genome organization and replication

For human parvovirus B19 (B19V) there is only one promoter, at 6 map units, but two alternative polyadenylation sites (Figure 5). Transcripts 1 and 2 encode VP1, and transcript 3 encodes NS1. Two small ORFs can also be accessed by these alternatively spliced mRNAs, depending upon the relative strength of the initiation codons. Transcripts 8 and 9 encode an 11 kDa protein containing three proline-rich regions that conform to consensus Src homology 3 (SH3) ligand sequences. Transcripts 1, 4, 6 and 8 are predicted to translate a 7.5 kDa polypeptide of unknown function.

## Biological properties

B19V causes Fifth Disease, polyarthropathia, anemic crises in children with underlying hematological diseases (e.g. sickle cell anemia or thalassemia) and intra-uterine infections (with hydrops fetalis in some cases).

## Species demarcation criteria in the genus

Members of each species are probably antigenically distinct, and natural infection is confined to a single host species. Species are $< 95\%$ related by non-structural gene DNA sequence.

## List of species in the genus *Erythrovirus*

| | | |
|---|---|---|
| *Human parvovirus B19* | | |
| Human parvovirus B19-A6 | [AY064475, AY064476] | (B19V-A6) |
| Human parvovirus B19-Au | [M13178] | (B19V-Au) |
| Human parvovirus B19-lali | [AY044266] | (B19V-LaLi) |
| Human parvovirus B19-V9 | [AJ223617, AJ242810] | (B19V-V9) |
| Human parvovirus B19-Wi | [M24682] | (B19V-Wi) |
| Human parvovirus B19-J35 | [AY386330] | (B19V-J35) |
| *Pig-tailed macaque parvovirus* | | |
| Pig-tailed macaque parvovirus | [AF221123] | (PmPV) |
| *Rhesus macaque parvovirus* | | |
| Rhesus macaque parvovirus | [AF221122] | (RmPV) |
| *Simian parvovirus* | | |
| Simian parvovirus (cynomolgus) | [U26342] | (SPV) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

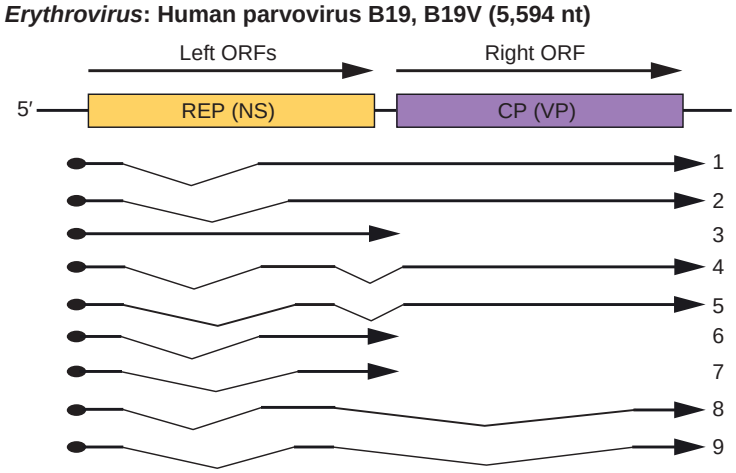


**Figure 5:** Gene organization and transcription scheme for members of the genus *Erythrovirus*, as shown for human parvovirus B19 (B19V). Genes are shown as boxes. The left ends of the mRNAs (thick lines) are the sites of the mRNA caps (filled circles), the right ends are the polyadenylation sites (arrows); introns are indicated by thin lines.

**List of other related viruses which may be members of the genus *Erythrovirus* but have not been approved yet as species**

| | | |
|---|---|---|
| Chipmunk parvovirus | [AF406967] | (ChpPV) |

# GENUS *DEPENDOVIRUS*

Type species *Adeno-associated virus-2*

## Distinguishing features

Populations of mature virions contain equivalent numbers of positive or negative strand ssDNA about 4.7 kb in size. The DNA molecules contain inverted terminal repeats of 145 nt, the first 125 nt of which form a palindromic sequence. Upon extraction, the complementary DNA strands usually form dsDNA. There are three mRNA promoters (map units 5, 19, 40) (Figure 6). For all currently accepted members of the genus *Dependovirus*, except for the duck and goose parvoviruses, efficient replication is dependent upon helper adenoviruses or herpesviruses. Under certain conditions (presence of mutagens or synchronization of cell replication with hydroxyurea), replication can also be detected in the absence of helper viruses. All isolates of adeno-associated virus share a common antigen as demonstrated by fluorescent antibody staining. Transplacental transmission has been observed for adeno-associated virus-1 (AAV-1) and vertical transmission has been reported for avian adeno-associated virus (AAAV).

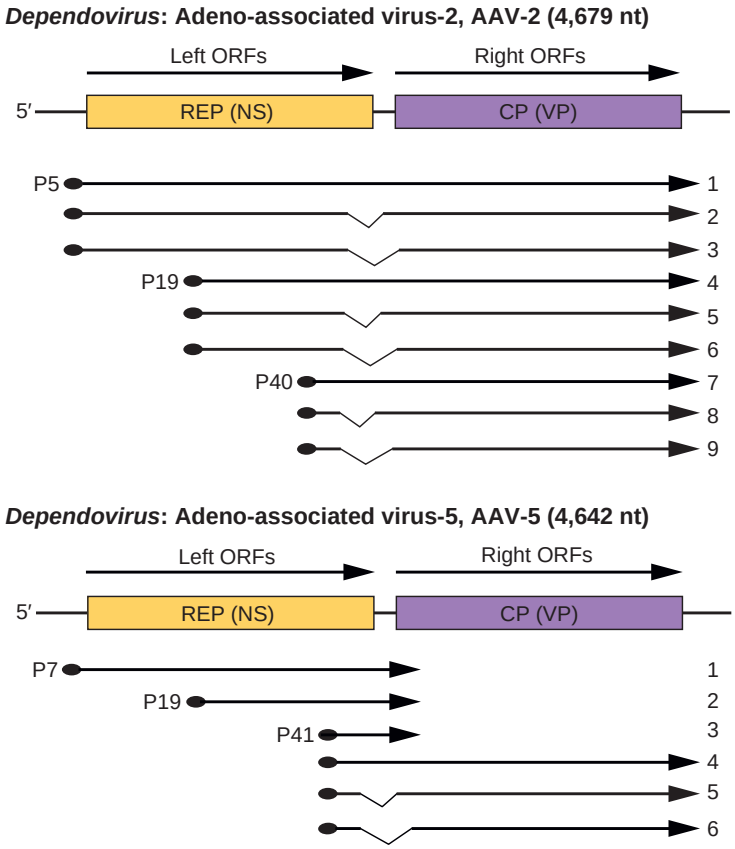


**Figure 6:** Gene organization and transcription scheme for members of the genus *Dependovirus*, as shown for adeno-associated virus 2 (AAV-2) and the nonprimate adeno-associated virus 2 (AAV-5). Genes are shown as boxes. The left ends of the mRNAs (thick lines) are the sites of the mRNA caps (filled circles), the right ends are the polyadenylation sites (arrows); introns are indicated by thin lines. In contrast to the primate AAVs, nonprimate AAVs also have a polyadenylation site in the middle of the genome. Both types of AAVs have 3 promoters.

## Genome organization and replication

Dependoviruses have three transcriptional promoters, at 5, 19 and 40 map units, which transcribe mRNAs in a temporally regulated fashion throughout infection (Figure 6). P5 transcripts are the first to be expressed, followed by those from P19, then those from P40. P5 transcript 1 encodes the non-structural proteins Rep78, and transcripts 2 and 3 encode Rep 68. These two forms of the Rep protein differ at their C-termini. Likewise, P19 transcripts encode two Rep forms, Rep52 from transcript 4 and Rep48 from trancripts 5 and 6. P40 transcripts encode the structural proteins, transcript 8 encoding VP1 and transcript 9 encoding VP2 and VP3 by an alternate translation initiation mechanism. The unspliced P40 transcript (7) does not appear to encode a functional protein.

RNAs generated from both the P7 (analogous to AAV-2 P5) and P19 promoters of all the non-primate members of the genus *Dependovirus* (exemplified by AAV-5 in Figure 6) are polyadenylated efficiently at a site lying within the intronic region in the centre of the genome, termed (pA)p. Because AAV5 P7- and P19-generated transcripts are polyadenylated at this site and not spliced, Rep78 and Rep52 are the only Rep proteins expressed as primary translation products during AAV-5 infection.

## Species demarcation criteria in the genus

Members of each species are antigenically distinct, as assessed by neutralization using polyclonal antisera, and natural infection is usually confined to a single host species. Generally, species are <95% related by non-structural gene DNA sequence.

## List of species in the genus *Dependovirus*

| | | |
|---|---|---|
| *Adeno-associated virus-1* | | |
| Adeno-associated virus-1 | [AF063497] | (AAV-1) |
| Adeno-associated virus-6 | [AF208704] | (AAV-6) |
| *Adeno-associated virus-2* | | |
| Adeno-associated virus-2 | [J01901] | (AAV-2) |
| *Adeno-associated virus-3* | | |
| Adeno-associated virus-3 | [AF028705] | (AAV-3) |
| *Adeno-associated virus-4* | | |
| Adeno-associated virus-4 | [U89790] | (AAV-4) |
| *Adeno-associated virus-5* | | |
| Adeno-associated virus-5 | [AF085716] | (AAV-5) |
| *Avian adeno-associated virus* | | |
| Avian adeno-associated virus | [AY186198] | (AAAV) |
| *Bovine adeno-associated virus* | | |
| Bovine adeno-associated virus | | (BAAV) |
| *Canine adeno-associated virus* | | |
| Canine adeno-associated virus | | (CAAV) |
| *Duck parvovirus* | | |
| Duck parvovirus | [U22967] | (BDPV) |
| *Equine adeno-associated virus* | | |
| Equine adeno-associated virus | | (EAAV) |
| *Goose parvovirus* | | |
| Goose parvovirus | [U25749] | (GPV) |
| *Ovine adeno-associated virus* | | |
| Ovine adeno-associated virus | | (OAAV) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Dependovirus* but have not been approved as species

| | | |
|---|---|---|
| Adeno-associated virus-7 | [AF513851] | (AAV-7) |
| Adeno-associated virus-8 | [AF513852] | (AAV-8) |
| Adeno-associated virus-9 | [AX753250] | (AAV-9) |
| Snake adeno-associated virus | [AY349010] | (SAAV) |

# Genus *Amdovirus*

Type species *Aleutian mink disease virus*

## Distinguishing features

Most features are shared with members of the genera *Parvovirus* and *Bocavirus*. Mature virions contain negative strand DNA of 4748 nt in length. Permissive replication is observed only in Crandell feline kidney cells, although restricted replication is observed, and may be antibody-dependent, in cells bearing Fc receptors (e.g. macrophages). Evidence of infection has been detected in most mustelids, skunks and raccoons. Virion structure differs slightly from members of the genera *Parvovirus* and *Bocavirus*, and resembles that of the genus *Dependovirus*. The primary difference is the presence of three mounds elevated above the capsid surface around the three-fold icosahedral axis of symmetry, similar to those observed for dependovirus virions. The VP1 N terminus is much shorter than those found for other members of the subfamily *Parvovirinae*, and there is no evidence of a phospholipase 2A enzymatic core.

## Genome organization and replication

AMDV generates six species of mRNAs, which are produced by alternative splicing and alternative polyadenylation of a pre-mRNA generated by a single promoter at the left end of the genome (Figure 7). Three different splicing patterns are used, and each type is found polyadenylated at either the distal 3′ end of the genome or at a proximal site (pA)p in the centre of the genome. The R1 class of mRNAs encode the viral replicator protein NS1. The R2 mRNA, which is the predominant RNA produced during infection, expresses the NS2 coding region and is also essentially the sole source of the viral capsid proteins VP1 and VP2. Caspase processing of the NS1 protein is required to produce a sub-species that locates to the nucleus. This processing is essential for completion of the viral life cycle.

## Antigenic properties

All isolates in the species appear to be antigenically indistinguishable.

## Biological properties

In susceptible adult hosts, pathogenic isolates cause a persistent, restricted infection associated with a progressive disorder of the immune system, including plasmacytosis, glomerulonephritis and hypergammaglobulinemia. Extremely high levels of antiviral antibody are directed at determinants on the virus capsid surface. In newborn animals, infection is permissive and causes a fulminant interstitial pneumonitis that is often fatal. Survivors develop an adult form of disease.

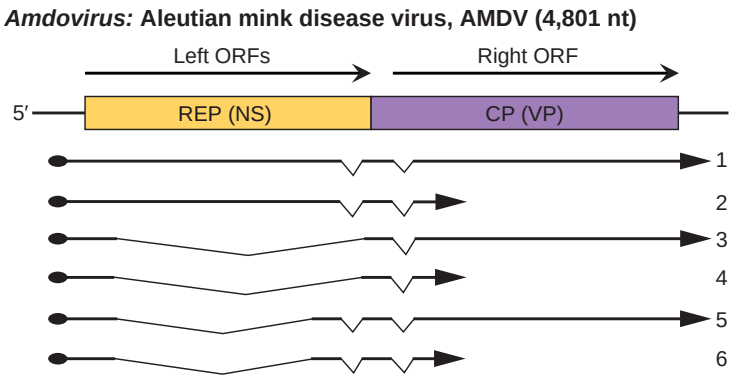


**Figure 7:** Gene organization and transcription scheme for the member of the genus *Amdovirus*, as shown for Aleutian mink disease virus (AMDV). Genes are shown as boxes. The left ends of the mRNAs (thick lines) are the sites of the mRNA caps (filled circles), the right ends are the polyadenylation sites (arrows); introns are indicated by thin lines (1 = R1; 2 = R1′; 3 = R2; 4 = R2′; 5 = Rx; 6 = Rx′).

## List of species in the genus *Amdovirus*

*Aleutian mink disease virus*
Aleutian mink disease virus-G [M20036] (AMDV-G)

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

# GENUS *BOCAVIRUS*

Type species ***Bovine parvovirus***

## Distinguishing features

The genome is ssDNA of 5.5 kb, with a monosense strategy and non-identical terminal palindromes. Sequence analysis of BPV and canine minute virus (CnMV) demonstrate that, while their NS1 and VP1 genes are 34% and 41% similar to one another, the two genomes are very distinct from all other clusters of viruses in the subfamily *Parvovirinae*. In addition, the bocavirus genome encodes a 22.5 kDa nuclear phosphoprotein, NP-1, that is distinct from any other parvovirus-encoded polypeptide.

## Genome organization and replication

Large ORFs within the left and right halves of the genome encode the NS and VP proteins, respectively, while a shorter ORF in the middle of the genome, overlapping the C-terminal sequence of the NS1 protein, encodes NP-1. All RNAs are generated from a single promoter at the left genome end at approximately 4.5 mu. BPV RNAs are alternatively spliced and alternatively polyadenylated (either at a site in the centre or at the 3′-end of the genome) to generate at least 8 mRNAs (Figure 8).

## Species demarcation criteria in the genus

Members of each species are probably antigenically distinct, and natural infection is confined to a single host species. Species are <95% related by non-structural gene DNA sequence. The four distinct genetic lineages of human bocavirus show a significant divergence, >8% amino acid and >10% nucleotide difference in the complete VP gene, but a low level of intraspecies diversity.

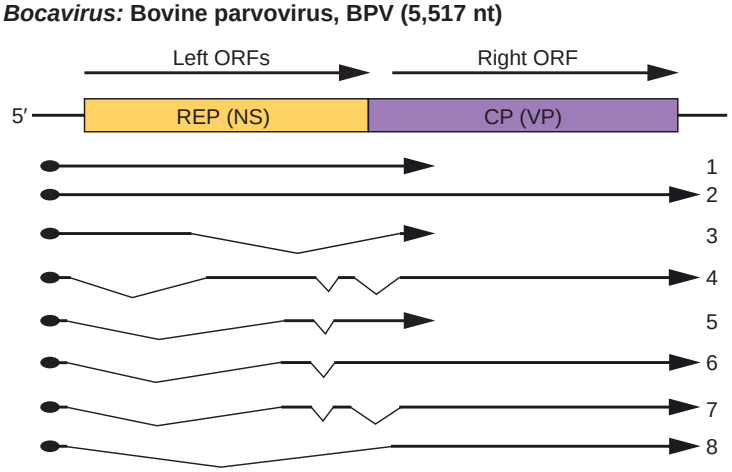


**Figure 8:** Gene organization and transcription scheme for members of the genus *Bocavirus*, as shown for bovine parvovirus (BPV). Genes are shown as boxes. The left ends of the mRNAs (thick lines) are the sites of the mRNA caps (filled circles), the right ends are the polyadenylation sites (arrows); introns are indicated by thin lines

## List of species in the genus *Bocavirus*

| | | |
|---|---|---|
| *Bovine parvovirus* | | |
| Bovine parvovirus | [M14363] | (BPV) |
| *Canine minute virus* | | |
| Canine minute virus | [AF495467] | (CnMV) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the subfamily *Parvovirinae* but have not yet been approved as a genus or a species

Human bocavirus 1 (HBoV-1) is primarily a respiratory virus, whereas human bocavirus 2 to 4 (HBoV-2, HBoV-3 and HBoV-4) are prevalent in stool samples.

| | | |
|---|---|---|
| Human bocavirus-1 | [DQ000496] | (HBoV-1) |
| Human bocavirus-2 | [FJ170278] | (HBoV-2) |
| Human bocavirus-3 | [EU918736] | (HBoV-3) |
| Human bocavirus-4 | [FJ973561] | (HBoV-4) |

## Unclassified vertebrate parvoviruses

Among vertebrate parvoviruses that may need to be reclassified into new genera or have not yet been classified, three groups can be discerned: PARV4 and related viruses, chicken parvovirus and related viruses, and a diverse group with no clear taxonomical structure. These groups lack significant sequence identity with members of the accepted genera in the subfamily *Parvovirinae*, and are also significantly different from each other.

PARV4 virus (PARV4) is widely distributed in injecting drug users in the United States and Europe. Genotypes 1 and 2 (formerly named PARV5, found in older coagulation concentrates) are phylogenetically distinct, as are PARV4 sequences detected in a study of sub-Saharan Africans (93%; equidistant from genotypes 1 and 2). Additionally, PARV4-like viruses, with a 60–65% nucleotide identity but identical genome organizations, were recently identified at high frequencies in porcine and bovine tissue samples. These viruses have a 5.3 kb genome and ITRs of about 150–200 nt. The largest capsid protein contains also a PLA2 motif and a conserved SAT sequence that may be expressed late during infection. A new genus has been proposed for this group.

| | | |
|---|---|---|
| Bovine hokovirus | [EU200669] | (BoHV) |
| PARV4 virus | [AY622943] | (PARV4V) |
| Porcine hokovirus | [EU200677] | (PoHV) |

The second group contains chicken and turkey parvoviruses, which have a 5 kb genome with a somewhat larger ITR (about 210 nt). However, the capsid proteins do not contain the PLA2 domain that most parvoviruses use for cell entry. A new genus has been proposed for this group.

| | | |
|---|---|---|
| Turkey parvovirus 260 | [GU214706] | (TuPV-260) |

Several parvoviruses from bovine and porcine tissues remain unclassified. Erythroviruses are the closest relatives of bovine parvovirus 3 (BPV3), with a 5.3 kb genome, but remain quite different. Bovine parvovirus 2 (BPV2) has a 5.6 kb genome with two ORFs (phylogenetically different from any other parvovirus). Porcine parvovirus 4 (PPV4) is closest to BPV2 but has an additional ORF in the middle of the genome. The only known porcine parvovirus 2 (PPV2) sequence is part of a dimeric replicative form. These unassigned viruses all contain a PLA2 domain.

| | | |
|---|---|---|
| Bovine parvovirus-2 | [[AF406966] | (BPV2) |
| Bovine parvovirus-3 | [AF406967] | (BPV3) |
| Porcine parvovirus-2 | [AB076669] | (PPV2) |
| Porcine parvovirus-4 | [GQ387499] | (PPV4) |

# SUBFAMILY *DENSOVIRINAE*

## Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Densovirinae*** |
| Genus | ***Iteravirus*** |
| Genus | ***Brevidensovirus*** |
| Genus | ***Densovirus*** |
| Genus | ***Pefudensovirus*** |

## Distinguishing features

Viruses assigned to the subfamily *Densovirinae* infect arthropods. The ssDNA genome of virions is either monosense (viruses in the genera *Iteravirus* and *Brevidensovirus*, and some unassigned densoviruses) or ambisense (viruses in the genera *Densovirus* and *Pefudensovirus*, and some unassigned densoviruses). Either strand may be packaged. There may be 1–5 structural proteins. Viruses multiply efficiently either in most of the tissues of larvae, nymphs and adults of the host species, without the involvement of helper viruses, or exclusively in the midgut. Cellular changes consist of hypertrophy of the nucleus with accumulation of virions therein to form dense, voluminous, intranuclear masses. The known host range includes members of the insect orders Dictyoptera, Diptera, Hemiptera, Homoptera, Lepidoptera, Odonata and Orthoptera. Some densoviruses infect and multiply in shrimps.

# GENUS *ITERAVIRUS*

Type species ***Bombyx mori densovirus***

## Distinguishing features

The ssDNA genome is about 5 kb in size with ITRs. Populations of virions encapsidate equal numbers of plus and minus strands. ORFs for both the structural and nonstructural proteins are located on the same strand.

## Genome organization and replication

There is apparently one mRNA promoter upstream of each ORF. There is a small ORF on the complementary strand of some of these viruses that may not be coding. The DNA has an inverted terminal repeat of 230 nt, the first 159 nt are palindromic and can form a J-shaped hairpin structure when folded. There are two ORFs for non-structural proteins and one ORF for four structural proteins.

## Species demarcation criteria in the genus

Members of each species are probably antigenically distinct, and natural infection is confined to a single host species. Species are <95% related by non-structural gene DNA sequence.

## List of species in the genus *Iteravirus*

*Bombyx mori densovirus*

| | | |
|---|---|---|
| Bombyx mori densovirus | [AY033435] | (BmDNV) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Iteravirus* but have not been approved as species

| | | |
|---|---|---|
| Casphalia extranea densovirus | [AF375296] | (CeDNV) |
| Dendrolimus punctatus densovirus | [AY665654] | (DpDNV) |
| Sibine fusca densovirus | | (SfDNV) |

## GENUS *BREVIDENSOVIRUS*

Type species ***Aedes aegypti densovirus***



### Distinguishing features

The genome is about 4 kb in size with terminal hairpins, but no ITRs. ORFs for the structural and nonstructural proteins are on the same strand. The brevidensoviruses are at least as different, in sequence, from other members of the subfamily *Densovirinae* as these are from any member of the subfamily *Parvovirinae*. Populations of virions encapsidate positive and negative strands, but a majority of strands are of negative polarity (85%).

### Genome organization and replication

A palindromic sequence of 146 nt is at the 3′-end of the genome and a different palindromic sequence of 164 nt is at the 5′-end. Both terminal sequences can fold to form a T-shaped structure. The genome does not contain any sequence recognizable as a PLA2 domain. There are mRNA promoters at map units 7, 7.5 and 60. There is a small ORF of unknown function on the complementary strand. PstDNV, infecting shrimps, has a weak sequence identity with other brevidensoviruses, probably partially because of different codon preferences of the hosts. However, the overall genome organization is similar.

### Species demarcation criteria in the genus

Members of each species are probably antigenically distinct, and natural infection is confined to a single host species. Species are <95% related by non-structural gene DNA sequence.

### List of species in the genus *Brevidensovirus*

| | | |
|---|---|---|
| *Aedes aegypti densovirus* | | |
| Aedes aegypti densovirus | [AY160976] | (AaeDNV) |
| *Aedes albopictus densovirus* | | |
| Aedes albopictus densovirus | [AY095351] | (AalDNV) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Brevidensovirus* but have not been approved as species

| | | |
|---|---|---|
| Anopheles gambiae densovirus | [EU233812] | (AgDNV) |
| Culex pipiens pallens densovirus | [EF579756] | (CppDNV) |
| Haemogogus equinus densovirus | [AY605055] | (HeDNV) |
| Penaeus stylirostris densovirus | [AF273215] | (PstDNV) |
| Simulium vittatum densovirus | | (SvDNV) |
| Toxorhynchites amboinensis densovirus | | (TaDNV) |
| Toxorhynchites splendens densovirus | [AF395903] | (TsDNV) |

# GENUS *DENSOVIRUS*

Type species ***Junonia coenia densovirus***

## Distinguishing features

The ssDNA genome is about 6 kb in size with long (>0.5 kb) inverted terminal repeats and ambisense organization (Figure 2). Populations of virions encapsidate equal amounts of positive and negative strands.

## Genome organization and replication

On one strand there are three ORFs which encode NS proteins on mRNAs transcribed from a promoter 10 map units from the left end. The four structural proteins are encoded on the complementary strand, on an mRNA transcribed from a promoter at 90 map units, towards the centre of the genome. The NS and VP transcripts have a short overlap in the centre of the genome. NS3 protein is produced by an unspliced mRNA, whereas NS1 and NS2 are produced by a spliced mRNA and leaky scanning translation. The four VP proteins (VP1-4), and NS1 and NS2 are produced by a leaky scanning translation initiation mechanism (Figure 9). The Junonia coenia densovirus genome has an inverted terminal repeat of 517 nt, the first 96 nt of which can fold to form a T-shaped structure of the type found in the ITR of AAV DNA.

## Species demarcation criteria in the genus

Members of each species are probably antigenically distinct, and natural infection is confined to a single host species. Species are <95% related by non-structural gene DNA sequence.

## List of species in the genus *Densovirus*

| | | |
|---|---|---|
| *Galleria mellonella densovirus* | | |
| Galleria mellonella densovirus | [L32896] | (GmDNV) |
| *Junonia coenia densovirus* | | |
| Junonia coenia densovirus | [S17265] | (JcDNV) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

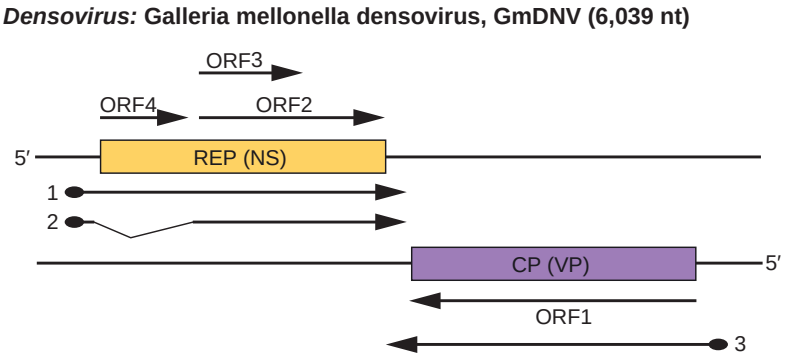


**Figure 9:** Gene organization and transcription scheme for members of the ambisense genus *Densovirus*, as shown for Galleria mellonella densovirus (GmDNV). Genes are shown as boxes as well as the ORFs that they contain. The starts of the mRNAs (thick lines) are the sites of the mRNA caps (filled circles), the ends are the polyadenylation sites (arrows); the intron is indicated by thin lines. ORF1 and ORF2/3 are translated by a leaky scanning mechanism from transcript 3 and 2, respectively.

### List other related viruses which may be members of the genus *Densovirus* but have not been approved as species

| | | |
|---|---|---|
| Diatraea saccharalis densovirus | [AF036333] | (DsDNV) |
| Helicoverpa armigera densovirus | | (HaDNV) |
| Mythimna loreyi densovirus | [AY461507] | (MlDNV) |
| Pseudoplusia includens densovirus | | (PiDNV) |

## GENUS *PEFUDENSOVIRUS*

Type species ***Periplaneta fuliginosa densovirus***

### Distinguishing features

The genome is about 5.5 kb in size, with ambisense organization (Figure 2) and relatively small ITRs (125–175 nt). The VP gene is split into a large and a small ORF (upstream) on the 5′ half of the complementary strand. Unlike other members of the family *Parvoviridae* that have proteins with a PLA2 domain, the pefudensoviruses have a PLA2 motif that is located in the C-terminal portion (centred 60–70 amino acid residues from the C-terminus) of the protein predicted to be translated from the small VP ORF.

### Genome organization and replication

The genes for the three non-structural proteins of pefudensoviruses are organized in the same way as those in members of the genus *Densovirus*, and are of similar sizes. The ORFs of the split VP gene are spliced in order to code for the largest VPs. Unlike all other members of the subfamilies *Parvovirinae* and *Densovirinae*, VP1 and VP2 have different N-termini. The N-terminus of VP2 lies within the VP-A region, whereas VP1 is encoded from transcript 4 from both the VP-A and VP-B regions. The smaller VP-B region also gives rise to some NS proteins (transcripts 5 and 6). The PLA2 motif in this region is localized in the intron of the VP-B region and is therefore only present in the transcripts that use the downstream splicing sites (connecting the 2 VP ORFs) (Figure 10). Several other densoviruses have a genome organization that is identical to that of PfDNV, but their sequence identity is very low. This may be due to the variation in insect orders to which the hosts of these viruses belong.

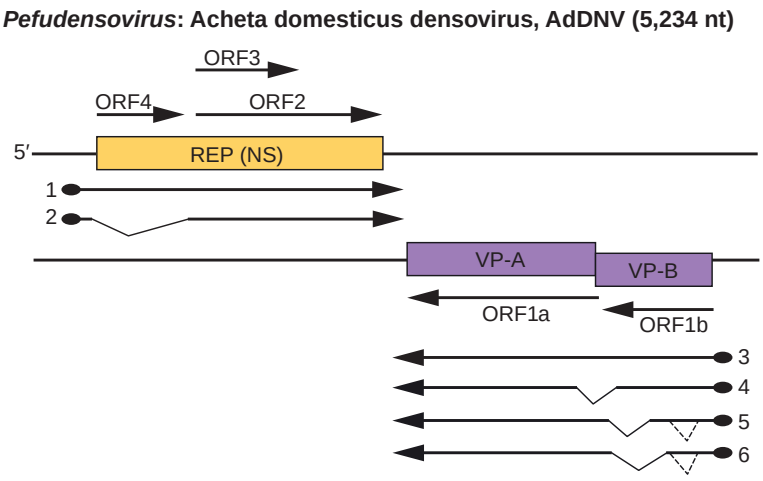


**Figure 10:** Gene organization and transcription scheme for members of the ambisense genus *Pefudensovirus*, as shown for Acheta domesticus densovirus (AdDNV). Genes are shown as boxes as well as the ORFs that they contain. The starts of the mRNAs (thick lines) are the sites of the mRNA caps (filled circles), the ends are the polyadenylation sites (arrows); the intron is indicated by thin lines. ORF1a/b and ORF2/3 are translated by a leaky scanning mechanism from transcript 4 and 2, respectively. Transcript 1 yields NS3 (ORF4), transcript 2 yields NS1 and NS2, transcript 3 yields VP2, transcript 4 yields VP1, VP3 and VP4 (possibly VP5), transcripts 5 and 6 yield also nonstructural proteins (ORF1b). There are some minor variations in the expression strategies of these viruses (e.g. an extra intron for Periplaneta fuliginosa densovirus (PfDNV) indicated by dashed lines).

## Species demarcation criteria in the genus

Members of each species are probably antigenically distinct, and natural infection is confined to a single host species. Species are <40% related by non-structural gene DNA sequence. VP1 and VP2 have different N-termini, i.e. VP2 is not fully contained within VP1 as for all other parvovirus genera.

## List of species in the genus *Pefudensovirus*

*Periplaneta fuliginosa densovirus*

| | | |
|---|---|---|
| Periplaneta fuliginosa densovirus | [AF192260] | (PfDNV) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Pefudensovirus* but have not been approved as species

| | | |
|---|---|---|
| Acheta domesticus densovirus | [AX344110] | (AdDNV) |
| Blattella germanica densovirus | [AY189948] | (BgDNV) |
| Planococcus citri densovirus | [AY032882] | (PcDNV) |

## List of other related viruses which may be members of the subfamily *Densovirinae* but have not yet been approved as a genus or a species

Among densoviruses infecting invertebrates, but not belonging to accepted genera, four groups can be discerned: the monosense Penaeus monodon DNV (PmDNV) and related viruses, Dysaphis plantaginea densovirus (DplDNV; ambisense) and related viruses, Culex pipiens densovirus and related viruses (ambisense), and a diverse group with no clear taxonomical structure. These virus groups lack significant sequence identity or genome organizational similarities among each other or with accepted genera.

The genome of PmDNV and related viruses is about 6.3 kb in size with terminal hairpins, but no ITRs. ORFs for the structural and nonstructural proteins are on the same strand. These densoviruses are at least as different in sequence from other members of the subfamily *Densovirinae* as they are from any member of the subfamily *Parvovirinae*. Populations of virions encapsidate mostly strands of negative polarity. The genome does not contain ITRs nor any sequence recognizable as a PLA2 domain. There are probably two mRNA promoters at map units 5 and 50, and the virus probably codes for a single 54 kDa capsid protein that lacks the PLA2 motif. The left and right hairpins of the genome are 136 and 170 nt long, respectively. The original name of these viruses was hepatopancreatic parvovirus ("HPV"), and a new genus has been proposed for this group.

| | | |
|---|---|---|
| Penaeus chinensis densovirus | [AY008257] | (PchDNV) |
| Penaeus merguiensis densovirus | [DQ458781] | (PmeDNV) |
| Penaeus monodon densovirus | [DQ002873] | (PmoDNV) |

Dysaphis plantaginea densovirus induces a low reproduction rate in rosy apple aphids but can produce a wing morph of the host to promote dispersal. Although the sequences for DplDNV and Myzus persicae densovirus (MpDNV) are still incomplete (about 65% sequence identity), a unique expression strategy is emerging. Like for the genus *Pefudensovirus* members, the VP coding sequence is split into two ORFs. However, the NS1 and NS2 ORFs are split as well, and spliced to yield in-frame coding sequences. A new genus has been proposed for this group.

| | | |
|---|---|---|
| Dysaphis plantaginea densovirus | [EU851411] | (DplDNV) |
| Myzus persicae densovirus | [AY148187] | (MpDNV) |

The third group contains so far a single virus, Culex pipiens densovirus (CpDNV), with a 6 kb genome. It shares some characteristics with lepidopteran viruses belonging to the genus *Densovirus*,

such as a single ORF for VP. However, it deviates with respect to the structure of ITRs, which are significantly shorter (285 nt), lack the GAC repeats assumed to function as an NS1-binding site, and the 68 terminal nt fold back to generate a J-shaped structure. The second difference concerns the split NS1 and NS2 coding sequences each in two ORFs, implying a splicing of the mRNA to put their N-terminal and C-terminal sequences in frame. A third significant difference lies in the mode of expression of NS genes, which requires two specific promoters, one (P7) to drive expression of NS3 and one (P17) to drive expression of NS1 and NS2. A new genus has been proposed for this group.

| | | |
|---|---|---|
| Culex pipiens densovirus | [FJ810126] | (CpDNV) |

Several densoviruses remain unassigned due to the lack of sequence information. Agraulis vanillae densovirus (AvDNV), Euxoa auxilliaris densovirus (EaDNV), Lymantria dispar densovirus (LdiDNV) and Pieris rapae densovirus (PrDNV) have lepidopteran hosts, whereas Leucorrhinia dubia densovirus (LduDNV) infects Odonata. LdiDNV was isolated from cell lines.

| | |
|---|---|
| Agraulis vanillae densovirus | (AvDNV) |
| Euxoa auxilliaris densovirus | (EaDNV) |
| Leucorrhinia dubia densovirus | (LduDNV) |
| Lymantria dispar densovirus | (LdiDNV) |
| Pieris rapae densovirus | (PrDNV) |



## Phylogenetic relationships within the family *Parvoviridae*

Only the NS1 gene product has conserved motifs among all parvoviruses. Nevertheless, many features are conserved among all parvo- and densoviruses. Figure 11 shows that a low sequence identity still can yield very similar structures. For instance, the VPs of PPV and MVM can be superimposed. Despite low sequence identity (52%), 97% of the $C_\alpha$s of residue pairs have similar positions (root-mean-square errors of 1.0 Å). It can be expected that proteins with sequence identities higher than 30% have quite similar structures. Even between PPV and GmDNV, 40% of their VPs residues align despite only 9% sequence identity (RMS error of 3.0 Å). Nevertheless, phylogenetic analysis (here the Phylip neighbour-joining method, Figure 11) is useful, in conjunction with genomic organizations, to distinguish the subfamiles, genera and possible new genera within the family.

## Similarity with other taxa

Previously, some Bombyx mori densoviruses (BmDNV2 and BmDNV3 isolated from the silkworm) were also classified among the parvoviruses. Although they also contained a linear, ssDNA genome, this is as far as the similarity goes. Their genome is split (hence their description as bidensoviruses), totals 14 kb instead of the 4–6 kb of the parvoviruses, and has different terminal structures. Moreover, they contain a polymerase motif, in contrast to parvoviruses, no PLA2 motif and have a different capsid composition. The conserved NS1 of parvoviruses, having similar helicase and rolling-circle replication motifs, does not have a bidensovirus counterpart.

## Derivation of names

*Adeno*: from Greek *aden*, "gland".
*Amdo*: from *A*leutian *m*ink *d*isease.
*Bocavirus*: from *bo*vine and *ca*nine
*Brevi*: from Latin *brevis*, "short".
*Denso*: from Latin *densus*, "thick, compact".
*Dependo*: from Latin *dependeo*, "to hang down".
*Erythro*: from Greek *erythros*, "red".
*Parvo*: from Latin *parvus*, "small".
*Pefu*: from *Pe*riplaneta *fu*liginosa densovirus.

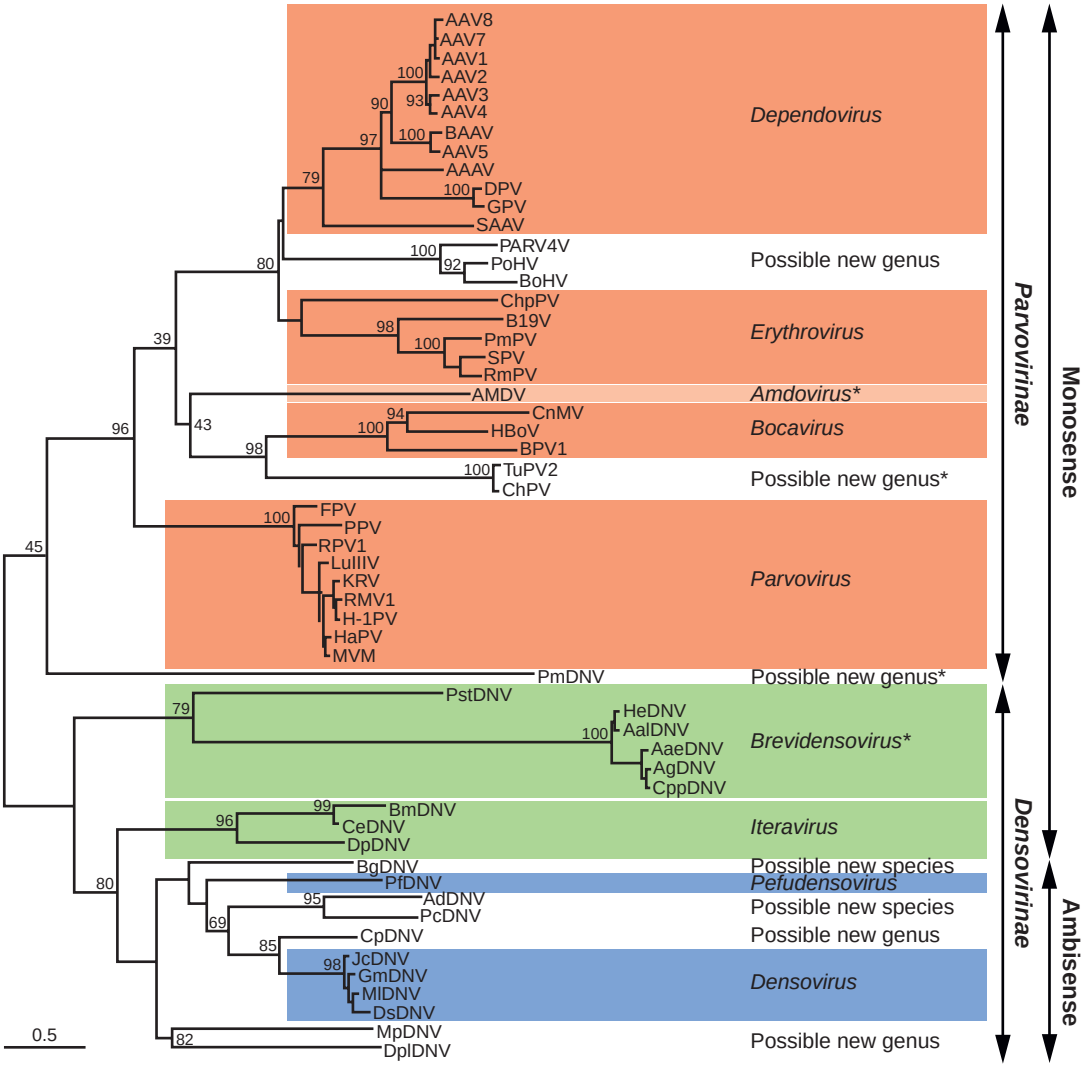


**Figure 11:** Phylogenetic relationship among the pleiotropic NS1 proteins of members of the family *Parvoviridae*. The tree was constructed with the programs included in the Phylip package at the http://mobyle.pasteur.fr/cgi-bin/portal.py website (ClustalW-multialign, Phylip distance matrix, PROTDIST, Neighbor-Joining method, and phylogenetic tree drawing). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The scale bar represents the rate of amino acid substitutions. This phylogenetic analysis distinguishes the two subfamilies, as well as the monosense and ambisense densoviruses, and recognizes the different genera as separate clades. Several other clades that are possible new genera, as described in the text, are also recognized. The asterisk indicates clades of viruses that do not contain the phospholipase A2 motif in their capsid proteins.

## Further reading

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## Contributed by

Tijssen, P., Agbandje-McKenna, M., Almendral, J.M., Bergoin, M., Flegel, T.W., Hedman, K., Kleinschmidt, J., Li, Y., Pintel, D.J. and Tattersall, P.

# FAMILY *CAULIMOVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Caulimoviridae*** |
| Genus | ***Caulimovirus*** |
| Genus | ***Petuvirus*** |
| Genus | ***Soymovirus*** |
| Genus | ***Cavemovirus*** |
| Genus | ***Badnavirus*** |
| Genus | ***Tungrovirus*** |

## Virion properties

### MORPHOLOGY

Virions are either isometric or bacilliform depending on the genus (Figure 1). There is no envelope.

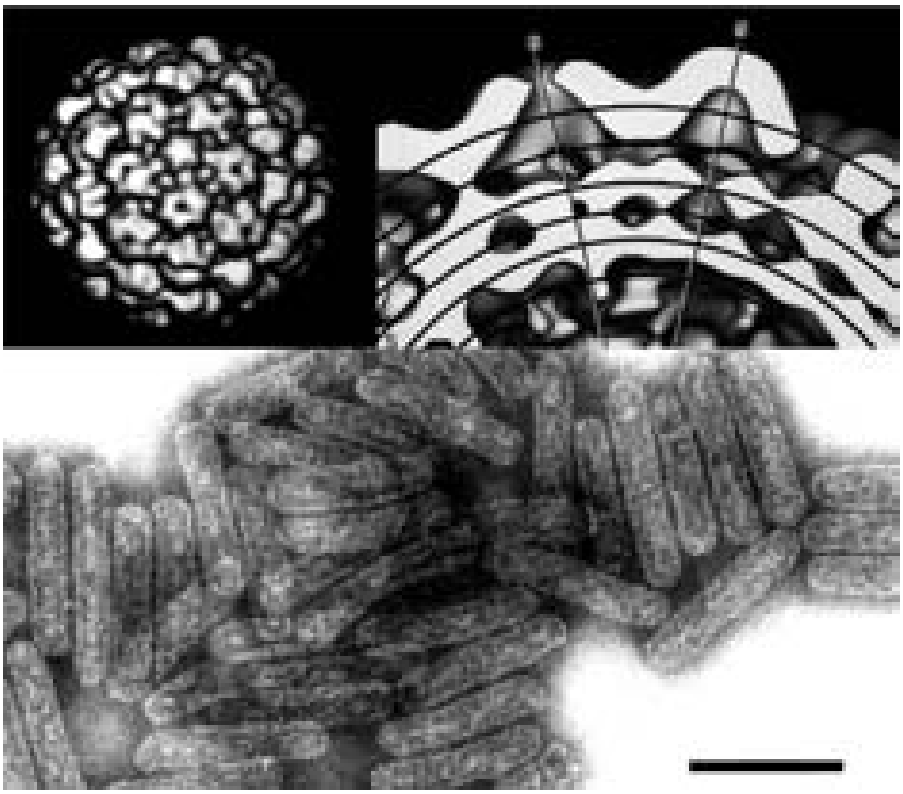

**Figure 1:** (Top left) Reconstruction of the surface structure of a cauliflower mosaic virus particle showing T = 7 symmetry. (Top right) Cutaway surface reconstruction showing multilayer structure. (From Cheng *et al.* (1992). *Virology*, **186**, 655–668). (Bottom) Negative contrast electron micrograph of particles of Commelina yellow mottle virus, stained with 2% sodium phosphotungstate, pH 7.0. The bar represents 10 nm.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virions have buoyant densities in CsCl of 1.37 g cm$^{-3}$ (genera with isometric virions) or in $Cs_2SO_4$ of 1.31 g cm$^{-3}$ (genera with bacilliform virions). $S_{20,w}$ is in the range of 200S to 220S. Particles are very stable between pH4 and pH9 and in high salt concentrations.

### Nucleic acid
Virions contain a single molecule of non-covalently closed circular dsDNA of 7.2–9.2 kbp. Each strand of the genome has discontinuities at specific places: the minus-strand has one discontinuity and the plus-strand has between one and three discontinuities.

### Proteins
Genomes contain between one and eight ORFs, depending on the genus. The virus-encoded proteins common to all genera are a movement protein, a coat protein, a multipurpose virion-associated protein, an aspartic protease and a reverse transcriptase (RT) with associated RNAse H1 activity.

### Lipids
None reported.

### Carbohydrates
The coat protein of cauliflower mosaic virus (CaMV) is glycosylated. No carbohydrates have been reported for the virions of other species.

## Genome organization and replication
By convention, numbering of the genome starts at the 5′ end of the minus-strand primer-binding site. One strand of DNA contains the coding sequence (plus-strand). The genome organization is dependent upon the genus (Figure 2) and is one of the main characteristics that distinguish the genera from each other.

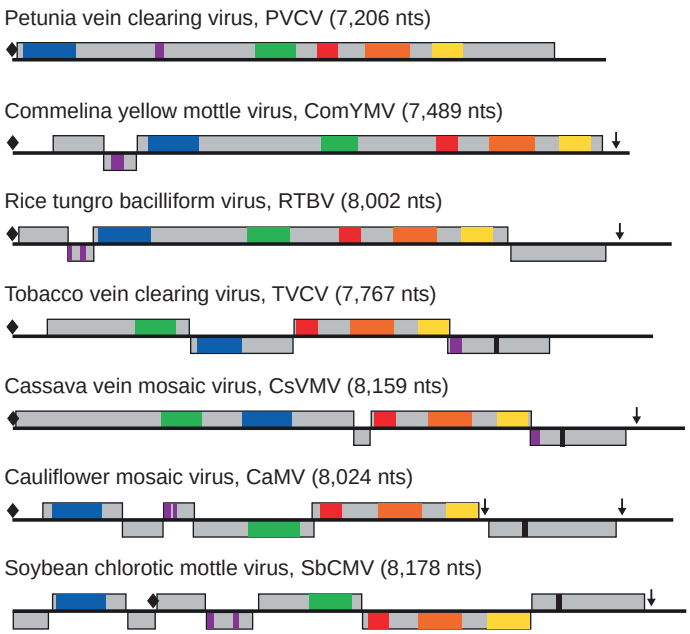


**Figure 2:** Comparison of the genome organizations of the different genera in the family *Caulimoviridae*. For all viruses except soybean mosaic virus (SbMV), the linearized maps begin at the 5′ nucleotide of the minus-strand primer-binding site (designated by a black diamond). For ease of comparison, the start point of the SbCMV genome is the beginning of ORF7. Light grey boxes mark open reading frames and regions within each ORF that are coloured are conserved protein domains as listed in the Pfam database (http://pfam.sanger.ac.uk/): blue is the viral movement protein domain (PF01107), corresponding to $L_{43}$–$E_{243}$ of the cauliflower mosaic virus (CaMV) ORF1 protein; red is the retropepsin (pepsin-like aspartic protease) domain (CD00303), corresponding to $K_{36}$–$Q_{120}$ of the CaMV ORF5 protein; orange is the reverse transcriptase domain (CD01647), corresponding to $K_{273}$–$G_{449}$ of the CaMV ORF5 protein; and yellow is the RNase H1 domain (CD06222), corresponding to $I_{547}$–$E_{673}$ of the CaMV ORF5 protein. Additionally, coiled-coil motifs that are characteristic of the virion-associated protein are marked purple, the conserved C-terminus of the coat protein, corresponding to $L_{261}$–$N_{429}$ of the CaMV ORF4 protein, is marked green, the translation transactivator active site is marked black and the position of RNA promoters is marked with an arrow.

Following entry into the cell, the virion is targeted to the nucleus by a nuclear localization signal (NLS) that is located in the N-terminus of the coat protein and is exposed on the surface of the virion. It is then thought that the virion docks at a nuclear pore, virion disassembly occurs and the DNA is imported into the nucleus utilizing the importin α pathway or perhaps other pathways in the case of rice tungro bacilliform virus (RTBV). The discontinuities in the genome are then sealed to give supercoiled DNA, which associates with histone proteins to form minichromosomes in the nucleus. These are transcribed asymmetrically by host DNA-dependent RNA polymerases to give a greater-than-genome length transcript (35S or 34S RNA) that has a terminal redundancy of about 35 to 270 nt, dependent upon the species. This transcript serves as a template (the pregenomic RNA) for reverse transcription to give the minus-strand DNA and as a polycistronic mRNA for expression of at least some of the ORFs.

The 5′ leader sequence of the pregenomic RNA of nearly all members of the *Caulimoviridae* is long and folds into a large and stable hairpin structure. Immediately preceding this hairpin structure is a small ORF (sORFA). Initiation of translation of ORF1 occurs by a ribosome shunt mechanism, whereby translation begins at sORFA but then the ribosome is shunted across the secondary structure to a landing site near the beginning of ORF1. Viruses in the genera *Caulimovirus* and *Soymovirus* produce a specific monocistronic mRNA (19S RNA) for ORF6; no sgRNAs have been reported for genera *Petuvirus*, *Soymovirus*, *Cavemovirus* and *Badnavirus*. ORF4 of RTBV is expressed from an RNA spliced from the pregenomic RNA.

The replication cycle, in contrast to that of retroviruses, is episomal and does not involve an integration phase. Minus-strand DNA synthesis is primed by host cytosolic $tRNA^{met}$ and synthesis of both strands is performed by the viral RT and RNAse H1. The site-specific discontinuities are at the priming sites for both minus- and plus-strand DNA synthesis and are made by the oncoming strand displacing the existing strand for a short distance and not ligating to form a closed circle.



## Antigenic properties

Virions range from moderate to efficient immunogens. There is pronounced antigenic variability within species in the genus *Badnavirus*. There are some serological cross-reactions between members of different genera.

## Biological properties

The host ranges of most species are narrow. Those in the genera *Petuvirus*, *Soymovirus* and *Cavemovirus* are restricted to dicotyledonous plants; tungroviruses infect monocotyledonous plants and badnaviruses infect either dicotyledonous or monocotyledonous plants. Many members of the family are spread by vegetative propagation.

The geographic range of many species is wide; most species in the genera *Tungrovirus* and *Badnavirus* are primarily tropical or subtropical with some temperate and sub-Antarctic species whereas most of the species in the genera *Petuvirus*, *Caulimovirus*, *Soymovirus* and *Cavemovirus* are found in temperate regions.

The symptoms caused by these viruses are variable and dependent on the virus species, host and climatic conditions. Mosaic or vein clearing symptoms predominate amongst members of the genera *Petuvirus*, *Caulimovirus*, *Soymovirus* and *Cavemovirus*, whereas interveinal chlorotic mottling and streaking is the most frequent symptom of those in the genera *Tungrovirus* and *Badnavirus*.

Most viruses in the family infect most cell types of their hosts although some in the genera *Tungrovirus* and *Badnavirus* are restricted to the vascular system. Virions occur in the cytoplasm and those of species in the genera *Petuvirus*, *Caulimovirus*, *Soymovirus* and *Cavemovirus* are associated with virus-encoded proteinaceous inclusion bodies.

Members of the genera *Caulimovirus*, *Petuvirus*, *Cavemovirus* and *Badnavirus* may have both endogenous (viral DNA integrated in the host nuclear genome) and exogenous forms. Integration is not

an obligatory step in the replication cycles of these viruses but rather the DNA has become captured in the host nuclear genome by non-homologous end-joining (also known as illegitimate recombination). Replication-competent endogenous caulimovirid sequences occur in *Musa balbisiana*, *Petunia hybrida* and *Nicotiana edwardsonii*. Replication-defective endogenous caulimovirid sequences are found in many other dicotyledonous and monocotyledonous plant species.

# Genus *Caulimovirus*

Type species ***Cauliflower mosaic virus***

## Distinguishing features

Members of this genus have particles and cytoplasmic inclusions similar to those of members of the genera *Soymovirus*, *Petuvirus* and *Cavemovirus* but differ from them in genome organization and phylogenetic placement using polymerase gene sequences. Caulimoviruses have six open reading frames and can be distinguished from their closest relatives, the soymoviruses, by the presence of only two ORFs between ORF1 (movement protein) and ORF4 (coat protein) instead of three. The position of the minus-strand primer-binding site also differs between the two genera, as does the presence of an intergenic region between ORF5 and 6.

## Virion properties

### Morphology

Isometric virions are 52 nm in diameter with an icosahedral T7 symmetry. The virion capsid consists of three concentric shells, built from 420 P4 proteins assembled as 60 hexavalent and 12 pentavalent capsomers. The inner cavity has a diameter of about 25 nm. P3 proteins are incorporated in the virion as a triskelion structure that cements three hexavalent or pentavalent capsomers together. The N-terminus of the P3 protein, containing two coiled-coil motifs with opposite handedness, is exposed at the surface of the virion. The C-terminus of the P3 protein, which has a nucleic acid-binding motif, is embedded inside the pores surrounding the capsomers and traverses the P4 protein layers to reach the genomic DNA.

### Physicochemical and physical properties

Virions have a buoyant density of 1.35–1.38 g cm$^{-3}$ in CsCl. Sedimentation coefficients are between 215 and 245 S.

### Nucleic acid

Virions contain a single molecule of non-covalently closed circular dsDNA of about 7.8–8.2 kbp. The minus-strand DNA has a single discontinuity and the plus-strand DNA, two or three discontinuities.

## Genome organization and replication

The genome contains six ORFs and large and small intergenic regions. The large intergenic region, containing the pregenomic RNA (35S) promoter, the RNA polyadenylation signal and the minus-strand primer-binding site, is located between ORF6 and ORF1. The small intergenic region, containing the 19S promoter, is located between ORF5 and 6. Protein P1 is the movement protein, P2 is the aphid transmission factor, P3 is the virion-associated protein, P4 is the coat protein precursor, P5 is the polymerase polyprotein (aspartic protease, RT and RNaseH1 enzymatic activities) and P6 is the translation transactivator/viral silencing suppressor. Protein P4 of CaMV is processed by the viral aspartic protease at both the N- and C-termini to yield three major polypeptides of ca. 44, 39 and 37 kDa. The N-terminus of the 44 kDa polypeptide is at alanine 77 and the C-terminus between positions 435 and 440.

Electron dense inclusion bodies (the viroplasm), which are composed primarily of P6, occur in the cytoplasm and are the site of viral DNA and protein synthesis, morphogenesis and storage of virus particles. Single electron lucent transmission bodies (TBs) are also found in the cytoplasm of each

infected cell and are composed of P2 and P3 proteins. These TBs are ingested by the aphid vector and in an interaction involving the N-terminus of the P2 protein, bind to the tip of the aphid stylet. The P3 protein is then released and the bound P2 protein then binds P3-decorated virions during subsequent intracellular probes by the aphid.

Two major capped and polyadenylated transcripts (35S and 19S RNAs) are produced. The 35S RNA serves as the template for reverse transcription and as a polycistronic mRNA for proteins P1 to P5. P6 is transcribed from the 19S RNA and enables re-initiation of translation of downstream ORFs in a polycistronic mRNA after stop codons are passed. Several spliced versions of the 35S RNA are generated, some containing ORF3 and downstream sequences and others, ORF1 and 2 fused in-frame. This splicing is thought to prevent excessive expression of P2, which is inhibitory to virus replication. As much as 70% of the total viral RNA population is spliced.

## Antigenic properties

The viruses are moderately to strongly immunogenic. Serological cross-reactivity between the isolates of different species is moderate to strong.

## Biological properties

The viruses are transmitted in a semi-persistent manner by aphids; LLDV has no known vector. Transmission requires a virus-encoded protein (aphid transmission factor) that is encoded by ORF2. All except SVBV and LLDV are mechanically transmissible. Seed transmission is not recorded.

Natural host ranges are narrow (restricted to a single plant family) although some species have been experimentally transmitted to hosts in two to four plant families. Hosts are limited to dicotyledonous plants.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Differences in host ranges
- Differences in polymerase (RT+RNAse H) nt sequences of more than 20%
- Differences in gene product sequences.

## List of species in the genus *Caulimovirus*

| | | |
|---|---|---|
| *Carnation etched ring virus* | | |
| Carnation etched ring virus-UK | [X04658 = NC_003498] | (CERV-UK) |
| *Cauliflower mosaic virus* | | |
| Cauliflower mosaic virus-Cabb-S | [V00141 = NC_001497] | (CaMV-CabbS) |
| *Dahlia mosaic virus* | | |
| Dahlia mosaic virus-USA | [AY309479*] | (DMV-US) |
| *Figwort mosaic virus* | | |
| Figwort mosaic virus-USA | [X06166 = NC_003554] | (FMV-US) |
| *Horseradish latent virus* | | |
| Horseradish latent virus-Denmark | [AY534728-33*] | (HRLV-DK) |
| *Mirabilis mosaic virus* | | |
| Mirabilis mosaic virus-Illinois | [AF454635 = NC_004036] | (MiMV_IL) |
| *Strawberry vein banding virus* | | |
| Strawberry vein banding virus-Czech Republic | [X97304 = NC_001725] | (SVBV-CR) |
| *Thistle mottle virus* | | |
| Thistle mottle virus-UK | | (ThMoV-UK) |

Species names are in italic script; names of isolates and synonyms are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
* sequences do not comprise the complete genome.

### List of other related viruses which may be members of the genus *Caulimovirus* but have not been approved as species

| | | |
|---|---|---|
| Aquilegia necrotic mosaic virus | | (ANMV) |
| Dahlia common mosaic virus | [EU090952-7] | (DCMV) |
| Eupatorium vein clearing virus | [EU569831 = NC_010738] | (EVCV) |
| Lamium leaf distortion virus | [EU554423 = NC_010737] | (LLDV) |
| Plantago virus 4 | | (PlV-4) |
| Rudbeckia flower distortion virus | [FJ493469 = NC_011920] | (RuFDV) |
| Sonchus mottle virus | | (SMoV) |

## Genus *Petuvirus*

Type species ***Petunia vein clearing virus***

### Distinguishing features

Petunia vein clearing virus (PVCV), the sole member of the genus, is distinguishable from all other members of the *Caulimoviridae* by its simple genome organization (one ORF) and phylogenetic placement using polymerase gene sequences.

### Virion properties

#### Morphology

Virions are isometric in shape.

#### Physicochemical and physical properties

No information available.

#### Nucleic acid

Virions contain a single molecule of non-covalently closed circular dsDNA of about 7.2 kbp.

### Genome organization and replication

The genome contains a single ORF encoding a large polyprotein and an intergenic region containing the pregenomic RNA promoter, a polyadenylation signal and the minus-strand primer-binding site. Protein domains characteristic of the movement protein, coat protein, aspartic protease, reverse transcriptase and RNaseH1 are present in the ORF1 polyprotein in that order. There is also a putative virion-associated protein homolog between the movement protein and coat protein domains of the polyprotein.

The 5′ leader sequence of the pregenomic RNA folds into a stable hairpin structure preceded by a small ORF. Thus a ribosome shunt mechanism of initiation of translation is assumed to be utilized. The polyprotein is processed by the aspartic protease to generate mature proteins. How the timing and level of expression of the different proteins is coordinated during the replication cycle is unknown. Infected cells contain electron dense inclusion bodies similar to those produced by infections of members of the genus *Caulimovirus*.

### Antigenic properties

No information available.

### Biological properties

Infection in *Petunia hybrida* is due to activation of replication-competent endogenous PVCV (ePVCV) sequences in the nuclear genome of the plant. Wounding (e.g. plant pruning) induces

infection. The virus is vertically transmitted to all progeny as part of the host's chromosomes and is also graft-transmissible to *Petunia parodii* and *Nicotiana glutinosa*. PVCV has no known insect vectors and it is not mechanically transmissible.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Petuvirus*

*Petunia vein clearing virus*
Petunia vein clearing virus-USA [U95208 = NC_001839] (PVCV-US)

Species name is in italic script; name of isolate is in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Petuvirus* but have not been approved as species

None reported.

## GENUS *SOYMOVIRUS*

Type species ***Soybean chlorotic mottle virus***

### Distinguishing features

Members of this genus have particles and cytoplasmic inclusions similar to those of species in the genera *Caulimovirus*, *Petuvirus* and *Cavemovirus* but differ from them in genome organization and phylogenetic placement using polymerase gene sequences. Soymoviruses have seven or eight ORFs and can be distinguished from their closest relatives in the genus *Caulimovirus* by the presence of three ORFs (ORF1b, ORF2 and ORF3) between the movement protein and coat protein-coding ORFs. The minus-strand primer-binding site is also located in ORF1b or in a small intergenic region immediately downstream of this ORF. Furthermore, there is no intergenic region between ORF5 and ORF6 as there is with members of the genus *Caulimovirus*.

### Virion properties

#### MORPHOLOGY

Virions are isometric and about 50 nm in diameter.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

When virion preparations of blueberry red ringspot virus-USA (BRRSV) are centrifuged in sucrose or CsCl gradients, two components are observed with buoyant densities in CsCl of 1.30 and 1.40 g cm$^{-3}$. Virions from the two fractions have no morphological differences. $A_{0.1\%,\,1cm,\,260nm}$ about 7.0.

#### NUCLEIC ACID

Virions contain a single molecule of non-covalently closed circular dsDNA of about 8.1–8.3 kbp. The minus-strand DNA has a single discontinuity and the plus-strand DNA, two discontinuities.

### Genome organization and replication

The genome contains seven or eight ORFs (ORFs 1a, 1b, 2–7). There is one large intergenic region between ORF6 and 7, in which is located the pregenomic RNA promoter and the polyadenylation signal. Additionally, for BRRSV and Cestrum yellow leaf curling virus (CmYLCV) there is a small intergenic region downstream of ORF1b. The location of the minus-strand primer-binding site is

either within ORF1b (soybean chlorotic mottle virus [SbCMV] and peanut chlorotic streak virus] or in the small intergenic region downstream of ORF1b [BRRSV and CmYLCV]). ORF1b and ORF7 are nonessential for replication and systemic infection and whether they are expressed is unknown. Furthermore, ORF7 is absent in CmYLCV. Protein P1a is the movement protein, the function of P2 is unknown, P3 is the virion-associated protein, P4 is the coat protein precursor, P5 is the polymerase polyprotein (aspartic protease, RT and RNaseH enzymatic activities) and P6 is the translation transactivator.

Two major RNA transcripts, the first representing the pregenomic RNA (*c*. 8.2 kbp) and the second, a monocistronic RNA (1.8 kbp) containing ORF6, have been observed for SbCMV. No putative promoter sequences could be identified upstream of the 5′-end of the smaller RNA species. The pregenomic RNA serves as a polycistronic mRNA and protein P6 enables expression of the downstream ORFs. Electron dense inclusion bodies (the viroplasm) are visible in infected cells and are likely to fulfil a similar role to those of members of the genus *Caulimovirus*. The 5′ leader sequence of the pregenomic RNA of SbCMV does not fold into a strong secondary structure as for other members of the family *Caulimoviridae*, and whether a ribosome shunt mechanism of initiation of translation of ORF1 is utilized requires further investigation.

## Antigenic properties

Virions are moderately immunogenic if fixed in 0.5% (v/v) formaldehyde. Serological relationships between species in this genus are unknown but no cross-reactivity with species in the genus *Caulimovirus* has been observed.

## Biological properties

Host ranges are narrow (one to two plant families). All but BRRSV are mechanically transmissible. Spread of the viruses in the field is observed although the vectors are unknown. BRRSV is spread by clonal propagation of its host.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Differences in host ranges
- Differences in polymerase (RT+RNAse H) nt sequences of more than 20%
- Differences in gene product sequences.

## List of species in the genus *Soymovirus*

| | | |
|---|---|---|
| *Blueberry red ringspot virus* | | |
| Blueberry red ringspot virus-USA | [AF404509 = NC_003138] | (BRRSV-US) |
| *Peanut chlorotic streak virus* | | |
| Peanut chlorotic streak virus-K1 | [U13988 = NC_001634] | (PCSV-K1) |
| *Soybean chlorotic mottle virus* | | |
| Soybean chlorotic mottle virus-Japan | [X15828 = NC_001739] | (SbCMV-JA) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Soymovirus* but have not been approved as species

| | | |
|---|---|---|
| Cestrum yellow leaf curling virus | [AF364175 = NC004324] | (CmYLCV) |

# Genus *Cavemovirus*

Type species ***Cassava vein mosaic virus***

## Distinguishing features

The members of this genus produce particles and cytoplasmic inclusions similar to those of species in the genera *Caulimovirus*, *Petuvirus* and *Soymovirus* but differ from them in genome organization and phylogenetic placement using polymerase gene sequences. The order of the movement protein and coat protein is also reversed to that of all other genera in the *Caulimoviridae*.

There are subtle differences in the genome organization of cassava vein mosaic virus (CsVMV) and tobacco vein clearing virus (TVCV), and the genetic distance between the two in the polymerase polyprotein gene is of a similar magnitude to different genera in the *Caulimoviridae*. A proposal to split these two species into different genera is currently under consideration.

## Virion properties

### Morphology

Virions are isometric and about 50 nm in diameter.

### Physicochemical and physical properties

TVCV virions have a buoyant density of 1.35 g cm$^{-3}$ in $Cs_2SO_4$. The sedimentation coefficient, $S_{20,w}$, of CsVMV is estimated to be about 246S.

### Nucleic acid

Virions contain a single molecule of non-covalently closed circular dsDNA of about 7.7–8.2 kbp.

## Genome organization and replication

Both CsVMV and TVCV have four ORFs and a large intergenic region, in which is located the pregenomic RNA promoter, the RNA polyadenylation signal and the minus-strand primer-binding site. ORF1 of CsVMV encodes a polyprotein with coat protein and movement protein domains in that order. In contrast, the coat protein and movement protein of TVCV are encoded by separate ORFs (ORFs 1 and 2). Furthermore, the CsVMV ORF1 protein has a 124-aa N-terminal extension of unknown function relative to the TVCV ORF1 protein. The putative ORF2 protein of CsVMV is small (8.8 kDa), has no known function and there is no homolog in the TVCV genome. For both viruses, the proteins encoded by ORF3 and 4 are the polymerase polyprotein (aspartic protease, RT and RNase H1 enzymatic activities) and the virion-associated protein/translation transactivator, respectively.

The 5′ leader sequence of the pregenomic RNA folds into a stable hairpin structure preceded by a small ORF and thus a ribosome shunt mechanism of initiation of translation of ORF1 is assumed to be utilized. The presence of a translation transactivator homolog in the genome would suggest a similar mechanism of expression of downstream ORFs in a multicistronic mRNA as utilized by members of the genus *Caulimovirus*.

## Antigenic properties

No information available.

## Biological properties

There is no conclusive evidence of vector or seed transmission of CsVMV. The only known host of this virus is cassava (*Manihot esculenta*). The virus is transmitted by vegetative propagation.

Attempts at mechanical, graft or aphid transmission of TVCV have been unsuccessful. Infection in the interspecific allohexaploid *Nicotiana edwardsonii* (*N. clevelandii* × *N. glutinosa*) is believed to originate from activation of replication-competent endogenous TVCV (eTVCV) sequences in the nuclear genome of the plant. The virus is vertically transmitted to all progeny as part of the host's chromosomes. TVCV infections have only ever been observed in *Nicotiana edwardsonii* although eTVCV sequences are present in *Nicotiana sylvestris*, *Nicotiana tabacum*, *Nicotiana tomentosiformis*, *Solanum habrochaites* and *Solanum lycopersicum* and related sequences have been detected in several other plant species in the *Solanaceae*.

### Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Differences in host ranges
- Differences in polymerase (RT + RNAse H) nt sequences of more than 20%
- Differences in gene product sequences.

### List of species in the genus *Cavemovirus*

| | | |
|---|---|---|
| *Cassava vein mosaic virus* | | |
| Cassava vein mosaic virus-Brazil | [U59751 = NC_001648] | (CsVMV-BR) |
| *Tobacco vein clearing virus* | | |
| Tobacco vein clearing virus-USA | [AF190123 = NC_003378] | (TVCV-US) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Cavemovirus* but have not been approved as species

None reported.

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## Genus *Badnavirus*

Type species ***Commelina yellow mottle virus***

### Distinguishing features

The genera *Badnavirus* and *Tungrovirus* are unique among the family *Caulimoviridae* in having bacilliform-shaped virions. Members of the genus *Badnavirus* can be distinguished from rice tungro bacilliform virus (RTBV), the sole member of the genus *Tungrovirus*, by genome organization, the lack of any RNA splicing during replication, the lack of dependency on a helper virus for vector transmission, and phylogenetic placement using polymerase gene sequences.

### Virion properties

#### Morphology

Virions are bacilliform with parallel sides and rounded ends (Figure 1). Virions are uniformly 30 nm in width. The modal particle length is 130 nm, but particles ranging in length from 60 to 900 nm are commonly observed. No projections or other capsid surface features have been observed by electron microscopy. The tubular portion of the virion has a structure based on an icosahedron cut across its 3-fold axis, with a structural repeat of 10 nm and nine rings of hexamer subunits per 130 nm length.

#### Physicochemical and physical properties

Purified virions have an $A_{260/280nm}$ ratio of 1.26 (uncorrected for light scattering).

### Nucleic acid

Virions contain a single molecule of non-covalently closed circular dsDNA of about 7.2–9.2 kbp. Each strand of the genome has a single discontinuity.

## Genome organization and replication

The genome contains three ORFs. The function of protein P1 is unknown, P2 is the virion-associated protein, P3 is a polyprotein with movement protein, coat protein, aspartic protease and RT/RNase H1 domains in that order.

A single, greater-than-genome length, terminally redundant pregenomic RNA is transcribed. No subgenomic RNAs have been observed. The pregenomic RNA serves as a polycistronic mRNA for translation of the three ORFs. By analogy to RTBV, translation of ORF1 is initiated by ribosome shunting and translation of ORF2 and 3 by leaky scanning. Consistent with a leaky scanning model of translation, the start codon of ORF1 and ORF2 are in unfavourable translation contexts and there is paucity of internal AUG codons in both ORFs. In banana streak MY virus and banana streak VN virus, ORF1 begins with a nonconventional start codon (CUG). Furthermore, ORF3 is in −1 translational frame relative to ORF2, which in turn is in a −1 translational frame relative to ORF1. ORFs 1 and 2 and 2 and 3 also have overlapping start and stop codons (ATGA).

Sites where the ORF3 polyprotein is cleaved by the viral aspartic protease have not been determined.

## Antigenic properties

Serological relationships between species members are weak to absent.

## Biological properties

Transmission is in a semi-persistent manner by mealybugs and for some members, by aphids or lacebugs. The virus does not multiply in its mealybug vector and there is no transovarial transmission. All motile life stages of vectors can acquire and transmit the virus. There is little information on the possible transmission of badnaviruses by other vector types. Seed transmission at a rate of 30–63% has been recorded for Kalanchoë top-spotting virus (KTSV). KTSV, Cacao swollen shoot virus and an unidentified badnavirus from sugarcane have been mechanically transmitted but attempts with other species have been unsuccessful, which may relate to the presence of inhibitory substances in the plant sap or low virus titres. Some species in woody hosts have been transmitted by dodder and grafting. Biological host ranges are narrow and restricted to one or two plant families.

Replication-competent endogenous badnaviral sequences (banana streak OL, banana streak GF and probably banana streak MY viruses) are present in *Musa balbisiana*. These endogenous sequences are responsible for causing infection in *M. acuminata* × *M. balbisiana* hybrids, especially following plant propagation by tissue culture. Many other plant species contain endogenous badnaviral sequences but most are probably replication-defective.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Differences in host ranges
- Differences in polymerase (RT + RNAse H) nt sequences of more than 20%
- Differences in gene product sequences
- Differences in vector specificities.

## List of species in the genus *Badnavirus*

| | | |
|---|---|---|
| *Aglaonema bacilliform virus* | | |
| Aglaonema bacilliform virus | | (ABV) |
| *Banana streak GF virus* | | |
| Banana streak GF virus-Ecuador | [AY493509 = NC_007002] | (BSGFV-EC) |

| | | |
|---|---|---|
| *Banana streak Mysore virus* | | |
| Banana streak MY virus-Australia | [AY805074 = NC_006955] | (BSMYV-AUS) |
| *Banana streak OL virus* | | |
| Banana streak OL virus-Nigeria | [AJ002234 = NC_003381] | (BSOLV-NI) |
| *Cacao swollen shoot virus* | | |
| Cacao swollen shoot virus-Agou1 | [L14546 = NC_001574] | (CSSV-AGOU1) |
| *Canna yellow mottle virus* | | |
| Canna yellow mottle virus-Italy1 | [EF156357*] | (CaYMV-IT1) |
| *Citrus mosaic virus* | | |
| (Citrus yellow mosaic virus) | | |
| Citrus mosaic virus-India | [AF347695 = NC_003382] | (CiMV-IN) |
| *Commelina yellow mottle virus* | | |
| Commelina yellow mottle virus-Guadeloupe | [X52938 = NC_001343] | (ComYMV-GU) |
| *Dioscorea bacilliform virus* | | |
| Dioscorea bacilliform Al virus-L85 | [X94576*] | (DBALV-L85) |
| *Gooseberry vein banding associated virus* | | |
| Gooseberry vein banding associated virus GB1 | [HQ852248] | (GVBAV-SCO) |
| *Kalanchoë top-spotting virus* | | |
| Kalanchoë top-spotting virus-USA | [AY180137 = NC_004540] | (KTSV-US) |
| *Piper yellow mottle virus* | | |
| Piper yellow mottle virus-Sri Lanka | [AJ626981*] | (PYMoV-SRL) |
| *Rubus yellow net virus* | | |
| Rubus yellow net virus-UK | [AF468454*] | (RYNV) |
| *Schefflera ringspot virus* | | |
| Schefflera ringspot virus-USA | | (SRV-US) |
| *Spiraea yellow leaf spot virus* | | |
| Spiraea yellow leaf spot virus-USA | [AF299074*] | (SYLSV_US) |
| *Sugarcane bacilliform IM virus* | | |
| Sugarcane bacilliform IM virus-Queensland | [AJ277091 = NC_003031] | (SCBIMV-QLD) |
| *Sugarcane bacilliform Mor virus* | | |
| Sugarcane bacilliform Mor virus-Morocco | [M89923 = NC_008017] | (SCBMV-MOR) |
| *Taro bacilliform virus* | | |
| Taro bacilliform virus-Papua New Guinea | [AF357836 = NC_004450] | (TaBV-PNG) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
* Sequences do not comprise the complete genome.

## List of other related viruses which may be members of the genus *Badnavirus* but have not been approved as species

| | | |
|---|---|---|
| Aucuba bacilliform virus | | (AuBV) |
| Banana streak VN virus | [AY750115 = NC_007003] | (BSVNV) |
| Bougainvillea chlorotic vein banding virus | [EU034539 = NC_011592] | (BCVBV) |
| Dioscorea bacilliform SN virus | [DQ822073 = NC_009010] | (DBSNV) |
| Dracaena mottle virus | [DQ473478 = NC_008034†] | (DrMV) |
| Mimosa bacilliform virus | | (MBV) |
| Pelargonium vein banding virus | [GQ428155 = NC_13262] | (PlVBV) |
| Pineapple bacilliform CO virus | [EU377666*] | (PBCOV) |
| Pineapple bacilliform ER virus | [EU377673*] | (PBERV) |
| Stilbocarpa mosaic bacilliform virus | [AF478961*] | (SMBV) |
| Sweetpotato badnavirus A | [FJ560943] | |
| Sweetpotato badnavirus B | [FJ560944 = NC_012728] | |
| Yucca bacilliform virus | [AF468688*†] | (YBV) |

* Sequences do not comprise the complete genome.
† Sequence amplified by PCR from total plant DNA extract; it has not been proved that the sequence derives from an exogenous viral genome.

# Genus *Tungrovirus*

Type species ***Rice tungro bacilliform virus***

## Distinguishing features

RTBV is the sole member of the genus *Tungrovirus*. Along with members of the genus *Badnavirus*, RTBV is readily distinguished from other genera in the *Caulimoviridae* by its bacilliform-shaped virions. Although the genome organization of RTBV is very similar to that of the genus *Badnavirus*, it differs by the presence of a fourth ORF (ORF4). This fourth ORF is expressed from a monocistronic RNA that is produced by splicing of the pregenomic RNA. RTBV can also be differentiated from members of the genus *Badnavirus* by phylogenetic placement using polymerase gene sequences.

## Virion properties

### Morphology

The virus particles are bacilliform of diameter 30 nm and predominant length 130 nm; longer particles, up to 300 nm, are found in some isolates. The particle structure is based on a $T = 3$ icosahedron cut across its three-fold axis, the tubular portion being made up of rings of hexamer subunits with a repeat distance of about 10 nm.

### Physicochemical and physical properties

The particles sediment with an $S_{20,w}$ of about 200S and have a buoyant density in CsCl of about 1.36 g ml$^{-1}$.

### Nucleic acid

Virions contain a single molecule of non-covalently closed circular dsDNA of about 8.0 kbp. Each strand of the genome has a single discontinuity.

## Genome organization and replication

The genome contains four ORFs, a large intergenic region between ORF4 and 1 and a small intergenic region between ORF3 and 4. The pregenomic RNA promoter, polyadenylation signal and minus-strand primer-binding site are all located in the large intergenic region. The function of protein P1 is unknown, P2 is the virion-associated protein, P3 is a polyprotein with movement protein, coat protein, aspartic protease and RT/RNase H1 domains in that order and the function of protein P4 is unknown.

A single, greater-than-genome length, terminally redundant pregenomic RNA is transcribed. The pregenomic RNA serves as a polycistronic mRNA for translation of ORFs 1-3. Translation of ORF1 is initiated by ribosome shunting and translation of ORF2 and 3 by leaky scanning. Consistent with a leaky scanning model of translation, the start codon of ORF1 is non-conventional (AUU), the start codon of ORF2 is in an unfavourable translation context and there is an absence of internal AUG codons in both ORFs. Furthermore, ORF3 is in −1 translational frame relative to ORF2, which in turn is in a −1 translational frame relative to ORF1. ORFs 1 and 2 and 2 and 3 also have overlapping start and stop codons (ATGA).

ORF4 is expressed from a monocistronic RNA that is produced by splicing of the pregenomic RNA. A 6300 nt intron is excised in a way that fuses the first small ORF (sORFA) in the leader sequence of the pregenomic RNA in frame to ORF4 at a position that is 22 codons upstream of the start codon of this ORF.

Protein precursors in the ORF3 polyprotein are processed through the action of the viral aspartic protease. The mature coat protein has been mapped to aa 477–791 (GenBank accession NP_056762), giving rise to a protein of 37.3 kDa. The aspartic protease has been mapped to aa 965–1085, giving rise to a protein of 13.8 kDa.

## Antigenic properties

RTBV is moderately immunogenic.

## Biological properties

RTBV is transmitted by leafhoppers in the genera *Nephotettix* and *Recilia*. Although able to replicate independently in its host, RTBV is only able to be transmitted when the leafhopper has acquired rice tungro spherical virus (genus *Waikavirus*) simultaneously or previously, suggesting that RTSV may contribute a helper component needed for transmission. RTBV is not mechanically transmissible. There is no evidence of seed transmission. Host range is limited to members of the *Poaceae* and *Cyperaceae*.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Tungrovirus*

*Rice tungro bacilliform virus*
Rice tungro bacilliform virus-Philippines [X57924 = NC_001914] (RTBV-PH)

Species name is in italic script; name of isolate is in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Tungrovirus* but have not been approved as species

None reported.

## Phylogenetic relationships within the family *Caulimoviridae*

Phylogenetic relationships within the family have not yet been fully resolved and some of the relationships that are deduced depend on the method of analysis. However, several systematics studies have concluded that the genus *Petuvirus* is sister to all other genera in the *Caulimoviridae* and that the genera *Badnavirus* and *Tungrovirus* form a monophyletic group, as do the genera *Soymovirus* and *Caulimovirus* (Figure 3).

## Similarity with other taxa

Members of the family *Caulimoviridae* have the conserved *gag-pol* replication core of all viral retroelements, suggesting a common ancestry. Phylogenetic analyses using conserved polymerase gene sequences suggest that the *Caulimoviridae* is sister to the *Metaviridae*. In the literature, members of the *Caulimoviridae* are frequently referred to as being plant-infecting pararetroviruses. The term "pararetrovirus" was coined to describe viral retroelements that do not integrate in the host genome as part of the replication cycle and which encapsidate dsDNA instead of ssRNA. However, the two "pararetrovirus" families, *Hepadnaviridae* and *Caulimoviridae*, are distantly related and a group containing these two families is polyphyletic.

## Derivation of names

*Badna*: from *ba*cilliform *DNA* viruses.
*Caulimo*: from *cau*liflower *mo*saic virus.
*Cavemo*: from *ca*ssava *ve*in *mo*ttle virus
*Petu*: from *petu*nia
*Soymo*: from *soy*bean chlorotic *mo*ttle virus
*Tungro*: from rice *tungro* bacilliform virus

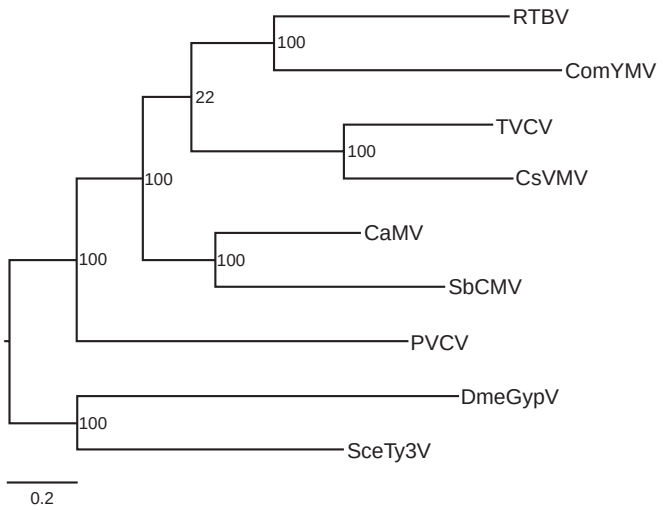


**Figure 3:** Evolutionary relationships within the family *Caulimoviridae* based on comparison of the polymerase polyprotein gene. To obtain the nucleotide sequence alignment, amino acid sequences homologous to $K_{36}$–$E_{673}$ of the cauliflower mosaic virus (CaMV) P5 protein (GenBank accession NP_056728) were first aligned with ClustalX and this alignment then used to generate a DNA alignment using the program Tranalign. Poorly aligned and highly variable regions in the alignment were then removed using the program Gblocks. Maximum likelihood analysis was done using the program RAxML and the GTR+Γ model of evolution to calculate the final tree topology. The dataset was partitioned into aspartic protease, reverse transcriptase and RNase H1 domains (see Figure 2 for domain boundaries), as well as tether regions between the domains. 1000 maximum likelihood bootstrap replicates were run (bootstrap values shown in nodes of branches) using the GTR+CAT approximation of GTR+Γ. Drosophila melanogaster Gypsy virus (DmeGypV; genus *Errantivirus*) and Saccharomyces cerevisiae Ty3 virus (SceTy3V; genus *Metavirus*) were included in the analyses as outgroups. Other acronyms are ComYMV (Commelina yellow mottle virus), CsVMV (cassava vein mosaic virus), PVCV (petunia vein clearing virus), SbCMV (soybean chlorotic mottle virus), RTBV (rice tungro bacilliform virus) and TVCV (tobacco vein clearing virus).



## Further reading

### Journals and books

Bousalem, M., Douzery, E. and Seal, S. (2008). Taxonomy, molecular phylogeny and evolution of plant reverse transcribing viruses (family *Caulimoviridae*) inferred from full-length genome and reverse transcriptase sequences. *Arch. Virol.*, **153**, 1085–1102.

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### Websites

Descriptions of Plant Viruses Online: http://www.dpvweb.net/index.php

## Contributed by

Geering, A.D.W. and Hull, R.

# Family *Hepadnaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Hepadnaviridae* |
| Genus | *Orthohepadnavirus* |
| Genus | *Avihepadnavirus* |

## Virion properties

### Morphology

Hepadnaviruses are spherical, occasionally pleomorphic, 42–50 nm in diameter, with no evident surface projections after negative staining. Projections are visible in cryo-EM pictures (Figure 1C). The outer, detergent-sensitive, envelope contains the surface proteins and surrounds an icosahedral nucleocapsid core that is composed of one major protein species, the core protein. The nucleocapsid encloses the viral genome (DNA), the viral DNA polymerase, and associated cellular protein(s), including protein kinase and chaperones that appear to play a role in the initiation of viral DNA synthesis.

In the case of hepatitis B virus (HBV), the majority of nucleocapsid cores are about 36 nm in diameter and contain 240 core protein subunits (triangulation number T = 4), while a minority are approximately 32 nm in diameter and consist of only 180 subunits (T = 3). Hepadnavirus infection induces overproduction of surface proteins that are secreted into the blood as pleomorphic lipoprotein particles together with virus. In the case of HBV, these form 17–22 nm spherical particles and filaments (Figure 1).

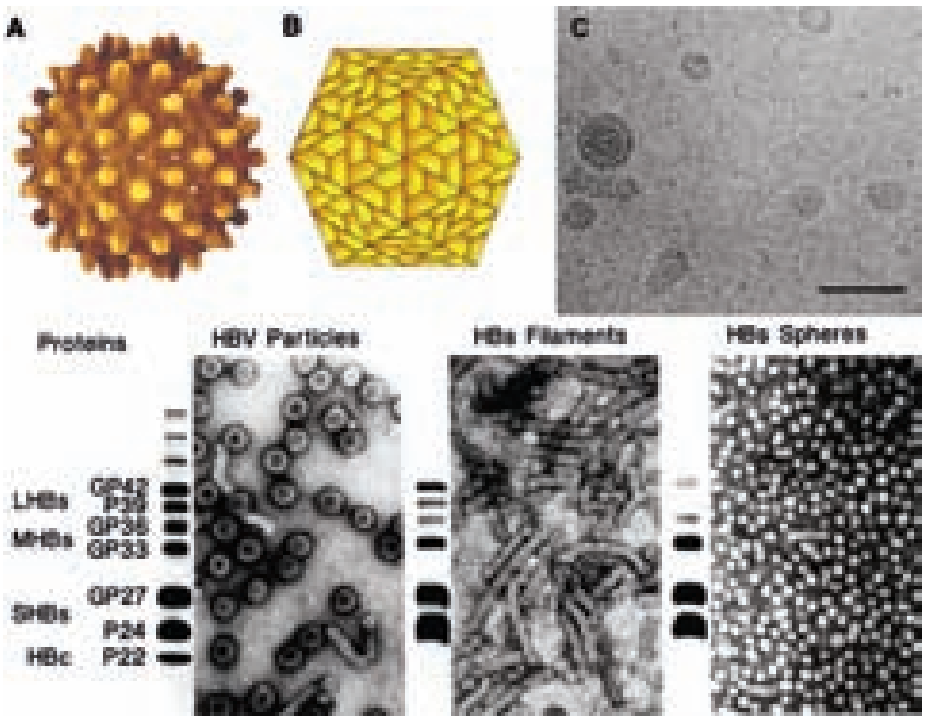


**Figure 1:** (A) Atomic resolution rendering of a particle of hepatitis B virus capsid (HBV) (Wynne, S.A., Crowther, R.A. and Leslie, A.G. (1999). *Mol. Cell.*, **3**, 771–780). (B) Diagram representing the T = 4 structure of an HBV core particle. (C) High resolution cryo-electron micrograph of normal (42–52 nm) isometric virus with icosahedral capsid or "core" surrounded by a coat of HBsAg, and of smaller (ca. 22 nm) spheres and rods composed of viral envelope proteins. The bar represents 65 nm. (Courtesy of B. Boettcher, J. Monjardino and R.A. Crowther.) (Bottom). Negative contrast electron micrographs of HBV virions (left) and virus-associated particles (centre and right), together with an SDS-PAGE protein profile of each particle form to the left of the relevant micrograph. LHBs, MHBs and SHBs refer to large, middle and small HB surface proteins, respectively. HBc, hepatitis B core proteins. GP, glycoprotein; P, protein. The identities of the slower migrating bands are unknown. (Courtesy of W. Gerlich.)

## Physicochemical and physical properties

The virion $S_{20,w}$ is approximately 280S. The buoyant density of virions in CsCl is approximately 1.25 g cm$^{-3}$. Estimates of the buoyant density of particles lacking cores are 1.18–1.20 g cm$^{-3}$. Virus-derived cores (lacking envelopes but containing nucleic acid) have densities of approximately 1.36 g cm$^{-3}$.

### Nucleic acid

The genome consists of a partially dsDNA that is held in a circular conformation by base pairing in a cohesive overlap between the 5′ ends of the two DNA strands. The length of the cohesive overlap is about 240 bp for the orthohepadnaviruses and 50 bp for the avihepadnaviruses. The size of the genome ranges from 3.0 to 3.3 kb in different family members; the viral DNA has an $S_{20,w}$ of about 14 and a G+C content of about 48%. One strand (negative sense, i.e. complementary to the viral mRNAs) is full-length, whereas the other varies in length. For both the orthohepadnaviruses and the avihepadnaviruses, the negative strand DNA has an 8–9 nt terminal redundancy. The 5′ end of the negative strand DNA is covalently attached to the terminal protein (TP) domain of the viral DNA polymerase, and the 5′ end of the positive sense DNA has a covalently attached 19 nt, 5′ capped oligoribonucleotide primer. The 3′ end of the positive strand terminates at a variable position in different molecules, creating a single stranded gap that may account for 60% of the HBV genome but is usually very short in avihepadnaviruses.

### Proteins

Virions and empty subviral particles may contain two or three surface proteins, with a common C-terminus but distinct N-termini due to different sites of translation initiation. Typically, virions contain a small transmembrane surface protein (SHBs), in the orthohepadnaviruses an intermediate size (MHBs) and a large (LHBs) protein that is myristylated at the N-terminus. In many cases, more than one form of each of the above proteins occurs due to alternative patterns of glycosylation. For HBV and WHV, virions and filaments are enriched in L proteins and empty spheres consist predominantly of S proteins, while for duck hepatitis B virus (DHBV), L and S proteins are distributed evenly between particle types.

The core protein has a large N-terminal domain and a small RNA-binding domain at the C-terminus. Core protein above a threshold concentration can self-assemble via dimers to complete nucleocapsids in the absence of other viral components.

The polymerase protein consists of an N-terminal domain (TP) with a DNA primer function, a spacer region of variable size, a reverse transcriptase and an RNase H domain. The TP domain is covalently attached to the 5′-end of the minus strand of viral DNA via a tyrosine residue, which serves as the primer for initiation of reverse transcription.

Orthohepadnaviruses contain a fourth ORF ("X" gene) situated downstream from the S gene and partly overlapping the cohesive 5′-terminal region. This codes for a non-structural protein that can function as a promiscuous transcriptional activator and, for the woodchuck hepatitis virus (whv), it has been shown to be required for efficient *in vivo* replication. Avihepadnaviruses have an ORF in a similar location, but it remains unclear whether it has a role in infection. At high expression levels in cell culture systems, the X proteins induce apoptosis.

Host proteins contained within nucleocapsids include heat shock protein Hsp 70 and heat shock protein Hsp 90, which, at least in the case of duck hepatitis B virus (DHBV), appear to be part of a multicomponent chaperone complex involved in replication and nucleocapsid assembly. A protein kinase has been detected in HBV nucleocapsids.

The core protein also exists in a secreted soluble form ("e" antigen) that is translated from an additional start codon 29 codons upstream of the core start codon. This additional precore sequence functions as a signal peptide. The "e" antigen is not essential but is conserved in all hepadnaviruses and seems to modulate the immune response.

### Lipids

Lipid constitutes 30–40% of the viral envelope or of the empty particles. It is derived from a host membrane compartment intermediate between the ER and Golgi, and includes phospholipids, cholesterol, cholesterol esters and triglycerides.

### Carbohydrates

Demonstrated in particles and virions of orthohepadnaviruses as N-linked glycans of the complex types. Many virus isolates also contain O-linked glycans in the M surface protein.

## Genome organization and replication

The hepadnavirus genome contains the following major ORFs; precore/core (preC/C), polymerase (P), env or surface (preS/S), and in the case of orthohepadnaviruses, a fourth ORF, the X gene (Figure 2).

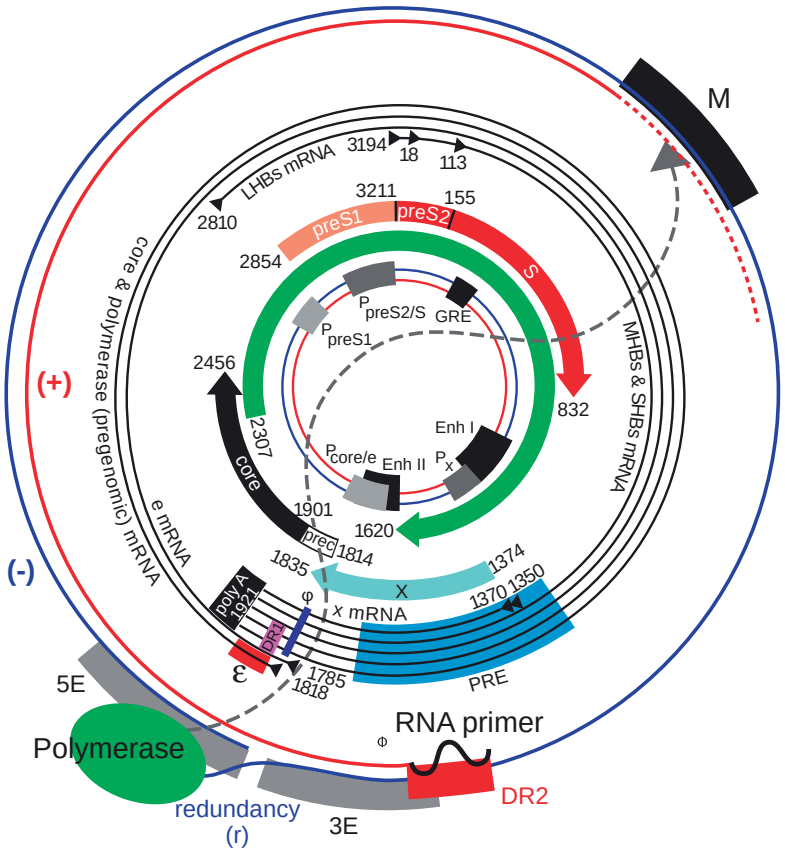


**Figure 2:** Genome organization and regulatory elements of orthohepadnaviruses are shown for a typical HBV isolate of genotype A. The outer circle represents the structure of relaxed circular, viral DNA found within virions, while the inner circle illustrates the structure and regulatory elements on cccDNA, the covalently closed circular DNA from which viral mRNAs are transcribed in the nucleus of the infected cell (red = positive strand; blue = negative strand). Numbering starts at the unique EcoRI restriction site located approximately at the junction of the preS1 and preS2 domains in the ORF for the viral envelope proteins. The regulatory elements on the DNA are depicted at their approximate positions. The promoters (P) are shown as grey boxes, and the enhancers (Enh), a glucocorticoid responsive element (GRE), and a CCAAT element (CCAAT) are depicted as black boxes. The basal core promoter is regulated by the negative regulatory element (NRE, not shown), which overlaps with Enh II. Liver-specific promoters are drawn in light grey; non-tissue-specific promoters are depicted as medium grey boxes. The ORFs are drawn as arrows with their corresponding start and termination sites. The viral mRNAs are depicted as black circles in the middle region. The black triangles represent their 5′ ends; the 3′ end is common and linked to an approximately 300 nt polyA tract. The regulatory elements on the RNAs are depicted as a red box (encapsidation signal ε), a black box (polyadenylation signal), in pink (DR1), in blue (phi) and in light blue (posttranscriptional regulatory element [PRE]). The genomic DNA is depicted as it is found in the virion. The minus DNA strand is drawn as a blue line with its terminal redundancy (r). The polymerase (green oval) is linked to the 5′ end of the minus strand. The plus-strand DNA is shown as a red line. The dotted red line represents the variation of the 3′ end of the plus strand DNA. The 5′ end of the plus strand is bound to its capped RNA primer, depicted as a black, wavy line. The dotted grey line between the polymerase and the 3′ end of the plus strand DNA reflects the fact that the polymerase is bound to the 5′ end of the minus strand DNA, but interacts with the variable 3′ end of the plus strand DNA for its elongation. The regulatory elements on the minus strand DNA are the DR2 (red box) and the M, 5E and 3E elements, which are required for circularization of the genome. Note that their position and size are approximate, since these elements are not yet completely characterized. (From Kann, M. (2002). Structure and molecular virology. In: *Hepatitis B virus Human Virus Guide*. (S. Locarnini and C.L. Lai, Eds.), ch 2. International Medical Press, London; with permission.)

The preC/C ORF codes for two distinct products: one is the core protein forming the protein shell of the nucleocapsid, the other, made by translation of the joint preC/C ORF, is the precore protein which is targeted into the cell's secretory pathway, processed at both ends and eventually found in the serum of infected individuals as e antigen. Both products are translated from genomic, terminally redundant, polyadenylated 3.5 kb transcripts with slightly different 5′-ends. The longer precore mRNAs contain the preC initiation codon, whereas the shorter core mRNA lacks it. The P ORF covers some 80% of the genome and encodes the viral replication enzyme P, which is also an indispensable component in the assembly process (see below). P protein is translated from the same genomic RNA that directs synthesis of core protein by internal initiation. The env or surface gene consists of three in-phase ORFs, termed in 5′- to 3′-direction, preS1, preS2 and S. S can be expressed separately to give the small or S protein; cotranslation of preS2/S yields the middle or M protein (orthohepadnaviruses), and that of the entire preS1/preS2/S gene the large or L protein. Thus, the S domain is common to all three forms of surface proteins. As for the preC/C ORF, this is achieved by the generation of mRNAs with staggered 5′ ends in which the initiator codons of the preS1, the preS2 or the S region are the first to be encountered by translating ribosomes. L protein is translated from a 2.4 kb mRNA, and M and S from a set of 2.1 kb transcripts. All viral transcripts are 3′-terminally colinear, ending after a unique polyadenylation signal located in the C gene. The X gene encodes a pleiotropic transcriptional activator that appears to be required for establishment of a normal infection with WHV, and has been implicated in one proposed mechanism for hepadnavirus carcinogenesis. The DNA sequence of HBV has two enhancer regions (ENHI and ENHII), a negative regulatory element NRE, four promoters (preC/C, preS1, preS2/S and X), two 11-base direct repeat sequences (DR1 and DR2), a polyadenylation signal (TATAAA), and putative glucocorticoid-responsive elements (GRE). The 5′ end of the negative strand is located within DR1, and the 5′-end of the positive strand is at the 3′ boundary of DR2.

Replication can be considered in two stages: an incoming or afferent arm in which the input viral genome enters the nucleus and is converted to covalently closed circular (ccc) DNA (cccDNA), and an outgoing or efferent arm in which RNA transcripts from the cccDNA are encapsidated and reverse transcribed within core particles in the cytoplasm, and the resulting genomic DNA is either transported to the nucleus or enveloped and secreted (Figure 3).

There is evidence that the infectious DNA-containing virion binds to its target cell via interaction of the L protein with cellular receptor(s) that are not yet fully characterized. The nucleocapsid is presumably delivered to the cytoplasm and transported to the nuclear pore where the genome is released to the nucleoplasm. Repair of the single stranded gap is carried out, though it remains uncertain whether this is achieved by the viral DNA polymerase or a host polymerase. Removal of the TP and oligoribonucleotide from the negative and positive strands, respectively, takes place, followed by DNA ligation, converting the viral genome to cccDNA. These steps are believed to involve the action of cellular enzymes. cccDNA, in the form of a histone-associated minichromosome, provides a stable template for transcription.

Genomic and sub-genomic RNAs (sgRNAs) are transcribed by host RNA polymerase II into a number of RNA size classes, some of which also show microheterogeneity at the 5′ end but all terminate at a common 3′-polyadenylation site. The RNAs of HBV and WHV contain a post-transcriptional regulatory element PRE, which allows for cytoplasmic transport without splicing. The largest of these RNAs is translated to form precore protein. A slightly shorter RNA encodes the core protein and (by internal initiation) the polymerase protein, and also serves as the template for reverse transcription. The polymerase protein, along with host chaperone proteins, associates with a specific encapsidation signal (ε), which is closed to the 5′ end of the pregenomic RNA. For efficient minus strand DNA synthesis epsilon has to interact with the 19 nt long phi signal, which locates 32 nt upstream of DR1 at the 3′ end of the RNA and this preassembly complex apparently triggers assembly of core protein dimers into complete nucleocapsids.

Concurrently, the different classes of sgRNAs are translated to produce the various surface gene products (L, M and S), which oligomerize and bud into the lumen of a post ER/pre-Golgi compartment to give rise to both empty subviral particles and virions.

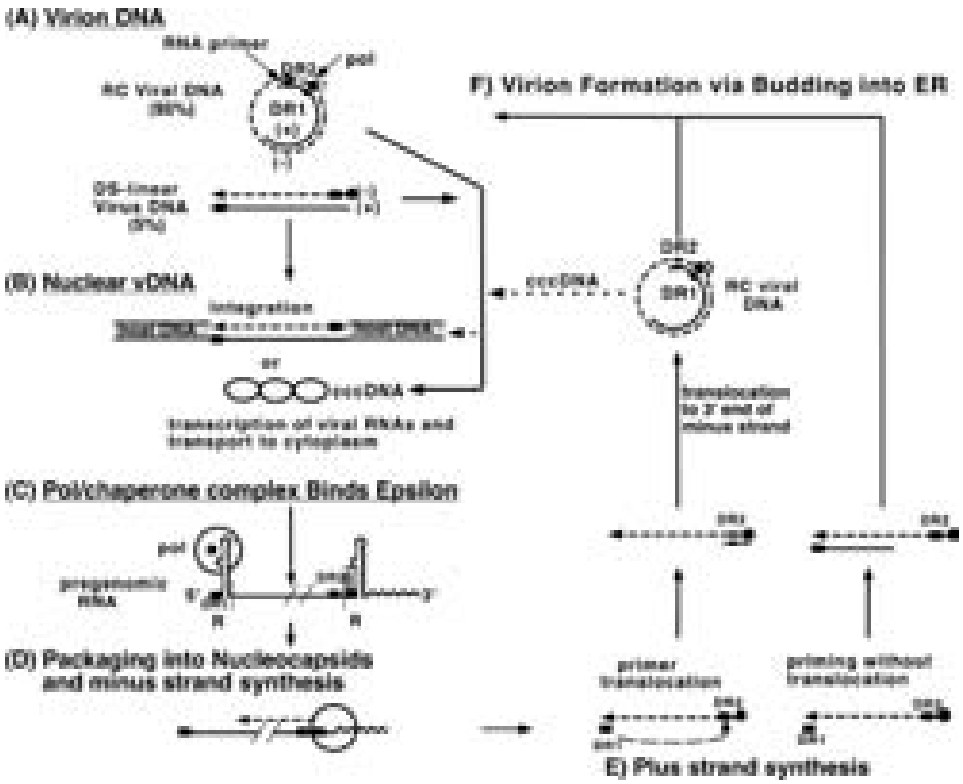


**Figure 3:** Hepadnavirus replication strategy. For details see text.

Reverse transcription of pregenomic RNA takes place within cytoplasmic immature cores. This process uses the TP domain of polymerase as primer for first strand synthesis, and a short, undigested, capped oligoribonucleotide derived from the 5′ end of the template RNA and extending through the proximal copy of DR1 as the second strand primer. Synthesis of both strands requires transfer reactions. Reverse transcription initiates with the copying of 4 nt from a bulge in ε. This product is then annealed to a complementary sequence at the 3′ copy of DR1, and it is from this site that synthesis of the full-length minus strand progresses. Plus strand synthesis involves a transfer of the RNA primer from the 3′ end of the minus strand to a remote site, DR2, which is near the 5′ end of the minus strand and identical in sequence to DR1. It is here that plus strand synthesis normally begins. Plus strand elongation requires a second translocation, from the 5′ to the 3′ end of the minus strand template, to form an open circular genome with a less than full-length (+) strand, maintained by overlapping cohesive ends. Nucleocapsids containing partly reverse transcribed DNA that have associated with cytoplasmically located preS domains of the L envelope protein may then bud into the lumen of multivesicular bodies as maturing virions, or alternatively may be transported to the nucleus, thereby increasing the pool of cccDNA. Although integration of viral DNA into the host genome is not required for replication and appears to be an infrequent event, integrated viral DNA, often containing deletions, inversions and duplications, is found in hepatocellular carcinoma (HCC) cells in culture and in patients, as well as in apparently normal livers from chronic carriers. An aberrant linear viral DNA, formed when plus strand synthesis initiates from an untranslocated primer, appears to be the precursor to the majority of integration events.

## Antigenic properties

Three principal antigens have been identified for hepadnaviruses, designated surface, core and e antigen. These are abbreviated HBsAg, HBcAg, HBeAg for the HBV-related antigens, DHBsAg, DHBcAg and DHBeAg for DHBV-related antigens, etc., while the corresponding antibodies are

designated anti-HBs, anti-HBc, anti-DHBs, anti-DHBc, etc. HBsAg is involved in neutralization. It cross-reacts to a limited extent with the analogous antigens of WHV and ground squirrel hepatitis virus (GSHV), but not with DHBsAg. PreS antigens and the HBsAg loop in the S protein bear specific neutralization determinants. S proteins are sufficient to stimulate protective immunity.

HBeAg and HBcAg proteins share common sequences and epitopes, but also contain epitopes that distinguish these two proteins from each other. HBeAg is a 16 kDa truncated derivative of HBcAg. It is found as a soluble antigen in the serum of patients. HBcAg has been found to cross-react more strongly with the WHV core antigen than is seen between the corresponding surface antigens. In much of the earlier literature, the term surface antigen or HBsAg is used arbitrarily to refer to either the antigenic specificity, various protein products of the preS1/preS2/S gene, or the empty 17–22 nm HBsAg-bearing particles. The term "antigen" should not be used if "protein" or "particle" is intended. Similar considerations apply to the use of "core antigen".

### Biological properties

All hepadnaviruses show narrow host specificity. *In vitro*, replication of many hepadnaviruses has only been demonstrated following transfection of tissue culture cells by cloned viral DNA, resulting in the production of infectious virus. Replication of several hepadnaviruses has been achieved following inoculation of primary hepatocytes with serum that contains virus.

Hepadnavirus infections *in vivo* possess characteristic features:

- They are markedly hepatotropic, although viral antigens and nucleic acids can also be detected in white blood cells (and in some extra-hepatic sites, e.g. pancreas, spleen and kidney with avihepadnaviruses).
- Infection may be transient or persistent, the outcome depending on factors such as host age and dose of inoculum. Persistent infection is more common in neonates and in immuno-compromised hosts. Persistent infections are typically life-long and can be accompanied by high levels of virions and subviral particles in the circulating blood.
- Empty subviral particles, composed of excess virus envelope material, are present in much greater numbers than complete virions in most individuals and at most stages of infection.
- Virus replication is generally thought to be non-cytopathic, and different degrees of ongoing liver damage in different individuals are thought to be governed by different degrees of immune-mediated damage to infected hepatocytes.
- In ortho-, but not avihepadnavirus infections, persistent virus infection confers a significantly increased rate of development of primary hepatocellular carcinoma, and a number of direct and indirect mechanisms have been described.

## GENUS *ORTHOHEPADNAVIRUS*

Type species ***Hepatitis B virus***

### Distinguishing features

Viruses of this genus infect mammals, with a narrow host range for each virus species. The only known natural host of HBV are humans and greater apes (chimpanzees, gorillas, orangutans and gibbons). Virions of HBV are 40–45 nm in diameter with a 32–36 nm internal nucleocapsid, and subviral HBV particles are typically spherical (16–25 nm diameter) and filamentous (20 nm diameter and variable in length). The genome of HBV is 3.2 kb with a cohesive overlap of 240 bp. The viruses have an S protein of approximately 226 amino acid residues (aa) as a major envelope protein, an M

protein of about 271 aa (which appears unnecessary for infection in experimental situations) and an L protein of about 400 aa.

The envelope, or surface, proteins are partially glycosylated, thus generating doublets in gel electrophoresis, e.g. for HBV, P24/GP27 for S, P39/GP42 for L, and, in the case of M, GP33/GP36 due to an additional glycosylation in the preS2 sequence. The HBV core protein is approximately 180 aa, and the virus encodes an HBx protein of 154 aa whose natural function in the virus life cycle is uncertain.

At least five antigenic specificities have been identified for HBsAg. A group determinant (a) is shared by virtually all HBsAg preparations. Mutations in this region have been found in immunized individuals who subsequently became infected, in HBV carriers, particularly in occult HBV infection without detectable HBsAg, and in infected individuals given immunotherapy. Two pairs of subtype determinants (d,y and w,r) have been demonstrated that are generally mutually exclusive (and thus usually behave as alleles). Antigenic heterogeneity of the w determinant, and additional determinants such as q, x or g have also been described. Thus, eight major serological subtypes are found (ayw, ayw2, ayw3, ayw4, ayr, adw2, adw4 and adr); they have distinct geographical distributions with some overlap. DNA sequence analysis has now replaced antigenic typing in defining viral genotypes and has distinguished genotypes, or clades, that differ between each other by 8–14% at the nucleotide level. Different genotypes also have different geographical distributions, and there is some, but not complete, correspondence between genotype and serological subtype. HBV strains infecting apes fall into species-specific genotypes, which have been suggested to reflect co-evolution of virus and host and not horizontal transmission between primate species.

Woolly monkey hepatitis B virus (WMHBV) is closely related in DNA sequence to HBV, with 20% sequence variation, but, unlike HBV, preferentially infects the woolly monkey and can be transmitted to the spider monkey; it transmits only poorly to the chimpanzee, which is highly susceptible to human HBV genotypes.

Different isolates of WHV show <3.5% nucleotide sequence variation. A virus of arctic ground squirrels (Arctic squirrel hepatitis virus, ASHV) differs from WHV and GSHV to about the same extent (about 15% nt changes) as these two latter viruses differ from each other.

## Biological properties

HBV may cause as a consequence of the host immune response to infection, acute and chronic hepatitis, and immune complex diseases like periarteritits nodosa, glomerulonephritis, and infantile papular acrodermatitis. Late sequelae are liver cirrhosis and hepatocellular carcinoma. An asymptomatic carrier state with high viremia may develop, particularly after perinatal infection or under immune suppression.

Horizontal transmission of HBV usually occurs by: (i) percutaneous contact with infected blood or body fluids, e.g. intravenous drug abuse or use of infected blood or blood products; (ii) sexual contact; (iii) perinatal transmission from an infected mother; and (iv) "inapparent horizontal" transmission, particularly between children in low socio-economic communities, thought to be due, at least in part, to unrecognized exposure to open skin breaks or mucous membranes. In communities with a high prevalence of infection, routes (iii) and (iv) predominate, while in low prevalence communities, infections are acquired later in life and involve particularly routes (i) and (ii).

Hepatitis occurs in woodchucks and squirrels infected with their respective viruses, and chronic infection leads to a risk of hepatocellular carcinoma even greater than that in chronic carriers of HBV. In the case of WHV, hepatocellular carcinoma frequently occurs within 2 years of infection.

Woolly monkey hepatitis B virus causes hepatitis in its host, but is not yet known to have a role in liver cancer.

## Species demarcation criteria in the genus

The species demarcation criteria in the genus are:

- Nucleotide sequence diversity; WHV/HBV 40%; GHSV/WHV 15%; WMHBV/HBV 20%; WMHBV/WHV 30%.
- Differences in host range: HBV infection is limited to primates, but HBV may infect primary hepatocyte cultures from *Tupaia belangueri* which is not a primate. GSHV infection has been experimentally transferred to chipmunks and woodchucks but not to several related ground squirrel species; WHV also has a narrow host range, being reported not to infect ground squirrels or other rodent species. WMHBV is transmitted to the spider monkey.
- Oncogenicity: HBV, WHV and GSHV have been associated with primary liver cancer in infected hosts. However, the proposed mechanisms are different in each case, and incidence and typical time scales differ, being highest with WHV and lowest with HBV.

A number of related viruses that are strong candidates for inclusion in this genus have been isolated from non-human primates (chimpanzees, gibbons, orangutan and gorilla) and from various rodent species (Arctic ground squirrel and Richardson's ground squirrel). As illustrated in Figure 4, these isolates are quite similar to assigned isolates of HBV, WHV or GSHV.

## List of species in the genus *Orthohepadnavirus*

| | | |
|---|---|---|
| *Ground squirrel hepatitis virus* | | |
| Ground squirrel hepatitis virus | [K02715] | (GSHV) |
| *Hepatitis B virus* | | |
| Hepatitis B virus - A | [X02763] | (HBV-A) |
| Hepatitis B virus - B | [D00330] | (HBV-B) |
| Hepatitis B virus - C | [AY123041] | (HBV-C) |
| Hepatitis B virus - D | [V01460] | (HBV-D) |
| Hepatitis B virus - E | [X75657] | (HBV-E) |
| Hepatitis B virus - F | [X69798] | (HBV-F) |
| Hepatitis B virus - G | [AF160501] | (HBV-G) |
| Hepatitis B virus - H | [AY090454] | (HBV-H) |
| Hepatitis B virus - gib I | [AJ131569] | (HBV-gib I) |
| Hepatitis B virus - gib II | [AJ131571] | (HBV-gib II) |
| Hepatitis B virus - gib III | [AJ131572] | (HBV-gib III) |
| Hepatitis B virus - gib IV | [AJ131573] | (HBV-gib IV) |
| Hepatitis B virus - gib V | [AJ131574] | (HBV-gib V) |
| Hepatitis B virus - orangutan | [AF193864] | (HBV-orangutan) |
| Hepatitis B virus - chHBV | [D00220] | (chHBV) |
| *Woodchuck hepatitis virus* | | |
| Woodchuck hepatitis virus | [J02442] | (WHV) |
| *Woolly monkey hepatitis B virus* | | |
| Woolly monkey hepatitis B virus | [AF046996] | (WMHBV) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Orthohepadnavirus* but have not been approved as species

| | | |
|---|---|---|
| Arctic squirrel hepatitis virus | [U29144] | (ASHV) |

### Phylogenetic relationships within the genus

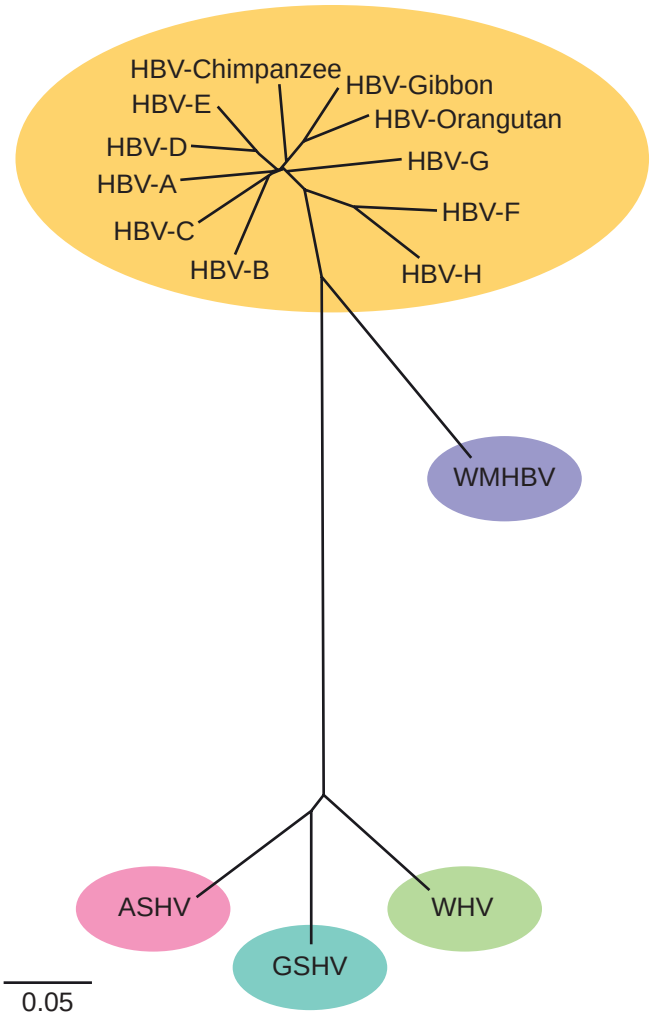


**Figure 4:** Phylogenetic tree of the genus *Orthohepadnavirus*. Complete genomes of hepatitis B virus (HBV) genotypes A (X02763), B (D00330), C (M12906), D (J02203), E (X75657), F (X69798), G (AF160501) and H (AY090454), and isolates found in chimpanzee (D00220), orangutan (AF193864) and gibbon (U46935), were aligned using Clustal W with orthohepadnavirus genomes from woolly monkey hepatitis B virus (WMHBV) (AF046996), woodchuck hepatitis virus (WHV) (J02442), ground squirrel hepatitis virus (GSHV) (K02715) and Arctic squirrel hepatitis virus (ASHV) (U29144). The alignment was tested with the neighbor-joining method. Calibration bar: substitutions per site. (Courtesy of Schaefer.)

## Genus *Avihepadnavirus*

Type species ***Duck hepatitis B virus***

### Distinguishing features

DHBV virions are spherical, 46–48 nm in diameter, with a nucleocapsid that is 35 nm in diameter and exhibits projections. Empty particles composed of excess envelope material are pleomorphic and up to 60 nm diameter. The single stranded gap in the virion DNA is usually very short, at about 12 nt. DHBV lacks an X gene containing a conventional initiation codon, but some other

avihepadnaviruses may have an X gene with a regular start codon. Virus particles have only the largest (36 kDa) and smallest (17 kDa) S proteins. Transmission is predominantly vertical. Heron hepatitis B virus (HHBV) differs from DHBV in that a highly conserved ORF is present upstream of C in a position analogous to the X gene of orthohepadnaviruses.

## Biological properties

DHBV is maintained in domestic duck flocks through vertical transmission from viremic ducks. The virus infects the developing liver *in ovo* and is not recognized sufficiently by the host immune response to produce hepatitis and liver disease, or to eliminate the virus. Liver cancer has not been associated with chronic infection. Transmission to neonates may also occur, leading to chronic infection. Transmission to adults generally leads to transient infection. The biology of HHBV infections in its natural host has not been studied.

## Species demarcation criteria in the genus

The species demarcation criteria in the genus are:

- Nucleotide sequence diversity: HHBV/DHBV 21.6%
- HHBV can be transmitted to herons but not to ducks.

A number of other characterized viruses have been isolated from geese and ducks, with sequences reportedly more closely related to that of DHBV than HHBV. A virus isolated from grey crowned cranes (*Balearica regulorum*), designated crane hepatitis B virus (CHBV), has sequences more closely related to DHBV than HHBV, and has been shown to infect primary duck hepatocytes. A virus closely related to HHBV has been isolated from white storks and designated stork hepatitis B virus (STHBV). Like HHBV, STHBV has a low infectivity for duck hepatocytes. Tentatively, STHBV may be assigned a variant of HHBV.

## List of species in the genus *Avihepadnavirus*

| | | |
|---|---|---|
| *Duck hepatitis B virus* | | |
| Duck hepatitis B virus | [K01834] | (DHBV) |
| Ross' goose hepatitis B virus | [M95589] | (RGHBV) |
| *Crane hepatitis virus* | [AJ44111] | (CHBV) |
| Heron hepatitis B virus | | |
| Heron hepatitis B virus | [M22056] | (HHBV) |
| Stork hepatitis B virus | [AJ251937] | (STHBV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Avihepadnavirus* but have not been approved as species

None reported.

## Phylogenetic relationships within the family *Hepadnaviridae*

Orthohepadnaviruses and avihepadnaviruses are distinguished by the following criteria:

- Low nucleotide sequence identity
- Differences in genome size (about 3.2 kb for orthohepadnaviruses and 3.0 kb for avihepadnaviruses)
- Larger core proteins and no M surface protein for the avihepadnaviruses
- Host range restricted to either mammals or birds, respectively.

## Similarity with other taxa

Reverse transcription, as an essential step in replication, is a common feature of hepadnaviruses, retroviruses and caulimoviruses. Hepadnaviruses and retroviruses also contain three major genes, each with the same function and in the same order (i.e. core-polymerase-pre S/S and *gag-pol-env* respectively). A fundamental distinction is that, with hepadnaviruses, the form of the genome in extracellular virions is DNA and reverse transcription takes place during the efferent or outgoing arm of the replication cycle, whereas the reverse holds true for retroviruses (with the exception of the spumaviruses, in which some infectious particles appear to contain a DNA genome). Retroviruses use tRNAs as primers for the DNA minus strand, whereas hepadnaviruses utilize a tyrosine in the polymerase itself. The polymerase protein of hepadnaviruses does not contain a protease or integrase function. Many other aspects are distinctly different in both virus families, partly due to the extremely small size of the hepadnaviral genome and the need to exploit this restricted genetic space efficiently by using the considerable overlap of both coding regions and regulatory elements.

## Derivation of names

*Avi*: from Latin *avis*, "bird".
*DNA*: deoxyribonucleic acid.
*Hepa*: from Greek *hepar*, "liver".
*Ortho*: from Greek *orthos*, "straight".

## Further reading

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## Contributed by

Mason, W.S., Gerlich, W.H., Taylor, J.M., Kann, M., Mizokami, T., Loeb, D., Sureau, C., Magnius, L. and Norder, H.

# Family *Metaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Metaviridae*** |
| Genus | ***Metavirus*** |
| Genus | ***Errantivirus*** |
| Genus | ***Semotivirus*** |

## Virion properties

### Morphology

*Metaviridae* is a family of retrotransposons that have been found in all studied lineages of eukaryotes. These viruses, primarily identified by their ability to induce mutations or by genome sequencing, replicate via virus-like intermediates referred to as a virus-like particles (VLPs). Members of the *Metaviridae* family are often referred to as LTR-retrotransposons of the Ty3-*gypsy* family. While there is good evidence that these particles are essential and direct intermediates in the life cycle of these viruses, only in one case, Drosophila melanogaster Gypsy virus (DmeGypV), do VLPs display infectivity according to the traditional virological definition. Viruses that generate VLPs or virions will be referred to collectively in this chapter as retrotransposons.

Morphology of particles is relatively poorly characterized and capsomeric symmetry is unknown. Members include species that produce primarily or exclusively intracellular particles [e.g., Saccharomyces cerevisiae Ty3 virus (SceTy3V)] so that collections of particles are heterogeneous with respect to stage of maturation. These intracellular particles will be referred to as virus-like particles (VLPs). Extracellular particles are enveloped with ovoid cores and will be referred to as virions (e.g., DmeGypV).

### Physicochemical and physical properties

In most systems virions are only crudely characterized biochemically.

### Nucleic acid

The genomes of retrotransposons in this family are positive strand RNAs. The genomic RNA is polyadenylated at the 3′ end; a cap structure has not yet been described, but may be presumed, given similarities of member elements with retroviruses. In addition to the RNA genome, some cellular RNAs may be randomly associated with particles including specific tRNAs in the case of virus replication which is primed by tRNAs. Particle fractions from cells are heterogeneous with respect to maturation and so are associated with intermediates and products of reverse transcription in addition to genomic RNA.

### Proteins

Proteins present in characterized VLPs include a major structural protein or capsid (CA), an aspartate protease (PR), reverse transcriptase containing an RNase H domain (RT-RH), and integrase (IN). For most viruses, these proteins are not yet characterized, but are predicted based on similarity of the protein sequence with those of proteins encoded by the internal domain. In most cases, the CA is not markedly similar to retroviral CA. VLPs of some retrotransposons in this family contain proteins with the metal finger characteristic of nucleocapsid (NC) of retroviruses. All viruses of the genus *Errantivirus* are distinguished by the presence of processed envelope proteins that apparently correspond to retroviral transmembrane (TM) and surface (SU) proteins. Only a limited number of viruses of the genera *Metavirus* and *Semotivirus* contain a putative *envelope* gene (*env*).

### Lipids

In the case of members that generate virions, the virion membrane appears to be derived from the membrane of the host cell.

### Carbohydrates

Carbohydrates have not been characterized, although their presence is inferred from sensitivity of the DmeGypV envelope precursor protein to digestion with endoglycosidase F.

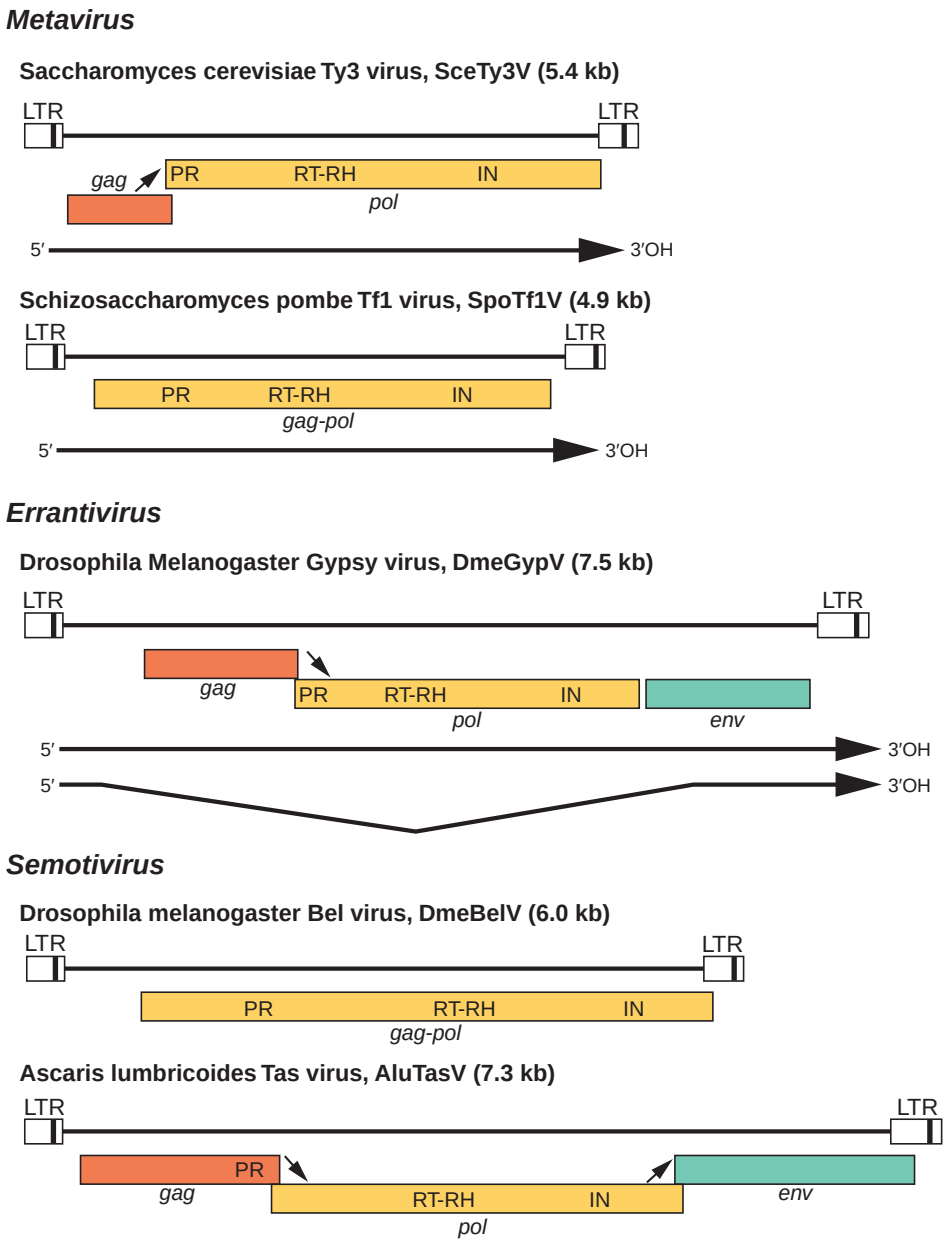


**Figure 1:** Genome organization of representative members of the *Metaviridae* family. The integrated genome of each virus contains Long Terminal Repeats (LTRs) flanking a central sequence. Black boxes within the LTRs depict sequences repeated at the 5′ and 3′ ends of the virus transcripts (R regions). Open boxes below the viruses indicate *gag*, *pol* and *env* ORFs. Not all viruses within the genera *Metavirus* and *Semotivirus* encode an Env-like protein. Arrows indicate sites of ribosomal frameshifting. Conserved aa sequences in *pol* that identify protease (PR), integrase (IN) and reverse transcriptase/RNAse H (RT-RH) are labeled. Individual mRNAs are depicted below the ORF diagrams as arrows. Transcription of the DmeBelV and AluTasV has not been studied.

## Genome organization and replication

The integrated form of these retrotransposons is composed of long terminal repeats (LTRs) flanking a central unique domain (Figure 1). The length of the viral genomes ranges from 4 kbp to more than 10 kbp. The LTRs are from 77 nt in the case of the Bombyx mori mag virus (BmoMagV) to greater than 2 kbp in length, in the cases of Drosophila virilis Ulysses virus (DviUlyV) and the Tribolium castaneum Woot virus (TcaWooV). Chromosomal copies of the viruses are flanked by short direct repeats of sequences derived from the insertion site. The length of the repeat is characteristic of the virus and ranges from 4 to 6 bp. The internal domain contains one to three ORFs.

The 3′ end of the final ORF can extend into the downstream LTR. In all cases, the order of domains encoded in the ORFs is inferred to be: 5′-CA-(NC where present)-PR-RT-RH-IN-3′. Where characterized, envelope proteins are encoded downstream of the IN domain by spliced mRNAs. These ORFs are referred to differently for different viruses and in this discussion, will be generally referred to as *gag*, *pol* and *env*. Thus viruses may have one *gag-pol* ORF, two (*gag* and *pol*) or three (*gag*, *pol* and *env*) ORFs.

Transcription of the genomic RNA is initiated in the upstream LTR and terminates at a position downstream of that site in the downstream LTR. This divides the long terminal repeats into regions represented uniquely in the 5′ end of the genomic RNA (U5), uniquely in the 3′ end of the genomic RNA (U3) or repeated at the 5′ and 3′ ends (R). Thus, the LTRs are comprised of U3-R-U5 regions analogous to those found in integrated retroviruses. By analogy with retroviruses, these species may carry two copies of the RNA genome per virion or particle; however, this has not yet been demonstrated, and dimerization functions have not yet been characterized.

Genomic RNA is translated into proteins required for particle formation, polyprotein maturation, reverse transcription and integration. Intracellular particle preparations show that particle fractions are comprised predominantly of species derived from the upstream portion of the ORF or where two or three ORFs are present from the first ORF. Where two ORFs occur, they usually overlap, and the second ORF is translated as a fusion protein of the first and second ORF translation products. The mechanism of frameshifting is not uniform among the member viruses. In case of the Schizosaccaromyces pombe Tf1 virus (SpoTf1V), the most completely characterized virus of this family containing one ORF, it appears that a polyprotein is produced and that later proteolytic events are responsible for a high ratio of major structural proteins to catalytic proteins. Little is known about where in the cell particle assembly occurs. PR is required for maturation of viral proteins. Catalytic proteins are PR, RT-RH and IN. Shortly after production of protein precursors, processed species are observed. Based on similarity of these metaviruses to retroviruses, it is likely that processing follows, and is dependent upon, intracellular assembly. Particle fractions are associated with genomic RNA and extrachromosomal DNA. RT activity associated with the particle fraction can be measured by exogenous assays.

Reverse transcription of genomic RNA of known members of this family is primed from either the 5′ end of the genomic RNA or from the 3′ end of a tRNA. In each case, the complementarity is overlapping, adjacent to, or just downstream of the U5 region of the genomic transcript. In cases in which the reverse transcription intermediates have been characterized (DmeGypV, Saccharomyces cerevisiae Ty3 virus (SceTy3V), and Schizosaccharomyces pombe Tf1 virus (SpoTf1V)), data are consistent with a species representing a minus-strand copy templated from the site of priming up to the 5′ end of the genomic RNA. This is a minor species. By analogy with retroviruses, this intermediate is probably transferred to the 3′ end of the genomic RNA, where an overlap of the R region minus strand represented in the cDNA, and the R region plus strand, represented at the 3′ end of the genomic RNA, allow transfer of the minus-strand strong stop which then acts to prime copying of the template plus-strand genomic RNA. Plus-strand priming probably occurs, as in retroviruses, from a polypurine tract or related sequence overlapping, adjacent to, or just upstream of the U3 region in the genomic RNA. This is consistent with priming from a site of cleavage by RH. Plus-strand, strong-stop species have been identified for some representatives (DmeGypV and SceTy3V) which are consistent with this position of priming and copying through to the first modified base in the primer tRNA. This family is heterogeneous with respect to the presence of extra terminal nt in the extrachromosomal replicated DNA and with respect to the presence of TG-CA inverted repeats at the ends of the integrated sequence.

## Biological properties

Activation of transposition of these viruses can cause disruption of host physiology depending on the site of insertion. Several members exhibit preferential patterns of insertion. It is notable that germline activation, which is a feature of some retroviruses, also occurs for some of these viruses. For example, SceTy3V transcription is induced by mating pheromone and transposition occurs after mating. In the case of the DmeGypV and the Drosophila melanogaster Zam virus (DmeZamV), transposition occurs in germline cells.

# Genus *Metavirus*

Type species ***Saccharomyces cerevisiae Ty3 virus***

## Distinguishing features

Saccharomyces cerevisiae Ty3 virus (SceTy3V) forms generally spherical, but irregular, intracellular particles of about 50 nm in diameter. These are observed as clusters or as individual particles in the cytoplasm of cells expressing high levels of SceTy3V RNA. Particles sediment as a heterodisperse population around 156S. The major particle-associated RNA species is a 5.2 kb, polyadenylated RNA. The primer of minus-strand reverse transcription is $tRNA^{iMet}$, which is complementary to a primer binding site (pbs). A minor 3.1 kb species is also observed, but the extent to which this is associated with particles has not been characterized. The 5.4 kb RNA contains two ORFs analogous to *gag* and *pol*, *GAG3* and *POL3*, which overlap in the +1 frame.

The genomic RNA is translated into Gag3 and Gag3-Pol3 polyproteins which are processed by SceTy3V PR. Mature proteins include CA (26 kDa), NC (15 kDa), PR (15 kDa), RT-RH and IN (58 kDa and 61 kDa, respectively), and an RT-RH-IN fusion protein of approximately 115 kDa. The molecular composition of the RT is not yet known. A protein of 10 kDa is predicted to be encoded between PR and RT but has not been identified. The integrated form of SceTy3V is 5.4 kbp in length and consists of an internal domain flanked by two LTRs (sigma elements) 340 bp in length. Insertions of SceTy3V are flanked by 5 bp direct repeats derived from insertion site cleavage and repair. SceTy3V is transcribed into a 5.2 kb genomic RNA. The $tRNA^{iMet}$ pbs has its 5′ end two nt downstream of the junction of the 5′ LTR with the internal domain. The full-length SceTy3V DNA molecule is 2 bp longer at each end than the integrated molecule, consistent with predictions based on the positions of the priming sequences. Two nt are removed from each 3′ end prior to integration. SceTy3V integrates within one or two nt of the transcription initiation site of genes transcribed by RNA polymerase III. SceTy3V is found in one to five copies in typical laboratory strains of *Saccharomyces cerevisiae*. In addition, there are approximately 30-40 copies of the isolated LTRs present. The latter presumably arose by recombination between the LTRs of complete viruses. SceTy3V transcription is induced by pheromone signal transduction. Although proteins are produced in cells undergoing signaling, DNA is not made in cells arrested in G1 of the cell cycle. Consequently, it is most likely that in natural populations, transposition only occurs after the fusion of mating cells to form diploids.

## Species demarcation criteria in the genus

Although the members of this family encode Pol proteins which are similar to those of SceTy3V, other properties of the members are distinct from SceTy3V. Several of the elements (Drosophila melanogaster micropia virus, DmeMicV; Lilium henryi del1 virus [LheDel1V]; and Schizosaccharomyces pombe Tf1 virus [SpoTf1V], Schizosaccharomyces pombe Tf2 virus [SpoTf2V] and Cladosporium fulvum T1 virus [CfuT1V]) encode a single long ORF from which major structural proteins, as well as Pol proteins, are expressed. In the most completely characterized case, SpoTf1V, the ORF is expressed as a single polyprotein, which is processed by a mechanism dependent on the SpoTf1V PR. Cells in stationary phase have the highest ratio of major structural to catalytic protein and products of reverse transcription accumulate concomitant with this transition. Members of this genus are also distinguished from SceTy3V by aspects of replication priming. SpoTf1V forms an RNA structure involving 89 bases at the 5′ end of the RNA, which is processed by RNase H to cleave within the structure between nt 11 and 12 from the 5′ end. This cleavage allows priming of SpoTf1V RT from the 3′ end of the 11 nt fragment annealed immediately downstream of the 5′-LTR. Other members of the genus (SpoTf2V and CfuT1V) have sequences consistent with a similar mechanism of self-priming. Thus these viruses are distinguished by self-priming, i.e., by an apparent lack of requirement for 3′-end processing. Individual species in the genus all have less than 50% identity in their Gag protein sequences compared to all other species. For example, although Drosophila melanogaster Mdg virus (DmeMdg1V) and Drosophila melanogaster 412 virus (Dme412V) each infects *Drosophila melanogaster*, their *gag* sequences are only 39% identical.

Two viruses, Drosophila buzzatti Osvaldo virus (DbuOsvaV) and Arabidopsis thaliana Athila virus (AthAthV), have an *env*-like gene. Based on this property it is possible to place these viruses within the *Errantivirus* genus. However, the envelope-like proteins of DbuOsvV and AthAthV are unrelated in sequence to that of the errantiviruses, and based on the sequence of their RT domain, cannot be placed within the *Errantivirus* genus.

## List of species in the genus *Metavirus*

| | | |
|---|---|---|
| *Arabidopsis thaliana Athila virus* | | |
| Arabidopsis thaliana Athila virus - At | [AC007209] | (AthAthV-At) |
| *Arabidopsis thaliana Tat4 virus* | | |
| Arabidopsis thaliana Tat4 - At | [AB005247] | (AthTat4V-At) |
| *Bombyx mori Mag virus* | | |
| Bombyx mori Mag virus - 200 X 300 | [X17219] | (BmoMagV-200X300 ) |
| *Caenorhabditis elegans Cer1 virus* | | |
| Caenorhabditis elegans Cer1 virus - Ce | [U15406] | (CelCer1V-Ce) |
| *Cladosporium fulvum T- 1 virus* | | |
| Cladosporium fulvum T- 1 virus - cf | [Z11866] | (CfuT1V-cf) |
| *Dictyostelium discoideum Skipper virus* | | |
| Dictyostelium discoideum Skipper virus - AX2 AX3 AX3 AX2 | [AF049230] | (DdiSkiV-AX2/3) |
| *Drosophila buzzatii Osvaldo virus* | | |
| Drosophila buzzatii Osvaldo virus - BU 30/4 | [AJ133521] | (DbuOsvV-BU30/4 ) |
| *Drosophila melanogaster Blastopia virus* | | |
| Drosophila melanogaster Blastopia virus - dm | [Z27119] | (DmeBlaV-dm) |
| *Drosophila melanogaster Mdg1 virus* | | |
| Drosophila melanogaster Mdg1 virus - dm | [X59545] | (DmeMdg1V-dm) |
| *Drosophila melanogaster Mdg3 virus* | | |
| Drosophila melanogaster Mdg3 virus - dm | [X95908] | (DmeMdg3V-dm) |
| *Drosophila melanogaster Micropia virus* | | |
| Drosophila melanogaster Micropia virus - CantonS | [X14037] | (DmeMicV-CantonS ) |
| *Drosophila melanogaster 412 virus* | | |
| Drosophila melanogaster 412 virus - dm | [X04132] | (Dme412V-dm) |
| *Drosophila virilis Ulysses virus* | | |
| Drosophila virilis Ulysses virus - dv | [X56645] | (DviUlyV-dv) |
| *Fusarium oxysporum Skippy virus* | | |
| Fusarium oxysporum Skippy virus - f. sp. lycopersici 42-87 | [L34658] | (FoxSkiV-42-87 ) |
| *Lilium henryi Del1 virus* | | |
| Lilium henryi Del1 virus - lh | [X13886] | (LheDel1V-lh) |
| *Saccharomyces cerevisia Ty3 virus* | | |
| Saccharomyces cerevisia Ty3 virus - AB950 | [M34549] | (SceTy3V-AB950 ) |
| *Schizosaccharomyces pombe Tf1 virus* | | |
| Schizosaccharomyces pombe Tf1 virus - NCYC 132 | [M38526] | (SpoTf1V-NCYC 132 ) |
| *Schizosaccharomyces pombe Tf2 virus* | | |
| Schizosaccharomyces pombe Tf2 virus - 972 | [L10324] | (SpoTf2V-972) |
| *Takifugu rubripes Sushi virus* | | |
| Takifugu rubripes Sushi virus - Tr | [AF030881] | (TruSusV-Tr) |
| *Tribolium castaneum Woot virus* | | |
| Tribolium castaneum Woot virus - A4/Ey | [U09586] | (TcaWooV-A4/Ey ) |
| *Tripneustis gratilla SURL virus* | | |
| Tripneustis gratilla SURL virus - Tg | [M75723] | (TgrSurV-Tg) |

Species names are in italic script; names of isolates and synonyms are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Metavirus* but have not been approved as species

None reported.

# Genus *Errantivirus*

Type species ***Drosophila melanogaster Gypsy virus***

## Distinguishing features

All elements contain a similar *env*-like ORF.

## Virion properties

Expression of DmeGypV results in production of enveloped irregular particles of approximately 100 nm in diameter and also much smaller non-enveloped particles. The *env* gene predicts a protein of 54 kDa. The actual protein has an apparent size of 66 kDa and is N-glycosylated as indicated by susceptibility to endoglycosidase F. More rapidly migrating molecules of 54 kDa and 28 kDa are also observed and are inferred to result from proteolytic processing by host enzymes, as is the case of members of the family *Retroviridae*.

## Genome organization and replication

DmeGypV is 7469 bp in length including two LTRs of 482 bp (Figure 1). It differs from most retroviruses and retrotransposons in that the termini are composed of AG...TT rather than TG...CA. The 11 nt immediately adjacent to the upstream U3 element and overlapping by one nt is complementary to $tRNA^{Lys}$. The DNA flanking the insertion includes 4 bp repeats, and the insertion site preference is for YRYRYR (where Y = purine and R = pyrimidine) sequence. The genomic RNA contains one ORF encoding the major structural protein and a second ORF overlapping in the −1 frame, encoding homologs of retroviral PR, RT-RH and IN. A third ORF, *env*, encoding a 54 kDa envelope protein, occurs in a spliced 2.1 kb mRNA. This protein is apparently N-glycosylated, processed into smaller species, and is analogous to retroviral envelope proteins by virtue of hydrophobic putative membrane spanning domains, localization to the viral membrane, similarity of processing sites for cleavage into trans-membrane and surface domains, and glycosylation. An envelope protein of similar sequence to that in DmeGypV has been identified for all members of this genus. The Env protein of DmeZamV is translated from a 1.7 kb spliced message. The envelope proteins of errantiviruses have been shown to have sequence similarity to the viral *env* gene of certain baculoviruses.

DmeGypV is transcribed into a 6.5 kb genomic RNA. A minus-strand strong stop species of approximately 242 nt (with RNA removed) has been identified. Plus-strand strong-stop DNA species of 479 nt, which are similar in length to the LTR, and a species longer by 15 to 18 nt, presumed to result from copying of the tRNA primer, have been observed.

## Biological properties

DmeGypV transposition is repressed by the activity of the *flamenco* gene. In females homozygous for the permissive allele of *flam*, the somatic follicle cells surrounding maternal germline cells appear to accumulate DmeGypV RNA and envelope protein. Transposition, however, is observed in the maternal germ cells, and this has led to the hypothesis that transposition is attributable to infection from surrounding follicle cells. A similar path of activity has also been suggested for DmeZamV and DmeIdeV. Infection has been demonstrated to result when susceptible strains are raised in the presence of DmeGypV particles mixed into their food. Incubation with antibodies against the Env protein decreased the level of infection, implicating Env in this process.

## Species demarcation criteria in the genus

At least one member of this group, DmeGypV, is infectious, and all members of the genus *Errantivirus* have a similar third ORF resembling *env*. While this property makes the errantiviruses candidates for inclusion into the family *Retroviridae* rather than the family *Metaviridae*, there is no sequence similarity between the *env* genes of these two groups, suggesting independent acquisition events. In addition, examination of phylogenetic relationship of these viruses based on their reverse transcriptase sequences (Figure 2) places the errantiviruses as an independent lineage distinct from the

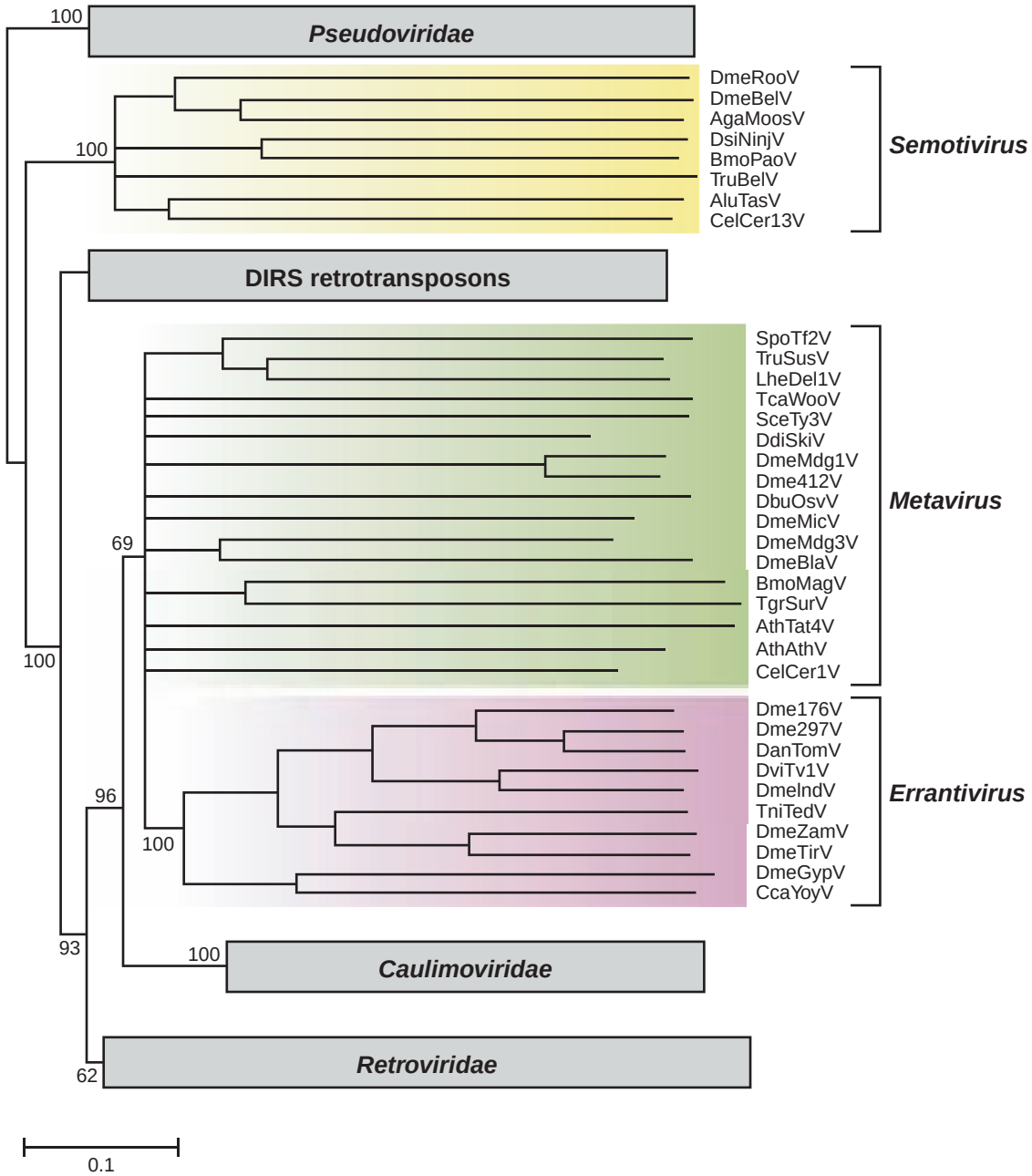


**Figure 2:** Phylogeny of the family *Metaviridae* and related groups based on their reverse transcriptase domain. The portion of the reverse transcriptase domain used in this analysis spans approximately 250 aa and includes the most conserved residues found in all retroelements. The phylogram is a 50% consensus tree of the viruses based on neighbor-joining distance algorithms and was rooted using sequences of members of the family *Pseudoviridae*. Bootstrap values (percentage of the time all elements are located on that branch) are shown for the major branches only. Viruses that are included in the various divisions of *Metaviridae* are indicated by the vertical lines to the right of each systematic name. Each group of viruses that are not part of the family *Metaviridae* is represented by a box with the length of the box related to the sequence diversity within that group. DIRS retrotransposons are mobile elements that utilize a reverse transcriptase closely related to that of the family *Metaviridae* but lack many structural features of this group and integrate by a different mechanism. The bar at the bottom represents divergence per site.

family *Retroviridae*. Individual species in the genus all have less than 50% identity in their Gag protein sequences compared to all other species. For example DmeZamV and DmeGypV are two species of viruses in this family from *Drosophila melanogaster*, but their *gag* sequences are only 35% identical.

### List of species in the genus *Errantivirus*

| | | |
|---|---|---|
| *Ceratitis capitata Yoyo virus* | | |
| Ceratitis capitata Yoyo virus - Med+ | [U60529] | (CcaYoyV-Med+) |
| *Drosophila ananassae Tom virus* | | |
| Drosophila ananassae Tom virus - da | [Z24451] | (DanTomV-da) |
| *Drosophila melanogaster Gypsy virus* | | |
| Drosophila melanogaster Gypsy virus - gy | [M12927] | (DmeGypV-gy) |
| *Drosophila melanogaster Idefix virus* | | |
| Drosophila melanogaster Idefix virus - dmi | [AJ009736] | (DmeIdeV-dmi) |
| *Drosophila melanogaster Tirant virus* | | |
| Drosophila melanogaster Tirant virus - dm | [X93507] | (DmeTirV-dm) |
| *Drosophila melanogaster Zam virus* | | |
| Drosophila melanogaster virus - dm | [AJ000387] | (DmeZamV-dm) |
| *Drosophila melanogaster 17.6 virus* | | |
| Drosophila melanogaster 17.6 virus - dm | [X01472] | (Dme176V-dm) |
| *Drosophila melanogaster 297 virus* | | |
| Drosophila melanogaster 297 virus - dm | [X03431] | (Dme297V-dm) |
| *Drosophila virilis Tv1 virus* | | |
| Drosophila virilis Tv1 virus - dv | [AF056940] | (DviTv1V-dv) |
| *Trichoplusia ni TED virus* | | |
| Trichoplusia ni TED virus - mutant FP- D | [M32662] | (TniTedV-FP-D) |

Species names are in italic script; names of isolates and synonyms are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Errantivirus* but have not been approved as species

None reported.

## GENUS *SEMOTIVIRUS*

Type species ***Ascaris lumbricoides Tas virus***

### Distinguishing features

The integrated form of Ascaris lumbricoides Tas virus (AluTasV) is 7.6 kbp in length and consists of an internal domain flanked by two LTRs 256 bp in length. Insertions of AluTasV are flanked by 5 bp direct repeats derived from the insertion sites. There are approximately 50 copies of AluTasV distributed in the genome of *A. lumbricoides*. RNA transcripts and VLPs have not been observed but can be inferred based on the similarity in structure and coding capacity to that of other members of the family *Metaviridae* or the family *Retroviridae*. Reverse transcription is primed by $tRNA^{arg}$ which anneals to the pbs 6 bp downstream of the 5′-LTR. AluTasV encodes three overlapping ORFs: the first encodes the major structural protein and PR, the second ORF overlapping in the −1 frame encoding RT-RH and IN, and the third overlapping in the +1 frame encoding the *env*. The Env-like protein encoded by AluTasV contains a transmembrane domain but exhibits no sequence similarity with the *env* gene of *Errantivirus* or *Retroviridae* suggesting its independent acquisition. The likely origin of the AluTasV third ORF is the glycoprotein gB gene of herperviruses.

### Species demarcation criteria in the genus

Members of this group have been identified in vertebrates, insects and nematodes. A reverse transcriptase phylogenetic tree (Figure 2) indicates that all members of this group are well separated

from members of the *Metavirus* and *Errantivirus* genera. Based on the sequence of the putative primer binding site, most viruses in this genus use either the acceptor stem of various $tRNA^{Arg}$ or $tRNA^{Gly}$ as the primer for minus-strand synthesis during reverse transcription. One continuous or two overlapping ORFs characterize the members of this group with the order of domains within the *pol* ORF (PR-RT-RH and IN) identical to that in the genera *Metavirus* and *Errantivirus*. Semotiviruses are particularly abundant in nematodes. The sequence of the *Caenorhabditis elegans* genome has revealed 13 families of viruses, but the majority of these are no longer active. An unusual feature of many of the *C. elegans* viruses is the presence of additional DNA between the 5′-LTR and the beginning of the first ORF. These additional sequences are variable within a family and completely different between families. One active group of sequences in the Caenorhabditis elegans Cer13 virus (CelCer13V) also contains a third Env-like domain between the *pol* encoded enzymatic domains and the 3′-LTR. This domain exhibits no sequence similarity with the domain in AluTasV, suggesting an independent acquisition. The likely origins of the CelCer13 Env-like domain is the G2 glycoprotein gene from phleboviruses. All individual species in the genus have less than 50% identity in their Gag protein sequences compared to all other species.

## List of species in the genus *Semotivirus*

| | | |
|---|---|---|
| *Anopheles gambiae Moose virus* | | |
| Anopheles gambiae Moose virus - Pink eye | [AF060859] | (AgaMooV-Pe) |
| *Ascaris lumbricoides Tas virus* | | |
| Ascaris lumbricoides Tas virus – al | [Z29712] | (AluTasV-al) |
| *Bombyx mori Pao virus* | | |
| Bombyx mori Pao virus – 703 | [L09635] | (BmoPaoV-703) |
| *Caenorhabditis elegans Cer13 virus* | | |
| Caenorhabditis elegans Cer13 virus - Bristol N2 | [Z81510] | (CelCer13V-BrN2) |
| *Drosophila melanogaster Bel virus* | | |
| Drosophila melanogaster Bel virus - white- zeste mottled | [U23420] | (DmeBelV-wzm) |
| *Drosophila melanogaster Roo virus* | | |
| Drosophila melanogaster Roo virus – roo | [AY180917] | (DmeRooV-roo) |
| *Drosophila simulans Ninja virus* | | |
| Drosophila simulans Ninja virus - white- chocolate | [D83207] | (DsiNinV-wc) |
| *Fugu rubripes Suzu virus* | | |
| Fugu rubripes Suzu virus – Fr | [AF537216] | (FruSuzV-Fr) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Semotivirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Metaviridae*

None reported.

## Phylogenetic relationships within the family

See Figure 2.

## Similarity with other taxa

Like viruses of the family *Pseudoviridae*, the viruses of the family *Metaviridae* are clearly related to the viruses of the family *Retroviridae*. All of these families are related by reverse transcription and a viral core structure made up of Gag-like proteins. All members of the families *Metaviridae*, *Pseudoviridae* and *Retroviridae* also share the following: a proviral form characterized by LTRs, protease, RNase H and integrase activities essential for multiplication, read through-mediated (Gag-Pol) *pol* gene expression and tRNA primers (in some species). An important and somewhat controversial question is therefore the extent of the relationship of the members of the family *Metaviridae* to those of

the family *Retroviridae*. Reverse transcriptase aa sequences are the most conserved sequences in retroelements and hence are the best character on which to base the phylogenetic relationship of these elements. Based on the phylogeny of their RT domains (Figure 2, see also Figure 4 in the chapter *Pseudoviridae*) members of the families *Metaviridae* and *Pseudoviridae* probably shared a common ancestor. The only major structural change between the genomes of members of these two groups was the movement of the IN domain from upstream to downstream of the RT-RH domain. The family *Metaviridae* is a numerous and diverse group of viruses distributed throughout eukaryotes. One lineage of the family *Metaviridae* in vertebrates appears to have given rise to the family *Retroviridae*.

The conversion of a lineage of the family *Metaviridae* into the family *Retroviridae* presumably occurred by the transduction of a gene encoding a ligand for cell-surface receptors or a cell fusion protein. The independent acquisition of a cell-surface receptor or fusion protein has occurred on at least five other occasions within the family *Metaviridae*. These include the *Errantivirus* genus in insects, Drosophila buzzatti Osvaldo virus (DbuOsvaV) and AthAthV within the genus *Metavirus* in insects and plants respectively, and AluTasV and CelCer13V within the genus *Semotivirus* in nematodes. The ease with which these viruses can gain, and presumably lose, an *env*-like gene means that this property is not always a reliable indicator of phylogenetic relationships. Finally, a second lineage in plants has become the family *Caulimoviridae* by the acquisition of a number of new genes that resulted in a number of changes to its life cycle (see description of *Caulimoviridae*).

## Derivation of names

*Erranti*: from Latin *errans*, "to wander".
*Meta*: from Greek *metathesis* for "transposition". Also to connote some uncertainty as to whether these are true viruses or not.
*Semoti*: from Latin *semotus*, "distant, removed". This prefix refers to the observation that based on the sequence of their RT domain, the viruses in this genus are distantly related to the other two genera of the family *Metaviridae*.

## Further reading

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## Contributed by

In reproducing this chapter in edited form from the Eighth ICTV Report, the editors gratefully acknowledge the contribution of the authors, Eickbush, T., Boeke, J.D., Sandmeyer, S.B. and Voytas, D.F.

# Family *Pseudoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Pseudoviridae*** |
| Genus | ***Pseudovirus*** |
| Genus | ***Hemivirus*** |
| Genus | ***Sirevirus*** |

## Virion properties

### Morphology

*Pseudoviridae* is a family of retrotransposable elements, primarily identified by genome sequencing. Pseudoviruses are often referred to as LTR-retrotransposons of the Ty1-copia family. They replicate via a virus-like intermediate referred to as a virus-like particle (VLP). VLPs do not display infectivity according to the traditional virological definition. However, there is good evidence that these particles are essential and direct intermediates in the life cycle of these elements. We will use the terms "virion" and "virus" here to conform to the usage in this volume.

Members of the family *Pseudoviridae* are typified by somewhat irregularly shaped VLPs that are round to ovoid, often with electron-dense centers. Although the VLPs are irregular in their native state, by expressing truncated forms of the major coat protein (Gag) icosahedral VLPs with regularity can be observed by cryo-electron microscopy (Figure 1). Saccharomyces cerevisiae Ty1 virus (Ty1) and Drosophila melanogaster copia virus (copia) both make similar looking particles, but Ty1 particles are cytoplasmic, whereas those of copia are nuclear. The typical mean radius of the VLPs is 30–40 nm. There is no envelope, although some members encode an *env*-like gene whose function is unknown.

### Physicochemical and physical properties

In most systems, virions are only partially characterized biochemically.

### Nucleic acid

The major virion RNA species consist of an LTR-to-LTR transcript of about 5–9 kb. In addition, most viruses package one or more host-derived primer tRNAs. The LTR-to-LTR transcript encodes one to two ORFs in most viruses, the equivalents of retroviral *gag* and *pol*; the second ORF is typically expressed at lower levels than the first. There is RNA, as well as various forms of DNA (intermediates) in the virion preparation. The RNA is 5–9 kb, positive sense, capped and polyadenylated; the DNA is 5.5–10 kbp. The linear ssRNA is packaged in virions, and the linear dsDNA "provirus" is integrated into the host genome.

### Proteins

Both Gag and Gag-Pol primary translation products are processed by the cognate protease into final products. The known *gag*-encoded proteins include analogs of retroviral capsid (CA) and

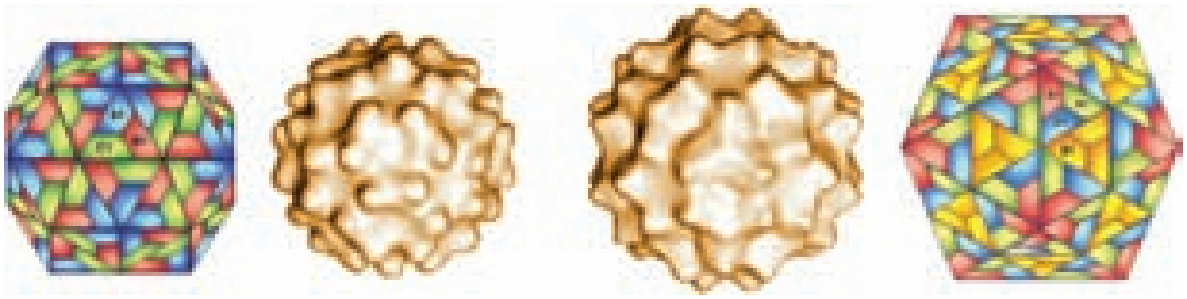

**Figure 1:** Saccharomyces cerevisiae Ty1 virus (SceTy1V) 1-381 virions; surface structure of two forms (T = 3, left; T = 4, right) determined by cryo-electron microscopy, with the corresponding diagrammatic models. (Courtesy of H. Saibil, adapted from *J. Virol.*, **71**, 6863–6868.)

nucleocapsid (NC), although the latter is only present in a subset of these viruses. The known *pol*-encoded proteins include the protease (PR), integrase (IN), and reverse transcriptase/RNase H (RT). All of these proteins appear to be required for replication.

### Lipids
None present.

### Carbohydrates
None present.

## Genome organization and replication

Most viruses encode a single ORF with similarity to retroviral *gag* and *pol* (Figure 2). The yeast Ty1, Ty2 and Ty4 viruses contain *pol* in the +1 frame relative to *gag*. For copia, *gag* is encoded on a spliced 2 kb mRNA, and differential splicing is the mechanism by which Gag and Gag-Pol stoichiometry is regulated. The mechanism(s) that regulates Gag and Gag-Pol expression for most single ORF viruses is unknown.

The virion-associated RT mediates the conversion of the LTR to LTR transcript into a full-length nucleic acid duplex containing full-length LTR sequences in the form of dsDNA. This DNA is then integrated into host DNA by the IN protein, where it becomes a part of the host genome and can persist there, essentially indefinitely. The integrated form (equivalent to the retroviral provirus) is then transcribed by host RNA polymerase II to generate new virus RNAs. In most viruses, the reverse transcription and integration processes closely mimic the replication of retroviral RNA, but there are some important exceptions.

## Antigenic properties

No information available.

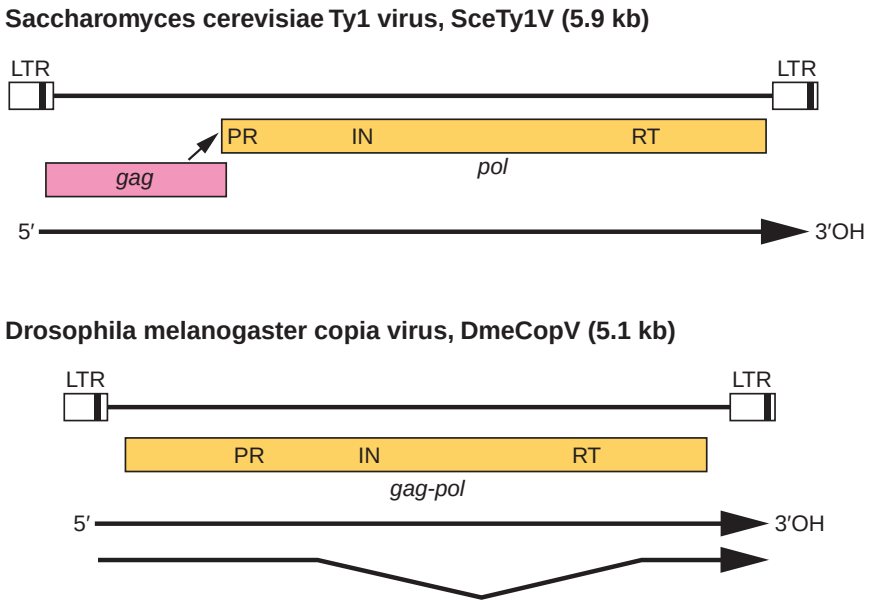


**Figure 2:** The genomic organization of Saccharomyces cerevisiae Ty1 virus (SceTy1V) (5.9 kb) and Drosophila melanogaster *copia* virus (DmeCopV) (5.1 kb). Black boxes within the LTRs depict sequences repeated at the 5′ and 3′ ends of the virus's transcripts (R regions); sequences 5′ of R represent U3, and sequences 3′ of R represent U5. Open boxes below the viruses indicate *gag* and *pol*. Conserved aa sequences in *pol* that identify protease (PR), integrase (IN) and reverse transcriptase/RNAse H (RT) are labeled. Individual mRNAs are depicted as arrows. Arrowhead indicates site of ribosomal frameshifting.

### Biological properties

Pseudoviruses are commonly referred to as LTR retrotransposons of the Ty1/*copia* family. They form an intrinsic and significant part of the genome of many eukaryotic species, especially plants. For most of these viruses, the virion is an essential part of their multiplication cycle, but is not infectious, in the traditional virological sense, under normal conditions.

### Species demarcation criteria in the family

In general, viruses inhabiting different host species will be considered different species, because they will have diverged through vertical descent from a common ancestor at least as much as the host species themselves (probably more so, due to the error-prone mechanism of replication). However, there are instances in the family *Pseudoviridae* and the family *Metaviridae* in which one finds two closely related viruses inhabiting the same host species, e.g. Ty1 and Ty2 of *S. cerevisiae*. In this pair of viruses, the RT aa sequences are quite similar. However, the capsid-encoding sequences are significantly diverged (e.g. <50% aa sequence identity; their DNA sequences fail to cross-hybridize). The question then arises whether these represent different species or more subtle variants. We have considered such viruses separate species if at least one of the major coding regions (e.g. capsid) is <50% identical to the reference aa sequence. For example, Ty1 and Ty2 Gag aa sequences are 49% identical.

## GENUS *PSEUDOVIRUS*

Type species ***Saccharomyces cerevisiae Ty1 virus***

### Virion properties

Virions are ovoid to spheroid strictly intracellular particles, sometimes observed as paracrystalline clusters in the cytoplasm in yeast cells. Particles are quite heterodisperse structurally. However, the C-terminal deletion mutant containing residues 1–381 of the Gag protein is less heterodisperse than the wild-type, and cryo-electron micrographs of these particles reveal a surface structure with icosahedral symmetry (Figure 1). The mean radius of the virions is 20–30 nm for wild type and 20 nm for the much better characterized and more uniform 1-381 mutant. The virions are not enveloped. The total Mr is estimated at 14 MDa and the sedimentation coefficient is estimated at about 200–300S for wild type and 115S for 1-381 mutant. The virions are sensitive to high temperature (65 °C).

The major virion RNA species consist of an LTR-to-LTR transcript of 5.6 kb. In addition Ty1 packages host-derived primer tRNAiMet. Ty1 particles contain two RNA molecules. This transcript encodes two overlapping ORFs, GAG and POL; the second expressed as the result of a +1 frameshift relative to the first. The major nucleic acid forms are RNA in the VLP, as well as various DNA forms (intermediates) in VLP preparations, and finally, after integration is complete, the integrated DNA "provirus". The genome size for Ty1 is RNA – 5.6 kb; DNA – 5.9 kb. There is linear ssRNA in the virion, and the provirus (integrated) consists of dsDNA.

The known GAG-encoded proteins are the CA and a short C-terminal peptide (the latter is only inferred to exist and has not been directly observed). No recognizable NC peptide has been identified, although the latter peptide performs similar functions. The known POL-encoded proteins are a protease (PR), integrase (IN) and reverse transcriptase/RNase H (RT). All of these proteins are required for replication.

### Genome organization and replication

The DNA form of the Ty1 genome consists of two 335 bp LTRs largely flanking a central coding region, although Ty1 is unusual in that GAG initiates within the U5 region of the 5′ LTR. The LTR sequences can be divided into three segments called U3, R and U5 (Figure 2). The U3 region is unique to the 3′ end of the RNA, the R region is repeated in the RNA and the U5

region is unique to the 5′ end of the RNA. Ty1 encodes a 5.6 kb ssRNA with two ORFs. The first ORF is GAG and encodes the major CA as well as a small C-terminal peptide; these two proteins are proteolytically derived from the GAG primary translation product. The second ORF, which overlaps GAG in the +1 frame, is POL and encodes protease (PR), integrase (IN), and reverse transcriptase/RNAse H (RT). These three POL proteins are derived by proteolysis of the GAG-POL precursor by the Ty1 PR; there is evidence that cleavage at the GAG-PR boundary obligatorily precedes the other cleavages. The GAG-POL precursor is expressed by an inefficient programmed frameshift that occurs within the sequence CUU AGG C (the indicated codon boundaries represent the GAG frame), which is necessary and sufficient to specify the +1 frameshift. A minor shorter transcript of about 2.2 kb is reported to be 5′ coterminal with the major transcript, but has not been fully characterized. A second minor transcript is reported to be 3′ coterminal with the major transcript and is most clearly observed in yeast strains with *spt3* mutations. These *spt3* mutations eliminate or greatly reduce the abundance of the full-length transcript.

The initial step in replication is transcription of the Ty1 virus to generate the full-length RNA described above. This RNA is encapsidated in the cytoplasm in a precursor particle consisting of unprocessed GAG and GAG-POL proteins. Action of the Ty1 PR then converts this into a mature virus particle. It is thought that this somehow activates the reverse transcription process.

The first step in the reverse transcription process is the extension of the tRNAiMet primer, which binds to the (−) strand primer binding site ((−)PBS) in the full-length RNA. The product of this extension is referred to as (−) strand strong stop DNA or (−)ssDNA by analogy with retroviruses. The (−)ssDNA is transferred to the 3′ end of the full-length RNA, where it can be further extended to generate a nearly full-length (−) strand DNA. Priming of the plus strand initiates at the (+) PPT1 (for polypurine tract 1) adjacent to the 3′ LTR, but the mechanism of this priming and the exact nature of the primer have not been determined. Extension yields a product, (+) ssDNA that corresponds to the similarly named retroviral intermediate. Transfer of (+)ssDNA to the left end of the (−) strand DNA sets up a primer-template that can be extended, in principle, to generate full-length duplex DNA. However, studies indicate that the (+) strand is not continuous because a second priming event occurring near the middle of the molecule at a site called (+) PPT2 results in a discontinuity in the middle of the plus strand. We refer to this final product of reverse transcription as the dsDNA form, although some experiments suggest that in many of these molecules there may be stretches of RNA rather than DNA in the (+) strand. The dsDNA is imported into the cell nucleus, possibly by a nuclear localization signal found at the C-terminus of IN.

The full length dsDNA is a substrate for the Ty1 IN, which inserts the dsDNA into a chromosomal target site, in the process generating a 5 bp target site duplication of the host target site DNA. Sequences located upstream of RNA polymerase III-transcribed genes represent strongly preferred targets for such Ty1 integration *in vivo*.

## Biological properties

Retrotransposon Ty1 is best thought of as a genome parasite of *Saccharomyces cerevisiae*. In typical wild strains of this yeast, 3–15 copies of Ty1 are found per haploid genome, whereas in typical laboratory isolates, there are 25–40 copies. In addition to these complete copies, the genome contains several hundred solo-LTRs (not associated with a central coding region) or fragments thereof. Ty1 appears to be restricted to this host species and to very closely related species of *Saccharomyces*, but is absent from more distant species of the genus. However, those species are likely to harbor related viruses. Transmission is likely to be exclusively vertical, and horizontally through conjugation. Ty1 and/or the closely related species, Ty2, has been found in virtually all isolates of *S. cerevisiae*, from all over the world. No cytopathic effects have been reported. Some strains contain large numbers of Ty1 virus particles and are otherwise normal in every way. However, overexpression of Ty1 proteins leads to slow growth, but this phenotype is poorly characterized.

## List of species in the genus *Pseudovirus*

| | | |
|---|---|---|
| *Arabidopsis thaliana Art1 virus* | | |
| Arabidopsis thaliana Art1 virus | [Y08010] | (AthArt1V) |
| *Arabidopsis thaliana AtRE1 virus* | | |
| Arabidopsis thaliana AtRE1 virus | [AB021263] | (AthAtRV) |
| *Arabidopsis thaliana Evelknievel virus* | | |
| Arabidopsis thaliana Evelknievel virus | [AF039373] | (AthEveV) |
| *Arabidopsis thaliana Ta1 virus* | | |
| Arabidopsis thaliana Ta1 virus | [X13291] | (AthTa1V) |
| *Brassica oleracea Melmoth virus* | | |
| Brassica oleracea Melmoth virus | [Y12321] | (BolMelV) |
| *Cajanus cajan Panzee virus* | | |
| Cajanus cajan Panzee virus | [AJ000893] | (CcaPanV) |
| *Glycine max Tgmr virus* | | |
| Glycine max Tgmr virus | [U96748] | (GmaTgmV) |
| *Hordeum vulgare BARE-1 virus* | | |
| Hordeum vulgare BARE-1 virus | [Z17327] | (HvuBV) |
| *Nicotiana tabacum Tnt1 virus* | | |
| Nicotiana tabacum Tnt1 virus | [X13777] | (NtaTnt1V) |
| *Nicotiana tabacum Tto1 virus* | | |
| Nicotiana tabacum Tto1 virus | [D83003] | (NtaTto1V) |
| *Oryza australiensis RIRE1 virus* | | |
| Oryza australiensis RIRE1 virus | [D85597] | (OauRirV) |
| *Oryza longistaminata Retrofit virus* | | |
| Oryza longistaminata Retrofit virus | [U72726] | (OloRetV) |
| *Physarum polycephalum Tp1 virus* | | |
| Physarum polycephalum Tp1 virus | [X53558] | (PpoTp1V) |
| *Saccharomyces cerevisiae Ty1 virus* | | |
| Saccharomyces cerevisiae Ty1 virus | [M18706] | (SceTy1V) |
| *Saccharomyces cerevisiae Ty2 virus* | | |
| Saccharomyces cerevisiae Ty2 virus | [M19542] | (SceTy2V) |
| *Saccharomyces cerevisiae Ty4 virus* | | |
| Saccharomyces cerevisiae Ty4 virus | [M94164] | (SceTy4V) |
| *Solanum tuberosum Tst1 virus* | | |
| Solanum tuberosum Tst1 virus | [X52387] | (StuTst1V) |
| *Triticum aestivum WIS-2 virus* | | |
| Triticum aestivum WIS-2 virus | [CT009735] | (TaeWis1V) |
| *Zea mays Hopscotch virus* | | |
| Zea mays Hopscotch virus | [U12626] | (ZmaHopV) |
| *Zea mays Sto-4 virus* | | |
| Zea mays Sto-4 virus | [AF082133] | (ZmaStoV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Pseudovirus* but have not been approved as species

None reported.

# GENUS *HEMIVIRUS*

Type species ***Drosophila melanogaster copia virus***

## Distinguishing features

For copia, Gag is encoded on a spliced 2 kb mRNA, and differential splicing is the mechanism by which Gag and Gag-Pol stoichiometry is regulated. As in the genus *Pseudovirus*, these viruses

encode *gag* and *pol* in one or two reading frames. The mechanism(s) that regulates Gag and Gag-Pol expression for most single ORF viruses remain to be determined.

Species in this genus typically use an initiator methionine tRNA as the primer for minus-strand DNA synthesis during reverse transcription. Hemiviruses, unlike members of the families *Retroviridae* and *Metaviridae* and genus *Pseudovirus*, use an initiator methionine tRNA half-molecule as a primer (or an Arg tRNA half-molecule in the case of Candida albicans Tca2 virus (Tca2)). This tRNA fragment is generated by cleaving the initiator tRNA in the anticodon stem. However, relatively little is known about the detailed mechanism of this reaction.

Both Gag and Gag-Pol primary translation products are processed by the cognate protease into final products. The known *gag*-encoded proteins include analogs of retroviral CA and NC. The known *pol*-encoded proteins include the protease (PR), integrase (IN) and reverse transcriptase/RNase H (RT). All of these proteins appear to be required for replication.

These viruses are found worldwide in fungi, algae and insects genomes. Their mode of transmission is unknown although presumed to be through vertical inheritance.

### List of species in the genus *Hemivirus*

| | | |
|---|---|---|
| *Aedes aegypti Mosqcopia virus* | | |
| Aedes aegypti Mosqcopia virus | [AF134899] | (AaeMosV) |
| *Candida albicans Tca2 virus* | | |
| Candida albicans Tca2 virus | [AF050215] | (CalTca2V) |
| *Candida albicans Tca5 virus* | | |
| Candida albicans Tca5 virus | [AF065434] | (CalTca5V) |
| *Drosophila melanogaster 1731 virus* | | |
| Drosophila melanogaster 1731 virus | [X07656] | (Dme1731V) |
| *Drosophila melanogaster copia virus* | | |
| Drosophila melanogaster copia virus | [X04456] | (DmeCopV) |
| *Saccharomyces paradoxus Ty5 virus* | | |
| Saccharomyces paradoxus Ty5 virus | [U19263] | (SceTy5V) |
| *Volvox carteri Lueckenbuesser virus* | | |
| Volvox carteri Lueckenbuesser virus | [U90320] | (VcaLeuV) |
| *Volvox carteri Osser virus* | | |
| Volvox carteri Osser virus | [X69552] | (VcaOssV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Hemivirus* but have not been approved as species

None reported.

## GENUS *SIREVIRUS*

Type species ***Glycine max SIRE1 virus***

### Distinguishing features

All members of this genus have thus far been identified only in plants. A reverse transcriptase phylogenetic tree separates the sireviruses from pseudoviruses and hemiviruses (Figure 4). Based on the sequence of putative primer binding sites, most species in this genus likely use the acceptor stem of an initiator methionine tRNA as the primer for minus-strand DNA synthesis during reverse transcription, similar to the pseudoviruses. Clusters of conserved PPTs are also present in many species, and highly conserved DNA sequence motifs of unknown function are present in the LTRs and internal domain.

A unique ORF and multiple gene expression mechanisms characterize members of this genus. For example, viruses such as Glycine max SIRE1 virus (SIRE1), Arabidopsis thaliana Endovir virus (Endovir) and Lycopersicon esculentum ToRTL1 virus (ToRTL1) have an extra ORF between *pol* and the 3′ LTR (Figure 3). This ORF has conserved transmembrane domains and is referred to as an *env*-like ORF. For SIRE1, env-like expression is regulated by stop codon suppression. However, stop codon suppression does not seem to be utilized by the other viruses (e.g. Endovir and ToRTL1), because the env-like ORF is separated from *pol* by non-coding sequence. In addition, based on ORF organization, two different Gag-Pol expression mechanisms are likely utilized by members of this genus. Some viruses such as SIRE1 and Endovir encode *gag* and *pol* in a single ORF. In contrast, for viruses such as Opie-2 and Prem-2, *pol* resides in a +1 frame (Figure 3). A unifying characteristic of this genus is that in all members, the *gag* gene is nearly twice the size of *gag* encoded by pseudoviruses and hemiviruses.

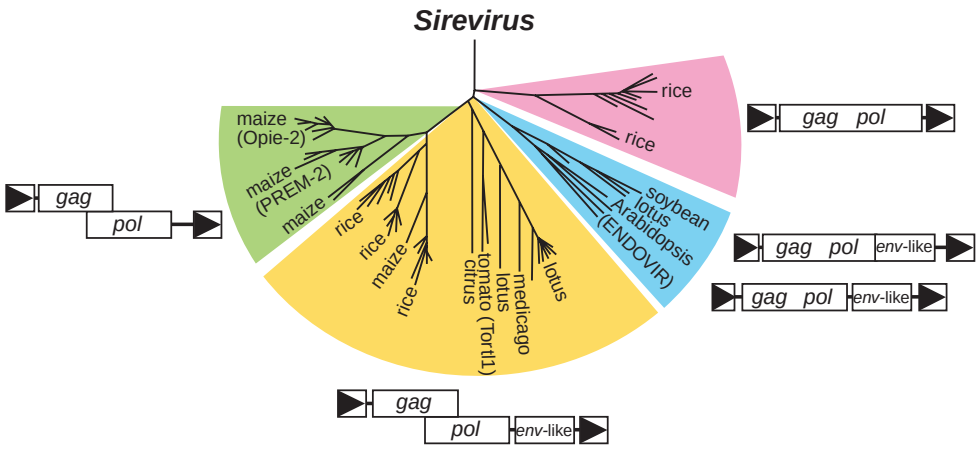


**Figure 3:** Examples of variability in the genome organization of members of the genus *Sirevirus*. Variability can be observed by the presence or absence of an *env*-like ORF, the organization of the *env*-like ORF relative to *pol*, and the organization of *gag* and *pol*.

## List of species in the genus *Sirevirus*

| | | |
|---|---|---|
| *Arabidopsis thaliana Endovir virus* | | |
| Arabidopsis thaliana Endovir virus | [AY016208] | (AthEndV) |
| *Glycine max SIRE1 virus* | | |
| Glycine max SIRE1 virus | [AF053008] | (GmaSIRV) |
| *Lycopersicon esculentum ToRTL1 virus* | | |
| Lycopersicon esculentum ToRTL1 virus | [U68072] | (LesToRV) |
| *Nicotiana debneyi Tnd1 virus* | | |
| Nicotiana debneyi Tnd1 virus | [AF059674] | (NdeTnd1V) |
| *Zea mays Opie-2 virus* | | |
| Zea mays Opie-2 virus | [U68408] | (ZmaOp2V) |
| *Zea mays Prem-2 virus* | | |
| Zea mays Prem-2 virus | [U41000] | (ZmaPr2V) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Sirevirus* but have not been approved as species

| | | |
|---|---|---|
| Oryza sativa Osr7 virus | [AP002538] | (OsaOsr7V) |
| Oryza sativa Osr8 virus | [AC021891] | (OsaOsr8V) |

## List of unassigned species in the family *Pseudoviridae*

*Phaseolus vulgaris Tpv2-6 virus*
Phaseolus vulgaris Tpv2-6 virus [AJ005762] (PvuTpvV)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

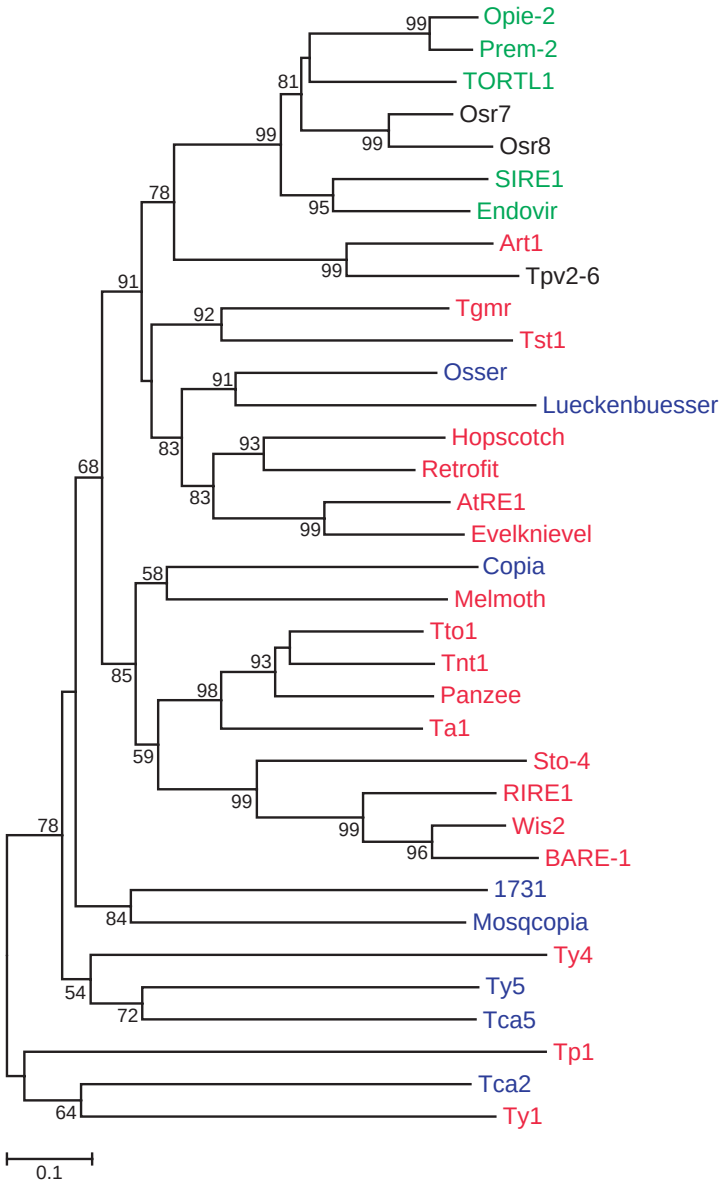


**Figure 4:** Phylogenetic tree based on the amino acid sequences of the reverse transcriptase domain of members of the family *Pseudoviridae*. The Pfam HMM reverse transcriptase model for *Pseudoviridae* retrotransposons (RVT_2 PF07727.6) was used to scan and locate the respective domain in each element. After the alignment, MEGA4 was used to construct the phylogenetic tree using the neighbor-joining distance method. Bootstrap support was calculated based on 1000 replicates (values shown where >50%) and the evolutionary distances were computed using the Poisson correction model. Members of the genus *Pseudovirus* are shown in red, *Hemivirus* in blue and *Sirevirus* in green. Tpv2-6 is an unassigned species and Osr7 and Osr8 have not yet been classified but clearly belong with the sireviruses. (Courtesy of A. Bousios, Institute of Agrobiotechnology, Centre for Research and Technology Hellas, Thessaloniki, 57001, Greece.)

## Phylogenetic relationships within the family

Phylogenetic analysis of the reverse transcriptase domain (Figure 4) does not support the current taxonomy, although the sireviruses clearly form a natural grouping.

## Similarity with other taxa

Like the families *Hepadnaviridae* and *Metaviridae*, the *Pseudoviridae* are clearly related to the family *Retroviridae*. All four families are linked by reverse transcription and a viral core structure made up of Gag-like proteins. Pseudoviruses are different from the other two families in that they have an unusual organization (PR-IN-RT-RH) of the gene *pol*. Members of the families *Pseudoviridae*, *Metaviridae* and *Retroviridae* also share the following: a proviral form characterized by LTRs: protease, reverse transcriptase, RNase H and integrase activities essential for multiplication; readthrough-mediated *gag-pol* gene expression (in some species); and tRNA primers (in most species).

An important and controversial question is the extent of the relationship between the families *Pseudoviridae*, *Metaviridae* and the *Retroviridae*. Because the genomic structures of viruses in families *Pseudoviridae* and *Metaviridae* are clearly related to, but typically simpler than the viruses in the family *Retroviridae*, many authors who have considered the problem have concluded that the families *Pseudoviridae* and *Metaviridae* represent more primitive groups; the family *Metaviridae* probably spawned the members of the family *Retroviridae* (presumably by incorporating genes encoding ligands for cell-surface receptors). This conclusion makes sense within the context of the enormous diversity of other types of retroelements, which are all clearly phylogenetically related by the presence of RT (Figure 5), but not all of which encode a virus-like intermediate. An alternative viewpoint that cannot be ruled out, but for which there is less support, is that members of the family *Metaviridae* represent degenerate forms of the family *Retroviridae*.

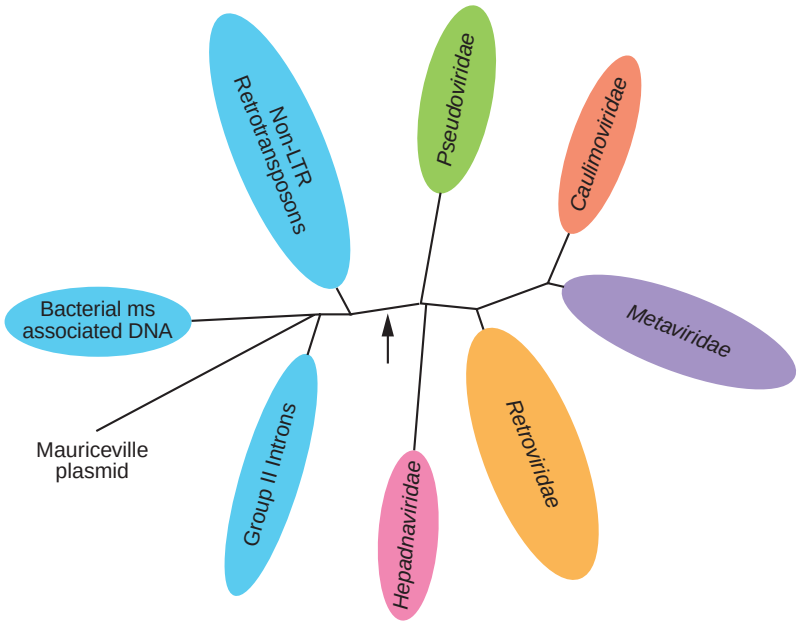


**Figure 5:** Unrooted phylogenetic tree of all class of reverse transcriptase containing viruses. While over 100 reverse transcriptase sequence were used to generate this phylogeny, to simplify visual comparison of the major topologies of the tree, viruses from the same class that are located on the same branch of the tree are indicated by an oval. The length of the oval corresponds to the most divergent viruses within that oval. The arrow indicates a possible root of the tree using RNA polymerase sequences.

## Derivation of names

*Hemi*: from Greek *hemi*, "half", referring to the half-molecule of tRNA used as a primer for reverse transcription.
*Pseudo*: from Greek *pseudo*, "false", to connote some uncertainty as to whether these are true viruses.
*Sire*: from the abbreviation of the species name: *Glycine max SIRE1 virus* (SIRE).

## Further reading

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## Contributed by

Boeke, J.D., Eickbush, T., Sandmeyer, S.B. and Voytas, D.F.

# FAMILY *RETROVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Retroviridae* |
| Subfamily | *Orthoretrovirinae* |
| Genus | *Alpharetrovirus* |
| Genus | *Betaretrovirus* |
| Genus | *Gammaretrovirus* |
| Genus | *Deltaretrovirus* |
| Genus | *Epsilonretrovirus* |
| Genus | *Lentivirus* |
| Subfamily | *Spumaretrovirinae* |
| Genus | *Spumavirus* |

## Virion properties

### MORPHOLOGY

Virions are spherical, enveloped and 80–100 nm in diameter. Glycoprotein surface projections are about 8 nm in length. The internal core constitutes the viral nucleocapsid. The apparently spherical nucleocapsid (nucleoid) is eccentric for members of the genus *Betaretrovirus*, concentric for members

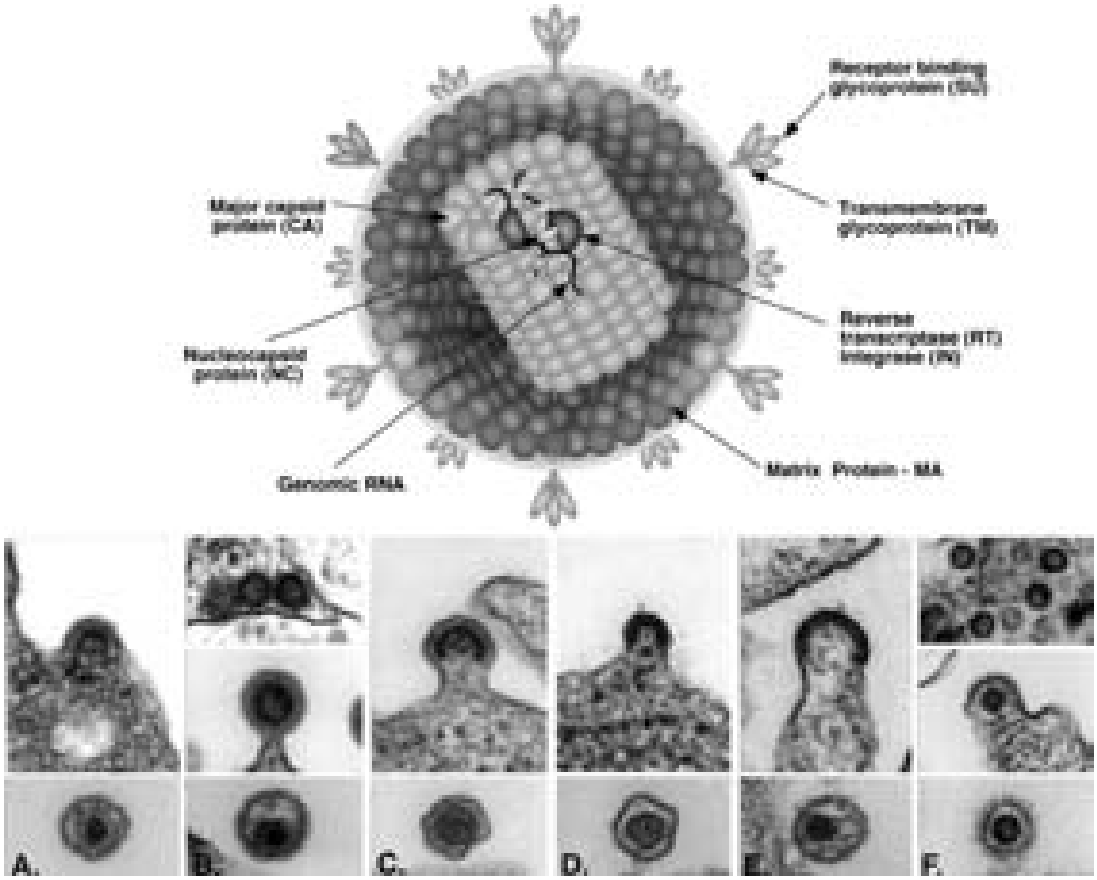


**Figure 1:** Structure of retrovirus particles. (Top) Schematic cartoon (not to scale) showing the inferred locations of the various structures and proteins. (Bottom) Panel (A): alpharetrovirus, avian leukosis virus (ALV); type "C" morphology. Panel (B): betaretrovirus, mouse mammary tumor virus (MMTV); type "B" morphology. Panel (C): gammaretrovirus, murine leukemia virus (MLV). Panel (D): deltaretrovirus, bovine leukemia virus (BLV). Panel (E): lentivirus, human immunodeficiency virus 1 (HIV-1). Panel (F): spumavirus, simian foamy virus (SFVcpz(hu); formerly called HFV). (Courtesy of M. Gonda, reproduced with permission from J.M. Coffin, S.H. Hughes and H. Varmus (Eds.) (1997). *Retroviruses.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.)

of the genera *Alpharetrovirus*, *Gammaretrovirus*, *Deltaretrovirus* and *Spumavirus*, and rod or truncated cone-shape for members of the genus *Lentivirus*.

Two distinct morphogenic pathways exist. Historically, a nomenclature based on electron microscopy classified members of the *Alpharetrovirus* and *Gammaretrovirus* genera, which assemble their immature capsids at the plasma membrane, as C-type viruses. Members of the *Betaretrovirus* genus in contrast were said to assemble A-type particles (immature capsids) in the cytoplasm which then budded with either a B-type (mouse mammary tumor virus, MMTV) or D-type (Mason-Pfizer monkey virus, MPMV) morphology.

### Physicochemical and physical properties

Virion buoyant density is 1.16–1.18 g cm$^{-3}$ in sucrose. Virion $S_{20,w}$ is approximately 600S in sucrose. Virions are sensitive to heat, detergents and formaldehyde. The surface glycoproteins may be partially removed by proteolytic enzymes. Virions are relatively resistant to UV light.

### Nucleic acid

The virus genome characteristic of members of the subfamily *Orthoretrovirinae* consists of a dimer of linear, positive sense, ssRNA, each monomer 7–13 kb in size. The RNA constitutes about 2% of the virion dry weight. The monomers are held together by hydrogen bonds. Each monomer of RNA is polyadenylated at the 3′ end and has a cap structure (type 1) at the 5′ end. The purified virion RNA is not infectious. Each monomer is associated with a specific molecule of tRNA that is base-paired to a region (termed the primer binding site) near the 5′ end of the RNA and involves about 18 nt at the 3′ end of the tRNA. Other host-derived RNAs (and small DNA fragments) found in virions are believed to be incidental inclusions. The virus genome characteristic of members of the subfamily *Spumaretrovirinae* is dsDNA, as reverse transcription is a late step in the viral life cycle of these viruses. The exact structure of the DNA has not been determined.

### Proteins

Proteins constitute about 60% of the virion dry weight. There are two envelope proteins: SU (surface) and TM (transmembrane) encoded by the viral *env* gene. Some members of the subfamily *Spumaretrovirina*e have a third Env protein, LP (leader peptide). There are 3–6 internal, non-glycosylated structural proteins (encoded by the *gag* gene). These are, in order from the amino terminus, (1) MA (matrix), (2) in some viruses a protein of undetermined function, (3) CA (capsid protein) and (4) NC (nucleocapsid). The MA protein is often acylated with a myristyl moiety covalently linked to the amino-terminal glycine. Other proteins are a protease (PR, encoded by the *pro* gene), a reverse transcriptase (RT, encoded by the *pol* gene) and an integrase (IN, encoded by the *pol* gene). In some viruses, a dUTPase (DU, role uncertain) is also present. Members of the *Spumaretrovirinae* encode only a single Gag protein which is cleaved once near the carboxyl-terminus in about half of the proteins. The complex retroviruses in the *Deltaretrovirus*, *Epsilonretrovirus*, *Lentivirus* and *Spumavirus* genera also encode non-structural proteins. Many of these viruses encode transcriptional transactivators, which are required for expression of the LTR promoters, or proteins required for RNA export from the nucleus.

### Lipids

Lipids constitute about 35% of the virion dry weight. They are derived from the plasma membrane of the host cell.

### Carbohydrates

Virions are composed of about 3% carbohydrate by weight. This value varies, depending on the virus. At least one (SU), and usually both, envelope proteins are glycosylated. Cellular glycolipids and some glycoproteins are also found in the viral envelope

## Genome organization and replication

Virions of members of the subfamily *Orthoretrovirinae* carry two copies of the RNA genome. Infectious viruses have four main genes coding for the virion proteins in the order: 5′-*gag-pro-pol-env*-3′. Some retroviruses contain genes encoding non-structural proteins important for the regulation of gene expression and virus replication. Others carry cell-derived sequences that are important

in pathogenesis. These cellular sequences are inserted either into a complete retrovirus genome (e.g. some strains of Rous sarcoma virus) or in the form of substitutions for deleted viral sequences (e.g. some isolates of murine sarcoma virus). Such deletions render the virus replication-defective and dependent on non-transforming helper viruses for production of infectious progeny. In many cases the cell-derived sequences form a fused gene with a viral structural gene that is then translated into one chimeric protein (e.g. Gag-Onc protein).

Entry into the host cell is mediated by interaction between the virion SU glycoprotein and specific receptors at the host cell surface, resulting in fusion of the viral envelope with the plasma membrane, either directly or following endocytosis. Receptors are cell surface proteins. Many have been identified. For human immunodeficiency virus (HIV), both the CD4 protein, which is an immunoglobulin-like molecule with a single transmembrane region, and a chemokine receptor (CCR5 or CXCR4), which spans the membrane seven times, are required for membrane fusion. The receptors for gammaretroviruses are involved in the transport of small molecules and have a complex structure with multiple transmembrane domains. For the avian leukosis viruses (ALVs), four receptors have been identified. That for subgroup A viruses is a small protein with a single transmembrane domain that is distantly related to a cell receptor for low-density lipoprotein, that for subgroup B viruses is related to the TNF-receptor family of proteins, that for subgroup C viruses is related to the mammalian butyrophilins, and that for subgroup J viruses is the chicken $Na^+/H^+$ exchanger protein.

The process of intracellular uncoating of viral particles is not understood. Subsequent early events are carried out in the context of a nucleoprotein complex derived from the capsid.

For members of the subfamily *Orthoretrovirinae*, replication starts with reverse transcription (by RT) of virion RNA into cDNA using the 3′ end of the tRNA as primer for synthesis of a negative sense cDNA transcript. The initial short product (to the 5′ end of the genome) transfers and primes further cDNA synthesis from the 3′ end of the genome by virtue of duplicated sequences at the ends of the viral RNA. cDNA synthesis involves the concomitant digestion of the viral RNA (RNase H activity of the RT protein). A product of this hydrolysis serves to prime positive sense cDNA synthesis on the negative sense DNA copies. In its final form, the linear dsDNA derived from the viral ssRNA genome contains long terminal repeats (LTRs) composed of unique sequences from the 3′ (U3) and 5′ (U5) ends of the viral RNA flanking a repeated sequence (R) found near both ends of the RNA. The process of reverse transcription is characterized by a high frequency of recombination due to the transfer of the RT from one template RNA to the other. Reverse transcription is thought to follow the same pathway in members of the subfamily *Spumaretrovirinae*, but the timing is different as it occurs during viral assembly and/or release from the cell. The mechanism of reverse transcription allows for high rates of recombination and genetic diversity for many of the retroviruses. The high rate of genetic variation *in vivo* can lead to formation of a quasispecies consisting of a large number of genetically diverse virions.

Retroviral DNA becomes integrated into the chromosomal DNA of the host, to form a provirus, by a mechanism involving the viral IN protein. The ends of the virus DNA are joined to cell DNA, involving the removal of two bases from the ends of the linear viral DNA and generating a short duplication of cell sequences at the integration site. Virus DNA can integrate at many sites in the cellular genome. However, once integrated, a sequence is apparently incapable of further transposition within the same cell. The map of the integrated provirus is co-linear with that of non-integrated viral DNA. Integration appears to be a prerequisite for virus replication. Different retroviruses can show distinct preferences in integration site selection, with HIV-1 tending to insert within gene sequences whereas murine leukemia viruses (MLV) prefer regions near the start of transcribed genes.

The integrated provirus is transcribed by cellular RNA polymerase II into virion RNA and mRNA species in response to transcriptional signals in the viral LTRs. In some genera, transcription is also regulated by virus-encoded transactivators. There are several classes of mRNA depending on the virus and its genetic map. An mRNA comprising the whole genome serves for translation of the *gag*, *pro* and *pol* genes (positioned in the 5′-half of the RNA). This results in the

formation of polyprotein precursors that are cleaved to yield the structural proteins, PR, RT and IN, respectively. A smaller mRNA consisting of the 5′ end of the genome spliced to sequences from the 3′ end of the genome, and including the *env* gene and the U3 and R regions, is translated into the precursor of the envelope proteins. In viruses that contain additional genes, other forms of spliced mRNA are also made; all these spliced mRNAs share a common sequence at their 5′ ends. Members of the subfamily *Spumaretrovirinae* are unique in that they make use of an internal promoter (IP), which is located in the *env* gene upstream of the accessory reading frames, for transcription of these distal genes. Most primary translation products in retrovirus infections are polyproteins that require proteolytic cleavage before becoming functional. The *gag*, *pro* and *pol* gene products are generally produced from a nested set of primary translation products. For *pro* and *pol*, translation involves bypassing translational termination signals by ribosomal frameshifting or by readthrough at the Gag-Pro and/or the Pro-Pol boundaries. Members of the subfamily *Spumaretrovirinae* synthesize Pol protein from its own mRNA rather than as a Gag-Pol fusion protein.

The retroviral genomic RNA contains sequences of varying lengths, usually located near the 5′ end between U3 and gag, which comprise a packaging signal (Ψ). Ψ is required for efficient encapsidation of the genome into particles, and is generally not present on the subgenomic mRNAs, a notable exception being the alpharetroviruses. In the case of the spumaviruses, Ψ does not appear to be in the 5′ end of the genome. In all cases, Ψ activity is not defined by the primary sequence, but by a complex folded structure.

Capsids assemble either at the plasma membrane (for a majority of the genera), or as intracytoplasmic particles (for members of the genera *Betaretrovirus* and *Spumavirus*) and are released from the cell by a process of budding. Budding appears to occur preferentially at specialized membrane microdomains known as lipid rafts. Virions of the spumaviruses and deltaretroviruses are highly cell-associated. Polyprotein processing of the internal proteins occurs concomitant with or just subsequent to the maturation of virions of members of the subfamily *Orthoretrovirinae*.

## Antigenic properties

Virion proteins contain type-specific and group-specific determinants. Some type-specific determinants of the envelope glycoproteins are involved in antibody-mediated virus neutralization. Group-specific determinants are shared by members of a serogroup and may be shared between members of different serogroups within a particular genus. There is evidence for weak cross-reactivities between members of different genera. Epitopes that elicit T-cell responses are found on many of the structural proteins. Antigenic properties are not used in classification of members of the family *Retroviridae*.

## Biological properties

Retroviruses are widely distributed as exogenous infectious agents of vertebrates. Endogenous proviruses that have resulted at some time from infection of germ line cells are inherited as Mendelian genes. They occur widely among vertebrates and can constitute up to 10% of genomic DNA. The vast majority have suffered inactivating mutations and cannot produce infectious virus. A few can exert significant biological effects following activation, either by replication in a manner indistinguishable from exogenous viruses or following recombination with replication-competent virus.

Retroviruses are associated with a variety of diseases. These include: malignancies, including certain leukemias, lymphomas, sarcomas and other tumors of mesodermal origin; mammary carcinomas and carcinomas of liver, lung and kidney; immunodeficiencies (such as AIDS); autoimmune diseases; lower motor neuron diseases; and several acute diseases involving tissue damage. Some retroviruses appear to be non-pathogenic. Transmission of retroviruses is horizontal via a number of routes, including blood, saliva, sexual contact, etc., and via direct infection of the developing embryo, or via milk or perinatal routes. Endogenous retroviruses are transmitted vertically by inheritance of proviruses.

# SUBFAMILY *ORTHORETROVIRINAE*

## Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | *Orthoretrovirinae* |
| Genus | *Alpharetrovirus* |
| Genus | *Betaretrovirus* |
| Genus | *Gammaretrovirus* |
| Genus | *Deltaretrovirus* |
| Genus | *Epsilonretrovirus* |
| Genus | *Lentivirus* |

# GENUS *ALPHARETROVIRUS*

Type species *Avian leukosis virus*

## Distinguishing features

Virus particles assemble at the plasma membrane and exhibit a "C-type" morphology. Approximate protein sizes are: MA 19 kDa; p10 10 kDa; CA 27 kDa; NC 12 kDa; PR 15 kDa; RT 68 kDa; IN 32 kDa; SU 85 kDa; and TM 37 kDa. The genome is about 7.2 kb in size (one monomer); its organization is illustrated in Figure 2. There are no known genes additional to *gag*, *pro*, *pol* and *env*. The tRNA primer is $tRNA^{Trp}$. The LTR is about 350 nt long, of which the U3 region is 250 nt, the R sequence is 20 nt and the U5 region is 80 nt. The viruses have a widespread distribution and include both exogenous (vertical and horizontal transmission) and endogenous viruses of chickens and some other birds. ALV isolates are classified into subgroups (e.g. -A, -J) by their distinct receptor usage. Distantly related endogenous sequences are found in birds and mammals. Virus infections are associated with malignancies and some other diseases such as wasting, and osteopetrosis. Many oncogene-containing members of the genus have been isolated.

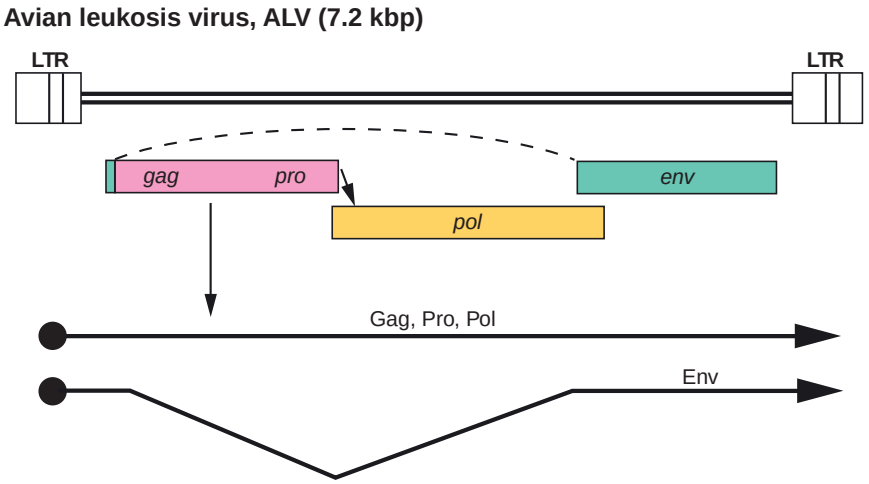


**Figure 2:** Alpharetrovirus genome expression. The 7.2 kbp avian leukosis virus (ALV) provirus genome is shown, with LTRs, protein-coding regions (*gag*, *pro*, *pol* and *env*) and transcripts (solid line arrows with protein names added) marked. The arrow between the *pro-pol* reading frames indicates a ribosomal frameshift.

## Species demarcation criteria in the genus

The list of species demarcation criteria is:

- Differences in genome sequence
- Differences in gene product sequences

- Differences in natural host range
- Different oncogenes that may be incorporated.

For example, isolates of avian leukosis virus can be readily distinguished from those of Rous sarcoma virus because they lack oncogene sequences while encoding *gag*, *pol* and *env*. The replication-defective alpharetroviruses can be distinguished from Rous sarcoma virus by the variable deletion of portions of the *gag*, *pol* and *env* genes and the presence of a unique oncogene in each species. Rous sarcoma virus strains encode the *src* oncogene, whereas avian myeloblastosis virus, for example, encodes the *myb* oncogene. Host range, defined by SU interaction with a specific receptor, is generally used in defining strains within a species.

### List of species in the genus *Alpharetrovirus*

| | | |
|---|---|---|
| **Replication-competent, non-oncogene-containing viruses** | | |
| *Avian leukosis virus* | | |
| Avian leukosis virus - RSA | [M37980] | (ALV-A) |
| Avian leukosis virus - HPRS103 | [Z46390] | (ALV-J) |
| **Replication-competent, oncogene-containing viruses** | | |
| *Rous sarcoma virus* | | |
| Rous sarcoma virus (Prague C) | [J02342] | (RSV-Pr-C) |
| Rous sarcoma virus (Schmidt-Ruppin B) | [AF052428] | (RSV-SR-B) |
| Rous sarcoma virus (Schmidt-Ruppin D) | [D10652] | (RSV-SR-D) |
| **Replication-defective viruses** | | |
| *Avian carcinoma Mill Hill virus 2* | | |
| Avian carcinoma Mill Hill virus 2 | [K02082] | (ACMHV-2) |
| *Avian myeloblastosis virus* | | |
| Avian myeloblastosis virus | [J02013] | (AMV) |
| *Avian myelocytomatosis virus 29* | | |
| Avian myelocytomatosis virus 29 | [J02019] | (AMCV-29) |
| *Avian sarcoma virus CT10* | | |
| Avian sarcoma virus CT10 | [Y00302] | (ASV-CT10) |
| *Fujinami sarcoma virus* | | |
| Fujinami sarcoma virus | [J02194] | (FuSV) |
| *UR2 sarcoma virus* | | |
| UR2 sarcoma virus | [M10455] | (UR2SV) |
| *Y73 sarcoma virus* | | |
| Y73 sarcoma virus | [J02027] | (Y73SV) |

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Alpharetrovirus* but have not been approved as species

None reported.

## GENUS *BETARETROVIRUS*

Type species ***Mouse mammary tumor virus***

### Distinguishing features

Virions of mouse mammary tumor virus (MMTV) exhibit a "B-type" morphology with prominent surface spikes and an eccentric condensed core. Other members of the genus have a "D-type" morphology with fewer dense surface spikes and a cylindrical core. Capsid assembly occurs within the cytoplasm (to yield structures previously termed "A-type" particles) prior to transport to, and budding from, the plasma membrane. Approximate protein sizes are: MA 10 kDa; p21 21 kDa; p8/p12 8 12 kDa; CA 27 kDa; NC 14 kDa; DU 30 kDa; PR 15 kDa; RT 50 kDa; IN 36 kDa; SU 52 kDa; and TM 36 kDa. The genome is 8–10 kb in size (one monomer); its organization in MMTV is illustrated in Figure 3.

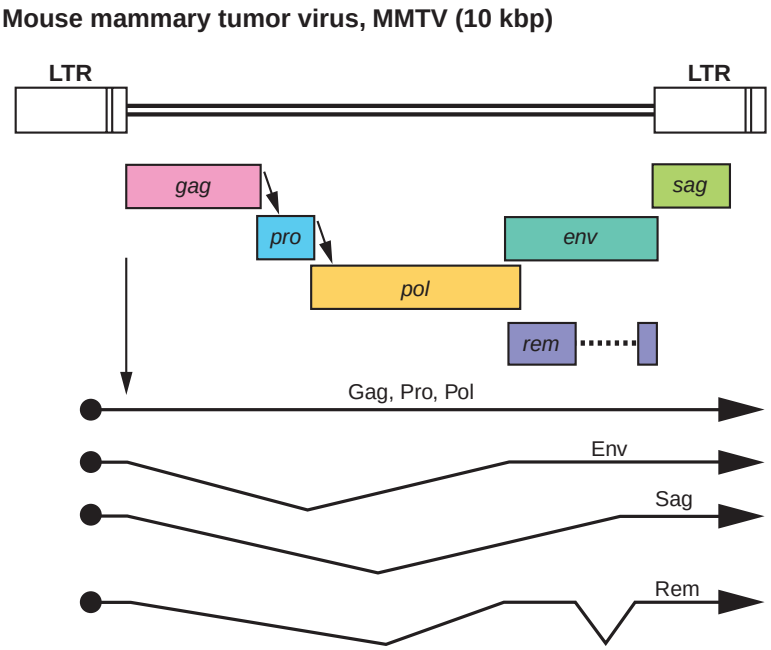


**Figure 3:** Betaretrovirus genome expression. The 10 kbp mouse mammary tumor virus (MMTV) provirus genome is shown, with LTRs, protein-coding regions (*gag*, *pro*, *pol*, *env*, *sag* and *rem*) and transcripts (solid line arrows with protein names added) marked. The arrows between the *gag-pro* and *pro-pol* reading frames indicate ribosomal frameshift sites.

In MMTV there are two additional genes: *sag*, whose product functions as a superantigen, is located at the 3′ end of the genome, overlapping U3 and *rem*, which encodes an RNA export protein and is translated from a doubly spliced mRNA overlapping the *env* gene. The *sag* gene is absent from other members of the genus, but several other viruses encode activities analogous to Rem. The tRNA primer is $tRNA^{Lys-3}$ for MMTV and $tRNA^{Lys-1,2}$ for other members of the genus. The LTR of MMTV is about 1300 nt long, primarily due to the *sag*-encoding U3 region of 1200 nt. The R sequence (15 nt) and the U5 region (95–120 nt) are of similar length in all members of the genus.

Viruses assigned to this genus include exogenous (milk-transmitted) and endogenous viruses of mice, as well as exogenous, horizontally transmitted and endogenous viruses of New and Old World primates and sheep. Murine viruses are associated with mammary carcinoma and T-lymphomas, whereas the exogenous primate viruses are associated with immunodeficiency diseases; Jaagsiekte sheep retrovirus is associated with pulmonary cancer of sheep. No oncogene-containing member is known.

## Species demarcation criteria in the genus

The list of species demarcation criteria is:

- Differences in genome sequence
- Differences in gene product sequences
- Differences in natural host range.

Several primate retroviruses have been described that appear to be divergent members of a single virus that arose from a recombination event in which the *env* gene of a primate gammaretrovirus was captured. The most divergent of these are the endogenous squirrel monkey retrovirus (SMRV) and langur virus (LNGV), which are unable to infect cells from the primate species of origin. Several serologically distinct strains exist within the species *Mason–Pfizer monkey virus*. The most divergent of these are the endogenous SMRV and LNGV, although the more closely related isolates Mason–Pfizer monkey virus, simian retrovirus-1 and simian retrovirus-2 are serologically distinct. MMTV is assigned to a separate species because of the unique *sag* coding region and a widely

divergent and distinct *env* gene. Jaagsiekte sheep retrovirus is also assigned to a separate species on the degree of nucleotide sequence divergence. A closely related virus (enzootic nasal tumor virus, ENTV) induces nasal adenocarcinoma in goats and sheep. Related endogenous proviruses have been identified in other mammalian species (rodents and primates).

### List of species in the genus *Betaretrovirus*

| | | |
|---|---|---|
| *Jaagsiekte sheep retrovirus* | | |
| Jaagsiekte sheep retrovirus | [M80216] | (JSRV) |
| (Ovine pulmonary adenocarcinoma virus) | | |
| Enzootic nasal tumor virus | [HM104174] | (ENTV) |
| *Langur virus* | | |
| Langur virus | | (LNGV) |
| *Mason–Pfizer monkey virus* | | |
| Mason–Pfizer monkey virus | [M12349] | (MPMV) |
| Simian retrovirus 1 | [M11841] | (SRV-1) |
| Simian retrovirus 2 | [M16605] | (SRV-2) |
| *Mouse mammary tumor virus* | | |
| Mouse mammary tumor virus | [M15122] | (MMTV) |
| *Squirrel monkey retrovirus* | | |
| Squirrel monkey retrovirus | [M23385] | (SMRV) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accessions [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Betaretrovirus* but have not been approved as species

None reported.

## GENUS *GAMMARETROVIRUS*

Type species ***Murine leukemia virus***

### Distinguishing features

Virions exhibit a "C-type" morphology with barely visible surface spikes. They have a centrally located, condensed core. Capsid assembly occurs at the inner surface of the membrane at the same time as budding. Approximate protein sizes are: MA 15 kDa; p12 12 kDa; CA 30 kDa; NC 10 kDa; PR

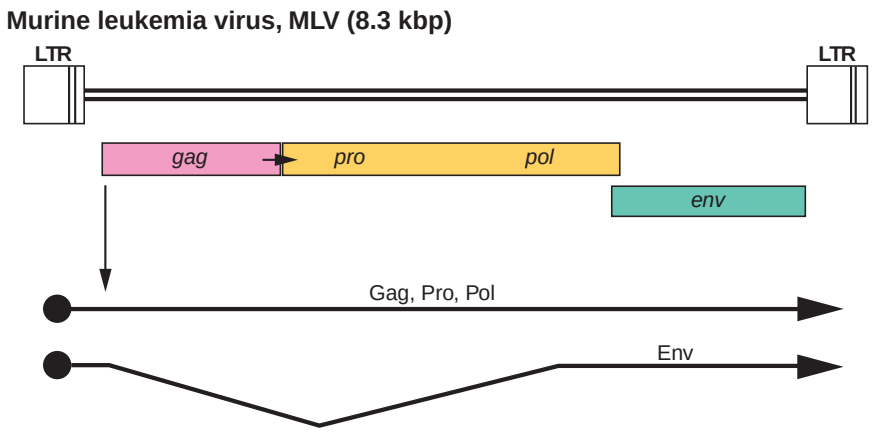


**Figure 4:** Gammaretrovirus genome expression. The 8.3 kbp murine leukemia virus (MLV) provirus is shown, with LTRs, protein-coding regions (*gag*, *pro*, *pol* and *env*) and transcripts (solid line arrows with protein names added) marked. The arrow between *gag-pro* indicates a ribosomal readthrough site.

14 kDa; RT 80 kDa; IN 46 kDa; SU 70 kDa; and TM 15 kDa. The genome is about 8.3 kb in size (one monomer); its organization is illustrated in Figure 4. The *pro-pol* region is translated following ribosomal readthrough at the *gag* gene termination codon. Some gammaretroviruses additionally translate the *gag* region from an upstream initiation codon, yielding a larger, glycosylated form of Gag protein, glyco-gag. There are no known additional genes. The tRNA primer is $tRNA^{Pro}$ ($tRNA^{Glu}$ is found in a few endogenous mouse viruses). The LTR is about 600 nt long, of which the U3 region is 500 nt, the R sequence is 60 nt and the U5 region is 75 nt.

The viruses are widely distributed; exogenous (vertical and horizontal transmission) and endogenous viruses are found in many mammals. The reticuloendotheliosis viruses comprise a few isolates from birds with no known corresponding endogenous relatives. The viruses are associated with a variety of diseases, including malignancies, immunosuppression, neurological disorders. Many oncogene-containing members of the mammalian and reticuloendotheliosis virus groups have been isolated. Viruses resulting from recombination between exogenous and endogenous viruses are frequently encountered.

## Species demarcation criteria in the genus

The list of species demarcation criteria is:

- Differences in genome sequence and viral oncogenes
- Differences in antigenic properties
- Differences in natural host range
- Differences in pathogenicity

There are mammalian, reptilian and avian (reticuloendotheliosis) viruses. The mammalian viruses include replication-competent viruses, which lack cell-derived oncogenes, and replication defective viruses, which have acquired a variety of oncogenes from their hosts.

Members of the species *Murine leukemia virus* can be distinguished from isolates of *Gibbon ape leukemia virus*, for example, by sequence divergence, distinct receptors for virus entry and only limited antigenic cross-reactivity in ELISA assays. Murine sarcoma viruses, which are invariably replication-defective, can be distinguished from the murine leukemia viruses and from one another by the presence of distinct cell-derived oncogenes (e.g. *mos* in isolates of *Moloney murine sarcoma virus* and *sis* in isolates of *Woolly monkey sarcoma virus*) and the characteristic loss of portions of *gag*, *pol* or *env*. A putative human gammaretrovirus closely related to an endogenous murine retrovirus has recently been discovered, xenotropic MLV-related retrovirus (XMRV).

## List of species in the genus *Gammaretrovirus*

| | | |
|---|---|---|
| **Replication-competent viruses** | | |
| *Feline leukemia virus* | | |
| Feline leukemia virus | [M18247] | (FeLV) |
| *Gibbon ape leukemia virus* | | |
| Gibbon ape leukemia virus | [M26927] | (GALV) |
| *Guinea pig type-C oncovirus* | | |
| Guinea pig type-C oncovirus | | (GPCOV) |
| *Murine leukemia virus* | | |
| Abelson murine leukemia virus* | [J02009] | (AbMLV) |
| AKR (endogenous) murine leukemia virus | [J01998] | (AKRMLV) |
| Friend murine leukemia virus | [M93134, Z11128] | (FrMLV) |
| Moloney murine leukemia virus | [J02255] | (MoMLV) |
| *Porcine type-C oncovirus* | | |
| Porcine endogenous retrovirus-C | [AM229312] | (PERV-C) |
| **Replication-defective viruses** | | |
| *Finkel-Biskis-Jinkins murine sarcoma virus* | | |
| Finkel–Biskis–Jinkins murine sarcoma virus | [K02712] | (FBJMSV) |
| *Gardner–Arnstein feline sarcoma virus* | | |
| Gardner-Arnstein feline sarcoma virus | | (GAFeSV) |
| *Hardy–Zuckerman feline sarcoma virus* | | |
| Hardy-Zuckerman feline sarcoma virus | | (HZFeSV) |

| | | |
|---|---|---|
| *Harvey murine sarcoma virus* | | |
| Harvey murine sarcoma virus | | (HaMSV) |
| *Kirsten murine sarcoma virus* | | |
| Kirsten murine sarcoma virus | | (KiMSV) |
| *Moloney murine sarcoma virus* | | |
| Moloney murine sarcoma virus | [J02266] | (MoMSV) |
| *Snyder–Theilen feline sarcoma virus* | | |
| Snyder–Theilen feline sarcoma virus | | (STFeSV) |
| *Woolly monkey sarcoma virus* | | |
| Woolly monkey sarcoma virus (Simian sarcoma virus) | [J02394] | (WMSV) |
| **Reptilian virus group** | | |
| *Viper retrovirus* | | |
| Viper retrovirus | | (VRV) |
| **Avian (reticuloendotheliosis) virus group** | | |
| *Chick syncytial virus* | | |
| Chick syncytial virus | | (CSV) |
| *Reticuloendotheliosis virus* | | |
| Reticuloendotheliosis virus (strain A) | [DQ237900] | (REV-A) |
| Reticuloendotheliosis virus (strain T) | | (REV-T) |
| *Trager duck spleen necrosis virus* | | |
| Trager duck spleen necrosis virus | | (TDSNV) |

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*A proposal to re-classify AbMLV among the Replication-defective viruses is under preparation.

### List of other related viruses which may be members of the genus *Gammaretrovirus* but have not been approved as species

| | | |
|---|---|---|
| Koala retrovirus | [AF151794] | (KoRV) |

## Genus *Deltaretrovirus*

Type species ***Bovine leukemia virus***

### Distinguishing features

Virions are similar to those of gammaretroviruses in terms of morphology and assembly. Approximate protein sizes are: MA 19 kDa; CA 24 kDa; NC 12-15 kDa; PR 14 kDa; RT, IN and SU 60 kDa; and TM 21 kDa. The genome is about 8.3 kb in size (one monomer); its organization is illustrated in Figure 5. There are non-structural genes, designated *tax* and *rex*, which are involved in regulation of synthesis and processing of virus RNA, in addition to *gag*, *pro*, *pol* and *env*. The tRNA primer is $tRNA^{Pro}$. The LTR is about 550–750 nt long, of which the U3 region is 200–300 nt, the R sequence is 135–235 nt and the U5 region is 100–200 nt.

The exogenous viruses (horizontal transmission) in this genus are found in only a few groups of mammals. No related endogenous viruses are known. Virus infections are associated with B or T cell leukemias or lymphomas as well as neurological disease (tropical spastic paraparesis or HTLV-associated myopathy) and exhibit a long latency with an incidence of much less than 100%. No oncogene-containing members of this genus have been identified.

### Species demarcation criteria in the genus

The list of species demarcation criteria is:

- Differences in genome sequence and viral oncogenes
- Differences in antigenic properties
- Differences in natural host range
- Differences in pathogenicity

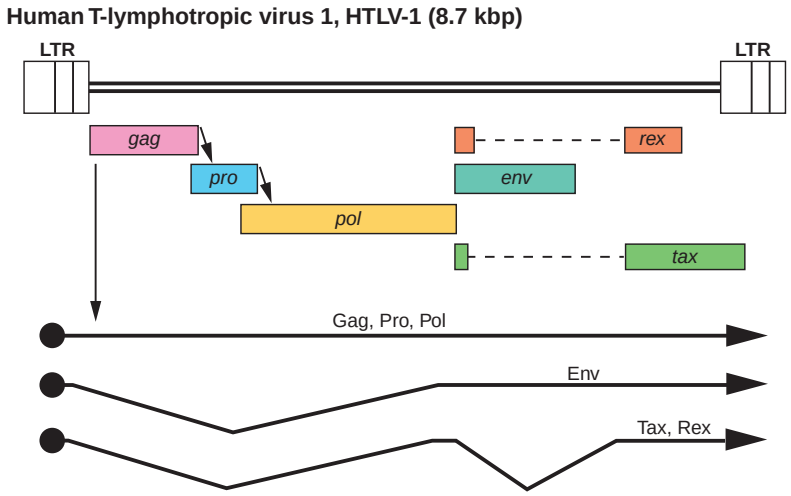


**Figure 5:** Deltaretrovirus genome expression. The 8.7 kbp human T-lymphotropic virus 1 (HTLV-1) provirus genome is shown, with LTRs, protein-coding regions (*gag*, *pro*, *pol*, *env*, *tax* and *rex*) and transcripts (solid line arrows with protein names added) marked. The arrows between the *gag-pro* and *pro-pol* reading frames indicate ribosomal frameshift sites.

Primate T-lymphotropic virus species are distinguished primarily on the basis of sequence differences, and each contains several subtypes. All viruses in the genus have a similar coding strategy, but only human T-lymphotropic virus 1 (HTLV-1) has been associated with human disease. HTLV-1 and simian T-lymphotropic virus 1 (STLV-1) are not clustered according to host species but rather according to geographic origin. All HTLV-1 subtypes described so far have most probably originated from separate interspecies transmissions from simians to humans.

### List of species in the genus *Deltaretrovirus*

| | | |
|---|---|---|
| *Bovine leukemia virus* | | |
| Bovine leukemia virus | [K02120] | (BLV) |
| *Primate T-lymphotropic virus 1* | | |
| Human T-lymphotropic virus 1 | [D13784] | (HTLV-1) |
| Simian T-lymphotropic virus 1 | [Z46900] | (STLV-1) |
| *Primate T-lymphotropic virus 2* | | |
| Human T-lymphotropic virus 2 | [M10060] | (HTLV-2) |
| Simian T-lymphotropic virus 2 | [U90557] | (STLV-2) |
| *Primate T-lymphotropic virus 3* | | |
| Human T-lymphotropic virus 3 | [DQ093792] | (HTLV-3) |
| Simian T-lymphotropic virus 3 | [Y07616] | (STLV-3) |

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Deltaretrovirus* but have not been approved as species

| | |
|---|---|
| Human T-lymphotropic virus 4 | [EF488483] |
| Simian T-lymphotropic virus 5 | [AY590142] |

## Genus *Epsilonretrovirus*

Type species ***Walleye dermal sarcoma virus***

### Distinguishing features

All members of the genus are exogenous retroviruses. They are complex retroviruses in that their genomes range from 11.7 to 12.8 kb in size and contain 1–3 ORFs, presumably encoding accessory

proteins, in addition to those encoding the structural proteins and enzymes of the virion (Figure 6). *Orf* a is present in all three walleye retroviruses and encodes the rv-cyclin protein, which functions in the control of transcription. The roles of the two additional ORFs (*orf b* and *orf c*) in walleye dermal sarcoma virus (WDSV) or the single ORF in snakehead retrovirus have not been determined. The LTRs of the fish retroviruses range from about 500 to 650 nt in length, of which the U3 region is about 450 nt, the R sequence is about 80 nt and the U5 region is about 75 nt. The primer used by the walleye retroviruses is $tRNA^{His}$, whereas the snakehead retrovirus uses $tRNA^{Arg}$. Phylogenetic analysis comparing the polymerase region shows that the walleye and perch retroviruses cluster and have diverged significantly from the snakehead retrovirus. Nevertheless, all piscine retroviruses to date appear to group with the mammalian type-C retroviruses.

## Species demarcation criteria in the genus

The list of species demarcation criteria is:

- Differences in genome sequence and viral oncogenes
- Differences in gene product sequence
- Differences in natural host range

The genus *Epsilonretrovirus* is comprised of three species of fish retrovirus, which are distinguished from one another on the basis of phylogenetic diversity. In addition, three viruses, snakehead retrovirus (SnRV), salmon swimbladder sarcoma virus (SSSV) and perch hyperplasia virus (PHV), are listed as probable members. As additional fish retroviruses are identified and characterized, both SnRV and SSSV may provide the basis for additional genera (Figure 9). PHV has been sequenced only within the *pol* gene, and its status remains tentative until further sequence information becomes available.

## List of species in the genus *Epsilonretrovirus*

| | | |
|---|---|---|
| *Walleye dermal sarcoma virus* | | |
| Walleye dermal sarcoma virus | [AF033822] | (WDSV) |
| *Walleye epidermal hyperplasia virus 1* | | |
| Walleye epidermal hyperplasia virus 1 | [AF014792] | (WEHV-1) |
| *Walleye epidermal hyperplasia virus 2* | | |
| Walleye epidermal hyperplasia virus 2 | [AF014793] | (WEHV-2) |

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

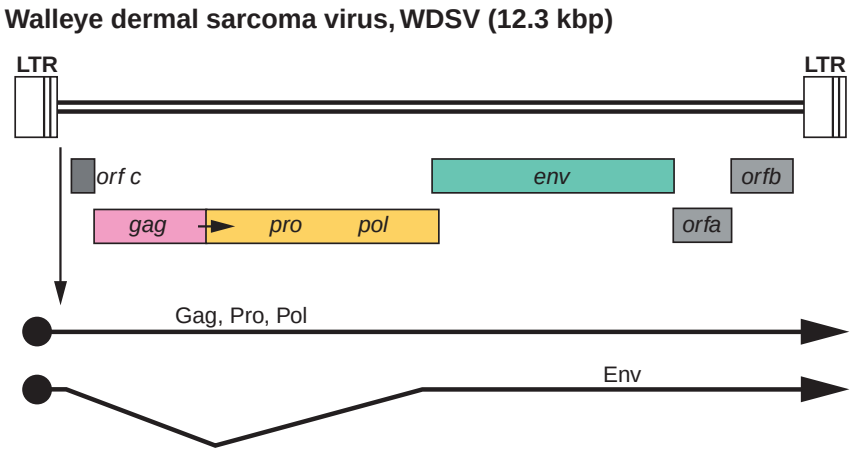


**Figure 6:** Epsilonretrovirus genome expression. The 12.3 kbp walleye dermal sarcoma virus (WDSV) provirus genome is shown, with LTRs, protein-coding regions (*gag*, *pro*, *pol*, *env*, *orf a*, *orf b* and *orf c*) and transcripts (solid line arrows with protein names added) marked. The arrow between *gag-pro* indicates a ribosomal readthrough site.

**List of other related viruses which may be members of the genus *Epsilonretrovirus* but have not been approved as species**

| | | |
|---|---|---|
| Perch hyperplasia virus | | (PHV) |
| Snakehead retrovirus | [U26458] | (SnRV) |
| Salmon swimbladder sarcoma virus | [DQ174103] | (SSSV) |

# GENUS *LENTIVIRUS*

Type species ***Human immunodeficiency virus 1***

## Distinguishing features

Virions have a distinctive morphology with a bar, or cone-shaped core (nucleoid). Viruses assemble at the cell membrane. Approximate protein sizes are: MA – 17 kDa; CA 24 kDa; NC 7–11 kDa; p6 6 kDa; PR 14 kDa; RT 66 kDa; DU (in all except the primate lentiviruses), 14 kDa; IN 32 kDa; SU 120 kDa; and TM 41 kDa. The genome is about 9.3 kb in size (one monomer); its organization is illustrated in Figure 7. Detailed structural data are available for HIV-1 MA, CA, NC, PR, RT, IN, SU and TM.

In addition to the structural *gag*, *pro*, *pol* and *env* genes, there are additional genes, depending on the virus (e.g. for HIV-1: *vif*, *vpr*, *vpu*, *tat*, *rev* and *nef*) whose products are involved in regulation of synthesis and processing of virus RNA and combating host restriction factors. Most are located 3′ to *gag-pro-pol* and, at least in part, 5′ to *env*; one (*nef* in human immunodeficiency virus 1, HIV-1) is 3′

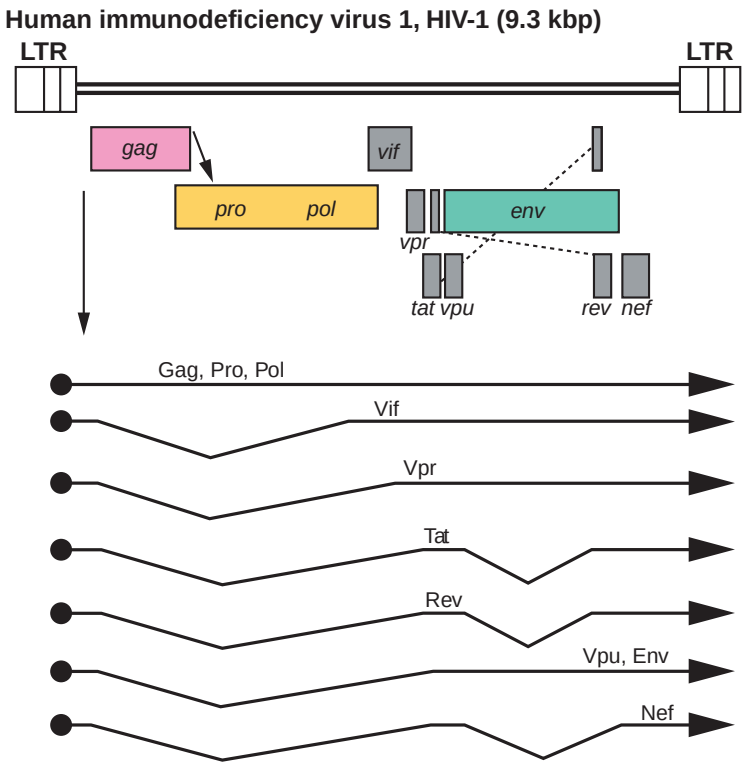


**Figure 7:** Lentivirus genome expression. The 9.3 kbp human immunodeficiency virus 1 (HIV-1) provirus is shown, with LTRs, protein-coding regions (*gag*, *pro*, *pol*, *env*, *vif*, *vpr*, *vpu*, *tat*, *rev* and *nef*) and transcripts (solid line arrows with protein names added) marked. The arrow between the *gag-pro* reading frames indicates a ribosomal frameshift site. The coding regions in other members of the genus may occupy different reading frames.

to *env*. For other viruses there may be additional non-structural genes (e.g. *vpx* in human immunodeficiency virus 2, HIV-2). The tRNA primer is $tRNA^{Lys-3}$. The LTR is about 600 nt long, of which the U3 region is 450 nt, the R sequence is 100 nt and the U5 region is 80 nt.

The viruses in the genus include exogenous viruses (horizontal and vertical transmission) of humans and many other mammals. Related endogenous viruses have been found in rabbit and lemur. The primate lentiviruses are distinguished by the use of a chemokine receptor and the CD4 protein as receptors and the absence of DU. Some groups have cross-reactive Gag antigens (e.g. ovine, caprine and feline lentiviruses). Viruses related to isolates of *Feline immunodeficiency virus* have been isolated from other large felids (e.g. puma lentivirus), and antibodies to Gag antigens in lions and other large felids indicate the existence of additional viruses related to FIV and ovine/caprine lentiviruses.

Some lentiviruses are associated with a variety of diseases, including immunodeficiencies, neurological disorders and arthritis, whereas others appear non-pathogenic. No oncogene-containing member of this genus has been isolated.

## Species demarcation criteria in the genus

The list of species demarcation criteria is:

- Differences in genome and gene product sequences
- Differences in antigenic properties
- Differences in natural host range
- Differences in pathogenicity

Five groups of lentiviruses can be clustered on the basis of the hosts they infect (primates, sheep and goats, horses, cats and cattle). Within the primate lentivirus group, HIV-1 is distinguished from HIV-2, for example, primarily on the basis of sequence divergence that exceeds 50% and the presence of the *vpx* gene in HIV-2. This reflects the different primate sources for HIV-1 and HIV-2 (chimpanzees and sooty mangabeys, respectively). There is limited cross-reactivity in ELISA tests based on Gag components, but essentially none in those based on *env* gene products.

## List of species in the genus *Lentivirus*

| | | |
|---|---|---|
| **Bovine lentivirus group** | | |
| *Bovine immunodeficiency virus* | | |
| Bovine immunodeficiency virus | [M32690] | (BIV) |
| *Equine infectious anemia virus* | | |
| Equine infectious anemia virus | [M16575] | (EIAV) |
| **Feline lentivirus group** | | |
| *Feline immunodeficiency virus* | | |
| Feline immunodeficiency virus (Oma) | [FIU56928] | (FIV-O) |
| Feline immunodeficiency virus (Petuluma) | [M25381] | (FIV-P) |
| *Puma lentivirus* | | |
| Puma lentivirus | [PLU03982] | (PLV-14) |
| **Ovine/caprine lentivirus group** | | |
| *Caprine arthritis encephalitis virus* | | |
| Caprine arthritis encephalitis virus | [M33677] | (CAEV) |
| *Visna/maedi virus* | | |
| Visna/maedi virus (strain 1514) | [M60609, M60610] | (VISNA) |
| **Primate lentivirus group** | | |
| *Human immunodeficiency virus 1* | | |
| Human immunodeficiency virus 1 (four groups, M, N, O and P, are recognized) | | (HIV-1) |
| Group M (main) has 9 discrete clades (A, B, C, D, F, G, H, J and K) and 15 circulating recombinant forms (RCF). Examples include: | | |
| M group Clade A | | |
| U455 | [M62320] | (HIV-1.U455) |

| | | |
|---|---|---|
| Clade B | | |
| ARV-2/SF-2 | [K02007] | (HIV-1.ARV-2/SF-2) |
| BRU (LAI) | [K02013] | (HIV-1.BRU (LAI)) |
| HXB2 | [K03455] | (HIV-1.HXB2) |
| Clade C | | |
| ETH2220 | [U46016] | (HIV-1.ETH2220) |
| Clade D | | |
| ELI | [X04414] | (HIV-1.ELI) |
| NDK | [M27323] | (HIV-1.NDK) |
| Clade F | | |
| 93BR020 | [AF005494] | (HIV-1.93BR020) |
| Clade H | | |
| 90CR056 | [AF0055494] | (HIV-1.90CR056) |
| N Group | | |
| CM_YBF106 | [AJ271370] | (HIV-1.YBF106) |
| O Group | | |
| pCMO2.3 | [AY618990] | (HIV-1.CMO2.3) |
| P Group | | |
| RBF168 | [GQ328744] | (HIV-1.RBF168) |
| *Human immunodeficiency virus 2* | | |
| Human immunodeficiency virus 2 | | (HIV-2) |
| At least 8 groups, representing independent transmissions from sooty mangabeys, are recognized. | | |
| Examples include: | | |
| Group A: | | |
| BEN | [M30502] | (HIV-2.BEN) |
| ISY | [J04498] | (HIV-2.ISY) |
| ROD | [M15390] | (HIV-2.ROD) |
| Group B: | | |
| D205 | [X61240] | (HIV-2.D205) |
| EHOA | [U27200] | (HIV-2.EHOA) |
| UC1 | [L07625] | (HIV-2.UC1) |
| *Simian immunodeficiency virus* | | |
| Simian immunodeficiency virus | | (SIV) |
| Isolates from at least 40 primate species are known. | | |
| Examples include: | | |
| African green monkey | | |
| African green monkey TYO | [X07805] | (SIV-agm.TYO) |
| African green monkey 155 | [M29975] | (SIV-agm.155) |
| African green monkey 3 | [M30931] | (SIV-agm.3) |
| African green monkey gr-1 | [M58410] | (SIV-agm.gr) |
| African green monkey Sab-1 | [U04005] | (SIV-agm.sab) |
| African green monkey Tan-1 | [U58991] | (SIV-agm.tan) |
| Chimpanzee | [X52154] | (SIV-cpz) |
| Mandrill | [M27470] | (SIV-mnd) |
| Pig-tailed macaque* | [M32741] | (SIV-mne) |
| Red-capped mangabey | [AF028607] | (SIV-rcm) |
| Rhesus macaque* (*Maccaca mulatta*) | [M19499] | (SIV-mac) |
| Sooty mangabey SIV-H4 | [X14307] | (SIV-smm) |
| Stump-tailed macaque* | [M83293] | (SIV-stm) |
| Sykes monkey | [L06042] | (SIV-syk) |

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
* Indicates cross-species transmissions of SIV-smm in captivity.

## List of other related viruses which may be members of the genus *Lentivirus* but have not been approved as species

None reported.

# SUBFAMILY *SPUMARETROVIRINAE*

## Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Spumaretrovirinae*** |
| Genus | ***Spumavirus*** |

# GENUS *SPUMAVIRUS*

Type species ***Simian foamy virus***

## Distinguishing features

Virions exhibit a distinctive morphology with prominent surface spikes and a central, uncondensed core. Capsid assembly occurs in the cytoplasm prior to budding into the endoplasmic reticulum or from the plasma membrane. Capsid budding requires the presence of Env protein. No cleavage of Gag protein precursors into MA, CA, NC subunits is detectable in infectious virions. The Gag protein is cleaved once near the carboxyl-terminus. Approximate protein sizes are: Gag precursor 71 kDa; N-terminal Gag cleavage product 68 kDa; Pol precursor 127 kDa; RT 85 kDa; IN 40 kDa; Env precursor 130 kDa; SU 80 kDa; TM 48 kDa; LP 18 kDa; Tas 35 kDa; Bet 60 kDa; and Env-Bet fusion protein 170 kDa. The genome is about 11.6 kb in size; its organization is illustrated in Figure 8. The genomic organization is identical to that of other members of the family *Retroviridae*, as is the mechanism of reverse transcription, which allows inclusion of these viruses into the family. There are two genes (*tas* and *bet*) that are expressed in cells in addition to *gag*, *pol* and *env*. Tas is a DNA-binding protein with transactivating function. The exact function of the other accessory protein (Bet) is unknown, but it may be involved in viral latency or act as an inhibitor of the APOBEC3 family. The tRNA primer is $tRNA^{Lys-1,2}$. The LTR of primate foamy viruses is about 1770 nt long, of which the U3 region is about 1400 nt, the R region is about 200 nt and the U5 region is 150 nt. In the bovine, equine and feline viruses, the LTR is 950–1400 nt. Spumaviruses make use of two start sites of transcription, R in the LTR and an internal promoter (IP) located upstream of the accessory reading frames within the *env* gene. The activity of both promoters is Tas-dependent. The additional major criteria distinguishing spumaviruses from members of the other genera are the expression of the Pol protein from a spliced subgenomic RNA and the presence of a large amount of reverse transcribed DNA in the virion, which is required for infection.

Viruses have a widespread distribution, and exogenous spumaviruses are found in many mammals. Phylogenetic analyses using sequences encoding IN are consistent with the hypothesis that simian foamy viruses may have co-evolved with their primate hosts. Human infections have been documented as a result of rare zoonotic transmissions from non-human primates, but human to human spread has not been observed. A distantly related endogenous virus has been reported. Many isolates cause characteristic "foamy" cytopathology in cell culture. No diseases have been associated with spumavirus infections. No oncogene-containing member of the genus has been found.

## Species demarcation criteria in the genus

The list of species demarcation criteria is:

- Differences in genome and gene product sequences
- Differences in natural host range

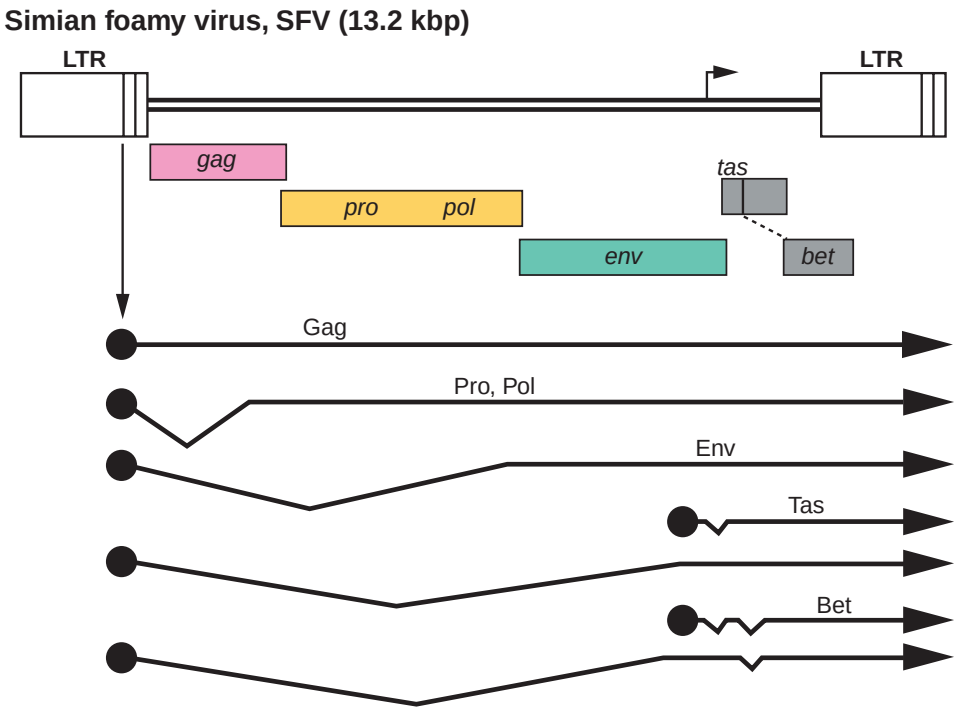


**Figure 8:** Spumavirus genome expression. The 13.2 kbp simian foamy virus (SFV) provirus is shown, with LTRs, the internal promoter, protein-coding regions (*gag*, *pro*, *pol*, *env*, *tas* and *bet*) and transcripts (solid line arrows with protein names added) marked.

## List of species in the genus *Spumavirus*

| | | |
|---|---|---|
| *African green monkey simian foamy virus* | | |
| African green monkey simian foamy virus (Simian foamy virus 3, SFV-3) | [M74895] | (SFVagm) |
| *Macaque simian foamy virus* | | |
| Macaque simian foamy virus (Simian foamy virus 1, SFV-1) | [X54482] | (SFVmac) |
| *Simian foamy virus* | | |
| Simian foamy virus, human isolate (Chimpanzee foamy virus, CFV) (Human foamy virus, HFV) (Prototype foamy virus, PFV) | [U21247] | (SFVcpz(hu)) |
| Simian foamy virus, chimpanzee isolate | [L25422] | (SFVcpz) |
| *Bovine foamy virus* | | |
| Bovine foamy virus | [U94514] | (BFV) |
| *Equine foamy virus* | | |
| Equine foamy virus | [AF201902] | (EFV) |
| *Feline foamy virus* | | |
| Feline foamy virus | [Y08851] | (FFV) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus but have not been approved as species

None reported.

## Phylogenetic relationships within the family *Retroviridae*

See Figure 9.

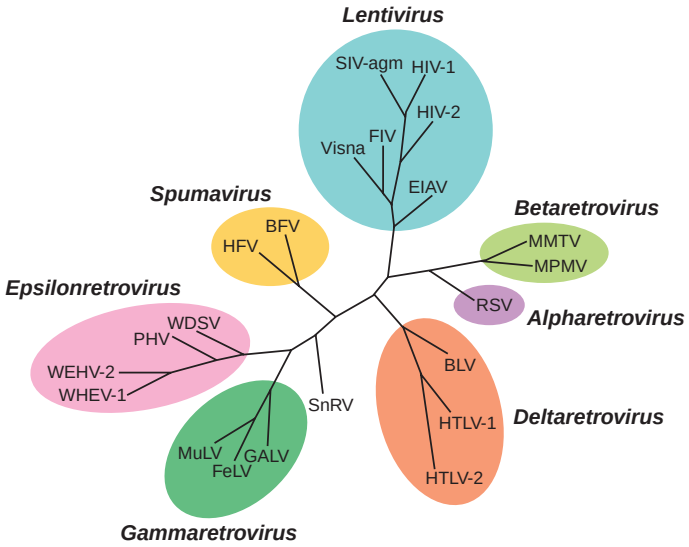


**Figure 9:** Phylogenetic analysis of conserved regions of the retrovirus polymerase gene (courtesy of Quackenbush, S and Casey, J.). An amino acid sequence alignment was constructed of residues in domains 1 to 4 and part of domain 5 of reverse transcriptase (Xiong, Y. and Eickbush, T.H. (1990). *EMBO J.*, **9**, 3353-3362). An unrooted neighbor-joining phylogenetic tree was constructed by using the PHYLIP package (Felsenstein, J. (1995). "PHYLIP [Phylogeny Inference Package] Version 3.57c." University of Washington, Seattle.)

## Similarity with other taxa

There is no nucleotide sequence similarity between members of the families *Hepadnaviridae*, *Caulimoviridae*, *Pseudoviridae* and *Metaviridae*, as well as with non-viral retroelements. However, there is similarity through the replication strategy with members of the family *Hepadnaviridae*.

## Derivation of names

*Lenti*: from Latin *lentus*, "slow".
*Ortho*: from Greek *orthos*, "straight".
*Retro*: from Latin *retro*, "backwards", refers to the activity of reverse transcriptase and the transfer of genetic information from RNA to DNA.
*Spuma*: from Latin *spuma*, "foam".

## Further reading

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## Contributed by

Stoye, J.P., Blomberg, J., Coffin, J. M., Fan, H., Hahn, B., Neil, J., Quackenbush, S., Rethwilm, A. and Tristem, M.

# Family *Birnaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Birnaviridae*** |
| Genus | ***Aquabirnavirus*** |
| Genus | ***Avibirnavirus*** |
| Genus | ***Blosnavirus*** |
| Genus | ***Entomobirnavirus*** |

## Virion properties

### Morphology

Viruses of the family *Birnaviridae* are non-enveloped, single-shelled particles with a diameter of about 65 nm (Figure 1). The capsid follows a T = 13 *laevo* icosahedral geometry and is made of a single capsid protein, VP2, clustered in trimers and forming 260 projections of about 4 nm at the surface of the particle.

### Physicochemical and physical properties

Virion Mr is about $55 \times 10^6$, $S_{20,W}$ is 435S; buoyant density in CsCl is 1.33 g cm$^{-3}$. Defective virions with interfering activity have been demonstrated to band at 1.30 g cm$^{-3}$. Viruses are stable at pH 3–9, resistant to heat (60 °C, 1 h), ether and 1% SDS at 20 °C, pH 7.5 for 30 min.

### Nucleic acid

Birnaviruses have a dsRNA genome which is made of two linear segments (A and B). The virions can package more than one complete genome copy that constitutes 8–10% of the particle by weight.

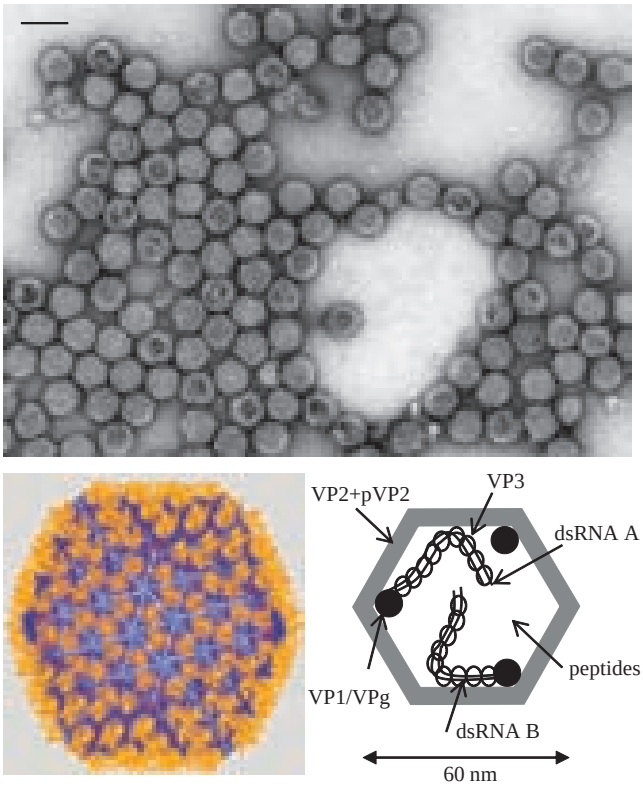


**Figure 1:** (Top) Negative contrast electron micrograph of infectious bursal disease virus (IBDV) particles (courtesy of J. Lepault). Bar represents 100 nm. (Bottom left) A three-dimensional model of the IBDV virion derived from X-ray crystallography (courtesy of F. Rey). (Bottom right) Diagrammatic representation of an IBDV particle, which has a single T = 13 icosahedral shell.

dsRNA

The larger segment (A) is 3.1–3.6 kbp long, and the length of the smaller segment (B) ranges from 2.8 to 3.3 kbp, depending on the genus. The genome RNA sequences have a nucleotide composition of 53–58% G+C, with the exception of those of Rotifer birnavirus (RBV) and Drosophila X virus (DXV), which are 44–47% G+C. The segments are completely base-paired, and the plus-sense strand is covalently linked to a viral protein (VPg) at its 5′ terminus, but has neither a polyadenylation signal nor a terminal polyA tract at its 3′ end. Detailed characterization of the genome extremities have been carried out on Infectious bursal disease virus (IBDV) and Infectious pancreatic necrosis virus (IPNV). The first 30 nucleotides (nt) of the 5′-noncoding regions in the two segments display conserved motifs in both viruses. In contrast, sequences differ in the 3′-noncoding region of both segments. Inverted terminal repeats are present in the 5′- and 3′-noncoding regions of both segments. These motifs and repeats indicate the presence of *cis*-acting signals that are important for regulation of transcription, replication and selection of segments for packaging. Whereas the 5′-noncoding regions of both segments vary in length between 100 and 120 nt, the 3′-noncoding regions are about 60–150 nt in length. The 5′-noncoding regions are followed by one long ORF after the first AUG codon in segment B. Segment A also encodes a large ORF, which is generally preceded by a small, overlapping ORF. For Tellina virus 1 (TV-1), Blotched snakehead virus (BSNV) and DXV, the small ORF in segment A does not overlap the initiation codon of the large ORF. For RBV, there is no evidence for the presence of a small ORF in segment A.

### Proteins

The polyprotein encoded by the large ORF in segment A is first processed during translation to generate preVP2, VP4 and VP3. Further processing of preVP2 occurs at its C-terminal domain to generate the mature capsid protein (VP2) and three or four peptides that remain in the particles. For IBDV, these peptides are 46, 7, 7 and 11 amino acid residues (aa) long. The capsid is formed by trimers of VP2 (417–442 aa). VP2 possesses a unique structural fold that is composed of two beta-barrels with a jelly-roll topology, oriented such that the beta-strands are tangential and radial to the virus particle. Inside the capsid, VP3 (238–309 aa) and the genomic RNA form thread-like ribonucleoprotein complexes that do not follow the icosahedral symmetry of the capsid. Different ratios of VP3 over VP2 have been reported in virions from members of different species.

The RNA-dependent RNA polymerase (RdRp), VP1 (844–1045 aa), is encoded by segment B. VP1 is found free in the viral particle and also covalently associated to the genome as VPg. The 2.5 Å resolution structure of IBDV VP1 reveals a characteristic rearrangement of motifs, from A–B–C to C–A–B, in the RNA polymerase catalytic palm domain, which is not found in viral RdRps from other dsRNA viruses. VP1 can guanylylate itself to produce VP1-pG and VP1-pGpG independently from its RNA polymerase activity. In the case of IBDV, VP1 has been shown to possess viral mRNA 5′-guanylyl transferase and capping activities.

VP4 (also called NS in IPNV, 212-244 aa) is a protease that cleaves its own N- and C- termini in the polyprotein and further processes preVP2. Its catalytic site is made of a serine-lysine dyad. The VP4 catalytic domain is structurally similar to the protease domain of bacterial ATP-dependent Lon proteases.

A nonstructural, positively charged polypeptide encoded by the small ORF of segment A has been designated VP5 (17 kDa in IBDV and 15 kDa in IPNV). This protein has been shown to be nonessential for replication of IBDV and IPNV. A second ORF, encoding an arginine-rich protein, has also been identified in genome segment A of DXV, BSNV and TV-1.

### Lipids

None reported.

### Carbohydrates

No N-linked glycosylation has been detected in any of the virion proteins. There is a report of O-linked glycosylation in IPNV VP2.

## Genome organization and replication

As explained above, birnaviruses have a bipartite genome. The organization of segment A is illustrated in Figure 2. Generally, it contains two ORFs: ORF2 encoding a large polyprotein of about

105–120 kDa, and an overlapping (IPNV, IBDV and Drosophila birnavirus (DBV)) or internal (DXV, BSNV and TV-1) ORF1, which encodes a protein of 15–27 kDa. Positions of the polyprotein cleavage sites to generate preVP2, VP4 and VP3 during translation have been determined experimentally for IBDV, IPNV, BSNV, DXV and TV-1 (Figure 2). For BSNV and TV-1, an additional polypeptide, named X, is encoded between the preVP2 and the VP4 domains. The processing of preVP2 to generate VP2 and the structural peptides, which occurs during particle assembly, has been characterized for IBDV, IPNV, BSNV and TV-1. Sequence alignments allow the prediction of the preVP2 cleavage sites for other birnaviruses.

A single cycle of replication takes about 18–22 h for IPNV and 4–8 h for IBDV. The mode of entry of viruses into cells is not well understood, and the information is fragmentary. For IBDV binding at the cell surface, proteins such as heat shock protein 90 and $\alpha 4\beta 2$ integrin have been proposed to serve as functional receptors in various types of chicken cells. Endosomal acidification is not a prerequisite for virus internalization in IPNV-infected cells. One of the small structural IBDV peptides, pep46 (a 46 aa amphiphilic peptide), and its homologs in other birnaviruses are able to induce pores in target membranes, suggesting a role in virus entry. After delivery into the cytoplasm, the virion RdRp becomes activated and produces two genome-length (24S) mRNA molecules from each of the 14S dsRNA genome segments. These mRNAs are capped, and they lack 3′ polyA sequences. Replicative intermediates have been identified in infected cells. Virus RNA is transcribed by a semi-conservative strand displacement mechanism *in vitro*. There is no information on minus strand RNA synthesis. The two mRNAs can be detected in infected cells by 3–4 h post infection (p.i.), and are synthesized in the same relative proportions throughout the replicative cycle (i.e. about twice as many A as B mRNA molecules). Virus-specific polypeptides can be detected at 4–5 h p.i. and are present in the same relative proportions until the end of the replication cycle. There are no specifically early or late proteins. The segment A mRNA is translated to yield a 105 kDa polyprotein



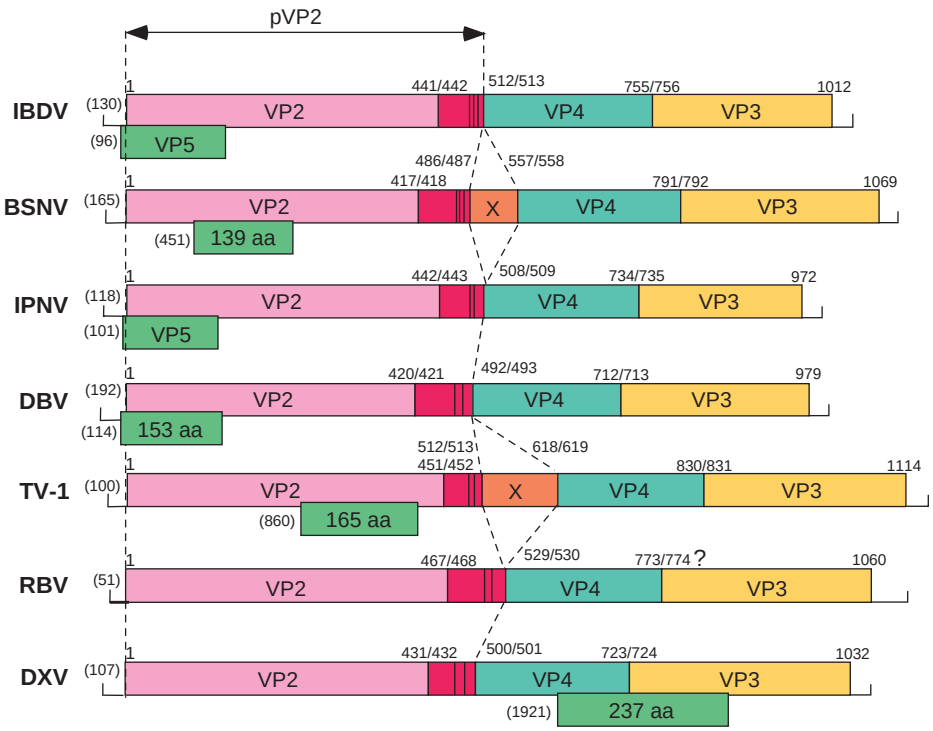


**Figure 2:** Schematic representation of the gene arrangement in genome segment A of birnaviruses representative of the family: infectious bursal disease virus (IBDV), blotched snakehead virus (BSNV), infectious pancreatic necrosis virus (IPNV), Drosophila birnavirus (DBV), Tellina virus 1 (TV-1), rotifer birnavirus (RBV) and Drosophila X virus (DXV). Polyprotein (ORF2) cleavage sites are indicated by a vertical bar and their positions by the P1/P′1 number. The location and size of the small ORFs (ORF1) are indicated below the polyprotein ORF. Numbers in parentheses at the 5′ ends indicate the length of the 5′-non-coding region.

that comprises the preVP2, VP4 (NS) and VP3 polypeptides, with the notable exception of BSNV and TV-1, which contain the X polypeptide between the preVP2 and VP4 domains (Figure 2). The VP4 protease co-translationally cleaves the polyprotein to generate three (or four in BSNV and TV-1) polypeptides (Figure 3). PreVP2 is later processed during virus assembly by a slow maturation cleavage to produce the mature VP2 and small structural peptides. This cleavage can be incomplete since traces of preVP2 are found in purified virus particles, although VP2 predominates. Virus assembly and maturation of the capsid protein preVP2/VP2 are concomitant and interdependent. In addition to VP4, the preVP2 processing requires the presence of VP3 and VP1. This requirement acts in favor of the existence of a large quaternary maturation complex formed by preVP2, VP4, VP3 and VP1. During infection, rigid tubes 55 nm in diameter are formed by preVP2. In the case of IBDV, additional tubules 25 nm in diameter, made of VP4, appear in late steps of the virus replication cycle.

The translation product of the 17 or 15 kDa ORF has been detected in IBDV or IPNV infected cells, respectively.

The mRNA from segment B is translated to a 94 kDa polypeptide that represents the viral RdRp (VP1, Figure 3). VP1 is found in virions in both a "free" and a genome-linked form (VPg). Virus particles assemble and accumulate in the cytoplasm. Encapsidation of the RdRp VP1 is mediated by its interaction with genome-associated protein VP3. The mechanism of virus release is unknown. In tissue culture, about half of the progeny virions remain cell-associated, and, depending on the multiplicity of infection, defective interfering particles are also formed.

Reverse genetics systems have been elaborated for IBDV and IPNV. *In vitro* transcribed viral cRNAs of segment A and B were found to be infectious, facilitating studies of birnavirus replication.

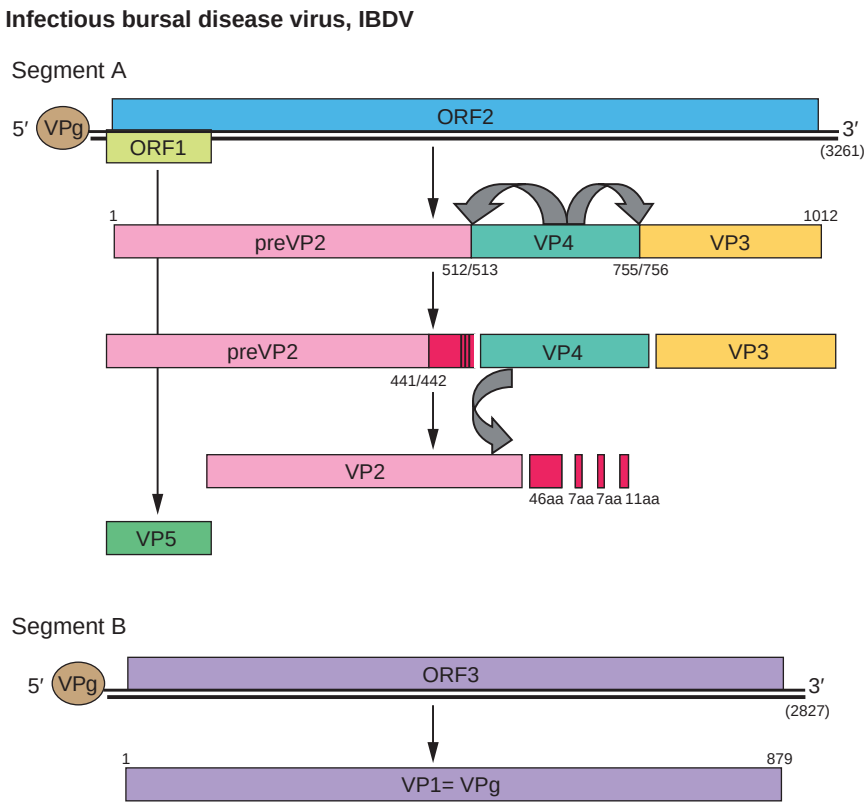


**Figure 3:** Schematic representation of the genome of infectious bursal disease virus (IBDV) illustrating processing of the encoded proteins. Numbers in parentheses indicate the nucleotide lengths of the two genomic segments.

### Antigenic properties

The capsid protein VP2 is the type-specific antigen and forms the virus-neutralizing epitopes. Anti-VP3 antibodies do not neutralize virus infectivity. There is no serological cross-reaction between the fish, avian and insect birnaviruses, or between the aquatic birnaviruses IPNV, BSNV, TV-1 and RBV.

### Biological properties

The natural hosts of IPNV are salmonid fish, although this virus or viruses antigenically-related to it have also been isolated from other freshwater and marine fishes, as well as from bivalve molluscs (Tellina virus 2 (TV-2)). The virus is transmitted both vertically and horizontally, and there are no known vectors. The geographic distribution is worldwide. IPNV can cause epizootics resulting in high mortality in hatchery-reared salmonid fry and fingerlings. The virus causes necrotic lesions in the pancreas and is also found, without lesions, in other organs such as kidney, gonad, intestine and brain. It is believed that infected adult fish become lifelong carriers without exhibiting overt signs of infection.

The natural hosts of IBDV are chickens and turkeys. Rarely, IBDV has been isolated from ducks and other domestic fowl. The mode of transmission is horizontal, and there are no known vectors. IBDV has a worldwide distribution. The virus affects the bursa of Fabricius in young chicks, causing B lymphocyte deficiency. Death can occur between 3 and 10 weeks of age, and is associated with inflammation in the bursa of Fabricius, formation of immune complexes, depletion of complement, and clotting abnormalities.

*Drosophila melanogaster* populations are the natural host of DXV. The mode of transmission is horizontal and there are no known vectors. The geographic distribution is unknown. Infected fruitflies become sensitive to $CO_2$. The target organs and histopathology are not known. DXV has also been isolated from populations of *Culicoides* spp.

BSNV was isolated from a cell line developed from a tropical fish species (*Channa lucius*), whereas TV-1 was identified in a bivalve mollusc (*Tellina tenuis*). RBV was isolated from a population collapse of the rotifer *Brachionus plicatilis*, which is cultivated for feeding the fry of marine fish in hatcheries. DBV was identified by deep sequencing of the small RNAs present in a cultured *Drosophila melanogaster* cell line.



## GENUS *AQUABIRNAVIRUS*

Type species ***Infectious pancreatic necrosis virus***

### Distinguishing features

Viruses in the genus thus far infect only fish, molluscs and crustaceans.

### Biological properties

Aquabirnaviruses have been isolated from a variety of aquatic animals in freshwater, brackish or seawater environments. The ubiquitous nature of these agents and, in some cases, the lack of any association with disease has led to difficulty in assigning nomenclature. The first reports of isolation of IPNV were limited to epizootics in cultured brook trout (*Salvalinus fontinalis*). Soon IPNV was found to be responsible for disease in a variety of salmonid fish, including members of the genera *Salmo*, *Salvalinus* and *Oncorhynchus*. The virus has also been associated with disease in Japanese eels (*Anguilla japonica*) where it causes a nephritis, in menhaden (*Brevoortia tryrannus*) where it causes a "spinning disease," and in yellowtail fingerlings (*Seriola quinqueradiata*) where it causes an ascites and cranial hemorrhage. In salmonid fish, IPNV causes acute gastroenteritis and destruction of the pancreas in the very young. A birnavirus has been associated with hematopoietic necrosis causing

high mortalities in turbot (*Scophthalmus maximus*) with renal necrosis, and birnaviruses have been isolated from clams exhibiting darkened gills and gill necrosis. A non-typical apoptosis has been observed in cultured cells infected by IPNV.

### Species demarcation criteria in the genus

Three species of aquabirnaviruses are distinguished, primarily on the basis of host species. However, this classification was established before the current, broad species definition was adopted by the ICTV. The extremely close genetic relationship between the species, illustrated by Figure 4, suggests that there may be a case for combining them into one. Aquabirnaviruses do, nevertheless, display considerable antigenic diversity. Based on reciprocal neutralization assays using polyclonal antisera and immunoassays with monoclonal antibodies, the genus has been grouped into nine cross-reactive serotypes: A1 (type strain West Buxton), A2 (type strain Sp), A3 (type strain Ab), A4 (type strain Hecht), A5 (type Tellina virus-2), A6 (type strain Canada 1), A7 (type strain Canada 2), A8 (type strain Canada 3) and A9 (type strain VR299). Capsid protein sequences correlate well with serological classification and geographical distribution. Six genogroups were defined on the basis of sequence similarities: while genogroup 1 clusters serotypes A1 and A9, genogroup 2 corresponds to serotypes A7 and A8, genogroup 3 to serotype A3, genogroup 4 to serotypes A5 and A6, genogroup 5 to serotype A2 and genogroup 6 to serotype A4 (Figure 4). Further sequence characterization of aquabirnaviruses in Asia and Australasia evidenced the existence of an additional genogroup (type Yellowtail ascites virus) and an enlarged genogroup 5 including the strain NZ10 and relatives.

### List of species in the genus *Aquabirnavirus*

| | | |
|---|---|---|
| *Infectious pancreatic necrosis virus* | | |
| Infectious pancreatic necrosis virus - West Buxton | [A:AF078668; B:AF078669] | (IPNV-WB) |
| *Tellina virus* | | |
| Tellina virus 2 | [A:AF342730] | (TV-2) |
| *Yellowtail ascites virus* | | |
| Yellowtail ascites virus - Y-6 | [A:AB006783; B:AY129662] | (YTAV-Y-6) |

Species names are in italic script; names of strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Aquabirnavirus* but have not been approved as species

| | | |
|---|---|---|
| Marine birnavirus - AY-98 | [B: AY123970] | (MABV-AY-98) |
| Marine birnavirus - H-1 | [B: AY129665] | (MABV-H-1) |

## GENUS *AVIBIRNAVIRUS*

Type species ***Infectious bursal disease virus***

### Distinguishing features

Viruses in the genus thus far infect only birds.

### Biological properties

IBDV causes an immunosuppressive disease in chickens by destruction of B lymphocytes in the bursa of Fabricius. Apoptosis has also been observed in this and other lymphoid organs. VP5 inhibits apoptosis at the early stage of viral infection in chicken embryonic fibroblast cells, whereas VP2 induces apoptosis in transfected mammalian cells. The latter finding correlates with evidence of apoptosis and B cell death in chickens infected with IBDV. The rapid depletion of B cells in the bursa of Fabricius leads to immunosuppression and increased susceptibility to other infections and

diseases. The virus is highly contagious and is of major importance to the poultry industry worldwide. Two serotypes (1 and 2) of IBDV have been identified by cross-neutralization assays. Serotype 1 strains are pathogenic in chickens, whereas serotype 2 strains are nonpathogenic.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Avibirnavirus*

*Infectious bursal disease virus*
Infectious bursal disease virus - strain P2 [A: X84034; B:X84035] (IBDV-P2)

Species names are in italic script; names of strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Avibirnavirus* but have not been approved as species

None reported.

## GENUS *BLOSNAVIRUS*

Type species ***Blotched snakehead virus***

### Distinguishing features

Viruses in the genus thus far infect only fish.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Blosnavirus*

*Blotched snakehead virus*
Blotched snakehead virus [A:AJ459382, B:AJ459383] (BSNV)

Species names are in italic script; names of strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Blosnavirus* but have not been approved as species

None reported

## GENUS *ENTOMOBIRNAVIRUS*

Type species ***Drosophila X virus***

### Distinguishing features

Viruses in this genus thus far infect only insects.

### Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Entomobirnavirus*

*Drosophila X virus*
Drosophila X virus [A:U60650, B:AF196645] (DXV)

Species names are in italic script; names of strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Entomobirnavirus* but have not been approved as species

None reported.

## List of other related viruses which may be members of the family *Birnaviridae* but have not been approved as species

| | | |
|---|---|---|
| Rotifer birnavirus | [A:FM995220, B:FM995221] | (RBV) |
| Tellina virus 1 | [A:AJ920335, B :AJ920336] | (TV-1) |
| Drosophila birnavirus | [A:GQ342962, B:GQ342963] | (DBV) |

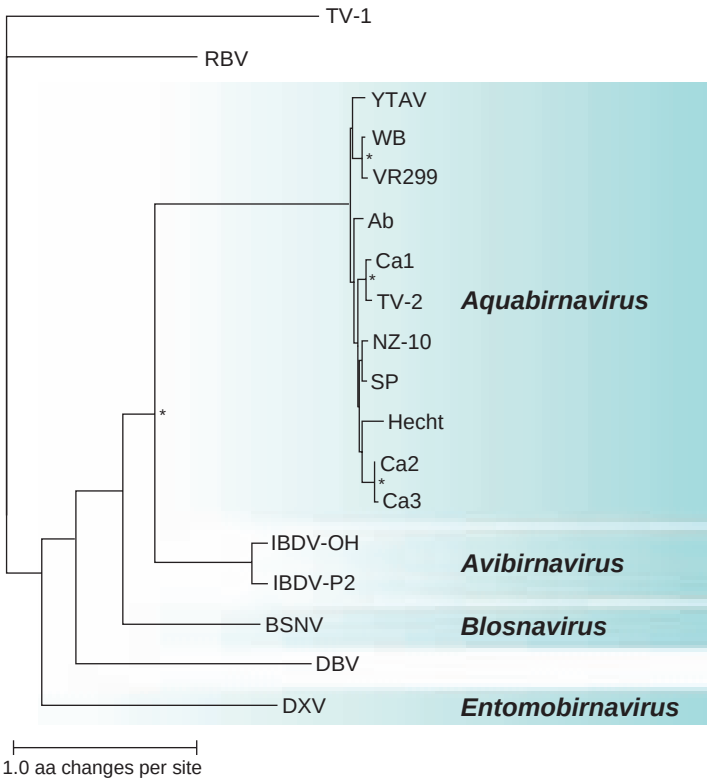


**Figure 4:** A distance tree representing the phylogenetic relationships of VP2 in the various genera and genetic clusters of the family. Alignment was performed with MUSCLE and phylogenetic analysis with PhyML using default parameters. Internal branches with bootstrap support above 80% are highlighted. The tree was inferred by maximum likelihood on the amino-acid sequences of the capsid protein. Tellina virus 1 (TV-1), Drosophila birnavirus (DBV) and rotifer birnavirus (RBV) are separate from each other and from the four genera. The protein accession numbers used for comparison were (top to bottom): TV-1: CAI74981, RBV: CAX33877, YTAV: BAA25005, WB: AF342727, VR299: AF343572, Ab: AF342729, Ca1: AF342732, TV-2: AF342731, NZ-10: ACG56371, SP: AF342728, Hecht: AF342730, Ca2: AF342733, Ca3: AF342734, IBDV-OH: AAC55351, IBDV-P2: CAA58851, BSNV: AJ459382, DBV: ACU32790, and DXV: AAB16798.

## Phylogenetic relationships within the family *Birnaviridae*

See Figure 4.

## Similarity with other taxa

Birnaviruses share no nucleic acid sequence similarity with other taxa. In the same way, the capsid protein VP2 has no sequence similarities with corresponding capsid proteins of any other virus family. However, the crystal structures of IBDV and IPNV VP2 shows that VP2 is folded into three distinct domains (base, shell and projection) disposed radially in the virus particle, the base and shell domains displaying high structural similarities with the capsid proteins of viruses belonging to the families *Nodaviridae* and *Tetraviridae,* which contain positive-strand ssRNA viruses. The VP4 protease has sequence and structural homologies with the Ser-Lys catalytic protease domain of bacterial ATP-dependent Lon proteases. Interestingly, the A-B-C to C-A-B motif rearrangement in the VP1 RdRps of birnaviruses, noted above, is also shared by the RdRps of Thosea asigna virus and Euprosterna elaeasa virus, insect viruses that have positive strain ssRNA genomes (genus *Betatetravirus*, family *Tetraviridae*), but not by RdRps of other dsRNA viruses. These unusual RdRps form a minor and deeply separated cluster in the viral RdRp phylogenetic tree. The structural relationships among the capsid proteins and the RdRps of birnavirus and viruses belonging to the family *Tetraviridae* suggest evolutionary links between positive-strand ssRNA and dsRNA viruses.

## Derivation of names

*Aqua*: from Latin *aqua*, "water".
*Avi*: from Latin *avis*, "bird".
*Birna*: from Latin prefix *bi*, "two", signifying the bisegmented nature of the viral genome as well as the presence of dsRNA; and *rna* from *r*ibo *n*ucleic *a*cid, indicating the nature of the viral genome.
*Entomo*: from Greek *entomon*, "insect".
*Blosna*: from *blo*tched *sna*kehead virus.



## Further reading

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## Contributed by

Delmas, B., Mundt, E., Vakharia, V.N. and Wu, J.L.

# Family *Chrysoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Chrysoviridae* |
| Genus | *Chrysovirus* |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Chrysovirus*

Type species *Penicillium chrysogenum virus*

## Virion properties

### Morphology

Virions are isometric, non-enveloped, about 400 Å in diameter, and the protein shell is 48 Å thick. The capsid comprises 60 protein subunit monomers arranged on a T = 1 icosahedral lattice; the most prominent features are 12 outward-protruding pentamers. The capsid protein has a high content of α helices, forming a repeated α-helical core indicative of gene duplication (or joined folds). Whereas the Penicillium chrysogenum virus (PcV) capsid protein has two motifs with the same fold, most dsRNA virus capsid subunits consist of dimers of a single protein with similar folds. The spatial arrangement of the α-helical core resembles that found in the L-A virus capsid protein, a fungal totivirus, suggesting a conserved basic fold. Due to the numerous interactions of the encapsidated genome with the inner surface of the capsid, specifically six interacting areas per monomer, the outermost dsRNA layer is arranged in an icosahedral cage, and is partially visualized as tubular densities corresponding to an A-form duplex. The genome ordering might constitute a framework for dsRNA transcription at the capsid interior, and/or has a structural role for capsid stability.

### Physicochemical and physical properties

Virion buoyant density in CsCl is in the range of 1.34–1.39 g cm$^{-3}$and $S_{20,w}$ is 145-150S.



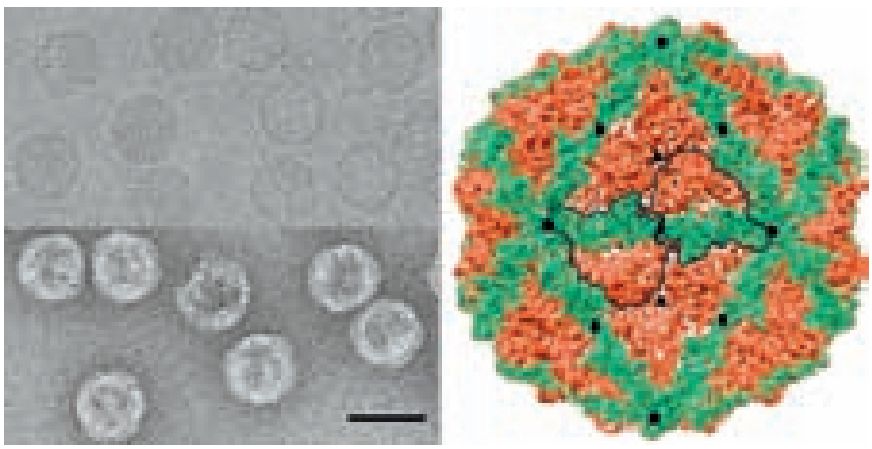

**Figure 1:** (Left) Electron micrographs of Penicillium chrysogenum virus (PcV), the type species of the genus *Chrysovirus*. Samples were negatively stained in 2% uranyl acetate (lower panel) or prepared unstained and vitrified (upper panel); the bar represents 50 nm. (Right) Three-dimensional cryo-electron microscopy reconstruction of PcV virions at 8 Å resolution. Surface-shaded virion capsid viewed along an icosahedral twofold axis. Boundaries for two capsid proteins are outlined in black; each subunit has two similar domains (red and green), suggesting ancestral gene duplication. Icosahedral symmetry axes are indicated.

### Nucleic acid

Virions contain four unrelated linear, separately encapsidated, dsRNA segments (2.4–3.6 kbp in size, Table 1). The largest segment, dsRNA-1, codes for the virion-associated RNA polymerase and dsRNA-2 codes for the major CP. Both dsRNAs 3 and 4 encode proteins of unknown function. Sequences at the 5′- and 3′-UTRs are highly conserved among the four-dsRNA segments. The 5′-UTRs are relatively long, between 140 and 400 nt (nucleotides) in length. In addition to the absolutely conserved 5′- and 3′-termini, a 40–75 nt region with high sequence identity is present in the 5′-UTR of all four dsRNAs (Box 1; Figure 2). A second region of strong sequence similarity is present immediately downstream from Box 1 (Figure 2). This consists of a stretch of 30–50 nt containing a reiteration of the sequence "CAA". The $(CAA)_n$ repeats are similar to the enhancer elements present at the 5′-UTRs of tobamoviruses.

### Proteins

The capsids are made up of a single major polypeptide species (110–112 kDa). Virion-associated RNA polymerase activity is present.

### Lipids

None reported.

### Carbohydrates

None reported.

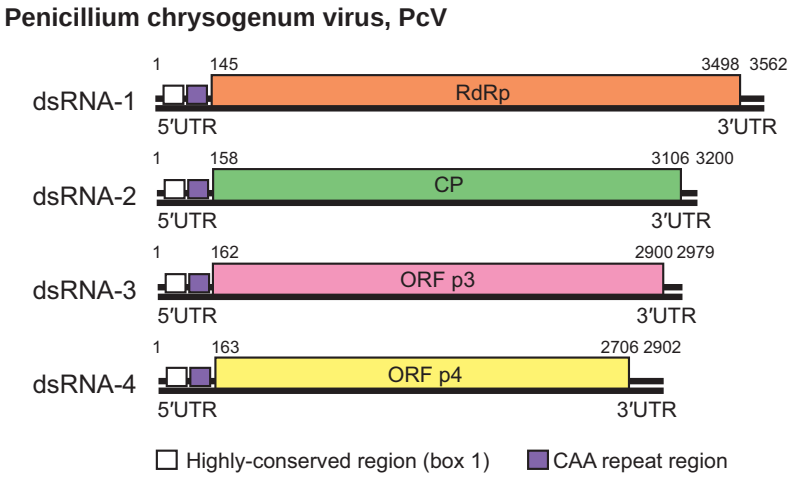


**Figure 2:** Genome organization of Penicillium chrysogenum virus (PcV). The genome consists of four dsRNA segments; each is monocistronic. The RdRp ORF (nt positions 145 to 3498 on dsRNA-1), the CP ORF (nt positions 158 to 3106 on dsRNA-2), the p3 ORF (nt positions 162 to 2900 on dsRNA-3) and the p4 ORF (nt positions 163 to 2706 on dsRNA-4) are represented by rectangular boxes.

**Table 1:** List of the dsRNA segments of Penicillium chrysogenum virus (PcV), with their size (bp), calculated size of their encoded proteins and function

| Segment | Size (bp)[a] | Size of encoded protein (Da)[b] | Function of protein |
|---|---|---|---|
| dsRNA1 | 3562 | 128,548 (1117 aa) | RdRp |
| dsRNA2 | 3200 | 108,806 (982 aa) | CP |
| dsRNA3 | 2976 | 101,458 (912 aa) | Unknown |
| dsRNA4 | 2902 | 94,900 (847 aa) | Unknown |

[a]Size determined by sequencing full-length cDNA clones of the indicated genome segments.
[b]Size determined from the predicted aa sequences derived from full-length cDNA clones of the indicated genome segments; The number of aa residues in the encoded viral proteins are placed in parentheses.

## Genome organization and replication

PcV has a multipartite genome comprising four unrelated linear dsRNA segments (Figure 2). Each is monocistronic; dsRNA-1 codes for the RdRp and dsRNA2 codes for the major CP. Although the proteins P3 and P4, coded for by dsRNA-3 and dsRNA-4, respectively, are of unknown function, protein database searches reveal that PcV P3 sequence shares a "phytoreo S7 domain" with a family consisting of several phytoreovirus P7 proteins known to be viral core proteins with nucleic acid binding activities. Interestingly, the N-terminal regions of PcV P3 (and corresponding P3 proteins of other chrysoviruses listed in Tables 2 and 3) share significant sequence similarity with comparable N-terminal regions of the putative RdRps encoded by chrysovirus dsRNA1s. The PcV P4 (and comparable proteins of other chrysoviruses) contains the motifs that form the conserved core of the ovarian tumor gene-like superfamily of predicted cysteine proteases.

Assignment of numbers 1–4 to PcV dsRNAs was made according to their decreasing size. Following the same criterion used for PcV, the dsRNAs associated with other chrysoviruses including Helminthosporium victoriae virus 145S (HvV145S), cherry chlorotic rusty spot associated chrysovirus (CCSRACV), Amasya cherry disease associated chrysovirus (ACDACV) and Cryphonectria nitschkei chrysovirus 1 (CnV1) (see below) were accordingly assigned the numbers 1–4. Sequence comparisons, however, indicated that dsRNAs 3 of Hv145SV, CCSRACV, ACDACV and CnV1 are in fact the counterparts of PcV dsRNA4 rather than dsRNA3. Likewise, dsRNA4 of these four chrysoviruses are the counterparts of PcV dsRNA3. Since PcV was the first chrysovirus to be characterized at the molecular level and to avoid confusion, the protein designations P3 and P4 as used for PcV are adopted and referred to as chryso-P3 and chryso-P4. Thus, whereas the chryso-P3 protein represents the gene product of PcV dsRNA3, it comprises the corresponding gene product of HvV145S dsRNA4, and so on.

The virion-associated RdRp catalyzes *in vitro* end-to-end conservative transcription of each dsRNA to produce mRNA by a conservative mechanism. Virions accumulate in the cytoplasm.



## Biological properties

The viruses are associated with latent infections of their fungal hosts. There are no known natural vectors. The chrysoviruses are transmitted intracellularly during cell division and sporogenesis (vertical transmission) and by cell fusion following hyphal anastomosis between compatible fungal strains (horizontal transmission).

## Antigenic properties

Virions are efficient immunogens.

## Species demarcation criteria in the genus

The criteria to differentiate species in the genus *Chrysovirus* are:

- host range
- size of dsRNA segments
- length of 5′-UTR
- serological relationships.

## List of species in the genus *Chrysovirus*

| | | |
|---|---|---|
| *Helminthosporium victoriae virus 145S* | | |
| Helminthosporium victoriae virus 145S | [AF297176, AF297177, AF297178, AF297179] | (HvV145S) |
| *Penicillium brevicompactum virus* | | |
| Penicillum brevicompactum virus | | (PbV) |
| *Penicillium chrysogenum virus* | | |
| Penicillium chrysogenum virus - ATTC 9480 | [AF296439, AF296440, AF296441, AF296442] | (PcV-ATCC9480) |

| | | |
|---|---|---|
| *Penicillium cyaneo-fulvum virus* | | |
| Penicillium cyaneo-fulvum virus | | (Pc-fV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Chrysovirus* but have not been approved as species

| | | |
|---|---|---|
| Amasya cherry disease associated chrysovirus | [AJ781163, AJ781164, AJ781165, AJ781166] | (ACDACV) |
| Cherry chlorotic rusty spot associated chrysovirus | [AJ781397, AJ781398, AJ781399, AJ781400] | (CCRSACV) |
| Cryphonectria nitschkei chrysovirus 1 | [DQ865186, DQ865187, DQ865188, DQ865189] | (CnV1) |
| Fusarium oxysporum chrysovirus 1 | [EF152346, EF152347, EF152348] | (FoCV1) |

## List of unassigned species in the family *Chrysoviridae*

None reported.

## Phylogenetic relationships within the family

BLAST searches of PcV RdRp amino acid sequence showed that it has significantly high sequence similarity (37–50% identity and 55–67% sequence similarity) to the RdRps of CnV1, Hv145SV, FoCV1, ACDACV and CCRSACV. Phylogenetic analysis based on the complete deduced amino acid sequences of the RdRps of chrysoviruses and selected totiviruses confirmed that approved as well as probable members of the family *Chrysoviridae* (listed above) are most closely related to each other (Figure 3). These results suggest that the probable chrysoviruses CnV1, FoCV1, ACDACV and CCRSACV should be placed under the genus *Chrysovirus* in the family *Chrysoviridae*. High similarities (BLAST hits of 2e-11 or lower) were also found between PcV RdRp amino acid sequence and several members of the family *Totiviridae* including Ustilago maydis virus H1 (UmV-H1), Saccharomyces cerevisiae virus L-A (ScV-L-A), Trichomonas vaginalis virus (TVV) and Helminthosporium victoriae virus 190S (HvV190S). Interestingly, no significant hits were evident

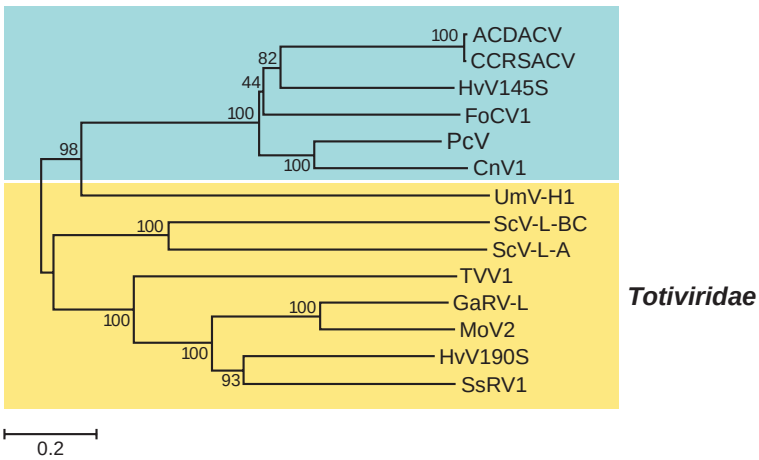


**Figure 3:** Neighbor-joining phylogenetic tree constructed based on the complete amino acid sequences of RdRps of members and probable members of the family *Chrysoviridae* and selected viruses from the family *Totiviridae*. The amino acid sequences were aligned using the program CLUSTAL W. See text for names and abbreviations of chrysoviruses. The following viruses in the family *Totiviridae* were included in the phylogenetic analysis (abbreviations in parenthesis): Gremmeniella abietina RNA virus L (GaRV-L), Helminthosporium victoriae virus 190S (HvV190S), Magnaporthe oryzea virus 2 (MoV2), Saccharomyces cerevisiae virus L-A (ScV-L-A), and ScV-L-BC, Sphaeropsis sapinea RNA virus 1 (SsRV1), Trichomonas vaginalis virus 1 (TVV1) and Ustilago maydis virus H1 (UmV-H1). The phylogenetic tree was generated using the MEGA4 phylogenetic package. Bootstrap values as a percent of 2000 replicates are indicated at each node.

with any of the viruses in the family *Partitiviridae*, confirming the decision to remove the chrysoviruses from the family *Partitiviridae* and their placement in the newly created family *Chrysoviridae*.

## Similarity with other taxa

Despite differences in the nature of the dsRNA genome between members in the families *Chrysoviridae* and *Totiviridae* (segmented versus nonsegmented genome), sequence and phylogenetic analyses of RdRp sequences suggest that chrysoviruses are most similar to viruses in the family *Totiviridae* (Figure 3).

## Derivation of name

*Chryso*: from the specific epithet of *Penicillium chrysogenum*.

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## Contributed by

Ghabrial, S.A. and Castón, J.R.

# Family *Cystoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Cystoviridae* |
| Genus | *Cystovirus* |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Cystovirus*

Type species *Pseudomonas phage phi6*

## Distinguishing features

The virion is enveloped and contains a segmented dsRNA genome. The innermost protein capsid is a polymerase complex responsible for genome packaging, replication and transcription.

## Virion properties

### Morphology

The enveloped virions are spherical, about 85 nm in diameter and covered by spikes (Figure 1). The envelope surrounds an isometric nucleocapsid, about 58 nm in diameter. The nucleocapsid surface shell (if present) follows T = 13 isocahedral symmetry. Turret-like extrusions of the underlying polymerase complex span the nucleocapsid surface shell layer at the five-fold symmetry positions (Figure 1). The dodecahedral polymerase complex is about 50 nm in diameter. In the polymerase complex major capsid protein dimers are arranged on a T = 1 icosahedral lattice.

### Physicochemical and physical properties

The molecular mass of Pseudomonas phage phi6 virion is $99 \times 10^6$ and nucleocapsid $40 \times 10^6$. Virion $S_{20,w}$ is about 405S. The buoyant density of the virion is 1.27 g cm$^{-3}$ in CsCl, 1.22 g cm$^{-3}$ in $Cs_2SO_4$ and 1.24 g cm$^{-3}$ in sucrose. Virions are sensitive to detergents, ether and chloroform but stable between pH 6.0 and 9.5.

### Nucleic acid

Virions contain three segments of linear, double stranded RNA: L (6.4–7.1 kb), M (3.6–4.7 kb), and S (2.6–3.2 kb). The complete genome is 12.7–15.0 kb and has a guanine + cytosine content of approximately 56%. All the genome segments are enclosed in a single particle and each virion contains a single copy of the genome. The genome constitutes approximately 10% of the virion weight.

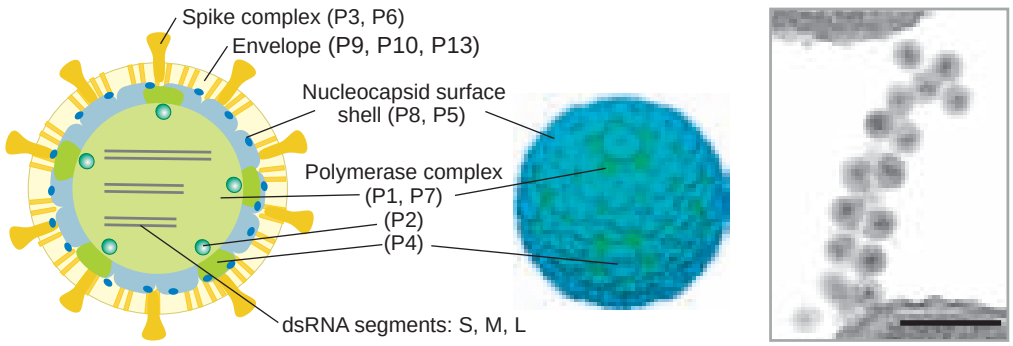


**Figure 1:** (Left) Schematic presentation of cystovirus particle (Pseudomonas phage phi6) with location of virion proteins. (Middle) Three-dimensional reconstructions of the nucleocapsid. (Right) Thin-section electron micrograph of Pseudomonas phage phi6 particles attached to the pilus receptor. The bar represents 200 nm.

### Proteins

Proteins constitute about 70% of the virion weight. The viral genome (Figure 2) encodes structural (Figure 1) and non-structural proteins.

The envelope contains three integral membrane proteins: P6, P9 and P10. Additional membrane proteins with unknown function are found from some members of the genus. Receptor binding spike is anchored to the envelope via fusogenic protein P6. The spike complex is composed of one (P3) to three polypeptides (P3a, P3b, P3c). Protein P8 forms the nucleocapsid surface shell (Figure 1) or is part of the envelope. Protein P5 is a lytic enzyme associated on the nucleocapsid surface. Major capsid protein P1 (120 copies per virion) is involved in single stranded RNA binding. Protein P2 is the viral replicase and transcriptase and is located in the interior of the P1 shell. The turret-like extrusions of the polymerase complex are made by hexamers of P4 protein. P4 is a nucleoside triphosphatase required for genome packaging and transcription. Minor capsid protein P7 is an assembly factor.

Non-structural protein P12 is a morphogenetic protein that is involved in envelope assembly. Non-structural proteins J and Hb of Pseudomonas phage phi8 regulate the stability of viral message RNAs. Different members of the genus may also encode additional non-structural proteins with unknown function.

### Lipids

Virions are composed of 20% lipids by weight. There is enough lipid to cover about one-half of the envelope surface area (the rest being protein). Viral lipids are derived from host plasma membrane. The lipid compositions of viral envelope and host plasma membrane are similar.

### Carbohydrates

None reported.

## Genome organization and replication

The overall genetic organization is similar in all proposed members of the genus. Genes are clustered into functional groups (Figure 2). Distinct noncoding regions at the termini of the three genome segments contain signals for genome packaging and replication.

Virions adsorb to pili or in some viruses directly to the outer membrane of the host bacterium (Figure 3). Viral envelope fuses with the host outer membrane and the nucleocapsid associated lytic enzyme locally digests the peptidoglycan layer. Viral polymerase complex delivery into the host cytoplasm involves an endocytic-like process at the host plasma membrane. The viral genome is transcribed by virion-associated RNA-dependent RNA polymerase within the polymerase complex. Early in the infection approximately equal amounts of messenger RNA molecules are produced from L, M and S. Later in the infection cycle transcripts of M and S typically predominate. This temporal control relies either on host factors or viral nonstructural proteins. Transcription is semiconservative and produces full-length copies of the genome segments.

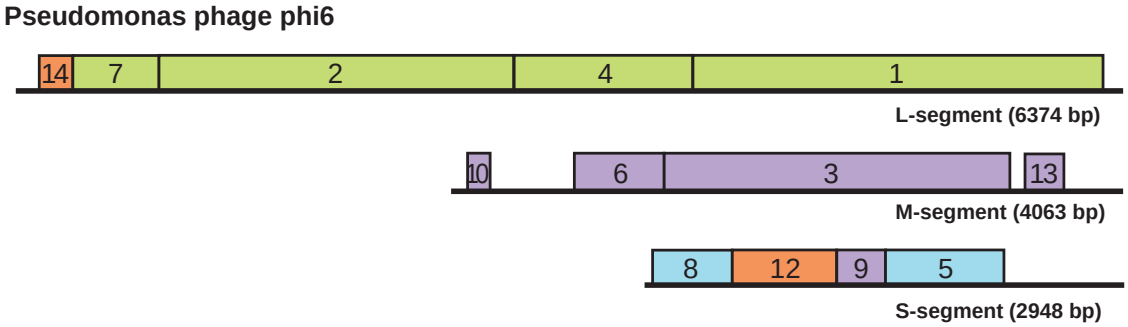


**Figure 2:** Genome organization of Pseudomonas phage phi6. The gene and protein numbers are the same. Genes encoding constituents of the polymerase complex and nucleocapsid are in green and blue, respectively. Genes encoding envelope associated proteins are in purple, and non-structural proteins in orange.

The produced messenger RNA molecules are polycistronic. Translation of L transcripts produces the early proteins, which assemble to form empty polymerase complexes (Figure 3). These package the three positive strand transcripts. Negative strand synthesis then takes place inside the polymerase complex. RNA packaging and replication induce structural changes in the polymerase complex (expansion). Transcription by these polymerase complexes produces messages for late protein synthesis. The nucleocapsid surface shell (if present) assembles on the polymerase complex (Figure 3) and inactivates transcription. Nucleocapsid acquires protein P5 and the envelope. Spikes are assembled on the envelope surface. Mature virions are release upon virus-induced host cell lysis.

## Antigenic properties

No information available.

## Biological properties

Cystoviruses are lytic bacteriophages that induce host cell lysis at the end of the viral reproduction cycle. Natural hosts are gram negative plant pathogenic bacteria.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Cystovirus*

*Pseudomonas phage phi6*
Pseudomonas phage phi6 [L: M17461; M: M17462; S: M12921] (phi6)

Species names are in italic script; names of strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.



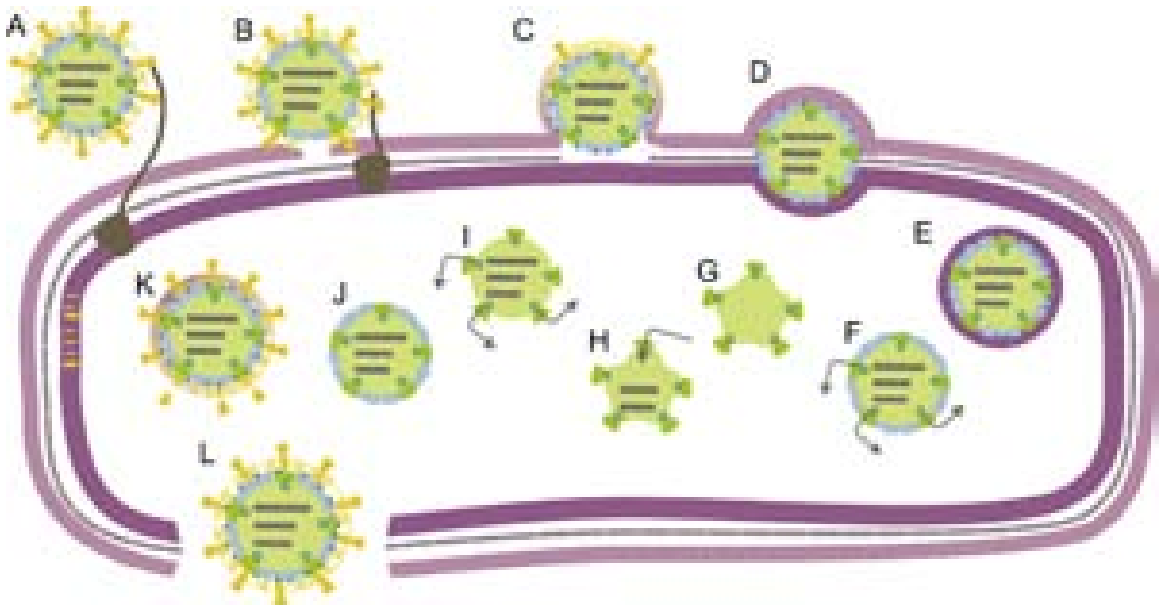


**Figure 3:** Schematic of the Pseudomonas phage phi6 life cycle. (A) Adsorption. (B) Envelope fusion. (C) Peptidoglycan digestion. (D–E) Endocytotic uptake of nucleocapsid. (F) Early transcription. (G) Polymerase complex assembly. (H) ssRNA packaging and replication. (I) Late transcription. (J) Nucleocapsid shell assembly. (K) Translocation of the viral envelope and assembly of spikes. (L) Host cell lysis and release of mature virions.

## List of other related viruses which may be members of the genus *Cystovirus* but have not been approved as species

| | | |
|---|---|---|
| Pseudomonas phage phi7 | | (phi7) |
| Pseudomonas phage phi8 | [L:AF226851; M:AF226852; S:AF226853] | (phi8) |
| Pseudomonas phage phi9 | | (phi9) |
| Pseudomonas phage phi10 | | (phi10) |
| Pseudomonas phage phi11 | | (phi11) |
| Pseudomonas phage phi12 | [L:AF408636; M:AY039807; S:AY034425] | (phi12) |
| Pseudomonas phage phi13 | [L:AF261668; M:AF261667; S:AF261666] | (phi13) |
| Pseudomonas phage phi14 | | (phi14) |
| Pseudomonas phage phi2954 | [L:FJ608823; M:FJ608824; S:FJ608825] | (phi2954) |

## Phylogenetic relationships within the family

Not applicable.

## Similarity with other taxa

In terms of genome replication strategy, cystoviruses resemble eukaryotic double stranded RNA viruses. The structure, organization and functions of the polymerase complex containing the genome are the major similarities among members of families *Cystoviridae*, *Reoviridae*, *Totiviridae*, *Partitiviridae* and *Picobirnaviridae*. The T = 13 architecture of the surrounding capsid layer is also shared by cystoviruses and reoviruses.

## Derivation of name

*Cysto*: from Greek *kystis*, "bladder", "sack".

## Further reading

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## Contributed by

Poranen, M.M. and Bamford, D.H.

# Family *Endornaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Endornaviridae* |
| Genus | *Endornavirus* |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Endornavirus*

Type species *Oryza sativa endornavirus*

## Virion properties

### Morphology
None reported. Endornaviruses do not produce virions.

### Physicochemical and physical properties
None reported.

### Nucleic acid
The linear dsRNA genomes of these viruses range in length from about 14 kbp to about 17.6 kbp. Each characterized genome includes a site-specific break (nick) in the coding strand about 1.2–2.7 kbp from the 5′ terminus.

### Proteins
None yet characterized. RdRp activity has been detected in cytoplasmic vesicles, which also contain the genomic dsRNA. Endornaviruses lack virion proteins.

### Lipids
None yet characterized. A lipid membrane that is probably derived from the host bounds the cytoplasmic vesicles.

### Carbohydrates
None yet characterized. Carbohydrate, possibly a glycolipid, has been detected in preparations of the cytoplasmic vesicles in plants infected with endornaviruses.

## Genome organization and replication

Each characterized genome encodes a single long polypeptide that crosses the break in the coding strand. These polypeptides include aa sequences typical of viral RNA helicases (Hels), UDP-glucosyltransferases (UGTs) and RNA-dependent RNA polymerases (RdRps). The polypeptides of Oryza sativa endornavirus (OsEV), Oryza rufipogon endornavirus (OrEV) and Phytophthora endornavirus 1 (PEV1) are about 4600 aa residues long, and those of Helicobasidium mompa endornavirus 1 (HmEV1) and Vicia faba endornavirus (VfEV) are about 5500 aa residues long. RNA replication occurs in cytoplasmic vesicles where RdRp activity has been detected in association with the genomic dsRNA. The cytoplasmic vesicles, sometimes called "virus-like particles," are bounded by a unit membrane and are believed to be functionally equivalent to the replication complexes of positive strand RNA viruses. Endornavirus RNA has been found in every tissue and at every developmental stage and is maintained at an almost constant concentration (20–100 copies/cell) except in the pollen of some species.

## Antigenic properties

Monoclonal antibodies raised against purified cytoplasmic vesicles from plants infected with VfEV allowed the VfEV-associated male sterility in the progeny of crosses to be detected. The antibodies recognized an epitope that contains sugars, possibly a glycolipid.

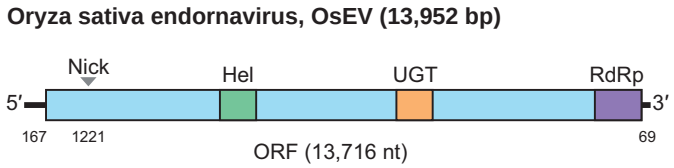


**Figure 1:** A genome map for an isolate of *Oryza sativa endornavirus*. A triangle marks the position of the break in the coding strand (1221 nucleotide from the 5′ end of the coding strand). Hel, UGT and RdRp indicate the positions of viral RNA helicase, UDP-glucosyltransferase and RNA-dependent RNA polymerase domains, respectively.

## Biological properties

Endornaviruses are found in some plants, fungi and oomycetes. Natural infections of endornaviruses have been confirmed in some varieties of cultivated rice (*Oryza sativa*), wild rice (*Oryza rufipogon*), broad bean (*Vicia faba*) and kidney bean (*Phaselous vulgaris*). Other plants that may be infected by species from this family include barley, bottle gourd, Malabar spinach, melon and pepper. Plant endornaviruses are transmitted through seed via both ova and pollen. No horizontal spread has been observed in the field and no potential vectors have been identified. No attempt to transmit the viruses other than through seed has succeeded, although the deduced phylogeny suggests that inter-species transmission has occurred in the past. Plant endornaviruses are not mechanically transmissible. None is associated with disease symptoms except for VfEV, which induces cytoplasmic male sterility.

A large dsRNA in the V670 strain of *Helicobasidium mompa*, the violet root rot fungus, has been identified as a hypovirulence factor, and shown by sequencing to be an endornavirus (*Helicobasidium mompa endornavirus 1*). Similarly, a 13.9 kbp dsRNA isolated from a *Phytophthora* sp. from Douglas fir (*Pseudotsuga s*p.) was identified as a member of the genus (*Phytophthora endornavirus 1*) by sequencing.

## Species demarcation criteria in the genus

At present, species are distinguished on the basis of their host-range and sequence differences. Each recognized endornavirus species was isolated from a different host species. The genomic nucleotide sequences of different endornavirus species are only 30% to 75% identical.

## List of species in the genus *Endornavirus*

| | | |
|---|---|---|
| Infecting plants | | |
| *Oryza sativa endornavirus* | | |
| Oryza sativa endornavirus - Nipponbare | [D32136] | (OsEV-Nip) |
| *Oryza rufipogon endornavirus* | | |
| Oryza rufipogon endornavirus W-1714 | [AB014344] | (OrEV-W-1714) |
| *Vicia faba endornavirus* | | |
| Vicia faba endornavirus - 447 | [AJ000929] | (VfEV-447) |
| *Phaseolus vulgaris endornavirus* | | |
| Phaseolus vulgaris endornavirus - Black Turtle Soup | [AB185246] | (PvEV-BTS) |
| Infecting fungi | | |
| *Helicobasidium mompa endornavirus 1* | | |
| Helicobasidium mompa endornavirus 1-670 | [AB218287] | (HmEV1-670) |
| Infecting oomycetes | | |
| *Phytophthora endornavirus 1* | | |
| Phytophthora endornavirus 1 - Oregon | [AJ877914] | (PEV1-OR) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Endornavirus* but have not been approved as species

| | | |
|---|---|---|
| Infecting fungi | | |
| Gremmeniella abietina type B RNA virus XL | [DQ399289] | (GaBRV-XL) |

## Phylogenetic relationships within the family

Phylogenetic relationships within the family are depicted in Figure 2.

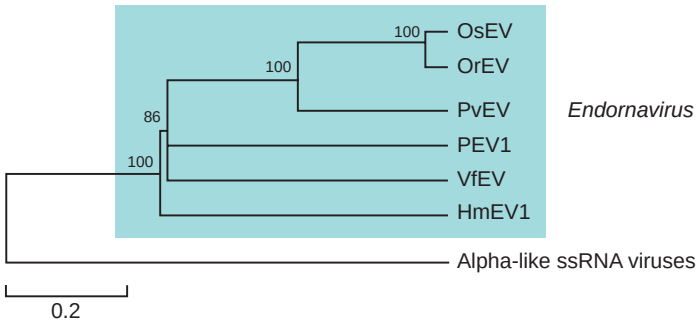


**Figure 2:** Phylogenetic positions of endornaviruses. About 470 aa of the RdRp regions of six endornaviruses and 10 alpha-like ssRNA viruses were analyzed by using the ClustalX and MEGA2 (Molecular Evolutionary Genetics Analysis) programs, and the resulting neighbor-joining (NJ) tree is shown. A bootstrap test was performed with 100 resamplings.

## Similarity with other taxa

Comparisons and analyses of RdRp and helicase sequences indicate that endornaviruses are related to viruses of the "alpha-like" supergroup.

## Derivation of name

*Endorna*: from *endo*, Greek "within", and *RNA*.

## Further reading

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## Contributed by

Fukuhara, T. and Gibbs, M.J.

# FAMILY *PARTITIVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Partitiviridae*** |
| Genus | ***Partitivirus*** |
| Genus | ***Alphacryptovirus*** |
| Genus | ***Betacryptovirus*** |
| Genus | ***Cryspovirus*** |

## Virion properties

### MORPHOLOGY

Virions are isometric, non-enveloped, 30–43 nm in diameter (Figure 1). Each virion contains 120 copies of a single capsid protein (CP) arranged as 60 dimers with T = 1 icosahedral symmetry. Dimeric surface protrusions are frequently observed on viral capsids.

### NUCLEIC ACID

Virions contain two unrelated, linear dsRNA segments (1.4–2.4 kbp in size). The two segments of the individual viruses are usually of similar size. The dsRNA segments are packaged in separate particles.

### PROTEINS

There is a single major CP and a separately translated RdRp. Virion-associated RNA polymerase activity is present.

### LIPIDS

None reported.

### CARBOHYDRATES

None reported.



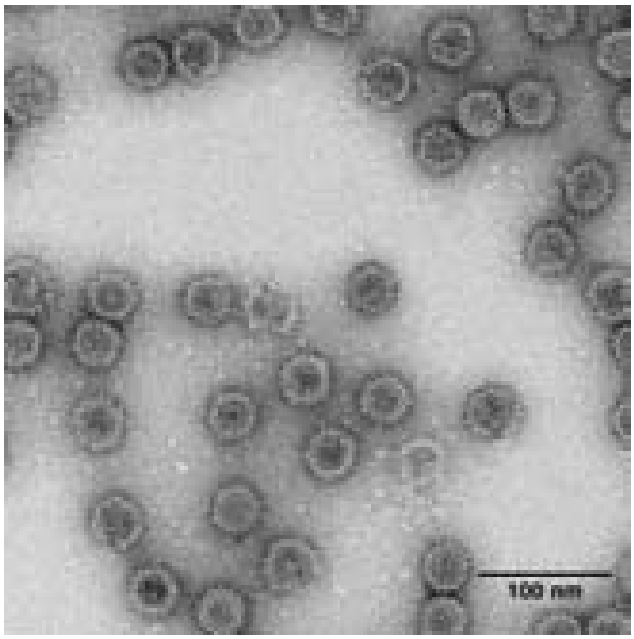


**Figure 1:** Transmission electron micrograph of negatively stained virions of an isolate of *Penicilllium stoloniferum virus S*, a representative species of the genus *Partitivirus*.

### Genome organization and replication

The genome consists of two linear dsRNA segments; the smaller usually codes for the CP and the larger usually codes for the virion-associated RNA polymerase. Each dsRNA is monocistronic. *In vitro* transcription/replication occurs by a semi-conservative mechanism. Virions accumulate in the cytoplasm.

### Antigenic properties

Virions are efficient immunogens. No serological relationships between the fungal viruses and the plant viruses in the family *Partitiviridae* have been detected.

### Biological properties

The viruses are associated with latent infections of their fungal, protozoan and plant hosts. There are no known natural vectors. The fungal partitiviruses are transmitted intracellularly during cell division, hyphal anastomosis and sporogenesis. In some ascomycetes (e.g. *Gaeumannomyces graminis*), virus is usually eliminated during ascospore formation. Experimental transmission of purified fungal partitiviruses has been reported by transfection of virions into fungal protoplasts. The plant cryptoviruses are transmitted by ovule and by pollen to the seed embryo. There is no graft transmission and apparently no cell-to-cell transport, except at cell division; seed transmission is the only known mode for the transmission of alpha- and betacryptoviruses.

## Genus *Partitivirus*

Type species ***Atkinsonella hypoxylon virus***

### Distinguishing features

Members of the genus *Partitivirus* infect only fungi and are transmitted intracellularly during cell fusion, cell division and sporogenesis. All members have two dsRNA segments of similar sizes that are individually encapsidated in separate particles.

### Virion properties

#### Morphology

The structures of Penicillium stoloniferum viruses F and S (PsV-F and PsV-S) have been determined by X-ray crystallography and/or electron cryomicroscopy, respectively (Figure 2). The outer diameter of the capsid is about 35–40 nm. The most prominent features of the capsid are 60 arch-like protrusions that decorate a spherical shell (Figure 2A–C). Each particle contains 120 CP molecules arranged with icosahedral symmetry. The tertiary structure of each CP molecule consists of two distinct domains, one of which forms the continuous, 3-nm thick, capsid, and the other that, with the corresponding domain of a neighboring CP, comprises the arch (Figure 1E–F). The asymmetric unit of the icosahedron consists of two related CP molecules, CPA and CPB. The 60 CPA molecules are organized as flower-like pentamers, each centered about one of the 12 vertices of the icosahedral capsid (Figure 2A). The 60 CPB molecules are arranged as trimers at the twenty icosahedral 3f axes (Figure 2A). The CPA-CPB dimer, defined by two monomers that form an arch, is most stable based on buried surface areas and likely functions as the assembly precursor (Figure 2E–F). The two CP molecules in this dimer assume nearly identical conformations and are related by almost-perfect local 2f symmetry. Each dimer is stabilized by extensive structure swapping between the monomers and the additional intersubunit interactions mediated by the arch tip. Unlike the assembly pathway that has been described for reoviruses and other larger dsRNA viruses, in PsV-F and PsV-S assembly is likely to proceed from dimers of CP dimers, each of which adopts a striking, smooth-edged diamond shape (Figure 2D). The N-terminal region (ca. 40 aa) of the CP polypeptide extends radially inwards and interacts with the dsRNA viral genome. Given the large number of basic residues within this region, it may participate in RNA packaging during particle assembly, and/or play a role during viral transcription. Small pores in the capsid shell at the icosahedral 5f and 3f axes may facilitate the export of RNA transcripts. The dsRNA genome appears as concentric layers in icosahedrally averaged maps.

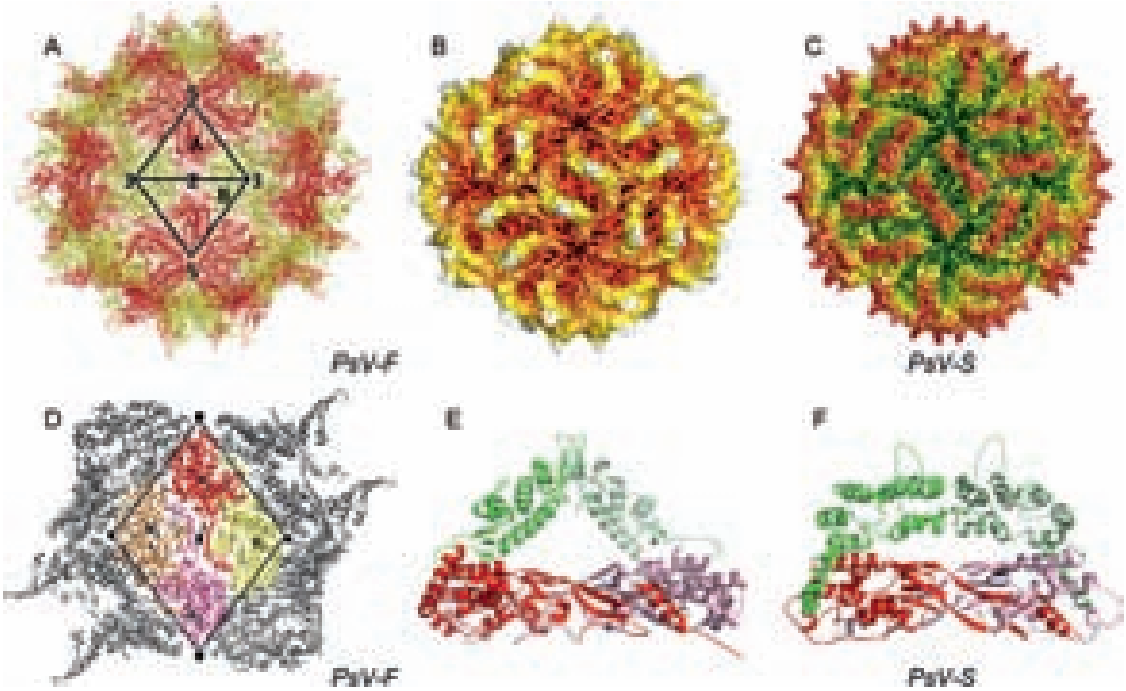


**Figure 2:** Structures of two partitiviruses. (A) Crystal structure of an isolate of *Penicillium stoloniferum virus F*. Two asymmetric units of the icosahedron are highlighted along with the icosahedral symmetry elements that define the boundaries of these units. One CPA monomer (in red) and one CPB monomer (in yellow) from the same CP dimer are labelled. (B-C) Cryo-EM reconstructions of PsV-F and -S, both at ~4.5 Å resolution, and rendered with radial, color mapping. (D) Putative capsid assembly unit consists of a dimer of CP dimers (red–yellow, labelled $A_1$-$B_1$ and pink–orange, labelled $A_2$-$B_2$) that occupy a diamond-shaped area defined by black lines. (E) A PsV-F CP dimer. In this and the next panel (F), the arch and shell domains are highlighted green/light green and red/magenta, respectively, and the two subunits are distinguished as green or light green and red or magenta. (F) A PsV-S CP dimer. The atomic coordinates for this model were generated from a homology model derived from the PsV-F structure and constrained by its fit to the PsV-S cryo-EM reconstruction.



### Physicochemical and physical properties

Virion Mr is estimated to range from 6 to $9 \times 10^6$. $S_{20,w}$ values range from 101 to 145S. Particles lacking nucleic acid have an $S_{20,w}$ of 66–100S. Virion buoyant density in CsCl is 1.29–1.30 and 1.34–1.36 g $cm^{-3}$ for particles without and with nucleic acid, respectively. Components with other density values and sedimentation rates are found in preparations of some viruses and are believed to be replicative intermediates. These consist of particles containing ssRNA and particles with both ssRNA and dsRNA. Virus purification is usually carried out at neutral pH.

### Nucleic

Virions contain two unrelated, linear dsRNA segments, 1.4–2.4 kbp in size, which are separately encapsidated. The dsRNA segments of the individual viruses are of similar size. Additional dsRNA segments (satellite or defective) may be present.

### Proteins

There is a single major CP sized 44–77 kDa. The sizes of the RNA-dependent RNA polymerase, as deduced from nucleotide sequence analysis, range from 60 to 87 kDa. Virion-associated RNA polymerase activity is present.

## Genome organization and replication

Atkinsonella hypxylon virus (AhV), an isolate of the type species of the genus *Partitivirus*, has a bipartite genome consisting of dsRNA1 and dsRNA2 (Figure 3). Each is monocistronic: dsRNA1 codes for the RdRp and dsRNA2 codes for the major CP. The virion-associated RdRp catalyzes *in vitro* end-to-end transcription of each dsRNA to produce mRNA by a semi-conservative mechanism. Virions accumulate in the cytoplasm. A model for the replication strategy of PsV-S is shown in Figure 4.

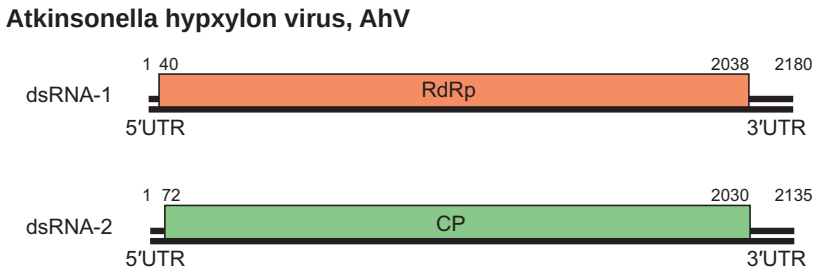


**Figure 3:** Genome organization of Atkinsonella hypxylon virus (AhV). The RdRp ORF (nt positions 40-2038 on dsRNA1) and the CP ORF (nt positions 72-2030 on dsRNA2) are represented by rectangular boxes.

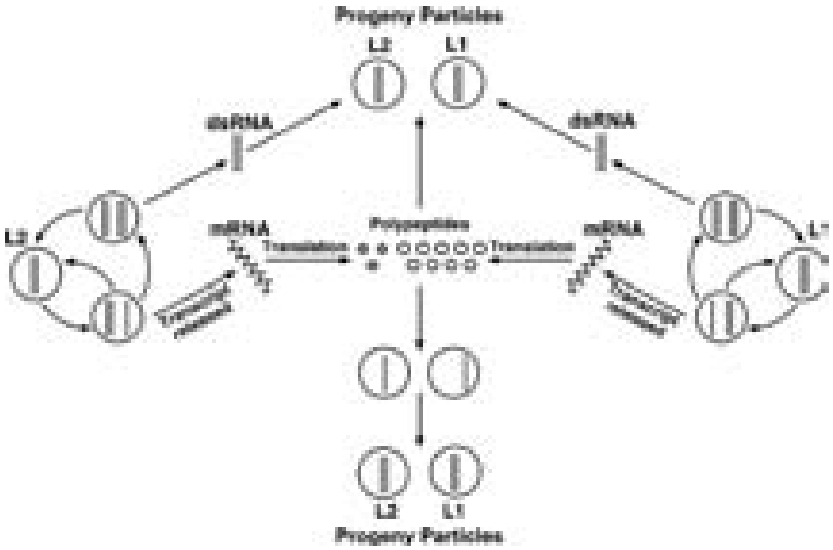


**Figure 4:** Model for replication of Penicillium stoloniferum virus S (PsV-S). The open circles represent CP subunits and the closed circles represent RNA polymerase subunits. Solid lines represent RNA strands whereas wavy lines represent mRNA.

## Species demarcation criteria in the genus

The criteria to differentiate species in the genus are:

- Host species in which the viruses naturally occur; partitiviruses lack natural vectors and they are confined to the fungal host species from which they were first isolated
- Sizes of dsRNA segments and encoded proteins
- Protein sequence similarity. Amino acid sequence similarity >40% between RdRps of viruses from different species in the same phylogenetic cluster and <40% between members of species in different clusters (see Figure 8; phylogenetic tree)

## List of species in the genus *Partitivirus*

| | | |
|---|---|---|
| *Agaricus bisporus virus 4* | | |
| Agaricus bisporus virus 4 | | (AbV-4) |
| *Aspergillus ochraceous virus 1* | | |
| Aspergillus ochraceous virus 1-FA0611 | [EU118277, EU118278] | (AoV1-FA0611) |
| *Atkinsonella hypxylon virus* | | |
| Atkinsonella hypxylon virus – 2H | [L39125, L39126, L39127] | (AhV-2H) |
| *Ceratocystis resinifera virus 1* | | |
| Ceratocystis resinifera virus 1 | [AY603051, AY603052] | (CrV1) |

| | | |
|---|---|---|
| *Discula destructiva virus 1* | | |
| Discula destructiva virus 1 – 247 | [AF316992, AF316993, AF316994, AF316995] | (DdV1-247) |
| *Discula destructiva virus 2* | | |
| Discula destructiva virus 2 – 331 | [AY033436, AY033437] | (DdV2-331) |
| *Fusarium poae virus 1* | | |
| Fusarium poae virus 1 – A11 | [AF047013, AF015924] | (FpV1-A11) |
| *Fusarium solani virus 1* | | |
| Fusarium solani virus 1 | [D55668, D55668] | (FsV1) |
| *Gaeumannomyces graminis virus 019/6-A* | | |
| Gaeumannomyces graminis virus 019/6-A | | (GgV-019/6-A) |
| *Gaeumannomyces graminis virus T1-A* | | |
| Gaeumannomyces graminis virus T1-A | | (GgV-T1-A) |
| *Gremmeniella abietina RNA virus MS1* | | |
| Gremmeniella abietina RNA virus MS1 – C5 | [AY089993, AY089994, AY089995] | (GaRV-MS1-C5) |
| *Helicobasidium mompa virus* | | |
| Helicobasidium mompa virus | [AB025903] | (HmV) |
| *Heterobasidion annosum virus* | | |
| Heterobasidion annosum virus | [AF473549] | (HaV) |
| *Ophiostoma partitivirus 1* | | |
| Ophiostoma partitivirus 1 | [AM087202, AM087203] | (OPV1) |
| *Penicillium stoloniferum virus F* | | |
| Penicillium stoloniferum virus F | [AY738336, AY738337] | (PsV-F) |
| *Penicillium stoloniferum virus S* | | |
| Penicillium stoloniferum virus S | [AY156521, AY156522] | (PsV-S) |
| *Pleurotus ostreatus virus 1* | | |
| Pleurotus ostreatus virus 1 – South Korea | [AY533036, AY533038] | (PoV1-SKor) |
| *Rhizoctonia solani virus 717* | | |
| Rhizoctonia solani virus 717 | [AF133290, AF133291] | (RhsV-717) |
| *Rosellinia necatrix virus 1* | | |
| Rosellinia necatrix virus 1 – W8 | [AB113347, AB113348] | (RnV1-W8) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.



### List of other related viruses which may be members of the genus *Partitivirus* but have not been approved as species

| | | |
|---|---|---|
| Botryotinia fuckeliana partitivirus 1 | [AM491609, AM491610, AM491611] | (BfPV1) |
| Ceratocystis polonica partitivirus 1 | [AY247204, AY247205] | (CpPV1) |
| Helicobasidium mompa partitivirus V1-1 | [AB110979] | (HmV-V1-1) |
| Helicobasidium mompa partitivirus V1-2 | [AB110980] | (HmV-V1-2) |

## GENUS *ALPHACRYPTOVIRUS*

Type species ***White clover cryptic virus 1***

### Distinguishing features

Members of the genus *Alphacryptovirus* infect plants and are transmitted from cell to cell during cell division. All members have two dsRNA segments that are believed to be individually encapsidated in separate particles.

### Virion properties

#### MORPHOLOGY

Virions are isometric, 30 nm in diameter. Particles lack fine structural detail, appearing rounded, usually penetrated by stain to give a ring-like appearance.

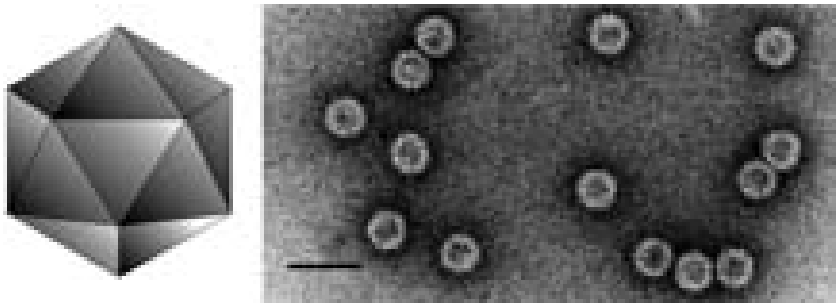

**Figure 5:** (Left) Diagrammatic representation of an alphacryptovirus capsid. (Right) Negative contrast electron micrograph of particles of an isolate of White clover cryptic virus 1, the type species of the genus *Alphacryptovirus*. The bar represents 50 nm. (From Ghabrial *et al.*, Family *Partitiviridae*. In: M.H.V. van Regenmortel *et al.* (Eds.), *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses*, Academic Press, San Diego, CA (2000), pp. 503-513; reproduced with permission).

### Physicochemical and physical properties

Density in CsCl is 1.392 g cm$^{-3}$.

### Nucleic acid

The virions typically contain two dsRNA segments of about 1.5 and 2.0 kbp in size. The dsRNA segments are thought to be separately packaged.

## Genome organization and replication

The genome typically consists of two dsRNA segments, whereas the larger one encodes the RdRp of about 73 kDa and the smaller one the CP of about 54 kDa. Conserved nucleotide sequence motifs of about seven to 12 nucleotides are located in the 5′-non translated region of dsRNA1 and dsRNA2 of beet cryptic virus 1 (BCV-1), Vicia cryptic virus (VCV), and white clover cryptic virus 1 (WCCV-1) as well as carrot cryptic virus (CaCV). In addition, these viruses contain conserved sequence motifs of 20 nucleotides in the 3′-non-translated region of dsRNA1 and 10 nucleotides in the 3′-non-translated region of dsRNA2, respectively. Both dsRNAs of the above mentioned viruses carry poly(A) stretches at their 3′ end, which may be interrupted by other nucleotides.

## Antigenic properties

Some viruses in the genus are serologically related; none is related to viruses in the genus *Betacryptovirus*. There are no known serological relationships with fungal viruses in the genus *Partitivirus*.

## Species demarcation criteria in the genus

The criteria to differentiate species in the genus are:

- Host range
- Size of dsRNA segments
- Serological relationships

Species in the genus are not serologically related (serological differentiation index of 5 or greater). Electrophoretic profiles of the genomic RNAs are distinct.

## List of species in the genus *Alphacryptovirus*

| | | |
|---|---|---|
| *Alfalfa cryptic virus 1* | | |
| Alfalfa cryptic virus 1 | | (ACV-1) |
| *Beet cryptic virus 1* | | |
| Beet cryptic virus 1- Hungary | [EU489061, EU489062] | (BCV-1-Hun) |
| *Beet cryptic virus 2* | | |
| Beet cryptic virus 2 | | (BCV2) |

| | | |
|---|---|---|
| *Beet cryptic virus 3* | | |
| Beet cryptic virus 3 | [S63913] | (BCV3) |
| *Carnation cryptic virus 1* | | |
| Carnation cryptic virus 1 | | (CCV1) |
| *Carrot temperate virus 1* | | |
| Carrot temperate virus 1 | | (CteV1) |
| *Carrot temperate virus 3* | | |
| Carrot temperate virus 3 | | (CteV3) |
| *Carrot temperate virus 4* | | |
| Carrot temperate virus 4 | | (CteV4) |
| *Hop trefoil cryptic virus 1* | | |
| Hop trefoil cryptic virus 1 | | (HTCV1) |
| *Hop trefoil cryptic virus 3* | | |
| Hop trefoil cryptic virus 3 | | (HTCV3) |
| *Radish yellow edge virus* | | |
| Radish yellow edge virus | | (RYEV) |
| *Ryegrass cryptic virus* | | |
| Ryegrass cryptic virus | | (RGCV) |
| *Spinach temperate virus* | | |
| Spinach temperate virus | | (SpTV) |
| *Vicia cryptic virus* | | |
| Vicia cryptic virus - Germany | [AY751737, AY75138] | (VCV-DE) |
| *White clover cryptic virus 1* | | |
| White clover cryptic virus 1 | [AY705784, AY705785] | (WCCV1) |
| *White clover cryptic virus 3* | | |
| White clover cryptic virus 3 | | (WCCV3) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.



### List of other related viruses which may be members of the genus *Alphacryptovirus* but have not been approved as species

| | | |
|---|---|---|
| Black raspberry cryptic virus | [EU082132] | (BrCV) |
| Carrot cryptic virus | [FJ550604, FJ550605] | (CaCV) |
| Cucumber cryptic virus | | (CuCV) |
| Fragaria chiloensis cryptic virus | [DQ093961, DQ355440, DQ355439] | (FCCV) |
| Pepper cryptic virus 1 | [DQ361008] | (PCV1) |
| Pinus sylvestris cryptovirus | [AY973825] | (PSCV) |
| Pyrus pyrifolia cryptic virus | [AB012616] | (PpV) |
| Raphanus sativus cryptic virus 2 | [DQ218036, DQ218037, DQ218038] | (RsCV2) |
| Raphanus sativus cryptic virus 3 | [FJ461349, FJ461350] | (RsCV3) |
| Rose cryptic virus 1 (Rosa multiflora cryptic virus) | [EU413666, EU413667, EU413668] | (RoCV1) |

## GENUS *BETACRYPTOVIRUS*

Type species ***White clover cryptic virus 2***

### Distinguishing features

Members of the genus *Betacryptovirus* infect plants and are transmitted from cell to cell during cell division. All members have two dsRNA segments that are believed to be individually encapsidated in separate particles.

### Virion properties

#### MORPHOLOGY

Virions are isometric, 38 nm in diameter. Particles show prominent subunits, but their precise geometrical arrangement is not clear. The particles are rounded and are not penetrated by stain.

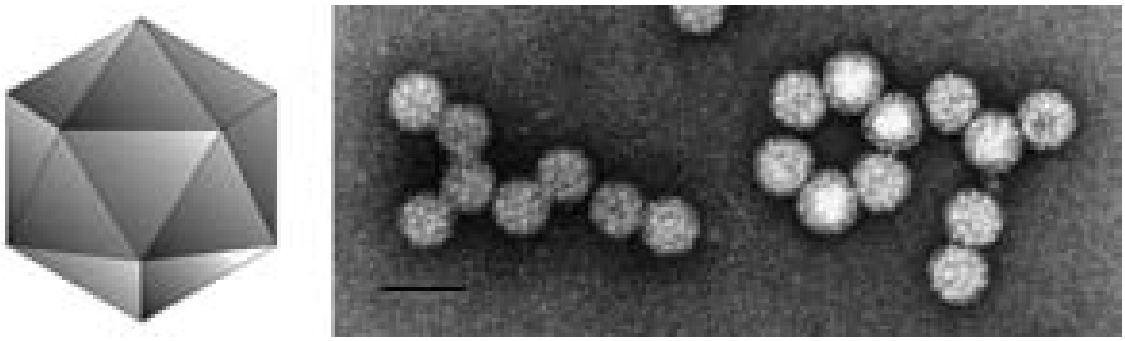

**Figure 6:** (Left) Diagrammatic representation of a betacryptovirus capsid. (Right) Negative contrast electron micrograph of particles of an isolate of White clover cryptic virus 2, the type species of the genus *Betacryptovirus*. The bar represents 50 nm. (From Ghabrial *et al.*, Family *Partitiviridae*. In: M.H.V. van Regenmortel *et al.* (Eds.), *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses*, Academic Press, San Diego, CA (2000), pp. 503-513; reproduced with permission).

### Physicochemical and physical properties

Virion buoyant density in CsCl is 1.375 g cm$^{-3}$.

### Nucleic acid

Viral nucleic acid comprises two dsRNA segments, which are about 2.1 and 2.25 kbp.

## Genome organization and replication

The genome typically consists of two dsRNA segments, whereas the larger one encodes the RdRp and the smaller one the putative CP.

## Antigenic properties

Some viruses in the genus are serologically related; none is related to viruses in the genus *Alphacryptovirus*.

## Species demarcation criteria in the genus

The criteria to differentiate species in the genus are:

- Host range
- Size of dsRNA segments
- Serological relationships

Species in the genus are not serologically related or are distantly related (serological differentiation index of 5 or greater). Electrophoretic profiles of the genomic RNAs are distinct.

## List of species in the genus *Betacryptovirus*

| | |
|---|---|
| *Carrot temperate virus 2* | |
| Carrot temperate virus 2 | (CTeV2) |
| *Hop trefoil cryptic virus 2* | |
| Hop trefoil cryptic virus 2 | (HTCV2) |
| *Red clover cryptic virus 2* | |
| Red clover cryptic virus 2 | (RCCV2) |
| *White clover cryptic virus 2* | |
| White clover cryptic virus 2 | (WCCV2) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

**List of other related viruses which may be members of the genus *Betacryptovirus* but have not been approved as species**

| | |
|---|---|
| Alfalfa cryptic virus 2 | (ACV2) |

# GENUS *CRYSPOVIRUS*

Type species ***Cryptosporidium parvum virus 1***

## Distinguishing features

Members of the genus *Cryspovirus* infect apicomplexan protozoa of the genus *Cryptosporidium* and are largely transmitted by intracellular means during cell division and gamete fusion. Their genomes comprise two dsRNA segments of similar sizes that are individually encapsidated in separate particles.

## Virion properties

### MORPHOLOGY

Virions are isometric and nonenveloped, about 31 nm in diameter as visualized by negative staining and transmission electron microscopy (Figure 7). The capsids appear single layered and thin, with short protrusions on their surfaces.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virions have a buoyant density of 1.39–1.44 g cm$^{-3}$ on CsCl gradients.

### NUCLEIC ACID

Virions contain two unrelated, linear dsRNA segments, 1.4 and 1.7 kbp in size, which are separately encapsidated. Additional dsRNA segments (satellite or defective) have not been reported.



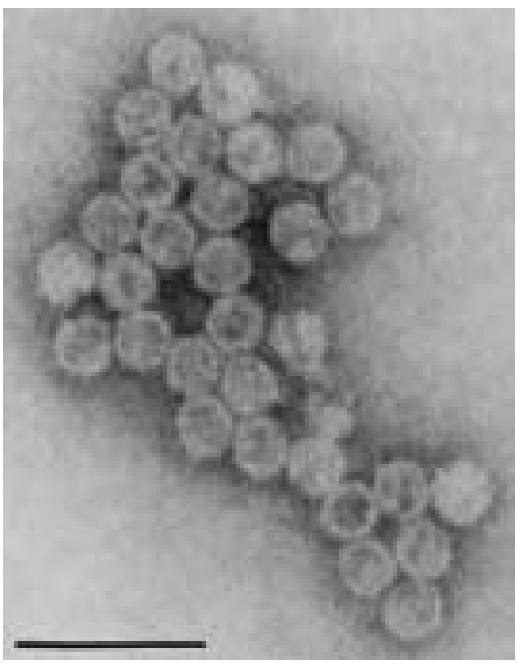

**Figure 7:** Electron micrograph of Cryptosporidium parvum virus 1 particles. Particles were gradient-purified from *C. parvum* strain KSU-1, negatively stained with uranyl acetate and visualized by transmission electron microscopy. Bar represents 100 nm. (Image is reproduced from Khramtsov and Upton (2000) with permission of the American Society for Microbiology.)

### Proteins

There is a single major CP sized 37 kDa and an RNA-dependent RNA polymerase sized 62 kDa. Virion-associated RNA polymerase activity is present.

## Genome organization and replication

Each genome segment is monocistronic: dsRNA1 codes for the RdRp and dsRNA2 codes for the major CP. The virion-associated RdRp catalyzes *in vitro* end-to-end transcription of each dsRNA to produce mRNA by a semi-conservative mechanism.

## Antigenic properties

Detection of the major CP by immunologic assays such as dot blotting has been reported as a means for identifying cryspovirus-positive *Cryptosporidium* isolates.

## Biological properties

Infections of the *Cryptosporidium* host cells appear to be persistent and largely avirulent. Although *Cryptosporidium* species are pathogens of humans and other vertebrates, there are so far no well-established examples in which parasite pathogenicity is either positively or negatively modulated by cryspovirus infection. Virions are disseminated in nature within *Cryptosporidium* oocysts, which are shed profusely from *Cryptosporidium*-infected animals. A correlation between cryspovirus genome levels and parasite fecundity in terms of oocyst excretion has been reported.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Cryspovirus*

*Cryptosporidium parvum virus 1*

| | | |
|---|---|---|
| Cryptosporidium parvum virus 1 – KSU-1 | [U95995, U95995] | (CSpV1-KSU1) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Cryspovirus* but have not been approved as species

| | | |
|---|---|---|
| Cryptosporidium felis virus | [DQ193520] | (CSfV) |
| Cryptosporidium hominis virus | [DQ193518] | (CShV) |
| Cryptosporidium meleagridis virus | [DQ193519] | (CSmV) |

## List of related viruses which may be members of the family *Partitiviridae* but have not been approved as species

| | | |
|---|---|---|
| Pyrus pyrifolia virus | [AB012616] | (PPV) |

## Phylogenetic relationships within the family

Phylogenetic analysis based on amino acid sequences of RdRps of members of the family *Partitiviridae* led to the identification of four clusters, two of which are large and comprise mostly members of the genus *Partitivirus* (Figure 8). One large cluster with strong bootstrap support includes an isolate of the type species AhV and related viruses (partitivirus I). The second cluster (partitivirus II) includes the well-characterized partitiviruses PsV-S and PsV-F. Members of cluster I have CPs and RdRps sized 70–77 and 77–87 kDa, respectively, whereas members of the partitivirus cluster II have CPs and RdRps sized 44–50 and 60–63 kDa, respectively. The genome segments of the

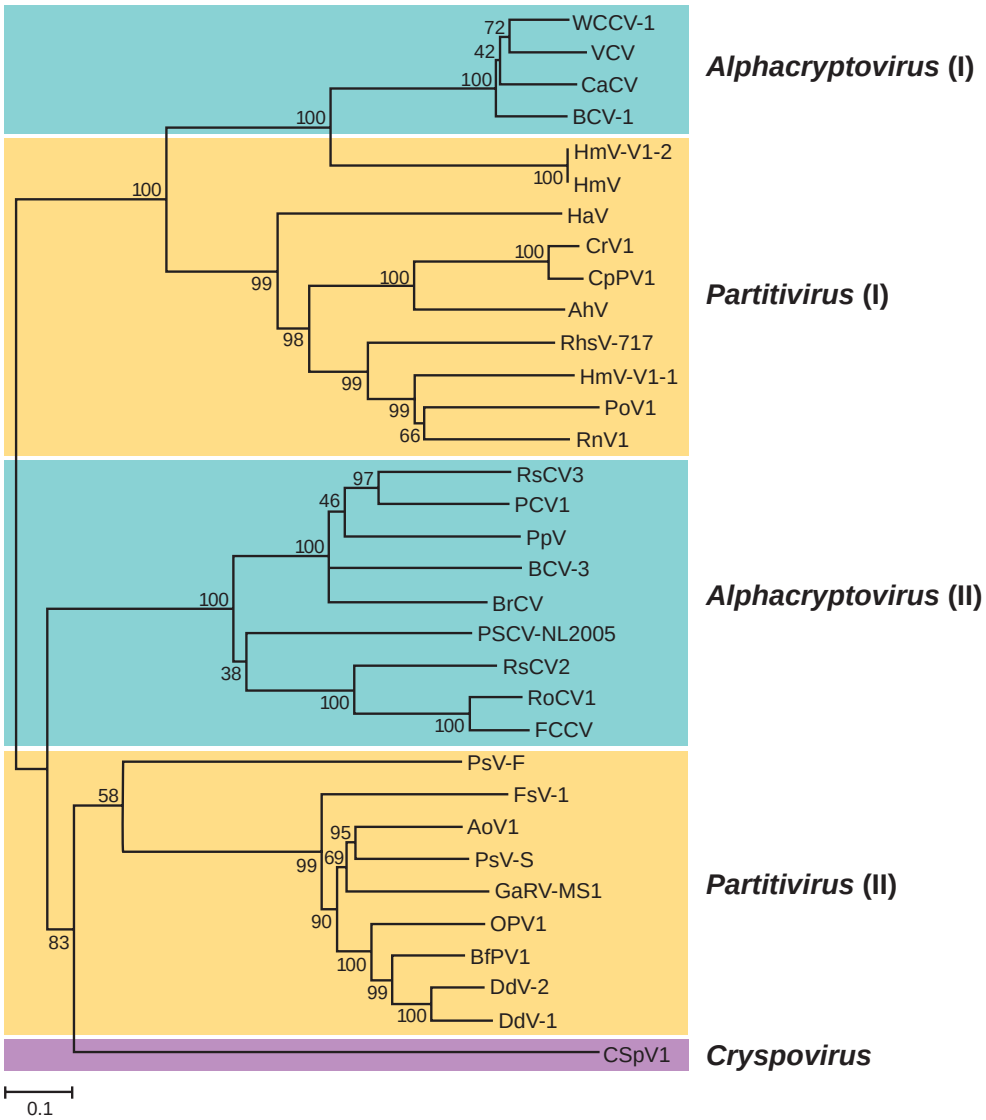


**Figure 8:** Neighbor-joining phylogenetic tree constructed based on the complete amino acid sequences of RdRps of members and probable members of the family *Partitiviridae*. The amino acid sequences were aligned using the program CLUSTAL W. For virus names and abbreviations, see lists in text. The phylogenetic tree was generated including codon positions using the MEGA4 phylogenetic package. Bootstrap percentages out of 1000 replicates are indicated at the nodes.

two clusters are also differently sized, consistent with their different encoded protein sizes. Thus the genus *Partitivirus* can justifiably be divided into two new genera to reflect these two clusters. CSpV1, the only member of the genus *Cryspovirus*, is more closely related to viruses grouped into the partitivirus cluster II than to any other members of the family *Partitiviridae*. The remaining two RdRp clusters (Figure 8) comprise members of the genus *Alphacryptovirus* and probable alphacryptoviruses (see lists in the text above). One small cluster (alphacryptovirus I) consists of WCCV-1 (an isolate of the type species of the genus *Alphacryptovirus*) and two other approved alphacryptoviruses (BCV-1 and VCV) and a probable alphacryptovirus (CaCV). Interestingly, this small cluster of plant partitiviruses (cryptoviruses) is more closely related to the fungal partitiviruses in cluster partitivirus I than to the second cluster of alphacryptoviruses and probable alphacryptoviruses (alphacryptovirus II). The genus *Alphacryptovirus* can also thus justifiably be divided into two new genera to reflect these two clusters. No sequences have yet been reported for members of the genus *Betacryptovirus*, exempting them from this current analysis.

## Similarity with other taxa

Members of the family *Partitiviridae* have properties similar to members of the family *Picobirnaviridae*, e.g., the genomes are bisegmented, and the capsids are small (<45 nm in diameter) with 120-subunit T = 1 symmetry and share similar structural details. The picobirnaviruses, however, are phylogenetically distinct and infect vertebrates rather than plant, fungi and protozoa; they probably have other basic differences including co-packaging of both genome segments and capacity for extracellular transmission.

## Derivation of names

*Crypto*: from Greek *crypto*, "hidden, covered, or secret".
*Cryspo*: from the host genus name, *Cryptosporidium*.
*Partiti*: from Latin *partitius*, "divided".

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## Contributed by

Ghabrial, S.A., Nibert, M.L., Maiss, E., Lesker, T., Baker, T.S. and Tao, Y.J.

# Family *Picobirnaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Picobirnaviridae*** |
| Genus | ***Picobirnavirus*** |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Picobirnavirus*

Type species ***Human picobirnavirus***

## Virion properties

### Morphology

Virions are isometric, non-enveloped, 33–37 nm in diameter. Virus particles have a spherical triacontahedral (30-sided) organization (Figure 1), with a unique capsid layer surrounding the genomic dsRNA segments. Owing to the absence of a cell culture system for propagating picobirnaviruses, knowledge of virion protein composition comes from virion-like particles produced by recombinant expression of the capsid precursor gene (ORF 3) of segment 1 of rabbit picobirnavirus (PBV). The particle is made of 60 symmetric CP dimers. The structure reveals an intricate interface with the N-terminal residues exchanged between the two subunits of the dimer. Two domains can be recognized in CP, namely a shell domain and a projection domain that forms the protrusions that stand out in the three-dimensional reconstruction (Figure 1, left).

### Physicochemical and physical properties

Virion buoyant density in CsCl is in the range of 1.38–1.4 g cm$^{-3}$.

### Nucleic acid

Virions contain two unrelated, linear dsRNA segments (named 1 and 2). The larger segment 1 is about 2.4–2.6 kbp long and possesses three ORFs. The smaller segment 2 is 1.5–1.9 kbp long and is monocistronic. It encodes the viral RNA-dependent RNA polymerase (RdRp).

### Lipids

Not present.

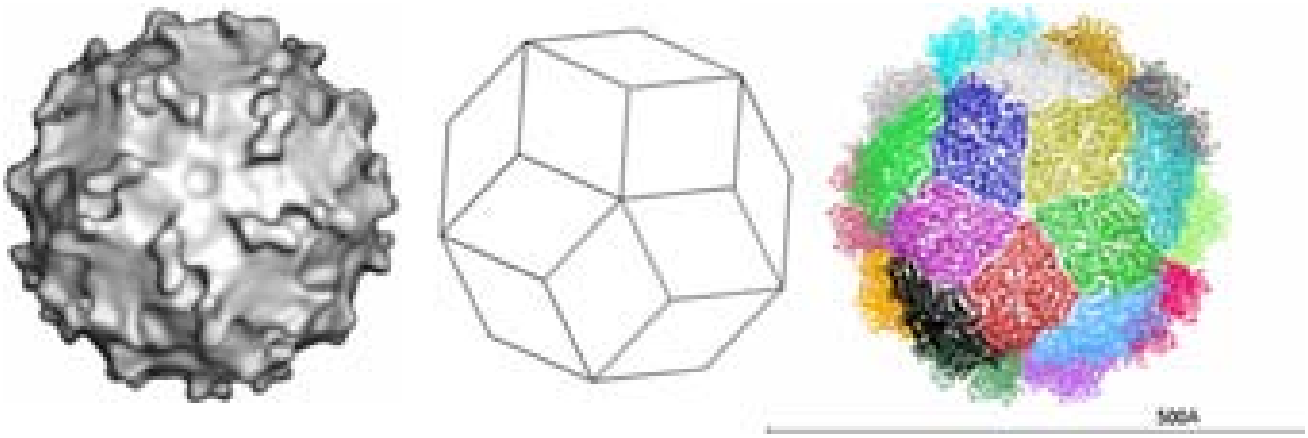

**Figure 1:** Structure of the PBV particle. (Left) Surface rendering from a three-dimensional reconstruction viewed down a slightly misoriented five-fold axis. Note the presence of 60 dimeric protrusions. (Middle) Diagram representing a triacontahedron, a convex polyhedron made of 30 rhombic faces or diamond tiles. (Right) Triacontahedral design of the particle, with each of the tiles formed by two CP dimers colored differently (Courtesy of J. Lepault and S. Duquerroy for the left and right panels, respectively.)

dsRNA

## Genome organization and replication

Only two complete sequences of genome segment 1, from human and rabbit PBVs, are available in sequence databases. The genome organization of segment 1 of human PBV is illustrated in Figure 2. Segment 1 possesses two large ORFs of 224 (ORF2) and 590 (ORF3) codons preceded by a short one (ORF1) of 39 codons. The three ORFs overlap at eight (ORF1–ORF2 junction) and one (ORF2–ORF3 junction) nucleotides (nt), in each case excluding the stop codon of the preceding ORF. While the functionality of ORF1 is unclear and ORF2 encodes a protein with an unknown function, more information is available for ORF3. This encodes the precursor of CP. For rabbit PBV, ORF3 expression results in the synthesis of a precursor that is autocatalytically cleaved to generate a large peptide and mature CP (Figure 3). Cleavage occurs between amino acid residues 65 and 66. Segment 2 encodes a RdRp, whose core component contains the canonical A–B–C motif arrangement of the palm subdomain present in conventional nucleic acid polymerases.

## Antigenic properties

No data are available.

## Biological properties

### Natural host range

PBVs are widely distributed geographically among humans and mammals in general, and have also been reported in birds and reptiles. PBVs have been identified from fecal specimens owing to

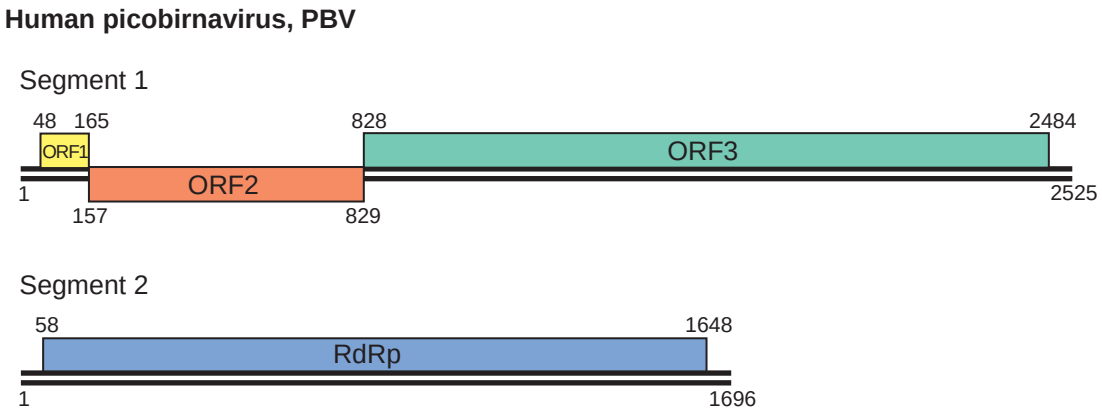


**Figure 2:** Schematic representation of the gene arrangement in genome segments 1 and 2 of human picobirnavirus. Segment 1 possesses three ORFs. ORF3 encodes the CP precursor. Segment 2 encodes an RNA-dependent RNA polymerase (RdRp). Numbers indicate the positions and lengths of the ORFs.

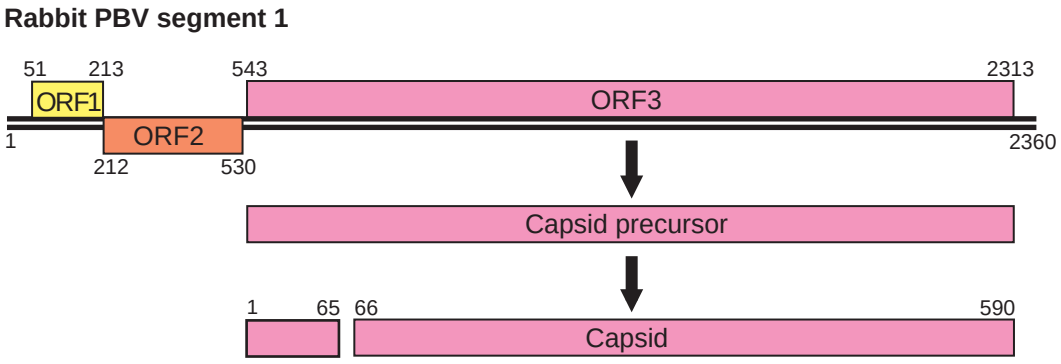


**Figure 3:** Schematic representation of the gene arrangement in genome segment 1 of rabbit PBV and processing of the CP precursor. Numbers indicate the nucleotide the amino acid positions (the latter in bold type).

large amounts of virus occasionally shed through feces. The viruses have also been detected in raw sewage samples.

### Pathology

The pathogenicity of PBVs has not been established. Studies conducted with immunocompromised persons suggest that PBVs are opportunistic pathogens that may cause diarrhea. PBVs have been detected in stool samples from children with diarrhoea as well as in immunocompromised patients, and they have also been detected in individuals lacking symptoms of gastroenteritis.

### Transmission

Genome sequencing of PBVs originating from different species suggests effective animal-to-animal transmission. The data do not provide evidence of virus clusters specific to a host species.

## Species demarcation criteria in the genus

Only two sequences of capsid protein are yet available in databases. The capsid protein of rabbit picobirnavirus displays 25% amino acid sequence identity with its human counterpart, showing that they are phylogenetically distant. The PBVs have been divided into two genogroups defined on the basis of nucleotide sequence similarities in the genomic segment that encodes the RdRp (segment 2). The reference viruses for genogroups I and II are HY005102 and 1-CHN-97, respectively. Genogroup II PBVs are identified on a less frequent basis than genogroup I viruses. Segment 2 of new human (VS10) and bovine (bovine/RUBV-P/IND/2005) isolates showed 55–65% identity at the amino acid sequence level to human prototype strains HY005102 and 1-CHN-97. These observations suggest that PBVs exhibit a high level of genetic diversity that is not reflected by the two prototype viruses. Viruses are distinguished on the basis of their host, the country in which they were identified and the year of isolation.

## List of species in the genus *Picobirnavirus*

| | | |
|---|---|---|
| *Human picobirnavirus* | | |
| Human picobirnavirus - Hy005102 | [RNA 1: AB186897, RNA 2: AB186898] | (human PBV-Hy005102) |
| *Rabbit picobirnavirus* | | |
| Rabbit picobirnavirus - R5-9 | [RNA 1: AJ244022] | (rabbit PBV-R5-9) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Picobirnavirus* but have not been approved as species

| | |
|---|---|
| Bovine picobirnavirus | [RNA 2: GQ221268] |
| Chicken picobirnavirus | |
| Dog picobirnavirus | [RNA 2: FJ164032] |
| Foal picobirnavirus | |
| Giant anteater picobirnavirus | |
| Guinea pig picobirnavirus | |
| Hamster picobirnavirus | |
| Porcine picobirnavirus | [RNA 2: EU104358] |
| Rat picobirnavirus | [RNA 2: EU814972] |
| Snake picobirnavirus | [RNA 2: EU814971] |

## Phylogenetic relationships within the family

Phylogenetic relationships within the family are shown in Figure 4.

## Similarity with other taxa

The crystal structure of a partitivirus, Penicillium stoloniferum virus F (PsV-F, also a dsRNA virus) also features 60 symmetric dimers, with an overall organization similar to that of the PBV particle.

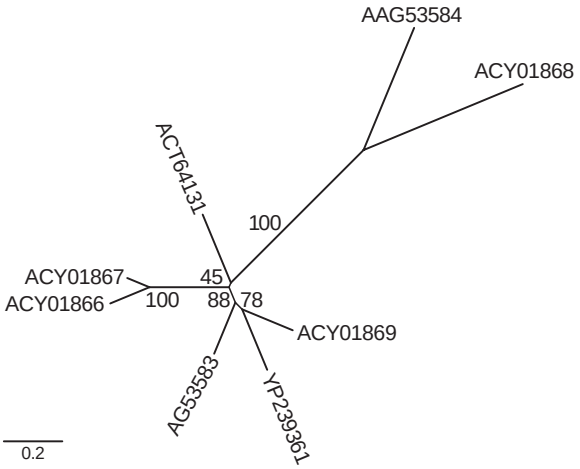


**Figure 4:** A distance tree representing the phylogenetic relationships among the RNA-dependent RNA polymerases (RdRps) from genetic clusters in the family. The tree was constructed using the software Mega4 and the NJ algorithm, with the option complete deletion, which ensures that only the positions represented in all sequences of the ClustalW multiple alignment were used for the tree calculation. Numbers indicate bootstrap percentages obtained for each node. Sequences are represented by their protein accession numbers. All derive from human picobirnavirus isolates except ACT64131, which corresponds to a bovine isolate. Two genogroups have been defined thus far on the basis of RdRp sequences. The prototype strains are HY005102 (YP_239361) and 1-CHN-97 (AAG53583) for genotype 1 and 4-GA-91 (AAG53584) for genotype 2.

The PsV-F capsid appears slightly smaller than its PBV counterparts. Also, instead of the projections observed in PBV, it exhibits an "arch" that protrudes at the center of each dimer. In contrast to what is observed in PBVs, no maturation cleavage occurs during partitivirus particle formation.

There is no similarity between PBVs and birnaviruses, as assessed from differences in their genome organizations, capsid structures and encoded proteins.

## Derivation of name

*Picobirna*: from Greek *pico*, "small"; Latin prefix *bi*, "two", signifies the bisegmented nature of the viral genome as well as the presence of dsRNA; and *rna*, abbreviation of *r*ibo*n*ucleic *a*cid, indicating the nature of the genome.

## Further reading

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**Websites**
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WIKIMEDIA: http://species.wikimedia.org/wiki/Picobirnaviridae

## Contributed by

Delmas, B.

# Family *Reoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Reoviridae*** |
| Subfamily | ***Spinareovirinae*** |
| Genus | ***Orthoreovirus*** |
| Genus | ***Aquareovirus*** |
| Genus | ***Oryzavirus*** |
| Genus | ***Fijivirus*** |
| Genus | ***Mycoreovirus*** |
| Genus | ***Cypovirus*** |
| Genus | ***Idnoreovirus*** |
| Genus | ***Dinovernavirus*** |
| Genus | ***Coltivirus*** |
| Subfamily | ***Sedoreovirinae*** |
| Genus | ***Orbivirus*** |
| Genus | ***Rotavirus*** |
| Genus | ***Seadornavirus*** |
| Genus | ***Phytoreovirus*** |
| Genus | ***Cardoreovirus*** |
| Genus | ***Mimoreovirus*** |

## Virion properties

### Morphology

Virus particles of members of the family *Reoviridae* (collectively called reoviruses) have icosahedral symmetry but may appear spherical in shape. The protein capsid is organized as one, two or three concentric layers of capsid proteins, which surround the linear dsRNA segments of the viral genome, with an overall diameter of 60–80 nm (Figure 1).

The 15 genera of reoviruses are divided between two subfamilies. The subfamily *Spinareovirinae* contains viruses that have relatively large spikes or turrets situated at the 12 icosahedral vertices of either the virus or core particle. The subfamily *Sedoreovirinae* includes viruses that do not have large surface projections on their virions or core particles, giving them an almost spherical or "smooth" appearance.

The terminology that has been used to describe reovirus particles with different numbers of capsid layers varies among the genera. The current nomenclature will therefore be explained in each case. The transcriptionally active core particle of the spiked viruses (subfamily *Spinareovirinae*) appears to contain only a single complete capsid layer (which has been interpreted as having T = 1 or T = 2 symmetry), to which the projecting spikes or turrets are attached. In most cases, the core is surrounded (in the complete virion) by an incomplete protein layer (with T = 13 symmetry) that forms the outer capsid, which is penetrated by the projections on the core surface. These virus particles are therefore usually regarded as double-shelled.

One exception is the cypoviruses, which have transcriptionally active but fully intact virions with only a single capsid shell that are equivalent to the core particles of viruses from other genera. However, virus particles of most cypoviruses are characteristically occluded (either singly or multiply) within the matrix of proteinaceous crystals called polyhedra. These are composed primarily (>90%) of the viral polyhedrin protein.

In contrast, virions of the non-spiked viruses (subfamily *Sedoreovirinae*) have an inner protein layer, which may be relatively fragile, having structural similarities to the innermost shell of the spiked viruses (interpreted as having T = 2 symmetry). However, in transcriptionally active core particles, the subcore is surrounded and reinforced by a complete core-surface layer, which has T = 13 symmetry. These double-layered cores have no surface spikes and (in intact virions) are surrounded by

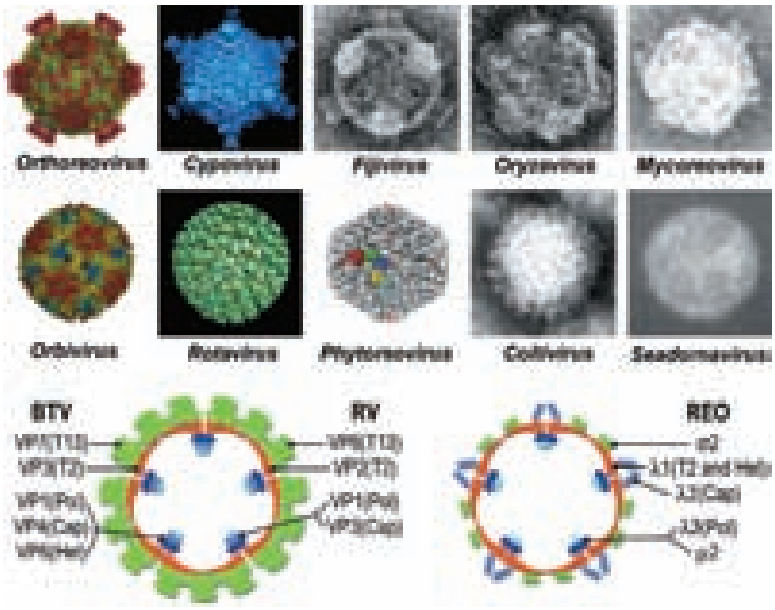


**Figure 1:** (Top and center) A comparison of two distinct core particle morphologies (spiked and unspiked) present amongst members of different genera of the family *Reoviridae*. *Orbivirus*: a 3D model from x-ray crystallography of the core particle of an isolate of bluetongue-1 virus. *Orthoreovirus:* a 3D model from x-ray crystallography studies of a core particle of an isolate of mammalian orthoreovirus 3. *Cypovirus*: a 3D cryoEM reconstruction of a particle of an isolate of Cypovirus 5, at 25 Å resolution. *Rotavirus*: a 3D cryoEM reconstruction of a double shelled particle of an isolate of rotavirus A (SiRV-A/SA11), at 25 Å resolution. *Fijivirus*: an electron micrograph of a core particle of an isolate of maize rough dwarf virus. *Phytoreovirus*: a 3D cryoEM reconstruction of the double shelled particle of an isolate of rice dwarf virus, at 25 Å resolution (highlighted in colour are a contiguous "group of 5 trimers" found in each asymmetric unit). *Coltivirus*: an electron micrograph of a negatively stained double shelled particle of an isolate of Colorado tick fever virus. *Oryzavirus*: an electron micrograph of a negatively stained core particle of an isolate of rice ragged stunt virus. *Mycoreovirus*: an electron micrograph of a negatively stained core particle of mycoreovirus 1 (Rosallinia necatrix mycoreovirus-1). *Seadornavirus*: an electron micrograph of a negatively stained core particle of an isolate of Banna virus. The reconstructions and electron micrographs are not shown to exactly the same scale. The outer capsid morphologies of members of the different genera of the family *Reoviridae* are more variable and may appear smooth, or with surface projects, or may even be absent. (Bottom) A diagrammatic representation of the core particles (on the left) of an orbivirus (BTV), or rotavirus (RV), which have a well defined capsomeric structure but lack large surface projections at the five-fold icosahedral axes, as compared to the turreted (spiked) core particle (on the right) of an orthoreovirus (Reo). (Courtesy of J. Diprose.)

a further outer capsid shell, giving rise to three-layered virus particles that are equivalent to the two-layered particles of members of the subfamily *Spinareovirinae*.

The innermost protein layer of reovirus particles has an internal diameter of approximately 50–60 nm and surrounds the 9, 10, 11 or 12 linear dsRNA genome segments. In the smooth-cored genera, the enzymatically active minor proteins of the virion are attached to the inner surface of the central space at the five-fold axes of symmetry. These include the RNA-dependent RNA polymerase (RdRp, which functions as a transcriptase and replicase), NTPase, helicase, and capping and transmethylase enzymes. However, in the spiked genera, some of these enzymatic proteins form turrets on the surface of the core. These hollow projections appear to act as conduits for the exit of nascent mRNA synthesized by core-associated enzymes.

Particles of some genera can leave infected cells by budding (e.g. genera *Orbivirus* and *Seadornavirus*) or can bud into the endoplasmic reticulum during morphogenesis (genus *Rotavirus*), acquiring an envelope derived from cellular membranes. However, in most cases, the envelope appears to be transient and is not usually considered to be part of the intact virion. In some genera, the protein components of the outer capsid shell can be modified by proteases (such as trypsin or chymotrypsin) to form infectious or intermediate subviral particles (ISVPs). ISVP formation may

occur intracellularly (within endocytic vesicles, which represent an entry route for virus particles taken in from the cell surface), extracellularly (e.g. in the intestinal lumen following ingestion or peroral inoculation, or in the host's blood stream), or *in vitro*, following treatment with proteases (including those present in the saliva of insect vector species). The virion-to-ISVP transition can significantly increase, and may even be essential for, infectivity of these viruses.

### Physicochemical and physical properties

The virion Mr is about $12 \times 10^7$. The buoyant density in CsCl is 1.36–1.39 g cm$^{-3}$. Virus infectivity is moderately resistant to heat, organic solvents (e.g. ether) and non-ionic detergents (depending on both virus strain and detergent). The pH stability of virions varies among the genera.

### Nucleic acid

Reovirus particles can contain 9, 10, 11 or 12 segments of linear dsRNA, depending on the genus. The individual Mr of these RNA molecules ranges from 0.2 to $3.0 \times 10^6$. The total Mr of the genome is $12–20 \times 10^6$. The RNA constitutes about 15–20% of the virion dry weight. The positive strands of each duplex have a 5′-terminal type 1 cap structure ($^{7m}$GpppN$^{2'Om}$pNp ), which was first discovered in the cypoviruses. There are data to suggest that negative strands may have phosphorylated 5′ termini. However, in some cases (e.g. bluetongue virus (BTV), genus *Orbivirus*), the negative strand has been shown to be poorly labeled (with the same efficiency as the positive strand) by treatment with polynucleotide kinase and [$\gamma$-$^{32}$P]ATP, suggesting that it may also have a blocked 5′ structure. Both RNA strands have a 3′-OH group, and viral mRNAs lack 3′-polyA tails. The viral dsRNA species are present within virus particles in equimolar proportions, representing one copy of each genome segment per virion. Intact virions of some genera also contain significant amounts of short ssRNA oligonucleotides.

Reovirus RNA is usually regarded as non-infectious. However, recent developments involving the introduction of viral mRNAs into susceptible cells have succeeded in recovering fully viable virus particles, thus providing further research opportunities utilizing reverse genetic technologies.

### Proteins

At least three internal virion structural proteins have enzyme activities involved in RNA synthesis and capping, including a conservative RdRp or Pol (which may function as a transcriptase, i.e. involved in positive strand synthesis on a dsRNA template, or a replicase, i.e. involved in negative strand synthesis on a positive strand ssRNA template), nucleotide phosphohydrolase, guanylyltransferase, two distinct transmethylases, dsRNA unwinding (i.e. helicase) activity and pyrophosphatase. Some of the minor proteins may also play a structurally significant role as components of the virion, together with at least three major capsid proteins. The virion structural proteins range in size from 15 to 155 kDa and constitute about 80–85% of the dry weight of virions.

### Lipids

Mature virions lack a lipid envelope. Depending on the genus, a myristyl residue may be covalently attached to one of the virion proteins. Coltiviruses, rotaviruses and orbiviruses have an intermediate in virus morphogenesis or release, which may have a lipid envelope that is subsequently lost or removed. However, this may help to explain why virus particles are in some cases associated with membrane fractions in infected cell lysates.

### Carbohydrates

In some genera, one of the outer capsid proteins can be glycosylated with high mannose glycans or O-linked N-acetylglucosamine. A small non-structural (NS) viral protein may also be glycosylated.

## Genome organization and replication

The viral RNA species are mostly monocistronic, although some segments have second functional, in-frame initiation codons or additional protein-coding ORFs. Proteins are encoded on one strand only of each duplex (the mRNA species). The mode of entry of viruses into cells varies between genera but usually results in loss of outer capsid components. Transcriptionally active particles derived from the parental virions (cores, represented by single or double layered particles, from subfamily *Spinareovirinae* or *Sedoreovirinae*, respectively) are released into the cell cytoplasm

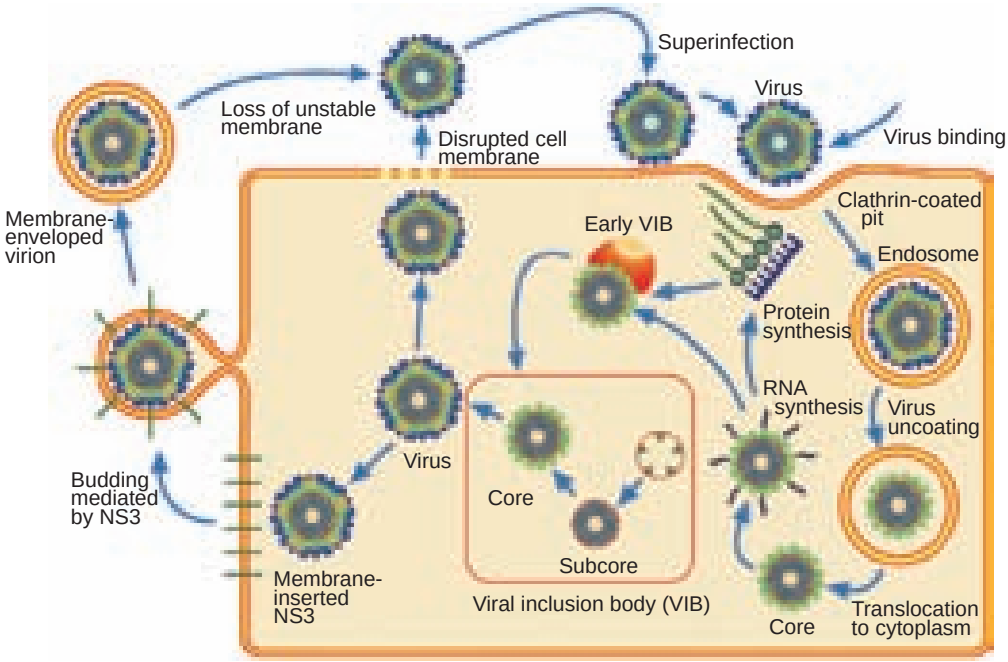


**Figure 2:** Typical virus replication cycle of a reovirus (presented for an orbivirus).

(Figure 2). Repetitive asymmetric transcription of full-length mRNA species from each dsRNA segment occurs within these particles throughout the course of infection. The mRNA products, which are produced in larger copy numbers from the smaller segments, are extruded from the icosahedral apices of these particles. Structures that have been called viroplasms or virus inclusion bodies (VIBs) occur in localized areas of the infected cell cytoplasm. They appear to be the sites of viral mRNA synthesis, genome replication and particle assembly. VIBs have a granular and moderately electron-dense appearance when viewed by electron microscopy, and usually contain nascent subviral particles. Outer capsid components appear to be added to progeny virus particles at the periphery of the VIB and are thought to stop further mRNA synthesis.

The mechanism of genome assembly and synthesis remains largely uncharacterized. For orthoreoviruses and rotaviruses, evidence has been obtained that sets of capped mRNAs and certain NS proteins are incorporated into assortment complexes that are considered to be the precursors of progeny virus particles. These mRNAs are then used as templates for a single round of minus strand synthesis, thereby reforming the dsRNA genome segments of a progeny virus particle. The various species of mRNAs in the cell cytoplasm are present in non-equimolar ratios. However, the dsRNA genome segments are usually packaged in exactly equimolar ratios (i.e. one copy of each genome segment per particle). The selection of viral mRNAs for packaging is therefore thought to be highly specific, involving recognition signals on each mRNA species. Genome segment re-assortment, involving the selection and packaging of mRNAs from different parental strains, occurs readily in cells that are co-infected with different viruses of the same species, which presumably share the same packaging signals.

The RNA segments have conserved terminal sequences at both ends, which may be involved as recognition signals for the viral transcriptase and replicase functions. These sequences may also be essential for selection and incorporation of the RNAs into the nascent progeny particles, and may play some role in efficient initiation of translation of the viral mRNAs. In many cases, sequences near to the 5′ and 3′ termini of the positive sense viral RNAs share extensive complementarity, interrupted by short sequences predicted to form stem loops and other secondary structures. These findings and mounting experimental evidence suggest that the viral mRNAs contain both primary sequences and higher-order structures that are involved in regulation of RNA function (i.e. translation, replication or packaging). A consistent feature of the secondary structures predicted for viral

positive sense RNAs is that the conserved 5′- and 3′-terminal sequences remain non-hybridized. Indeed, non-hybridized 3′-terminal sequences have been shown to be required for efficient negative-strand synthesis. The dsRNA within assembled particles has been shown, in at least some genera, to be packaged as a series of concentric and highly organized shells, which also have elements of icosahedral symmetry.

In addition to the parental virus-derived subviral particles (smooth cores), progeny cores (single or double layered particles from subfamily *Spinareovirinae* or *Sedoreovirinae*, respectively) also synthesize mRNAs, providing an amplification step in replication. Depending on the genus, some NS proteins are involved in translocation of virus particles within cells or virus egress by budding. Many cypoviruses also form polyhedra, which are large crystalline protein matrices that occlude virus particles (either singly or multiply) and which appear to be involved in transmission between individual insect hosts. The steps involved in virion morphogenesis and virus egress from cells vary according to genus. The only known examples of non-enveloped viruses that induce cell–cell fusion and syncytium formation in virus-infected cells are members of the family *Reoviridae*. In the case of fusogenic orthoreoviruses, syncytium formation promotes a rapid lytic response and release of progeny virions.

## Antigenic properties

The viruses that infect vertebrate hosts generally possess both serogroup- (virus species) specific antigens, and (within each species or serogroup) more variable serotype-specific antigens. The viruses that infect plants and insects only may show greater uniformity and less antigenic variation in their proteins, possibly due to the lack of neutralizing antibodies in the host and therefore the absence of antibody selective pressure on neutralization-specific antigens. No antigenic relationship has been found between the viruses in different genera. Some viruses bind erythrocytes (i.e. hemagglutinate).

## Biological properties

The biological properties of the viruses vary according to genus. Some viruses replicate only in certain vertebrate species (orthoreoviruses and rotaviruses) and are transmitted between hosts by respiratory or fecal–oral routes. Other vertebrate viruses (orbiviruses, coltiviruses and seadornaviruses) replicate in both arthropod vectors (e.g. biting midges, mosquitoes or ticks) and vertebrate hosts. Plant viruses (phytoreoviruses, fijiviruses and oryzaviruses) replicate in both plants and arthropod vectors (leafhoppers). Viruses that infect insects (cypoviruses) are transmitted by contact or fecal–oral routes.

## Genus and species demarcation criteria in the family

The number of genome segments (usually 9, 10, 11 or 12) is in most cases characteristic of viruses within a single genus, although the genus *Mycoreovirus* currently contains viruses with both 11 and 12 genome segments. Host (and vector) range and disease symptoms are also important indicators that help to identify viruses from different genera. Capsid structure (number of capsid layers, the presence of spiked or unspiked cores, and the symmetry and structure of the outer capsid) can also be significant. The level of sequence divergence, particularly in the more conserved genome segments and proteins (for example as detected by comparisons of RdRp or inner capsid shell proteins and the segments from which they are translated) can be used to distinguish members of different genera. Available data suggests that isolates from different genera usually have <26% amino acid identity in comparisons between their RdRps, while within a single genus identities are usually >33%. However, the RdRp of *Rotavirus B* isolates shows a high level of amino acid sequence divergence from that of other rotaviruses (<21% identity).

The prime determinant for inclusion of virus isolates within a single virus species is their ability to exchange genetic information during co-infection, by genome segment re-assortment, thereby generating viable progeny virus strains. However, data providing direct evidence of segment re-assortment between isolates are only available for viruses in a few genera. The following methods are therefore commonly used (preferably in combination) to examine levels of similarity between isolates and to predict their possible compatibility:

- Cross-hybridization assays (northern or dot blot), with probes made from viral RNA or cDNA. Stringency conditions may be selected so that viruses within a species will show hybridization.
- Nucleotide and amino acid sequence analysis (viruses within different species should have low levels of sequence similarity among the cognate genome segments).
- Serological comparisons of antigens or antibodies using either polyclonal antisera or monoclonal antibodies against conserved antigens. Methods used may include ELISA, complement fixation and agar gel immunodiffusion. Closely related isolates and serotypes generally belong to the same species.
- Analysis of electropherotype by agarose gel electrophoresis (AGE) but not by PAGE. Virus isolates within the same species will show a relatively uniform electropherotype. However, a major deletion/insertion event may sometimes result in two distinct electropherotypes within a single species, and similarities can exist between more closely related species.
- Identification of the conserved terminal regions of the genome segments. These are usually conserved across all segments within a species although some closely related species can also have identical terminal sequences on at least some segments.

These criteria apply throughout the family. Additional or more specific criteria are provided in the section for each genus, where applicable.

# SUBFAMILY *SPINAREOVIRINAE*

## Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Spinareovirinae*** |
| Genus | ***Orthoreovirus*** |
| Genus | ***Aquareovirus*** |
| Genus | ***Oryzavirus*** |
| Genus | ***Fijivirus*** |
| Genus | ***Mycoreovirus*** |
| Genus | ***Cypovirus*** |
| Genus | ***Idnoreovirus*** |
| Genus | ***Dinovernavirus*** |
| Genus | ***Coltivirus*** |

# GENUS *ORTHOREOVIRUS*

Type species ***Mammalian orthoreovirus***

## Distinguishing features

Orthoreoviruses infect only vertebrates and are spread by respiratory or fecal–oral routes. All members of the genus have a well-defined capsid structure, as observed by electron microscopy and negative staining, with 12 spikes or turrets situated on the surface of the core particle at the icosahedral vertices. They also contain 10 linear dsRNA molecules that include three large (L), three medium (M) and four small (S) size-class segments. They have a characteristic protein profile with three λ, three μ and four σ primary translation products, as well as additional small gene products that are encoded by polycistronic segments. Members of all of the five species, except *Mammalian orthoreovirus*, induce syncytium formation.

## Virion properties

### MORPHOLOGY

Virion morphology and construction is illustrated in Figure 3. Virions are icosahedral with a roughly spherical appearance and possess a double-layered protein capsid, the different layers of which are discernible by negative staining and electron microscopy (Figure 3A). Higher-resolution images have been obtained by cryo-electron microscopy (cryoEM) and image reconstruction of

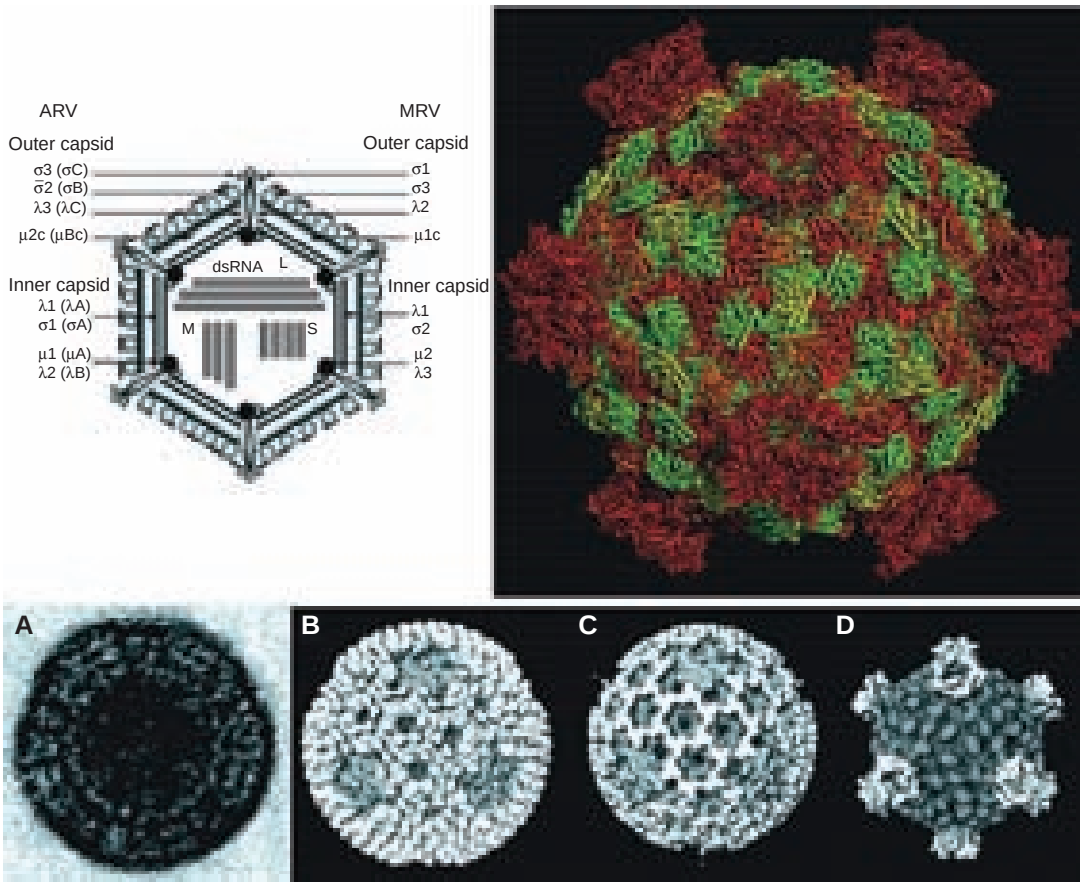


**Figure 3:** (Top left) Diagrammatic representation of an orthoreovirus particle in cross-section. The locations and identities of the virus structural proteins are indicated using the nomenclature scheme for both mammalian orthoreovirus (MRV) and avian orthoreovirus (ARV). The protein components of the inner and outer capsids are indicated (Duncan, R. (1999). *Virology*, **260**, 316-328; Palmer, E.L. and Martin, M.L. (1977). *Virology*, **76**, 109-113). (Top right) Computer-generated image of the inner capsid of mammalian orthoreovirus 1 (MRV-1), based on X-ray crystallography data. (Bottom) Electron micrograph of a negatively stained MRV-1 particle (panel A). Image reconstructions from cryoEM of MRV-1 virions (panel B), infectious subviral particles (ISVPs) (panel C) and cores (panel D). All particles are viewed from the three-fold axis of rotational symmetry. (Courtesy of M. Nibert and T. Baker.)

mammalian orthoreovirus (MRV) and avian orthoreovirus (ARV) particles. These are similar with a central compartment (about 48 nm in diameter) containing the dsRNA genome segments, surrounded by an inner capsid that has T = 1 symmetry (60 nm diameter: composed of 120 copies of protein λ1(Hel)) and an outer capsid (85 nm diameter) that has T = 13 (laevo) symmetry. The innercapsid of the orthoreoviruses is equivalent to the T = 1 core-particle of the rotaviruses and the subcore of the orbiviruses (which is also composed of 120 molecules (of VP3) interpreted as having T = 2 pseudo icosahedral symmetry). The surface of the complete orthoreovirus particle is covered by 600 finger-like projections arranged in 60 hexameric and 60 tetrameric clusters that surround solvent channels, which extend radially into the outer capsid layer (Figure 3B).

Intact virions also contain large, open depressions with a flower-shaped structure at the five-fold axes, resulting in an angular capsid profile when viewed in the three-fold orientation (Figure 3A, 3B). ISVPs, which are generated by partial removal of the outer capsid proteins (Figure 3C), are approximately 80 nm in diameter. The flower-shaped structures at the five-fold axes of the ISVPs may contain an extended form of the viral attachment protein, σ1, which protrudes as a 40 nm fibre from the vertices. MRV core particles generated by more extensive removal of the outer capsid proteins (Figure 3D) have also been examined by X-ray crystallography and have 150 ellipsoidal nodules (protein σ2) on their surface and distinctive turrets located at the five-fold axes. These projections,

which are altered conformations of the flower-shaped structures observed on intact virions (composed of protein λ2(Cap), the viral capping enzyme) are about 10 nm in length, possessing central channels 5–8 nm in diameter extending into the central compartment.

### Physicochemical and physical properties

The virion Mr is about $130 \times 10^7$ with a buoyant density in CsCl of 1.36 g cm$^{-3}$ (1.38 g cm$^{-3}$ for ISVPs, 1.43 g cm$^{-3}$ for core particles). The virion, ISVP and core $S_{20,w}$ values are about 730S, 630S and 470S, respectively. Virions are remarkably stable and withstand extremes of ionic conditions, temperatures up to 55 °C, pH values between 2 and 9, lipid solvents, and detergents. Exposure to UV irradiation reduces infectivity.

### Nucleic acid

All orthoreoviruses have 10 linear dsRNA segments that range from $0.60 \times 10^6$ to $2.60 \times 10^6$ Mr. The total Mr of the MRV-3 (strain Dearing) genome is about $1.5 \times 10^7$ (23,549 bp) and constitutes approximately 11.5% of the virion mass. Based on their resolution by gel electrophoresis, the genomic dsRNAs are grouped into three size classes commonly referred to as large (L1–L3, about 3.9–3.8 kbp), medium (M1–M3, about 2.3–2.2 kbp) and small (S1–S4, about 1.6–0.9 kbp). The gel mobilities of certain genome segments are characteristic of the five distinct species of orthoreoviruses. In comparison to isolates of the type species *Mammalian orthoreovirus*, most isolates of ARV, Nelson Bay orthoreovirus (NBV) and reptilian orthoreovirus (RRV) display retarded genome segment migration of their polycistronic S1 genome segments. Baboon orthoreovirus (BRV) and the Muscovy duck isolates of ARV (ARV-Md) have truncated polycistronic S1 genome segments that migrate as the S4 genome segment by PAGE.

Complete virus particles contain numerous oligonucleotides (2–20 nt) representing approximately 25% of the total RNA content. Three-quarters of these are abortive reiterative 5′-terminal transcripts, produced by the reovirus core-associated transcriptase and capping enzymes, while the remainder are oligoadenylates. The 5′ terminus of the positive-sense RNA strand of each genome segment contains a dimethylated Cap 1 structure ($^{m7}$GpppG$^{m2'OH}$). The genomic RNAs lack polyA tails and do not contain covalently linked proteins. Genomic dsRNA segments contain 5′- and 3′-terminal sequences of 4 or 5 bp that are conserved in all 10 genome segments within a particular virus species. The 3′-terminal consensus sequence (UCAUC-3′) is also conserved between orthoreovirus species, at least as assessed from the available sequences of the four S-class genome segments. The 5′-terminal conserved sequences vary and may be useful for assigning new isolates to one of the five species or subgroups thereof (Table 1).

### Proteins

The orthoreovirus structural proteins are designated in terms of their relative sizes and size classes: λ1, λ2, λ3; μ1, μ2; and σ1, σ2, σ3. In ARV, these proteins are referred to as λA, λB, λC; μA, μB; and σA, σB, σC (Table 2). The following discussion refers to the nomenclature scheme for prototype strain MRV-3.

The stabilizing lattice of the outer capsid is composed of 200 interlocking trimers of the 76 kDa μ1 protein. The μ1 subunits also interact with monomers of the σ3 protein, which represent finger-like projections on the surface of the virion. Pentameric subunits of the λ2 protein make up the

**Table 1:** Conserved terminal sequences (positive strand) of orthoreovirus genome segments

| Virus species | Serotype or strain | 5′ end | 3′ end |
|---|---|---|---|
| *Mammalian orthoreovirus* | MRV-1La* | 5′-GCUA | UCAUC-3′ |
| *Avian orthoreovirus* | ARV-138** | 5′-GCUUUUU | UCAUC-3′ |
| *Nelson Bay orthoreovirus* | NBV | 5′-GCUUUA | UCAUC-3′ |
| *Baboon orthoreovirus* | BRV | 5′-GUAAAUUU | UCAUC-3′ |
| *Reptilian orthoreovirus* | RRV-Py | 5′-GUUAUUUU | UCAUC-3′ |

*Also MRV-2Jo, MRV-3De and MRV-4Nd.
**Also ARV-176 and ARV-Md.

**Table 2:** Genome segments and protein products of mammalian orthoreovirus-3De

| Genome segment | Size (bp) | Proteins (structure/ function)* | Size (kDa) | Protein copies per particle | Location | Function |
|---|---|---|---|---|---|---|
| L1 | 3854 | λ3 (Pol) | 142 | 12 | core | RNA polymerase |
| L2 | 3916 | λ2 (Cap) | 144 | 60 | core spike | Guanylyl transferase, methyl transferase turret protein |
| L3 | 3896 | λ1 (Hel) | 143 | 120 | core | Inner capsid structural protein, binds dsRNA, NTPase, helicase |
| M1 | 2304 | μ2 | 83 | 12 | core | NTPase, required for inclusion body development, probable polymerase subunit, cell tropism, modulation of cellular interferon response |
| M2 | 2203 | μ1 | 76 | 600 | outer capsid | Multimerizes with σ3 and cleaved to μ1C and μ1N during viral entry, assumes T = 13 symmetry in the outer capsid |
| | | μ1C (T13)<br>δ<br>ϕ<br>μ1N | 72<br>59<br>13<br>4 | | | μ1C further cleaved to δ and ϕ during the entry process, myristoylated N-terminus, membrane penetration, apoptosis |
| M3 | 2235 | μNS / μNSC | 80/75 | 0 | NS | Binds ssRNA and cytoskeleton, nucleates viral inclusion bodies, phosphoprotein, μNSC (unknown function) from alternate translation start site |
| S1 | 1416 | σ1 | 49 | 36 | outer capsid | Viral attachment protein, homotrimer, hemagglutinin, type-specific antigen, cell tropism, pathways of viral spread in vivo, virulence |
| | | σ1s | 16 | O | NS | Viral spread *in vivo*, cell cycle arrest |
| S2 | 1331 | σ2 | 47 | 150 | core | Inner capsid structural protein, weak dsRNA-binding, morphogenesis? |
| S3 | 1189 | σNS | 41 | 0 | NS | ssRNA-binding, inclusion body development, genome packaging? |
| S4 | 1196 | σ3 | 41 | 600 | outer capsid | dsRNA-binding, multimerizes with μ1, nuclear and cytoplasmic localization, translation control, modulation of cellular interferon response, zinc-binding |

*Protein structure/function: Pol, RNA polymerase ; Cap, capping enzyme (guanylyltransferase and transmethylase); T13, virus structural protein with T = 13 symmetry; Hel, protein with helicase activity.

flower-like structures and turrets at the vertices of viral particles and cores, respectively. The λ2 structures interact with subunits of the tetrameric σ3 clusters and with the μ1 lattice and represent essential structural components of the outer capsid. This essentially outer capsid protein (CP) remains associated with core particles, unlike the other outer CPs. The fourth component of the outer capsid, the σ1 protein, exists as 12 homotrimers associated with the vertices of virions and ISVPs. It may assume either a retracted or extended conformation. The λ1 (120 copies) and σ2 proteins (150 copies) represent the major structural proteins of the inner capsid. The final two structural proteins of the virus, λ3 and μ2, are present at about 12 copies per virion and located on the inside of the inner capsid. The λ3 protein forms 7 nm projections that extend toward the interior of the core, underlying the 12 vertices of the capsid. The μ2 protein may be associated with these λ3 structures.

### Lipids

Mature virions lack a lipid envelope. The major outer capsid lattice protein, μ1, and its μ1N cleavage product are N-terminally myristoylated. The small NS proteins responsible for syncytium formation induced by the fusogenic orthoreoviruses are either N-terminally myristoylated or palmitoylated at internal cysteine residues. These acylations are essential for the membrane fusion activity of the proteins.

### Carbohydrates

Convincing evidence that any of the orthoreovirus proteins are glycosylated has not been reported. Moreover, no carbohydrate has been observed in the structures of any of the mammalian reovirus proteins that have been determined by X-ray crystallography (λ1, λ2, λ3; μ1; and σ1, σ 2, σ3).

## Genome organization and replication

The genome consists of ten segments of linear dsRNA, which are packaged in equimolar ratios (one copy of each within each virion). The segments possess terminal non-translated regions (NTRs) that are shorter at the 5′ terminus (12–32 bp for MRV-3De) than at the 3′ terminus (35–85 bp). The major ORFs vary in length from 1059 to 3867 bp. The MRV S1 segment is bicistronic, encoding the 49 kDa σ1 protein and the 14 kDa σ1s protein from a second overlapping ORF (Table 2). The S1 genome segments of ARV and NBV are functionally tricistronic, encoding the viral attachment protein σC, a membrane-associated protein (p17) of unknown function, and a fusion-associated small transmembrane (FAST) protein (p10) responsible for virus-induced syncytium formation. The RRV S1 genome segment is bicistronic, encoding a σC viral attachment protein homolog and a novel FAST protein (p14). The truncated S1 genome segment-equivalent (S4, 1124 bp) of Muscovy duck reovirus (ARV-Md) encodes a σC viral attachment protein homolog and a p10 protein that shares limited sequence similarity to the p10 FAST proteins of NBV and other ARV isolates. The truncated S1 genome segment-equivalent of BRV (S4, 887 bp), contains two sequential 140–141 codon ORFs, one of which encodes a third unique FAST protein (p15) and the other a novel NS protein (p16) of unknown function (Figure 4).

The overall course of infection involves adsorption, low pH-dependent penetration and uncoating to core particles, asymmetric transcription of capped, non-polyadenylated mRNAs via a fully conservative mechanism (the nascent strand is displaced), translation, assembly of positive strands into progeny subviral particles, conversion of positive strands to dsRNA, and further rounds of mRNA transcription and translation. The efficiency of translation of the various orthoreovirus mRNA species varies over a 100-fold range, while the proportions of the mRNA species found in infected cells vary inversely to their proportionate size. The final stage of the replication cycle involves the assembly of the outer capsid onto progeny subviral particles to form infectious virions. Based on studies of MRV replication, virion morphogenesis is thought to proceed along a pathway involving a series of assembly intermediates. Progeny particles accumulate in paracrystalline arrays in the perinuclear region of the cytoplasm and are released when infected cells lyse late in the replication cycle. The exception to the above generalized replication cycle involves the formation of multinucleated syncytia by ARV, BRV, RRV and NBV. Syncytia formation commences 10–12 h post infection, resulting in a more rapid lytic response and enhanced kinetics of virus release.

The functions and properties of specific viral proteins influence various stages of the MRV replication cycle (Table 2). The MRV σ1 viral attachment protein determines the cell and tissue tropism of the virus strain and has hemagglutination activity. The σ1 protein binds cell-surface carbohydrate

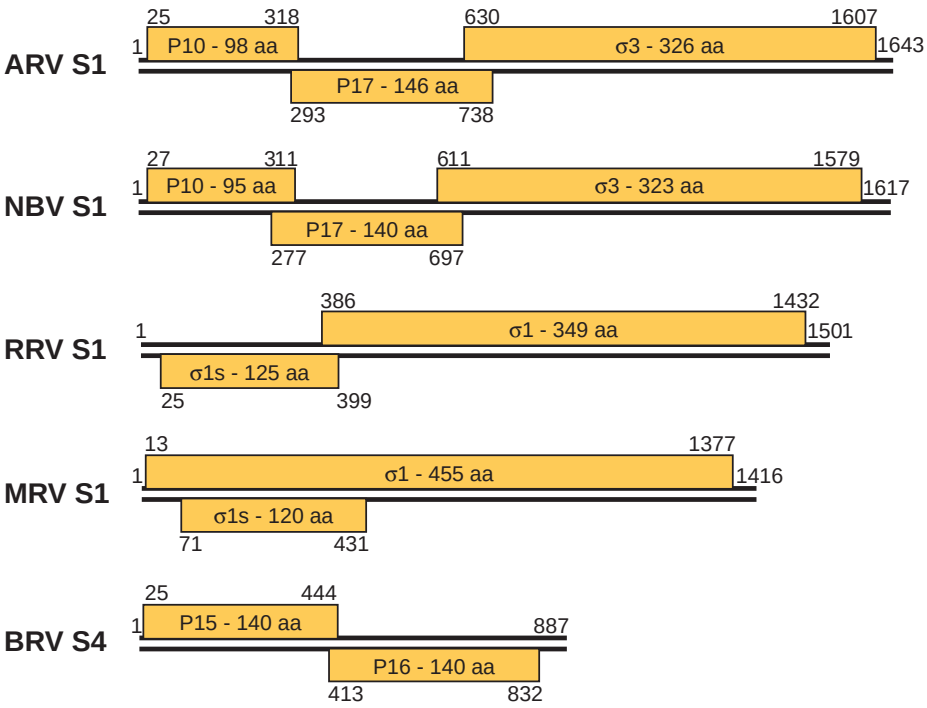


**Figure 4:** Gene organization of the polycistronic genome segments of the five species of orthoreoviruses. The solid line indicates the dsRNA, and the numbers refer to the first and last nucleotides of the genome segment, along with the nt positions of the various ORFs (excluding the termination codons) indicated by the rectangles. The identities of the gene products encoded by the various ORFs are indicated within the rectangles. The virus species and the genome segment are indicated on the left. The code for the abbreviations can be found in the list of species.



and junctional adhesion molecule-A. The μ1 protein is N-terminally myristoylated and forms a complex with σ3 in solution that triggers cleavage of μ1 to μ1N and μ1C. The μ1C fragment is further proteolytically cleaved into δ and ϕ polypeptides during virus entry into cells and is responsible for membrane penetration. The μ1 protein also influences strain-specific differences in capsid stability, transcriptase activation, apoptosis and neurovirulence. In the case of ARV, the μ1 homolog (μB) has been implicated in strain-specific differences in macrophage infection. In addition to interacting with μ1 and forming the outer capsid layer of the virion, the σ3 protein is a dsRNA-binding protein involved in translation regulation, altering the activity of protein kinase R (PKR), and modulating the interferon response. The λ2 core spike is the guanylyl transferase involved in mRNA capping, while the λ1 and σ2 major inner capsid proteins both bind dsRNA. The λ1 protein may also function as a helicase and an RNA triphosphatase. The minor inner CP λ3 is the viral polymerase, while the second minor inner CP μ2, along with the major inner CP λ1, is involved in the NTPase activity associated with core particles.

There are also at least three NS proteins encoded by the MRV genome: μNS, σNS, and σ1s. The μNS and σNS proteins are produced in high abundance during infection and, together with σ3, associate with mRNA to form virus mRNA-containing complexes, which are presumed to be precursors of progeny virus assembly.

The σNS protein binds ssRNA, and the μNS protein associates with the cytoskeleton. Core protein μ2 stabilizes microtubules within viral inclusions and associates with μNS, which is an organizing center for inclusion formation. Co-expression of μNS and σNS proteins in mammalian cells from cloned viral cDNAs yields punctate structures resembling intracytoplasmic inclusions of virally infected cells. The σ1s protein is a small, basic protein expressed in cells infected by all three MRV serotypes. This protein is required for MRV dissemination in infected mice and contributes to cell-cycle arrest during MRV

infection. The relationship between these σ1s-mediated properties is currently unknown. It is dispensable for growth in cell culture but is involved in cell cycle arrest at the G2/M checkpoint.

Replication strategies used by ARV, NBV, RRV and BRV are similar to that described for MRV, with some notable exceptions. The truncated viral attachment protein of ARV, RRV and NBV, σC (35 kDa), exists as a multimer with a coiled-coil domain similar to that of MRV but possesses no hemagglutination activity. BRV is unique in that the S-class genome segments encode no homolog of the ARV, NBV, RRV or MRV viral attachment proteins. The dsRNA-binding domain of the MRV major sigma-class outer CP σ3 is not conserved in the homologous σB proteins of ARV, NBV or BRV. As with the MRV σ2 protein, the major sigma-class core protein of ARV, σA, displays dsRNA-binding activity. The ARV σA core protein may function analogously to the σ3 major outer CP of MRV by regulating PKR activity and the interferon response. ARV, NBV, RRV and BRV encode an additional FAST (fusion-associated small transmembrane) protein responsible for syncytium formation. The p10 FAST proteins of ARV and NBV share sequence and structural similarities, but are unrelated to the p15 and p14 FAST proteins of BRV and RRV, respectively. All of these FAST proteins are small, basic, acylated, transmembrane proteins and induce fusion in transfected cells in the absence of other viral proteins.

## Antigenic properties

The serotype-specific antigen of the orthoreoviruses is protein σ1 (σC of the avian species), which is recognized by neutralizing antibodies. Antigenic recognition of this protein is the basis for three major serotypes of MRV and 5–11 serotypes of ARV. Ndelle virus was isolated from a mouse and originally classified as an orbivirus. Recent sequence data revealed that Ndelle virus is actually an orthoreovirus and is closely related to MRV-1 and MRV-3. However, neutralizing antibodies against the three major MRV serotypes do not neutralize Ndelle virus, indicating that it represents a fourth MRV serotype. The MRV σ1 and σ1s proteins elicit strain-specific and cross-reactive cytotoxic T-cell activities. The MRV proteins λ2 and σ3 are species-specific antigens, similar to the λB and σB proteins of ARV (Figure 3). The considerable sequence similarity that exists between different isolates in the same orthoreovirus species, but not among species, is reflected by the limited antigenic cross-reactivity detected among species. The most extensive antigenic similarity between species subgroups occurs between ARV and NBV, which is in accordance with the increased amino acid sequence identity between these species.

## Biological properties

Transmission is by an enteric or respiratory route, no arthropod vectors are involved, and infection is restricted to a variety of vertebrate species (baboons, bats, birds, cattle, humans, monkeys, sheep, snakes, swine and rodents). Orthoreovirus distribution is worldwide. Human orthoreoviruses generally do not produce symptoms, but may cause upper respiratory tract illness and possibly enteritis in infants and children (albeit rare). In mice, orthoreovirus infection can cause diarrhea, runting, oily hair syndrome, hepatitis, jaundice, myocarditis, myositis, pneumonitis, encephalitis and hydrocephalus. A variety of symptoms may be associated with orthoreovirus infection of domestic animals including upper and lower respiratory illnesses and diarrhea. In monkeys, orthoreoviruses cause hepatitis, extrahepatic biliary atresia, meningitis and necrosis of ependymal and choroid plexus epithelial cells. The prototype BRV isolate was obtained from baboons with meningoencephalomyelitis. RRV isolates from snakes were obtained from animals displaying neurological symptoms. The outcome of ARV infection in birds may range from inapparent to lethal, depending on the virus strain and the age of the host. Systemic infection results in virus dissemination to numerous tissues. Disease presentations in chickens include feathering abnormalities, gastroenteritis, hepatitis, malabsorption, myocarditis, paling, pneumonia, stunted growth and weight loss. In turkeys, ARVs cause enteritis. Birds that survive an acute systemic infection may develop obvious joint and tendon disorders (tenosynovitis) that resemble the pathology of rheumatoid arthritis in humans. ARVs do not infect mammals.

MRV and ARV induce the biochemical and morphologic hallmarks of apoptosis in cultured cells. MRV infection leads to activation of nuclear factor kappa B (NF-κB), a family of transcription factors known to play important roles in regulating cellular stress responses, including apoptosis. The μ1 cleavage

fragment ϕ, which is released following disassembly, is an important trigger of NF-κB activation, but the precise mechanism is unclear. Apoptosis induced by reovirus requires both extrinsic (death-receptor) and intrinsic (mitochondrial) signaling pathways linked by the small Bcl-2 family member, Bid. As with MRV, ARV-induced apoptosis requires virus disassembly but not viral transcription.

Recent studies indicate that MRV preferentially replicates in a lytic manner in transformed cells. The basis for this cell tropism has been suggested to result from an activated Ras pathway in transformed cells on modulation of PKR activity and regulation of the translation machinery. These observations have led to the development of orthoreoviruses as an oncolytic agent for cancer therapy.

## Species demarcation criteria in the genus

The orthoreoviruses include five species. The classification is supported by experiments showing re-assortment of genome segments between isolates of the same species but not between those of different species. In addition to the other general criteria used throughout the family, members of a species in the genus *Orthoreovirus* may be identified by:

- Extensive sequence identity between the proteins encoded by homologous genome segments (for conserved core proteins, >85% amino acid sequence identity within a species versus <65% identity between species; for the more divergent outer CPs, >55% identity within a species and <35% between species)
- Extensive sequence identity between homologous genome segments (for most genome segments, >75% nucleotide sequence identity within a species versus <60% between species)
- Analysis of electropherotype by agarose gel electrophoresis but not by PAGE (some similarities can exist between closely related species)
- Similar organization of the polycistronic genome segment
- Identification of host species and clinical signs

## List of species in the genus *Orthoreovirus*

| | | |
|---|---|---|
| *Mammalian orthoreovirus* | | |
| Mammalian orthoreovirus 1 Lang | [L1: M24734, L2: AF378003, L3: AF129820, M1: AF461682, M2: AF490617, M3: AF174382, S1: M14779, S2: M17598, S3: M14325, S4: M13139] | (MRV-1La) |
| *Avian orthoreovirus* | | |
| Avian orthoreovirus S1133 {chicken isolate} | [S1: L39002, S2: AF104311, S3: U20642, S4: U95952] | (ARV-1133) |
| *Baboon orthoreovirus* | | |
| Baboon orthoreovirus | [S1: AF059719, S2: AF059723, S3: AF059727, S4: AF406787] | (BRV) |
| *Nelson Bay orthoreovirus* | | |
| Nelson Bay orthoreovirus | [S1: AF218360, S2: AF059718, S3: AF059726, S4: AF059722] | (NBV) |
| *Reptilian orthoreovirus* | | |
| Reptilian orthoreovirus-Python | [S1: AY238887, S3: AY238886] | (RRV-Py) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct ®, www.sciencedirect.com.

*Mammalian orthoreovirus* includes all the nonfusogenic orthoreoviruses, with three major serotypes (MRV-1, MRV-2 and MRV-3) representing numerous isolates, and a fourth serotype with only one isolate, Ndelle reovirus (MRV-4Nd). Amino acid sequence identities of the sigma-class major outer CPs and core proteins of various MRV serotypes range from 90 to 97%.

*Avian orthoreovirus* contains numerous isolates from commercial poultry flocks, including chickens, Muscovy ducks, turkeys and geese, and includes several different serotypes. Sequence diversity is

more extensive among the various ARV isolates than among MRV isolates (54–95% in the sigma-class major outer CP).

*Nelson Bay orthoreovirus* contains an atypical syncytium-inducing mammalian reovirus isolated from a flying fox. The sequence similarity between NBV and ARV exceeds that between NBV and the other species subgroups. ARV and NBV also share more extensive antigenic similarity than other species, possess more similar conserved terminal genome segment sequences, display a similar gene organization of the polycistronic S1 genome segment, and encode homologous p10 fusion proteins (Table 2). These observations indicate that NBV is more closely related to ARV isolates than to other mammalian or reptilian reovirus isolates. Although ARV and NBV clearly share a more recent evolutionary past than the other reovirus species, in view of the extent of sequence divergence (59–61% identity in the sigma-class core protein and only 29–36% identity in the sigma-class major outer CP) and the absence of evidence for re-assortment between the ARV and NBV isolates, these isolates are considered as two separate species.

*Baboon orthoreovirus* contains a single isolate, BRV. This atypical mammalian isolate induces syncytium formation but shares little sequence (16–32% amino acid sequence identity between homologous S-class gene products) or antigenic similarity with the other fusogenic species. BRV contains a truncated, fusion-inducing, polycistronic S1 genome segment-equivalent (the S4 genome segment) with a distinct gene organization, a fusion protein (p15) with no sequence or sequence-predicted structural similarity to the fusion proteins of ARV or NBV, and a unique 5′-terminal consensus sequence. This isolate clearly represents a distinct species of the orthoreoviruses.

*Reptilian orthoreovirus* represents the RRVs. Sequence information is available for the polycistronic S1 genome segment and the S-class genome segment encoding the sigma-class major outer CP of an RRV isolate from a python (RRV-Py). Several additional isolates have been obtained from other snakes and iguanas, but no sequence information is currently available. RRV contains the conserved 3′-terminal pentanucleotide sequence of the orthoreoviruses (UCAUC-3′) but possesses a unique 5′-terminal conserved sequence (5′-GUUA) (Table 1). The S1 genome segment of RRV-Py is bicistronic, encoding a viral attachment protein homolog and a novel p14 FAST protein that induces syncytium formation. Amino acid sequence identities between the RRV sigma-class major outer CP and the homologous protein of other species subgroups are 16–25%, clearly indicating that RRV represents a distinct species of orthoreoviruses.

### List of other related viruses which may be members of the genus *Orthoreovirus* but have not been approved as species

None reported.

### Phylogenetic relationships within the genus

The five species of orthoreoviruses represent evolutionarily distinct lineages, as illustrated by phylogenetic analysis using the amino acid sequences of the sigma-class major outer CP, for which the greatest number of sequences from diverse isolates is available (Figure 5). Identical phylogenetic relationships are generated by comparison of the NS proteins (data not shown).

## Genus *Aquareovirus*

Type species ***Aquareovirus A***

### Distinguishing features

Aquareoviruses physically resemble orthoreoviruses but possess 11 dsRNA genome segments. They infect a variety of aquatic animals, including finfish and crustacea. Aquareoviruses replicate in cell cultures of piscine and mammalian origins, at temperatures between 15 and 25 °C. Large syncytia are produced as a typical cytopathic effect of infection by a majority of aquareoviruses.

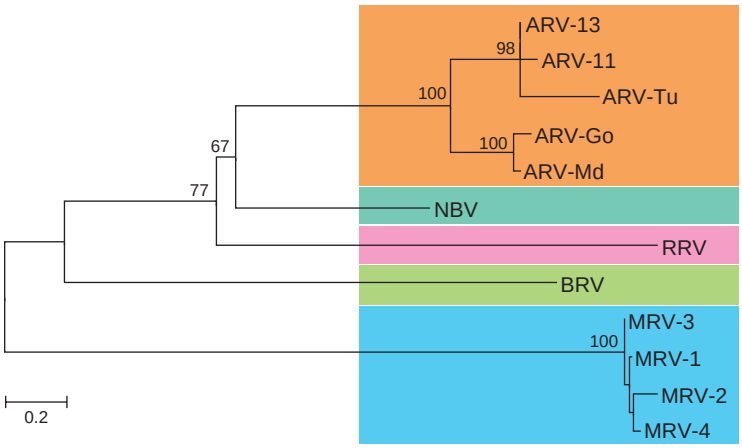


**Figure 5:** Phylogenetic relationships between the five orthoreovirus species using the aa sequences of the sigma-class major outer CPs of various isolates. Sequences were aligned using ClustalX and the unrooted neighbor-joining tree constructed using MEGA4 (Dayhoff distances and 10,000 bootstrap replicates). Accession numbers used were ARV-13 (AF059271), ARV-11 (U20642), ARV-Tu (AF4645799), ARV-Md (AJ006476), ARV-Go (AY114138), BRV (AF059723), MRV-1 (X61586), MRV-2 (X60066), MRV-3 (HM159622), MRV-4 (AF368037), NBV (AF059722) and RRV (AY238886).

## Virion properties

### Morphology

Aquareovirus particles are spherical in appearance with diameter of about 80 nm composed of multiple capsid layers (Figure 6, upper and middle panels). The outermost layer, formed by VP5–VP7 heterodimers, consists of 600 subunits (200 trimers) arranged on an incomplete T = 13 icosahedral lattice, with an overall structural organization identical to those of MRV and ARV. A distinguishing feature on the outer layer is the five-fold proximal depressions, resulting from missing peripentonal trimers (Figure 6, upper left panel, P1 position indicated by arrows).

The shaded surface view of the aquareovirus core structure (Figure 6, upper right panel), shows the innermost capsid shell, which is about 600 Å in diameter. Twelve VP1 pentameric turrets decorate the shell of 120 VP3 monomers, which are arranged with icosahedral symmetry that is interpreted as T = 1 (comparable to the sub-core of the orbiviruses and the innermost capsid shell of the rotaviruses), and are clamped together by 120 VP6 monomers.

Removal of VP7 generates ISVPs, which have a smooth surface formed by a network of VP5 trimers (Figure 6, bottom panel). The atomic model of ISVPs contains six conformers of four proteins: two of VP3, two of VP6, one VP1 on the core, and one VP5 on the coat.

The aquareovirus particle morphology is strikingly similar to that of the orthoreovirus ISVP. A noticeable morphology distinction between aquareovirus and orthoreoviruses is that aquareovirus particles lack the hemagglutinin spike protein σ1 observed in orthoreoviruses.

### Physicochemical and physical properties

The virion buoyant density in CsCl is 1.36 g cm$^{-3}$ with a sedimentation coefficient of about 550S. Virus infectivity is stable between pH 3 and pH 10. Virus infectivity is not affected by treatment with ether or chloroform. Exposure to UV irradiation reduces infectivity. None of the viral proteins is removed from the particle by treatment with 3 mM EDTA or cesium salts. Aquareoviruses held at 4, 16 or 23 °C in minimal essential medium (MEM) with 5% serum showed no significant reduction in infectivity over a period of 28 days. However, all virus infectivity is lost after incubation at 45 °C for 7 days. Virus infectivity is rapidly inactivated by heating to 56 °C.

### Nucleic acid

The aquareovirus genome is composed of 11 segments of dsRNA that are packaged in equimolar ratios. The Mr of the dsRNA segments range from 0.4 to 2.6 ×10$^6$. The total Mr of the golden shiner

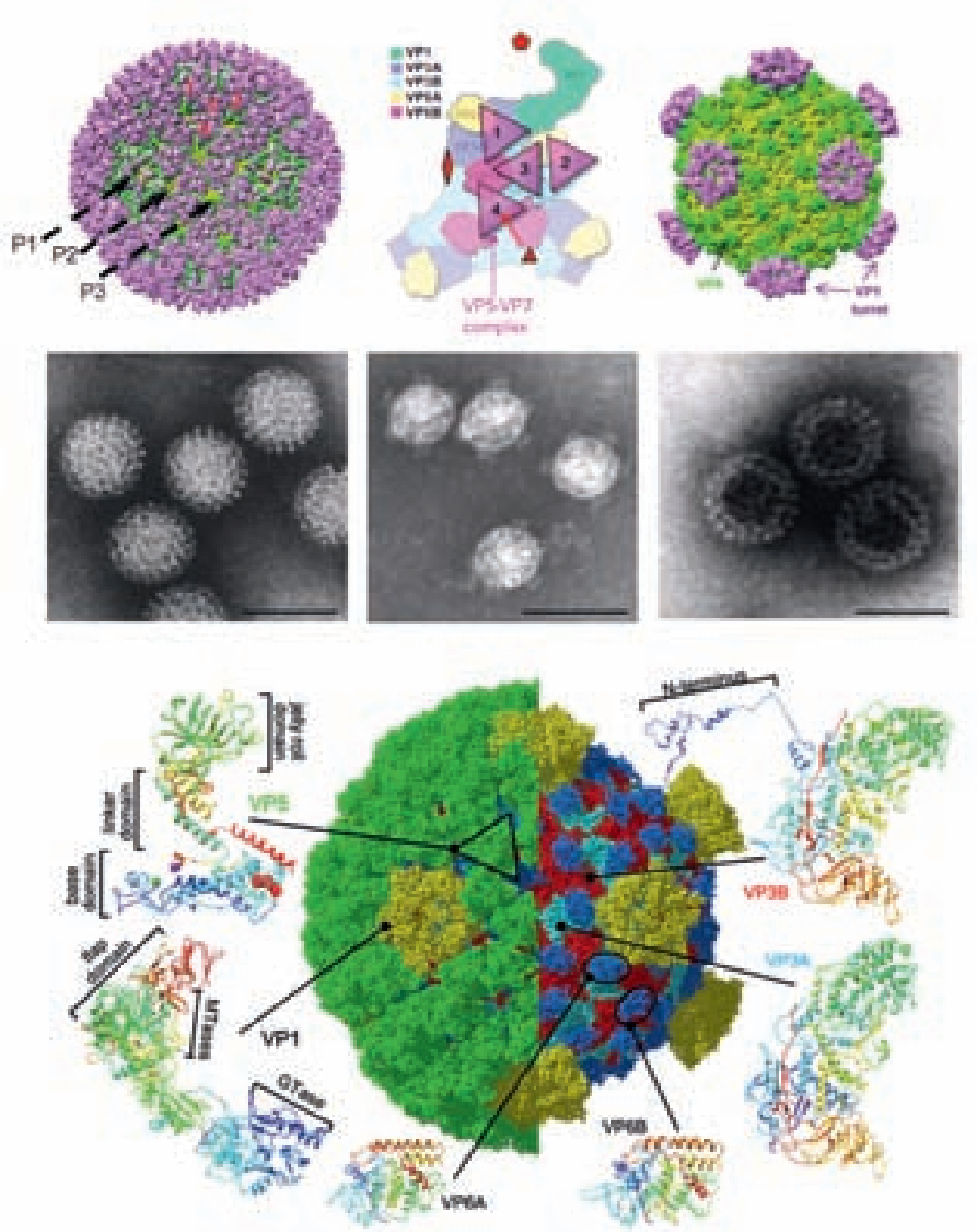


**Figure 6:** (Top panel) Structural representation of grass carp reovirus virion and core by cryoEM. Triangles represent the VP5–VP7 complex on the virion (from Cheng, L., Fang, Q., Shah, S., Atanasov, I. C. and Zhou Z. H. (2008). *J. Mol. Biol.*, **382**, 213–222). (Middle panel) Transmission electronmicroscopy (TEM) of negatively stained grass carp reovirus (GCRV) particles. From left to right: intact virion, core and top components (empty particles). The scale bar represents 100 nm (from Fang, Q., Seng, E. K., Ding, Q. Q. and Zhang, L. L. (2008). *Arch. Virol.*, **153**, 675–682). (Bottom panel) Complete atomic model of grass carp infectious subviral particle. In the right hand side of the CryoEM reconstruction the removal of the VP5 coat reveals the core proteins. Ribbon models of the atomic structures of the six conformers from four structural proteins are shown in the periphery. The black triangle encloses a VP5 trimer (from Zhang *et al.* (2010). *Cell*, **141**, 472–482).

**Table 3:** Conserved terminal sequences (positive strand) of aquareovirus genome segments

| Virus species | Serotype or strain | 5′ end | 3′ end |
|---|---|---|---|
| *Aquareovirus A* | CHSRV | 5′-GUUUUA$^{U}/_{G}$ | $^{A}/_{U}$UCAUC-3′ |
| *Aquareovirus C* | GSRV/GSRV | 5′-GUUAUU$^{U}/_{G}$ | $^{A}/_{U}$UCAUC-3′ |
| *Aquareovirus G* | AGCRV | 5′-GUUUUA$^{U}/_{A}$ | $^{U}/_{A}{}^{U}/_{A}$UCAUC-3′ |

reovirus (GSRV) is about $1.5 \times 10^7$ (23,695 bp). The genomic RNAs are named segment 1 (Seg1) to segment 11 (Seg11) in order of increasing electrophoretic mobility in 1% agarose gels. The genome segments migrate as three size classes. There are three large (Seg1 to Seg3, about 3.9–3.8 kbp), three medium (Seg4 to Seg6, about 2.3–2.0 kbp) and five small segments (Seg7 to Seg11, about 1.4–0.8 kbp). Six distinct species (*Aquareovirus A* to *Aquareovirus F*) were originally identified by reciprocal RNA–RNA hybridization studies, but can also be distinguished by nucleotide sequence analyses. The genome segment migration pattern (electropherotype), as analyzed by electrophoresis in 1% agarose gel, is consistent within a single species but shows significant variation among species. However, viruses within a single species can show variations in electropherotype, when their dsRNA genome segments are analyzed by electrophoresis in high percentage (>6%) polyacrylamide gels.

The G+C content of aquareoviruses ranges between 52 and 60%. The complete genomic sequences of GSRV and grass carp reovirus (GCRV) have been determined from cloned cDNAs, along with several genome segments of other aquareovirus isolates. Genomic dsRNA segments contain 7 nt at the 5′ terminus and 6 nt at the 3′ terminus, which are conserved in all 11 genome segments within a particular virus species (Table 3). The 5′- and 3′-conserved terminal sequences of isolates of *Aquareovirus C* are 5′-GUUAUU$^{U}/_{G}$-3′ and 5′-$^{A}/_{U}$UCAUC-3′, compared to 5′-GUUUUA$^{U}/_{G}$-3′ and 5′-$^{A}/_{U}$UCAUC-3′ in *Aquareovirus A*.

### Proteins

Virions of *Aquareovirus A* isolates contain seven structural proteins: VP1, 130 kDa; VP2, 127 kDa; VP3, 126 kDa; VP4, 73 kDa; VP5, 71 kDa; VP6, 46 kDa; VP7, 35 kDa. VP1, VP2, VP3 and VP6 form the core of the virus particle. VP3 and VP6 are more abundant than VP1 and VP2. VP1 is present in greater copy numbers than VP2. VP6 and VP3 probably form nodules and the spherical shell of the core, respectively. VP1 is thought to form turret-like structures present at the five-fold axis. VP2 is present in very small amounts per virion and is thought to be present beneath the five-fold axis.

VP7, VP4 and VP5 are present in the outer coat of the virion. All three proteins are removed by prolonged trypsinization, resulting in release of core particles. VP7 is the most external protein. VP5 is the next most accessible protein after VP7. Removal of VP7 by trypsin may expose some regions of VP5 critical for efficient entry into cells.

### Lipids

Aquareoviruses have no known lipid components.

### Carbohydrates

VP7 of *Aquareovirus A* isolates may be glycosylated.

## Genome organization and replication

Twelve primary gene products have been identified for isolates of *Aquareovirus A* (Table 4). However, observed variations in dsRNA electropherotype suggest that viruses from different species may have proteins with significant differences in size. Each genome segment of *Aquareovirus A* isolates encodes only one primary translation product, with the exception of Seg11, which encodes two primary translation products. In addition to the seven structural proteins, five non-structural proteins of unknown function are encoded. In isolates of *Aquareovirus C* and *Aquareovirus G*, it is Seg7 that encodes two proteins, from non-overlapping and out-of-phase ORFs (Figure 7).

**Table 4:** Genome segments and protein products of striped bass reovirus (species *Aquareovirus-A*)

| Genome segment | Size (kbp) | Protein nomenclature | Protein size (kDa) | Protein location |
|---|---|---|---|---|
| Seg1 | 3.8 | VP1 | 130 | Inner capsid (core) |
| Seg2 | 3.6 | VP2 | 127 | Inner capsid (core) |
| Seg3 | 3.3 | VP3 | 126 | Inner capsid (core) |
| Seg4 | 2.5 | VP4 | 97 | Non-structural |
| Seg5 | 2.4 | VP5 | 71 | Inner capsid (core) |
| Seg6 | 2.2 | VP4 | 73 | Inner capsid (core) |
| Seg7 | 1.5 | NS4 | 28 | Non-structural |
| Seg8 | 1.4 | VP6 | 46 | Inner capsid (core) |
| Seg9 | 1.2 | NS2 | 39 | Non-structural |
| Seg10 | 0.9 | VP7 | 34 | Major outer capsid |
| Seg11 | 0.8 | NS3 | 29 | Non-structural |
| | | NS5 | 15 | Non-structural |

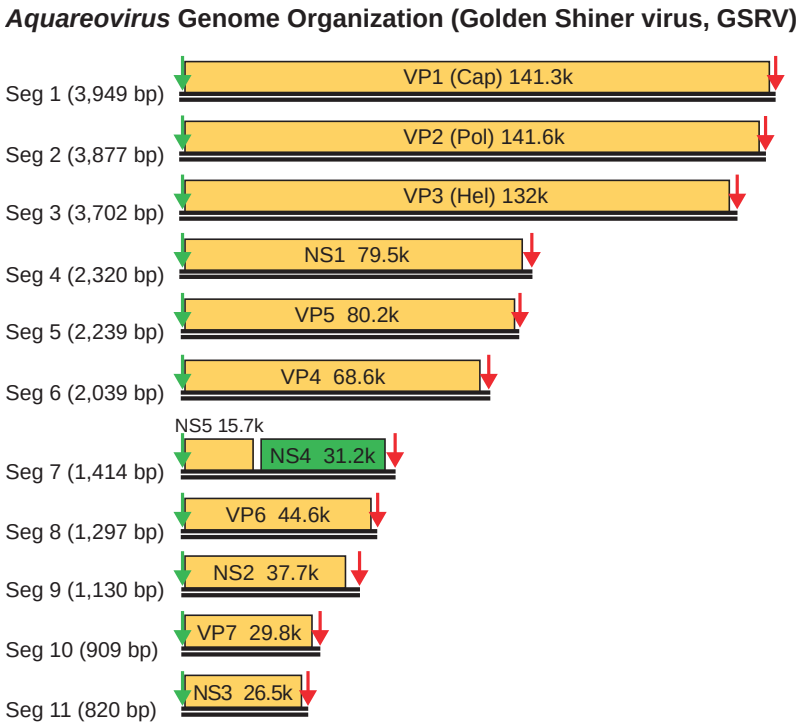


**Figure 7:** Genome organization of the 11 dsRNA segments of golden shiner virus (species *Aquareovirus C*). Each segment has a single ORF, except Seg7 which contains two ORFs. The green arrows indicate the upstream conserved terminal sequence (+ve 5′-GUUAUU$^{U}/_{G}$ .) while the red arrows indicate the downstream conserved terminal sequence (+ve ..$^{A}/_{U}$UCAUC-3′).

## Antigenic properties

Aquareovirus outer CPs lack hemagglutinating activity. Viruses possess type-specific and group-specific antigenic determinants. Members within a single species may be antigenically related. Members of different species are antigenically distinct. Minor antigenic cross-reactivity has only been demonstrated between members of *Aquareovirus A* and *Aquareovirus B*. Distinct serotypes probably exist within each species. The major outer CP of isolates of *Aquareovirus A* (VP7) is not the major neutralizing antigen. There is no antigenic relationship between aquareoviruses and MRVs.

## Biological properties

### Host range

Aquareoviruses have been isolated from poikilothermic vertebrates as well as invertebrates (hosts include fish, molluscs, etc.) obtained from both fresh and sea water. The viruses replicate efficiently in fish and mammalian cell lines at temperatures ranging from 15°C to 25°C. They produce a characteristic cytopathic effect consisting of large syncytia. Generally, the viruses are of low pathogenicity in their host species. However, GCRV is highly pathogenic in grass carp. The infectivity of aquareoviruses is enhanced by treatment with trypsin or chymotrypsin, which correlates with digestion of the outer capsid protein VP7. The most infectious stage of the virus is produced by a 5-min treatment with trypsin. However, prolonged trypsin treatment almost completely abolishes infectivity, reflecting release of core particles.

## Species demarcation criteria in the genus

In addition to the other general criteria used throughout the family, members of a species in the genus *Aquareovirus* may be identified by:

- Northern hybridization assays under conditions (stringency) that do not allow >17% mismatch. Only isolates within the same species will show hybridization.
- Sequence analysis: In genome segment 10 which encodes the major outer CP (VP7), viruses from different species have <55% nucleotide identity (36% amino acid identity in the VP7). In the RdRp isolates of the same species have >95% aa identity, while the corresponding values between species are 57–74%.

Seven species (*Aquareovirus A* to *Aquareovirus* G) and some unassigned viruses have been recognized on the basis of RNA-RNA hybridization.

## List of species in the genus *Aquareovirus*

| | | |
|---|---|---|
| *Aquareovirus A* | | |
| Chum salmon reovirus CS | [AF418294-304] | (CSRV) |
| Other strains: American oyster reovirus 13p2, Angel fish reovirus, Atlantic salmon reovirus HBR, AS and TS, Chinook salmon reovirus DRC, Masou salmon reovirus MS, Smelt reovirus, Striped bass reovirus | | |
| *Aquareovirus B* | | |
| Chinook salmon reovirus B | | (GRCV) |
| Other strains: Chinook salmon reovirus LBS, YRC and ICR, Coho salmon reovirus CSR, ELC and SCS | | |
| *Aquareovirus C* | | |
| Golden shiner reovirus | [AF403398-408] | (GSRV) |
| Other types: Grass carp reovirus | | |
| *Aquareovirus D* | | |
| Channel catfish reovirus | | (CCRV) |
| *Aquareovirus E* | | |
| Turbot reovirus | | (TRV) |
| *Aquareovirus F* | | |
| Chum salmon reovirus PSR | | (PSRV) |
| Other strains: Coho salmon reovirus SSR | | |
| *Aquareovirus G* | | |
| American grass carp reovirus | [EF589098-108] | (AGCRV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct ®, www.sciencedirect.com.

## List of other related viruses which may be members of the genus *Aquareovirus* but have not been approved as species

| | | |
|---|---|---|
| Chub reovirus | | (CHRV) |
| Golden ide reovirus | [Seg2: AF450323, Seg5: AF450324] | (GIRV) |
| Hard clam reovirus | | (HCRV) |
| Landlocked salmon reovirus | | (LSRV) |
| Tench reovirus | | (TNRV) |

### Relationship with orthoreoviruses

The highest level of amino acid sequence identity detected between the RdRp of aquareoviruses and a member of a distinct reovirus genus was 41% (to MRV, a member of the genus *Orthoreovirus*), supporting the hypothesis that these genera are closely related (derived from a common ancestor, estimated ca. 510 million years ago [MYA]). Although this value of amino acid sequence identity is higher than that separating most genera (usually <30%), classification of the aquareoviruses and orthoreoviruses as members of two distinct genera is based on multiple parameters and not simply genetic relatedness. For example, the aquareoviruses can infect many marine and freshwater species, whereas the orthoreoviruses primarily infect mammals, birds and reptiles. The common origin of these viruses, and of their respective hosts, suggests co-speciation of the viruses with their respective hosts.

## GENUS *ORYZAVIRUS*

Type species ***Rice ragged stunt virus***

### Virion properties

#### MORPHOLOGY

Intact rice ragged stunt virus (RRSV) particles appear to be icosahedral in symmetry and double-shelled. The particle diameter is in the range of 75–80 nm and surface A-spikes (approximately 10–12 nm wide and 8 nm in length) are attached to the end of B-spikes situated at the five-fold axes of the viral core. The subviral or core particles have an estimated diameter of 57–65 nm (Figure 8) and possess 12 B-type spikes, 8–10 nm in height, 23–26 nm wide at the base and 14–17 nm wide at the top. In negatively-stained preparations of RRSV, B-spiked subviral particles have been seen but

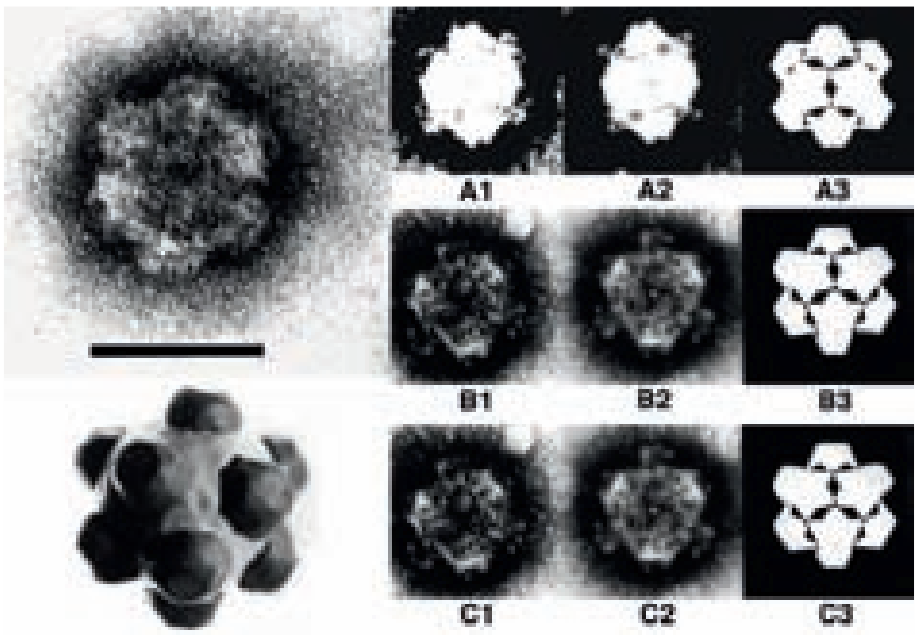


**Figure 8:** (Top left) Electron micrograph of rice ragged stunt virus (RRSV) particles (courtesy of R.G. Milne). (Bottom left) schematic of RRSV particle; (right panel) micrographs of the virus showing 2-, 3- and 5-fold symmetries (A1, B1 and C1, respectively) images of the same rotated by increments of 180° (A2), or 120° (B2), or 72° (C2) and proposed models of the 2-, 3- and 5-fold symmetries (A3, B3 and C3 respectively) (courtesy of E. Shikata). The bar represents 50 nm.

intact double-shelled particles are not seen without pretreatment with fixative. Echinochloa ragged stunt virus (ERSV) particles are slightly larger than RRSV particles.

### Physicochemical and physical properties

RRSV particles sediment as a single component and are stable at pH 6.0–9.0. They are stable in 0.1 M $MgCl_2$. The B spikes dissociate from the core particle in 0.5 M $MgCl_2$ and the entire particle is disrupted in 2 M $MgCl_2$. The particles retain infectivity after 7 days at 4 °C and after 10 min at 50 °C but lose their infectivity after 10 min at 60 °C. They retain infectivity after three cycles of freezing and thawing. The particles contain an RdRp.

### Nucleic acid

The oryzavirus genome consists of 10 linear dsRNA segments. The genomes of RRSV and ERSV have similar sizes and segment profiles (RRSV Mr $18.15 \times 10^6$ (26,066 bp); ERSV Mr $17.78 \times 10^6$), with segments ranging in size from 1,162 to 3,849 bp. The genomic dsRNAs are termed Seg1 to Seg10, in order of increasing electrophoretic mobility in 7.5% polyacrylamide gels. The entire genome of RRSV has been sequenced; Seg4 and Seg10 are larger than they appear from migration in polyacrylamide gels, suggesting that they may migrate in the position 3 and 9 respectively during agarose gel electrophoresis (AGE). The conserved terminal sequences of the ERSV genome segments are identical to those of RRSV (5′-GAUAAA...(G)GUGC-3′) and differ from those of phytoreoviruses or fijiviruses. RRSV RNAs hybridize weakly with their counterparts in ERSV but not with segments of the phytoreovirus rice dwarf virus (RDV).

### Proteins

RRSV particles are composed of five major, highly immunoreactive structural proteins, with estimated sizes of 33, 39, 43, 70 and 120 kDa, and at least five minor structural proteins (49, 60, 76, 90 and 94 kDa). Three more proteins (31, 63 and 88 kDa) have also been identified by *in vitro* translation of RRSV genomic dsRNA, and designated as non-structural proteins. RRSV S5, S8 and S9, respectively, encode a 90 kDa minor structural protein (possibly a guanylyltransferase), a 67 kDa major structural protein, which is further self-processed to 46, 43 and 26 kDa proteins, and a 38 kDa major structural protein. P9 is thought to be involved in vector transmission. RRSV segments S7 and S10 encode non-structural proteins of about 68 and 32 kDa, respectively. RRSV S4 probably encodes an RdRp and a second protein of unknown function. ERSV particles have four major structural proteins (127, 123, 63 and 34 kDa) and three minor proteins (103, 50 and 49 kDa). The reported differences in morphology of the outer capsids of RRSV and ERSV could be at least partially due to differences in the sizes of these structural proteins.

### Lipids

None reported.

### Carbohydrates

There is no evidence for the glycosylation of oryzavirus proteins.

## Genome organization and replication

The genome organization is well characterized only for RRSV (Table 5). The dsRNA genome segments contain a single large ORFs (in one strand of the pair) except S4, which contains two large ORFs. The proteins encoded by S3, S8 and S9 are major components of the RRSV particle, but those encoded by segments S7 and S10 are not found in the virion. Seg8 codes for a polyprotein that appears to autocatalytically cleave into at least two polypeptides one of which is a major structural protein. The larger protein encoded by Seg4 appears to be an RdRp. The tentative functions of the proteins encoded by the other segments are shown in Table 5. The viruses induce viroplasms in the cytoplasm of infected cells.

## Antigenic properties

RRSV and ERSV cross-react in serological tests. Polyclonal antisera raised against RRSV particle preparations react most strongly with P3, P8 and P9 (both the native state and the state resulting from *in vitro* production), suggesting that they are highly immunogenic. P5 is weakly immunogenic.

**Table 5:** Genome segments and protein products of rice ragged stunt virus

| Genome segment | Size (bp) | Protein nomenclature | Protein Mr predicted (kDa) | Protein Mr apparent (kDa) | Function (location) |
|---|---|---|---|---|---|
| Seg1 | 3849 | P1 | 137.7 | 137 | Virus core associated (B Spike) |
| Seg2 | 3810 | P2 | 133.1 | 118 | (Inner core capsid) |
| Seg3 | 3699 | P3 | 130.8 | 130 | (Major core capsid) |
| Seg4 | 3823 | P4A (Pol) | 141.4 | 145 | RDR polymerase |
| | | P4B | 36.9 | | (Unknown) |
| Seg5 | 2682 | P5 (Cap) | 91.4 | 90 | Capping enzyme/ guanyltransferase |
| Seg6 | 2157 | P6 | 65.6 | | |
| Seg7 | 1938 | NS7 | 68 | 66 | (Nonstructural) |
| Seg8 | 1814 | P8 | 67.3 | 67 | Precursor Protease (major capsid) |
| | | P8A/ P8B | 25.6/41.7 | 47/44 | |
| Seg9 | 1132 | P9 | 38.6 | 37 | Vector transmission (spike) |
| Seg10 | 1162 | NS10 | 32.3 | 32 | Non-structural |

Glutathione-S-transferase-NSP7 fusion protein is highly immunogenic, and antibodies against this protein are useful in ELISA for the detection of RRSV in infected plants and insects.

## Biological properties

Oryzaviruses infect plants in the family Gramineae, causing diseases in rice (RRSV) and species of *Echinochloa* (barnyard grasses and millets; ERSV). They are transmitted by, and replicate in, phloem-feeding, viruliferous delphacid planthoppers (RRSV: *Nilaparvata lugens*; ERSV: *Sogatella longifurcifera and S. vibix*). RRSV is ingested when the hopper feeds on rice plants, usually at the seedling stage. The minimum acquisition access period for the vector is about 3 h, the latent period is about 9 days, and the minimum inoculation access time is about 1 h. Planthopper nymphs are more efficient vectors than adults, but all forms of the insect can act as vectors. Any individual viruliferous hopper gives intermittent transmission. The virus is not passed though the egg.

Oryzaviruses appear to replicate in fibrillar viroplasms within the cytoplasm of phloem, or phloem-associated, plant cells and in cells of the salivary glands, fat body, gut and brain of the planthopper. The phloem cells proliferate to form galls on the plant. RRSV has been reported in southeastern and far-eastern Asian countries, where it affects rice yields (generally 10–20% loss, but up to 100% in severely affected areas). ERSV has been reported in Taiwan.

## Species demarcation criteria in the genus

In addition to the general criteria used throughout the family, species in the genus *Oryzavirus* differ in vector (planthopper) and host plant species.

## List of species in the genus *Oryzavirus*

*Echinochloa ragged stunt virus*
{Sogatella longifurcifera, S. vibix}
{Graminae: Echinochloa}

| | | |
|---|---|---|
| Echinochloa ragged stunt virus | | (ERSV) |
| *Rice ragged stunt virus* | | |
| {Nilaparvata lugens} | | |
| {Graminae: Rice} | | |
| Rice ragged stunt virus - Thailand | [Seg1: AF020334; Seg2: AF020335; Seg3:AF020336; Seg4: U66714; Seg5: U33633; Seg6: AF020337, Seg7: U66713; Seg8: U46682; Seg9: L38899; Seg10: U66712] | (RRSV-Tai) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ], insect vector and host names { } and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Oryzavirus* but have not been approved as species

None reported.

### Phylogenetic relationships within the genus

No information.

## GENUS *FIJIVIRUS*

Type species ***Fiji disease virus***

### Distinguishing features

Fijivirus particles have a double-shelled, icosahedral structure, with a spherical rather than angular appearance and short surface spikes (A spikes) on each of the 12 vertices of the icosahedron. The outer shell is fragile and easily breaks down, leaving the inner shell bearing 12 B spikes. There are 10 genome segments. The viruses replicate in delphacid planthoppers. Nilaparvata lugens reovirus (NLRV) has the above properties but replicates only in insects, whereas other fijiviruses can also replicate in phloem cells of susceptible plants of the families Gramineae (in which they induce small tumors or enations), or Liliaceae.

### Virion properties

#### MORPHOLOGY

Virions are double-shelled, spherical, 65–70 nm in diameter with A spikes of about 11 nm in length and breadth, at the 12 vertices on the icosahedra (Figure 9, left). Unless pre-fixed, viruses readily break down *in vitro* to give cores, about 55 nm in diameter, with 12 B spikes, about 8 nm long and 12 nm in diameter (Figure 9, right). Some treatments (shaking with butan-1-ol or incubation with 1.9 M $MgCl_2$) produce smooth subcores (Figure 9, center).

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

The physicochemical properties of the virions have not been determined.

#### NUCLEIC ACID

Fijiviruses have 10 dsRNA segments that are numbered in order of increasing electrophoretic mobility during PAGE. Some segments do not migrate in order of their Mr and may migrate in a reverse order during (1%) agarose gel electrophoresis (AGE). Examples include segments 2 and 3 of NLRV and Mal de Rio Cuarto virus (MRCV) and segments 8 and 9 of oat sterile dwarf virus (OSDV). The conserved terminal sequences are shown in Table 6. Within the genus, only the 3′-terminal sequence GUC-3′ is conserved. Adjacent to the conserved terminal oligonucleotide sequences, each genome segment possesses inverted repeats, which are several bases long, similar to those in phytoreovirus and oryzavirus RNAs, although the sequences involved differ in these other genera. Characteristic of the genus is the low G+C content of the genomic RNAs, mostly around 34–36%.

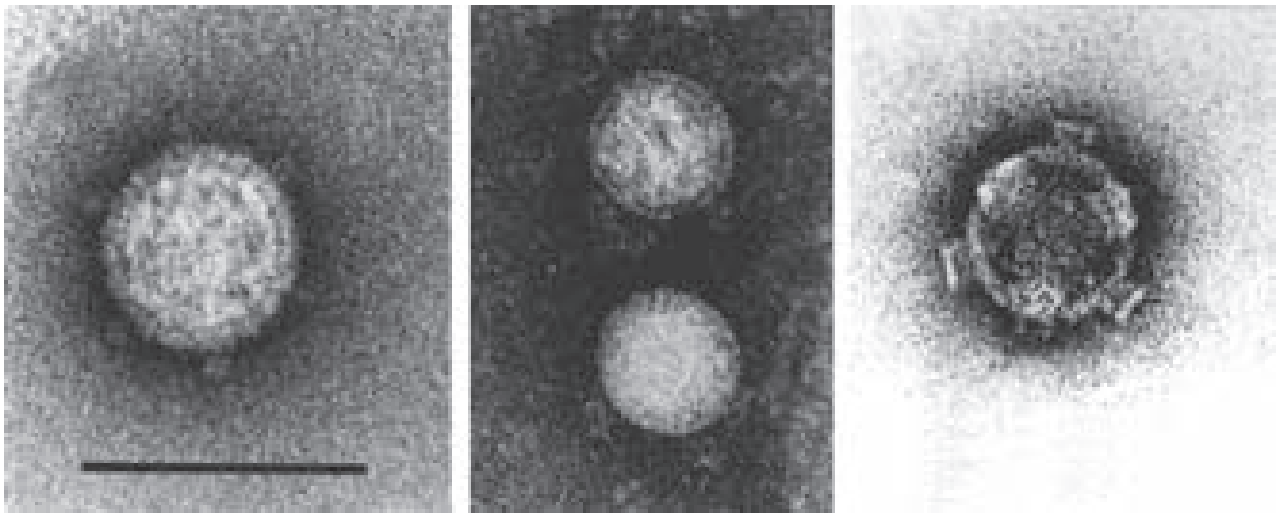

**Figure 9:** (Left) Negative contrast electron micrograph of maize rough dwarf virus (MRDV) virions stained with uranyl acetate showing "A" spikes; (center) smooth subcores derived from MRDV on staining with neutral phosphotungstate; (right) "B" spikes on virus-derived MRDV cores stained with uranyl acetate (courtesy of R. G. Milne). The bar represents 100 nm.

**Table 6:** Conserved terminal sequences (positive strand) of *Fijivirus* genome segments

| Virus species | Strain | 5′ end | 3′ end |
|---|---|---|---|
| *Fiji disease virus* | FDV | 5′-AAGUUUUU | CAGCNNNNGUC-3′ |
| *Maize rough dwarf virus* | MRDV | 5′-AAGUUUUUU | UGUC-3′ |
| *Mal de Rio Cuarto virus* | MRCV | 5′-AAGUUUUU | CAGCUNNNGUC-3′ |
| *Nilaparvata lugens reovirus* | NLRV | 5′-AGU | GUUGUC-3′ |
| *Oat sterile dwarf virus* | OSDV | 5′-AACGAAAAAAA | UUUUUUUUAGUC-3′ |
| *Rice black streaked dwarf virus* | RBSDV | 5′-AAGUUUUU | AGCUNN($^{C}/_{U}$)GUC-3′ |
| (not classified) | SRBSDV | 5′-AAGUUUUU | CAGCUGAUGUC-3′ |

The sizes and groupings of the 10 dsRNA species are characteristic and distinctive for the five groups of fijiviruses that are recognized.

### Proteins

Six polypeptides, numbered respectively I to VI (139, 126, 123, 111, 97 and 64 kDa), can be detected by SDS PAGE of purified MRDV. The B-spiked cores contain peptides I, II and III, while the smooth core contains peptides I and II. The B spikes should therefore be composed of peptide III. Peptides IV–VI form the outer capsid. During infection of most (possibly all) fijiviruses, tubules about 90 nm in diameter accumulate in the cytoplasm. Sometimes these are incompletely closed and form scrolls. They are presumably composed of a non-structural protein whose function and genome segment assignment are unknown.

Three major proteins (130, 120 and 56 kDa) and three minor ones (148, 65 and 51 kDa) can be detected by SDS PAGE of purified virions of Rice black streaked dwarf virus (RBSDV). The 120 kDa protein is the B spike protein. Smooth subcore particles consist of 148, 130 and 65 kDa proteins. The 56 kDa protein is the major component of the outer capsid shell and the 51 kDa protein is a partial degradation of it. In NLRV virions, three major proteins (140, 135 and 65 kDa), three intermediate (160, 110 and 75 kDa), and one minor protein (120 kDa) can be resolved. The 135 kDa protein is the B spike. The 65 kDa protein is the major component of the outer capsid shell and the 140 kDa protein is the major core protein. In addition to the above structural proteins, there is an A spike but its protein has not yet been identified.

**Table 7:** Genome segments and protein products of Fiji disease virus. The equivalent segment numbers for other members of the genus *Fijivirus* are also shown

| Genome segment | Size (bp) | Protein Mr predicted (kDa) | Location (function)* | Homologous segment in other members of the genus | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | MRCV | MRDV | OSDV | RBSDV | NLRV |
| Seg1 | 4532 | 170.6 | Core (RNA polymerase) | 1 | na | na | 1 | 1 |
| Seg2 | 3820 | 137.0 | Major core | 3 | na | na | 2 | 3 |
| Seg3 | 3623 | 135.5 | Outer shell (possible B spike) | 2 | na | na | 4 | 2 |
| Seg4 | 3568 | 133.2 | Unknown | 4 | na | na | 3 | 4 |
| Seg5 | 3150 | 115.3 | Unknown | 5 | na | na | 5 | |
| Seg6 | 2831 | 96.8 | Unknown | 6 | na | na | 6 | |
| Seg7 | 2194 | 41.7 | Non-structural (possible tubule protein) | 7 | 6 | 7 | 7 | 10** |
| | | 36.7 | Unknown | | | | | |
| Seg8 | 1959 | 68.9 | Core protein (possible NTP-binding) | 8 | 7 | 9 | 8 | 7 |
| Seg9 | 1843 | 38.6 | Viroplasm | 9 | 8 | 10 | 9 | |
| | | 23.8 | Non-structural protein? | | | | | |
| Seg10 | 1819 | 63.0 | Major outer capsid | 10 | 10 | 8 | 10 | 8 |

*The probable function of some of the proteins has been deduced from the equivalent genome segment of other virus species.
**Genome Seg10 of NLRV does not contain a second ORF.



The Fiji disease virus (FDV) VP9a equivalent in MRCV is designated P9-1. This protein was shown to establish cytoplasmic inclusion bodies resembling viral inclusion bodies, after transfection of *Spodoptera frugiperda* insect cells. P9-1 self-associates, giving rise to high molecular weight complexes when expressed in bacteria. P9-1 binds ssRNA and possesses an ATPase activity.

### Lipids
Not known.

### Carbohydrates
Not known.

## Genome organization and replication

Genome organizations and coding assignments of fijiviruses are summarized in Table 7. Most of the genome segments are monocistronic. Some segments possess two ORFs but expression of the second ORF has not been demonstrated *in vivo* in insect or plant cells. For viruses other than NLRV, replication occurs in the cytoplasm of phloem-related cells in association with viroplasms composed partly of fine filaments. NLRV does not have a counterpart to the ORF2 present in FDV Seg7 (and the corresponding segments of other plant-infecting fijiviruses), and this may reflect its inability to replicate in plant hosts.

### Antigenic properties
Some proteins of the viruses in group 2 (MRCV, MRDV, Pangola stunt virus (PaSV) and RBSDV) are distantly related but homologous proteins from viruses of other species in the genus are serologically unrelated.

## Biological properties

All the plant-infecting fijiviruses induce hypertrophy of the phloem (both expansion and multiplication of cells), leading to vein swellings and sometimes galls (enations or tumors) derived from

phloem cells, especially on the backs of leaves. MRDV in maize induces longitudinal splitting of the roots. Other effects include the suppression of flowering, plant stunting, increased production of side shoots, and induction of a dark green coloring. In insect hosts, no particular tissue tropism or severe disease is recognized. Viruses are transmitted propagatively by delphacid planthoppers (Hemiptera, Delphacidae, e.g. *Perkinsiella*, *Laodelphax*, *Toya*, *Sogatella*, *Javesella*, *Ribautodelphax*, *Dicranotropis*, *Delphacodes*, *Sogatella* and *Unkanodes*). Following virus acquisition from infected plants, the latent period is about two weeks, and leads to a lifelong capacity for virus transmission to plants. No transovarial or seed transmission of virus has been identified. Mechanical transmission from plant to plant can be demonstrated only with difficulty. Virus is spread by offsets in vegetatively propagated crops (e.g. pangolagrass and sugarcane). Viruses can overwinter in diapausing planthoppers, in certain weed species and in autumn-sown cereals.

Generally, fijiviruses are widespread in nature, although they are apparently absent from North America and have not been reported from Africa or confirmed from India. FDV has been reported from Australia and the Pacific islands. RBSDV occurs in Japan, Korea and China. PaSV occurs in northern countries of South America, Oceania, Taiwan and northern Australia, and OSDV occurs in northern Europe. Garlic dwarf virus (GDV) has been found only in southern France. MRDV is found in Scandinavia and in areas bordering the northern and eastern Mediterranean. MRCV occurs in Argentina.

NLRV was found in the planthopper *Nilaparvata lugens*, which occurs in south-east Asia. Experimentally it infects a second hopper, *Laodelphax striatellus*. There is no evidence that NLRV can multiply in rice plants, a natural host of *N. lugens*, but the virus is transmitted from hopper to hopper through contaminated rice plants and moves through the phloem and/or xylem of rice plants once injected by the viruliferous hoppers.

## Species demarcation criteria in the genus

Of the seven fijivirus species, the four members of group 2 are relatively closely related to one another. Further information about these viruses may eventually necessitate a revision of their species status. In particular, MRDV and RBSDV may be considered sufficiently closely related to constitute a single species.

The conserved terminal sequences of genome segments do not differ greatly between fijivirus species (Table 6). In addition to the other general criteria used throughout the family, members of a species in the genus *Fijivirus* may be identified by:

- Sequence analysis: members of different species usually have <40% amino acid identity in comparisons of proteins corresponding to those encoded by RBSDV segments 7, 8, 9 and 10). In comparisons among the genome segments coding for the major capsid protein, viruses from different groups have <55% nucleotide identity (but identities are much higher within group 2).
- Cross-hybridization of less conserved genome segments (Segs-10 of MRDV and RBSDV, encoding a highly conserved major outer shell protein, share 94% nucleotide sequence and so are not suitable). Hybridization using RBSDV Seg5 and Seg6 cDNA probes to detect the homologous sequences is more than 20 times more sensitive than hybridization using their counterparts from MRDV.
- Serological cross-reactions: viruses in different groups do not cross-react; those in group 2 do so to a limited extent that is dependent on the proteins being compared.
- The identity or family of the plant host species (if any) together with the insect vector and its host.

## List of species in the genus *Fijivirus*

**Fijivirus group 1**

*Fiji disease virus*

| | | |
|---|---|---|
| Fiji disease virus {*Perkinsiella saccharicida*, *P. vastatrix*, *P. vitiensis*: *Graminae*} | [Seg1: AY029520, Seg2: AF049704, Seg3: AF359556, Seg4: AF049705, Seg5: AY029521, Seg6: AF356083, Seg8: AY297693, Seg9: AF050086, Seg10: AY297694] | (FDV) |

| | | |
|---|---|---|
| **Fijivirus group 2** | | |
| *Rice black streaked dwarf virus* | | |
| Rice black streaked dwarf virus-ZJ {*Laodelphax striatellus, Ribautodelphax albifascia, Unkanodes sapporona: Graminae*} | [Seg1: AJ294757, Seg2 AJ409145, Seg3: AJ293984, Seg4: AJ409146, Seg5: AJ409147, Seg6: AJ409148, Seg7: AJ297427, Seg8: AJ297431, Seg9: AJ297430, Seg10: AJ297433] | (RBSDV-ZJ) |
| *Maize rough dwarf virus* | | |
| Maize rough dwarf virus-Italy {*Ribautodelphax notabilis*: *Graminae*} | [Seg6: X55701, Seg7: L76562, Seg8: L76561, Seg10: L76560] | (MRDV-IT) |
| *Mal de Rio Cuarto virus* | | |
| Mal de Rio Cuarto virus – Argentina {*Delphacodes kuscheli: Graminae*} | [Seg1: AF499925, Seg2: AF499926, Seg3: AF499928, Seg4: AF395873, Seg6: AF499927, Seg7: AY923115, Seg8: AF395872, Seg9: DQ023312, Seg10: AY607586] | (MRCV-ARG) |
| *Pangola stunt virus* | | |
| Pangola stunt virus {*Sogatella furcifera S. kolophon*: *Graminae*} | | (PaSV) |
| **Fijivirus group 3** | | |
| *Oat sterile dwarf virus* | | |
| Oat sterile dwarf virus {*Javesella pellucida, J. discolor, J. dubia, J. obscurella, Dicranotropis hamata: Graminae*} | [Seg7: AB011024, Seg8: AB011025, Seg9: AB011026, Seg10: AB011027] | (OSDV) |
| **Fijivirus group 4** | | |
| *Garlic dwarf virus* | | |
| Garlic dwarf virus {unknown: *Liliaceae*} | | (GDV) |
| **Fijivirus group 5** | | |
| *Nilaparvata lugens reovirus* | | |
| Nilaparvata lugens reovirus {*Nilaparvata lugens, Laodelphax striatellus:* no plant hosts | [Seg1: D49693, Seg2: D49694, Seg3: D49695, Seg4: D49696, Seg5: D49697, Seg6: D49698, Seg7: D49699, Seg8: D26127, Seg9: D49700, Seg10: D14691] | (NLRV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ], insect vector and host names { } and assigned abbreviations ( ) are also listed.



### List of other related viruses which may be members of the genus *Fijivirus* but have not been approved as species

| | | |
|---|---|---|
| Southern rice black streaked dwarf virus | [Seg1: FN563983, Seg2: FN563984, Seg3: FN563985, Seg4: FN563986, Seg5: FN563987, Seg6: FN563988, Seg7: EU784841, Seg8: EU784842, Seg9: EU784843, Seg10: EU784840] | (SRBSDV) |

### Phylogenetic relationships within the genus

Phylogenetic relationships within the genus are shown in Figure 10.

## GENUS *MYCOREOVIRUS*

Type species ***Mycoreovirus 1***

### Distinguishing features

Virions have a relatively featureless outer capsid as viewed by negative staining and electron microscopy, whereas the core particles have 12 icosahedrally arranged surface turrets or spikes. The genome is composed of 11 or 12 segments of dsRNA. The members of the genus that have been described all infect fungi.

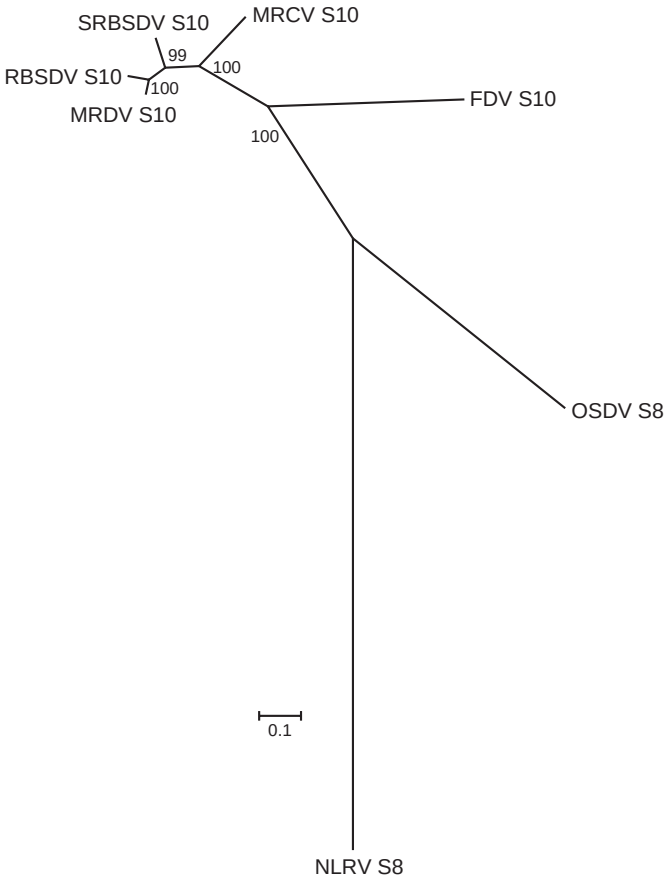


**Figure 10:** Neighbor-joining phylogenetic tree constructed using the amino acid sequences of outer capsid proteins of fijiviruses. (Fiji disease virus, FDV (Seg10) [AY297694]; maize rough dwarf virus, MRDV (Seg10) [L76560]; rice black streaked dwarf virus, RBSDV (Seg10) [D00606]; oat sterile dwarf virus, OSDV (Seg8) [AB011025]; Nilaparvata lugens reovirus, NLRV (Seg8) [D26127]; mal de Rio Cuarto virus, MRCV (Seg10) [AY607586]; southern rice black streaked dwarf virus, SRBSDV (Seg10) [EU784840]). Sequences were aligned using ClustalX and the tree constructed using MEGA4 (Dayhoff distances and 10,000 bootstrap replicates).

## Virion properties

### Morphology

Particles are non-enveloped. Electron microscopy and negative staining of mycoreovirus virions with aqueous uranyl acetate indicates that they are double shelled, spherical in appearance (icosahedral symmetry) and approximately 80 nm in diameter. The viral core (estimated as 50 nm in diameter) has 12 icosahedrally arranged surface projections (turrets or B-spikes) (Figure 11). Particles are disrupted by 2% phosphotungstic acid (pH 7.0).

### Physicochemical and physical properties

Not determined.

### Nucleic acid

The genome consists of 11 (group 1) or 12 (group 2) dsRNA segments that are numbered in order of reducing molecular weight or increasing electrophoretic mobility following agarose gel electrophoresis. The total genome of Cryphonectria parasitica mycoreovirus-1 (CpMRV-1) contains 23,436 bp, with the length of individual segments ranging from 732 bp to 4127 bp, showing a 3-3-2-3 electrophoretic profile following either 11% PAGE or 1% agarose gel electrophoresis (AGE). In contrast, the genomic RNA of Rosellinia necatrix mycoreovirus-3 (RnMRV-3) shows a 3-3-6 electrophoretic profile following 5% PAGE. As with other members of the family *Reoviridae*, the genome segment

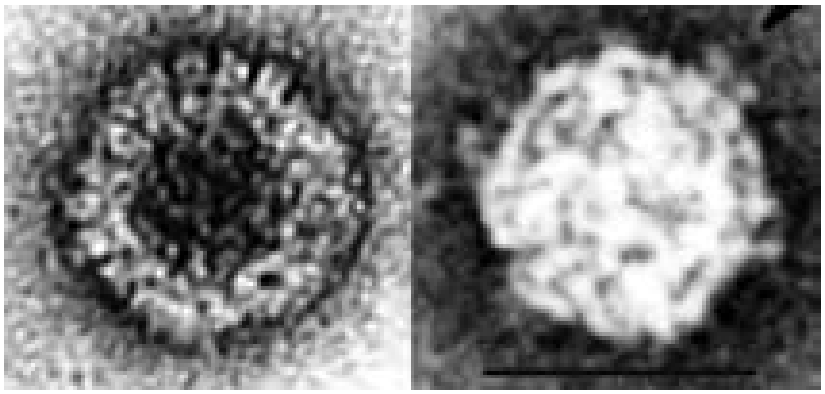

**Figure 11:** (Left) Electron micrograph of virus particles of Cryphonectria parasitica mycoreovirus-1 (CpMYRV-1) after purification by sucrose gradient centrifugation, stained with 1% uranyl acetate (courtesy of B. Hillman). (Right) Core particle of Rosellinia necatrix mycoreovirus-3 (RnMYRV-3) showing icosahedral arrangement surface projections (turrets or spikes), stained with 1% uranyl acetate (courtesy of C. Wei). The bar (right panel) represents 50 nm.

**Table 8:** Conserved terminal sequences (positive strand) of mycoreovirus genome segments

| Virus species | Serotype or strain | 5′ end | 3′ end |
|---|---|---|---|
| *Mycoreovirus 1* | CpMYRV-1-9B21 | 5′-GAUCA | CGCAGUCA-3′ |
| *Mycoreovirus 3* | RnMYRV-3-W370 | 5′-ACAAUUU | UGCAGAC-3′ |



migration patterns during AGE (or low percentage (<5%) PAGE) are considered likely to be characteristic of each virus species. Terminal sequences of the genome segments are shown in Table 8.

### Proteins

Protein sequences of Cryphonectria parasitica mycoreovirus-1 (CpMYRV-1) were deduced from sequences of the viral genomic RNAs. Their putative functions are shown in Table 9. Proteins of the 11 and 12 segmented mycoreoviruses are currently named as VP1 to VP11 or VP12, based on the molecular weight of the genome segment (segment number) from which they are translated. Proteins of CpMYRV-1 were expressed in baculovirus, and the capping enzyme was identified by an autoguanylation assay as VP3 (encoded by Seg3). A series of progressive N-terminal and C-terminal deletion mutants were also made to localize the auto-guanylation active-site of VP3 to amino acid residues 133–667. Within this region, a sequence was identified (residues 170–250) that has relatively high sequence similarity to homologues in the two other mycoreoviruses, CpMYRV-2 and Rosellinia necatrix mycoreovirus-3 (RnMYRV-3), as well as two coltiviruses, Colorado tick fever virus and Eyach virus. Site-directed mutagenesis of conserved residues revealed that H233, H242, Y243, F244 and F246, but not K172 or K202, play critical roles in guanylyltransferase activities.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

The sizes and predicted ORFs for the 11 segments of CpMYRV-1 and the 12 segments of RnMYRV-3 are shown in Tables 9 and 10, respectively. On the basis of the available sequence data for several of the genome segments and the overall similarity of mycoreoviruses to other members of the family *Reoviridae*, it is assumed that many aspects of the genome organization and replication are also

**Table 9:** Genome segments and protein products of Cryphonectria parasitica mycoreovirus-1

| Genome segment | Size (bp) | Protein nomenclature | Protein size aa (kDa) | Structure/function |
|---|---|---|---|---|
| Seg1 | 4127 | VP1 | 1354 (151.8) | RdRp (Pol); sequence similarity to coltivirus VP1 |
| Seg2 | 3846 | VP2 | 1238 (138.5) | Sequence similarity to coltivirus VP2 |
| Seg3 | 3251 | VP3 | 1065 (120.8) | Guanylyltransferase (CaP) |
| Seg4 | 2269 | VP4 | 721 (79.8) | Sequence similarity with RnMYRV-1-W370 Seg4 and coltivirus Seg4 |
| Seg5 | 2023 | VP5 | 648 (72.8) | |
| Seg6 | 2056 | VP6 | 650 (73.4) | Sequence similarity with RnMYRV-1-W370 Seg6 and coltivirus Seg10 |
| Seg7 | 1536 | VP7 | 482 (54.1 | |
| Seg8 | 1539 | VP8 | 470 (51.2 | |
| Seg9 | 1072 | VP9 | 298 (32.9) | Sequence similarity with RnMYRV-1-W370 Seg11 |
| Seg10 | 975 | VP10 | 248 (27.8 | |
| Seg11 | 732 | VP11 | 102 (11.5) | |

**Table 10:** Genome segments and protein products of Rosellinia necatrix mycoreovirus-3

| Genome segment | Size (bp) | Protein nomenclature | Protein size aa (kDa) | Structure/function |
|---|---|---|---|---|
| Seg1 | 4143 | VP1 | 1360 (153.4) | |
| Seg2 | 3773 | VP2 | 1226 (138.5) | Shows some sequence similarity to coltivirus VP2 |
| Seg3 | 3310 | VP3 | 1086 (121.9) | |
| Seg4 | 2259 | VP4 | 725 (78.7) | |
| Seg5 | 2089 | VP5 | 646 (72.3) | |
| Seg6 | 2030 | VP6 | 634 (71.5) | |
| Seg7 | 1509 | VP7 | 482 (55.1) | |
| Seg8 | 1299 | VP8 | 325 (36.5) | |
| Seg9 | 1226 | VP9 | 380 (41.6) | |
| Seg10 | 1171 | VP10 | 310 (33.6) | |
| Seg11 | 1003 | VP11 | 282 (31.1) | |
| Seg12 | 943 | VP12 | 265 (29.2) | |

similar. On this basis it is likely that the viral core contains transcriptase complexes that synthesize mRNA copies of the individual genome segments, which are exported and translated to produce viral proteins within the host cytoplasm. These positive sense RNAs also are likely to form templates for negative strand synthesis during progeny virus assembly and maturation. As with other reoviruses, most of the mycoreovirus genome segments appear to represent single genes, with a large ORF and relatively short terminal non-coding regions.

## Antigenic properties

Not available.

## Biological properties

RnMYRV-1 is found in the mycelium of a strain of the white root rot fungus *Rosellinia necatrix*. The virus itself appears to make the fungus hypovirulent and may represent a useful biological control for the damage caused by the wild-type fungus. The uninfected fungus can be regenerated by hyphal tip culture. CpMYRV-1 and CpMYRV-2 are found in the mycelium of the filamentous fungus that causes chestnut blight disease (*Cryphonectria parasitica*). Purified particles of CpMYRV-1 can be used to infect protoplasts of virus-free mycelium. Infection with CpMYRV-1 greatly reduces virulence of the fungal strain and may represent a useful biological control for the disease.

## Species demarcation criteria in the genus

See the general criteria used throughout the family.

## List of species in the genus *Mycoreovirus*

| | | |
|---|---|---|
| **Group 1** (11 genome segments) | | |
| *Mycoreovirus 1* | | |
| Cryphonectria parasitica mycoreovirus-1 (9B21) | [Seg1: AY277888, Seg2: AY277889, Seg3: AY277890, Seg4:AB179636, Seg5: AB179637, Seg6: AB179638, Seg7: AB179639, Seg8: AB179640, Seg9: AB179641, Seg10: AB179642, See11: AB179643] | (CpMYRV-1 / 9B21) |
| *Mycoreovirus 2* | | |
| Cryphonectria parasitica mycoreovirus-2 (C18) | [Seg3: DQ902580] | (CpMYRV-2 / C18) |
| **Group 2** (12 genome segments) | | |
| *Mycoreovirus 3* | | |
| Rosellinia necatrix mycoreovirus-3 (W370) (Rosellinia anti-rot virus) | [Seg1: AB102674, Seg2: AB098022, Seg5: AB098023, Seg6: AB073277, Seg7: AB073278, Seg8: AB073279, Seg9: AB073280, Seg10: AB073281, Seg11: AB073282, Seg12: AB073283] | (RnMYRV-3 / W370) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed

## List of other related viruses which may be members of the genus *Mycoreovirus* but have not been approved as species

None reported.

## Phylogenetic relationships within the genus and with other genera of family *Reoviridae*

Both *C. parasitica* mycoreoviruses (CpMYRV-1 and CpMYRV-2) contain 11 genome segments (Group 1), but only have approximately 29% amino acid sequence identity in the capping enzyme, confirming their identity as distinct species.

There are also some clear indications of homology in the larger genome segments of the Group 1 mycoreoviruses to RnMYRV-3 (Group 2, 12 segment genome). The highest amino acid sequence identity was detected in the polymerase sequence (39% identity).

Previous phylogenetic analyses, using the RdRp amino acid sequence (see section on the family *Reoviridae*), have shown that, in most cases, members of different reovirus genera exhibit amino acid sequence identities of less than 30% although aquareoviruses and orthoreoviruses can show up to 42% aa identity.

CpMYRV-1 shows about 29% amino acid sequence identity with the polymerase of members of the genus *Coltivirus*.

# Genus *Cypovirus*

Type species ***Cypovirus 1***

## Distinguishing features

Cypovirus particles may be singly or multiply occluded by a virus-coded polyhedrin protein, which forms polyhedra within the cytoplasm of infected cells. Cypoviruses only infect, and are pathogenic for, arthropods. Virions have a single capsid shell with surface spikes, and have transcriptase and capping enzymes that are active without particle modification. They can retain RNA polymerase activity despite particle disruption into 10 distinct RNA protein complexes, each representing a single genome segment and a transcriptase complex. Consequently, transcriptase activity is resistant to repeated freeze–thawing, which disrupts the particle structure. The transcriptase activity may show very pronounced dependence on the presence of S-adenosyl-L-methionine or related compounds, although this dependence may be reduced by repeated freeze–thawing.

## Virion properties

### Morphology

Virus particles have a single-layered capsid, composed of a central capsid shell of 57 nm diameter, which extends to 71.5 nm (determined by cryoEM) when the 12 surface spikes or turrets that are situated on the icosahedral five-fold vertices are included. These surface projections are hollow and have previously been estimated to be up to 20 nm in length and 15–23 nm wide (by conventional microscopy and negative staining). They also appear to have a section near the tip that can be lost or removed. The virus particle has a central compartment about 35 nm in diameter. Cypovirus virions are structurally comparable to the core particles of members of other genera within the family *Reoviridae,* particularly genera containing viruses spiked cores (*Orthoreovirus*, *Aquareovirus*, *Idnoreovirus* and *Oryzavirus)* (Figures 12 and 13). The virus particles contain three major structural proteins that have been identified as: the capsid shell protein (CSP), 120 copies per particle, equivalent to the VP3(T2) protein of bluetongue virus, and orthoreovirus lambda 1; large protrusion protein (LPP, 120 copies, comparable to orthoreovirus lambda 3) and turret protein (TP, 60 copies, comparable to orthoreovirus lambda 2). The virion also contains transcriptase enzyme complexes attached to the inner surface of the capsid shell at the icosahedral five-fold vertices.

Cypovirus particles may be occluded by a crystalline matrix of polyhedrin protein, forming a polyhedral inclusion body. These polyhedra have a symmetry (e.g. cubic, icosahedral or irregular) that is influenced by both the virus strain (polyhedrin sequence) and the host. The polyhedrin

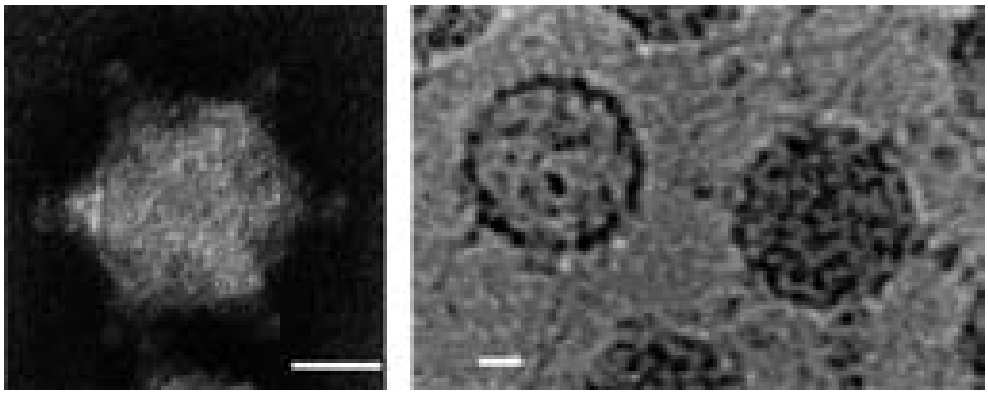

**Figure 12:** (Left) Negative contrast electron micrograph of a non-occluded virion of Orgyia pseudosugata cypovirus 5 (OpCPV-5). (Right) Negative contrast electron micrograph of empty and full "occluded" virions (purified from polyhedra) of OpCPV-5, stained with uranyl acetate (courtesy of C. L. Hill). The bars represent 20 nm.

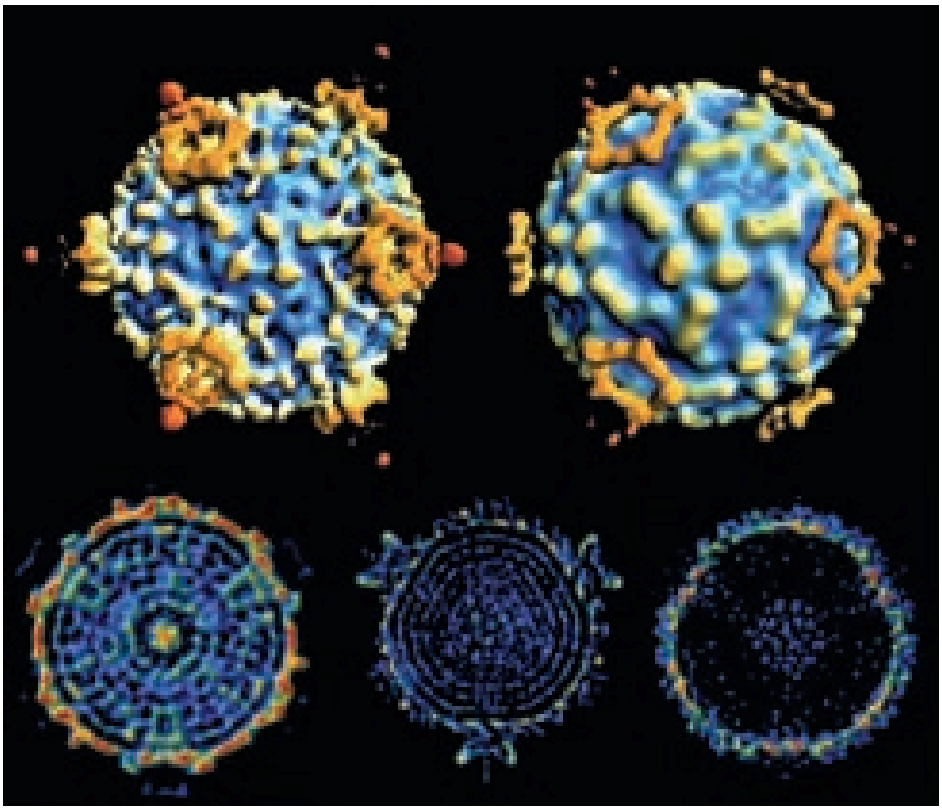

**Figure 13:** CryoEM reconstructions of Orgyia pseudosugata cypovirus 5 (OpCPV-5) virions, to 26 Å resolution: (top left) non-occluded virion; (top right) occluded virion; (bottom left) cross-section of a full occluded virion; (bottom center) cross-section of a full non-occluded virion; (bottom right) cross-section of an empty virion. The cross-sections show evidence of dsRNA packaged as distinct layers and suggest localization of the transcriptase complexes at the five-fold axes of symmetry. (Courtesy of C. L. Hill.)



protein appears to be arranged as a face-centered cubic lattice with center-to-center spacing varying between 4.1 and 7.4 nm.

### Physicochemical and physical properties

The virion Mr is about $5.4 \times 10^7$. The buoyant density in CsCl is 1.44 g cm$^{-3}$ for virions, approximately 1.30 g cm$^{-3}$ for empty particles, and 1.28 g cm$^{-3}$ for polyhedra. The $S_{20,W}$ is approximately 420S for virions and 260S for empty particles. Polyhedra vary considerably in size and Mr and do not have a single characteristic S value. Polyhedra may occlude many virus particles or only single particles depending on the virus strain. Large empty polyhedra (apparently containing no virions) have also been observed.

Cypoviruses retain infectivity for several weeks at −15 °C, 5 °C or 25 °C. The virus retains full enzymatic activity (dsRNA-dependent ssRNA polymerase and capping activity) after repeated freeze–thawing (up to 60 cycles). However, it appears likely that this results in the breakdown of the virus particle into ten active and distinct enzyme/template complexes. Each complex contains one genome segment and a complete transcriptase complex, derived from the virion capsid and including one of the spike structures from the vertices of the icosahedron. Polymerase activity is therefore a poor indicator of virion integrity. Within the family *Reoviridae*, the ability to retain enzyme function despite particle breakdown may be unique to the cypoviruses.

Cations have relatively little effect on the virus structure. Heat treatment of virions at 60 °C for 1 h leads to degradation and release of genomic RNA. Virus particles are relatively resistant to treatment with trypsin, chymotrypsin, ribonuclease A, deoxyribonuclease or phospholipase. Virion

enzyme functions also show some resistance to treatment with proteinase K. However, this may reflect the retention of enzyme activities despite particle disruption, particularly during the early stages of digestion. Cypovirus particles are resistant to detergents such as sodium deoxycholate (0.5–1%) but are disrupted by 0.5–1% SDS, which releases the genomic dsRNA. Treatment with Triton X-100, NP40 or urea also causes disruption of the virus particle structure. One or two fluorocarbon treatments have little effect on virus infectivity, and treatment with ethanol leads to release of RNA from virions. Viruses and polyhedra are readily inactivated by UV-irradiation. It has been reported that UV also releases the dsRNA template from individual genome segment/transcriptase complexes. Polyhedra remain infectious for years at temperatures below 20 °C. Virions can be released from polyhedra by treatment with carbonate buffer at pH >10.5 but are disrupted at pH <5. High pH treatment completely dissolves the polyhedral protein matrix, as in the mid-guts of permissive insects. This process is partly due to increased solubility of polyhedrin at high pH but is also aided by alkaline-activated proteases associated with polyhedra.

### Nucleic acid

Polyhedra (but not virions) contain significant amounts of adenylate-rich oligonucleotides. In the majority of cases, cypovirus particles contain ten linear dsRNA genome segments. However there is evidence to indicate that in some cases the virus particles may also contain an eleventh small segment (e.g. Trichoplusia ni cypovirus 15, TnCPV-15). In Bombyx mori cypovirus 1 (BmCPV-1), the genome segments vary in size from 4190 to 944 bp with a total genome size of 24,809 bp. In other isolates that have not yet been sequenced, the sizes of the genome segments have been estimated by electrophoretic comparisons and have calculated Mrs that vary from $0.42 \times 10^6$ to $3.7 \times 10^6$ (0.6 to 5.6 kbp) and a total genome Mr that varies from $19.3 \times 10^6$ to $22.0 \times 10^6$ (29.2 to 33.3 kbp).

The pattern of size distribution of the genome segments (Table 11) varies widely between different cypoviruses (e.g. the smallest dsRNA has an estimated size that varies between 530 and 1440 bp). These size differences have formed a basis for the recognition and classification of distinct species (electropherotypes) of cypoviruses (with patterns of dsRNA migration, which differ significantly in the migration of at least three genome segments, and frequently in the majority of segments, as analyzed by electrophoresis using 1% agarose or 3% SDS-PAGE). The genome segment migration patterns of members of species *Cypovirus 1*, *Cypovirus 12* and *Cypovirus 14* have some overall similarity, although in each case at least three segments show significant migrational differences during agarose gel electrophoresis. These viruses also show significant serological cross-reactions. More recently, it has been shown that members of different cypovirus species can also be distinguished on the basis of RNA sequence comparisons (e.g. by comparison of genome segment 10: the polyhedrin gene).

The termini of the coding strands are common or very closely related for each of the different genome segments within members of the species *Cypovirus 1*, but differ from those reported for other cypovirus species (Table 12). Choristoneura fumiferana cypovirus 16 (CfCPV-16) shows high levels of overall sequence variation when compared to members of species *Cypovirus 1*, *Cypovirus 2*, *Cypovirus 5*, *Cypovirus 14* or *Cypovirus 15* and is therefore considered to be in a different species (*Cypovirus 16*), although it has a similar 5′ and different 3′ end to representatives of species *Cypovirus 5*. These data demonstrate that different cypovirus electropherotypes are likely to, but may not always, have different conserved RNA terminal sequences.

### Proteins

Cypovirus particles generally contain five to six distinct proteins, two to three with sizes of more than 100 kDa. For BmCPV-1, the structural proteins have sizes in kDa (and are identified as): 148 (VP1), 136 (VP2), 140 (VP3), 120 (VP4), 64 (VP6) and 31 kDa (VP7) (see Table 13). Polyhedra also contain a 25–37 kDa polyhedrin protein (28.5 kDa for BmCPV-1) that constitutes about 95% of the polyhedra protein dry weight. Due to the very high level of variation between different cypoviruses, it is unlikely that their homologous proteins will be identifiable simply by their migration order during PAGE.

## LIPIDS

Cypoviruses are not known to contain any lipids in either virus particles or polyhedra.

### Carbohydrates

The polyhedrin protein is glycosylated.

**Table 11:** Cypovirus genome segment size distribution (kbp) determined by sequence analyses or estimated from electrophoretic comparisons of the genomic dsRNA of cypoviruses 1 to 20

| Genome segment number | Cypovirus types (1 to 20) | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** |
| Total genome | **24.8** | 25.5 | 26.7 | 27.5 | 26.3 | 27.2 | 25.6 | 27.0 | 24.1 | 27.6 | 25.5 | 26.1 | 25.2 | **25.3** | **24.9** | | **24.6** | **24.7** | **23.9** | **22.0** |
| 1 | **4.19** | 4.06 | 4.29 | 4.17 | 4.17 | 4.17 | 4.32 | 4.54 | 4.32 | 4.31 | 4.60 | 4.43 | 4.26 | **4.33** | **4.36** | | **3.87** | **4.17** | **4.17** | **3.70** |
| 2 | **3.86** | 4.06 | 4.12 | 4.17 | 4.17 | 4.06 | 4.15 | 4.54 | 4.18 | 4.31 | 4.40 | 4.12 | 4.26 | **4.06** | **4.19** | | **3.75** | **3.79** | **3.76** | **3.65** |
| 3 | **3.85** | 3.83 | 4.12 | 4.17 | 4.17 | 4.00 | 4.02 | 4.40 | 4.07 | 4.02 | 4.40 | 4.12 | 4.03 | **3.92** | **3.88** | | **3.58** | **3.79** | **3.64** | **3.60** |
| 4 | **3.26** | 3.65 | 3.69 | 3.90 | 3.69 | 3.72 | 3.81 | 3.92 | 3.62 | 4.02 | 3.83 | 3.67 | 3.60 | **3.34** | **3.31** | | **3.30** | **3.25** | **3.27** | **3.10** |
| 5 | **2.85** | 2.21 | 3.60 | 2.43 | 3.22 | 2.73 | 2.54 | 3.69 | 2.34 | 2.50 | 1.98 | 3.30 | 3.20 | **3.16** | **2.26** | | **2.40** | **2.88** | **2.11** | **2.20** |
| 6 | **1.80** | 1.93 | 2.29 | 2.17 | 2.17 | 2.36 | 2.27 | 1.90 | 1.72 | 2.29 | 1.98 | 2.00 | 1.60 | **1.78** | **1.86** | | **1.90** | **1.80** | **1.89** | **1.75** |
| 7 | **1.50** | 1.79 | 2.15 | 1.95 | 2.06 | 2.23 | 2.02 | 1.30 | 1.72 | 2.29 | 1.35 | 1.44 | 1.40 | **1.39** | **1.78** | | **1.85** | **1.47** | **1.70** | **1.40** |
| 8 | **1.33** | 1.56 | 1.08 | 1.72 | 1.21 | 1.63 | 1.08 | 1.19 | 0.78 | 1.69 | 1.27 | 1.27 | 1.14 | **1.25** | **1.23** | | **1.50** | **1.42** | **1.28** | **1.40** |
| 9 | **1.19** | 1.38 | 0.83 | **1.47** | 0.88 | 1.40 | 0.85 | 0.88 | 0,69 | 1.21 | 0.98 | 1.13 | 0.98 | **1.14** | **1.16** | | **1.50** | **1.18** | **1.18** | **1.25** |
| 10 | **0.99** | 0.98 | 0.60 | 1.44 | **0.88** | 0.90 | 0.53 | 0.65 | 0.69 | 0.99 | 0.71 | 0.64 | 0.78 | **0.96** | **0.90** | **1.171** | **0.90** | **0.93** | **0.87** | **0.85** |
| 11 | | | | | | | | | | | | | | | **0.20*** | | | | | |

Sizes in bold are derived from sequence analysis of the genome segment. Previously published estimates of genome segment sizes for members of *Cypovirus 2* to *Cypovirus 13* have been adjusted in line with base pair values derived from sequencing studies of cDNA copies of genome segments from BmCPV-1.
*TnCPV-15 has been reported to contain an 11th small genome segment (200 bp).

**Table 12:** Conserved terminal sequences (positive strand) of cypovirus genome segments

| Virus species | Strain | 5′ end | 3′ end |
|---|---|---|---|
| *Cypovirus 1* | BmCPV-1 | 5′-AGUAA | GUUAGCC-3′ |
| | DpCPV-1 | 5′-AGUAA | GUUAGCC-3′ |
| | LdCPV-1 | 5′-AGU$^{A}/_{G}{}^{A}/_{G}$ | $G^{U}/_{C}$UAGCC-3′ |
| *Cypovirus 2* | IiCPV-2 | 5′-AGUUUUA | UAGGUC-3′ |
| *Cypovirus 4*[*] | ApCPV-4 | 5′-AAUCGACG | GUCGUAUG-3′ |
| *Cypovirus 5* | OpCPV-5 | 5′-AGUU | UUGC-3′ |
| *Cypovirus 14* | LdCPV-14 | 5′-AGAA | CAGCU-3′ |
| *Cypovirus 15* | TnCPV-15 | 5′-AUUAAAAA | GC-3′ |
| *Cypovirus 16*[**] | CfCPV-16 | 5′-AGUUUUU | UUUGUGC-3′ |
| (not classified) | UsCPV-17 | 5′-AGAACAAA | UACACU-3′ |
| (not classified) | ObCPV-18 | 5′-AGUAAA$^{G}/_{U}/A^{C}/_{U}$ | $^{U}/_{C}{}^{A}/_{C}$GUUAGCU-3′ |
| (not classified) | ObCPV-19 | 5′AACAAA$^{A}/_{U}{}^{A}/_{U}$ | $^{A}/_{U}G^{A}/_{U}$UUUGC-3′ |
| (not classified) | SuCPV-20 | 5′-AGAAAAC | CAUGGC-3′ |
| (not classified) | MvCPV-21 | 5′-AUAUAAUU | AGUUAGU-3′ |

*Based on genome Seg9 only.
**Based on genome Seg10 only.

**Table 13:** Genome segments and protein products of Bombyx mori cypovirus 1 (strain I)

| Genome segment | Size (bp) | Protein[1] nomenclature ([2]) | Size (kDa) | Function (location) |
|---|---|---|---|---|
| Seg1 | 4190 | VP1 (VP1) | 148 | Major core CP (virion) |
| Seg2 | 3854 | VP2 (VP2) | 136 | RdRp (virion) |
| Seg3 | 3846 | (VP3) | 140 | (virion) |
| Seg4 | 3262 | VP3 (VP4) | 120 | Possible Mtr (virion) |
| Seg5 | 2852 | NS1 (NS5) | 101 | Non-structural, contains auto cleavage aa sequence, similar to FMDV $2A^{pro}$ |
| | | NS2 (NS5a) | 80[*] | |
| | | NS6 (NS5b) | 23[*] | |
| Seg6 | 1796 | VP4 (VP6) | 64 | Leucine zipper ATP/GTP binding protein (virion) |
| Seg7 | 1501 | NS3 | 50 (61[*]) | Non-structural, with "structural" cleavage products |
| | | NS4 | 58[*] | |
| | | (VP7) | | |
| Seg8 | 1328 | VP5 or P44 (NSP8) | 44 | Unknown (shows anomalous migration during PAGE, with apparent size 55 kDa) |
| Seg9 | 1186 | NS5 (NSP9) | 36 | Non-structural, dsRNA binding |
| Seg10 | 944 | Polyhedrin (Pod) | 28.5 | Polyhedron matrix protein (Pod) |

Size of genome segments and encoded proteins determined by sequence analysis of the genome segments.
*Sizes of some proteins estimated from electrophoretic migration.
[1]Protein nomenclature suggested by McCrae and Mertens (McCrae, M.A. and Mertens, P.P.C. (1983). *In vitro* translation studies on and RNA coding assignments for cytoplasmic polyhedrosis viruses. In: Compans, R.W. and Bishop, D.H.L. (eds.), *Double-stranded RNA Viruses*, NewYork: Elsevier Science, pp. 35–41.)
[2]Alternative nomenclature suggested by Hagiwara *et al.* (Hagiwara, K., Rao, S., Scott, S.W. and Carner, G. R. (2002). Nucleotide sequences of segments 1, 3 and 4 of the genome of Bombyx mori cypovirus 1 encoding putative capsid proteins VP1, VP3 and VP4, respectively. *J. Gen. Virol.*, **83**, 1477–1482.)

## Genome organization and replication

For BmCPV-1, the coding assignments are indicated in Table 13. The cognate genes of other cypoviruses are not known. The large variations in the sizes of genome segments between most cypoviruses (apart from members of *Cypovirus 1*, *Cypovirus 12* and *Cypovirus 14*) indicate that these assignments will not apply to other cypovirus species. Genome segment coding assignments generated by *in vitro* translation of individual denatured genome segment RNAs have been published for members of *Cypovirus 1* and *Cypovirus 2*. These data and subsequent sequencing studies indicate that, in many cases, polyhedrin may be encoded by the smallest segment.

Unlike orthoreoviruses, cell entry and initiation of cypovirus replication in insect cells does not require modification of virions for activation of core-associated transcriptase enzymes. Uptake appears to be a relatively inefficient process in cell culture, which can be very significantly improved by the use of liposomes. Virus replication and assembly occur in the host cell cytoplasm, although there is some evidence that viral RNA synthesis may also occur in the nucleus. Replication is accompanied by the formation of viroplasms (viral inclusion bodies or virogenic stroma) within the cytoplasm. Viroplasms contain large amounts of virus proteins and virus particles. How genome segments are selected for packaging and assembly into progeny particles is not known. The importance of the terminal regions in this process is indicated by the packaging and transcription of a mutant Seg10 from an isolate of *Cypovirus 1* that contained only 121 bp from the 5′ end and 200 bp from the 3′ end. Particles are occluded within polyhedra apparently at the periphery of the virogenic stroma, from about 15 h post infection onwards. The polyhedrin protein is produced late in infection and in large excess compared to other viral proteins. It is not known how polyhedrin synthesis is regulated.

## Antigenic properties

Serological cross-comparisons of cypovirus structural and polyhedrin proteins support the use of genomic dsRNA electropherotypes as one of the species parameters for the genus *Cypovirus.* Virus isolates within a single electropherotype exhibit high levels of antigenic cross-reaction (in both polyhedrin and virion structural proteins), as well as efficient cross-hybridization of denatured genomic RNA, even under high-stringency conditions. In contrast, there is evidence of little or no serological cross-reaction between viruses representing different electropherotypes. Exceptions are members of *Cypovirus 1* and *Cypovirus 12*, which show low level serological cross-reactions but have some overall similarity in electropherotype patterns and show a low level of cross-hybridization of their genome segments. *Cypovirus 14* members also show some similarity in RNA electropherotype patterns to viruses in both *Cypovirus 1* and *Cypovirus 12* and may therefore also show some antigenic relationship and RNA sequence similarity with these viruses.

## Biological properties

Cypoviruses have only been isolated from arthropods. Attempts to infect vertebrates or vertebrate cell lines have failed. In addition, cypovirus replication is inhibited at 35 °C. Even susceptible insect larvae treated with cypoviruses fail to develop infections at temperatures ≥35 °C. Cypoviruses are normally transmitted by ingestion of polyhedra on contaminated food materials. The polyhedra dissolve within the high pH environment of the insect gut and release occluded virus particles, which then infect the cells lining the gut wall. Virus infection in larvae is generally restricted to the columnar epithelial cells of the midgut, although goblet cells may also become infected. Cypovirus replication in the fat body has been reported. In larvae, the virus infection spreads throughout the midgut region. In some species the entire gut is occasionally infected. The production of very large numbers of polyhedra gives the gut a characteristically creamy-white appearance. In infected cells the endoplasmic reticulum is progressively degraded, mitochondria enlarge and the cytoplasm becomes highly vacuolated. In most cases, the nucleus shows few pathological changes. An exception is one little-studied cypovirus strain that produces inclusion bodies within the nucleus. In the later stages of infection, cellular hypertrophy is common and microvillae are reduced or completely absent. Very large numbers of polyhedra are released by cell lysis into the gut lumen and excreted. The gut pH is lowered during infection and this prevents dissolution of progeny polyhedra in the gut fluid.

The majority of cypovirus infections produce chronic disease, often without extensive larval mortality. Consequently, many individuals reach the adult stage even though heavily diseased. However,

cypovirus infections produce symptoms of starvation due to changes in the gut cell structure and reduced adsorptive capacity. Infected larvae stop feeding as early as 2 days post infection. Larval body size and weight are often reduced and diarrhea is common. The larval stage of the host can be significantly increased (about by 1.5 times the normal generation time). The size of infected pupae is frequently reduced, and the majority of diseased adults are malformed. They may not emerge correctly, and may be flightless. Infected females may exhibit a reduced egg-laying capacity.

Virus can be transmitted on the surface of eggs, producing high levels of infection in the subsequent generation. However, provided the egg surface is disinfected, no transovarial transmission has been observed. The infectious dose increases dramatically in the later larval instars. Different virus strains vary significantly in virulence. Larvae can recover from cypovirus infection, possibly because the gut epithelium has considerable regenerative capacity and because infected cells are shed at each larval molt.

## Species demarcation criteria in the genus

Cypoviruses are currently classified within 16 species, most of which were initially characterized by their distinctive dsRNA electropherotype patterns. Cross-hybridization analyses of the dsRNA, serological comparisons of cypovirus proteins and, more recently, comparison of RNA sequences have confirmed the validity of this classification and have identified new virus species. However, relatively few cypoviruses have been characterized, suggesting that there may be many more distinct species that are as yet unidentified.

The system of nomenclature currently used to identify different cypovirus isolates takes account of both the virus species and the host species from which the virus was originally isolated (e.g. BmCPV-1). The relationships between different cypoviruses within a single electropherotype or species, or with other cypovirus types, are not fully understood at the molecular level. Sequence analyses of genome segments from distinct isolates have shown high levels of identity within a single species. For example, the different isolates of *Cypovirus 5* that have been analyzed show >98% identity in genome Seg10 (the polyhedrin gene), whereas isolates of *Cypovirus 1* show 80–98% nt sequence identity in this gene. In contrast, comparisons of unrelated types showed only low levels of sequence identity (20–23%). Studies of genomic RNA from different cypovirus isolates have demonstrated that, although there may be slightly higher conservation in the largest genome segments (possibly as a result of functional constraints), the level of variation is relatively uniform across the whole genome. This contrasts with reoviruses that infect vertebrates, perhaps because there is no neutralizing antibody response in the host insects of cypoviruses and consequently no selective pressure to promote variation in outer capsid proteins and the genome segments from which they are translated.

In addition to the other general criteria used throughout the family, members of a species in the genus *Cypovirus* may be identified by:

- Similar electrophoretic migration of at least seven genome segments, as analyzed using either an agarose, or a low percentage (3%) polyacrylamide gel system. Viruses of different species have significant migrational differences in at least three genome segments.
- A high degree of nucleotide or amino acid sequence conservation (estimated >80% for the former).
- Cross-hybridization of genome segments under high stringency conditions (designed to detect >90% identity).

## List of species in the genus *Cypovirus*

| | | |
|---|---|---|
| *Cypovirus 1* | | |
| Bombyx mori cypovirus 1 | [Seg1: AF323781, Seg2: AF323782, Seg3: AF323783, Seg4: AF323784, Seg5: AB035733, Seg6: AB030014, Seg7: AB030015, Seg8: AB016436, Seg9: AF061199, Seg10: D37768] | (BmCPV-1) |
| *Cypovirus 2* | | |
| Aglais urticae cypovirus 2 | | (AuCPV-2) |

| | | |
|---|---|---|
| *Cypovirus 3* | | |
| Anaitis plagiata cypovirus 3 | | (ApCPV-3) |
| *Cypovirus 4* | | |
| Antheraea assamensis cypovirus 4 | [Seg9: AF374299] | (AaCPV-4) |
| *Cypovirus 5* | | |
| Euxoa scandens cypovirus 5 | [Seg10: J04338] | (EsCPV-5) |
| *Cypovirus 6* | | |
| Aglais urticae cypovirus 6 | | (AuCPV-6) |
| *Cypovirus 7* | | |
| Mamestra brassicae cypovirus 7 | | (MbCPV-7) |
| *Cypovirus 8* | | |
| Abraxas grossulariata cypovirus 8 | | (AgCPV-8) |
| *Cypovirus 9* | | |
| Agrotis segetum cypovirus 9 | | (AsCPV-9) |
| *Cypovirus 10* | | |
| Aporophyla lutulenta cypovirus 10 | | (AlCPV-10) |
| *Cypovirus 11* | | |
| Heliothis armigera cypovirus 11 | | (HaCPV-11) |
| *Cypovirus 12* | | |
| Autographa gamma cypovirus 12 | | (AgCPV-12) |
| *Cypovirus 13* | | |
| Polistes hebraeus cypovirus 13 | | (PhCPV-13) |
| *Cypovirus 14* | | |
| Lymantria dispar cypovirus 14 | [Seg1: AF389452, Seg2: AF389453, Seg3: AF389454, Seg4: AF389455, Seg5: AF389456, Seg6: AF389457, Seg7: AF389458, Seg8: AF389459, Seg9: AF389460, Seg10: AF389461] | (LdCPV-14) |
| *Cypovirus 15* | | |
| Trichoplusia ni cypovirus 15 | [Seg1: AF291683, Seg2: AF291684, Seg3: AF291685, Seg4: AF291686, Seg5: AF291687, Seg6: AF291688, Seg7: AF291689, Seg8: AF291690, Seg9: AF291691, Seg10: AF291692, Seg11*: AF291693] | (TnCPV-15) |
| *Cypovirus 16* | | |
| Choristoneura fumiferana cypovirus 16 | [Seg10, U95954] | (CfCPV-16) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*TnCPV-15 has been reported as having 11 distinct genome segments.
Full table available online on Science Direct ®, www.sciencedirect.com.

## List of other related viruses which may be members of the genus *Cypovirus* but have not been approved as species

In addition to many other lepidopteran cypoviruses that have been described (but are otherwise uncharacterized), there are hymenopteran cypoviruses. One isolate from a freshwater daphnid has been reported. In total, more than 230 cypoviruses have been described. The total potential number of species is unknown but, based on the number and diversity of insect species, is thought likely to be considerably greater than 16.

| | | |
|---|---|---|
| Uranotaenia sapphirina cypovirus 17 | [Seg10: AY876384] | (UsCPV-17) |
| Culex restuans cypovirus 17 | [Seg10: DQ212785] | (CrCPV-17) |
| Operophtera brumata cypovirus 18 | [Seg5: DQ192245, Seg6: DQ192246, Seg7: DQ192247, Seg8: DQ192248, Seg9: DQ192249, Seg10: DQ192250] | (ObCPV-18) |
| Operophtera brumata cypovirus 19 | [Seg2: DQ192251, Seg5: DQ192252, Seg9: DQ192253, Seg10: DQ192254] | (ObCPV-19) |
| Simulium ubiquitum cypovirus 20 | [Seg10: DQ834386] | (SuCPV-20) |
| Maruca vitrata cypovirus 21 | | (MvCPV-21) |
| Heliothis armigera cypovirus ("B" strain) | | (HaCPV-B) |
| Maruca vitrata cypovirus (A strain) | | (MvCPV-A) |
| Maruca vitrata cypovirus (B strain) | | (MvCPV-B) |
| Plutella xylostella cypovirus | | (PxCPV) |

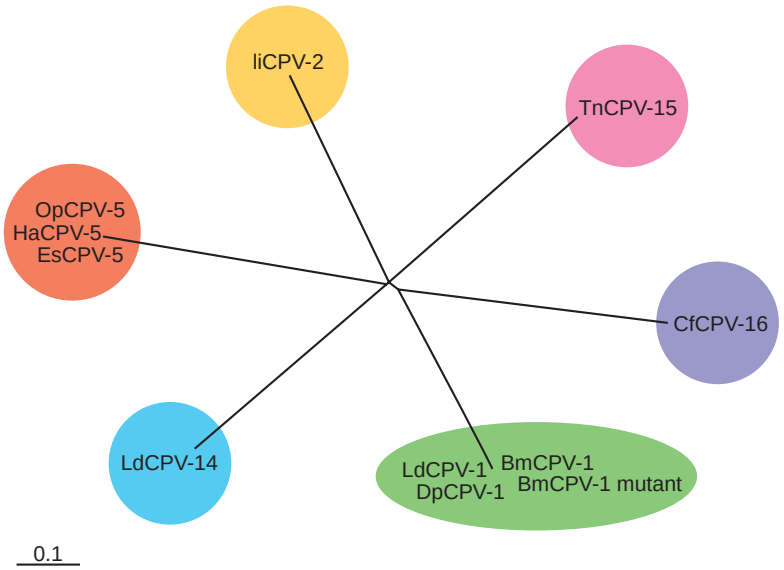


**Figure 14:** Phylogenetic tree for polyhedrin proteins from 11 cypovirus isolates. Sequences were aligned using ClustalX and the tree constructed in MEGA4 using the neighbour-joining method and the P-distance algorithm. Branching is supported by bootstrap values >85%.

### Phylogenetic relationships within the genus

The available sequence data for members of *Cypovirus 1*, *Cypovirus 2*, *Cypovirus 5*, *Cypovirus 14*, *Cypovirus 15* and *Cypovirus 16* allow a comparison of some genes of these viruses, showing not only that different cypovirus species are quite distantly related but also (at least for the viruses analyzed) that there is a high level of conservation within a single species. For example, a comparison of polyhedrin genes shows only 20–23% sequence identity between the members of different cypovirus species, but 89–98% identity between different isolates of a single species (Figure 14). These data indicate that sequence analyses and comparisons are effective methods for distinguishing and identifying the members of cypovirus species.

## GENUS *IDNOREOVIRUS*

Type species ***Idnoreovirus 1***

### Distinguishing features

Idnoreovirus virions have a roughly spherical outer capsid, which may have small icosahedrally arranged surface projections when viewed by negative staining and electron microscopy. The core particles have 12 icosahedrally arranged surface turrets or spikes, which appear similar to those of the cypoviruses. The genome is composed of 10 segments of linear dsRNA. All of the members of the genus that have been described infect insects.

### Virion properties

#### MORPHOLOGY

Particles are non-enveloped. Unlike the cypoviruses, there are no polyhedra, and the virus particles have a clearly defined outer capsid layer (Figure 15). Electron microscopy and negative staining of virions (e.g. with aqueous uranyl acetate) shows that they are double shelled, roughly spherical in appearance (with icosahedral symmetry), with an estimated diameter of about 70 nm. Core particles (estimated diameter of ca. 60 nm) display 12 icosahedrally arranged, prominent surface projections

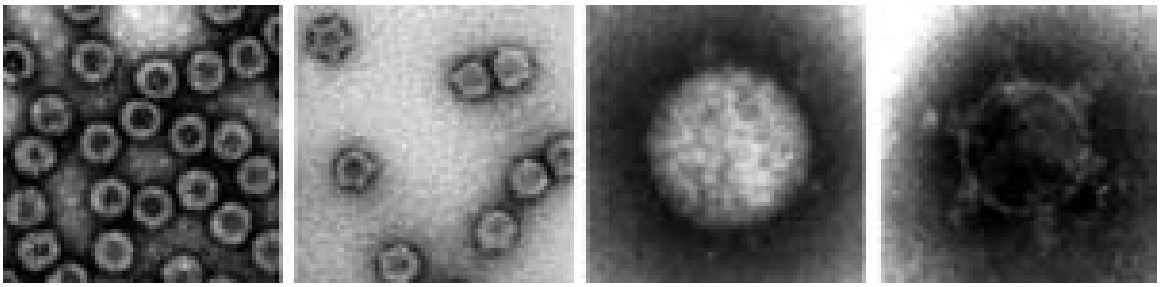

**Figure 15:** Electron micrographs of purified virus particles (far left) and core particles (second left) of Hyposoter exiguae idnoreovirus-2 (HelRV-2), stained with uranyl acetate (courtesy of A. Makkay and D. Stoltz). Electron micrographs of a virus particle (second right) and core particle (far right) from purified preparations of Dacus oleae idnoreovirus-4 (DoIRV-4), stained with sodium phosphotungstate (courtesy of M. Bergoin). DoIRV-4 virions have small icosahedrally arranged surface projections (estimated 12 in number). The DoIRV-4 cores have twelve large icosahedrally arranged spikes or turrets, which (like those of the cypoviruses) may lose a portion near to the tip.

(ca. 15 nm in length – identified as turrets or spikes), which may lose a portion near the tip and at least in some cases appear to be tubular. In particles where stain has entered the central space, there appears to be material (considered likely to be protein) associated with the spike structure on the inside of the inner capsid shell (Figure 15, far-right panel).

### Physicochemical and physical properties

Limited studies of some viruses in the genus indicate that particles are resistant to freon (trichlorotrifluoroethane) and CsCl. They may also be resistant to chymotrypsin. Intact particles and cores of Diadromus pulchellus idnoreovirus-1 (DpIRV-1) have densities of 1.370 g cm$^{-3}$ and 1.385 g cm$^{-3}$, respectively, and intact virions and empty particles of Dacus oleae idnoreovirus-4 (DoIRV-4) have a density of about 1.38 g cm$^{-3}$ and 1.28 g cm$^{-3}$, respectively, as determined by CsCl gradient centrifugation. The outer capsid layer of Hyposoter exiguae idnoreovirus-2 (HeIRV-2) was disrupted by brief exposure to 0.4% sodium sarcosinate, releasing the virus core.

### Nucleic acid

The genome usually consists of 10 dsRNA segments that are numbered in order of reducing molecular weight (or increasing electrophoretic mobility) during agarose gel electrophoresis (AGE). By analogy with other members of the family *Reoviridae*, the genome segment migration patterns during AGE are likely to be characteristic for each idnoreovirus species.

The total genome of DpIRV-1 (the current type member) contains an estimated 25.15 kbp of dsRNA, with individual segments that range between about 4.8 to 0.98 kbp, showing a 5-5 electrophoretic migration pattern by 1% AGE. However, the virions of DpIRV-1 may be unusual in the genus, since they can sometimes also contain an eleventh, 3.33 kbp dsRNA segment, the presence of which is related to, and may help determine, the sex and ploidy of the individual wasp host. This additional dsRNA (migrating between Seg3 and Seg4) contains sequences similar to, and therefore possibly derived by, duplication of an incomplete Seg3 (3.8 kbp).

The genome segments of HeIRV-2 range in estimated size from about 3.9 to 1.35 kbp, with a 4-6 electrophoretic migration pattern by 12.5% polyacrylamide gel electrophoresis (PAGE). DoIRV-4 contains an estimated 23.4 kbp of dsRNA, with the estimated lengths of individual segments ranging from about 3.8 to 0.7 kbp and a 5-3-2 electrophoretic migration pattern by 7% PAGE. Ceratitis capitata idnoreovirus-5 (CcIRV-5) has a 3-3-4 genome segment migration pattern by 6% PAGE, and has clear similarities to Drosophila melanogaster idnoreovirus-5 (DmIRV-5), as analyzed by 0.5% agarose-2% polyacrylamide gels, suggesting that, despite some serological differences, these viruses belong to the same species (*Idnoreovirus – 5*). It is unclear how closely Drosophila S virus (which causes the "S" phenotype in *D. simulans*) is related to the other *Drosophila*-derived idnoreoviruses. It is therefore currently listed as a possible member of the genus.

**Table 14:** Conserved terminal sequences (positive strand) of idnoreovirus genome segments

| Virus species | Serotype or strain | 5′ end | 3′ end |
|---|---|---|---|
| *Idnoreovirus - 1* | DpIRV-1 | 5′-(A/U/G)CAAUUU | (variable)-3′ |
| (not classified) | ObIRV | 5′-AA($^{A}/_{C}$)($^{A}/_{U}$)AA | AGGUU-3′ |

The total genome of Operophtera brumata idnoreovirus (ObIRV) contains 23,647 kbp, with genome segments that range in size from 4.17 to 1.51 kbp, giving an electrophoretic migration pattern of 4-2-4. This is the only complete genome sequence available and appears to represent a new species. All 10 genome segments of ObIRV contain five fully-conserved bases at the 3′ termini of their positive sense RNA strands, which are different from those found in other species that have been characterized within the family *Reoviridae*. There is also significant conservation at the 5′ termini (four of the six terminal bases). In each case, the two terminal bases are complementary (5′AA to 3′TT).

In contrast, initial sequencing studies suggest that the 3′ termini of DpIRV-1 genome segments are more variable than those of other species in the family *Reoviridae*, with little sign of conservation. However, conserved sequences were detected at the 5′ termini (Table 14), and these are different from those found in other reoviruses. No sequence data are currently available for other members of this genus.

### Proteins

Native viral proteins of idnoreoviruses have not been characterized extensively. However, sequencing of the ObIRV genome indicates that it encodes a total of 10 proteins, ranging in size from 49.6 to 155.7 kDa. The composition of these proteins was deduced from the sequence of the viral genome, as indicated in Table 15, where they are named as VP1 to VP10 based on the molecular weight of the genome segment (segment number) from which they are translated.

The genome of DpIRV-1 encodes 11 proteins with size ranging from 21 to 140 kDa. Three of these proteins appeared to be glycosylated (ca. 21, 15 and 35 kDa).

### Lipids

None reported.

### Carbohydrates

Three proteins from DpIRV-1 appeared to be glycosylated (ca. 21, 15 and 35 kDa).

## Genome organization and replication

On the basis of the overall similarity of idnoreoviruses to other members of the family *Reoviridae*, it is assumed that many aspects of the genome organization and replication are similar. Thus, it is likely that the virus core contains transcriptase complexes that synthesize mRNA copies of the individual genome segments. These mRNAs are likely to be exported and translated to produce viral proteins in the host cytoplasm. These positive sense RNAs are also likely to form templates for negative strand synthesis during progeny virus assembly and maturation. Each of the genome segments that has been sequenced represents a single gene, with a single large ORF and relatively short terminal NCRs (Table 15).

## Antigenic properties

Unknown.

## Biological properties

The idnoreoviruses can infect insect species, where in many cases they appear to cause few pathological effects. However, they may significantly alter the biological properties of the individual host. Drosophila S virus appears to be associated with the S phenotype in *D. simulans*. The presence of an additional 3.33 kbp-dsRNA segment in DpIRV-1 is related to, and may help determine, the sex

**Table 15:** Genome segments and protein products of Operophtera brumata idnoreovirus

| Genome segment | Size (bp) | Protein nomenclature | Protein size aa (kDa) | Structure/putative function |
|---|---|---|---|---|
| Seg1 | 4170 | VP1 | 1358 (155.7) | RNA-dependent RNA polymerase |
| Seg2 | 3780 | VP2 | 1207 (137) | |
| Seg3 | 3595 | VP3 | 1161 (133) | T2 subcore shell |
| Seg4 | 3362 | VP4 | 1091 (122.7) | |
| Seg5 | 2106 | VP5 | 620 (69.2) | |
| Seg6 | 1935 | VP6 | 594 (68.4) | |
| Seg7 | 1606 | VP7 | 499 (57.1) | |
| Seg8 | 1584 | VP8 | 467 (51.6) | |
| Seg9 | 1547 | VP9 | 437 (49.6) | |
| Seg10 | 1509 | VP10 | 467 (53.9) | |

The sizes of dsRNA segments and their putative translation products have been determined by sequence analyses.

and ploidy of the host. This segment may play a role in the biology of this wasp species, possibly by providing information necessary for larval development.

## Species demarcation criteria in the genus

See the general criteria used throughout the family.

## List of species in the genus *Idnoreovirus*

*Idnoreovirus 1*
Diadromus pulchellus idnoreovirus-1 (Diadromus pulchellus reovirus) [Seg2: X82049; Seg3: X80481; Seg7: X82048; Seg8: X82047; Seg9: X82046; Seg10: X82045; Additional segment: X80480] (DpIRV-1)

*Idnoreovirus 2*
Hyposoter exiguae idnoreovirus-2 (Hyposoter exiguae reovirus) (HeIRV-2)

*Idnoreovirus 3*
Musca domestica idnoreovirus-3 (Musca domestica reovirus) (Housefly virus) (MdIRV-3)

*Idnoreovirus 4*
Dacus oleae idnoreovirus-4 (Dacus oleae reovirus) (DoIRV-4)

*Idnoreovirus 5*
Ceratitis capitata idnoreovirus-5 (Ceratitis capitata I virus) (CcIRV-5)
Drosophila melanogaster idnoreovirus-5 (Drosophila F virus) (DmIRV-5)

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Idnoreovirus* but have not been approved as species

Drosophila S virus (DSV)
Operophtera brumata idnoreovirus [Seg1: DQ192235; Seg2: DQ192236; Seg3: DQ192237; Seg4: DQ192238; Seg5: DQ192239; Seg6: DQ192240; Seg7: DQ192241; Seg8: DQ192242; Seg9: DQ192243; Seg10: DQ192244] ObIRV

### Similarity with other taxa

There is no evidence of significant sequence homology between idnoreovirus genes and those of the other members of the family *Reoviridae*, other than that of the RNA-dependent RNA polymerase. The RdRp of ObIRV has 20–27% amino acid sequence identity to those of viruses in other genera of the subfamily *Spinareovirinae*.

## Genus *Dinovernavirus*

Type species ***Aedes pseudoscutellaris reovirus***

### Distinguishing features

The dinovernavirus genome consists of nine segments of dsRNA. Aedes pseudoscutellaris reovirus (APRV) is currently the only member of this genus and was isolated from persistently infected *Aedes pseudoscutellaris* cells (AP61).

### Virion properties

#### Morphology

Purified APRV particles, isolated using an Iodixanol (Optiprep®) gradient and analyzed by TEM, had the morphology typical of cores of turreted reoviruses (Figure 16). In particular, the morphology of APRV appears similar to that observed for cypovirus particles (which are single-shelled). It is therefore considered likely that APRV is also single-shelled. The mean diameter of the particle is approximately 49–50 nm, with a central section that is 36–37 nm. Turrets were visible projecting from the particle surface. These also appear to have sections near the tip that can become detached (Figure 16) in a manner similar to that observed previously with cypoviruses.

#### Physicochemical and physical properties

At 4 °C, APRV is stable for long periods, even non-purified in cell culture lysate, which is a convenient way for medium-term storage. Heating to 55 °C significantly reduces infectivity. The virus is stable upon treatment with freon, which can be used for purification of virus particles from cell lysate. Virion infectivity is not affected by treatment with 1% deoxycholate, but is abolished by treatment with sodium dodecyl sulfate. Viruses can be stored for long periods at −80 °C, and

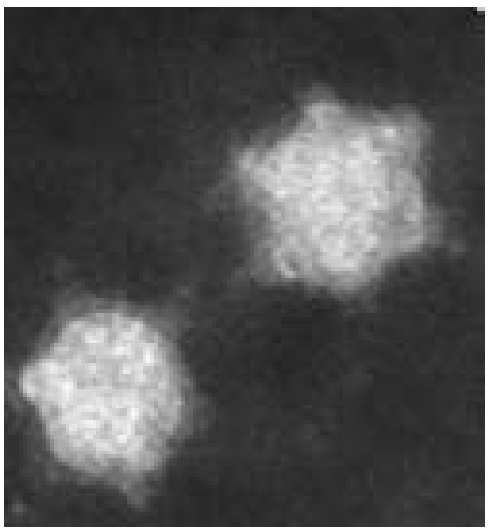

**Figure 16:** Negative contrast electron micrograph of particles of Aedes pseudoscutellaris reovirus purified using Iodixanol (Optiprep®) gradient (courtesy of H. Attoui).

infectivity can be further protected by addition of 50% fetal calf serum. Infectivity is retained at pH values between 6 and 8. Between pH 4 and 5 or between pH 9 and 10, infectivity is reduced by a factor of 10. Virion morphology (observed by electron microscopy) was considerably distorted at pH values lower than 5 and virions were completely disrupted at pH values lower than 3.5.

### Nucleic acid

The dinovernavirus genome consists of nine linear segments of dsRNA, which numbered in order of reducing Mr, or increasing electrophoretic mobility during agarose gel electrophoresis (AGE). The genome comprises 23,355 bp, with segment lengths that range between 3817 and 1147 bp. Analysis of genomic RNAs by 1% AGE shows a 5-1-3 migration pattern (electropherotype) (Figure 17).

The positive strands of all nine segments of the APRV genome have conserved sequences in the 5′ and 3′ non-coding regions (NCRs) (5′-AGUU$^{A}/_{U}$AA$^{A}/_{C}{}^{A}/_{C}$----------$^{U}/_{G}$UUnnn$^{C}/_{U}$nn$^{A}/_{U}$AGU-3′, where n = any nucleotide; Table 16). Comparisons of these conserved termini with those of viruses in the genera *Cypovirus*, *Oryzavirus* and *Fijivirus* (Table 16) showed that only the first three nucleotides in the 5′ NCR (AGU) are conserved between APRV, cypoviruses and NLRV (species *Nilaparvata lugens reovirus*, genus *Fijivirus*). The 3′ termini of APRV differ from those of the cypoviruses, NLRV and RRSV (species *Rice ragged stunt virus*, genus *Oryzavirus*).

In contrast to those of cypoviruses and fijiviruses, the first and last nucleotides of the APRV genome segments are complementary (A and U). The mean G+C content of the APRV genome is 34.4%, compared to 34.8% for NLRV, 44.7% for RRSV and 43% for cypoviruses.

### Proteins

APRV proteins were inferred from the ORFs in the dsRNA segments. Each segment encodes a single protein (Table 17).

### Lipids

None reported.

### Carbohydrates

None reported.



## Genome organization and replication

APRV persistently infects the AP61 cell line, with an estimated 6–8 particles per cell. Treatment of cells with 2-aminopurine showed a 10-fold increase in the number of viral particles, as shown by qPCR. The virus replicates to high titres in C6/36 cells and considerable amounts (over 40% of progeny) are liberated into the culture medium.

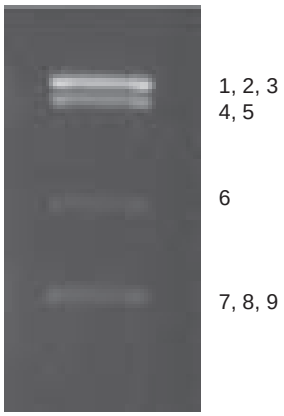


**Figure 17:** Agarose gel electrophoretic profiles of genome segments of Aedes pseudoscutellaris (propagated in C6/36 cells) in 1% agarose gel. These migration patterns (electropherotype) are thought to be characteristic of each virus species.

**Table 16:** Conserved terminal sequences (positive strand) of dinovernavirus genome segments and comparison to those of cypoviruses, fijiviruses, oryzaviruses and idnoreoviruses

| Genus | Virus | 5′ end | 3′ end |
|---|---|---|---|
| *Dinovernavirus* | APRV | 5′-AGUU$^{A}/_{U}$AA$^{A}/_{C}{}^{A}/_{C}$ | $^{A}/_{U}$AGU-3′ |
| *Cypovirus* | CPV1 | 5′-AGUAAA | GUUAGCC-3′ |
| | CPV2 | 5′-AGUUU | GAGUUUGC-3′ |
| | CPV15 | 5′-AUUAAAAA | GC-3′ |
| | CPV4 | 5′-AAUCGACG | GUCGUAUG-3′ |
| *Fijivirus* | NLRV | 5′-AGU | GUUGUC-3′ |
| | MRCV | 5′-AAGUUUUUU | GUC-3′ |
| | FDV | 5′-AAGUUUUUU | GUC-3′ |
| | RBSDV | 5′-AAGUUUUUU | GUC-3′ |
| *Oryzavirus* | RRSV | 5′-GAUAAA | GUGC-3′ |
| *Idnoreovirus* | ObIRV | 5′-AA$^{A}/_{C}{}^{A}/_{U}$AA | AGGUU-3′ |

**Table 17:** Genome segments and protein products of Aedes pseudoscutellaris reovirus

| Genome segment | Size (bp) | Protein nomenclature | Protein size aa (kDa) | Structure/putative function |
|---|---|---|---|---|
| Seg1 | 3817 | VP1 | 134 | Unknown |
| Seg2 | 3752 | VP2 | 143 | RdRp |
| Seg3 | 3732 | VP3 | 136 | Unknown |
| Seg4 | 3375 | VP4 | 116 | Unknown |
| Seg5 | 3227 | VP5 | 121 | Unknown |
| Seg6 | 1775 | VP6 | 62 | Unknown |
| Seg7 | 1171 | VP7 | 39.4 | Unknown |
| Seg8 | 1151 | VP8 | 39.8 | Unknown |
| Seg9 | 1147 | VP9 | 32 | Unknown |

Extracts of mammalian cells inoculated with APRV fail to support replication, indicating that this virus does not grow in mammalian cells. The RNA extracted from the blood of mice inoculated with APRV also remained negative (by RT-PCR) from 0 to 12 days post-injection.

## Antigenic properties

None reported.

## Biological properties

APRV was isolated from persistently infected AP61 cells. AP61 cells collected from different sources were consistently found to contain the same APRV strain. Virus-like particles were also identified in the original AP61 line by electron microscopy. Care should be taken when using AP61 cells to propagate any other mosquito-borne arboviruses, particularly dsRNA viruses, as these readily become contaminated with APRV. Upon infection with other dsRNA viruses, the dormant or persistent state of APRV is activated to a productive infection, releasing APRV in larger amounts into the cell culture supernatant, along with other mosquito-borne dsRNA viruses (such as orbiviruses and seadornaviruses) that were used to infect the cells.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Dinovernavirus*

*Aedes pseudoscutellaris reovirus*
{Aedes pseudoscutellaris mosquitoes}
Aedes pseudoscutellaris reovirus [Seg1: DQ087276., Seg2: DQ087277., Seg6: DQ087278., Seg7: DQ087279., Seg8: DQ087280., Seg9: DQ087281] (APRV)

Species names are in italic script; names of isolates are in roman script. Sequence accession numberss [ ], arthropod vector and host names { } and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Dinovernavirus* but have not been approved as species

None reported.

## Phylogenetic relationships with other taxa

The morphology of purified APRV is very similar to that of the single shelled cypoviruses. Based on electron microscopy analysis, it was initially thought that APRV would represent a new cypovirus from mosquitoes. However, unlike the cypoviruses, APRV particles are non-occluded and full-length sequence analyses confirmed that it only has nine genome segments. These analyses also demonstrated a genetic distance between APRV and the cypoviruses that is consistent with membership of distinct genera. Indeed, a similar relationship was also demonstrated with the oryzaviruses and fijiviruses (Table 18).

It is interesting to note that the VP1 of APRV (1189 amino acid residues) shows a significant match (22% identity, with a probability of $2e^{-6}$) over a sequence between residues 756 and 1173 (a 418 residue fragment) to a minor structural protein of a ssDNA parvo-like virus isolated from the silkworm. Observation of significant similarities between dsRNA viruses and DNA viruses is rather unusual. APRV VP3 also shows some similarity to the histidine kinase of Heliothis zea virus, which is in genus *Nudivirus*.

**Table 18:** Amino acid identity values between APRV, cypoviruses, fijiviruses and oryzaviruses

| APRV proteins | CPV1 - proteins | | RRSV- proteins | | NLRV- proteins | |
|---|---|---|---|---|---|---|
| | (position) | [% identity] | (position) | [% identity] | (position) | [% identity] |
| **VP1** | **P138** (768-1157) | [23] | **P1** (963-1208) | [22] | | |
| **VP2(Pol)** | **V2** (23-1206) | [26] | **P4** (68-1030) | [23] | **P165.9** (599–624) | [22] |
| **VP3** | **V1** (6-1302) | [21] | **P3** (398-645) | [21] | **P106.4** (339–698) | [21] |
| **VP4** | **NS1** (795-874) | [28] | **P5** (727-789) | [31] | | |
| **VP5** | **V3** (38-1045) | [22] | **P2** (589-1102) | [19] | | |
| **VP6** | **V4** (1-555) | [22] | **NS7** (399-591) | [23] | **P73.5** (399–591) | [23] |
| VP7 | | | | | | |
| VP8 | | | | | | |
| **VP9** | | | | | **NS35.2** (169–218) | [31] |

# Genus *Coltivirus*

Type species ***Colorado tick fever virus***

## Distinguishing features

The coltivirus genome consists of 12 segments of dsRNA. During replication, viruses are found in the cell cytoplasm, associated with granular matrices (viral inclusion bodies: VIB), arrays of filaments or tubules and fine kinky threads. Immunofluorescent staining reveals nucleolar fluorescence. Viruses are transmitted to vertebrate hosts by tick vectors.

## Virion properties

### Morphology

Coltivirus particles are 60–80 nm in diameter having two concentric capsid shells with a core that is about 50 nm in diameter. Electron microscopic studies, using negative staining, have shown that particles have a relatively smooth surface capsomeric structure and icosahedral symmetry (Figure 18). Particles are found intimately associated with filamentous structures and granular matrices in the cytoplasm. The majority of the viral particles are non-enveloped, but a few acquire an envelope structure during their passage through the endoplasmic reticulum.

### Physicochemical and physical properties

The buoyant density of the virus in CsCl is 1.38 g cm$^{-3}$. Viruses are stable between pH 7 and 8, but lose infectivity at pH 3.0. At 4 °C, the virus is stable for long periods when stored in presence of 50% fetal calf serum in 0.2 M Tris-HCl pH 7.8. Heating to 55 °C considerably decreases the viral infectivity. Coltiviruses are fairly stable upon treatment with non-ionic detergents, sodium lauroyl sarcosine, or freon but the viral infectivity is abolished by treatment with sodium deoxycholate or sodium dodecyl sulfate. Moderate ultrasonic oscillation treatment does not destroy infectivity and can be used in virus purification. Viruses can be stored for long periods at −80 °C, and infectivity is further protected by addition of 50% fetal calf serum.

### Nucleic acid

The genome consists of 12 dsRNA segments that are numbered in order of decreasing size, or increasing electrophoretic mobility during agarose gel electrophoresis. The genome comprises approximately 29,000 bp, with segment lengths that range between 4350 and 675 bp. The genomic

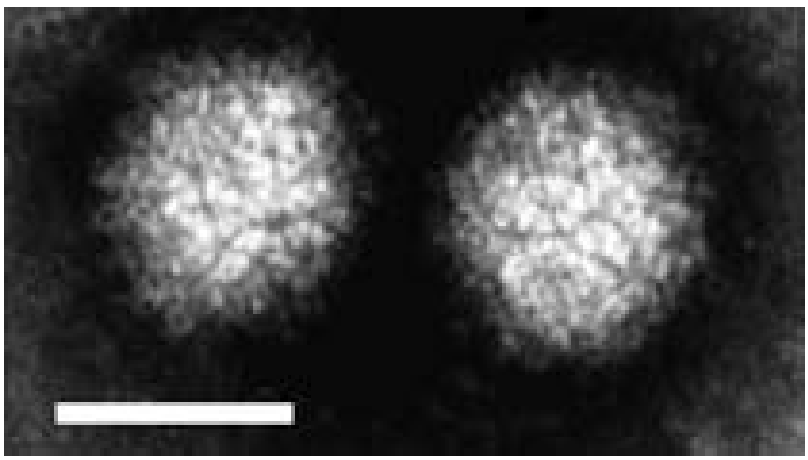

**Figure 18:** Negative contrast electron micrograph of particles of Colorado tick fever virus (CTFV) (courtesy of F. A. Murphy). The bar represents 50 nm.

RNA of Colorado tick fever virus (CTFV) migrates in three size classes (Figure 19) during 1% agarose gel electrophoresis (AGE): the large (long) or L-segments (Seg1 to Seg4), the medium length or M-segments (Seg5 to Seg10) and the small (short) or S-segments (Seg11 and Seg12). The dsRNA genome of Eyach virus (EYAV) has not yet been analyzed by AGE. The terminal 5′- and 3′-sequences of the coltivirus genome are conserved (Table 19).

RNA cross-hybridization analysis shows that CTFV isolates have remained relatively homogenous, and distinct CTFV serotypes have been difficult to distinguish (although some sequence variation does occur, for example in Seg4 and Seg6). The overall nt sequence similarity between genome segments of different CTFV isolates, ranges between 90% and 100%. However, the degree of similarity between homologous segments from CTFV and EYAV isolates ranges from 55% to 86%. Seg7 from EYAV is partially homologous to Seg6 of CTFV, although the protein it encodes also shows similarities to a sarcolemmal-associated protein from the European rabbit *Oryctolagus cunniculis*, which is thought to be one of its major hosts. Coltivirus mRNAs are usually regarded as non-infectious. However fully functional and infectious viruses have been recovered by the introduction of all 12 mRNAs into BSR cells.

### Lipids
None reported.

### Carbohydrates
None reported.

## Genome organization and replication
CTFV genome segments 1 to 8 and 10 to 11, each encodes a single protein. However, Seg9 contains a leaky stop codon and produces two proteins when translated in a cell-free system or in cells transfected with a plasmid containing the full length Seg9. The shorter 38 kDa protein, (VP9ter) is the early-termination translation product, while the read-through protein (VP9rdt) is 67.3 kDa (Table 20). The proteins of EYAV have not been characterized by translation.



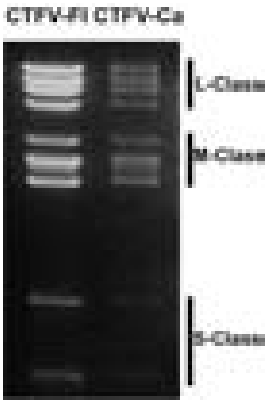


**Figure 19:** Electrophoretic profiles of the genome segments of Colorado tick fever virus isolate Florio (CTFV-Fl) and California hare virus (CTFV-Ca) in 1% agarose gel. Genome migration patterns (electropherotype) are thought to be characteristic of each virus species.

**Table 19:** Conserved terminal sequences (positive strand) of coltivirus genome segments

| Virus species | Strain | 5′ end | 3′ end |
|---|---|---|---|
| *Colorado tick fever virus* | CTFV-Fl | 5′-$^{G}/_{C}$ACAUUUUGU | UGCAGU$^{G}/_{C}$-3′ |
| *Eyach virus* | EYAV-Fr578 | 5′-GACA$^{A}/_{U}$UU | $^{A}/_{U}$UG$^{C}/_{U}$AGUC-3′ |

**Table 20:** Genome segments and protein products of Colorado tick fever virus

| Genome segment | Size (bp) | Protein nomenclature | Protein size (kDa)* | Protein structure/ function |
|---|---|---|---|---|
| Seg1 | 4350 | VP1 | 125 (163) | RdRp |
| Seg2 | 3909 | VP2 | 117 (136) | Methyltransferase, cell-receptor |
| Seg3 | 3586 | VP3 | 113 (135) | RNA replication factors |
| Seg4 | 3157 | VP4 | 100 (112) | |
| Seg5 | 2432 | VP5 | 90 (84) | Guanylyl transferase |
| Seg6 | 2141 | VP6 | 82 (78) | Nucleotide binding, NTPase |
| Seg7 | 2133 | VP7 | 75 (76) | RNA replication factors |
| Seg8 | 2029 | VP8 | 60 (74) | |
| Seg9 | 1884 | VP9ter/VP9rdt | 42 and 60 (38 and 67) | |
| Seg10 | 1880 | VP10 | 55 (69) | Kinase, helicase |
| Seg11 | 998 | VP11 | 34 (28.5) | |
| Seg12 | 675 | VP12 | 25 (20.4) | RNA replication factors |

*As determined by translation, or calculated from nt sequences (in brackets).

In cells infected by CTFV, granular matrices are produced which contain virus-like particles. These structures appear similar to VIBs produced during orbivirus infections. In addition, bundles of filaments (tubules), characterized by cross-striations, are found in the cytoplasm and, in some cases, in the nucleus of infected cells. These may also be comparable to the tubules found in orbivirus infected cells. There is no evidence for virus release prior to cell death and lysis, after which more than 90% of virus particles remain cell associated. Immuno-fluorescence staining shows that viral proteins accumulate in the cytoplasm and could be detected from 12 h post infection. Nucleolar fluorescence was also observed. Mosquito cells infected by EYAV show syncitial foci. Electron microscopy of EYAV-infected mouse brain shows similar intracellular structures to those observed in CTFV-infected cells.

## Antigenic properties

There is little cross-reaction in virus neutralization tests between isolates of CTFV (from North America) and EYAV (from Europe). CTFV-Ca from a hare, collected in California in 1976, shows some one-way cross-reaction in serum neutralization tests with EYAV, but is clearly distinguishable. Distinct serotypes of both CTFV and EYAV have been reported.

## Biological properties

Coltiviruses have been isolated from several mammalian species (including humans), as well as ticks and mosquitoes which serve as arthropod vectors. The tick species that have been implicated include *Dermacentor andersoni*, *D. occidentales*, *D. albipictus*, *D. parumapertus*, *Haemaphysalis leporis-palustris*, *Otobius lagophilus*, *Ixodes sculptus*, *I. spinipalpis*, *I. ricinus* and *I. ventalloi.*

Ticks can become infected with CTFV after ingestion of a blood meal from an infected vertebrate host. Both adult and nymphal ticks become persistently infected and provide an overwintering mechanism for the virus. CTFV is transmitted trans-stadially but not trans-ovarially. Some rodent species have prolonged viraemia (more than 5 months) which may also facilitate overwintering and virus persistence. Humans most frequently become infected with CTFV when bitten by the adult wood tick *D. andersoni* but probably do not act as a source of re-infection for other ticks.

Transmission from person to person has been recorded as the result of blood transfusion. The prolonged viraemia observed in humans and rodents is thought to be due to the intra-erythrocytic location of virions, protecting them from immune clearance.

CTFV is characterized in humans by an abrupt onset of fever, chills, headache, retro-orbital pains, photophobia, myalgia and generalized malaise. Abdominal pain occurs in about 20% of patients. Rashes are uncommon (less than 10%). A diphasic, or even triphasic, febrile pattern has been observed, usually lasting for 5–10 days. Severe forms of the disease, involving infection of the central nervous system, or hemorrhagic fever, or both, have been infrequently observed (nearly always in children under 12 years of age). A small number of such cases are fatal. Congenital infection with CTFV may occur, although the risk of abortion and congenital defects remains uncertain. Antibodies to EYAV have been found in patients with meningoencephalitis and polyneuritis but a causal relationship to the virus has not been established.

CTFV causes leucopoenia in adult hamsters and in about two-thirds of infected humans. Suckling mice, which usually die at 6–8 days post infection, suffer myocardial necrosis, necrobiotic cerebellar changes, widespread focal necrosis and perivascular inflammation in the cerebral cortex, degeneration of skeletal myofibers, hepatic necrosis, acute involution of the thymus, focal necrosis in the retina and in brown fat. The pathologic changes in mice due to CTFV infection (in skeletal muscle, heart and brain), are consistent with the clinical features of human infection, which may include meningitis, meningo-encephalitis, encephalitis, gastro-intestinal bleeding, pneumonia and myocarditis.

CTFV occurs in forest habitats at 4000–10,000 ft elevation in the Rocky Mountain region of North America. Antibodies to the virus have been detected in hares in Ontario and a virus isolate has been reported from Long Island, New York. EYAV appears to be widely distributed in Europe. An Eyach-like virus has been recently also been identified from ticks in the USA.



## Species demarcation criteria in the genus

In addition to the other general criteria used throughout the family, members of a species in the genus *Coltivirus* may be identified by:

- RNA cross-hybridization assays: within a single species, RNA sequence that exhibit more than 74% similarity will hybridize at 36°C below the Tm of the fully base-paired duplex.
- Sequence analysis: Nucleotide identity of >89% in the conserved Seg12; amino acid identities of >55%, >57% and >60% respectively in VP6, VP7 and VP12 (the most variable proteins).

## List of species in the genus *Coltivirus*

| | | |
|---|---|---|
| *Colorado tick fever virus* | | |
| {Ixodidae ticks: rodents, humans} | | |
| Colorado tick fever virus-Florio | [Seg1: AF133428, Seg2: AF139758, Seg3: AF139759, Seg4: AF139760, Seg5: AF139761, Seg6: AF139762, Seg7: AF139763, Seg8: AF139764, Seg9: AF000720, Seg10: AF139765, Seg11: U72694, Seg12: U53227] | (CTFV-Fl) |
| *Eyach virus* | | |
| {Ixodidae ticks: possibly humans } | | |
| Eyach virus-France-578 | [Seg1: AF282467, Seg2: [AF282468] Seg3: AF282469, Seg4: AF282470, Seg5: AF282471, Seg6: AF282472, Seg7: AF282473, Seg8: AF282474, Seg9: AF282475, Seg10: AF282476, Seg11: AF282477, Seg12: AF282478] | (EYAV-Fr578) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ], arthropod vector and host names { } and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Coltivirus* but have not been approved as species

Salmon River virus* (SRV)

*Has been reported as a serotype distinct from CTFV.

### Phylogenetic relationships within the genus and evolutionary links between coltiviruses and aquareoviruses

The level of aa identity between homologous proteins of CTFV and EYAV ranges from 55% to 88%, with similarity ranging between 72 and 93%. The most divergent proteins are those encoded by Seg6, Seg7 and Seg12 with aa identities of 55%, 57% and 63%, respectively. Highest identity values were found in VP1 (86–99%), which is the viral RNA-dependent RNA polymerase (Pol or RdRp).

The amino acid sequence (residues 370–490) of EYAV VP7 showed similarities (identity: 24%, similarity: 50%) to the sarcolemmal-associated protein of the European rabbit *Oryctolagus cuniculus*, a major host of EYAV. A corresponding region of the homologous VP6 protein of CTFV shows no significant match with this rabbit protein. This suggests that a recombination event has occurred between viral and host RNAs leading to the formation of a novel genome segment with mixed ancestry. However, the level of similarity (ca. 50%) to sarcolemmal-associated protein, suggests that this event occurred a very long time ago, possibly involving an ancestor of the European rabbit. Sequence analysis and phylogenetic studies suggest that EYAV is derived from an ancestral virus that was introduced into Europe during the migration of ancestors of the lagomorphs (hares, rabbits) from North America through Asia. Lagomorph ancestors first appeared during the Eocene epoch (57.8–36.6 MYA) in what was then North America. They are thought to have first migrated into Asia during the Oligocene epoch (34–23 MYA) and by the high Miocene epoch (23–25 MYA) they were common in Europe.

Sequence comparisons to other members of the family *Reoviridae* suggest that there has been an evolutionary jump, involving a change in the number of genome segments, between the aquareoviruses (11 segments) and coltiviruses (12 segments). Segments 7 of Aquareovirus C and G encode two proteins, from two distinct ORFs, which are homologues of two coltivirus proteins encoded separately by genome segments 9 and 12.

## SUBFAMILY *SEDOREOVIRINAE*

### Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Sedoreovirinae*** |
| Genus | ***Orbivirus*** |
| Genus | ***Rotavirus*** |
| Genus | ***Seadornavirus*** |
| Genus | ***Phytoreovirus*** |
| Genus | ***Cardoreovirus*** |
| Genus | ***Mimoreovirus*** |

## GENUS *ORBIVIRUS*

Type species ***Bluetongue virus***

### Distinguishing features

Virions have a relatively featureless outer capsid as viewed by negative staining and electron microscopy and a genome composed of 10 segments of dsRNA. Core particles have characteristic ring-shaped capsomers. Replication is accompanied by production of viral tubules and viral inclusion

bodies (VIB), and may be accompanied by formation of flat hexagonal crystals/arrays of the major outer core protein (VP7 (T13)) in the cytoplasm of infected cells. Viruses are transmitted between vertebrate hosts by a variety of hematophagous arthropods.

## Virion properties

### Morphology

Virions of bluetongue virus (BTV) are approximately 90 nm in diameter. Core particles have a maximum diameter of 73 nm, and sub-cores have a maximum diameter of 59 nm and an internal diameter of 46 nm (Figure 20). The virion is spherical in appearance but has icosahedral symmetry. Although mature virions lack a lipid envelope, they can leave the host cell by budding through the plasma membrane. During this process, they transiently acquire an unstable membrane envelope. Unpurified virus is often associated with cellular membranes. By conventional electron microscopy, the surface of intact virions is indistinct (Figure 20). However, the outer capsid does have

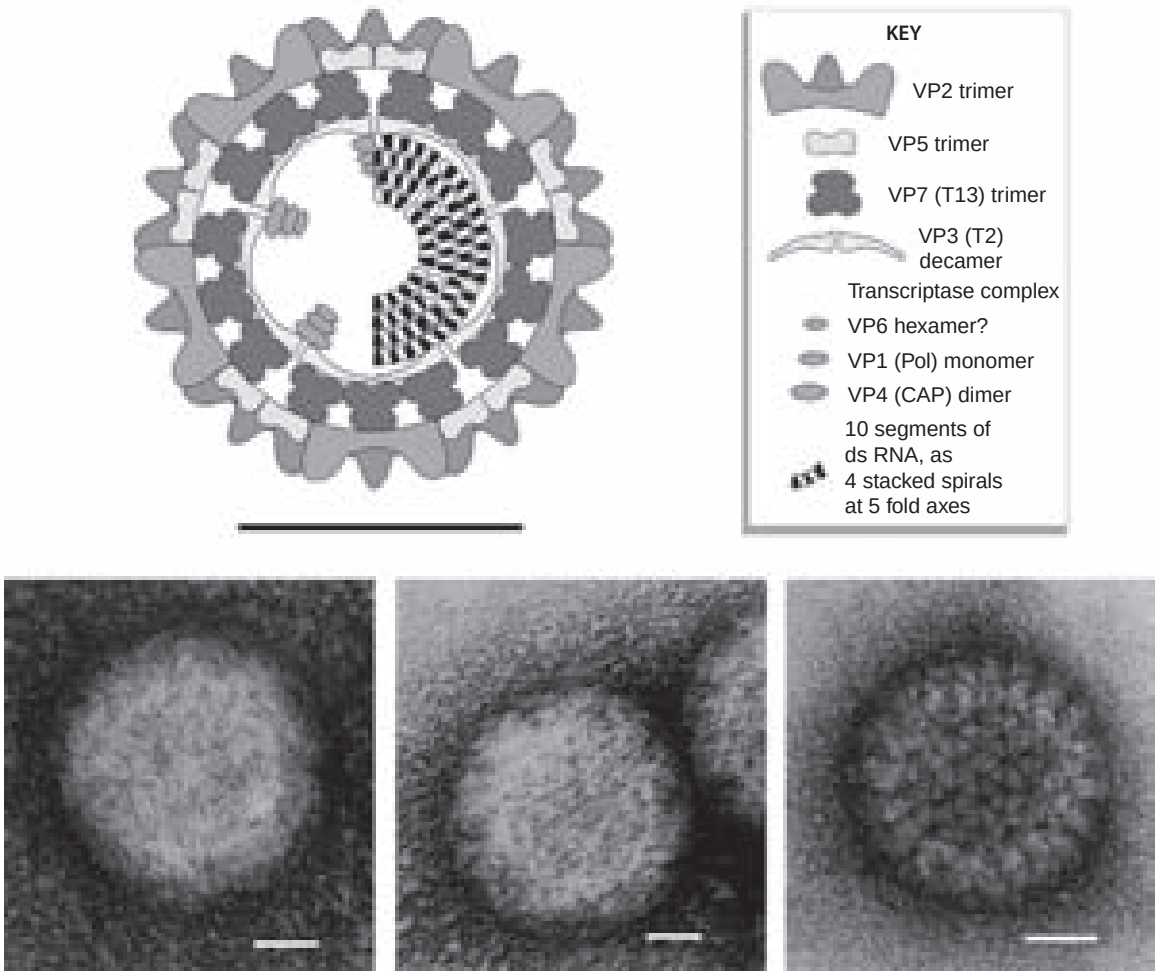


**Figure 20:** (Top) Diagram of the bluetongue virus (BTV) particle structure, constructed using data from biochemical analyses, electron microscopy, cryoEM and X-ray crystallography (courtesy of P.P.C. Mertens and S. Archibald). (Bottom) Electron micrographs of African horse sickness virus (AHSV) serotype 9 particles stained with 2% aqueous uranyl acetate (left) virus particles, showing the relatively featureless surface structure. (Center) Infectious subviral particles (ISVP), containing chymotrypsin cleaved outer capsid protein VP2 and showing some discontinuities in the outer capsid layer. (Right) core particles, from which the entire outer capsid has been removed, to reveal the structure of the VP7(T13) core surface layer and showing the ring shaped capsomeres (courtesy of P. P. C. Mertens).

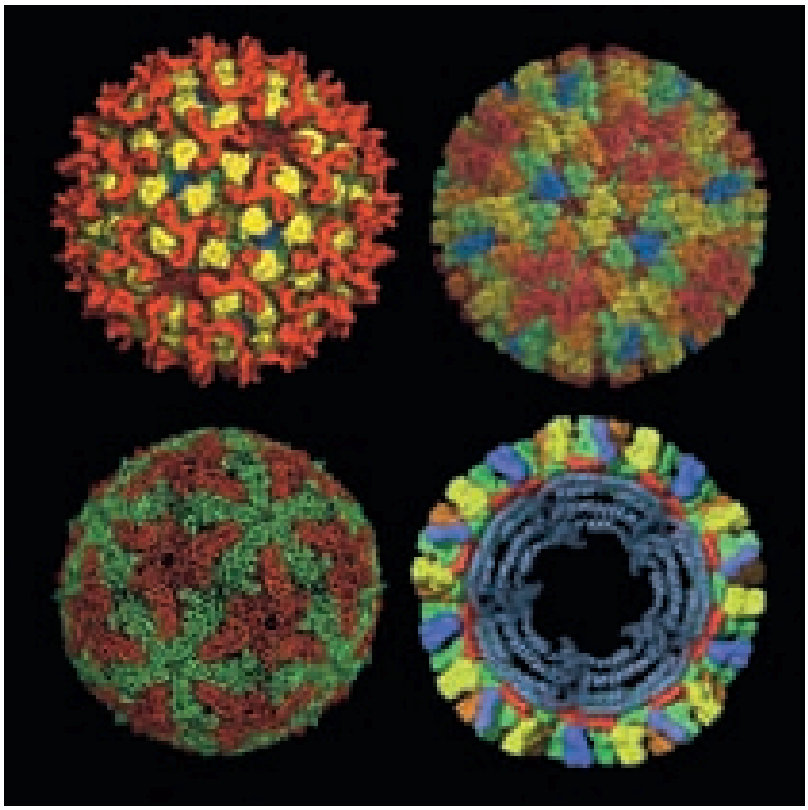

**Figure 21:** (Top left) The outer capsid layer of bluetongue virus (BTV) (from cryoEM) showing trimers of VP2 in red and trimers of VP5 in yellow, superimposed on the underlying X-ray crystallography structure for the BTV core. (Top right) The structure of the BTV core as determined by X-ray crystallography of the native core particle. The outer core surface, composed of 260 trimers of VP7 arranged with T = 13 *l* symmetry. The chemically identical but structurally different trimers are named and colored in order of increasing distance from the five-fold axes of symmetry (P [red], Q [orange], R [green], S [yellow] and T [blue] situated at the three-fold axes). (Bottom left) The BTV 1 subcore shell (from X-ray crystallography) is composed of 120 copies of VP3, arranged with T = 2 symmetry. The chemically identical but structurally different molecules are shown: "A" (green: surrounding the five-fold axis) and "B" (red: surrounding the three-fold axis). (Bottom right) Model cross-section of the BTV core showing packaging of the dsRNA as four concentric shells (courtesy of D. I. Stuart, J. Grimes, P. Gouet, J. Diprose, R. Malby, P. Roy, B. P. V. Prasad and P. P. C. Mertens).

an ordered structure, with icosahedral symmetry and sail-shaped surface projections that can be observed on virions where the particle structure is maintained (e.g. using cryoEM; Figure 21). When the outer capsid layer is removed, it is possible to view the surface layer of the core particle, which is composed entirely of capsomeres of VP7 (T = 13) arranged as hexameric rings (pentameric at the five-fold axes; Figures 20 and 21). These rings, which are readily observed by conventional electron microscopy, give rise to the name of this genus. The core particle also contains a complete inner capsid shell (the subcore layer), which surrounds the 10 dsRNA genome segments and minor structural proteins. The minor core proteins (the transcriptase complexes) are attached to the inner surface of the subcore at the 5-fold symmetry axes (Figure 20). Assembly of the subcore layer appears to control the overall assembly, size and symmetry of the particle.

### Physicochemical and physical properties

The virion Mr is about $10.8 \times 10^7$, and the core Mr is about $6.7 \times 10^7$. Their buoyant densities in CsCl are $1.36\,g\,cm^{-3}$ (virions) and $1.40\,g\,cm^{-3}$ (cores). The $S_{20,W}$ is 550S (virions) and 470S (cores). Virus infectivity is stable at pH 8–9 but virions exhibit a marked decrease in infectivity outside the pH range 6.5–10.2. In part, this may be related to the loss of outer coat proteins, particularly at the lower pH range. The sensitivity of the outer capsid proteins and their removal by cation treatment (e.g. by treatment with $MgCl_2$, or CsCl) varies markedly with both pH and virus strain. At low pH

values (less than 5.0), virions and cores are both disrupted. Unlike orthoreoviruses, virus infectivity is abolished at pH 3.0. In blood samples, serum or albumin, viruses held *in vitro* at less than 15 °C may remain infectious for decades. Purified BTV-1 virions held at 4 °C in 0.1 M Tris/HCl pH 8.0 showed no significant reduction in infectivity after 1 year. Crystals of core particles are very stable when kept at 29 °C. Virus infectivity is rapidly inactivated on heating to 60 °C. In general, orbiviruses are considered to be relatively resistant to treatment with solvents or detergents, although the sensitivity to specific detergents varies with virus species. However, sodium dodecyl sulfate will disrupt the particle and destroy its infectivity. Freezing reduces virus infectivity by about 90%, possibly due to particle disruption. However, once virus is frozen and held at −70 °C, infectivity remains stable.

### Nucleic acid

The genomic RNA represents 12% or 19.5% of the total molecular mass of virus particles or cores, respectively. The genome is composed of 10 linear dsRNA segments that are packaged in exactly equimolar ratios, one of each segment per particle. The genomic RNA is packaged as a series of ordered concentric shells within the VP3 layer of the subcore (Figure 21). Four layers of RNA, each of which has elements of icosahedral symmetry, can be detected by X-ray crystallography of the BTV core. Within the central space of the subcore, there appears to be an association between the dsRNA molecules and the protein density at the five-fold axes of symmetry (at the vertices of the icosahedron), which is thought to represent the transcriptase complexes (TCs). From the five-fold axis, the RNA, in the outmost layer, appears to spiral away from the five-fold axes outward around the TC for two turns until it clashes with an icosahedrally related neighbor. At this point, it is thought to move inward forming the next concentric shell of RNA. The genomic RNA contains 5′-terminal Cap 1 structures (7mGpppG$^{(2\text{-}Om)}$ ).

For BTVs, the genome segments range in size from 3954 to 822 bp (total size is 19.2 kbp, total Mr of $13.1 \times 10^6$). There is no evidence for short ssRNA oligonucleotides in intact virions. The genomic RNAs are numbered in order of increasing electrophoretic mobility in 1% agarose gels and in order of decreasing Mw. For BTVs, the segments migrate as three size classes: three large (Seg1–3: 3.9–2.8 kbp), three medium (Seg4–6: 2.0–1.6 kbp) and four small segments (Seg6–10: 1.2–0.8 kbp). For other members of the genus, different sizes and size classes exist. For an individual virus species, the dsRNA sizes from different isolates or serotypes are usually comparable, such that a uniform segment migration pattern is observed when the genomic RNAs of normal isolates are analyzed by agarose gel electrophoresis. However, variations in primary sequence cause significant variations in rate and order of migration of genome segments during polyacrylamide gel electrophoresis (PAGE), particularly in high percentage gels (>5% polyacrylamide). Earlier BTV genome segment nomenclature based on PAGE is inconsistent, and the migration of Seg5 and Seg6 is often reversed. In the majority of the orbivirus genome segments that have been analyzed, there is only a single major ORF, which is always on the same strand (see conserved terminal sequences below). However, the ORF may have more than one functional initiation site near to the 5′ end of the RNA, resulting in production of two related proteins.

For BTV-10, the 5′-NTRs of the positive RNA strands range from 8 to 34 bp, and the 3′-NTRs are 31 to 116 bp in length. For other serotypes and viruses, the lengths may be different. However, in general the 5′-NTRs are shorter than the 3′-NTRs. The NTRs of almost all the orbivirus genome segments that have been sequenced (Table 21) contain two conserved base pairs at either terminus (+ve 5′-GU  AC-3′). The NTRs of BTV include terminal sequences of 6 bp that are usually identical for all 10 dsRNA segments (although some variation does occur) and that are conserved between different BTV isolates. Other orbiviruses have terminal sequences that are comparable to those of BTVs, but that are not always identical and that may not be conserved in all 10 segments (Table 21).

BTV mRNAs are usually regarded as non-infectious. However, fully functional and infectious viruses have been recovered by the introduction of all 10 mRNAs into BSR cells.

### Proteins

There are seven virus structural proteins (VP1 to VP7; Table 22). These proteins constitute 88% and 80.5% of the dry weight of virions and cores, respectively. In BTV, the outer capsid consists of 180 copies of the 111 kDa sail-shaped VP2 protein arranged as trimeric triskelion structures, and 360 copies

**Table 21:** Conserved terminal sequences (positive strand) of orbivirus genome segments

| Virus species | Strain | 5′ end | 3′ end |
|---|---|---|---|
| *African horse sickness* | | 5′-GUU($^{A}/_{U}$)A($^{A}/_{U}$) | AC($^{A}/_{U}$)UAC-3′ |
| *Bluetongue virus* | | 5′-GUUAAA | ($^{A}/_{G}$)CUUAC-3′ |
| *Chobar Gorge virus* | | 5′-GUUUA($^{A}/_{U}$) | ($^{A}/_{G}$)($^{G}/_{C}$)($^{A}/_{C}$)UAC-3′ |
| *Epizootic hemorrhagic disease virus* | | 5′-GUUAAA | ($^{A}/_{G}$)CUUAC-3′ |
| *Equine encephalosis virus* | | 5′-GUU($^{U}/_{A}$) | A ($_{U/A/G}$)($_{A/U/C}$)GUUAC-3′ |
| *Eubenangee virus* | | 5′-GU($^{U}/_{A}$)($^{A}/_{U}$)AA | ($^{A}/_{C}$)($_{U/A/C}$)UAC-3′ |
| *Great Island virus* | | 5′-GUAAA($^{A}/_{U}$)($_{A/U/C}$) | ($^{A}/_{G}$)($_{A/U/C}$)($^{C}/_{G}$)($_{C/A/G}$)AC-3′ |
| *Ieri virus*[*] | | 5′-GUU($^{U}/_{A}$)AA | ($_{A/G/C}$)($_{G/A/C}$)NUAC-3′ |
| *Palyam virus* | | 5′-GU($^{A}/_{U}$)AAA | ($^{A}/_{G}$)CUUAC-3′ |
| *Peruvian horse sickness virus* | | 5′-GUUAAAA | ($^{A}/_{G}$)($^{C}/_{G}$)($^{A}/_{G}$)UAC-3′ |
| *St Croix River virus* | | 5′-($^{A}/_{G}$)UAAU($_{G/A/U}$) | ($_{G/A/U}$)($^{C}/_{U}$)($^{C}/_{A}$)UAC-3′ |
| *Umatilla virus* | | 5′-GUUU($^{A}/_{U}$)A | A($^{G}/_{A}$)GAUAC-3′ |
| *Wad Medani virus* | | 5′-GU($^{A}/_{U}$)($^{A}/_{U}$)AA | N($_{G/A/C}$)CUAC-3′ |
| *Yunnan orbivirus* | | 5′-GUUAAA($^{A}/_{U}$) | N($_{G/A/C}$)($^{A}/_{G}$)UAC-3′ |
| *Warrego virus* | MRV | 5′-GUAAA($^{A}/_{U}$) | ($_{A/C/U}$)C($^{U}/_{A}$)UAC-3′ |
| *Wallal virus* | MUDV | 5′-GUAUA($^{A}/_{U}$) | A($^{C}/_{A}$)($^{A}/_{G}$)C($_{U/A/C}$)UAC-3′ |
| (not classified) | ANDV[**] | 5′-GUUAAA | ($^{A}/_{U}$)CUUAC-3′ |

*Based on genome segments 3 to 10.
**Based on genome segments 2 to 10.

of an interdispersed and underlying VP5 protein (59 kDa), arranged as 120 trimers (Figures 20 and 21). The electrophoretic migration order and nomenclature of proteins may vary in members of other *Orbivirus* species. Both VP2 and VP5 of BTV are attached to VP7. The surface of the core particle consists entirely of 780 copies of VP7, which are arranged with T = 13 *l* symmetry, as a network of hexameric and pentameric rings (in a near-perfect example of quasi-equivalence; Figure 21). The VP7 trimers of the core surface can bind dsRNA molecules, although the functional significance of this binding remains undetermined. Beneath the VP7 layer, the subcore capsid shell is composed of 120 copies of VP3 arranged with T = 2 symmetry, displaying geometrical or pseudo quasi-equivalence (Figure 21). The VP3 (T2) capsid shell encloses the 10 dsRNA segments (Figure 21), as well as the three minor structural proteins. The latter include: the 150 kDa VP1(Pol), which is the RdRp; the 76 kDa VP4(Cap), which forms functional dimers and has both guanylyl-transferase and two methyl-transferase (Mtr) activities [Mtr 1 (forming the 7-methyl guanosine of the cap structure) and Mtr 2 (forming the 2-O-methyl guanosine, as the terminal nucleotide of the RNA chain)]; and the 36 kDa VP6/VP6a(Hel), which binds ssRNA or dsRNA and has both helicase and NTPase activities.

A 7 Å resolution of the structure of the infectious BTV virion (BTV-1SA) by cryoEM revealed structural information concerning the VP2 and VP5 outer coat proteins. The VP2 triskelion is composed of three tip domains branching from a central hub domain. The hub domain contains three putative sialic acid-binding pockets. Experimental data indicate that sugar-moiety-binding is important for BTV infection. The VP5 membrane penetration trimer, located between the VP2 trimers, has a central coiled-coil α-helical bundle, similar to the fusion proteins of many enveloped viruses. Weak interactions between the VP5 trimer and the VP2 trimer were detected in the cryoEM density map. Similar interactions were also detected with the underlying core surface layer of VP7 trimers. It has been suggested that the surface of VP5 could unfurl, like an umbrella, during penetration and shedding of the coat to release the transcriptionally active core particle.

X-ray diffraction studies indicate that the minor proteins are attached as a TC to the inner surface of the subcore layer [VP3 (T = 2)] at the five-fold symmetry axes (at the vertices of the icosahe-

dron). However, because there is only a single TC at each position, they do not have full icosahedral symmetry and it has not yet been possible to determine their organization at the atomic level.

The VP7 protein of some viruses, such as African horse sickness virus (AHSV), can also form flat hexagonal crystals/arrays, typically up to 5 m in diameter, within the cytoplasm of the infected cell. These are composed of flat sheets of hexameric rings, which appear similar to the rings of trimers seen in the core-surface layer.

There are three distinct non-structural viral proteins produced in cells infected with BTV or other orbiviruses. The 64 kDa NS1(TuP) protein forms tubules that vary in length up to 4 m. Although NS1 tubules have an unknown function, they are regarded as a characteristic feature of orbivirus replication. These tubules may have a ladder-like structure, as observed for BTV and epizootic hemorrhagic disease virus (EHDV) (68 and 52 nm in diameter), or they may be finer (23 nm in diameter) and have a reticular cross-weave pattern, as for AHSV.

The 41 kDa NS2(ViP) protein can be phosphorylated and is an important component of the matrix of VIBs, which are the site of virus replication and assembly. VIBs also contain relatively large amounts of the virus core proteins. NS2(ViP) consists of two domains joined by a hinge region and assembles into large multimeric complexes. NS2(ViP) has ssRNA-binding activity, suggesting that it plays an active role in replication. In conjunction with other virus proteins, it is believed to be involved in the recruitment of viral mRNA for encapsidation. The NS3/NS3a proteins are two small, non-structural membrane proteins (25 and 24 kDa) translated from different in-frame initiation sites on a single ORF, and are involved in the release of virus particles from cells. This function may be essential for dissemination of progeny virus, particularly from insect vector cells, which can become persistently infected and do not show cytopathic effect or high levels of cell death. In the process of particle release, the NS3 proteins are also released from the cell.

### Lipids

Orbivirus particles may be intimately associated with membraneous cell debris. Mature virions can acquire a membrane envelope by budding through the cell membrane during the process of cell exit, producing membrane enveloped virus particles (MEVPs). However, this membrane is thought to be transient or unstable and the virus particles are usually considered to be non-enveloped.

### Carbohydrates

The BTV VP5 protein may be glycosylated. NS3 and NS3a synthesized in mammalian cells can become glycosylated, forming high molecular weight products.

## Genome organization and replication

BTV genome segments are usually monocistronic but the Seg9 and Seg10 mRNAs are translated from either of two in-frame AUG codons. Coding assignments are shown in Table 22. The significance of the two forms of the Seg9 and Seg10 gene products (NS3, NS3A; VP6, VP6A) is not known. In some cases, other virus proteins form morphologically defined structures in infected cells [e.g. the flat hexagonal crystals/arrays formed of VP7 of AHSVs], but these are of unknown functional significance. Great Island virus was found to have two overlapping, out-of-phase ORFs, the longest of which codes for VP6(Hel). The second and shorter ORF codes for a 22 kDa protein (identified as VP6[dBP]), which has similarities to known dsRNA-binding proteins.

Virus adsorption involves components of the outer capsid, although cell entry may also involve VP7(T13). VP2 (and possibly also VP5) is involved in determination of virulence. VP5 may be involved in penetration of the cell membrane (release from endosomes into the cytoplasm), and the expressed protein can induce cell fusion. The outer capsid layer is lost during the early stages of replication. The transcription frequency of mRNA from individual genome segments varies, with more copies produced from the smaller segments.

Some details of the processes of virus replication are lacking. VIBs are considered to be the sites of morphogenesis of transcriptionally active virus cores containing dsRNA. The smallest particles containing RNA, which are observed in VIBs, appear to represent progeny subcore particles. The outer core protein [VP7] is added within the VIB and the outer-capsid proteins at the periphery of the VIB.

**Table 22:** Genome segments and protein products of bluetongue virus serotype 10

| Genome segment | Size (bp) | ORF (bp) | Protein* | Protein size (kDa) | Protein copy number/ particle | Function (location) |
|---|---|---|---|---|---|---|
| Seg1 | 3954 | 12-3917 | VP1(Pol) | 149.6 | 10 | RdRp |
| Seg2 | 2926 | 20-2887 | VP2 | 111.1 | 180 | Outer layer of the outer capsid, controls virus serotype, cell attachment protein, involved in determination of virulence, readily cleaved by proteases. Most variable protein. Reacts with neutralizing antibodies. Trimer |
| Seg3 | 2770 | 18-2720 | VP3(T2) | 103..3 | 120 | Forms the innermost protein capsid shell subcore capsid layer, T = 2 symmetry, controls overall size and organization of capsid structure, RNA binding, interacts with minor internal proteins |
| Seg4 | 2011 | 9-1970 | VP4(Cap) | 76.4 | 20 | Dimer, Mtr 1 and Mtr 2, capping enzyme (guanylyl-transferase) |
| Seg5 | 1769 | 35-1690 | NS1(TuP) | 64.4 | 0 | Forms tubules of unknown function in the cell cytoplasm. These are characteristic of orbivirus replication |
| Seg6 | 1638 | 30-1607 | VP5 | 59.2 | 360 | Inner layer of the outer capsid, may be glycosylated, helps determine virus serotype, variable protein. Trimer |
| Seg7 | 1156 | 18-1064 | VP7(T13) | 38.5 | 780 | Forms outer core surface, which can bind dsRNA, T = 13 symmetry, in some species (AHSV) it can form flat hexagonal crystals, involved in cell entry and core particle infectivity in adults and cells of vector insects, reacts with "core neutralizing" antibodies, immuno dominant virus species specific antigen. Trimer |
| Seg8 | 1124 | 20-1090 | NS2(ViP) | 41.0 | 0 | Important viral inclusion body matrix protein, ssRNA binding, phosphorylated. May be associated with outer capsid |
| Seg9 | 1046 | 16-999 | VP6(Hel) VP6a | 35.8 | 60 | ssRNA and dsRNA binding, Helicase, NTPase |
| Seg10 | 822 | 20-706 | NS3 | 25.6 | 0 | Glycoproteins, membrane proteins, involved in cell exit. In some species (AHSV) these are variable proteins and are involved in determination of virulence |
| | | | NS3a | 24.0 | 0 | |

*Protein structure/function: Pol, RNA polymerase ; Cap, capping enzyme (guanylyltransferase and transmethylase); T2, inner virus structural protein with T = 2 symmetry; T13, inner virus structural protein with T = 13 symmetry; Hel, protein with helicase activity; ViP, viral inclusion body or viroplasm matrix protein; TuP, virus tubule protein. Viruses from other species within the genus may have proteins with significant differences in sizes.

Virus particles are transported within the cell by specific interaction with the cellular cytoskeleton and can be released from the cell prior to lysis through interaction with membrane-associated NS3 proteins (Figure 2). There is also some evidence of specific association between NS1 tubules and intact virions in the cell cytoplasm. In most mammalian cells, replication of orbiviruses leads to shut-off of host protein synthesis and usually results in cell lysis and the release of virus particles. However, in persistently infected insect cells (or gamma delta T cells), there is no evidence for shut-off of host protein synthesis, extensive cell lysis or cytopathic effect. In some viruses (such as AHSV), NS3 is involved in determination of virulence for the mammalian host and, by controlling virus dissemination within the insect, may at least partially determine their ability to transmit the virus (vector competence). Virus particles can leave viable mammalian cells by two distinct mechanisms: extrusion (involving cell membrane damage) and budding. Only budding has been observed in cells of the BTV vector *Culicoides variipennis*, resulting in particles that have a membrane envelope, although this is unstable and is rapidly lost. Continuous release of virus particles from infected cells and re-infection appear to be features of orbivirus replication.

## Antigenic properties

The main virus serogroup- (species-) specific antigen is the immunodominant outer core protein VP7. Monoclonal or polyclonal antibodies against VP7 can neutralize core particle infectivity, but do not attach to, or neutralize, undamaged virus particles or ISVPs in aqueous suspension, indicating that VP7 is not exposed on the intact virion surface. Other viral proteins are also conserved between virus species (in particular core proteins, NS1 and NS2). Some of these antigens may also show cross-reactions with viruses in other species, particularly those regarded as closely related. These cross-reactions are usually at a significantly lower level than with other viruses from the same virus species and may be one-way. Such relationships between species are also demonstrated by comparisons of the RNA sequences of conserved segments (for example, homologs of BTV Seg3, coding for inner core protein VP3. These data indicate that orbiviruses may be divided into at least four groups. Group A contains: AHSV, BTV, EHDV, equine encephalosis virus (EEV), Eubenangee virus (EUBV), Palyam virus (PALV), Wallal virus (WALV) and Warrego virus (WARV). Group B contains: Chenuda virus (CNUV), Ieri virus (IERIV), Wad Medani virus (WMV) and Great Island virus (GIV). Group C contains Corriparta virus (CORV). Group D contains Wongorr virus (WGRV). Insufficient comparisons have been made to conclusively assign all of the species of the genus *Orbivirus* to these groups.

Each species of the genus *Orbivirus* includes a number of serotypes that can be identified and distinguished in serum neutralization assays of intact virus particles (primarily via the specificity of interactions between neutralizing antibodies and the outer CPs). VP2 is the main neutralization antigen of BTV. VP5 is also involved in determination of virus serotype, possibly by imposing conformational constraints on VP2. The VP2 and VP5 proteins of BTV exhibit the greatest antigenic and sequence variation (Figure 22). In other viruses (such as GIV), the relative sizes of the outer CPs (VP4 and VP5) are very different and their individual roles may also be different. There is evidence that VP2 of BTV and AHSV (particularly in association with VP5) and VP7 can act as protective antigens.

In AHSV, the small nonstructural proteins, NS3 and NS3a, are also variable and may be divided into three groups ($\alpha$, $\beta$ and $\gamma$) based on sequence analysis. Preliminary serological evidence suggests that NS3 cross-reacts poorly between these groups. NS3 can also be involved in determination of virulence (for AHSV), possibly as a result of its involvement in release of virus particles from cells (budding) and its consequent effect on virus dissemination. Recent sequencing studies of BTV NS3 also indicate that it can be highly variable. NS3 variation does not correlate with virus serotype.

## Biological properties

The specific infectivity of purified (disaggregated) BTV particles is equivalent to a particle infectivity ratio of approximately 1000:1 in both mammalian and insect cell systems. However, core particle infectivity varies from being 1000-fold less than that of intact virions (baby hamster kidney cells)

to non-infectious (Chinese hamster ovary cells) in mammalian systems, depending on the cell line used. However, in some insect cells (KC cells, derived from *Culicoides sonorensis*) and adult vector insects, core particles are only slightly less infectious than intact virions (particle infectivity ratio of 1900:1). Treatment of virus with chymotrypsin or trypsin results in production of ISVPs, in which VP2 is cleaved. BTV ISVPs lack hemaglutinating activity, as well as the tendency to aggregate, but have a significantly elevated infectivity for adults of insect vectors and some insect cell lines (a particle infectivity ratio of approximately 13:1 for KC cells).

Different orbiviruses infect a wide range of vertebrate hosts including ruminants (domesticated and wild), equids (domesticated and wild), rodents, bats, marsupials, birds, sloths and primates, including humans. Orbiviruses can also replicate in, and are primarily transmitted by, arthropod vectors (gnats, mosquitoes, phlebotomines or ticks, depending on the virus). Trans-stadial transmission in ticks has been demonstrated for some viruses. Infection of vertebrates *in utero* may also occur. Orbiviruses, particularly those transmitted by short-lived vectors (gnats, mosquitoes and phlebotomines), are only enzootic in areas where adults of the competent vector species persist and are present all, or most, of the year. Orbiviral RNA has been detected in *Culicoides* larvae recovered from outbreak areas, although trans-ovarial transmission has not been confirmed by the recovery of infectious virus. Orbiviruses have also been detected in cell lines derived from tick eggs. BTV and EHDV are distributed worldwide between about 50° north and 30° south in the Americas and between 40° north and 35° south in the rest of the world. These limits have recently expanded to 53° north (e.g. in Europe), possibly as the result of climate change.

There is also evidence for persistence of these viruses over winter in the absence of overt disease. Mechanisms for persistence in the vertebrate host species even at low levels (including vertical transmission) may be of particular importance. Virus distribution also depends on the initial introduction into areas containing susceptible vertebrate hosts and competent vector species. For this reason, not all serotypes of each virus (e.g. BTV) are present at locations where some serotypes are endemic.

Orbivirus infection of arthropods has little or no evident effect. Infection in vertebrates can vary from inapparent to fatal, depending on both the virus and the host. Some BTV strains cause death in sheep, others cause a variety of pathologies, including hemorrhagic conditions, lameness, oedema, a transitory cyanotic appearance of the tongue (giving rise to the species name), nasal and mouth lesions, etc.; still others cause no overt pathology. BTV infection of cattle may show no signs of disease but can involve long-lived viraemias. AHSV, EHDV and EEV can cause severe pathology in their respective vertebrate hosts. Mortality rates in serologically naive populations can be over 98% (AHSV).

## Species demarcation criteria in the genus

In addition to the other general criteria used throughout the family, members of a species in the genus *Orbivirus* may be identified by:

- High levels of serological cross reaction using either polyclonal sera or monoclonal antibodies against conserved antigens such as VP7. For example, in competition ELISA at a test serum dilution of 1/5, a positive serum will show >50% inhibition of color formation, while a negative control serum, or a serum that is specific for a different species, will normally produce <25% inhibition of color compared to a no antibody control. Distinct but related species may show low level serological cross-reaction, which may be only one-way.
- Sequence analysis: In the conserved Seg3 (encoding the major subcore structural protein, VP3), viruses within the same species will normally have >76% nucleotide identity while those in different species usally have <74% identity. These differences are also reflected in the amino acid sequences of the viral proteins.
- Relatively efficient cross-hybridization of conserved genome segments (those not encoding outer capsid components or other variable proteins) under high stringency conditions (>85% identity).
- Identification of common vector or host species and the clinical signs produced. For example BTV is transmitted only by certain *Culicoides* species and will infect cattle and sheep, producing clinical signs of varying severity, but is not thought to infect horses. The reverse is true of AHSV.

## List of species in the genus *Orbivirus*

*African horse sickness virus*
(9 serotypes)
{*Culicoides*: equids, dogs, elephants, camels, cattle, sheep, goats, predatory carnivores and (in special circumstances) humans}
- African horse sickness virus 1 — [Seg1: AM883164, Seg2: AM883165, Seg3: AM883166, Seg4: AM883167, Seg5: AM883168, Seg6: AM883169, Seg7: AM883170, Seg8: AM883171, Seg9: AM883172, Seg10: AM883173] — (AHSV-1)

*Bluetongue virus*
(26 serotypes)
- Bluetongue virus 10 — [Seg1: X12819, Seg2: M11787, Seg3: M22096, Seg4: Y00421, Seg5: Y00422, Seg6: D12532, Seg7: X06463, Seg8: D00500, Seg9: D00509, Seg10: AF044372] — (BTV-10)

*Changuinola virus*
(12 serotypes)
{phlebotomines, culicine mosquitoes: humans, rodents, sloths}
- Changuinola virus — (CGLV)
- Other strains: Almeirim virus, Altamira virus, Caninde virus, Gurupi virus, Irituia virus, Jamanxi virus, Jari virus, Monte Dourado virus, Ourem virus, Purus virus, Saraca virus

*Chenuda virus*
(7 serotypes)
{ticks: seabirds}
- Chenuda virus — (CNUV)
- Other strains: Baku virus, Essaouira virus, Huncho virus, Kala Iris virus, Mono Lake virus, Sixgun city virus

*Chobar Gorge virus*
(2 serotypes)
{ticks: bats}
- Chobar Gorge virus — (CGV)
- Other strain: Fomede virus

*Corriparta virus*
(6 serotypes/strains*)
{culicine mosquitoes: humans, rodents}
- Corriparta virus MRM1 — [Seg3: AF530086] — (CORV-MRM1)
- Other strains: Acado virus, CS109, V654, V370, Jacareacanga virus

*Epizootic hemorrhagic disease virus*
(7 serotypes/strains*)
{*Culicoides*: cattle, sheep, deer, camels, llamas, wild ruminants, marsupials}
- Epizootic hemorrhagic disease virus 1 — [Seg1:AM744977, Seg2: AM744978, Seg3:AM744979, Seg4:AM744980, Seg5:AM744981, Seg6: AM744982, Seg7:AM744983, Seg8: AM744984, Seg9:AM744985,Seg10: AM744986] — (EHDV-1)
- Other strain: Ibaraki virus (EHDV-2)

*Equine encephalosis virus*
(7 serotypes)
{*Culicoides*: equids}
- Equine encephalosis virus 1 — [Seg1: FJ183384, Seg2: FJ183385, Seg3: FJ183386, Seg4: FJ183387, Seg5: FJ183388, Seg6: FJ183389, Seg7: FJ183391, Seg8: FJ183390, Seg9: FJ183392, Seg10: FJ183393] — (EEV-1)

*Eubenangee virus*
(4 serotypes)
{*Culicoides*, anopheline and Culicine mosquitoes: unknown hosts}

| | | |
|---|---|---|
| Eubenangee virus | [Seg3: AF530087] | (EUBV) |
| Other strains: Ngoupe virus, Pata virus, Tilligerry virus | | |
| *Great Island virus* | | (GIV) |
| (36 serotypes/strains*) | | |
| {*Argas*, *Ornithodoros*, *Ixodes* ticks: seabirds, rodents, humans} | | |
| Great Island virus | [Seg1: HM543465, Seg2: HM543466, Seg3: HM543467, Seg4: HM543468, Seg5: HM543469, Seg6: HM543470, Seg7: HM543471, Seg8: HM543472, Seg9: HM543473, Seg10: HM543474] | (GIV) |
| Other strains: Above Maiden virus, Arbroath virus, Bauline virus, Broadhaven virus, Cape Wrath virus, Colony virus, Colony B North virus, Ellidaey virus, Foula virus, Great Saltee Island virus, Grimsey virus, Inner Farne virus, Kemerovo virus, Kenai virus, Kharagysh virus, Lipovnik virus, Lundy virus, Maiden virus, Mill Door virus, Mykines virus, North Clett virus, North End virus, Nugget virus, Okhotskiy virus, Poovoot virus, Rost Island virus, St Abb's Head virus, Shiant Islands virus, Thormodseyjarlettur virus, Tillamook virus, Tindholmur virus, Tribec virus, Vearoy virus, Wexford virus, Yaquina Head virus | | |
| *Ieri virus* | | |
| (3 serotypes) | | |
| {mosquitoes: birds} | | |
| Ieri virus | | (IERIV) |
| Other strains: Gomoka virus, Arkonam virus | | |
| *Lebombo virus* | | |
| {culicine mosquitoes: humans, rodents} | | |
| Lebombo virus 1 | | (LEBV-1) |
| *Orungo virus* | | |
| (4 serotypes) | | |
| {culicine mosquitoes: humans, camels, cattle, goats, sheep, monkeys} | | |
| Orungo virus 1 | | (ORUV-1) |
| *Palyam virus* | | |
| (13 serotypes/strains*) | | |
| {*Culicoides*, culicine mosquitoes: cattle, sheep} | | |
| Kasba virus (Chuzan virus) | [Seg1: AB018086, Seg2: AB014725, Seg3: AB014728, Seg4: AB018087, Seg5: AB018089, Seg6: AB014726, Seg7: AB014727, Seg8: AB018090, Seg9: AB018088, Seg10: AB018091] | (KASV) |
| Other strains: Abadina virus, Bunyip creek virus, CSIRO village virus, D'Aguilar virus, Gweru virus , Kindia virus, Marrakai virus, Marondera virus , Nyabira virus, Palyam virus, Petevo virus, Vellore virus | | |
| *Peruvian horse sickness virus* | | |
| {mosquitoes: horses } | | |
| Peruvian horse sickness virus-1 | [Seg1: DQ248057, Seg2: DQ248058, Seg3: DQ248059, Seg4: DQ248060, Seg5: DQ248064, Seg6: DQ248061, Seg7: DQ248065, Seg8: DQ248063, Seg9: DQ248062, Seg10: DQ248066] | (PHSV-1) |
| *St Croix River virus* | | |
| {ticks: unknown hosts} | | |
| St Croix River virus-1 | [Seg1: AF133431, Seg2: AF133432, Seg3: AF145400, Seg4: AF145401, Seg5: AF145402, Seg6: AF145403, Seg7: AF145404, Seg8: AF145405, Seg9: AF145406, Seg10: AF145407] | (SCRV-1) |
| *Umatilla virus* | | |
| (4 serotypes) | | |
| {culicine mosquitoes: birds} | | |
| Umatilla virus | | (UMAV) |
| Other strains: Llano Seco virus, Minnal virus, Netivot virus | | |
| *Wad Medani virus* | | |
| (2 serotypes) | | |
| {Boophilus, Rhipicephalus, Hyalomma, Argas ticks: domesticated animals} | | |
| Wad Medani virus | | (WMV) |
| Other strain: Seletar virus | | |

| | | |
|---|---|---|
| *Wallal virus* | | |
| (3 serotypes/strains*) | | |
| {*Culicoides:* marsupials} | | |
| Wallal virus | [Seg3: AF530084] | (WALV) |
| Other strains: Mudjinbarry virus, Wallal K virus | | |
| *Warrego virus* | | |
| (3 serotypes/strains*) | | |
| {*Culicoides*, anopheline and culicine mosquitoes: marsupials} | | |
| Warrego virus | [Seg3: AF530083] | (WARV) |
| Other strains: Mitchell river virus, Warrego K virus | | |
| *Wongorr virus* | | |
| (8 serotypes/strains*) | | |
| {*Culicoides*, mosquitoes: cattle, macropods} | | |
| Wongorr virus V195 | [Seg3: U56990] | (WGRV-V195) |
| Other strains: Paroo river virus, Picola virus, MRM13443, V199, V595, V1447 | | |
| *Yunnan orbivirus* | | |
| (2 serotypes) | | |
| {Mosquitoes: cattle, sheep, donkeys} | | |
| Yunnan orbivirus 1 | [Seg1: AY701509, Seg2: AY701510, Seg3: AY701511, Seg4: AY701512, Seg5: AY701513, Seg6: AY701514, Seg7: AY701515, Seg8: AY701516, Seg9: AY701517, Seg10: AY701518] | (YUOV-1) |

Species names are in italic script; names of isolates are in roman script. Sequence accessions [ ], arthropod vector and host names { } and assigned abbreviations ( ) are also listed.
*In some species the serological relationship between strains has not been fully determined.
Full table available online on Science Direct ®, www.sciencedirect.com.



### List of other related viruses which may be members of the genus *Orbivirus* but have not been approved as species

| | |
|---|---|
| Andasibe virus {mosquitoes: unknown hosts} | (ANDV) |
| Codajas virus {mosquitoes: rodents} | (COV) |
| Ife virus {mosquitoes: rodents, birds, ruminants} | (IFEV) |
| Itupiranga virus {mosquitoes: unknown hosts} | (ITUV) |
| Japanaut virus {mosquitoes: unknown hosts} | (JAPV) |
| Kammavanpettai virus {unknown vectors: birds} | (KMPV) |
| Lake Clarendon virus {ticks: birds} | (LCV) |
| Matucare virus {ticks: unknown hosts} | (MATV) |
| Tembe virus {mosquitoes: unknown hosts} | (TMEV) |
| Tracambe virus {mosquitoes: unknown hosts} | (TRV) |

### Phylogenetic relationships within the genus

The phylogenetic relationships within the genus are illustrated in Figures 22 and 23 (pp. 604 and 605).

## GENUS *ROTAVIRUS*

Type species ***Rotavirus A***

### Distinguishing features

Rotaviruses infect only vertebrates and are transmitted by a fecal–oral route. When viewed by negative contrast electron microscopy (Figure 24), virus particles have a wheel-like appearance from which the genus derives its name (Latin *rota*, "wheel"). The triple-layered capsid encloses a genome of 11 linear dsRNA segments and is formed in a unique morphogenic pathway, which involves acquisition of a transient lipid envelope during budding of immature particles into the endoplasmic reticulum (ER).

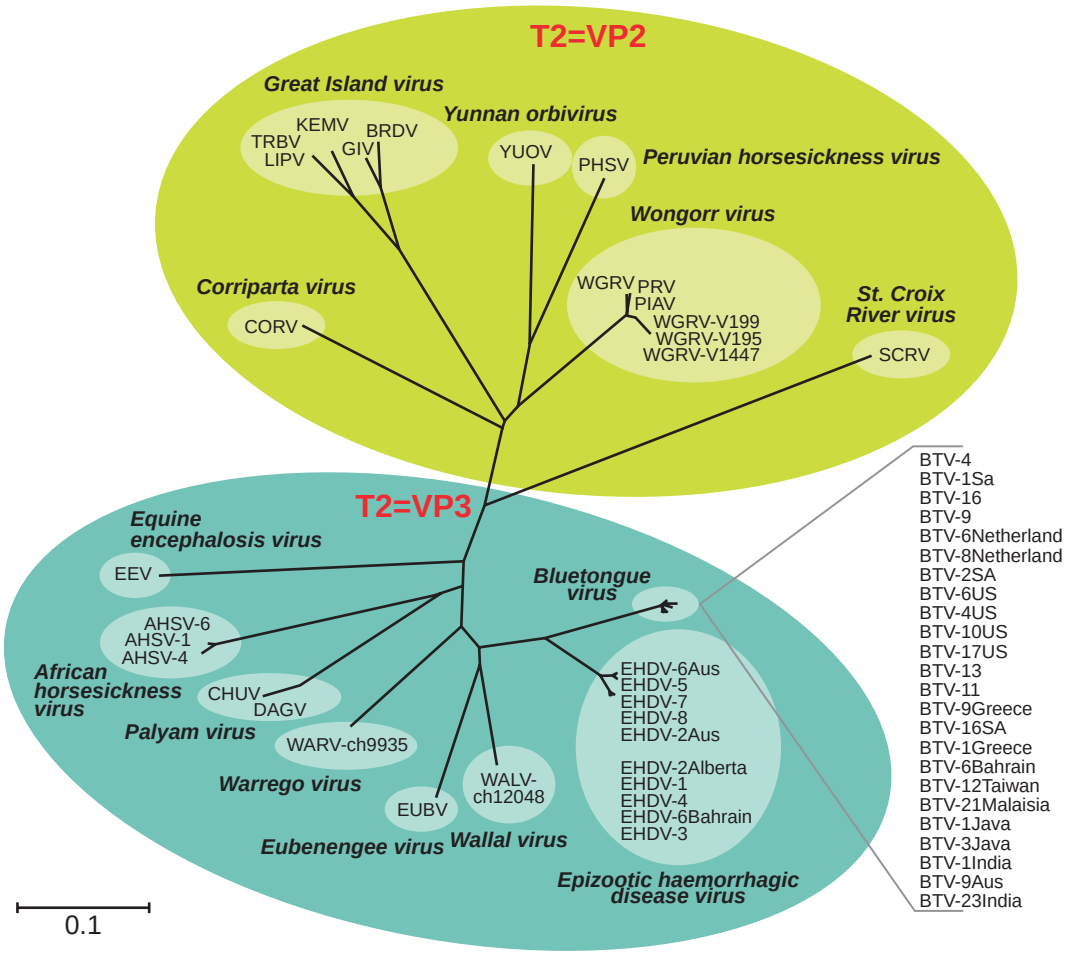


**Figure 22:** Phylogenetic tree of the T2 subcore shell proteins of members of the genus *Orbivirus*. The tree shows two groups: a mosquito-borne/tick-borne group where the second largest viral protein (VP2) forms the "T2" sub-core capsid layer; and a *Culicoides*-borne group where the third largest viral protein (VP3) forms the T2-layer. Sequences were aligned using ClustalX and the tree constructed in MEGA4 using the neighbor-joining method and the P-distance algorithm. Branching is supported by bootstrap values >85%.

## Virion properties

### Morphology

The data for simian rotavirus A/SA11 (SiRV-A/SA11) represent a paradigm for other viruses within the genus. The mature infectious virion has an overall diameter of about 100 nm, is made up of three concentric protein layers and lacks a lipid-containing envelope (Figure 24). The detailed topology of these layers and their protein components has been revealed using cryoEM, followed by image processing of viral and subviral particles, as well as virus-like particles formed using recombinant baculoviruses that express specific rotavirus structural proteins (Figure 25). The innermost layer of the virion, composed of VP2, is about 3.5 nm thick. This layer is comparable to the internal capsid layer of members of other genera within the family *Reoviridae* (e.g. the VP3 (T2) layer of the orbiviruses). The VP2 layer (T = 1; 60 asymmetric dimers of VP2) surrounds the genomic dsRNAs and two structural proteins, the RdRp VP1 and the capping enzyme VP3, which are organized as a series of up to 12 enzymatic complexes, tethered to the inner surface of VP2 near the five-fold axes of symmetry (Figures 24 and 25).

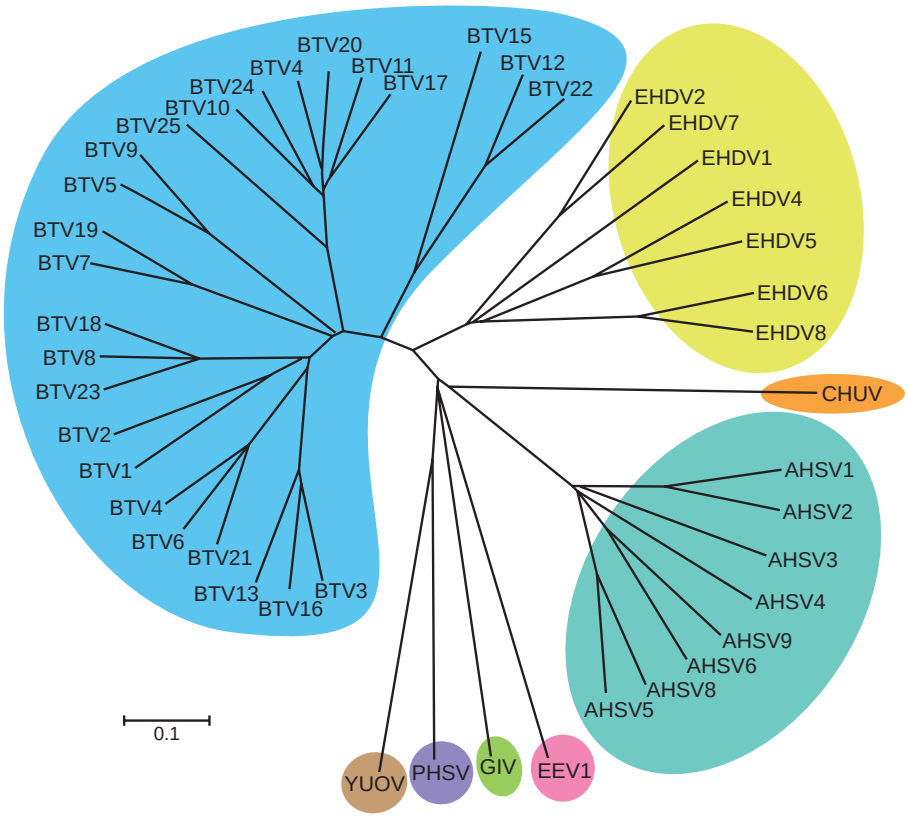


**Figure 23:** Phylogenetic tree, constructed using complete amino acid sequences of the cell attachment protein (VP2 of the *Culicoides*-borne viruses, VP3 of the mosquito-borne viruses and VP4 of Great Island virus, which is tick-borne). In bluetongue virus (the *Orbivirus* type species) VP2 is the larger of the two outer CPs and is the most variable and primary neutralization antigen. Amino acid sequences were aligned using Clustal X and the trees were constructed using the neighbor-joining method and P-distance in MEGA4 (courtesy of H. Attoui). Names and abbreviations correspond to those used in the list of species. Sequences were aligned using ClustalX and the tree constructed in MEGA4 using the neighbor-joining method and the P-distance algorithm. Branching is supported by bootstrap values >85%.

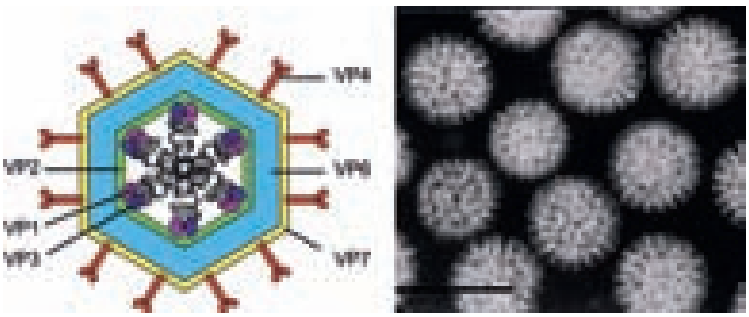

**Figure 24:** Rotavirus morphology. (Left panel) Cartoon representation of a rotavirus triple-layered particle, with proteins labeled. Black corkscrews represent segments of genomic dsRNA. The precise locations of VP1 and VP3 have not been determined. (Right panel) Electron micrograph of rotavirus particles viewed by negative staining. Bar represents 100 nm. (Provided by B. V. V. Prasad.)

The assembled genomic dsRNAs, along with the enzymatic complexes and VP2 layer, comprise the rotavirus core, which has a diameter of about 51 nm. Each of the two outer layers of the rotavirus virion is organized with T = 13 (laevo) icosahedral symmetry. Spanning these layers is a uniquely characteristic set of 132 large channels that link the outer surface with the inner VP2 protein layer. The intermediate capsid layer is composed of 780 copies of VP6, arranged as 260 trimeric morphological units positioned at the local and strict three-fold axes of the icosahedral lattice (Figure 25). The VP6 layer forms the outer surface of the double-layered particle (DLP; ca. 70.5 nm in diameter) and is directly comparable to one of the capsid layers of viruses of some other genera within the *Reoviridae* (e.g. the VP7 (T13) layer of the orbiviruses). Two proteins (VP4 and VP7) form the outermost layer of the rotavirus virion (ca. 75 nm in diameter, not including spikes) and are required for infectivity (Figure 25). The glycoprotein VP7 makes up the surface of the outermost shell, which is arranged as 260 trimers stabilized by $Ca^{2+}$ bound in the inter-subunit interfaces. VP7 trimers cap the trimeric pillars of VP6 and grip them with amino-terminal arms. Projecting from the VP7 layer are 60 trimeric spikes formed by VP4. Trypsin cleavage of VP4 generates amino-terminal (VP8*) and carboxyl-terminal (VP5*) fragments of VP4 and primes virions for infectivity. Primed VP4 spikes are about 20 nm long and extend about 12 nm from the surface of the outer VP7 layer, giving a final maximum particle diameter of about 100 nm. The foot of the VP4 spike has three-fold symmetry and interacts extensively with VP6. Distal to the foot, the spike lacks three-fold symmetry. A stalk formed primarily by VP5* connects the foot to a paired body, atop which two heads formed by the VP8* receptor-binding domain sit. Each VP5* monomer contains a β-barrel domain, tipped by a hydrophobic apex, which is buried in the cleaved spike. Similar to enveloped virus fusion proteins, during entry VP4 undergoes a rearrangement that is linked to membrane penetration. The hydrophobic apices of VP5* are exposed as the β-barrel domains fold back and a trimeric coiled-coil zips up.

### Physicochemical and physical properties

The rotavirus virion has a density of 1.36 g $cm^{-3}$ in CsCl and sediments at 520–530S in sucrose. Virus infectivity is dependent upon the presence of the VP4-VP7 outermost protein layer, the integrity of which requires $Ca^{2+}$. Treatment of virions with $Ca^{2+}$-chelating agents, such as EGTA or EDTA, destabilizes VP7 trimers, leading to loss of the outer capsid. Infectivity is not affected by exposure of infectious virions to pH ranges from 3 to 9 and, in the presence of 1.5 mM $CaCl_2$, by storage for months at 4 °C or even 20 °C. Infectivity is also relatively thermostable at 50 °C but can be lost by repeated cycles of freezing and thawing. Infectivity is generally resistant to fluorocarbon extraction,

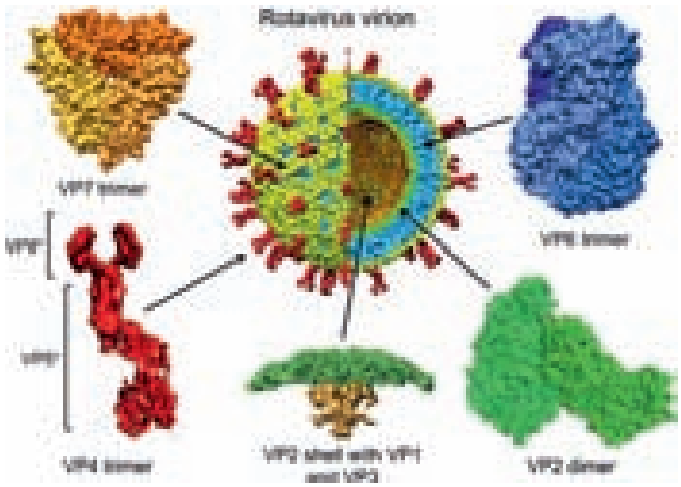


**Figure 25:** Structure and location of protein components of the rotavirus virion. A cutaway view of a cryoEM image reconstruction of a rotavirus virion at 9.5 Å resolution (center) is a reference for enlarged, high-resolution images of specific virus components. The particle and components are colored as follows: VP4 spikes (red), VP7 layer (yellow), VP6 layer (blue) and VP2 layer (green). A portion of VP2 that extends into the interior of the core (the "hub") and transcriptional enzymes VP1 and VP3 are colored gold in the enlarged image of a five-fold vertex (bottom, center). The VP8* and VP5* cleavage products of the VP4 spike are indicated. The trimeric foot and dimeric stalk and head of VP4 can be seen. PDB files 3KZ4 (VP6 and VP2) and 3GZT (VP7) were used to make images. (Courtesy of B. V. V. Prasad.)

treatment with solvents such as ether and chloroform, or non-ionic detergents such as deoxycholate, all of which reflect the absence of a lipid-containing envelope on the mature particle. However, infectivity is lost by treatment with sodium dodecyl sulfate (0.1%), or a number of disinfectants such as betapropiolactone, chlorine, formalin and phenols. In addition, 95% ethanol, which disrupts the outer protein layer of the virion, represents an effective disinfectant. The VP4-mediated hemagglutinin activity of the infectious virion is lost rapidly at 45 °C or as a result of freezing and thawing. Some variation has been observed in the physicochemical properties and stability of intact virions of different rotavirus strains. For example, not all human rotaviruses exhibit hemagglutinin activity, and they tend to lose the proteins of their outer layer more easily than some animal strains. In re-assortment studies, some of this variation has been attributed to the parental origin of the VP4 component in the virus particle.

Double-layered particles (DLPs) are non-infectious, have a density of 1.38 g cm$^{-3}$ in CsCl and sediment at 380–400S in sucrose. These are equivalent to cores of the orbiviruses. Single-layered rotavirus particles (cores) can be produced by treatment of DLPs with either chaotropic agents such as sodium thiocyanate or high concentrations of $CaCl_2$. Cores have a density of 1.44 g cm$^{-3}$ in CsCl, sediment at 280S in sucrose, and are readily disrupted by incubation in hypotonic solutions, and are structurally equivalent to sub-cores of the orbiviruses.

### Nucleic acid

The rotavirus genome consists of 11 discrete segments of linear dsRNA, which are organized with dodecahedral symmetry in the core. For SiRV-A/SA11, the segments range in size from 3302 to 667 bp and have a combined size of 18,555 bp. When resolved by gel electrophoresis, the segments of *Rotavirus A* strains (not including isolates from avian species) typically display a 4:2:3:2 distribution pattern. The genome segments are numbered segment 1 (Seg1) to segment-11 (Seg11) in order of increasing mobility during electrophoresis, although the migration order of cognate segments, particularly in the Seg7 to Seg9 triplet, does vary. In some *Rotavirus A* isolates, deviation from the 4:2:3:2 RNA migration pattern is indicative of a concatemerization event, in which up to an additional 1800 bp is packaged into viable virions, due to the partial duplication of a genome segment. Rotavirus genomic RNA sequences are A+U rich (58–67%). The segments are completely base-paired, and the plus-strand contains a 5′-terminal cap structure ($m^7GpppG^{(m)}GC$), but lacks a polyadenylation signal near its 3′ end. In contrast to members of the genus *Orthoreovirus*, there is no evidence for the presence of single-stranded oligonucleotides within the virion. Two levels of terminal sequence conservation are evident in *Rotavirus A* isolates. Firstly, all genome segments share short, conserved 5′ and 3′ termini. The 5′-terminal conserved region has a consensus sequence of 5′-(GGCUUUUAAA  )-3′, and the 3′ terminus has the consensus sequence 5′-(  AUGUGACC)-3′ (Table 23). Immediately internal to these terminal regions at each end of the different segments, there is a second region of conservation of at least 30–40 nt, which is segment-specific. These two levels of sequence conservation may be indicative of *cis*-acting signals that are important for controlling transcription, replication and segment selection for packaging. The 5′ nontranslated regions (NTRs) vary in length, but are typically less than 50 nt and are followed by at least one long ORF beginning with the first AUG. Some segments contain additional in-frame (Seg7, Seg9 and Seg10) or out-of-frame (Seg11) ORFs. However, only in the case of Seg9 and Seg11 are alternate start codons used to initiate synthesis of more than a single primary translational product from each segment. The 3′ NTRs vary in length from 17 nt (Seg1) to 182 nt (Seg10).

**Table 23:** Conserved terminal sequences (positive strand) of rotavirus genome segments

| Virus species | Strain | 5′ end | 3′ end |
|---|---|---|---|
| *Rotavirus A* | SA11 | 5′-GGC($^{U}/_{A}$)$_2$U($^{A}/_{U}$)$_4$ | ($^{A}/_{U}$)U($^{G}/_{A}$)UG($^{A}/_{G}$)CC-3′* |
| *Rotavirus B* | WH-1 | 5′-GG($^{U}/_{C}$)($^{A}/_{U}$)N($^{A}/_{U}$)$_5$** | ($^{A}/_{U}$)$_3$($^{A}/_{G}$)$_2$A($^{C}/_{A}$)CC-3′ |
| *Rotavirus C* | Bristol | 5′-GCC($^{A}/_{U}$)$_7$ | UGUGGCU-3′ |
| *Rotavirus D* | 05V0049 | 5′-GG($^{U}/_{C}$)($^{A}/_{U}$)$_4$AA($^{A}/_{U}$) | ($_{U/A/C}$)U($_{G/A/U}$)($^{U}/_{C}$)GACC-3′ |
| (not classified) | NADRV-J19 | 5′-GG($_{CAU}$)A($_{CAU}$) | ($^{A}/_{G}$)UA($^{U}/_{C}$)ACCC-3′ |

*For segment 5, the 3′-terminal sequence is CUGUGAACC.
**Rarely, a G or C is found at position 6, 8, or 10.

Detailed genomic information for *Rotavirus B*, *Rotavirus C*, *Rotavirus D* and novel adult diarrhea rotaviruses (NADRVs) is sparse. However, complete sequence information is available for all genome segments of at least one isolate for each species. In all cases, the genome is made up of 11 segments, which have broadly similar properties to SiRV-A/SA11 in terms of length, presence of ORFs and conserved terminal sequences.

**Proteins**

Thirteen primary gene products have been defined for SiRV-A/SA11. The majority of genome segments encode a single protein, but two segments (Seg9 and Seg11) each encode two primary translation products. In the case of Seg9, two initiation codons in the same reading frame may be used, giving largely overlapping forms of the protein product VP7. Gene 11 contains two out-of-frame ORFs, translation of which results in two unrelated non-structural proteins, NSP5 and NSP6 (Table 24).

The nomenclature system employed for rotavirus proteins numbers them according to their migration rates upon SDS-PAGE, starting with the slowest (i.e. highest molecular weight). Structural proteins are given the prefix "VP", whereas non-structural proteins are given the prefix "NSP". Six structural proteins have been identified, and their approximate locations within the virion have been defined. The viral core, which encapsidates the dsRNA genome, is composed of three proteins. Two of the core proteins (VP1 and VP3) are directly associated with the genome, while the third (VP2) makes up the core shell, the innermost protein layer of the capsid. VP1, the largest viral protein (125 kDa), is a four-tunneled RdRp, responsible for both transcription and replication. Transcriptional activity can be detected in preparations of purified DLPs. Genome replication (negative strand synthesis) can be achieved *in vitro* using disrupted viral core preparations or recombinant VP1 protein. However, VP1 is active only in the presence of VP2. Viral core preparations also exhibit guanylyltransferase and methyltransferase activities. The VP3 component (98 kDa; the capping enzyme) of DLPs, binds ssRNA and forms adducts with GTP and S-adenosyl-L-methionine. VP1 and VP3 form a complex that is tethered near the inward protruding hubs at the five-fold vertices of the VP2 capsid layer (Figure 25). The amount of VP1 and VP3 in the virion is known to be low (≤12 molecules/particle) but has not been measured precisely. VP2 (102 kDa) is the most abundant protein of the viral core, with 120 molecules per virion. VP2 is required for encapsidation of VP1 and VP3 and has nonspecific ssRNA- and dsRNA-binding activity. By analogy with other reoviruses, it is likely that VP2 determines both the size of the particle as well as the structural organization of the outer capsid components, the internal enzymatic components and the RNAs. This important functional load is reflected in the highly conserved nature of VP2.

The intermediate protein layer of the virion is made up of 260 trimers of VP6 (45 kDa). The two remaining structural proteins of the virion, VP4 (87 kDa) and VP7 (36 kDa), of which there are 60 trimers and 260 trimers per virion, respectively, make up the outermost protein layer (Figure 25). The spike protein VP4 contains a trypsin cleavage site approximately one-third of the way along its length. Cleavage of the protein *in vitro* by treatment with trypsin produces two products, VP5* (60 kDa) and VP8* (28 kDa), enhances virus infectivity, and induces conformational changes that stabilize the spike structure. The VP8* cleavage product has hemagglutinin activity and contains a carbohydrate binding site (galectin-like fold). VP7 is the primary component of the outer shell of the virion. The VP7 glycoprotein is synthesized on the rough ER (RER) and co-translationally inserted in the ER membrane. As indicated earlier, Seg9 contains two in-frame initiation codons, both of which may be used to generate protein products. However, post-translational cleavage results in removal of the amino terminus of both forms of VP7, yielding identical protein products that are incorporated into the virion.

Six non-structural proteins are encoded by the viral genome. NSP1 (59 kDa), encoded by Seg5, is the most variable of all the rotavirus proteins within a single species, with as much as 65% sequence diversity observed between strains of *Rotavirus A*. The total length of this genome segment, as well as the size of the ORF, can vary among strains. Although highly variable overall, NSP1 has a conserved cysteine-rich motif near the amino terminus, which is organized in a manner characteristic of zinc-binding RING-domain proteins. NSP1 has been shown to bind both the 5′ end of viral RNA and zinc. As indicated by studies of rotavirus reassortants in a mouse model of infection, NSP1 has a role in viral pathogenesis and is a virulence determinant. NSP1 antagonizes the host innate immune response by inducing the degradation of interferon regulatory factors (IRF3, IRF5

**Table 24:** Genome segments and protein products of Simian rotavirus A/SA11

| Genome segment | Size (bp) | Protein* | Protein size (kDa) | Protein copy number/ particle | Location | Function |
|---|---|---|---|---|---|---|
| Seg1 | 3302 | VP1 (Pol) | 125.0 | ≤12 | core | RNA-dependent RNA polymerase, minor core component, activated by VP2 |
| Seg2 | 2690 | VP2 (T1) | 102.4 | 120 | innermost capsid | Core shell protein, RNA binding, sub-group specificity antigen |
| Seg3 | 2591 | VP3 (Cap) | 98.1 | ≤12 | core | Guanylyltransferase, methyltransferase, ssRNA binding, minor core component |
| Seg4 | 2362 | VP4<br>VP5*<br>VP8* | 86.8<br>60.0<br>28.0 | 180 | outer capsid | P-type neutralization antigen, viral attachment protein, homotrimer, spike, hemagglutinin, activated by trypsin cleavage (VP5*, membrane penetration; VP8*, carbohydrate binding) |
| Seg5 | 1611 | NSP1 | 58.7 | 0 | cytoplasm | Antagonist of interferon expression, putative viral E3 ubiquitin ligase, RNA binding, RING domain |
| Seg6 | 1356 | VP6 (T13) | 44.8 | 780 | intermediate capsid | Group and sub-group specificity antigen, trimeric, major virion protein |
| Seg7 | 1104 | NSP3 | 34. 6 | 0 | cytoplasm | Binds 3′ end of viral mRNA and cellular eIF4G, promotes circularization of viral mRNAs, surrogate of PABP, inhibits host translation |
| Seg8 | 1059 | NSP2 (ViP) | 36.7 | 0 | viroplasm | Octamer with NTPase, RTPase, and helix destabilizing activities, ssRNA binding, essential viroplasm component, interacts with NSP5 |
| Seg9 | 1062 | VP7 (1)<br>VP7 (2) cleaved form | 37.4<br>33.9 | 780 | outer capsid | G-type neutralization antigen, virion surface glycoprotein, forms $Ca^{2+}$-stabilized trimer |
| Seg10 | 751 | NSP4 | 20.3 | 0 | RER membrane | RER transmembrane glycoprotein, binds DLPs, essential for budding into ER and addition of outer capsid, age-dependent diarrhea-inducing enterotoxin, disrupts Ca2+ homeostasis |
| Seg11 | 667 | NSP5 | 21.7 | 0 | viroplasm | Phosphorylated, O-linked glycosylation, serine-threonine rich, RNA binding, essential viroplasm component, interacts with NSP2 |
| | | NSP6 | 11.0 | 0 | viroplasm | Product of second ORF, RNA binding, interacts with NSP5, non-essential viroplasm component |

*Protein structure/function: Pol, RNA polymerase ; Cap, capping enzyme (guanylyltransferase and transmethylase); T1, inner virus structural protein with T = 1 symmetry with two molecules in the icosahedral asymmetric unit (interpreted in the orbiviruses as T = 2 symmetry); T13, inner virus structural protein with T = 13 symmetry; ViP, viral inclusion body or viroplasm matrix protein. Viruses from other species within the genus may have proteins with significant differences in sizes.

and IRF7) required for expression of type I interferon, possibly by acting as an E3 ubiquitin ligase. NSP1 may also play a role in host range restriction, suggesting that its activity may vary depending on the host species.

NSP2 (35 kDa) contains a HIT-like fold and self-assembles into a stable octamer, the functional form of the protein. It binds nonspecifically to ssRNA, has NTPase, RTPase and helix destabilizing activities, and interacts with NSP5 to form viroplasms (equivalent to VIBs generated by members of other genera within the family Reoviridae). Viruses that contain temperature-sensitive mutations in Seg8, which encodes NSP2, have an RNA-negative phenotype at the non-permissive temperature, indicating that NSP2 has a direct role in the mechanism of virus replication. It has been hypothesized that NSP2 functions as a molecular motor and plays an important role in viral RNA packaging.

NSP3 (34 kDa) is a multifunctional protein that forms dimers and has several binding partners. NSP3 binds specifically to the 3′-terminal conserved sequence of viral mRNAs via its amino terminus. The carboxyl-terminal region of NSP3 interacts with the eukaryotic translation initiation factor eIF4G, and its middle region binds RoXaN (*ro*tavirus *X* protein *a*ssociated with *N*SP3). In uninfected cells, eIF4G interacts with polyA-binding protein (PABP), which binds to the 3′-polyA tail of mRNAs and stimulates translation by circularizing host mRNAs. NSP3 is proposed to inhibit host cellular protein synthesis during infection by evicting PABP from its binding site on eIF4G. NSP3 may facilitate circularization of viral mRNA through its specific interactions with eIF4G and the 3′-conserved sequence of viral mRNAs, thereby promoting viral translation.

NSP4 (20 and 28 kDa) is synthesized on the RER as a transmembrane protein and may be post-translationally glycosylated. Both glycosylated and nonglycosylated forms of NSP4 are detected in infected cells. During later stages of virion maturation, membrane-associated NSP4 functions as a receptor for DLPs, aids in acquisition of VP4 and VP7 and budding through the ER membrane. NSP4 also functions as a viral enterotoxin that leads to $Ca2+$ release from internal stores in the ER and induction of age-dependent diarrhea in mice. An NSP4 cleavage product is secreted from infected cells and binds to integrins $\alpha1\beta1$ or $\alpha2\beta1$, triggering a signalling pathway that activates phospholipase C and elevates inositol 1,4,5-triphosphate, leading to $Ca2+$ release. This pathway is distinct from that of intracellularly expressed NSP4.

The two remaining non-structural proteins, NSP5 (26 kDa) and NSP6 (12 kDa), are encoded by two ORFs in the same viral gene. NSP5, which is serine-threonine rich, is post-translationally modified by both phosphorylation and glycosylation. The protein has ssRNA- and dsRNA-binding activities, forms dimers, and is essential for viroplasm formation and genome replication. Multiple phosphorylated isomers of NSP5 exist in the infected cell. Phosphorylation is stimulated by NSP2 *in vivo*, but mediated by cellular kinases and possibly an NSP5 autokinase activity. Highly phosphorylated forms of NSP5 localize to viroplasms; the significance of phosphorylation to NSP5 function is unknown. Interactions of NSP2 and NSP5 are responsible for viroplasm formation and localization of core proteins. Interactions of NSP2 and NSP5 with core proteins regulate progeny core formation. A carboxyl-terminal domain of NSP5 is required for multimerization, hyperphosphorylation and interactions with NSP6. A potential ORF encoding NSP6 is conserved among many, but not all, virus isolates. However, NSP6 protein expression has been demonstrated for only a few strains, and its function is not clearly defined. For SiRV-A/SA11, NSP6 (11 kDa) is phosphorylated and localizes to viroplasms, where it may play a regulatory role in the self-association of NSP5.

Information on proteins encoded by viruses of species other than *Rotavirus A* is less abundant and primarily drawn from sequence analysis of viral genes. The majority of rotaviruses are predicted to encode a single protein product per segment. It is clear that other rotavirus species have homologs of the proteins characterized for *Rotavirus A* viruses. However, high levels of variation are observed between protein homologs (or putative protein products) of different species (e.g. >84% for VP3 and >87% for VP4), in comparison to the variation observed among *Rotavirus A* proteins (e.g. <25% for VP2 and <45% for VP4) (Figure 26 and Table 25). In a few cases, proteins of species other than *Rotavirus A* have been analyzed. For example, the structure of *Rotavirus C* NSP2 has been determined. Although structurally quite similar, this protein was unable to complement the function of *Rotavirus A* NSP2 in SiRV-A/SA11-infected cells in which expression of the cognate NSP2 had been knocked down. Additionally, it has been shown that recombinant *Rotavirus C* VP1 will replicate

*Rotavirus A* RNA, or its own RNA, but only in the presence of its cognate VP2. *Rotavirus C* VP1 is also predicted to have a structure similar to SiRV-A/SA11 VP1. Further insights into the structure and function of viral proteins from species other than *Rotavirus A* await the establishment of suitable highly permissive cell culture systems and the expression and characterization of additional non-*Rotavirus A* recombinant proteins.

### Lipids

None reported. Immature particles acquire a transient membrane during budding into the ER.

### Carbohydrates

Three viral proteins have been shown to be glycosylated. In two cases (VP7 and NSP4), the sugar is N-linked to asparagine, and in the third (NSP5) it is O-linked to serine and/or threonine.

## Genome organization and replication

The complete RNA-protein coding assignments have been determined for several *Rotavirus A* isolates. The coding assignments for the SiRV-A/SA11 strain are given in Table 24. The replication cycle for many animal rotavirus strains, which is typically completed in 12–15 h at 37 °C, has been studied primarily in continuous cell cultures derived from monkey kidneys. There is little definitive information about the early steps in the replication cycle. VP4 is the viral attachment-protein but the cellular receptor has not been conclusively identified. Some rotavirus strains attach to the N-acetylneuraminic (sialic) acid residues on the cell surface, and there is evidence to suggest that several integrins and heat shock protein 70 serve as co-receptors. Current data suggest that rotaviruses may enter cells either by receptor-mediated endocytosis or by direct membrane penetration. In both cases, virus entry leads to loss of the outer VP4 and VP7 protein layer and release of a transcriptionally-active DLP into the cytoplasm. DLP-associated enzymes produce 5′-capped, non-polyadenylated mRNAs, which are synthesized using the full-length minus strand of each genome segment as a template. In part, gene expression is regulated by differences in the level of transcription occurring from each genome segment. The viral mRNAs serve two functions. Firstly, they act as templates for translation, producing viral proteins. Efficiency of mRNA translation regulates expression levels of the individual viral proteins. Secondly, viral mRNAs act as templates for minus-strand synthesis, producing dsRNA genome segments. The use of short interfering RNA technology has provided evidence that the pools of mRNAs that serve as templates for translation and replication are distinct. Virus assembly begins in cytoplasmic inclusions termed viroplasms, formation of which is mediated by NSP2 and NSP5. During early assembly steps, the 11 different viral mRNAs interact with one another and with VP1 and VP3. These interactions are followed by association with VP2, which triggers minus-strand synthesis and results in the formation of cores containing a complete set of 11 dsRNA segments. Cis-acting replication elements for minus-strand synthesis have been identified using an *in vitro* replication system, the most critical of which is the seven 3′-terminal nucleotides of the mRNAs. Following their assembly, VP6 is added to cores, forming DLPs. The next steps in the morphogenesis of progeny virions are unique to rotaviruses and involve recruitment of DLPs to the ER by the transmembrane glycoprotein NSP4. Budding of DLPs through the ER results in the transient acquisition of a lipid envelope and addition of VP4 and VP7 to form the outer virion shell.

## Antigenic properties

Three viral proteins (VP4, VP6 and VP7) of *Rotavirus A* isolates have been subjected to detailed antigenic characterization. VP6, which forms the intermediate capsid layer, is a highly conserved and highly immunogenic protein, carrying both virus group and sub-group (SG)-specific determinants. It does not elicit the production of neutralizing antibodies, but it may play a role in the induction of protective immunity. VP6 has been the major target of diagnostic assays for rotaviruses and specifies the group (i.e. the species or candidate species listed for this genus).

Within *Rotavirus A* isolates, VP6 bears the epitopes of the SG specificities that allow antigenic classification of rotavirus into SG I, SG II, both SG I and II, or neither SG, according to reactivity with two monoclonal antibodies. More than a decade ago, a binary classification system reminiscent of the one used for the classification of influenza A viruses was established. This system was based on antigenic reactivities of the two outer capsid proteins, VP4 and VP7, which independently elicit

neutralizing antibodies. Thus, rotavirus strains were classified into P serotypes (VP4 is protease sensitive) and G serotypes (VP7 is a glycoprotein). Classification of rotaviruses into P (VP4) or G (VP7) serotypes is performed by cross-neutralization assays using hyperimmune sera raised to prototype viruses or laboratory-engineered mono-reassortants. So far, 14 G serotypes and 14 P serotypes have been identified. As the ease of nucleic acid sequencing has increased, antigenic classification has slowly been replaced by a classification system of rotaviruses into P (VP4) and G (VP7) genotypes that is based on nucleotide sequence similarities of VP4 and VP7 genes. G serotype designations largely coincide with G genotype designations. There is greater discrepancy among P serotype and genotype designations. Thus, a dual nomenclature has been adopted for VP4 antigenic and genetic classification. The P serotype, when known, is denoted by a number. The P genotype is denoted by a number within squared brackets, which immediately follows the P serotype. When the P serotype is not known, only the P genotype is used.

Recently, a uniform classification and nomenclature system, based on nucleotide identities of the 11 rotavirus genome segments and phylogenetic dendrograms, has been established by the Rotavirus Classification Working Group for *Rotavirus A*. This classification scheme currently comprises 23 G (VP7), 32 P (VP4), 14 I (VP6; *i*nner capsid), 6 R (VP1; *R*NA-dependent RNA polymerase), 6 C (VP2; *c*ore), 7 M (VP3; *m*ethyltransferase), 16 A (NSP1; interferon *a*ntagonist), 6 N (NSP2; *N*TPase), 8 T (NSP3; *t*ranslation enhancer), 12 E (NSP4; *e*nterotoxin), and 8 H (NSP5/6; p*h*osphoprotein) genotypes.

In the case of species other than *Rotavirus A*, little information is available at present on the extent of antigenic diversity.

## Biological properties

Rotaviruses can prove difficult to cultivate *in vitro*, with highly permissive growth generally restricted to epithelial cell lines derived from monkey kidneys. Infection can be enhanced by pretreatment of virus with trypsin. The restriction of virus growth *in vitro* parallels the *in vivo* situation, in which virus replication is typically restricted to the terminally differentiated enterocytes lining the tips of the microvilli in the small intestine. However, recent evidence suggests that some rotavirus infections may cause antigenemia and viremia due to replication at secondary sites in the host.

There are several mechanisms of pathogenesis, including the destruction of enterocytes, leading to malabsorption and an osmotic diarrhea. Prior to the appearance of histologic changes, a watery diarrhea is often seen, which is thought to be secretory, possibly induced by the action of the rotavirus enterotoxin NSP4. It has been proposed that the destruction of enterocytes causes a loss of the permeability barrier between the gut lumen and the vasculature, resulting in the osmotic pull of fluid from the circulation into the gut and the watery characteristic of rotavirus-induced diarrhea. Rotaviruses infect a wide range of avian and mammalian species, with disease being restricted in the great majority of cases to the young. Isolates of *Rotavirus A*, *Rotavirus B* and *Rotavirus C* can infect humans, with *Rotavirus A* being responsible for the majority of seasonal endemic diarrheal disease in young children. Isolates of *Rotavirus B* have caused sporadic epidemic outbreaks of gastroenteritis in adults. *Rotavirus C* has been associated with self-limiting outbreaks of gastroenteritis in humans, primarily in the young.

## Species demarcation criteria in the genus

The rotaviruses are currently divided into five species (*Rotavirus A* to *Rotavirus E*), and three additional groups that have not yet been classified (Rotavirus F, Rotavirus G and NADRV). Viruses within different species are thought to be unable to reassort their genome segments under normal circumstances, and each species may therefore represent a separate gene pool.

In addition to the other general criteria used throughout the family, members of a species in the genus *Rotavirus* may be identified by:

- Serological cross-reactivity by ELISA, using either polyclonal sera or monoclonal antibodies against VP6 (or its homolog in isolates of other species).

- Sequence analysis of conserved genome segments (e.g. Seg1 and Seg6). Viruses within the same species will normally have >75% nucleotide in Seg6. However, this is not a consistent method for differentiating all species, as avian and mammalian isolates of *Rotavirus A* may have only about 60% identity.
- Host range. For example, *Rotavirus E* isolates have to date only been found in pigs. *Rotavirus D* isolates and the Rotavirus F and Rotavirus G groups have only been isolated from avian species.

### List of species in the genus *Rotavirus*

| | | |
|---|---|---|
| *Rotavirus A* | | |
| Simian rotavirus A/SA11 | [Seg1: X16830, Seg2: X16831, Seg3: X16062, Seg4: X14204, Seg5: X14914, Seg6: X00421, Seg7: X00355, Seg8: J02353, Seg9: K02028, Seg10: KO1138, Seg11: X07831] | (SiRV-A/SA11) |
| *Rotavirus B* | | |
| Rotavirus B | [Seg1: EU490413, Seg2: AY539859, Seg3: EU490416, Seg4: AY539857, Seg5: AY539862, Seg6 AY539858, Seg7: AY539860, Seg8: AY539861, Seg9: AY539856, Seg10: AY539864, Seg11: AY539863] | (RV-B/WH-1) |
| *Rotavirus C* | | |
| Human rotavirus C/Bristol | [Seg1: AJ304859, Seg2: AJ303139,Seg3: X79442, Seg4: X96697, Seg5: X59843, Seg6: AJ132203, Seg7: AJ132204, Seg8: X77257, Seg9: AJ132205, Seg10: M81488, Seg11: X83967] | (HRV-C/Bristol) |
| *Rotavirus D* | | |
| Chicken rotavirus 05V0059 | [Seg1: GU733443, Seg2: GU733444, Seg3: GU733445, Seg4: GU733446, Seg5: GU733447, Seg6: GU733448, Seg7: GU733449, Seg8: GU733450, Seg9: GU733451, Seg10: GU733452, Seg11: GU733453] | (AvRV-D/05V0059) |
| *Rotavirus E* | | |
| Porcine rotavirus E/DC-9 | | (PoRV-E/DC-9) |

### List of other related viruses which may be members of the genus *Rotavirus* but have not been approved as species

| | | |
|---|---|---|
| Rotavirus F | | (RV-F) |
| Chicken rotavirus F/A4 | | (AvRV-F/A4) |
| Rotavirus G | | (RV-G) |
| Chicken rotavirus G/555 | | (AvRV-G/555) |
| NADRV | | (NADRV) |
| Human NADRV/ J19 | [Seg1: DQ113897, Seg2: DQ113898, Seg3: DQ113900, Seg4: DQ113899, Seg5: DQ113901, Seg6: DQ113902, Seg7: DQ113903, Seg8: DQ113904, Seg9: DQ113905, Seg10: DQ113906, Seg11: DQ113907] | (Hu NADRV/J19) |

### Phylogenetic relationships within the genus

## GENUS *SEADORNAVIRUS*

Type species ***Banna virus***

### Distinguishing features

The seadornavirus genome consists of 12 segments of dsRNA. During replication, viruses are found in the cell cytoplasm. Viruses are transmitted to vertebrate hosts by mosquito vectors.

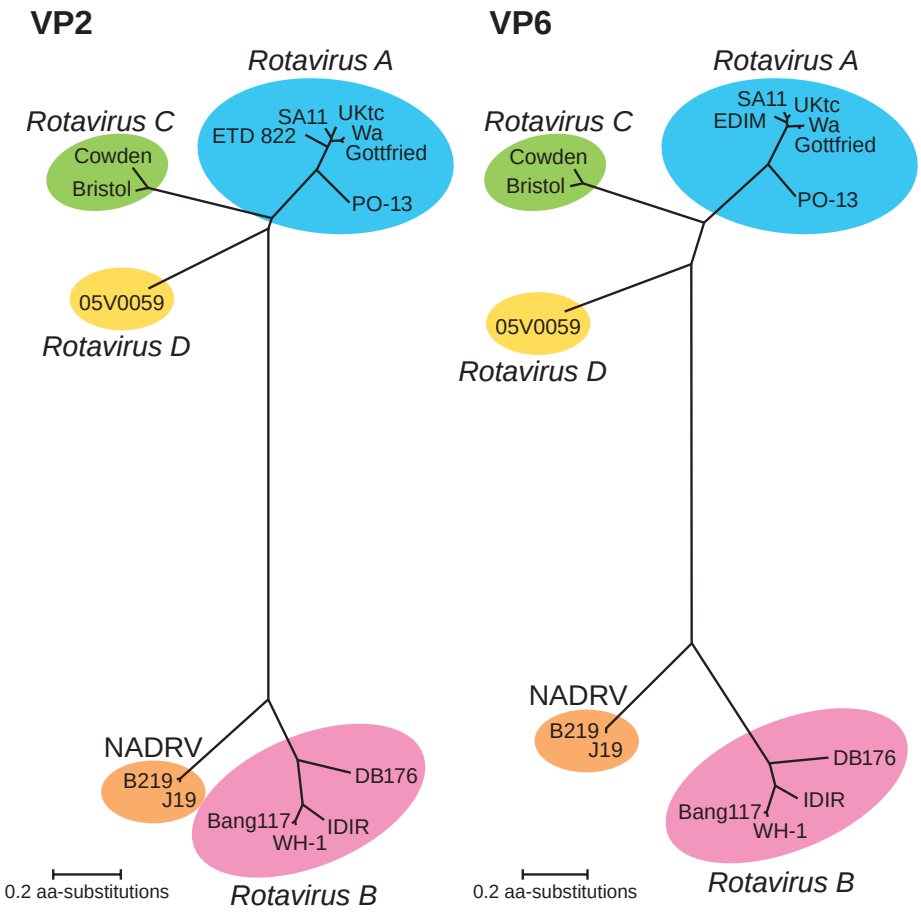


**Figure 26:** Phylogenetic tree comparing amino acid sequences of the rotavirus inner core shell protein VP2 (left) and intermediate capsid protein VP6 (right). Isolates and accession numbers used are: Bo/UKtc (P17462,P18610), Si/SA11 (CAA34733, AAO32085), Hu/Wa (X14942, P03530), Po/Gottfried (ADE44250, P16593), Mu/ETD_822 (ACY95261), Mu/EDIM (AAC57838), Av/PO-13 (BAA24147, BAA03836), Hu/WH-1 (AAT09117, AAT09116), Hu/Bang117 (ADF57895, ADF57898), Bo/DB176 (ADC53105, ADC53099), Mu/IDIR (AAA17401, QO1754), Hu/Bristol (CAC44890, CAA42504), Po/Cowden (P26191, AAA47097), Av/05V0049 (ADN06424, ADN06428), Hu/J19 (YP_392491, AAZ03490), and Hu/B219 (ABR32123, ABA60393). Sequences were aligned and trees calculated (neighbor-joining method) using MEGA 4 (courtesy of J. Matthijnssens). Abbreviations used to indicate host species are: Av, avian; Bo, bovine; Hu, human; Mu, murine; Po, porcine; Si, simian. Sequences were aligned using ClustalX and the tree constructed in MEGA4 using the neighbor-joining method and the P-distance algorithm. Branching is supported by bootstrap values >85%.

## Virion properties

### Morphology

Particles are non-enveloped with a diameter ranging between 60 and 70 nm having two concentric capsid shells with a core that is about 40–50 nm in diameter. Electron microscopic studies, using negative staining have shown that particles have a well-defined surface capsomeric structure and icosahedral symmetry (Figure 27).

### Physicochemical and physical properties

The buoyant density of the virus in CsCl is 1.36 g cm$^{-3}$. Viruses are stable around neutral pH, but lose infectivity at pH 3.0. At 4 °C, the virus is stable for long periods, even non-purified in cell culture lysate, which is a convenient way for medium-term storage. Heating to 55 °C considerably decreases the viral infectivity. Seadornaviruses are stable upon treatment with freon, which could be used for purification of viral particles from cell lysate. The viral infectivity is abolished by

**Table 25:** Percentage amino acid differences between the inner capsid protein (VP2) of isolates of *Rotavirus A*, *B*, *C* and *D*, and novel adult diarrhea rotavirus (NADRV)

| Accession no. | | *Rotavirus A* | | | | *Rotavirus B* | *Rotavirus C* | *Rotavirus D* | NADRV |
|---|---|---|---|---|---|---|---|---|---|
| | | **Hu/Wa CAA34733** | **Mu/ ETD_822 X14942** | **Av/PO-13 ACY95261** | **Hu/WH-1 BAA24147** | **Hu/Bristol AAT09117** | **Av/05V0049 CAC44890** | **Hu/J19 ADN06424** | **YP_392491** |
| *Rotavirus A* | Si/SA11 | 0 | 7.5 | 11.6 | 23.6 | 88.2 | 53.0 | 53.4 | 87.5 |
| | Hu/Wa | | 0 | 12.2 | 23.8 | 88.2 | 52.8 | 53.2 | 88.1 |
| | Mu/ETD_822 | | | 0 | 24.6 | 88.2 | 52.8 | 54.0 | 87.6 |
| | Av/PO-13 | | | | 0 | 89.3 | 51.2 | 52.9 | 88.3 |
| *Rotavirus B* | Hu/WH-1 | | | | | 0 | 89.8 | 89.0 | 52.9 |
| *Rotavirus C* | Hu/Bristol | | | | | | 0 | 55.0 | 89.6 |
| *Rotavirus D* | Av/05V0049 | | | | | | | 0 | 88.6 |
| NADRV | Hu/J19 | | | | | | | | 0 |

The abbreviations used to indicate host species are: Av, avian; Hu, human; Mu, murine; Si, simian. MEGA 4 software was used to calculate p-distances on the amino acid level.

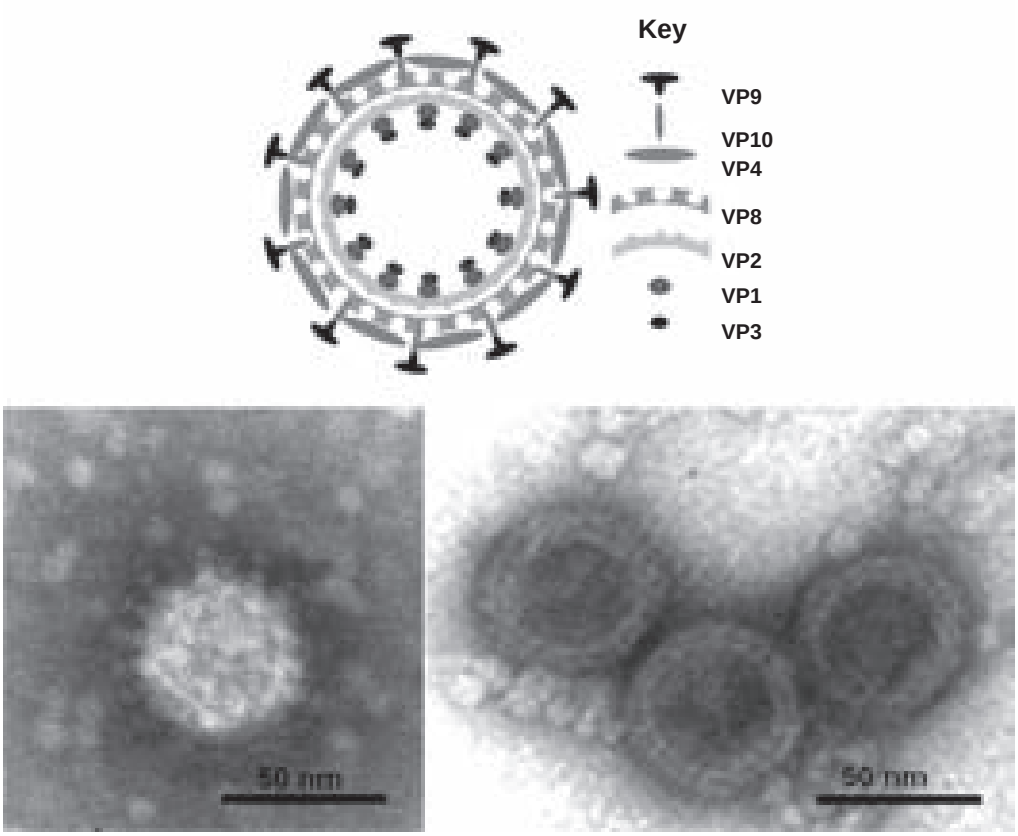


**Figure 27:** (Top) Diagram of the Banna virus (BAV) particle structure, constructed using data from biochemical analyses, electron microscopy and X-ray crystallography. (Bottom) Negative contrast electron micrograph of Banna virus particles: (left hand side) full BAV particles showing multiple of protein spikes; (right hand side) double layered cores of BAV (courtesy of H. Attoui).

treatment with sodium dodecyl sulfate. Viruses can be stored for long periods at −80 °C, and infectivity is further protected by addition of 50% fetal calf serum.

### Nucleic acid

The genome consists of 12 dsRNA segments that are numbered in order of reducing Mr, or increasing electrophoretic mobility during agarose gel electrophoresis. The genome comprises approximately 21,000 bp, with segment lengths that range between 3747 and 862 bp. The RNA genome segments of Banna virus (BAV), Kadipiro virus (KDV) and Liao ning virus (LNV) migrate as groups of 6-6, 6-5-1 and 6-2-3-1, respectively during 1% agarose gel electrophoresis (AGE) (Figure 28). These patterns are thought to be characteristic of each virus species.

From the full-genome sequence analyses of different Banna virus isolates, two distinct genotypes have been identified (based on Seg7 and Seg9) that correlate with virus serotype. Genotype A includes isolates BAV-Ch (China) and BAV-In6423 (Indonesia), while genotype B includes BAV-In6969 and BAV-In7043 (Indonesia). The proteins translated from Seg7 and Seg9 show 72% and 54% aa identity between genotypes A and B respectively, while all of the other segments appear to be more conserved, showing 83% to 98% identity.

Two genotypes of LNV have also been identified that correlate with virus serotype. Amino acid identity in the cell attachment outer capsid protein VP10 between these two genotypes was found to be 81%.

Much lower levels of aa sequence identity were detected between homologous proteins of different species: between BAV and KDV (24% to 42%), between BAV and LNV (18% to 41%) or between LNV and KDV (21% to 42%). In each case highest levels of aa identity were detected in the viral polymerase (VP1[Pol]).

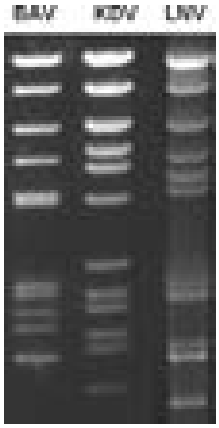


**Figure 28:** Migration patterns (electropherotypes) of genome segments from isolates of Banna virus (BAV), Kadipiro virus (KDV) and Liao ning virus (LNV) in 1% agarose gel. Electropherotype is thought to be characteristic of each virus species.

**Table 26:** Conserved terminal sequences (positive strand) of seadornavirus genome segments

| Virus species | Strain | 5′ end | 3′ end |
|---|---|---|---|
| *Banna virus* | BAV-In6423 | 5′-GUAU$^{A}/_{U}{}^{A}/_{U}$AA$^{A}/_{U}{}^{A}/_{U}$U | $^{A}/_{G}$C$^{C}/_{U}$GAC-3′ |
| *Kadipiro virus* | KDV-Ja7075 | 5′-GUAGAA$^{A}/_{U}{}^{A}/_{U}{}^{A}/_{U}$U | A$^{A}/_{C}{}^{C}/_{U}$GAC-3′ |
| *Liao ning virus* | LNSV-NE97-31 | 5′-GUUAU$^{A}/_{U}{}^{A}/_{U}{}^{A}/_{U}$ | $^{A}/_{C}{}^{U}/_{C}$CGAC-3′ |

The genome segments of BAV and KDV have a G+C content that varies between 37% and 39%, with 5′- and 3′-NTRs that vary between 76 and 200 nt respectively. The NTRs of all seaodornaviruses so far studied (Table 26) contain conserved base pairs at both termini (+ve 5′-GU GAC-3′).

Seadornavirus mRNAs are usually regarded as non-infectious. However, fully functional and infectious viruses have been recovered by the introduction of all twelve mRNAs into BSR cells.

### Proteins

Native proteins of BAV particles were characterized by mass spectrometry analysis and by radiolabeling of BAV infected cells. Their putative functions are indicated in Table 27.

Host–cell protein synthesis shut-off starts at 2 h post BAV infection of C6/36 cells (a mosquito cell line), and the shut-off is complete by 6 hpi. [$^{35}$S]-methionine added to C6/36 cell cultures at 6 hpi was incorporated almost exclusively into 12 protein bands (resolved by SDS PAGE, which are thought to represent the different viral proteins (one protein per genome segment). Most of these proteins have apparent molecular masses that agree with the theoretical sizes predicted by sequence analysis of the viral genome. The only exception is VP7, which migrates more slowly than expected.

Purified BAV-Ch virus particles contain seven structural proteins, each of which co-migrated with one of the radio-labeled proteins from infected cells (VP1, VP2, VP3, VP4, VP8, VP9 and VP10). Only five of these proteins were also detected in cores, indicating that the outer coat (like those of the non-turreted orbiviruses and rotaviruses) is composed of two proteins (VP4 and VP9). Analyses of BAV-Ch structural protein sequences by mass spectrometry confirmed the identity of the core (VP1, VP2, VP3, VP8, VP10) and outer capsid components (made of VP9 and VP4), demonstrating that VP5, VP6, VP7, VP11 and VP12 are non-structural proteins.

[$^{35}$S] methionine labeled BAV-Ch particles were purified and analyzed by SDS-PAGE and the ratios of the different structural proteins were calculated. VP2 and VP8 are the two most abundant proteins of the BAV core. The lower relative abundance and higher molecular weight of VP2 identifies

it as the subcore-shell T2 protein [equivalent to VP3 of bluetongue virus (BTV) and VP2 of rotavirus]. In contrast, VP8 is smaller and more abundant, identifying it as the core-surface T13 protein. VP8 and VP2 have a molar ratio of 6.5 in purified BAV-Ch particles, identical to the ratio of 780/120 previously detected between the subcore and core-surface proteins of both BTV and rotaviruses. On this basis, the numbers of the VP8 and VP2 molecules in the BAV core are assumed to be 780 and 120 per particle, respectively, allowing the average copy number of the other protein components of virus particles or cores to be calculated: 24 copies for VP1(Pol), 12 copies for the VP3(Cap), 333 copies for the VP4, 310 copies for VP9 and VP10.

The structure of the BAV outer-capsid protein VP9 was determined by X-ray crystallography at 2.6Å resolution, revealing a trimeric molecule, held together by an N-terminal helical bundle, reminiscent of coiled-coil structures found in fusion-active proteins such as HIV gp41. The major domain of VP9 contains stacked β sheets with marked structural similarities to the rotavirus receptor binding protein VP8. Anti-VP9 antibodies neutralize viral infectivity, and, remarkably, pretreatment of cells with trimeric VP9 increased viral infectivity, indicating that VP9 is involved in virus attachment to cell surface and subsequent internalization. Sequence similarities were also detected between BAV VP10 and the VP5 portion of rotavirus VP4, suggesting that the receptor binding and internalization apparatus, which is a single gene product activated by rotavirus proteolysis, is the product of two separate genome segments in BAV.

### Lipids
None reported.

### Carbohydrates
None reported.

## Genome organization and replication

The 12 genome segments are all monocistronic (Table 27). Seadornavirus isolates replicate in mosquito cell lines, and considerable amounts of virus (over 40% of progeny) are liberated into the culture medium prior to cell death and gross CPE, which usually occurs by 40 h post infection with BAV, and 72 h post infection with KDV. BAV was also found to replicate in BSR cells (a clone of BHK-21). Liao ning virus is the only seadornavirus known to replicate in a wide variety of mammalian cells, including primary cell cultures.

**Table 27:** Genome segments and protein products of Banna virus-Indonesia-6423

| Genome segment | Size (bp) | Protein (copy number per particle) | Protein size (kDa)* | Structure/function |
|---|---|---|---|---|
| Seg1 | 3747 | VP1(Pol) [24] | 137 | RdRp |
| Seg2 | 3048 | VP2(T2) [120] | 108 | Inner layer of core, T2 protein |
| Seg3 | 2400 | VP3(Cap) [12] | 82 | Capping enzyme |
| Seg4 | 2038 | VP4 [333] | 64 | Outer coat protein |
| Seg5 | 1716 | VP5-NS [0] | 56 | Non-structural |
| Seg6 | 1671 | VP6-NS [0] | 48 | Non-structural |
| Seg7 | 1136 | VP7-NS [0] | 35 | Protein kinase |
| Seg8 | 1119 | VP8 [780] | 32 | T13, outer layer of core |
| Seg9 | 1101 | VP9 [310] | 31 | Outer coat cell attachment protein |
| Seg10 | 977 | VP10 [310] | 29 | Stalk base for VP10 |
| Seg11 | 867 | VP11-NS [0] | 21 | Non-structural |
| Seg12 | 862 | VP12-NS [0] | 24 | dsRNA-binding |

*Calculated from the sequenced segments.

Large electron-dense structures occur in the cytoplasm of BAV-Ch infected cells, which correspond to the viral inclusion bodies (VIB) thought to be the main site of replication and particle assembly of other reoviruses. Particles (ca. 50 nm in diameter) with a smooth surface were detected mainly at the periphery of the VIB, although some particles were observed within the VIB matrix. Virus particles were also detected within large vacuoles that were dispersed throughout the infected cell cytoplasm. These vacuoles contained multiple double-layered vesicles, lined with viral particles (ca. 50 nm in diameter) at their inner surface and it is possible that this reflects some involvement of cellular membrane structures or organelles in virus morphogenesis, transport or replication (as previously reported for the rotaviruses). Virus entry into cells by endocytosis was suggested by the detection of virus particles in pits at the cell surface. Virions were also observed near the cell membrane, which appeared to be budding from the cell surface.

## Antigenic properties

BAV from China, Vietnam and Indonesia, KDV from China and Indonesia and Liao ning virus from China are classified as three distinct species and show little cross-reaction in neutralization tests.

## Biological properties

Seadornaviruses have been isolated from humans and mosquitoes, which serve as vectors. The mosquito species that have been implicated include *Culex vishnui*, *C. fuscocephalus*, *Anopeles vagus*, *Anopheles aconitus*, *Anopheles subpictus* and *Aedes dorsalis*. Experimentally, the viruses were also found to replicate in adult mice and were detected in infected mouse blood 3 days post infection. LNV kills adult mice causing a hemorrhagic syndrome. BAV was isolated from the serum and cerebrospinal fluids of human patients showing neurological manifestations. The pathology provoked by this virus is only poorly described. KDV has only been isolated from mosquitoes.

BAV and KDV occur in tropical and subtropical regions, where other mosquito-borne viral disease especially Japanese encephalitis and dengue have been reported as endemic. Despite the isolation of BAV from infected human patients, no surveys have been reported concerning the detection and prevalence of antibodies to these viruses in human sera. Liao ning virus has been isolated from mosquitoes.

## Species demarcation criteria in the genus

In addition to the other general criteria used throughout the family, members of a species in the genus *Seadornavirus* may be identified by:

- RNA cross-hybridization assays: within a single species: RNA sequence that exhibit more than 74% similarity will hybridize at 36 °C below the Tm of fully base-paired duplex.
- Serological comparisons by neutralization assays. Hyperimmune ascitic fluids against genotype A viruses of BAV, do not efficiently cross-neutralize those of genotype B. However, isolates within a single genotype show high levels of cross-neutralization. There is no cross-neutralization or cross-reactivity between members of the species *Banna virus* and *Kadipiro virus*.
- Sequence analysis: In the conserved Seg12, viruses within the same species will normally have >89% nucleotide identity. Within the polymerase (the most conserved protein) isolates of different species have <50% aa identity.

## List of species in the genus *Seadornavirus*

*Banna virus*
{*Culex* and *Anopheles* mosquitoes: humans}

| | | |
|---|---|---|
| Banna virus-China | [Seg1: AF134525, Seg2: AF13526, Seg6: AF13527, Seg7: AF052035, Seg8: AF052034, Seg9: AF0520333, Seg10: AF052032, Seg11: AF052031, Seg12: AF052030] | (BAV-Ch) |

| | | |
|---|---|---|
| *Kadipiro virus* | | |
| {*Culex* mosquitoes} | | |
| Kadipiro virus-Java-7075 | [Seg1: AF133429, Seg2: AF134509,Seg3: AF134510, Seg4: AF134511, Seg5: AF134512, Seg6: AF134513, Seg7: AF052023, Seg8: AF052022, Seg9: AF052021, Seg10: AF052020, Seg11: AF052019, Seg12: AF019909] | (KDV-Ja7075) |
| *Liao ning virus* | | |
| {*Aedes dorsalis*: mosquito} | | |
| Liao ning virus (NE97-12) | [Seg1: AY701339, Seg2: AY701340,Seg3: AY701341, Seg4: AY701342, Seg5: AY701343, Seg6: AY701344, Seg7: AY701345, Seg8: AY701346, Seg9: AY701347, Seg10: AY701348, Seg11: AY701349, Seg12: AY701350] | (LNV-NE97-12) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ], arthropod vector and host names { } and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Seadornavirus* but have not been approved as species

| | |
|---|---|
| Banna virus ACH | (BAV-ACH) |
| Banna virus HN59 | (BAV-HN59) |
| Banna virus HN131 | (BAV-HN131) |
| Banna virus HN191 | (BAV-HN191) |
| Banna virus HN295 | (BAV-HN295) |
| Banna virus LY1 | (BAV-LY1) |
| Banna virus LY2 | (BAV-LY2) |
| Banna virus LY3 | (BAV-LY3) |
| Banna virus M14 | (BAV-M14) |
| Banna virus TRT2 | (BAV-TRT2) |
| Banna virus TRT5 | (BAV-TRT5) |
| Banna virus WX1 | (BAV-WX1) |
| Banna virus WX2 | (BAV-WX2) |
| Banna virus WX3 | (BAV-WX3) |
| Banna virus WX8 | (BAV-WX8) |

These isolates of seadornaviruses have been isolated in many provinces in China including Beijing, Gansu, Yuannan, Hainan, Henan, Shanshi, Xinjiang and recently from Liao ning. Viruses other than Banna, Kadipiro and Liao-ning viruses are still uncharacterized and have been temporary designated Banna virus isolates. Their serological relationship to Banna virus was not fully explored. These isolates are probably distinct from *Banna virus*, and at least some should represent new species within genus *Seadornavirus*.

### Phylogenetic relationships within the genus

## GENUS *PHYTOREOVIRUS*

Type species ***Wound tumor virus***

### Distinguishing features

Phytoreovirus particles have icosahedral symmetry with a distinctive angular appearance and possess 12 dsRNA species. They are transmitted by cicadellid leafhoppers to susceptible plant species, replicating in both hosts and vectors.

### Virion properties

#### MORPHOLOGY

Virions of rice dwarf virus (RDV) are icosahedral, appear to be double-shelled and about 70 nm in diameter (Figure 30). The outer layer of RDV contains 260 trimers of P8 (46 kDa): a total of 780 molecules, arranged with T = 13 *l* symmetry (Figure 30). The relative location of the neighboring

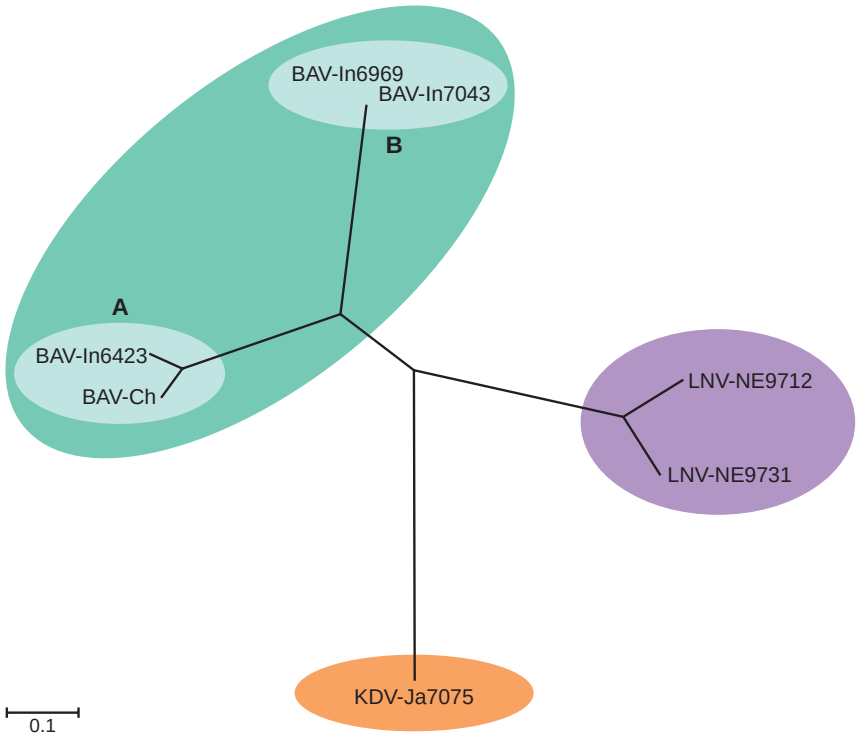


**Figure 29:** Phylogenetic tree for homologous genome segments from members of the species *Banna virus*, *Kadipiro virus* and *Liao ning virus*. The tree is based on outer-capsid and cell attachment protein sequences (encoded by Seg9 (VP9) of BAV, its homologous Seg11 (VP11) of KDV and Seg10 (VP10) of LNV). This tree also shows the two genotypes (serotypes) of BAV (A and B). The amino acid identity between VP9 of BAV and VP11 of KDV ranges between 14% and 16%. Between VP9 of BAV and VP10 of LNV amino acid identity ranges from 17% to 19%. Between VP11 of KDV and VP10 of LNV amino acid identities ranged from 17% to 18%. Between the two genotypes of BAV, it ranges from 41% to 43%. Within a given genotype of BAV it ranges between 90 and 96%. Between the two genotypes (serotypes) of LNV amino acid identity is 81%. Sequences were aligned using ClustalX and the tree constructed in MEGA4 using the neighbor-joining method and the P-distance algorithm. Branching is supported by bootstrap values >85%.



capsomers on the icosahedral particle is such that they form pentameric or hexameric rings. The inner capsid layer is reported to be a complete protein shell, composed of 60 dimers of P3 (114 kDa), a total of 120 molecules, arranged with a suggested T = 1 icosahedral symmetry (Figure 30). The outer capsid P8 trimers bind more tightly at the threefold positions of the single-layered core. The RDV particle structure appears to be comparable to that of core or double layered particles of some other genera (*Orbivirus* and *Rotavirus* respectively). Ordered structures are visible in the periphery of the RNA region.

Wound tumor virus (WTV) is reported to possess three protein shells, including an outer amorphous layer, an internal layer of distinct capsomers and a smooth core that is about 50 nm in diameter but lacking spikes.

### Physicochemical and physical properties

The Mr of phytoreoviruses is about $75 \times 10^6$. The virion $S_{20,w}$ is about 510. The optimal stability of particles is at pH 6.6. The buoyant density of RDV is 1.39–1.42 g cm$^{-3}$ and the virion is unstable losing P8 in CsCl. $CCl_4$ removes P2 from the RDV virion.

### Nucleic acid

Phytoreoviruses have 12 genome segments of linear dsRNA, numbered according to their migration during PAGE. However, their relative sizes based on RNA sequence data indicate that Seg4 and Seg5, or Seg9 and Seg10, may migrate in the reverse order during agarose gel electrophoresis (Table 28). The RNA constitutes about 22% of the virion dry weight. The dsRNA Mr is in the range

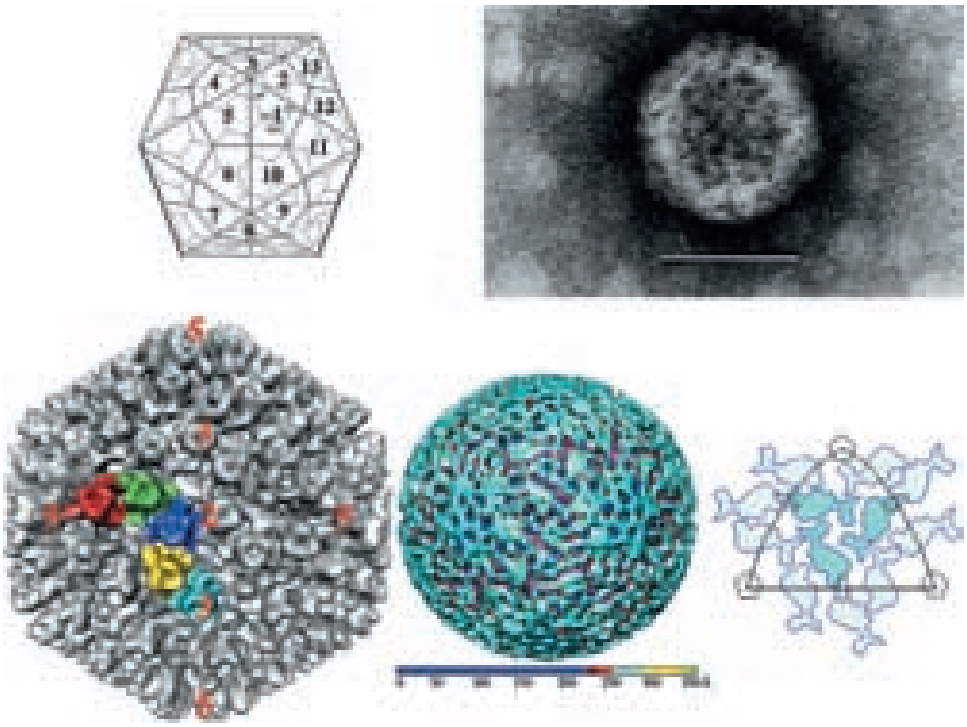

**Figure 30:** (Top left) Schematic diagram representing a T = 13 capsid structure. (Top right) Negative contrast electron micrograph of rice gall dwarf virus particles, negatively stained with phosphotungstic acid. The bar represents 50 nm. (Bottom left) Electron cryo-microscopic image and 25 Å resolution 3D structure of the double shelled rice dwarf virus (RDV). (Bottom center) Inner shell computationally extracted with 59 nm diameter. It exhibits T = 1 lattice. Dashed triangle designates one triangular face of the icosahedron. (Bottom right) Schematic diagram of fish-shaped density distribution within a triangle in a T = 1 lattice. (Courtesy of Hong Zhou and Wah Chiu, from Lu *et al.* (1998). *J. Virol.*, **72**, 8541–8549.)

0.3 to $3.0 \times 10^6$, with characteristic sizes for each virus. For WTV Seg4 = 2565bp; Seg5 = 2613bp; Seg6 = 1700bp; Seg7 = 1726bp; Seg8 = 1472bp; Seg9 = 1382bp; Seg10 = 1172bp; Seg11 = 1128bp; Seg12 = 851bp. G+C content is 38–44% and 41–48% for the genomic segments of WTV and RDV respectively. The positive strand of each genome segment, of all viruses in the genus, contains the conserved sequence 5′-GG($^{U}/_{C}$)A---UGAU-3′ except for RDV Seg9 which has 5′-GGUA---CGAU-3′ (Table 29). These genus-specific terminal sequences are situated adjacent to inverted repeats, which are 6–14 bases long. These sequences differ for each RNA segment. Individual isolates of RDV can frequently be distinguished by electrophoretic profiles of at least one of the 12 genomic segments in PAGE. RDV particles encapsidate the genomic dsRNA in supercoiled form.

### Proteins

Phytoreoviruses have six or seven structural proteins in the range 45 to 160 kDa. RDV has six structural proteins (P1(Pol), P2, P3, P5(Cap), P7, and P8). For WTV the seven CPs are organized in three shells consisting of an amorphous outer layer of two CPs, an inner shell of two CPs and a core of three CPs. Protein constitutes about 78% of the particle dry weight. Removal of the outer shell is not required for activation of the virus transcriptase and associated enzymes. Removal of RDV P2 abolishes the ability to infect vector cell monolayers but virus particles without P2 retain viral transcriptase activity and can infect vector insects by an injection method. P1 is the transcriptase/polymerase and binds to genomic dsRNA. P7 has non-specific nucleic acid binding activity. P3 binds to P3, P7 and P8. P7 binds to P1 and P8. P5 is probably a guanylyl transferase and has GTP, ATP and UTP binding activities. P3 and P8 form virus-like particles in transgenic rice plants. P8 interacts with rice glycolate oxidase, a typical enzyme of peroxisomes. P7 was found to contain dsRNA-binding domains. Of the non-structural proteins, Pns4 is phosphorylated and is associated

**Table 28:** Genome segments and protein products of rice dwarf virus, Akita isolate

| Genome segment | Size (bp) | Non-coding regions (bp) 5′ – 3′ | Protein* | Protein size (kDa)** | Function and location (Number per particle) |
|---|---|---|---|---|---|
| Seg1 | 4423 | 35–53 | P1 (Pol) | 164.1 (170) | Core, RNA polymerase |
| Seg2 | 3512 | 14–147 | P2 | 123.0 (130) | Outer capsid, essential for vector transmission |
| Seg3 | 3195 | 38–97 | P3 | 114.3 (110) | Major core (120) |
| Seg4 | 2468 | 63–221 | Pns4 | 79.8 (83) | Non-structural (Phosphorylated) |
| Seg5 | 2570 | 26–138 | P5 (Cap) | 90.5 (89) | Core, guanylyltransferase |
| Seg6 | 1699 | 48–121 | Pns6 | 57.4 (56) | Non-structural |
| Seg7 | 1696 | 25–150 | P7 | 55.3 (58) | Core, nucleic acid binding protein |
| Seg8 | 1427 | 23–138 | P8 (T13) | 46.5 (43) | Major outer capsid (780) (trimer) |
| Seg9 | 1305 | 24–225 | Pns9 | 38.9 (49) | Non-structural |
| Seg10 | 1321 | 26–233 | Pns10 | 39.2 (35) | Non-structural (silencing suppressor) |
| Seg11 | 1067 | 29–492 | Pns11a | 20.0 (23) | Non-structural (nucleic acid binding protein) |
| | | 5–492 | Pns11b | 20.8 (24) | |
| Seg12 | 1066 | 41–86 | Pns12 | 33.9 (34) | Non-structural |
| | | 312–475 | | Pns12OPa | |
| | | 336–475 | Pns12OPb | 9.6 (7) | |

*Protein structure/function: Pol, RNA polymerase ; Cap, capping enzyme (guanylyltransferase and transmethylase); T13, inner virus structural protein with T = 13 symmetry.
**Calculated from nt sequences (size determined by SDS PAGE in brackets).

**Table 29:** Conserved terminal sequences (positive strand) of phytoreovirus genome segments

| Virus species | Strain | 5′ end | 3′ end |
|---|---|---|---|
| *Rice dwarf virus* | RDV | 5′-GG$^{U}/_{C}$AAA | $^{U}/_{C}$GAU-3′ |
| *Rice gall dwarf virus* | RGDV | 5′-GGU/CA$^{A}/_{U}$UUU | UGAU-3′ |
| *Wound tumor virus* | WTV | 5′-GGUAUU | UGAU-3′ |
| (not classified) | Homalodisca vitripennis reovirus | 5′-GGC$^{G}/_{A}$ | $^{U}/_{C}$GAU-3′ |

with large cytoplasmic fibrils and formed novel minitubules in infected cultured cells of its leafhopper insect vector, as revealed by immunofluorescence and immunoelectron microscopy. Early in infection, Pns4 was detected at the periphery of the viroplasm, and it was then observed on amorphous or fibrillar inclusions, which were identified as bundles of minitubules, at later stages of infection. Pns10 protein was found to possess an antisilencing activity, which suppresses the host RNA silencing machinery. Pns11 has nonspecific nucleic acid binding activity and Pns 12 can be phosphorylated and is one of the early proteins expressed in cultured insect cells.

### Lipids
None known.

### Carbohydrates
None known.

## Genome organization and replication

The coding strand of each dsRNA has a single ORF, except for Seg11 and Seg12 of RDV (Table 28), Seg9 of rice gall dwarf virus (RGDV) and Seg9 of WTV. RDV Seg11 has two in-frame initiation codons, thus resulting in two ORFs. RDV Seg12, RGDV Seg9 and WTV Seg9 possess a second, small out-of-frame and over-lapping ORF, downstream within the major ORF. No evidence has yet been obtained for the expression of this second ORF. Five structural and five NS WTV proteins have been assigned to their respective genome segments. RDV Seg1 encodes the putative transcriptase. Genus-specific and segment-specific sequence motifs appear to be necessary for successful replication, translation and encapsidation. Laboratory strains having internal deletions in some segments, but intact termini, replicate and compete favorably with wild-type virus, although the proteins expressed are aberrant, and the ability of the viruses to be transmitted by vectors may be lost. Virus replication occurs in the cytoplasm of infected cells in association with viroplasms. WTV and RGDV are confined to phloem tissues of the plant host, whereas RDV can also multiply elsewhere.

## Antigenic properties

The three recognized phytoreoviruses are antigenically distinct. Epitopes representing the outer surface are unrelated to each other, while the inner surface epitopes of the capsid of RDV and RGDV will cross-react.

## Biological properties

Plant hosts are either dicotyledonous (WTV), or graminaceous (RDV and RGDV). WTV was originally identified in northeastern USA in the leafhopper *Agalliopsis novella*. The virus was recently found in New Jersey, USA, in a single periwinkle (*Catharanthus*) plant set out as bait for mycoplasmas in a blueberry (*Vaccinium*) field. The experimental plant host range of WTV is wide and encompasses many dicotyledonous plants. The name of this virus derives from the fact that infected plants develop phloem-derived galls (tumors) at wound sites, notably at the emergence of side roots.

RDV and RGDV have narrow and overlapping host ranges. RDV causes severe disease in rice crops in South-East Asia, China, Japan and Korea, Nepal and the Philippines. RGDV has been reported in Thailand, Malaysia and China. RDV induces white flecks and streaks on leaves, with stunting and excessive production of side shoots. RDV is the only plant reovirus that is not limited to the phloem. Plants infected with RDV are stunted and fail to bear seeds. Since the virus is widespread among rice plants in southern China and other Asian countries, it is considered likely to be the cause of a significant overall reduction in rice production. RDV does not provoke enlargement or division of infected cells and does not induce galls, enations, or tumors. RGDV was found in a rice field in Thailand and induces stunting, shoot proliferation, a dark green color and enations in rice.

Phytoreoviruses induce no marked disease in the insect vectors. Virus replication occurs in the cytoplasm of infected cells in association with viroplasm. In the vector, there are no particular tissue tropisms. However, RDV induces abnormalities in fat body cells and mycetocytes. They are all transmitted propagatively by cicadellid leafhoppers (Hemiptera, Cicadellidae, e.g., *Agallia*, *Agalliopsis*, *Nephotettix* and *Recilia*). Virus is acquired from plants shortly after feeding. The latent period in leafhoppers is about 10–20 days. Thereafter, infected insects have a lifelong ability to transmit virus to plants. Phytoreoviruses are also transmitted transovarially in their insect vectors. Experimental data suggest that phytoreoviruses are not mechanically transmissible from plant to plant. No seed transmission has been reported.

## Species demarcation criteria in the genus

In addition to the other general criteria used throughout the family, members of a species in the genus *Phytoreovirus* may be identified by:

- Sequence analysis: Nucleotide sequence identities amongst RDV isolates from different countries are more than 90% (Table 30). Amino acid identities are >80% within species and <56% between species.

- Cross-hybridization using conditions designed to detect >80% similarity.
- Host plant species; dicotyledons (WTV), or the family Graminae (RDV and RGDV).

## List of species in the genus *Phytoreovirus*

| | | |
|---|---|---|
| *Rice dwarf virus* | | |
| Rice dwarf virus-Akita {Nephotettix cincticeps, N. nigropictus, Recilia dorsalis: Gramineae} | [Seg1: D90198, Seg2: AB263418, Seg3: X54620, Seg4: X54622, Seg5: D90033, Seg6: M91653, Seg7: D10218, Seg8: D10219, Seg9: D10220, Seg10: D10221, Seg11: D10249, Seg12: D90200] | (RDV-A) |
| *Rice gall dwarf virus* | | |
| Rice gall dwarf virus-Thailand {Nephotettix cincticeps, N. nigropictus, N. virescens, N. malayanus, Recilia dorsalis: Gramineae} | [Seg1: AB254451, Seg2: D86439, Seg3: D13774, Seg4: AB254452, Seg5: D76429, Seg6, AB254454, Seg7: AB254453, Seg8: D13410,Seg9: D01047, Seg10: D13411, Seg11: AB030009, Seg12: AB254455] | (RGDV-TH) |
| *Wound tumor virus* | | |
| Wound tumor virus {Agallia constricta, A. quadripunctata, Agalliopsis novella: many dicotyledons} | [Seg4: M24117, Seg5: J03020, Seg6: M24116, Seg7: X14218, Seg8: J04344, Seg9: M24115, Seg10: M24114, Seg11: X14219,Seg12: M11133] | (WTV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ], insect vector and host names { } and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Phytoreovirus* but have not been approved as species

| | | |
|---|---|---|
| Homalodisca vitripennis reovirus {Hemiptera: *Homalodisca vitripennis*} | [Seg1: FJ497789, Seg2: FJ497790, Seg3: FJ497791, Seg4: FJ497792, Seg5: FJ497793, Seg6: FJ497794, Seg7: FJ497795, Seg8: FJ497796, Seg9: FJ497797, Seg10: FJ497798, Seg11: FJ497799, Seg12: FJ497800] | (HoVRV) |
| Tobacco leaf enation phytoreovirus* {tobacco: dicotyledon} | [Seg5: AY587757, Seg7: AY587758, Seg8: AY587759, Seg10: AY587760, Seg11: AY587761, Seg12: AY587762] | (TLEV) |

*First phytoreovirus isolated from Africa.

## Phylogenetic relationships within the genus

See Table 30 and Figure 31.

**Table 30:** Percentage nucleotide differences in genome Seg8 amongst different phytoreoviruses

| Species (virus) | RDV | | | | RGDV | WTV |
|---|---|---|---|---|---|---|
| *Rice dwarf virus (RDV-B)* | 0.0 | 1.8 | 2.4 | 5.2 | 44.8 | 47.4 |
| *Rice dwarf virus (RDV-S)* | | 0.0 | 2.7 | 5.2 | 45.0 | 47.1 |
| *Rice dwarf virus (RDV-A)* | | | 0.0 | 5.5 | 44.4 | 47.4 |
| *Rice dwarf virus (RDV-China)* | | | | 0.0 | 45.4 | 46.5 |
| *Rice gall dwarf virus (RGDV)* | | | | | 0.0 | 44.9 |
| *Wound tumor virus (WTV)* | | | | | | 0.0 |

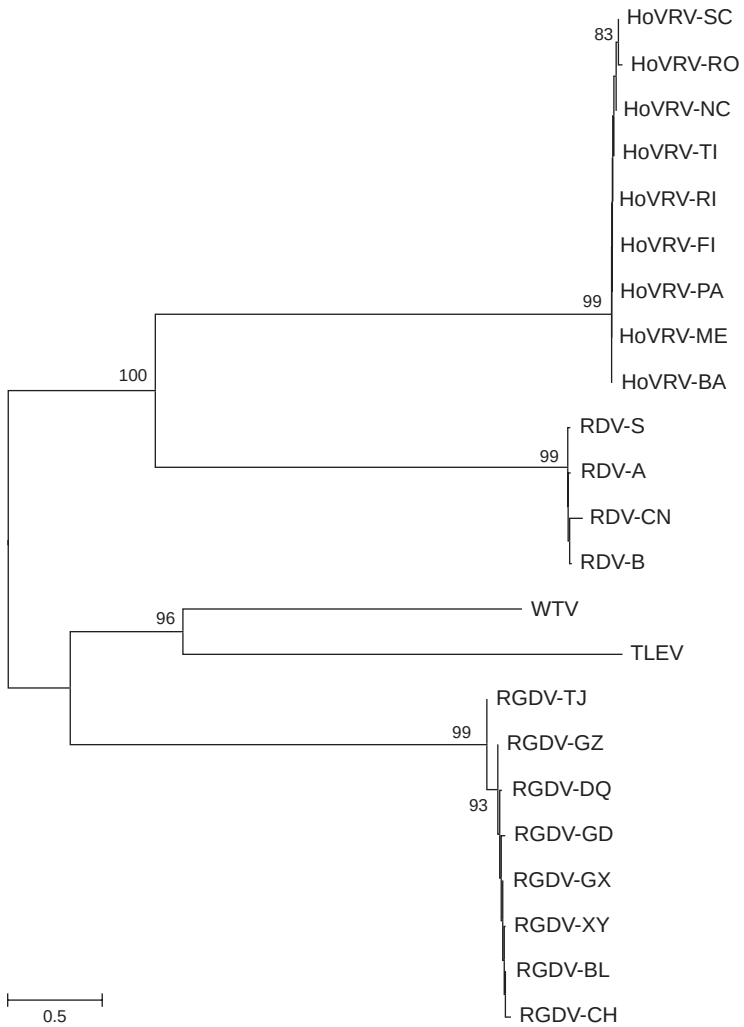


**Figure 31:** Phylogenetic (neighbor-joining) tree based on phytoreovirus Seg8 sequences from the following accession numbers HoVRV-BA (GU362071), HoVRV-FI (FJ497796), HoVRV-ME (GU369689), HoVRV-NC (GU384990), HoVRV-PA (GU370369), HoVRV-RI (GU350428), HoVRV-RO (GU395195), HoVRV-SC (GU390596), HoVRV-TI (GU437834), RDV-A (D10219), RDV-B (D00536), RDV-CN (U36565), RDV-S (D13773), RGDV-BL (AY999077), RGDV-CH (AY999078), RGDV-DQ (AY999079), RGDV-GD (AY216767), RGDV-GX (DQ364683), RGDV-GZ (AY999080), RGDV-TJ (D13410), RGDV-XY (AY999081), TLEV (AY587759), WTV (J04344). Tree was produced in MEGA4 (maximum composite likelihood distances) with 10,000 booststrap replicates (values shown when >60%).

# Genus *Cardoreovirus*

Type species ***Eriocheir sinensis reovirus***

## Distinguishing features

The cardoreovirus genome consists of 12 segments of linear dsRNA. All three members or possible members of the genus (Eirocheir sinensis reovirus (ESRV), Macropipus depurator reovirus and Carcinus mediterraneus reovirus (CMRV)) have been isolated from crabs. Intact virus particles show capsomeric structures by negative staining and transmission electron microscopy (TEM) (Figure 32). Replication is accompanied by production of filaments, tubules and viral inclusion bodies (VIB).

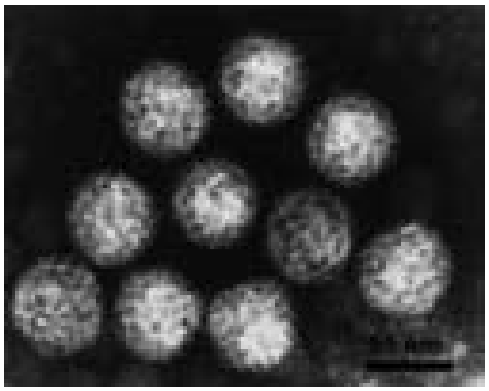

**Figure 32:** Negative contrast electron micrograph of CsCl purified particles of Eriocheir sinensis reovirus. (From Zhang *et al.* (2004). *J. Fish Dis.*, **27**, 687–692.)

## Virion properties

### Morphology

Purification of ESRV or CMRV particles on CsCl, and analysis by TEM and negative staining, revealed intact icosahedral particles about 70 nm in diameter with a morphology typical of non-turreted reovirus cores (Figure 32). In particular, the morphology of ESRV was similar to that observed for the seadornaviruses and orbiviruses. Negative staining showed subunits 8 to 9 nm in diameter on the surface of the particles. Empty particles have a hexagonal electron-dense (stained) centre, 50 nm in diameter, surrounded by an 8 to 10 nm thick capsid. Some of the empty capsids appear as two concentric layers of similar thickness. The double layered core is surrounded by a further outer-capsid layer. Cores of particles containing RNA are about 55 nm in diameter.

### Physicochemical and physical properties

The buoyant density of ESRV viral particles in CsCl is 1.39 g cm$^{-3}$ for full particles and 1.29 g cm$^{-3}$ for empty particles.

### Nucleic acid

The genome consists of 12 segments of linear dsRNA that are numbered in order of reducing Mr, or increasing electrophoretic mobility during agarose gel electrophoresis (AGE). The genome comprises approximately 23,000 bp. The AGE electropherotype follows a 3-4-2-3 pattern. The sizes of the 12 segments are estimated as: 3.7, 3.2, 2.8, 1.85, 1.6, 1.6, 1.4, 1.2, 1.1, 0.95, 0.9 and 0.75 kb. The terminal sequences of ESRV Seg1 are 5′-GGAUUUAAAA .AUAACAGAC-3′.

### Proteins

Genome segment 1 of ESRV is the only segment so far sequenced. It encodes an RdRp of 1217 amino acid with an estimated molecular weight of 138 kDa. The structural proteins of CMRV can be separated by SDS-PAGE and six proteins have been identified by silver staining, with molecular weights of 120, 94, 76, 44, 32 and 24 kDa.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

Replication of cardoreoviruses occurs in the cytoplasm of crab cells (there is no available cell culture system which support growth of cardoreoviruses), where normal virions can associate in rosettes. During the early stages of infection, virions appear within viral inclusion bodies (VIB),

which are dispersed in several regions of the cytoplasm. Viruses accumulate in rosettes around VIBs. Filaments and tubular structures have also been observed in the cytoplasm of infected cells. Infected tissues appear to be progressively destroyed and their normal structures are replaced by disorganized cell debris and cells derived from blood or connective tissues.

## Antigenic properties

None reported.

## Biological properties

Cardoreoviruses have only been isolated from diseased crabs. Experimental inoculation of the viruses into healthy crabs caused symptoms of infection which included absence of aggressiveness, increasing weakness and lack of appetite. Infected crabs survived from 7 to 20 days. Connective tissue of many organs including the hepatopancreas, digestive tract, gills and hematopoietic organs showed severe damage. The most obvious lesions were observed in connective tissue surrounding tubules of the hepatopancreas, although all cellular types were progressively destroyed. Connective tissue was replaced by isolated cells, degenerative cells and debris which did not exhibit any organization. Inside these necrotic areas, nodules appeared which consisted of debris surrounded by aggregated haemocytes. Epithelial cells of the digestive tract, gills and hepatopancreas did not seem to be affected by the virus.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Cardoreovirus*

*Eriocheir sinensis reovirus*
{Chinese mitten crab}
Eriocheir sinensis reovirus [Seg1: AY542965] (ESRV)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ], host names { } and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Cardoreovirus* but have not been approved as species

Carcinus mediterraneus reovirus (isolate W2) (CMRV)
Macropipus depurator reovirus (isolate P) (MDRV)

## Phylogenetic relationships within the genus and with other members of subfamily *Sedoreovirinae*

Since the first crab virus was reported in 1966, numerous viruses from crabs have been described. Most have been associated with marine crabs and only a few have been reported from freshwater crustaceans. The three crab reovirus isolates described (ESRV, Macropipus depurator P virus and CMRV) have been isolated from three different crab species (*Eriocheir sinensis*, *Macropipus depurator* and *Carcinus mediterraneus* respectively). All three viruses have multi-segmented dsRNA genomes made of 12 segments of linear dsRNA but so far only one segment of ESRV has been sequenced and the relationship between the three viruses is uncertain. Their genome electrophoretic profiles are distinct from the other members of family *Reoviridae* with 12 segmented genomes, namely the phytoreoviruses (plant viruses) and seadornaviruses (insect-borne arboviruses). In comparisons of the ESRV RdRp with other reoviruses only a distant relationship with the seadornaviruses was identified (aa identity as low as 27%), suggesting that they have a distant but common origin.

# Genus *Mimoreovirus*

Type species ***Micromomonas pusilla reovirus***

## Distinguishing features

The mimoreovirus genome consists of 11 segments of linear dsRNA. The only known representative of this genus is Micromonas pusilla reovirus (MpRV) which has been isolated from the marine protist *Micromonas pusilla*. MpRV does not grow in mammalian or fish cells lines but grows in *Micromonas pusilla* LAC38 strain.

## Virion properties

### Morphology

The virus particles, isolated on Percoll® gradients, from supernatants of infected *Micromonas pusilla* protest, have an average diameter of 90–95 nm (Figure 33), which is larger than any previously described member of family *Reoviridae.* Some damaged particles showed an outer layer of protein (ca. 15 nm thick) surrounding a more compact internal structure (ca. 75 nm diameter).

MpRV particles purified on CsCl gradients have an average diameter of 75 nm, suggesting that they have lost the outer capsid proteins, although this size is similar to that of whole particles of other reoviruses. Twin bands of viruses were recovered from CsCl gradients. The denser layer has traces of a protein of an identical size as the VP1. The protein content of these particles is similar to those of non-turreted intact particles such as rotaviruses, orbiviruses and seadornaviruses.

MpRV particles that were treated with $CaCl_2$ then purified on Percoll® gradients have a diameter of 50 nm, showing that they have lost outer capsid proteins and may have lost other components from the underlying capsid layer. These particles have a smooth outline (no turrets; Figure 33), similar that observed for orbivirus (sub-core particles), rotaviruses and seadornaviruses.

The outer layer of the MpRV particle appears to represent a pseudo-envelope (or an additional coat) formed by the VP1 protein. Transient envelope structures have been described for orbiviruses, coltiviruses, rotaviruses and seadornaviruses as a consequence of budding of virus particles from the cell membrane or budding into the endoplasmic reticulum during morphogenesis. MpRV may be the first member of family *Reoviridae* to possess a constitutive pseudo-envelope structure or an additional protein coat.

### Physicochemical and physical properties

Thermostability of the virus was tested at temperatures ranging from −196 °C (liquid nitrogen) to 95 °C. The virus was inactivated at temperatures above 35 °C. Freezing at temperatures below −20 °C preserved virus infectivity. Treatment with sodium dodecyl sulfate (0.1–0.5%) abolished infectivity, while treatment with non-ionic detergents such as Tween 80, NP40 and triton X-100 (0.1–1%) did not affect infectivity. Exposure to acetone or alcohol inactivated the virus while treatment with diethyl ether, chloroform or Vertrel XF preserved infectivity. These organic solvents might therefore be useful during purification. Exposure to acidic conditions (pH $< 5$) inactivated the virus, while pH between 7 and 10 did not affect virus infectivity.

Incubation of infected *Micromonas pusilla* in the absence of light inhibited viral replication.

### Nucleic acid

The genome consists of 11 dsRNA segments that are numbered in order of reducing Mr, or increasing electrophoretic mobility during agarose gel electrophoresis (AGE). The total genome length of MpRV is 25,563 bp, with segment lengths that range between 5792 bp and 741 bp. Electrophoretic analysis (1% AGE) of genomic RNA shows a 1-1-2-5-2 migration pattern (Figure 34). AGE profiles are thought to be characteristic of each virus species.

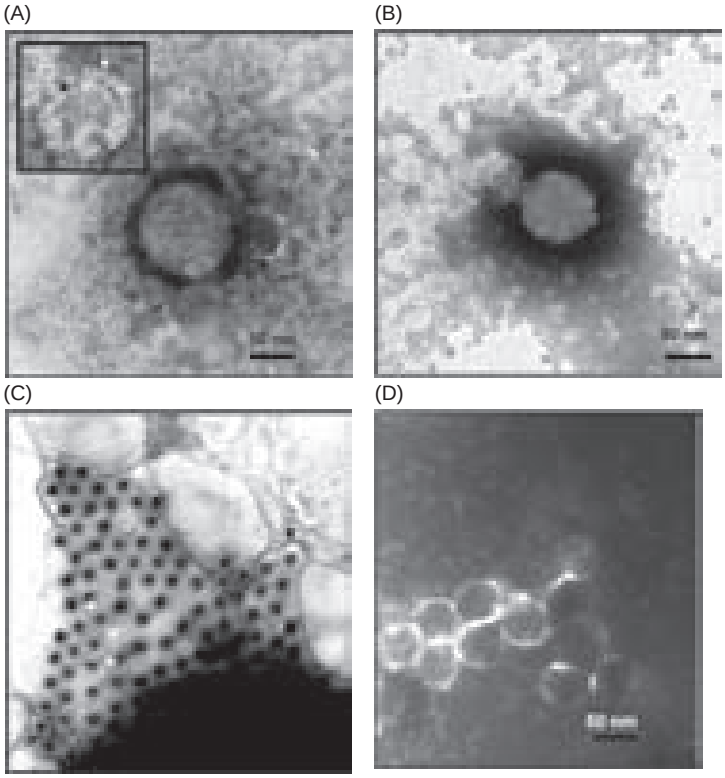


**Figure 33:** (A) MpRV virus particle purified on Percoll®, with a diameter of 95nm. A damaged particle is shown at the upper left corner, showing an outer layer about 15nm thick (indicated by a white arrow), surrounding a more compact structure with a diameter of about 75nm (indicated by a black arrow). (B) A virus particle purified by CsCl gradient centrifugation, with a diameter of about 75nm. (C) Particles pelleted from the clarified lyaste of infected Micromonas pusilla. Some particles (indicated by arrow) have a larger diameter. (D) Particles generated by treating whole MpRV particles (purified on Percoll®) with 1.5MCaCl2 and subsequent purification on CsCl gradient, generating cores (or sub-cores) with smooth outline. (Courtesy of H. Attoui and C. Brussaard.)

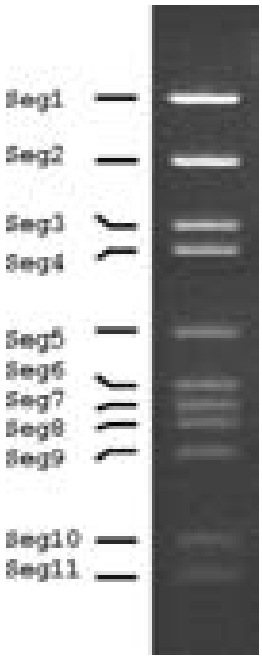


**Figure 34:** Agarose gel electrophoretic profile (electropherotype) of genome segments of MpRV in 1% agarose gel. These migration patterns (electropherotype) are thought to be characteristic of each virus species.

Sequence analysis of the MpRV genome has shown that each genome segment contains a single large ORF. The only exception is Seg5, which contains an ORF spanning nucleotides 44 to 2005 that is interrupted by an in-frame "leaky" TGA stop codon, at position 1571–1573. The G+C content of the MpRV genome segments is between 41 and 50% (the highest value found in Seg5). The conserved terminal sequences of the 11 segments are: 5′-GAAGA$^{A}/_{U}$ . $^{A}/_{G}$AAAGUC-3′.

### Proteins

The protein content of virions purified using CsCl gradient centrifugation has been determined. Eight structural proteins were detected, with Mr of 200, 150, 120, 107, 67, 53, 35 and 32 kDa. The relative position of these proteins is not yet known and the role of the various structural and non-structural proteins is yet to be characterized although some putative assignments can be made based on sequence comparisons (see Genome organization and replication, below).

### Lipids

None reported.

### Carbohydrates

VP1 might be glycosylated based on it relatedness to mucin.

## Genome organization and replication

Each of the 11 genome segments encodes a single protein except Seg5, where a "readthrough" inferred from sequence analysis may result in the production of two related proteins. The shorter 53.1 kDa protein, (VP5ter) is the early-termination translation product, while the readthrough protein (VP5rdt) is 68.9 kDa. The other proteins encoded by the different genome segments are identified as VP1-VP4 and VP6-VP11 respectively. There are thus 12 translation products (Table 31).

Sequence analysis showed that VP1 amino acids 88 to 255 showed 24% identity with the minor capsid protein sigma-1 (a hemagglutinin and responsible for cell attachment) of MRV and its equivalent sigma-c (22% identity, aa 172-321 of the VP1) of the Pulau reovirus.

VP1 shows significant aa identity with hemagglutinins from viral and non-viral origins, including those of the bacterial pathogens *Burkholderia* species (aa identity 20%, similarity 40%) and

**Table 31:** Genome segments and protein products of Micromonas pusilla reovirus

| Genome segment | Size (bp) | Protein | Protein size (kDa), calculated from RNA sequences | Structure/putative function |
|---|---|---|---|---|
| Seg1 | 5792 | VP1 | 201.35 | Outer layer/pseudo-envelope |
| Seg2 | 4175 | VP2 | 154.69 | RNA-dependent RNA polymerase (RdRp or Pol) |
| Seg3 | 3129 | VP3 | 116.27 | Sub-core "2" layer |
| Seg4 | 2833 | VP4 | 102.64 | Unknown |
| Seg5 | 2027 | VP5(tr) / VP5(rdt) | 53.17 / 68.93 | Similar to rotavirus VP4 outer capsid protein |
| Seg6 | 1687 | VP6 | 59.0 | Unknown |
| Seg7 | 1556 | VP7 | 55.41 | Show similarity to cypovirus NS1 |
| Seg8 | 1449 | VP8 | 51.69 | Show similarity to rotavirus NSP2 |
| Seg9 | 1296 | VP9 | 44.31 | Show similarity to nucleotide binding core-protein of fijiviruses |
| Seg10 | 878 | VP10 | 24.69 | Unknown |
| Seg11 | 741 | VP11 | 22.25 | Unknown |

*Staphylococcus* species (aa identity 19%, similarity 38%) and the yeasts *Candida albicans* (identity 20%, similarity 39%) and *Saccharomyces cerevisiae* (identity 20%, similarity 37%). It also matched large-DNA-virus proteins, such as: (i) those of the family *Phycodnaviridae* including the Paramecium bursaria Chlorella virus (PBCV) Vp260 (24% identity, 39% similarity) which is a surface glycoprotein; (ii) those of family *Herpesviridae* including the equine herpesvirus glycoprotein 2 (gp2: identity 20%, similarity 32%) which is an envelope protein; (iii) those of bacteriophages, including the envelope protein of Acholeplasma phage L2 (identity 26%, similarity 46%). All of these glycoproteins are found in envelopes or cell wall structures.

MpRV VP1 has a high serine and threonine content (≥11% each) compared to 1 to 7.5 % for other amino acids. This is characteristic of glycoproteins and in particular for mucin and mucin-like proteins and cell wall adhesins. Such serine and threonine rich proteins are usually heavily O-glycosylated. Amino acid sequence repeats were identified within VP1. Interestingly each repeat was found to align best with a protein sequence immediately N-terminal to it in the VP1. The repeated sequences were not fully identical to the matching sequences. This suggests what has previously been described as sequence duplication in viral genes, followed by distinct and diverging evolution of both the parental and the daughter repeat sequences.

In summary, VP1 is considered likely to form the outermost surface of the virus, possibly representing an extra coat in comparison to other reoviruses.

Seg2 of MpRV encodes VP2, which is thought to be the viral RdRp (or Pol). RdRp core motifs identified in VP2, include the motif SG (position 801-802) and the motif GDD (position 835-837). A partial match (aa 647–962, identity 21%) was also found within the enzyme "core" region, with the RdRp of human rotavirus C (accession number CAC44891), another 11 segmented dsRNA virus belonging to family *Reoviridae*.

Seg3 of MpRV encodes VP3, which by analogy with other reoviruses, appears likely to represent the structural protein which forms the inner capsid shell (sub-core). In other reoviruses this layer has been shown to have pseudo T = 2 icosahedral symmetry (also described as a modified T = 1 symmetry) and the sub-core shell proteins is also identified as the T2 protein. VP3 was found to partially (aa 229–311, identity 26%) match the P3(T2) of rice dwarf virus (phytoreovirus), lambda-1(T2) of MRV-3 (aa 50-145, identity 20%). Lambda-1 of MRV-3 also possesses NTPase and helicase activities).

Seg5 of MpRV encodes VP5, which was found to partially match (aa 214–318, 21% identity) the outer capsid spike protein VP4 of rotavirus A. It also showed 24% identity with the killer toxin protein (accession number S51548) of the dsRNA M28 satellite of Saccharomyces cerevisiae L-A virus.

Seg7 of MpRV encodes VP7, which partially matches (aa 130–209, 32% identity) the non-structural protein NS1 of cypovirus type 1, while VP8 of MpRV (encoded by Seg8) partially matches (aa 42–66, 28% identity) NSP2 of human rotavirus A (NSP2 has a dsRNA helix destabilisation activity, binds RNA and is an NTPase).

Seg9 of MpRV encodes VP9, which partially matches (aa 269–338, 28% identity) the protein encoded by Seg7 of Nilaparvata luguens reovirus (genus *Fijivirus*), which is a core protein with nucleotide-binding activity.

No significant matches were found for VP4, VP6, VP10 and VP11.

## Antigenic properties

None reported.

## Biological properties

MpRV was found in a sample of the marine protist *Micromonas pusilla*, along with a larger dsDNA virus particle (size 100–140 nm). However, the larger virus type was removed by passage through a 0.1 Am pore-size filter and end-point dilution. Ten virus clones were obtained, all showing

comparable particle size and host specificity as well as similar behavior on infection. It did not infect insect or mammalian cells and, out of six strains of *M. pusilla*, only strain LAC38 supported replication of the virus, suggesting a high degree of strain specificity. LAC38 originates from Norwegian coastal waters, while the other five strains were isolated from different locations in the English Channel, English coastal waters, or the Gulf of Maine in the United States.

The growth of *M. pusilla* was inhibited by addition of MpRV within 24 h. The number of free virus particles started to increase at 36 h post infection. A decline in algal cell numbers was also observed by 40 h post infection, with the percentage of dead algal cells steadily increasing, to match an increase in virus released from the host cells (>60% dead cells). The estimated burst size was 460–520 virus particles per lysed algal cell.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Mimoreovirus*

| | | |
|---|---|---|
| *Micromonas pusilla reovirus* | | |
| {Marine protist} | | |
| Micromonas pusilla reovirus | [Seg1: DQ126101, Seg2: DQ126102, Seg3: DQ126103, Seg4: DQ126104, Seg5: DQ126105, Seg6: DQ126106, Seg7: DQ126107, Seg8: DQ126108, Seg9: DQ126109, Seg10: DQ126110, Seg11: DQ126111] | (MpRV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ], host { } and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Mimoreovirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Reoviridae*

None.

## List of other related viruses which may be members of the family *Reoviridae* but have not been approved as species

| | | | |
|---|---|---|---|
| Viruses of *Arthropoda* | | | |
| Cimex lactularius reovirus | {*Cimex lactularius* (Hemiptera: bed bug)} | (ClRV) | 11 segments, icosahedral double-shelled capsid about 50 nm diameter |
| Viruses of *Crustacea* | | | |
| Porcelio dilatatus reovirus | {*Porcelio dilatatus* (Isopoda: terrestrial crustacean)} | (PdRV) | Uncharacterized |
| Viruses of *Arachnida* | | | |
| Buthus occitanus reovirus | {*Buthus occitanus* (Scorpionidae: scorpion)} | (BoRV) | Uncharacterized |

Source or host species { }, assigned abbreviation ( ) and characteristics are also given.

## Phylogenetic relationships in the family

The *Reoviridae* represents the largest family of dsRNA viruses. It contains 15 genera of viruses having genomes composed of 9, 10, 11 or 12 segments of linear dsRNA. Member viruses have been isolated from a wide range of mammals, birds, reptiles, fish, crustaceans, marine protists, insects, ticks, arachnids, plants and fungi and include a total of 75 virus species.

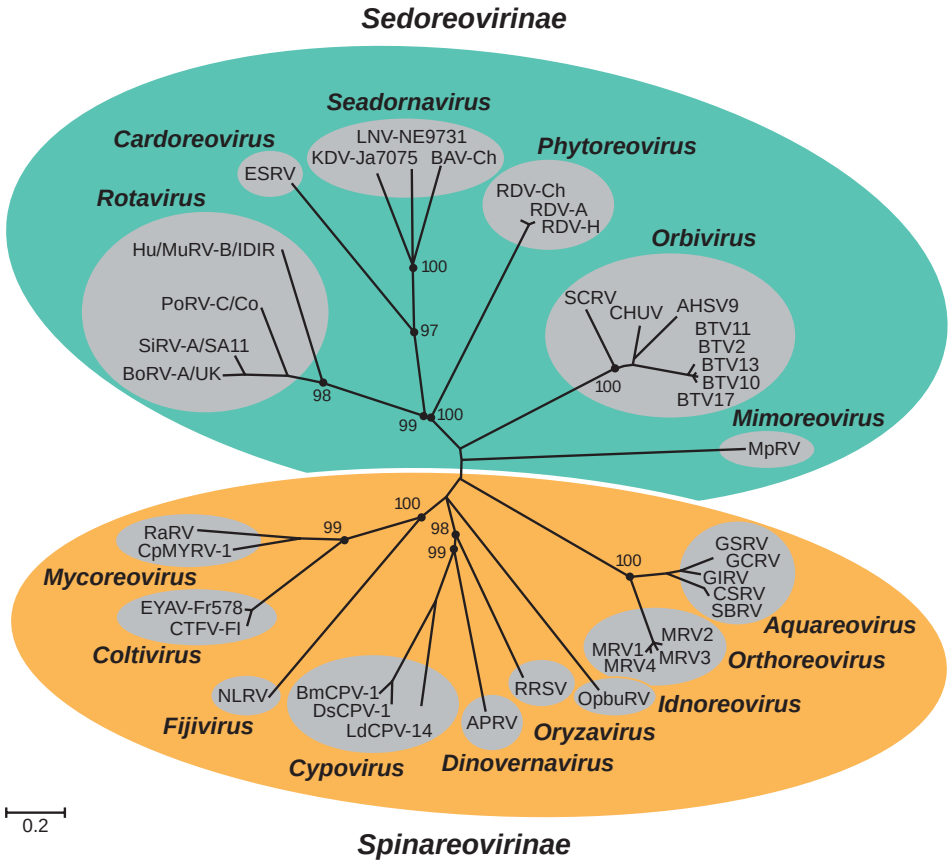


**Figure 35:** Neighbor joining tree constructed with the AA sequences of putative RdRp of representative viruses from the following genera of the family *Reoviridae* [accession numbers]: *Seadornavirus*, *Banna virus*: isolate BAV-Ch [AF168005], *Kadipiro virus*: isolate KDV-Ja7075 [AF133429], *Liao ning virus*: isolate LNV-NE9731 [AY317099]. *Coltivirus*, *Colorado tick fever virus*, isolate CTFV-Fl [AF134529], *Eyach virus*, isolate EYAV-Fr578 [AF282467]. *Orthoreovirus*, *Mammalian orthoreovirus*, serotype-1 (MRV-1) [M24734], serotype-2 (MRV-2) [M31057], serotype-3 (MRV-3) [M31058], serotype-4 (MRV-4) also known as Ndelle virus [AF368033]. *Aquareovirus*, *Aquareovirus C*, isolate golden shiner virus (GSRV) [AF403399], grass carp reovirus (GCRV) [AF260512], *Aquareovirus A*, isolate striped bass reovirus (SBRV) [AF450318], isolate chum salmon reovirus (CSRV) [AF418295], *Aquareovirus G isolate* golden ide reovirus (GIRV) [AF450323]. *Orbivirus*, *African horse sickness virus*, serotype-9 (AHSV-9) [U94887], *Bluetongue virus*, serotype-2 (BTV-2) [L20508], serotype-10 (BTV-10) [X12819], serotype-11 (BTV-11) [L20445], serotype-13 (BTV-13) [L20446], serotype-17 (BTV-17) [L20447], species *Palyam virus*, isolate CHUV [Baa76549], *St Croix River virus*, isolate SCRV[AF133431]. *Rotavirus*, *Rotavirus A*, strain BoRV-A/UK [X55444], strain SiRV-A/SA11 [AF015955], *Rotavirus B*, strain Hu/MuRV-B/IDIR [M97203], *Rotavirus C*, strain PoRV-C/Co [M74216], *Fijivirus*, species *Nilaparvata lugens reovirus*, strain NLRV-Iz [D49693]. *Phytoreovirus*, species *Rice dwarf virus*, isolate RDV-Ch [U73201], isolate RDV-H [D10222], isolate RDV-A [D90198]. *Mycoreovirus*, species *Mycoreovirus-1*, isolate CpMYRV-1 [AY277888], species *Mycoreovirus-3*, isolate RnMYRV-3 [AB102674]. *Oryzavirus*, isolate *Rice ragged stunt virus*, strain RRSV-Th [U66714]. *Cypovirus*, *Bombyx mori cytoplasmic polyhedrosis virus*-1 strain BmCPV-1 [AF323781], *Dendrlymus punctatus cytoplasmic polyhedrosis virus-1* strain DsCPV-1 [AAN46860], *Lymantria dispar cytoplasmic polyhedrosis virus-14* strain LdCPV-14 [AAK73087]. *Dinovernavirus*, species Aedes pseudoscutellaris reovirus, isolate APRV [DQ087276]. *Cardoreovirus*, species *Eriocheir sinensis reovirus*, isolate ESRV [AY542965]. *Mimoreovirus*, *Micromonas pusilla reovirus*, isolate MPRV [DQ126101]. Values at the nodes represent bootstrap confidence levels (500 replications).

Phylogenetic analyses, using amino acid sequences of the RNA-dependent RNA polymerase, have shown that the different genera of reoviruses exhibit amino acid sequence identities of less than 30%. There are two exceptions: (i) *Rotavirus B*, shows only 22% identity with other rotaviruses, (ii) *Aquareovirus* and *Orthoreovirus*, show an amino acid sequence identity of up to 42%. In most cases the phylogenetic tree of the polymerase (shown in Figure 35), clearly identifies the turreted viruses and non-turreted viruses as members of separate clades. However, it is noteworthy that the functional core of the RdRp contains similar motifs across the entire family (Table 32).

**Table 32:** Comparison of sequences surrounding the conserved RdRp motifs of reoviruses

| Motifs | I | IV,1,A | V,2,B | VI,3,C | D |
|---|---|---|---|---|---|
| Consensus | grrtRiI | D.s.wd. | SGe.aTs.a....nla | qvqGDDtlm.ikdg | he.n.sK.s |
| BTV (515) | PIKATRTI 72 | DYSEYDTH 119 | SGENSTLIANSMHNMA 21 | EQYVGDDTLFYTKLD 22 | HEASPSKTM (804) |
| Rotavirus (455) | PGRRTRII 57 | DVSQWDSS 63 | SGEKQTKAANSIANLA 19 | IRVDGDDNYAVLQFN 20 | RMNAKVKAL (669) |
| RDV (643) | AWRPVRPI 73 | DCTSWDQT 76 | SGRLDTFFMNSVQNLI 20 | FQVAGDDAIM.VYDG 24 | HIINPQKTV (890) |
| Reovirus S3 (521) | VQRRPRSI 56 | DISACDAS 89 | SGSTATSTEHTANNST 31 | YVCQGDDGLM.IIDG 21 | GEEFGWKYD (772) |
| NLRV (646) | IDRRGRII 60 | DMSGMDAH 90 | SGLFATSGQHT.MFLV 20 | NYVMGDDIFQNIKNG 24 | IDGNYSKYS (894) |
| RRSV (500) | IGRRQRAI 62 | DASVQASV 83 | SGQPFTTVHHTFTLSN 1 | LTVQGDDTRT.INYG 15 | VSDWGFKVS (735) |
| BAV (557) | LVRGTRAK 74 | DTSQWGQI 62 | SGELTTQTRNTTTNIS 25 | DNKVGDDSVEVLRVV 24 | HLEISAKRTI (415) |

The regions covering the putative polymerase module in RRSV P4 (aa 500 to 735) and other reoviruses were analyzed using the GCG program PILEUP and further aligned manually taking into account the polymerase motifs presented and aligned by Poch *et al.* (1989) (A–D), Bruenn (1991) (1–3) and Koonin (1992) (I, IV–VI).

References:
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Overall phylogenetic analyses support the co-speciation hypothesis that reoviruses have co-evolved with their respective hosts and/or arthropod vectors. Although reoviruses form a single monophyletic group, they have been evolving for over about 550 million years, resulting in sequence divergence to near randomness and clear structural differences between the more distantly related genera. However, the replication mechanisms used and certain structural parameters of the internal proteins (of the subcore shell and polymerase complexes) remain fundamentally and remarkably similar. Evolutionary analyses suggest that the non-turreted viruses may represent an ancestral lineage from which the turreted viruses have evolved.

Closer relations between certain genera have been identified by structural analyses of the viral proteins, and may be indicated by the presence of signature sequences. For example, comparisons of polymerase, capping enzyme and capsid protein sequences, as well as structural analyses of outer capsid proteins, suggest that an evolutionary jump has occurred between the seadornaviruses and rotaviruses, within the *Sedoreovirinae.* This is thought to have involved gene duplication and rearrangement, changing the number of genome segments (from 11 for the rotavirus to 12 for the seadornaviruses).

Closer relationships have also been identified between the aquareoviruses and orthoreoviruses within the *Spinareovirinae*, with homologous proteins exhibiting up to 42% amino acid identity and particle morphology that is super-imposable (revealed by cryoEM). Gene duplication followed by rearrangement, also appears to have occurred between the aquareoviruses and coltiviruses, increasing the number of segments from 11 (aquareoviruses) to 12 (coltiviruses).

## Similarities with other taxa

Although there is little evidence for nucleotide or amino acid sequence similarities with other families of dsRNA viruses, it may be significant that some (e.g. the families *Cystoviridae* and *Totiviridae*) also have particles in which the inner shell is characteristically composed of 120 copies of a triangular protein, arranged in a manner similar to that of the members of the family *Reoviridae*. This protein provides an apparently simple yet elegant mechanism of assembling the inner icosahedral capsid shell, which has been described alternatively as having T = 1 or T = 2 symmetry, although it is important to note these are different academic interpretations of a similar particle architecture. These similarities may also indicate a common, if distant, ancestry and again suggest that these viruses may even have diversified and evolved along with their host species.

## Derivation of names

*Cardo*: from *Carcinus* (crab) and *do*deca, from Ancient Greek *dodeka*, "12".
*Cypo*: from *cy*toplasmic *po*lyhedrosis.
*Dinoverna*: from *d*ouble stranded *i*nsect *novem* ("nine" in Latin) segmented *RNA* viruses.
*Fiji*: from the country where the virus was first isolated.
*Idno*: from *i*nsect-*d*erived *n*on-*o*ccluded (in contrast to the cypoviruses).
*Mimo:* from *Micromonas*, the genus name of the type member.
*Myco*: from *myco*, Latin for "fungus".
*Orbi*: from *orbis*, Latin for "ring" or "circle", in recognition of the ring-like structures observed in micrographs of the surface of core particles.
*Ortho*: from *orthos*, Greek for "straight".
*Oryza*: from the genus name of rice, the host of the type member.
*Phyto*: from *phyton*, Greek for "plant".
*Reo*: from *r*espiratory *e*nteric *o*rphan, due to the early recognition that the viruses caused respiratory and enteric infections, and the (incorrect) belief that they were not associated with disease and so were considered orphan viruses.
*Rota*: from *rota*, Latin for "wheel".
*Seadorna*: from *S*outh-*E*astern *A*sia *do*deca *RNA* viruses.
*Sedo*: from *sedo*, Latin for "smooth".
*Spina*: from *spina*, Latin for "spike".

## Further reading

A supplementary list of references is available online on Science Direct®, www.sciencedirect.com.

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## Contributed by

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# FAMILY *TOTIVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Totiviridae*** |
| Genus | ***Totivirus*** |
| Genus | ***Victorivirus*** |
| Genus | ***Giardiavirus*** |
| Genus | ***Leishmaniavirus*** |

## Virion properties

### MORPHOLOGY

Virions in this family are isometric with no lipid or carbohydrate content reported and no surface projections. There is a close similarity in many aspects of the virus particles (Figures 1, 2) of members of the family *Totiviridae* and the cores of dsRNA viruses of higher organisms.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virion buoyant density in CsCl is 1.33–1.43 g cm$^{-3}$. Additional components with different sedimentation coefficients are found in preparations of some viruses in the genus *Totivirus*. These consist of particles containing satellite or defective dsRNA.

### NUCLEIC ACID

Virions contain a single molecule of linear uncapped dsRNA, 4.6–7.0 kbp in size.

### PROTEINS

Virions contain a single major CP, of 70–100 kDa. Virion-associated RNA polymerase activity is present.

### LIPIDS

None reported.

### CARBOHYDRATES

None reported.

## Genome organization and replication

The genome contains two large, usually overlapping, ORFs: the 5′-proximal ORF encodes the major CP (Gag) and the 3′-proximal ORF encodes an RdRp. Virion-associated RdRp catalyzes *in vitro*

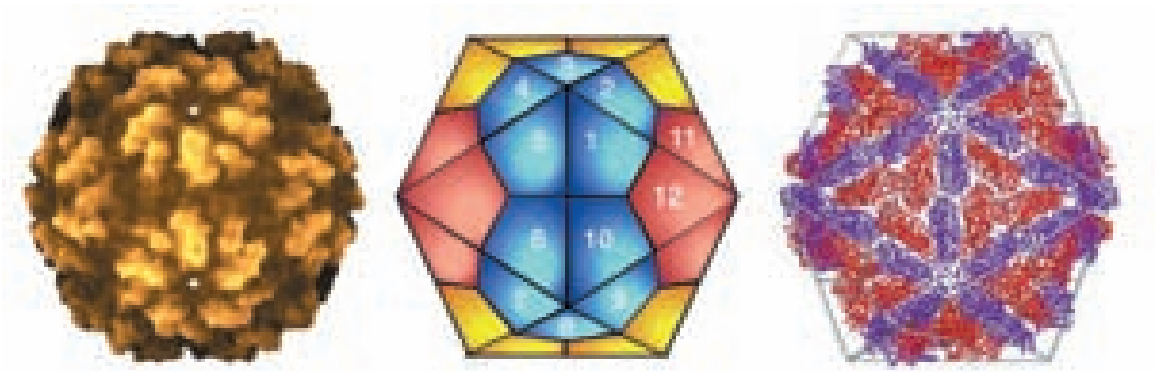


**Figure 1:** (Left) Reconstruction of the atomic resolution structure of the *Saccharomyces cerevisiae virus L-A* (ScV-L-A) virion. (Center) Schematic representation of a T = 1 capsid structure. (Right) The ScV-L-A particle viewed down an icosahedral two-fold axis with the Cα positions traced. Red Gag molecules contact the three-fold axes, but not the two-fold or five-fold axes. Purple molecules surround the five-fold axes and contact the two-fold axes. Thus, two kinds of Gag molecules with identical covalent structure are in distinct environments in the viral particle.

end-to-end transcription of dsRNA to produce mRNA for CP and RdRp, by a conservative mechanism. When provided with viral (+) strand template, the RdRp specifically binds (+) strands and catalyzes (−) strand synthesis to form dsRNA. When provided with viral dsRNA, the RdRp carries out *in vitro* transcription in a template-specific reaction. The polymerase is usually expressed as a gag-pol-like fusion protein containing two ORFs.

### Antigenic properties

Not reported.

### Biological properties

These viruses are associated with latent infections of their fungal or protozoan hosts.

## GENUS *TOTIVIRUS*

Type species ***Saccharomyces cerevisiae virus L-A***

### Distinguishing features

Virus replicates in *Saccharomyces cerevisiae* and supports the replication of one of several satellite dsRNAs (called M dsRNAs) encoding a secreted toxin and immunity to that toxin (killer toxins).

### Virion properties

#### MORPHOLOGY

Virions are 40 nm in diameter and icosahedral with T = 1 with a dimer of the major CP as the asymmetric unit (Figures 1,2). Pores seen in the capsid structure presumably function in the uptake of nucleotides and the release of viral mRNA.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virion Mr is estimated as $12.3 \times 10^6$. Buoyant density in CsCl is 1.40–1.43 g cm$^{-3}$ and $S_{20,w}$ is 160–190S. Additional components with different sedimentation coefficients and buoyant densities are present in virus isolates with satellite or defective RNAs. Particles lacking nucleic acid have an $S_{20,w}$ of 98–113S.

#### NUCLEIC ACID

Virions contain a single linear molecule of uncapped dsRNA (4.6–6.7 kbp). Some virus isolates contain additional satellite dsRNAs which encode "killer" proteins; these satellites are encapsidated separately in capsids encoded by the helper virus genome. Some virus isolates may contain (additionally or alternatively) defective dsRNAs which arise from the satellite dsRNAs; these additional dsRNAs are also encapsidated separately in capsids encoded by the helper virus genome. The complete RNA sequences of Saccharomyces cerevisiae virus L-A (ScV-L-A) (4,579 bp), Saccharomyces cerevisiae virus L-BC (La) (ScV-L-BC) and Ustilago maydis virus H1 (UmV-H1) dsRNAs are available. The positive strand has two large overlapping ORFs; the length of the overlap for ScV-L-A is 130 nt. The first ORF encodes the viral major CP with a predicted size of 76–81 kDa. In the case of ScV-L-A, the two reading frames together encode, via a translational frameshift, the putative RdRp as a fusion protein (analogous to gag-pol fusion proteins of the retroviruses) with a predicted size of 170 kDa. Sites essential for encapsidation, and replication and ribosomal frameshifting have been defined.

#### PROTEINS

Virions contain a single major CP of 73–88 kDa. The ScV-L-A Gag removes the 5′ cap structure of host mRNA and covalently attaches it to His154, an activity necessary for expression of viral mRNA. This necessity is relieved by mutation of the host *SKI1/XRN1* 5′ exoribonuclease specific for uncapped RNAs (e.g., viral (+) strands). RNA polymerase (replicase-transcriptase) is present. In ScV-L-A virions, RNA polymerase occurs as 1–2 molecules of the 170 kDa fusion protein. The Pol domain of the

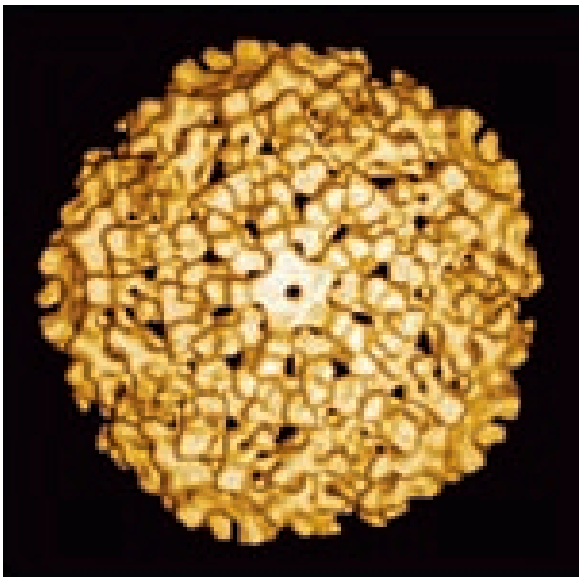

**Figure 2:** Cryo-electron microscopic reconstruction of Saccharomyces cerevisiae virus L-A (ScV-L-A) at 16Å resolution. The view shown is along a five-fold axis of the icosahedral particles. The virions show the "T = 2" symmetry found in the cores of all dsRNA viruses.

Gag-Pol fusion protein has three single stranded RNA binding activities. The N-terminal 1/4 of Pol is necessary for packaging viral (+) strands and includes one of the RNA binding activities.



## Genome organization and replication

ScV-L-A virus has a single 4.6 kbp dsRNA segment with two ORFs (Figure 3). The 5′ ORF is *Gag* and encodes the major CP that can bind and covalently remove the 5′-cap structure from mRNAs. The 3′ ORF, *Pol*, encodes the RdRp, and has ssRNA binding activity. *Pol* is expressed only as a Gag-Pol fusion protein formed by a −1 frameshift in the 130 bp overlap region between the two ORFs. The −1 ribosomal frameshift is produced by a 72 bp region that has a 7 bp slippery site and an essential pseudoknot structure. Covalent linkage of 5′-cap structures (7meGDP) from cellular mRNAs to His154 of Gag is necessary for viral expression, apparently by decoying the Ski1p exoribonuclease from degrading the capless viral (+) strands. The efficiency of frameshifting is critical for viral replication.

## Antigenic properties

Virions are efficient immunogens.

## Biological properties

### Transmission

Virions remain intracellular and are transmitted during cell division, sporogenesis and cell fusion

### Host range

ScV-L-A depends for its multiplication on host genes *MAK3*, *MAK10* and *MAK31*. The *MAK3* gene encodes an N-acetyltransferase that acetylates the N-terminus of the major CP and Mak10p and Mak31p are complexed with Mak3p. Reduced levels of 60S ribosomal subunits result in lower levels of ScV-L-A virus and loss of $M_1$ dsRNA, due to poor translation of the viral poly(A)$^-$ mRNA. The *S. cerevisiae* antiviral gene, *SKI1*, encodes a 5′ to 3′ exoribonuclease specific for uncapped RNAs (such as viral mRNAs). The *SKI2*, *3*, *6*, *7*, and *8* system blocks expression of non-poly(A) (e.g., viral) mRNAs. Ski2p is an RNA helicase, homologous to nucleolar human homologs. Ski6p is a 3′ to 5′ exoribonuclease involved in 60S ribosomal subunit biogenesis. If an *SKI* gene is defective, ScV-L-A

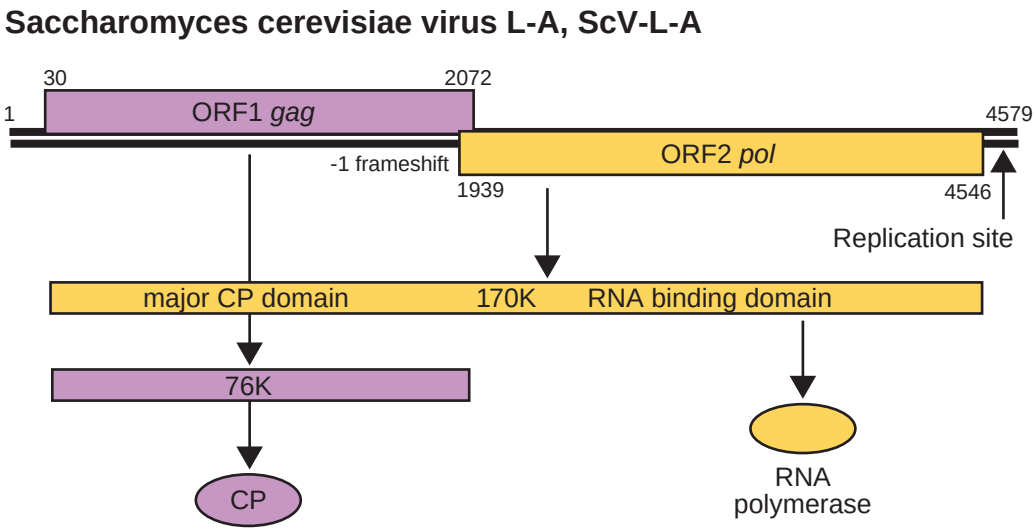


**Figure 3:** Genome organization of Saccharomyces cerevisiae virus L-A (ScV-L-A). The virion-associated RNA polymerase catalyzes *in vitro* end-to-end transcription of dsRNA by a conservative mechanism to produce mRNA for CP. In the case of ScV-L-A, all of the positive strand transcripts are extruded from the particles. The positive strand of satellite RNA $M_1$, or deletion mutants of L-A or $M_1$, on the other hand, often remain within the particle where they are replicated to produce two or more dsRNA molecules per particle (headful replication). The positive ssRNA of ScV-L-A is the molecule encapsidated to form progeny virus particles. The encapsidation signal on ScV-L-A or $M_1$ positive sense ssRNA is a 24 nt stem-loop sequence located 400 nt from the 3′ end in each case. The *Gag* protein must be acetylated (by the cellular Mak3p) for assembly and packaging to proceed. These particles have a replicase activity that synthesizes the negative strand on the positive strand template to produce dsRNA, thus completing the replication cycle. Replication requires an internal site overlapping the packaging signal, and a specific 3′-end sequence and secondary/tertiary structure. Virions accumulate in the cytoplasm.

becomes pathogenic; but only the M dsRNA causes a cytopathogenic effect. Cells become cold sensitive and temperature sensitive for growth.

## Species demarcation criteria in the genus

According to the virus species definition, viruses found only in distinct host species are for that reason different species. Totiviruses generally replicate stably within the cell as the cells grow. Different virus strains are expected to segregate relative to each other as the cells grow, whereas different virus species should be stably co-maintained. Viruses of the same species should be similarly affected by host chromosomal mutations. Viruses that can recombine or exchange segments with each other to give viable progeny should be considered the same species. Although these biological criteria are the prime determinants of species, sequence criteria also are used. Less than 50% sequence identity at the protein level generally reflects a species difference. None of the above criteria is absolute, but totiviruses described so far leave little doubt about species demarcation. For example, ScV-L-A and ScV-L-BC are only 30% identical in the 717 aa region of highest similarity. More important, they are stably compatible with each other in the same yeast strain, and respond differently to chromosomal mutations. Mutations resulting in loss of ScV-L-BC do not result in loss of ScV-L-A and vice versa.

## List of species in the genus *Totivirus*

| | | |
|---|---|---|
| *Saccharomyces cerevisiae virus L-A* | | |
| Saccharomyces cerevisiae virus L-A | [J04692] | (ScV-L-A) |
| *Saccharomyces cerevisiae virus L-BC (La)* | | |
| Saccharomyces cerevisiae virus L-BC | [U01060] | (ScV-L-BC) |
| *Ustilago maydis virus H1* | | |
| Ustilago maydis virus H1 | [U01059] | (UmV-H1) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

**List of other related viruses which may be members of the genus *Totivirus* but have not been approved as species.**

None reported.

# GENUS *VICTORIVIRUS*

Type species ***Helminthosporium victoriae virus 190S***

## Distinguishing features

The RdRp of HvV190S is expressed independent of CP as a separate non-fused virion-associated minor protein. This is also predicted for the RdRps of other members of the genus *Victorivirus*. The victorivirus CPs are unique among members of the family *Totiviridae* in having an Ala/Gly/Pro-rich region near their C termini. Victoriviruses replicate in the filamentous fungal host species in which they naturally occur.

## Virion properties

### MORPHOLOGY

Virions are isometric, approximately 40 nm in diameter, with icosahedral symmetry (Figure 4). The capsid of HvV190S is made up of 60 asymmetric CP dimers arranged in a T = 1 (so-called "T = 2" layer). Although the HvV190S capsid shows relatively smoother outer surfaces compared to the ScV-L-A capsid, its quaternary organization is remarkably similar to the ScV-L-A as well as to the cores of the larger dsRNA viruses of plants and animals: the CP-A-subunits cluster around the five-fold axes and CP-B-subunits around the threefold axes.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

The buoyant density of HvV190S virions in CsCl is 1.43 g cm$^{-3}$, and (as its name implies) the sedimentation coefficient is 190S ($S_{20,w}$, in Svedberg units).



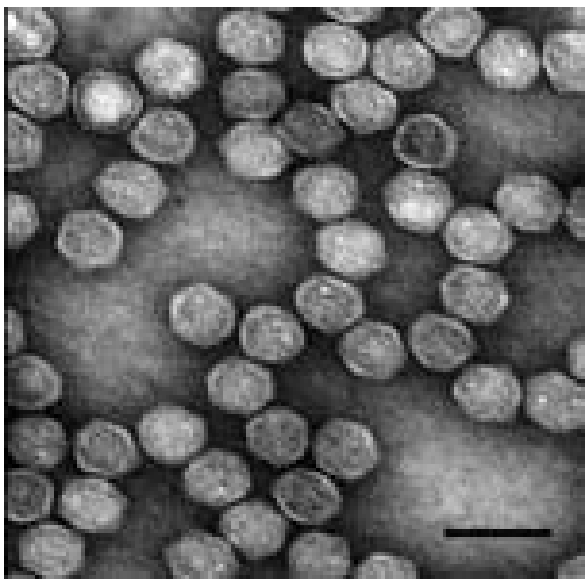

**Figure 4:** Negative contrast electron micrograph of an isolate of *Helminthosporium victoriae virus 190S*, the type member of the genus *Victorivirus*.

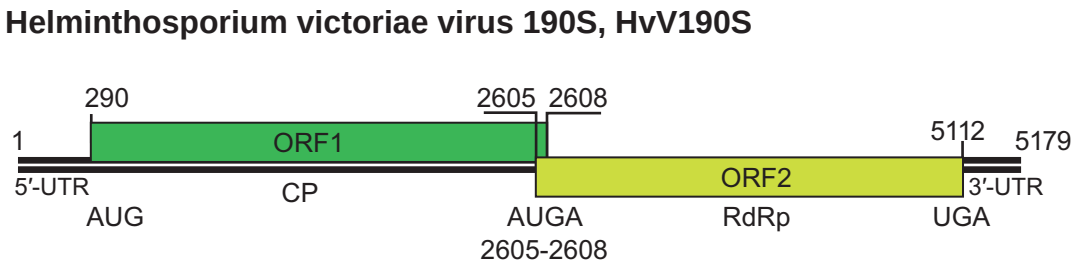


**Figure 5:** Genome organization of Helminthosporium victoriae virus 190S. The genomic plus strand includes two large, overlapping ORFs, with the 5′ one (ORF1) encoding the CP and the 3′ one (ORF2) encoding the RdRp. The stop codon of ORF1 overlaps the start codon of ORF2 in the tetranucleotide sequence AUGA.

### Nucleic acid

The genome of victoriviruses is a single molecule of uncapped dsRNA, consistently sized near 5 kbp, which contains two large ORFs. The upstream ORF codes for the CP and the downstream ORF codes for the RdRp.

### Proteins

The HvV190S CP ORF encodes a single primary translation product of 88 kDa (p88). Two additional differently sized forms of CP, designated p83 and p78, are derived from p88 via proteolysis at its C-terminus. *In vivo* and *in vitro* phosphorylation studies have indicated that p88 and p83 are phosphoproteins, whereas p78 is not. Two particle types, designated 190S-1 and 190S-2, are present in purified HvV190S preparations. These two particle types are thought to represent different stages in the virus life cycle. The 190S-1 particles contain p88 and p83, and the 190S-2 particles contain p88 and p78. The virion-associated RdRp of HvV190S is present as a separate nonfused minor protein of 92 kDa.

## Genome organization and replication

The plus strand of the dsRNA genome of HvV190S encompasses two large ORFs with the 5′-proximal ORF encoding the CP and the 3′-proximal ORF encoding the RdRp. The termination codon of the CP ORF overlaps the initiation codon of the RdRp ORF in the tetranucleotide sequence AUGA (Figure 5). The AUGA overlap region, or a very similar structure, is characteristic of the CP/RdRp junction region of all other members of the genus Victorivirus. ORF 2 in all victoriviruses, with possibly one exception, is in the −1 frame relative to ORF1. The 5′ end of the HvV190S genomic plus strand is uncapped, contains a relatively long (289 nt) 5′ leader with two minicistrons, and is predicted to be highly structured when in single stranded form. These structural features of the 5′ UTR suggest that ORF1 (with its AUG present in suboptimal context according to the Kozak criteria) is translated via a cap-independent mechanism. The HvV190S RdRp is a separate, nonfused virion associated minor protein that is expressed by an internal initiation mechanism; a coupled termination–reinitiation mechanism has been proposed based on current evidence. In *in vitro* reactions, the RdRp activity associated with HvV190S virions catalyzes end-to-end transcription of dsRNA, by a conservative mechanism, to produce mRNA for CP translation.

## Biological properties

Victoriviruses are transmitted intracellularly: vertically during host cell division and sporogenesis and horizontally during cell–cell fusion via hyphal anastomosis between compatible fungal strains. Victoriviruses that infect ascomycetous fungi appear to be largely excluded during ascospore formation.

## Species demarcation criteria in the genus

The amino acid sequence identity in pairwise comparisons of either the CP or the RdRp gene product of the nine species is no more than 60%. In addition, each of these species was isolated from a different filamentous fungus, with the exception of one pair. That pair, SsRV1 and SsRV2, is stably co-maintained in the same fungal host and adheres to the 60% maximum identity criterion.

## List of species in the genus *Victorivirus*

| | | |
|---|---|---|
| *Chalara elegans RNA virus 1* | | |
| Chalara elegans RNA virus 1 | [AY561500] | (CeRV1) |
| *Coniothyrium minitans RNA virus* | | |
| Coniothyrium minitans RNA virus | [AF527633] | (CmRV) |
| *Epichloe festucae virus 1* | | |
| Epichloe festucae virus | [AM261427] | (EfV 1) |
| *Gremmeniella abietina RNA virus L1* | | |
| Gremmeniella abietina RNA virus L1 | [AF337175] | (GaRV-L1) |
| *Helicobasidium mompa totivirus 1-17* | | |
| Helicobasidium mompa totivirus 1-17 | [AB085814] | (HmTV1-17) |
| *Helminthosporium victoriae virus 190S* | | |
| Helminthosporium victoriae virus 190S | [U41345] | (HvV190S) |
| *Magnaporthe oryzae virus 1* | | |
| Magnaporthe oryzae virus 1 | [AB176964] | (MoV1) |
| *Sphaeropsis sapinea RNA virus 1* | | |
| Sphaeropsis sapinea RNA virus 1 | [AF038665] | (SsRV1) |
| *Sphaeropsis sapinea RNA virus 2* | | |
| Sphaeropsis sapinea RNA virus 2 | [AF039080] | (SsRV2) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Victorivirus* but have not been approved as species

| | | |
|---|---|---|
| Botryotinia fuckeliana totivirus 1 | [AM491608] | (BfTV1) |
| Magnaporthe oryzae virus 2 | [AB300379] | (MoV2) |



# GENUS *GIARDIAVIRUS*

Type species ***Giardia lamblia virus***

## Distinguishing features

Giardia lamblia virus (GLV) is a dsRNA virus that replicates in growing *Giardia lamblia* and is released into the medium without lysing the host cells. It is the only member of the family *Totiviridae* for which transfection has succeeded. Virions are isometric, 36 nm in diameter (Figure 6).

## Virion properties

### MORPHOLOGY

See Figure 6.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virion buoyant density in CsCl is 1.368 g cm$^{-3}$.

### NUCLEIC ACID

Virions contain a single molecule of dsRNA, 6277 bp in size.

### PROTEINS

Virions contain a single major CP of 98 kDa and a viral RNA polymerase of 190 kDa.

## Genome organization and replication

The virus is found in the nuclei of infected *G. lamblia*. Virus replicates without inhibiting the growth of *G. lamblia* trophozoites. Virus is also extruded into the culture medium and the extruded virus

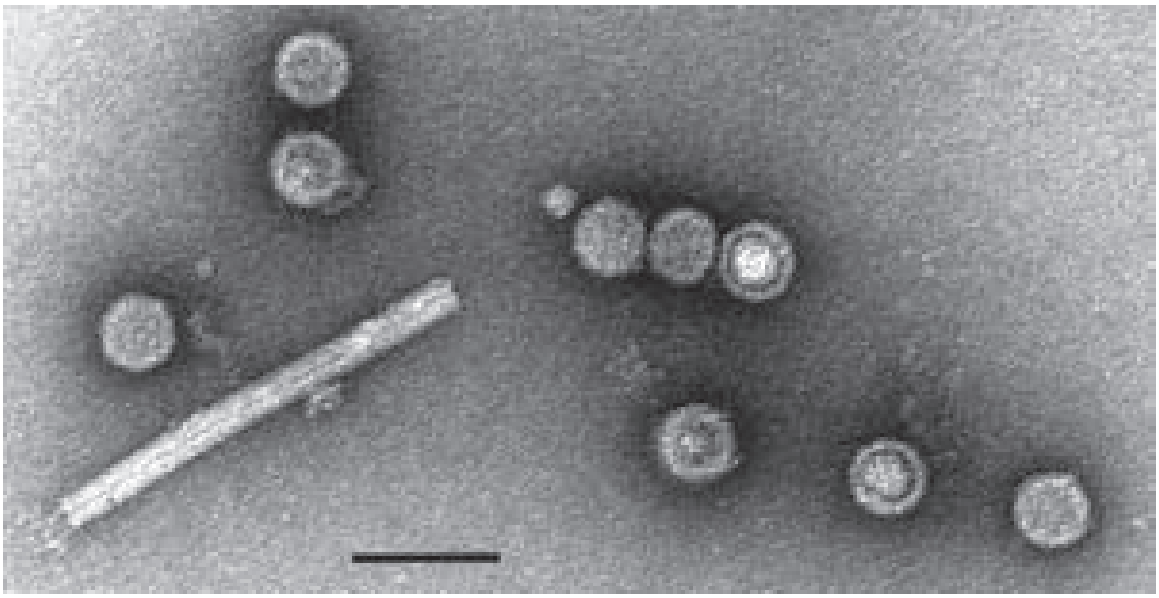

**Figure 6:** Negative contrast electron micrograph of particles of an isolate of *Giardia lamblia virus*. TMV (rod-shaped) is included as an internal size marker. The bar represents 100 nm.

can infect many virus-free isolates of the protozoan host. There are isolates of the protozoan parasite, however, that are resistant to infection by GLV. A single stranded copy of the viral dsRNA genome is present in infected cells. The concentration of the ssRNA observed during the time course of GLV infection is consistent with a role as a viral replicative intermediate or mRNA. The ssRNA is not capped or polyadenylated.

## Biological properties

The virus infects many isolates of *G. lamblia*, a flagellated protozoan human parasite. The virus does not seem to be associated with the virulence of the parasite. It is not observed in the cyst form of the parasite, and it is not known whether it can be carried through the transformation between cyst and trophozoite. The virus is infectious as purified particles and can infect uninfected *G. lamblia*.

## Species demarcation criteria in the genus

Biological criteria, as for the genus *Totivirus*, are the prime determinants of species. In addition, less than 50% sequence identity at the protein level generally reflects a species difference. None of these criteria is absolute, but totiviruses described so far leave little doubt about species demarcation.

## List of species in the genus *Giardiavirus*

| | | |
|---|---|---|
| *Giardia lamblia virus* | | |
| Giardia lamblia virus | [L13218] | (GLV) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Giardiavirus* but have not been approved as species

| | | |
|---|---|---|
| Trichomonas vaginalis virus 1 | [U08999] | (TVV1) |
| Trichomonas vaginalis virus 2 | [AF127178] | (TVV2) |
| Trichomonas vaginalis virus 3 | [AF325840] | (TVV3) |

# Genus *Leishmaniavirus*

Type species ***Leishmania RNA virus 1 - 1***

## Virion properties

### Morphology

Virions are isometric, 33 nm in diameter, with no envelope or surface projections (Figure 7).

### Physicochemical and physical properties

Virion buoyant density in CsCl is 1.33 g cm$^{-3}$.

### Nucleic acid

Virions contain a single molecule of linear uncapped dsRNA, 5.3 kbp in size. The complete 5284 nt sequence is available.

### Proteins

Virions contain a single major CP of 82 kDa.

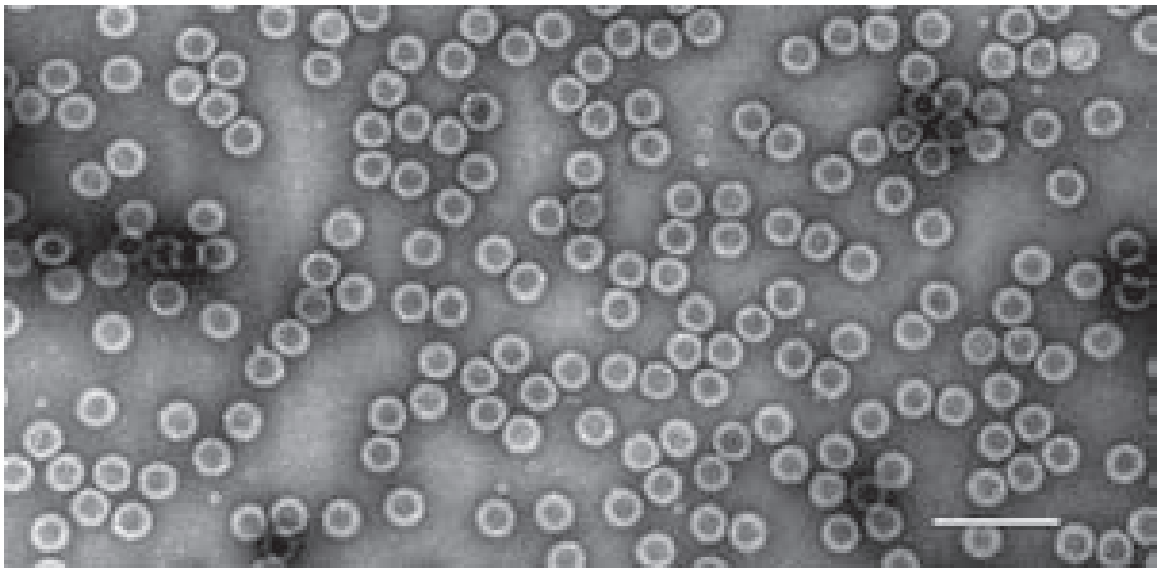

**Figure 7:** Negative contrast electron micrograph of particles of an isolate of *Leishmania RNA virus 1 - 1*. The bar represents 100 nm.

## Genome organization and replication

The positive strand contains three ORFs (Figure 8). The predicted aa sequence of ORF3 has motifs characteristic of viral RdRp. ORF2 encodes the major CP and overlaps ORF3 by 71 nt, suggesting a +1 translational frameshift to produce a *gag-pol*-like fusion protein with a predicted size of 176 kDa. Sequencing data support the idea that the abundant ssRNA found in infected cells is the message sense RNA.

## Biological properties

Leishmania RNA virus 1- 1 (LRV-1-1) is found in infected *Leishmania braziliensis* strain CUMC1. Viruses infecting several other strains of *L. braziliensis* and *L. guyanensis* are possibly strains of LRV-1-1. A single strain of *L. major* is known to be infected with LRV-1-1-like virus. The latter is designated LRV-2-1 in order to distinguish it from the viruses infecting new world strains of *Leishmania*.

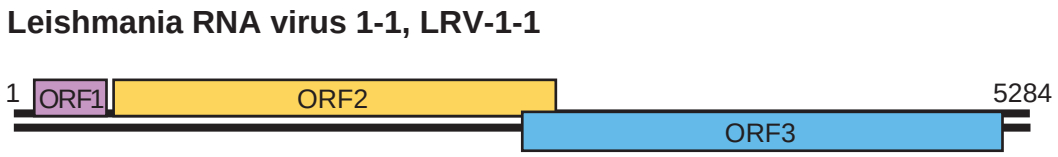


**Figure 8:** Genome organization of Leishmania RNA virus 1 - 1 (LRV-1-1).

## Species demarcation criteria in the genus

Biological criteria, as for the genus *Totivirus*, are the prime determinants of species. In addition, less than 50% sequence identity at the protein level generally reflects a species difference. None of these criteria is absolute, but totiviruses described so far leave little doubt about species demarcation.

## List of species in the genus *Leishmaniavirus*

| | | |
|---|---|---|
| *Leishmania RNA virus 1 - 1* | | |
| Leishmania RNA virus 1 - 1 | [M92355] | (LRV-1-1) |
| *Leishmania RNA virus 1 - 2* | | |
| Leishmania RNA virus 1 - 2 | [AF230881*] | (LRV-1-2) |
| (Leishmania RNA 2) | | (LR2) |
| *Leishmania RNA virus 1 - 3* | | |
| Leishmania RNA virus 1 - 3 | | (LRV-1-3) |
| *Leishmania RNA virus 1 - 4* | | |
| Leishmania RNA virus 1 - 4 | [U01899] | (LRV-1-4) |
| (Leishmania B virus) | | (LBV) |
| *Leishmania RNA virus 1 - 5* | | |
| Leishmania RNA virus 1 - 5 | | (LRV-1-5) |
| *Leishmania RNA virus 1 - 6* | | |
| Leishmania RNA virus 1 - 6 | | (LRV-1-6) |
| *Leishmania RNA virus 1 - 7* | | |
| Leishmania RNA virus 1 - 7 | [AF230882*] | (LRV-1-7) |
| *Leishmania RNA virus 1 - 8* | | |
| Leishmania RNA virus 1 - 8 | [AF230883*] | (LRV-1-8) |
| *Leishmania RNA virus 1 - 9* | | |
| Leishmania RNA virus 1 - 9 | [AF230884*] | (LRV-1-9) |
| *Leishmania RNA virus 1 - 10* | | |
| Leishmania RNA virus 1 - 10 | [AF230885*] | (LRV-1-10) |
| *Leishmania RNA virus 1 - 11* | | |
| Leishmania RNA virus 1 - 11 | [AF230886*] | (LRV-1-11) |
| *Leishmania RNA virus 1 - 12* | | |
| Leishmania RNA virus 1 - 12 | | (LRV-1-12) |
| *Leishmania RNA virus 2 - 1* | | |
| Leishmania RNA virus 2 - 1 | [U32108] | (LRV-2-1) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

## List of other related viruses which may be members of the genus *Leishmaniavirus* but have not been approved as species

None reported.

## List of other related viruses which may be members of the family *Totiviridae* but have not been approved as species

| | | |
|---|---|---|
| Eimeria brunetti RNA virus 1 | [AF356189] | (EbRV1) |
| Infectious myonecrosis virus | [AY570982] | (IMNV) |

## Phylogenetic relationships within the family

Phylogenetic analysis based on amino acid sequences of the RdRps of members of the family *Totiviridae* (Figure 9) showed that members of each of three recognized genera, *Totivirus*, *Victorivirus* and *Leishmaniavirus*, form separate distinct clusters. The fourth recognized genus (*Giardiavirus*), with a single species, clusters with the unassigned virus IMNV. It is also clear from such analysis that the Trichomonas vaginalis viruses (TVV1, TVV2 and TVV3) constitute a monophyletic cluster distinguishable from all other viruses in the family, including GLV, the prototype member of the genus *Giardiavirus*, to which TVVs had been previously assigned on a tentative basis. A proposal to create a new genus for these *Trichomonas* viruses is under consideration by the ICTV-EC. It is of interest that members of the genus *Victorivirus* that infect filamentous fungi are more closely related to some protozoan viruses (leishmaniaviruses and *Trichomonas* viruses as well as the unassigned virus EbRV1) than to members of the genus *Totivirus* that infect yeast and smut fungi. This further justifies the placement of viruses that infect filamentous fungi, which were once included in the genus *Totivirus*, in their own separate genus, *Victorivirus*.

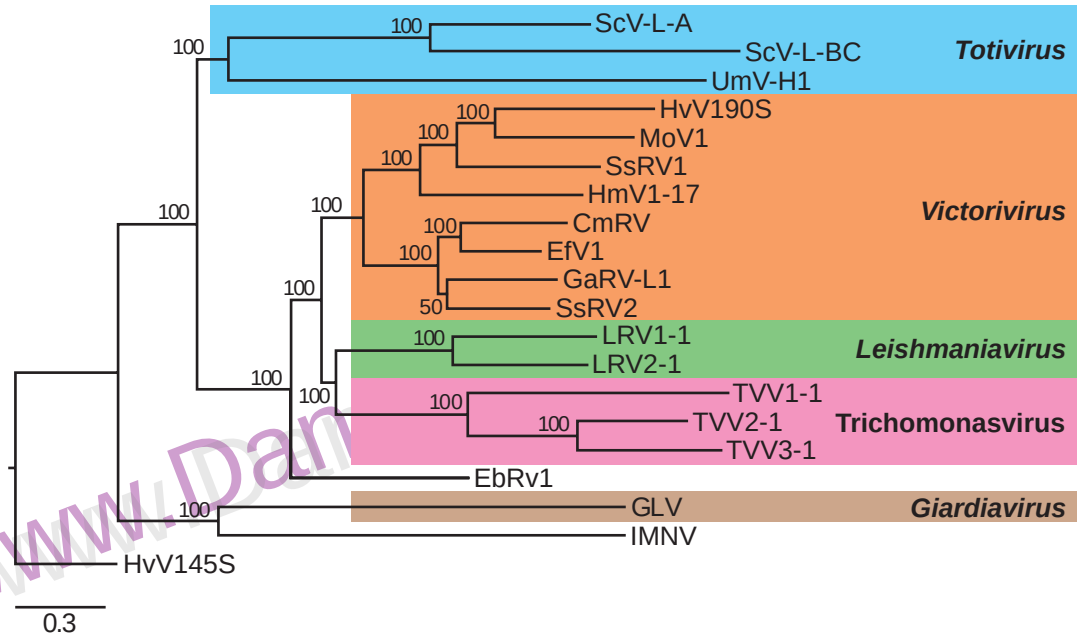


**Figure 9** Phylogenetic relationships among members of the family *Totiviridae*. A Bayesian tree was derived from the RdRp ORF amino acid sequences of each analyzed virus. The multiple sequence alignment was generated using MUSCLE v3.7 without Gblocks curation; the phylogenetic tree was generated using MrBayes v3.1.2 (10,000 generations with sampling every 100 and first 100 generations discarded); and the tree was rendered using TreeDyn v198.3. All four steps were performed using the "à la carte" option at www.phylogeny.fr/ with other default settings. The tree was refined for presentation using FigTree v1.3.1 obtained from http://tree.bio.ed.ac.uk/software/figtree/. The tree is rooted on outgroup virus HvV145S (Helminthosporium victoriae virus 145S; GenBank accession no. YP_052858) from the family *Chrysoviridae*, and the scale bar indicates the number of substitutions per position in the alignment. Approved and probable members of the family *Totiviridae* included in the tree are (GenBank accession no. in parenthesis): ScV-L-A, Saccharomyces cerevisiae virus L-A (NP_620495.1); ScV-L-BC, Saccharomyces cerevisiae virus L-BC (NP_042581.1); UmV-H1, Ustilago maydis virus H1 (NC_003823.1); HvV190S, Helminthosporium victoriae virus 190S (AAB94791.2); MoV1, Magnaporthe oryzae virus 1 (YP_122352.1); HmV1-17, SsRV1, Sphaeropsis sapinea RNA virus 1 (NP_047558.1); Helicobasidium mompa totivirus 1-17 (NP_898833.1); CmRV, Coniothyrium minitans RNA virus (YP_392467.1); EfV1, Epichloe festucae virus 1 (CAK02788.1); GaRV-L1, Gremmeniella abietina RNA virus L1 (AAK11656.1); SsRV2, Sphaeropsis sapinea RNA virus 2 (NP_047560.1); LRV1-1, Leishmania RNA virus 1 - 1 (NP_041191.1); LRV2-1, Leishmania RNA virus 2 - 1 (NP_043465.1); TVV1-1, Trichomonas vaginalis virus 1-1 (AAA62868.1); TVV2-1, Trichomonas vaginalis virus 2-1 (AAF29445.1); TVV3-1, Trichomonas vaginalis virus 3-1 (NP_659390.1); EbRV1, Eimeria brunetti RNA virus 1 (NP_108651.1); GLV, Giardia lamblia virus (NP_620070.1); and IMNV, penaeid shrimp infectious myonecrosis virus (YP_529549.1). Viruses are clustered and labeled as follows: genus *Totivirus* (cyan), genus *Victorivirus* (orange), genus *Leishmaniavirus* (green), proposed new genus of Trichomonas viruses (magenta), and genus *Giardiavirus* (brown). Probable family members EbRV1 and IMNV are indicated in gray.

## Similarity with other taxa

The RdRp of each virus has the consensus sequences typical of the RdRps of (+) ssRNA and dsRNA viruses. The capsid structures have the "T = 2" structure with 120 monomers, typical of the cores of all dsRNA viruses but of no other viruses. The replication and transcription strategies of the L–A virus resemble those of other dsRNA viruses.

## Derivation of names

*Giardia*: derived from the name of the host.
*Leishmania*: derived from the name of the host.
*Toti*: from Latin *totus*, "whole" or "undivided".
*Victori*: derived from the species name of the host, *Helminthosporium victoriae*.

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## Contributed by

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# ORDER *MONONEGAVIRALES*

## Taxonomic structure of the order

| | |
|---|---|
| Order | ***Mononegavirales*** |
| Family | ***Bornaviridae*** |
| Genus | ***Bornavirus*** |
| Family | ***Filoviridae*** |
| Genus | ***Marburgvirus*** |
| Genus | ***Ebolavirus*** |
| Family | ***Paramyxoviridae*** |
| Subfamily | ***Paramyxovirinae*** |
| Genus | ***Rubulavirus*** |
| Genus | ***Avulavirus*** |
| Genus | ***Respirovirus*** |
| Genus | ***Henipavirus*** |
| Genus | ***Morbillivirus*** |
| Subfamily | ***Pneumovirinae*** |
| Genus | ***Pneumovirus*** |
| Family | ***Rhabdoviridae*** |
| Genus | ***Vesiculovirus*** |
| Genus | ***Lyssavirus*** |
| Genus | ***Ephemerovirus*** |
| Genus | ***Novirhabdovirus*** |
| Genus | ***Cytorhabdovirus*** |
| Genus | ***Nucleorhabdovirus*** |
| Genus | ***Metapneumovirus*** |

## Virion properties

### MORPHOLOGY

The virions are large enveloped structures with a prominent fringe of peplomers, 5–10 nm long and spaced 7–10 nm apart, in all except the members of the family *Bornaviridae*. The morphologies are variable and individual particles frequently exhibit pleomorphism, but in general distinguish the families: 90 nm diameter spherical particles with a 50 nm diameter electron-dense core and without peplomers in the family *Bornaviridae*; simple, branched, U-shaped, 6-shaped or circular filaments of uniform diameter (about 80 nm) extending up to 14,000 nm are characteristic of viruses classified in the family *Filoviridae*, although purified virions are bacilliform and of uniform length (e.g. 790 nm in the case of Marburg virus); filamentous, pleomorphic or spherical structures of variable diameter are characteristic of viruses belonging to the family *Paramyxoviridae*; and regular bullet-shaped or bacilliform particles are characteristic of the member viruses of the family *Rhabdoviridae*. The ribonucleoprotein core has a diameter of 13–20 nm, which in viruses belonging to the families *Filoviridae* and *Rhabdoviridae* is organized into a helical nucleocapsid of about 50 nm in diameter. Differences in helical pitch of the ribonucleoprotein core distinguish the *Paramyxovirinae* from the *Pneumovirinae*.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virion Mr is 300–1000 $\times 10^6$. $S_{20,W}$ is 550–1045S (plant rhabdoviruses have larger $S_{20,W}$ values). Virion buoyant density in CsCl 1.18–1.22 g cm$^{-3}$. Virus infectivity is rapidly inactivated by heat treatment at 56 °C, or following UV- or X-irradiation, or exposure to lipid solvents.

### NUCLEIC ACID

Virions contain one molecule of linear, non-infectious, negative sense, ssRNA, 8.9–19 kb in size, Mr of 3–5 $\times 10^6$ which comprises about 0.5 to 2.0% of the particle weight. The viral RNA lacks a capped 5′ terminus, or a covalently associated protein. The 3′ terminus of viral RNA lacks a poly(A) tract. The 5′- and 3′-terminal regions exhibit significant inverse complementarity, and there are conserved motives in the terminal regions of all four families. Full-length positive sense (anti-genomic) RNAs are found in infected cells. The genome comprises a linear sequence of genes, with limited overlaps in some viruses, and with short terminal non-coding regions. The virus genes are expressed as

individual transcription units, each bounded by short transcription start and termination sequences. The transcription start and termination sequences show considerable similarities within the families with conserved motifs present in all families. The non-transcribed intergenic regions range from two to several hundred nucleotides. The predominant pattern is that each individual virus mRNA encodes a single protein but exceptions are seen; genetic information may be encoded in all three reading frames in the P genes of respiroviruses and morbilliviruses and the M2 genes of the *Pneumovirinae* encode two proteins from different reading frames. Splicing of some mRNA and overlapping start/stop signals are characteristic of bornaviruses. In the subfamily *Paramyxovirinae* of the family *Paramyxoviridae*, but not the subfamily *Pneumovirinae*, the number of nucleotides in the genome is divisible by six ("the rule of six"), presumably reflecting a nucleocapsid structural constraint.

### Proteins

There are a limited number of proteins in relation to the large particle size. The 5–7 structural proteins comprise envelope glycoprotein(s), a matrix protein, a major RNA-binding protein, other nucleocapsid-associated protein(s), plus a large molecular weight polymerase protein, and in some viruses several non-structural proteins which may be phosphorylated. The matrix protein is non-glycosylated in all except the bornaviruses. The matrix protein of Borna disease virus is N-glycosylated and expressed on the surface of virions. Enzymatic activities associated with the virions may include transcriptase, polyadenylate transferase, mRNA transferase and neuraminidase.

### Lipids

Virions are composed of about 15–25% lipids, their composition reflecting that of the host cell membrane from which the virions bud. Generally, phospholipids represent about 55–60%, and sterols and glycolipids about 35–40% of the total lipids. Glycoproteins may have a covalently associated fatty acid proximal to the lipid envelope.

### Carbohydrates

Virions are composed of about 3% carbohydrate by weight. The carbohydrates are present as N- and O-linked glycan chains on surface proteins and on glycolipids. When made in mammalian cells the oligosaccharide chains are generally of the complex type, in insect cells they are of the non-complex types.

## Genome organization and replication

In the families *Filoviridae*, *Paramyxoviridae* and *Rhabdoviridae*, the site of multiplication is the cytoplasm, with the exception of viruses classified in the genus *Nucleorhabdovirus.* The 3′ end (leader) region of the genomes contains the promoter element which directs the virus RdRp to initiate transcription. Discrete mRNAs are transcribed by sequential interrupted synthesis. Transcription is polar, with a gradient of attenuation. The order of the genes on the genome encoding the structural proteins is conserved, though individual members may carry additional genes located between the structural genes (Table 1). The presence of these additional genes may affect the relative levels of the mRNAs from the structural genes. The mRNAs are capped and polyadenylated, with polyadenylation occurring as a result of iterative transcription from a short poly U tract at the end of each gene. Generally, genes do not overlap, the exceptions being the M2 and L genes of pneumoviruses, the VP30 and VP24 of the Marburgviruses and the VP35/VP40, GP/VP30 and VP24/L of ebolaviruses. The P genes of *Paramyxovirinae* encode multiple proteins. In members of the respirovirus, morbillivirus and Henipavirus genera, the C proteins arise from the use of an alternative reading frame accessed by a standard AUG initiation codon, and additional C-related proteins in the respiroviruses by the use of non-AUG start codons. Members of the avulavirus and rubulavirus genera do not encode a C protein. In all members of the subfamily *Paramyxovirinae*, except human parainfluenzavirus type 1, a proportion of the P mRNA produced by the virus is altered by an editing process in which one or more additional guanine residues, not represented by cytosine residues in the template genome, are inserted at a specific site in the mRNA during transcription. This process results in translation of the edited mRNA utilizing a different reading frame following the position of the inserted base(s) to direct the synthesis of the additional V and W proteins. In the avulaviruses the faithfully transcribed mRNA encodes the V protein and the P protein is produced from an edited mRNA. A non-templated insertion event occurs during transcription of the glycoprotein gene of ebolaviruses generating both membranes-inserted and secreted forms of the glycoprotein. The M2 mRNA of the members of the *Pneumovirinae* sub-family encode a second protein from an additional ORF accessed by a coupled

**Table 1:** A diagrammatic representation of the 3′ to 5′ arrangement of the transcriptional units in the genomes of viruses classified in the four families (*Bornaviridae*, *Filoviridae*, *Paramyxoviridae* and *Rhabdoviridae*) comprising the order *Mononegavirales*

| Family/ Subfamily | Genus | Virus | 3′←Gene order→5′ | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***Bornaviridae*** | *Bornavirus* | BDV | le | | | N | X/P | | M | | | | G | | | L | tr |
| ***Rhabdoviridae*** | *Vesiculovirus* | VSV | le | | | N | P | | M | | | | G | | | L | tr |
| | *Lyssavirus* | RabV | le | | | N | P | | M | | | | G | Ps | | L | tr |
| | *Cytorhabdovirus* | LNYV | le | | | N | P | 4b | M | | | | G | | | L | tr |
| | *Nucleorhabdovirus* | SYNV | le | | | N | P | Sc4 | M | | | | G | | | L | tr |
| | *Novirhabdovirus* | IHNV | le | | | N | P | | M | | | | G | NV | | L | tr |
| | *Ephemerovirus* | BEFV | le | | | N | P | | M | | | | G | Gns | | L | tr |
| ***Filoviridae*** | *Ebolavirus* | ZeboV | le | | | N | P | | M1 | | | | GP/ SP | | M2 | L | tr |
| | *Marburgvirus* | MarV | le | | | N | P | | M1 | | | | G | | M2 | L | tr |
| ***Paramyxoviridae*** | | | | | | | | | | | | | | | | | |
| *Paramyxovirinae* | *Avulavirus* | NDV | le | | | N | P/V | | M | F | | | H | | | L | tr |
| | *Henipavirus* | HeV | le | | | N | P/C/V | | M | F | | | H | | | L | tr |
| | *Morbillivirus* | MeV | le | | | N | P/C/V | | M | F | | | H | | | L | tr |
| | *Respirovirus* | SeV | le | | | N | P/C/V | | M | F | | | HN | | | L | tr |
| | *Rubulavirus* | MuV | le | | | N | V/P | | M | F | | SH | HN | | | L | tr |
| *Pneumovirinae* | *Metapneumovirus* | AMPV | le | | | N | P | | M1 | F | M2 | SH | G | | | L | tr |
| | *Pneumovirus* | HRSV | le | NS1 | NS2 | N | P | | M1 | SH | G | F | M2 | | | L | tr |
| **Unassigned viruses** | | JV | le | | | N | P/C/V | | M | F | | SH | G | | | L | tr |
| | | BeV | le | | | N | P/C/V | | M | F | | SH | G | | | L | tr |
| | | FDLV | le | | | N | P/V | | M | F | | | HN | | | L | tr |

Genes encoding polypeptides of presumed homologous function are aligned vertically. The genome organizations of currently unclassified viruses which encode additional proteins are also shown.
Abbreviations: viruses: AMPV, avian metapneumovirus; BDV, Borna disease virus; BeV, Beilong virus; BEFV, bovine ephemeral fever virus; FDLV, Fer de Lance virus; HeV, Hendra virus; HRSV, human respiratory syncytial virus; IHNV, infectious hemopoetic necriosis virus; JV, J virus; LNYV, lettuce necrotic yellows virus; MarV, Lake Victoria marburgvirus; MeV, measles virus; MuV, mumps virus; NDV, Newcastle disease virus; RabV, rabies virus; SeV, Sendai virus; SYNV, sonchus yellow net virus, VSV, vesicular stomatitis Indiana virus; ZeboV, Zaire ebolavirus.
Transcriptional units; le, non-coding leader region; NS, non-structural protein gene; N, nucleoprotein gene; U, gene of unknown function; P, phosphoprotein gene; V and C, dispensible non-structural protein genes; sc4 and 4b, genes of unknown function; M and M1, non-glycosylated matrix protein gene; (M) glycosylated matrix protein gene; F, fusion protein gene; SH, small hydrophobic protein gene; TM, transmembrane protein gene; G (or H or HN), glycosylated (or hemagglutinin or hemagglutinin/neuraminidase) attachment protein gene; M2, non-glycosylated (BDV excepted) envelope protein gene; Ps, pseudogene; NV, non-virion protein gene; Gns, presumptive duplicated G sequence; GP/SP, the glycoprotein gene of ebolavirus produces two products, the major secreted form from the unaltered mRNA and the structural form produced by transcriptional insertion of a single base in the mRNA; L, large (polymerase) protein gene; tr, non-coding trailer region.

translation process dependent on translation of the upstream ORF. Replication occurs by synthesis of a complete positive sense anti-genomic RNA. Genomic and anti-genomic RNAs are present as nucleocapsids. In the family *Bornaviridae*, the site of multiplication is the nucleus. Transcription of bornavirus genomes is complex, with splicing of mRNA and overlapping stop/start signals. The mRNAs of bornaviruses are capped, but synthesis is not inhibited by alpha-amanitin, suggesting that a cap-snatching mechanism is not involved. In the filoviruses, paramyxoviruses and rhabdoviruses, maturation of the independently assembled helical nucleocapsid occurs by budding through host membranes with investment by a host-derived lipid envelope containing transmembrane proteins. The process of assembly and maturation of bornaviruses is not known at present.

## Antigenic properties

The major neutralizing epitopes against which antibodies are directed lie within the membrane glycoproteins. Virus serotypes are defined by the surface antigens. Filoviruses are an exception in that they are poorly neutralized *in vitro*. In bornaviruses, antibodies to both the glycosylated matrix protein, which may function as an attachment protein, and the gp94 envelope protein neutralize infectivity.

## Biological properties

The host ranges vary from restricted to unrestricted. Filoviruses have only been isolated from primates and swine. Paramyxoviruses occur only in vertebrates and no vectors are known. Rhabdoviruses infect invertebrates, vertebrates and plants. Some rhabdoviruses multiply in both invertebrates and vertebrates, some in invertebrates and plants, but none in all three. In human hosts the pathogenic potential tends to be characteristic of the family: i.e. hemorrhagic fever (*Filoviridae*); respiratory and neurological diseases (*Paramyxoviridae*); mild febrile to fatal neurological diseases (*Rhabdoviridae*). Bornaviruses have been isolated from horses, cattle, sheep, rabbits, rats, cats, ostriches and man. The pathology associated with virus infection is variable. Infection of animals is associated with conditions ranging from behavioral disturbances to severe non-purulent encephalomyelitis. Cytopathology varies from none (bornaviruses and filoviruses) to rapidly lytic (rhabdoviruses and paramyxoviruses); syncytium formation is common in paramyxoviruses.

## Phylogenetic relationships within the order

Phylogenetic relationships between the families *Bornaviridae*, *Filoviridae*, *Paramyxoviridae* and *Rhabdoviridae* are illustrated in Figure 1.

## Derivation of names

*Borna*: from Borna, a town in Saxony.
*Cyto*: from Greek *kytos*, "cell".
*Ebola*: from the river Ebola, in Sudan and Zaire.
*Ephemero*: from Greek *ephemeros*, "short-lived".
*Filo*: from Latin *filo*, "thread-like".
*Lyssa*: from Greek *lyssa*, "rage, fury, canine madness".
*Marburg*: from the city of Marburg, in Germany.
*Meta*: from Greek *meta*, "after".
*Mono*: from Greek *monos*, "single".
*Morbilli*: from Latin *morbillus*, diminutive of *morbus*, "disease".
*Nega*: from negative sense RNA.
*Novi*: *no*n *vi*rion protein gene, characteristic of the genus.
*Nucleo*: from Latin *Nux*, *nucis* "nut".
*Paramyxo*: from Greek *para*, "by the side of", and *myxa*, "mucus".
*Pneumo*: from Greek *pneuma*, "breath".
*Respiro*: from Latin *respirare*, "to breathe".
*Rhabdo*: from Greek *rhabdos*, "rod".
*Rubula*: from Latin *ruber*, "red"; Rubula inflans, old name for mumps.
*Vesiculo*: from Latin *vesicula*, diminutive of *vesica*, "bladder", "blister".
*Virales*: from Latin *virales*, "viruses".

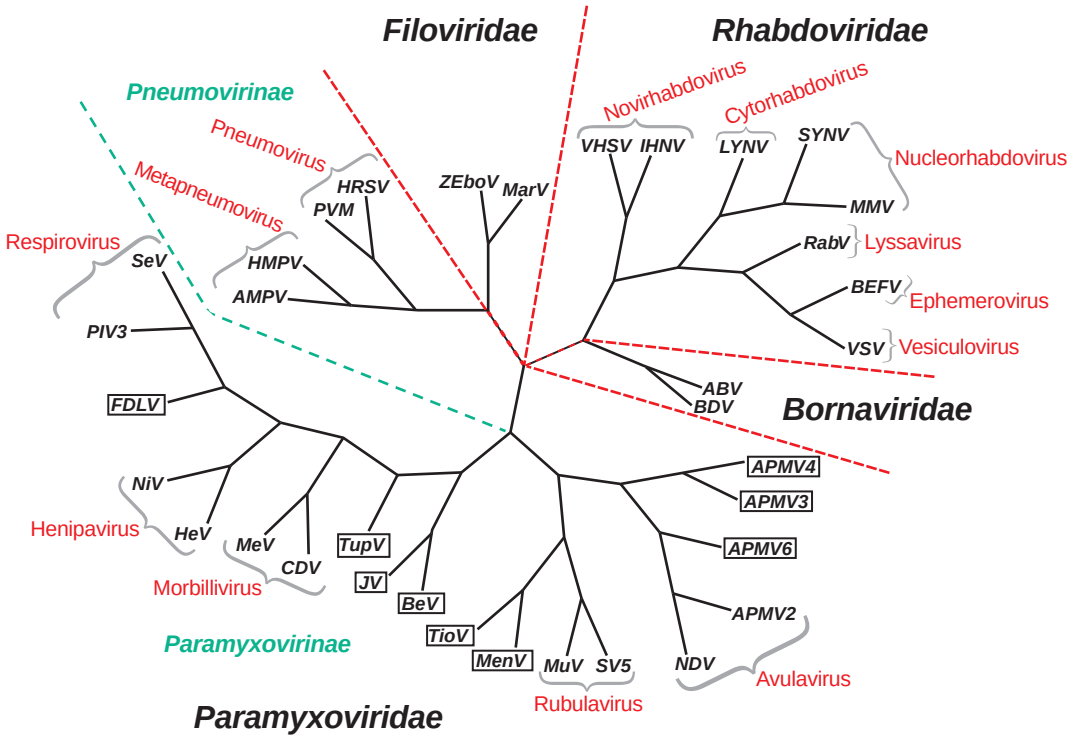


**Figure 1:** Unrooted phylogenetic tree of members of the order *Mononegavirales*. The tree was constructed using the CLUSTALX and PHYLIP programs with the sequences of the conserved domain III of the polymerase proteins (Poch *et al.*, 1989, 1990). Nine paramyxoviruses, not yet assigned to genera, are included (boxed): avian parainfluenza virus types 3 (APMV3), avian parainfluenza virus types 4 (APMV4), avian parainfluenza virus types 6 (APMV6), Beilong virus (BeV), Fer de Lance virus (FDLV), J virus (JV), Menangle virus (MenV), Tioman virus (TioV) and Tupaia paramyxovirus (TupV). Abbreviations: ABV, avian bornavirus; AMPV, avian metapneumovirus; APMV2, avian parainfluenza virus types 2; BDV, Borna disease virus; BEFV, bovine ephemeral fever virus; CDV, canine distemper virus; HeV, hendra virus; HMPV, human metapneumovirus; HRSV, human respiratory syncytial virus; IHNV, infectious hemorraghic necrosis virus; LNYV, lettuce necrotic yellows virus; MarV, Marburg virus; MeV, measles virus; MMV, maize mosaic virus; MuV - mumps virus; NDV, Newcastle disease virus; NiV, Nipah virus; PIV3, parainfluenza virus type 3; PVM, pneumonia virus of mice; RabV, rabies virus; SeV, Sendai virus; SV5, simian virus 5; SYNV, Sonchus yellow net virus; VSV, vesicular stomatitis Indiana virus; VHSV, viral hemorrhangic septicemia virus; ZeboV, Zaire ebolavirus.



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## Contributed by

Easton, A.J. and Pringle, C.R.

# Family *Bornaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Bornaviridae*** |
| Genus | ***Bornavirus*** |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Bornavirus*

Type species ***Borna disease virus***

## Virion properties

### Morphology

Electron microscopy studies of negatively stained infectious particles of an isolate of Borna disease virus (BDV) have shown that virions have a spherical morphology with a diameter of $90 \pm 10$ nm containing an internal electron-dense core (50–60 nm) (Figure 1).

### Physicochemical and physical properties

Virion Mr and the $S_{20,w}$ are not known. Partially purified BDV infectious particles have a buoyant density in CsCl of 1.16–1.22 g cm$^{-3}$, in sucrose of 1.22 g cm$^{-3}$, in renografin of 1.13 g cm$^{-3}$. Virus infectivity is rapidly lost by heat treatment at 56 °C. Virions are relatively stable at 37 °C, and only minimal infectivity loss is observed after 24 hrs incubation at 37 °C in the presence of serum. Virions are inactivated below pH 5.0, as well as by treatment with organic solvents, detergents, and exposure to UV radiation. Infectivity is completely and rapidly destroyed by chlorine-containing disinfectants or formaldehyde treatment.

### Nucleic acid

The genome consists of a single molecule of a linear, negative sense ssRNA about 8.9 kb in size and Mr of about $3 \times 10^6$). The RNA genome is not polyadenylated. Extracistronic sequences are found at the 3′ (leader) and 5′ (trailer) ends of the BDV genome. BDV 3′-terminal genomic sequences have a high A + U content with a U/A ratio of about 2:1. The ends of the BDV genome RNA exhibit partial inverted complementarity. Full-length plus-strand (antigenomic) RNAs are present in infected

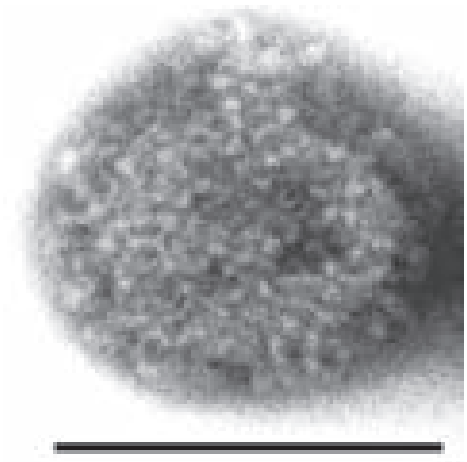

**Figure 1:** Negative contrast electron micrograph of a particle of Borna disease virus. The bar represents 100 nm. (Courtesy of Dr M. Eickmann.)



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cells and in viral ribonucleoproteins. Defective RNAs have not been identified in BDV-infected cells and tissues. BDV can be classified into two subtypes based on the complete genome sequences of several BDV strains. With the unique exception of strain No/98, all isolates of BDV to date (independent of year, species and area isolation) have approximately 95% homology at the nt level (subtype 1), while BDV strain No/98 shows only 85–86% nt sequence identity compared to other BDV strains (subtype 2). The nt changes are distributed fairly evenly over the entire genome of BDV. However, all strains predict the same BDV genomic organization and differ by only one nt in absolute genome size.

### Proteins

Six major ORFs are found in the BDV genome sequence (Figure 2). These ORFs code for polypeptides with predicted size of 40 kDa (p40), 24 kDa (p24), 10 kDa (p10), 16 kDa (p16), 56 kDa (p56) and 180 kDa (p180), respectively. Based on their positions in the viral genome and abundance in infected cells and virion particles, together with their biochemical and sequence features, p40, p24 and p16 BDV polypeptides correspond to the viral nucleoprotein (N), the phosphoprotein (P) transcriptional activator, and matrix (M) proteins, respectively, found in other negative sense ssRNA viruses. Two isoforms of the BDV N (p39 and p38) are found in BDV-infected cells. These two forms of the viral N appear to be encoded by two different mRNA species. Differential usage of two in-frame initiation codons present in the BDV p40 gene may also contribute to the production of BDV p39/38. BDV p39 contains both a nuclear localization signal (NLS) and a nuclear export signal (NES), whereas p38 harbors only the NES. BDV p24 is an acidic polypeptide (predicted I.P. of 4.8), that has a high Ser-Thr content (16%), with phosphorylation at serine residues which is mediated by both protein kinase C and casein kinase II. These features are consistent with those of the phosphoprotein (P) transcriptional activator found in other negative sense ssRNA viruses. BDV p24 contains a bipartite NLS in the sequence. In addition to P, a 16 kDa polypeptide (P′) is also translated from the second in-frame AUG codon in the P ORF. An additional ORF, p10, encodes a polypeptide of 10 kDa present in BDV-infected cells. BDV X starts within the same mRNA transcription unit, 46 nt upstream from p24 and overlaps, in a different frame, with the 71 N-terminal aa of p24. Recent study indicates that BDV X harbors a NLS in the N-terminus of the sequence.

Consistent with other negative sense ssRNA viruses, BDV p16, the putative BDV M protein, is a non-glycosylated M protein, associated at the inner surface of the viral membrane. BDV ORF4 (p56) overlaps, in a different frame, with the C-terminus of ORF p16, and is capable of encoding a 503 aa polypeptide with a predicted size of 56 kDa. Based on its sequence features, BDV p56 is the counterpart of the virus surface glycoproteins (G) found in other negative sense ssRNA viruses. The p56 gene directs the synthesis of three glycosylated polypeptides of about 84 or 94 kDa (GP-84/94, G), 43 kDa (GP-43, GP-C) and 45 to 55 kDa (GP-N). G corresponds to the full length of the p56 gene, whereas GP-C and GP-N represent the C-terminal subunit and the N-terminal subunit of ORF p56, respectively. Both GP-C and GP-N are associated with BDV infectious particles. Antibodies to p56 have neutralizing activity, suggesting that BDV p56 gene products play an important role in the early steps of BDV infection. BDV ORF5 (p180) is capable of encoding a polypetide with a predicted size of 180 kDa, whose deduced aa sequence displays strong homology to other negative sense ssRNA virus polymerases, members of the L protein family. An additional ORF predicted in mRNA species generated via RNA splicing would encode a variant BDV L with a predicted size of 190 kDa (BVp190). BDVp190 corresponds to BDVp180 with 153 aa added to its N-terminus. Recent evidence suggests that p190, rather than p180, is the active BDV L. BDV L contains the NLS in the sequence.

### Lipids

Not known.

### Carbohydrates

Only N-glycans, mannose-rich type and partially hybrid types.

## Genome organization and replication

The negative sense BDV RNA genome codes for at least six ORFs in the order 3′-N-P/X-M-G-L-5′. The genomic polarity has a very limited coding capability, and none of its predicted ORFs has a favorable translational start signal; further they are not flanked by putative transcription start and termination/polyadenylation signals. Therefore, it seems unlikely that BDV uses an ambisense coding strategy. BDV has the property, unique among known negative sense ssRNA animal viruses, of

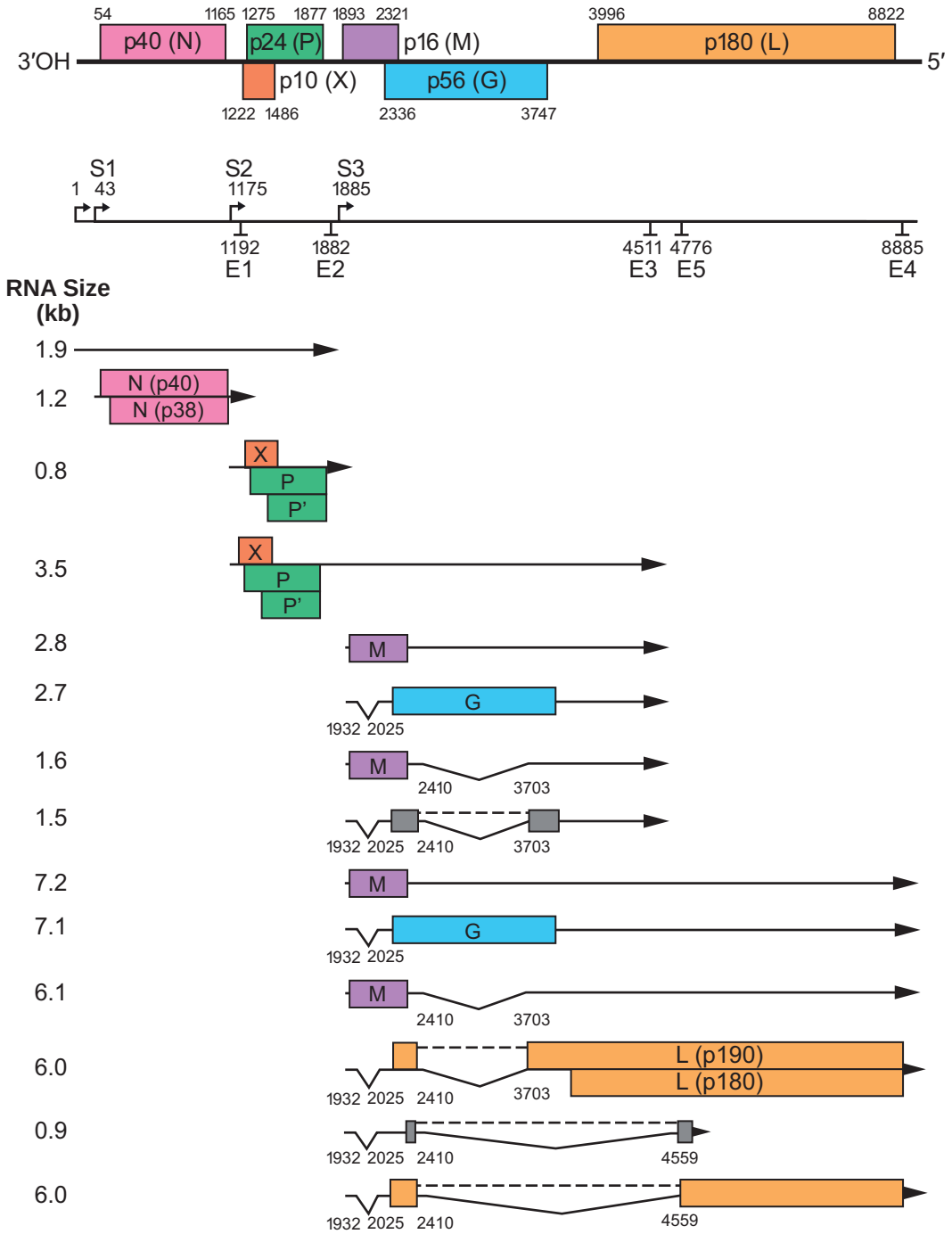


**Figure 2:** Genomic organization and transcriptional map of an isolate of Borna disease virus. ORFs are represented by boxes at the top. The location of transcription initiation and transcription termination sites are indicated by S and E, respectively. Positions of introns: I (nt 1932–2025), II (nt 2410–3703), and III (nt 2410–4559) are indicated. For details see text.

a nuclear site for genome transcription and replication. Full-length genome complementary RNA molecules (antigenomes) act as templates for new viral genome RNA synthesis. Genome and antigenome RNA molecules are neither capped nor polyadenylated. These RNAs exist as nucleocapsids in the nucleus of infected cells. It is unknown whether RNA species corresponding to the leader RNA are transcribed in BDV-infected cells.

BDV cell entry occurs by receptor-mediated endocytosis. The virus G protein has been implicated in entry. The identity of the BDV cellular receptor is unknown. In endosomes, low pH-dependent fusion occurs between viral and cellular membranes. This fusion event releases the BDV ribonucleoproteins (RNP) which are then transported to the cell nucleus where viral transcription and replication occur. Sequential and polar transcription results in decreasing molar quantity of BDV transcripts from the 3′- to the 5′-encoded cistrons. The viral mRNAs are polyadenylated, and their 5′ ends contain a blocking group, presumably a cap structure. Virus specific mRNA synthesis is not inhibited by α-amanitin, and sequences at the 5′ of the BDV mRNAs are homogeneous and genome-encoded. Thus, it is unlikely that transcription initiation of BDV mRNAs involves a cap-snatching mechanism similar to the one used by influenza viruses. Monocistronic viral mRNAs in BDV-infected cells are detected only for the N gene (Figure 2). The BDV G and L polymerase gene products are synthesized from downstream ORFs within polycistronic mRNAs. Mapping of BDV sgRNAs present in infected cells to the viral genome revealed that the BDV genome contains three transcription initiation sites (S signals), and four transcription termination/polyadenylation sites (E signals) (Figure 2). In addition, a putative E signal (E5) is found at nt 4776. The S signals contain a semi-conserved U-rich motif that is partially copied into the respective transcripts. A similar motif is not found within the S signals of previously described negative sense ssRNA viruses. BDV E signals consist of six or seven U residues preceded by a single A residue, resembling the E signal motif found in other negative sense ssRNA viruses. The BDV genome lacks the characteristic configuration of E signal/intergenic (IG) region/S signal, found at the gene boundaries of other negative sense ssRNA viruses. Instead, BDV transcription units and transcriptive signals frequently overlap (Figure 2). Two of the BDV primary transcripts are post-transcriptionally processed by the cellular RNA splicing machinery. Three introns (I, II and III) have been identified in the BDV genome. BDV introns I and II span nt 1932 to 2025 and 2410 to 3703, respectively, in the BDV antigenomic sequence (Figure 2). Splicing of intron I places the aa in position 13 of M next to a stop codon, whereas splicing of intron II, and I + II, results in a mRNA containing a predicted ORF that corresponds to the first 58 aa of G fused to a new C-terminus of 20 aa. RNA species resulting from splicing of intron II, and I + II, predicts also an additional ORF that would encode a variant BDV L protein with 153 aa added to the N-terminus. Intron III is generated by alternative 3′ splice site choice and spans nt 2410 to 4559 in the BDV antigenome (Figure 2). Splicing of introns II and III is regulated by the utilization of an alternative E signal (E5) and a putative *cis*-acting exon splicing suppressor signal located within the L gene. Transcripts lacking Intron III have the capacity to encode two new proteins with predicted size of 8.4 kDa (p8.4) and 165 kDa (p165). Whether these new predicted BDV polypeptides are synthesized in infected cells is unknown. BDV strain No/98 lacks the alternative 3′ splice site and thus cannot generate transcripts lacking intron III. RNA splicing can also modulate the efficiency of termination-reinitiation of translation and leaky scanning mechanisms, thus contributing to the regulation of the expression of BDV M, G and L gene products.

BDV-infected cells exhibit a heterogeneous pattern of viral antigen expression. BDV N, P and X polypeptides are expressed both in the nucleus and cytoplasm. N and P are the viral antigens expressed at higher levels, and they are expressed by the majority of the cells within an infected population. In contrast, only 1 to 10% of the infected cells express detectable levels of BDV G. Expression of full-length BDV G (GP-84/94) is restricted to the ER and nuclear envelope. The subcellular distribution of the BDV M is cytosolic and associated with cellular membranes. G is post-translationally modified by N-glycosylation. BDV G undergoes post-translational cleavage by the cellular protease furin, with the resulting GP-N and GP-C reaching the cell surface. Cleavage of G likely occurs in the trans-Golgi compartment. G, GP-N and GP-C are partially Endo H-sensitive and PNGase F-sensitive. The newly exposed N-terminus of GP-C is highly hydrophobic, and BDV-infected cells form extensive syncytia upon low-pH treatment. These findings suggest that GP-C is involved in pH-dependent fusion after internalization of BDV by receptor-mediated endocytosis. GP-N is most likely responsible for receptor binding.

The mechanisms involved in nucleocytoplasmic transport of viral RNP through the nuclear pore complex remain largely unknown. However, recent studies suggest that the nuclear import activity of BDV is mediated by the NLS-containing viral antigens, such as N, P, X and L, that form complexes in infected cells. In contrast, a nuclear export activity is found only in N protein. The NES of BDV N contains the canonical leucine-rich motif, and the nuclear export activity of the protein is mediated through the chromosome region maintenance protein (CRM1) pathway.

The assembly process and site of virus maturation have not been identified and budding of BDV particles from infected cells has been documented only from the surface of BDV-infected MDCK cells after treatment with n-butyrate. BDV RNP accumulate in the nucleus and, as with other negative sense ssRNA viruses, they are also infectious on the basis of an ability to direct synthesis of BDV macromolecules, as well as the production of BDV cell-associated infectivity upon transfection of BDV-susceptible cells. Thin sections of BDV-infected cells revealed the presence of intracytoplasmic virus-like particles with morphological characteristics similar to those described for partially purified cell-free BDV infectious particles. These particles showed no association with cisternae of the endoplasmic reticulum, the Golgi complex, or other intracytoplasmic membranes.

As for many other member of the order *Mononegavirales*, a reverse genetics system has recently been established for BDV.

## Antigenic properties

BDV possess a number of distinct antigenic determinants. The so-called soluble antigen (s-antigen) obtained from the supernatant after ultracentrifugation of ultrasonicated BDV-infected brain tissue, contains the viral N, P and M proteins. Serum antibodies from BDV-infected animals frequently recognize all the components of the s-antigen, but rarely recognize the viral G products. BDV field isolates from the same or different animal species, as well as viruses recovered from experimental infections with different histories of passages exhibit strong serological cross-reactivity. There is only one recognized serotype of BDV, but monoclonal antibodies have revealed minor antigenic differences among BDV isolates. Complement independent IgG-specific neutralizing antibodies have been documented in experimentally infected animals. Titers of neutralizing antibodies are usually very low and dependent on the infected host species. BDV G protein has been implicated in virus neutralization.

## Biological properties

Horses and sheep have been regarded as the main natural hosts of BDV. In these species BDV can cause a fatal neurologic disease, Borna disease (BD). Evidence indicates that the natural host range of BDV is wider than originally thought. Naturally occurring BDV infections have been documented in cattle, rabbits and cats. In addition, sporadic cases of natural infection with BDV have been reported in several other species, including donkeys, mules and llamas. Moreover, experimental infections have revealed a remarkable wide host range for BDV, from birds to rodents and non-human primates. BDV-induced neurobehavioral abnormalities in animals are reminiscent of some human neuropsychiatric disorders. Serological data and molecular epidemiological studies indicate that BDV can infect humans, and is possibly associated with certain neuropsychiatric disorders.

BDV is thought to be transmitted through salival, nasal, or conjunctival secretions. Infection may therefore result from direct contact with these secretions. Intranasal infection is the most likely route of natural infection, allowing BDV access to the central nervous system (CNS) by intraaxonal migration through the olfactory nerve. Cases of Borna disease (BD) are more frequent in some years than others and tend to occur in spring and early summer, suggesting arthropods as a potential vector. BDV has not been isolated from insects, but ticks have been implicated in the transmission of an infectious encephalomyelitis similar to BD affecting ruminants in the Middle East.

Asymptomatic naturally infected animals of different species have been documented in Europe, North America, Africa and Asia, suggesting that the prevalence and geographic distribution of BDV may have been underestimated. However, a definite natural reservoir of BDV has not been identified. Phenotypic differences have been described among different BDV field isolates, and among viruses with different histories of passages in animals and cultured cells. Despite its wide host range and phenotypic variation, molecular epidemiological data have shown a remarkable sequence conservation of BDV, not only within the same host species but also amongst sequences derived from different animal species.

BDV is highly neurotropic and has a non-cytolytic strategy of multiplication. BDV causes CNS disease in several non-human vertebrate species, which is characterized by neurobehavioral abnormalities that are often, but not always, associated with the presence of inflammatory cell infiltrates in the brain. BDV

exhibits a variable period of incubation, from weeks to years, and diverse pathological manifestations that depend on the genetics, age and immune status of the host, as well as route of infection and viral determinants. Classic BD is caused by a T cell-dependent immune mechanism. Inflammatory cells are found forming perivascular cuffs and also within the brain parenchyma. Both $CD4^+$ and $CD8^+$ T-cells are present in the CNS cell infiltrates and contribute to the immune-mediated pathology associated with BD. BDV can also induce distinct deficiencies in emotional and cognitive functions that are associated with specific neurochemical disturbances in the absence of lymphoid infiltration. Heightened viral expression in limbic system structures, together with astrocytosis and neuronal structural alterations within the hippocampal formation are the main histopathological hallmarks of BDV infection.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Bornavirus*

*Borna disease virus*
Borna disease virus-V [U04608] (BDV-V)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Bornavirus* but have not been approved as species

None reported.

## Similarity with other taxa

BDV has a genomic organization similar to that of other negative sense ssRNA viruses. The size of the BDV genome (ca. 8.9 kb) is significantly smaller than those of the other known members of the order *Mononegavirales*: *Rhabdoviridae* (ca. 11–15 kb), *Paramyxoviridae* (ca. 15 kb) and *Filoviridae* (ca. 19 kb). BDV replication and transcription take place in the nucleus. This is a unique feature among known negative sense ssRNA animal viruses, but shared with the plant nucleorhabdoviruses. Expression of the BDV genome is regulated by an overlap of transcription units and transcriptive signals, an overlap of ORFs, readthrough of transcription termination signals and differential use of translational initiation codons. There is precedent for use of each of these strategies by other members of the order *Mononegavirales*. However, the concurrent use by BDV of such a diversity of strategies for the regulation of its gene expression is unique among known negative sense ssRNA viruses. In addition, as with viruses belonging to the family *Orthomyxoviridae*, BDV uses RNA splicing to generate some of its mRNAs. This represents another unique feature in the order *Mononegavirales*. BDV has one single surface glycoprotein gene (G) which is responsible for viral attachment and fusion upon endocytosis and endosome acidification. This pH-dependent fusogenic activity of G requires its post-translational cleavage by the cellular protease furin. Thus, BDV G expression and function appear to be a unique feature in negative sense ssRNA viruses, representing a combination of the strategies adopted by rhabdoviruses and paramyxoviruses.

## Derivation of name

*Borna* refers to the city of Borna in Saxony, Germany, where many horses died in 1885 during an epidemic of a neurological disease, designated as Borna disease (BD), caused by the infectious agent presently known as Borna disease virus (BDV).

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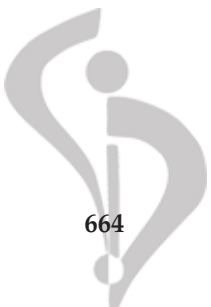
## Contributed by

In reproducing this chapter in edited form from the Eighth ICTV Report, the editors gratefully acknowledge the contribution of the authors, Schwemmle, M., Carbone, K.M., Tomonago, K., Nowotny, N. and Garten, W.



# FAMILY *FILOVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Filoviridae*** |
| Genus | ***Marburgvirus*** |
| Genus | ***Ebolavirus*** |

## Virion properties

### MORPHOLOGY

Virions are bacilliform in shape, but particles can also appear as branched, circular, U or 6-shaped and long filamentous forms. Spherical forms are rare to absent (Figure 1). This morphology is unusual for mammalian viruses and, thus, is considered characteristic for members of the family. Virions vary greatly in length but show a uniform width of about 80 nm. Family members differ in length of virions, but seem to be very similar in morphology. Peak infectivity has been associated with particles of about 665 nm in the case of marburgviruses and about 805 nm in the case of ebolaviruses.

Virions are composed of a central core formed by a nucleocapsid or ribonucleoprotein (RNP) complex, surrounded by a lipid envelope derived from the host-cell plasma membrane. Electron micrographs reveal an axial channel (about 10–15 nm in width) surrounded by a central dark layer (about 20 nm in width) and an outer helical layer (about 50 nm in width) with cross-striations of about 5 nm intervals (Figure 1). Spikes about 7 nm in diameter and spaced at intervals of about 10 nm are seen as globular structures on the surface of virions.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virions have a molecular weight of about $3.82 \times 10^8$. The buoyant density of virions in potassium tartrate is about $1.14\,g\,cm^{-3}$. The $S_{20,w}$ of bacilliform particles is 1.40S, but higher for longer particles. The nucleocapsid has a buoyant density in CsCl of about $1.32\,g\,cm^{-3}$.

### NUCLEIC ACID

Filovirus genomes are nonsegmented, single stranded, linear RNA molecules of negative polarity with lengths of about 19 kb: marburgvirus genomes are 19.1 kb in length and ebolavirus genomes are 18.9 kb. The $M_r$ of a genomic RNA is about $4.2 \times 10^6$ and the genome represents about 1.1% of the total virion mass. Genomic RNA is not polyadenylated at the 3′ end and there is no evidence for a 5′-terminal cap structure or a covalently-linked protein. Full-length nucleotide sequences of the genomes of members of all filovirus species have been determined.

### PROTEINS

Filovirus RNP complexes are composed of a genomic RNA molecule and four virion-associated structural proteins: NP (nucleoprotein), VP35 (RNA-dependent RNA polymerase cofactor), VP30 (transcriptional activator), and L (RNA-dependent RNA polymerase). The three remaining structural proteins are membrane-associated: $GP_{1,2}$ (spike glycoprotein) is a type 1 transmembrane and class I fusion protein, whereas the two non-glycosylated proteins VP40 and VP24 (primary and secondary matrix proteins) are associated with the inner side of the virion membrane. The sizes and functions of the proteins are shown in Table 1.

### LIPIDS

The viral envelope is derived from host cell membranes and is considered to have a lipid composition similar to that of the host-cell plasma membrane.

### CARBOHYDRATES

The spike glycoproteins of filoviruses are highly glycosylated with N-linked glycans of the complex, hybrid and oligomannosidic type, and O-linked glycans of the neutral mucin type. Glycans constitute >50% of the $GP_{1,2}$ total mass. Ebola virus (EBOV) $GP_{1,2}$ is sialylated to higher levels than Marburg virus (MARV) $GP_{1,2}$, and in certain cell lines the $GP_{1,2}$ of MARV completely lacks sialic acid. The $GP_{1,2}$ proteins of marburgviruses and ebolaviruses are acylated at their C-termini and form homotrimers.

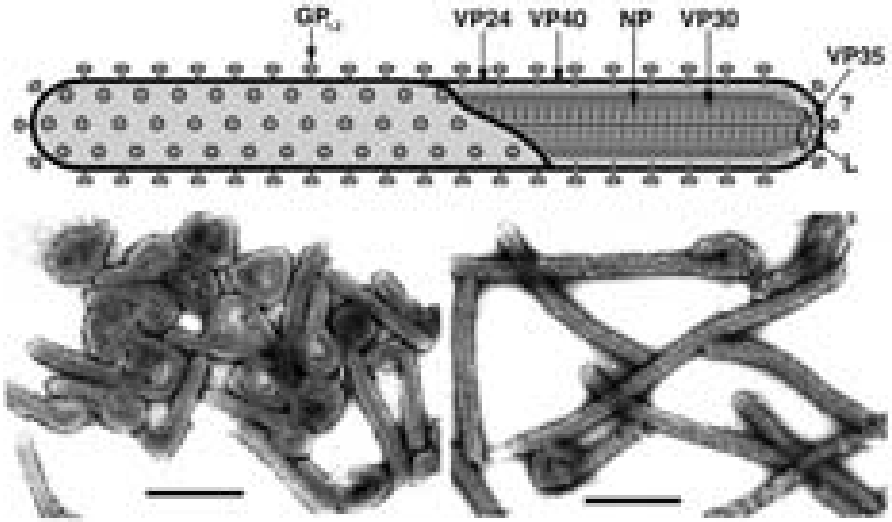



**Figure 1:** (Top) Diagram of a filovirion. (Bottom left) Negative contrast electron micrograph of Marburg virus (MARV) particles purified and concentrated by centrifugation from guinea pig serum, 7 days after intraperitoneal infection, and stained with 1% phosphotungstate. (Bottom right) Negative contrast electron micrograph of Ebola virus (EBOV) particles, from Vero cell culture supernatant, 4 days after infection. The bars represent 450 nm.

**Table 1:** Filovirus structural proteins

| Protein | Encoding gene | Function | Marburg virus (kDa) A/B | Ebola virus (kDa) A/B |
|---|---|---|---|---|
| NP | 1 (NP) | Nucleoprotein | 77.9/96.0 | 83.3/104.0 |
| VP35 | 2 (VP35) | RNA-dependent RNA polymerase cofactor (paramyxovirus P protein analog) | 31.0/32.0 | 38.8/35.0 |
| VP40 | 3 (VP40) | Primary matrix protein (paramyxovirus M protein analog) | 31.7/38.0 | 35.3/40.0 |
| $GP_{1,2}$ | 4 (GP) | Spike glycoprotein (paramyxovirus G protein analog) | 74.8/170.0 | 74.5/140.0 |
| VP30 | 5 (VP30) | Transcriptional activator | 31.5/28.0 | 29.7/30.0 |
| VP24 | 6 (VP24) | Secondary matrix protein | 28.8/24.0 | 28.3/24.0 |
| L | 7 (L) | RNA-dependent RNA polymerase | 267.2/>200.0 | 252.7/>200.0 |

A, size calculated from the deduced amino acid sequences of the corresponding ORFs; B, size estimated from SDS-PAGE analysis; a complete set of data is not yet available for ebolaviruses other than EBOV.

## Genome organization and replication

Filovirus genomes are characterized by the gene order: 3′-NP-VP35-VP40-GP-VP30-VP24-L-5′ (Figure 2). The extragenic sequences at the extreme 3′ (leader) and 5′ (trailer) ends of the genomes are conserved. They demonstrate a significantly high complementarity at their very ends. Genes are flanked by conserved transcriptional initiation and termination (polyadenylation) sites. Those sites contain the highly conserved pentamer 3′-UAAUU-5′. Most genes are separated by non-conserved intergenic sequences, but some genes overlap. Most of these overlaps are extremely short and limited to the highly conserved pentamer. There is a single gene overlap in marburgvirus genomes, but several (2–3) gene overlaps in ebolavirus genomes. The functional significance of these short overlaps is unclear, but transcriptional attenuation of the downstream gene has been postulated for other viruses of the order *Mononegavirales*. In addition, most genes possess relatively long 3′- and

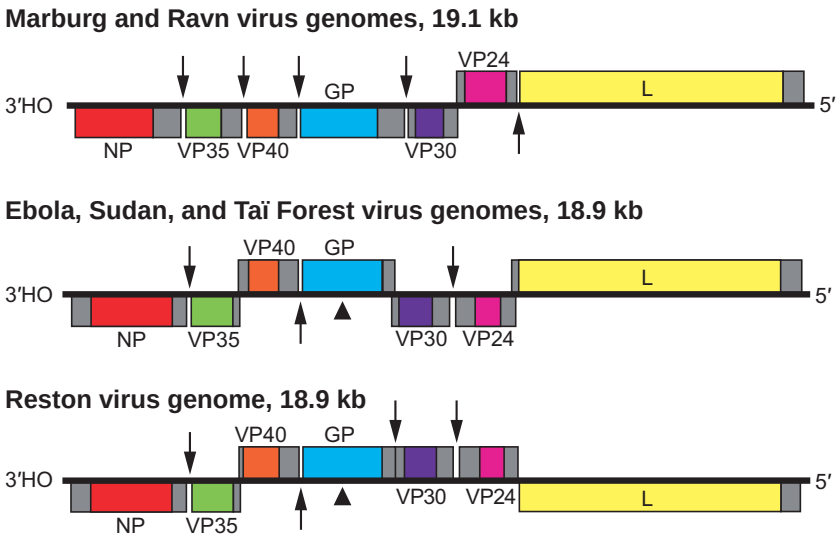



**Figure 2:** Diagram of filovirus genome organization. The genes that encode structural proteins are identified in the genomes and are drawn to scale. Colored boxes designate coding regions and gray boxes designate non-coding regions. Genes begin with conserved transcriptional initiation sites and end with conserved transcriptional termination sites (polyadenylation sites). Adjoining genes are either separated from one another by an intergenic region (arrows) or overlap. The slippery sequence of the ebolaviral GP gene is indicated by a black triangle. At the extreme 3′ and 5′ ends of the genomes are leader and trailer sequences, respectively, that are in part complementary.

5′-noncoding regions. In contrast to MARV, the GP genes of ebolaviruses possess three overlapping ORFs that can be joined through transcriptional polymerase stuttering (Figure 2).

The replication strategy of filoviruses is not well studied. Ultrastructural studies indicate an association of viral particles with coated pits for the initiation of infection, suggesting that filoviruses enter cells by endocytosis; molecular studies in part confirm this but are contradictory in terms of which endocytosis pathway is used. The spike glycoprotein mediates receptor binding and subsequent fusion. C-type lectins and integrins have been described as potential virion attachment factors, but the cell surface receptor remains elusive. Uncoating is presumed to occur in a manner analogous to that of other mononegaviruses. Transcription and genome replication take place in the cytoplasm and, in general, follow the models for members of the families *Paramyxoviridae* and *Rhabdoviridae*. Transcription starts at the conserved start site and polyadenylation occurs at a stretch of uridine residues within the termination site. The 5′-terminal non-coding sequences favor hairpin-like structures for all sgRNAs (mRNAs). Replication involves the synthesis of full-length positive-strand copies (antigenomes). During infection, massive amounts of nucleocapsids accumulate intracellularly and form intracytoplasmic inclusion bodies. Virions are released via budding from plasma membranes. The expression strategy of ebolaviral GP genes is distinct from that of marburgviral GP genes and involves transcriptional editing (Figure 2). The primary product of the unedited transcript (ORF1) yields a non-structural glycoprotein (pre-sGP), which is proteolytically cleaved to two secreted proteins, sGP and Δ-peptide. Editing results in the expression of either full-length $GP_{1,2}$ or a secondary secreted glycoprotein (ssGP). sGP, $GP_{1,2}$ and ssGP share the N-terminal 295 amino acid residues, but differ in their C-termini and, because of that, in quarternary structure.

## Antigenic and genetic properties

Filovirus infectivity is poorly neutralized *in vivo*, but at least two potent neutralizing antibodies have been described that are potent *in vitro* (Sudan virus: 16F6; Ebola virus: KZ52). There is only limited antigenic cross-reactivity between marburgvirions and ebolavirions. Marburgvirus and ebolavirus GP genes differ by >57%, and phylogenetic analysis clearly separates marburgviruses

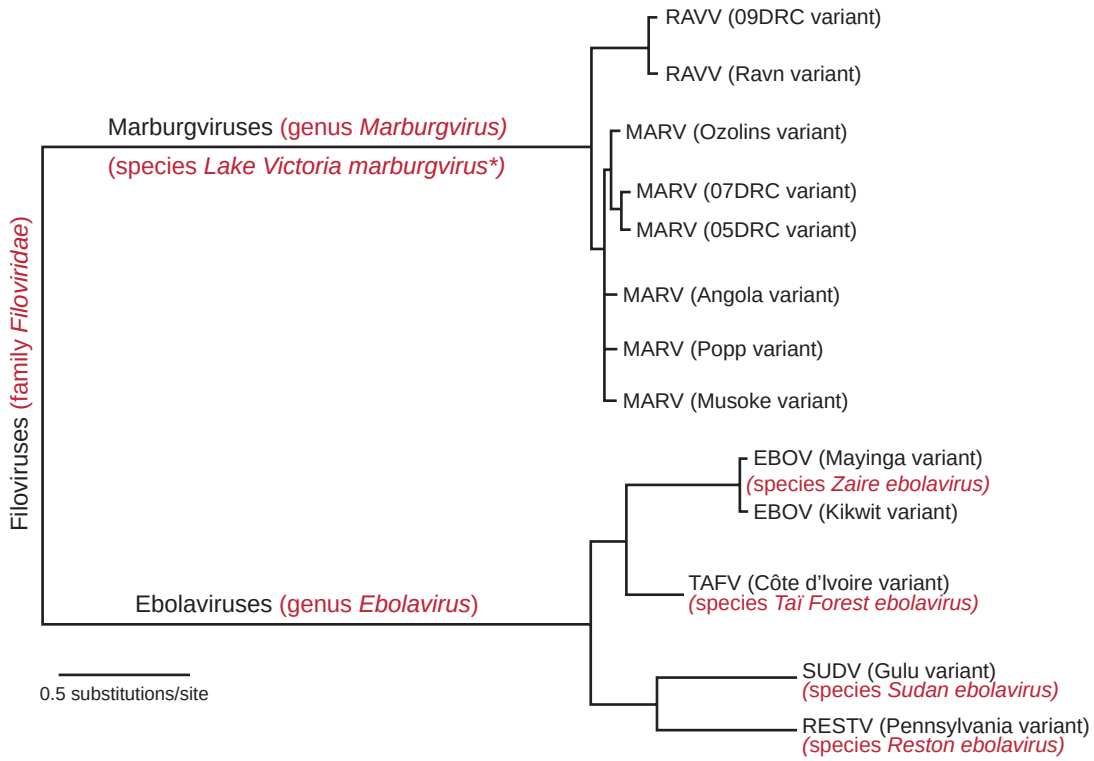



**Figure 3:** Phylogenetic tree comparing full-length marburgvirus and ebolavirus genomes by Bayesian analysis.

and ebolaviruses, justifying the establishment of two filovirus genera (*Marburgvirus* and *Ebolavirus*) (Figure 3). One marburgvirus (Marburg virus, species *Lake Victoria marburgvirus**) and four distinct ebolaviruses (Reston virus, species *Reston ebolavirus*; Sudan virus, species *Sudan ebolavirus*; Taï Forest virus, species *Taï Forest ebolavirus*; and Ebola virus, species *Zaire ebolavirus*) have been identified. They differ from one another by 37–41% at the nucleotide level. At least five genetic marburgvirus lineages exist. Virus genomes of four lineages (Marburg virus) differ from each other by 0–7.4%. Genomes from viruses of the fifth lineage (Ravn virus) reach 21% nucleotide difference compared to the others. The variation in genomic sequences has been shown to be low among isolates of individual filoviruses: <2%, for instance, in the case of Ebola virus isolates. There seems to be less or even no genetic variability between isolates from different patients of single outbreaks. All data indicate a remarkable degree of genetic stability over time.

## Biological properties

Filoviruses are endemic in Central Africa in an area approximately between the 10th parallel north and south of the Equator as indicated by the locations of known outbreaks, and in the Philippines (Reston virus only). Filoviruses are the only mononegaviruses that cause viral hemorrhagic fevers in primates.

The natural reservoirs of filoviruses remain to be identified, but infectious marburgviruses have been isolated from Egyptian rousettes (*Rousettus aegyptiacus*) and infectious Reston virus has been isolated from domestic pigs (*Sus scrofa*). There is no known connection of virus spread with any vector. The usual transmission pattern seen with large outbreaks of filovirus disease in humans begins with a focus of infection that disseminates to a number of contacts. Secondary and subsequent episodes of disease occur following close contact with patients; such infections usually occur in family members or medical personnel. The major route of interhuman transmission of filoviruses requires

direct contact with blood, body fluids, or injured skin (ritual embalming). Usage of contaminated syringes and needles is the main source for nosocomial infections. In the laboratory, nonhuman primates, mice, guinea pigs, hamsters and domestic pigs have been infected experimentally, but infection of rodents requires sequential adaptation.

## GENUS *MARBURGVIRUS*

Type species ***Lake Victoria marburgvirus****

### Distinguishing features

- Almost no antigenic cross-reactivity of marburgvirions with ebolavirions.
- Virion length is about 665 nm compared to about 805 nm for ebolavirions.
- Single gene overlap compared to several (2–3) overlaps in genomes of ebolaviruses.
- Spike glycoprotein expression does not involve editing during transcription of gene four.
- Gene four encodes only one protein, the spike glycoprotein $GP_{1,2}$.
- Genomes differ from those of ebolaviruses by ≥50% at the nucleotide level.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Marburgvirus*

*Lake Victoria marburgvirus**

| | | |
|---|---|---|
| Marburg virus - Musoke, Kenya, 1980 | [Z12132 = NC_001608] | (MARV-Mus) |
| Ravn virus - Ravn, Kenya, 1987 | [DQ447649] | (RAVV-Rav) |

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### Other related viruses which may be members of the genus *Marburgvirus* but have not been approved as species

None reported.

## GENUS *EBOLAVIRUS*

Type species ***Zaire ebolavirus***

### Distinguishing features

- Almost no antigenic cross-reactivity of ebolavirions with marburgvirions.
- Virion length is about 805 nm compared to about 665 nm for marburgvirions.
- Several (2–3) gene overlaps compared to a single overlap in genomes of marburgviruses.
- Spike glycoprotein expression involves editing during transcription of gene four.
- Gene four encodes four proteins, the spike glycoprotein $GP_{1,2}$ and three soluble glycoproteins (sGP/Δ-peptide and ssGP).
- Genomes differs from those of marburgviruses by ≥50% at the nucleotide level.

### Species demarcation criteria in the genus

Members of different species in the genus may be distinguished on the basis of glycoprotein gene sequence differences (≥30% amino acid difference), whole genome differences (≥30% nucleotide difference), cross-protection data (where available), number of gene overlaps (two in Reston

virus genomes, but three in all others) and differences in geographic origin (Taï Forest virus: Côte d'Ivoire; Reston virus: Philippines; Sudan virus: Sudan, Uganda; Ebola virus: Democratic Republic of the Congo, Gabon, Republic of the Congo).

## List of species in the genus *Ebolavirus*

*Taï Forest ebolavirus*
Taï Forest virus - Côte d'Ivoire, Côte d'Ivoire, 1994 [FJ217162] (TAFV-Côt)
*Reston ebolavirus*
Reston virus - Pennsylvania, USA, 1989 [AF522874 = NC_004161] (RESTV-Pen)
*Sudan ebolavirus*
Sudan virus - Boniface, Sudan, 1976 [FJ968794] (SUDV-Bon)
*Zaire ebolavirus*
Ebola virus - Mayinga, Zaire, 1976 [AF086833 = NC_002549] (EBOV-May)

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Ebolavirus* but have not been approved as species

Bundibugyo virus - Bundibugyo, Uganda, 2009 [FJ217161 = NC_014373] (BDBV-Bun)

This virus was recently proposed to be the member of a new ebolavirus species ("*Bundibugyo ebolavirus*") (Kuhn *et al*., 2010).

## List of unassigned species in the family *Filoviridae*

None.

## List of other related viruses which may be members of the family *Filoviridae* but have not been approved as species

Lloviu virus (LLOV)

It is expected that this virus will be proposed as the type species ("*Lloviu cuevavirus*") of a new genus ("*Cuevavirus*") in the family (Kuhn *et al*., 2010).

## Phylogenetic relationships within the family

Phylogenetic relationships are illustrated in Figure 3 on p. 668

## Similarity with other taxa

Comparison of filovirus genomes with other mononegaviruses demonstrates a similar structure and suggests comparable mechanisms of transcription and replication. Comparative sequence analyses of single genes indicate that filoviruses are phylogenetically quite distinct from members of other families of the order *Mononegavirales*. Limited homology exists between the carboxy-terminal part of filovirus $GP_{1,2}$ and the trans-membrane p15E-related glycoproteins of oncogenic retroviruses.

## Derivation of names

*Ebola*: from the Ebola river in Zaire/Democratic Republic of the Congo, where one of the first registered outbreaks of the disease was thought to have occurred.
*Filo*: from Latin *filum*, "thread", to indicate the morphology of virus particles.
*Marburg*: from Marburg an der Lahn, a town in Germany, where the first known outbreak of filovirus disease occurred.

## *Note

In 2010, the ICTV *Filoviridae* Study Group and other experts suggested to change the species name *Lake Victoria marburgvirus* to "*Marburg marburgvirus*" (Kuhn *et al*., 2010) and a proposal to this effect is currently under consideration by the ICTV.

## Further reading

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Martini, G.A. and Siegert, R. (Eds.) (1971). *Marburg Virus Disease*. Berlin: Springer-Verlag.

Pattyn, S.R. (Ed.) (1978). *Ebola Virus Haemorrhagic Fever*. Amsterdam: Elsevier/North-Holland Biomedical Press.

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## Contributed by

Kuhn, J.H., Becker, S., Ebihara, H., Geisbert, T.W., Jahrling, P.B., Kawaoka, Y., Netesov, S.V., Nichol, S.T., Peters, C.J., Volchkov, V.E. and Ksiazek, T.G.

# FAMILY *PARAMYXOVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Paramyxoviridae*** |
| Subfamily | ***Paramyxovirinae*** |
| Genus | ***Rubulavirus*** |
| Genus | ***Avulavirus*** |
| Genus | ***Respirovirus*** |
| Genus | ***Henipavirus*** |
| Genus | ***Morbillivirus*** |
| Subfamily | ***Pneumovirinae*** |
| Genus | ***Pneumovirus*** |
| Genus | ***Metapneumovirus*** |

## Virion properties

### MORPHOLOGY

Virions are 150 nm or more in diameter, pleomorphic, but usually spherical in shape, although filamentous and other forms are common. Virions consist of a lipid envelope surrounding a nucleocapsid. The envelope is derived directly from the host cell plasma membrane by budding and contains two or three transmembrane glycoproteins. These are present as homo-oligomers and form spike-like projections, 8–12 nm in length, spaced 7–10 nm apart (depending on the genus). One non-glycosylated membrane or matrix protein is associated with the inner face of the envelope. The viral nucleocapsid consists of a single species of viral RNA and associated proteins. It has helical symmetry and is 13–18 nm in diameter with a 5.5–7 nm pitch (depending on the subfamily); its length can be up to 1000 nm in some genera. Multiploid virions are found, although the vast majority of virions contain a single functional genome. The viral polymerase is packaged in the virion.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virion Mr is about $500 \times 10^6$, and much greater for multiploid virions. Virion buoyant density in sucrose is 1.18–1.20 g $cm^{-3}$. Virion $S_{20,w}$ is at least 1000S. Virions are very sensitive to heat, lipid solvents, ionic and non-ionic detergents, formaldehyde and oxidizing agents.

### NUCLEIC ACID

Virions contain a single molecule of linear, negative sense, ssRNA that is not infectious alone but is infectious in the form of the nucleocapsid. The RNA genome size varies substantially: 15,384 nt for Sendai virus (SeV); 15,600 nt for human parainfluenza virus 1 (HPIV1); 15,462 nt for human parainfluenza virus 3, (HPIV-3); 15,384 nt for mumps virus (MuV); 15,246 nt for parainfluenza virus 5 (PIV5, previously known as simian virus 5 [SV-5]); 15,450 nt for simian virus 41 (SV-41); 15,156 nt for Newcastle disease virus (NDV); 14,904–17,262 for other avulaviruses; 15,654 nt for human parainfluenza virus 2 (HPIV-2); 18,234 nt for Hendra virus (HeV); 18,246–15,252 nt for Nipah virus (NiV); 15,894 nt for measles virus (MeV); 15,690 nt for canine distemper virus (CDV); 15,882 nt for rinderpest virus (RPV); 15,702 nt for cetacean morbillivirus (CeMV); 15,191–15,226 nt for human respiratory syncytial virus (HRSV), 13,280–13,378 nt for human metapneumovirus (HMPV) and 19,212 nt for Beilong virus (BeiPV), an unclassified virus in the subfamily *Paramyxovirinae*. Genome lengths for all viruses in the subfamily *Paramyxovirinae* are multiples of 6, which is a requirement for the efficient replication of the members of the subfamily *Paramyxovirinae*, but does not apply to the members of the subfamily *Pneumovirinae*. Some virions may contain positive sense RNA. Thus, partial self-annealing of extracted RNA may occur. Intracellularly, or in virions, genome-size RNA is found exclusively as nucleocapsids. The genome RNA does not contain a 5′ cap, nor a covalently linked protein. The genome 3′ end is not polyadenylated.

### PROTEINS

Members of the subfamily *Paramyxovirinae* encode 7–10 proteins (5–250 kDa) of which 2–4 (or more) are derived from the 2–3 overlapping ORFs in the P locus (Figure 2). Pneumoviruses encode 9–11

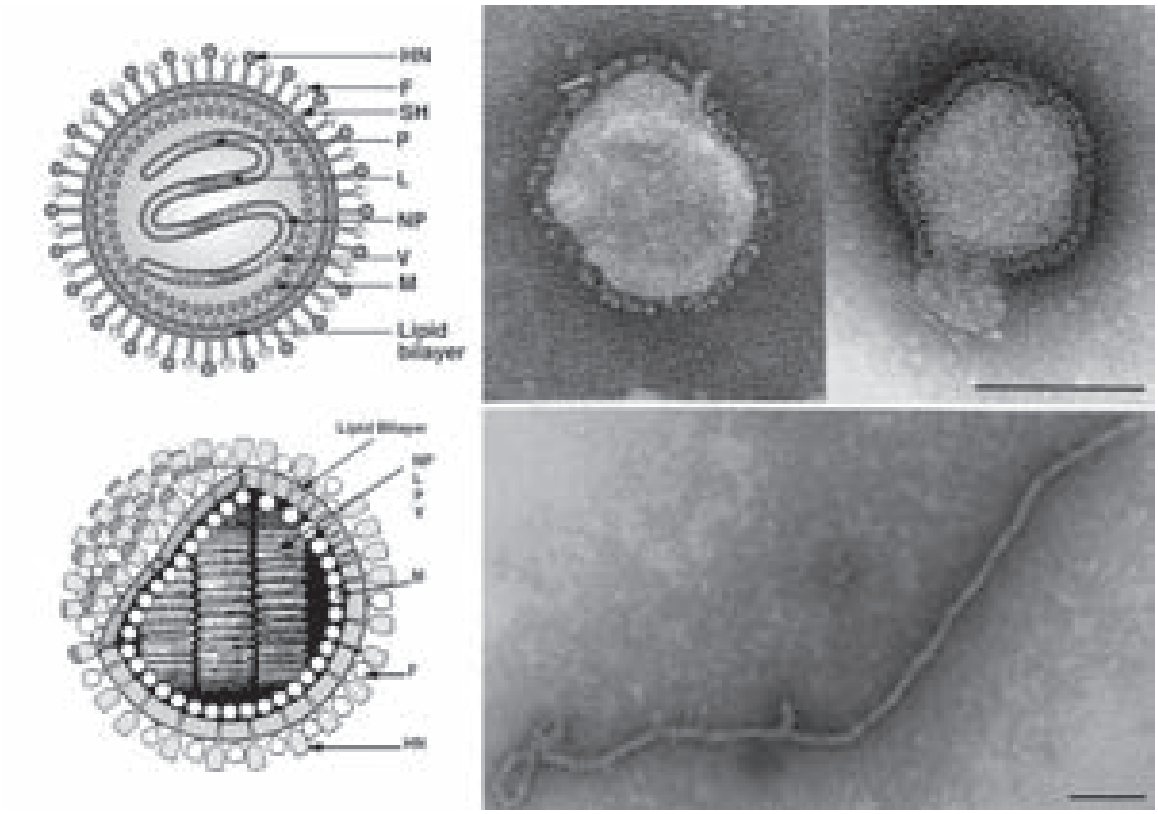


**Figure 1:** (Right) Negative contrast electron micrographs of intact parainfluenza virus 5 (PIV5, previously known as simian virus 5 [SV5]) particles (genus *Rubulavirus*) (top) and the PIV5 nucleocapsid after detergent lysis of virions (bottom) (courtesy of G.P. Leser and R.A. Lamb). The bars represent 100 nm. (Left top and bottom) Schematic diagrams of PIV5 particles in cross-section (N) (formerly NP), nucleocapsid; P, phosphoprotein; L, large polymerase protein; V, cysteine rich protein that shares its N-terminus with P sequence and for PIV5 is found in virions; M, matrix or membrane protein; F, fusion protein; HN, hemagglutinin-neuraminidase; SH, small hydrophobic protein). (Adapted from Kingsbury, D.W. (1990). Paramyxoviridae: the viruses and their replication. In: *Virology*, 2nd edn (B.N. Fields and D.M. Knipe, Eds.), Raven Press, New York; and from Scheid, H. (1987). In: *Animal Virus Structure* (M.V. Nermut, and A.C. Steven, Eds.), Elsevier, Amsterdam; with permission.)



proteins of 4.8–250 kDa, including two proteins encoded by overlapping ORFs in the M2 locus. Virion proteins common to all genera include: three nucleocapsid-associated proteins, i.e., an RNA-binding protein (N) (formerly NP), a phosphoprotein (P) and a large polymerase protein (L); three membrane-associated proteins, i.e., an unglycosylated inner membrane or matrix protein (M); and two glycosylated envelope proteins, comprising a fusion protein (F) and an attachment protein (G, or H, or HN). The F protein is synthesized within an infected cell as a precursor ($F_0$) that is activated following cleavage by cellular protease(s) to produce the virion disulfide-linked $F_1$ and $F_2$ subunits (order: amino $F_2$-S-S-$F_1$ carboxyl). Variable proteins include putative non-structural proteins (C, NS1, NS2), a cysteine-rich protein that binds zinc (V) (in the subfamily *Paramyxovirinae* only) that can be structural or non-structural depending on the virus, a small integral membrane protein (SH), a transcription processivity factor (M2-1, formerly called 22K protein) which previously was thought to be a second M-like protein, and a non-abundant protein (M2-2) involved in the balance between genome replication and transcription. Virion enzyme activities (variously represented among the genera) include an RNA-dependent RNA transcriptase, mRNA guanylyl and methyl transferases, and a neuraminidase. A protein kinase is associated with many members but it is probably of cellular origin.

### LIPIDS

Lipids in the viral envelope are derived from host cell plasma membrane.

### Carbohydrates

Virions are composed of 6% carbohydrate by weight; composition is dependent on the host cell. Fusion and attachment proteins are glycosylated by N-linked carbohydrate side chains. In the subfamily *Pneumovirinae* the attachment protein (G) is heavily glycosylated by O-linked as well as N-linked carbohydrate side chains. The SH protein of respiratory syncytial virus contains polylactosaminoglycan.

## Genome organization and replication

The genome organization is illustrated in Figure 2 for viruses representing the seven genera of the family. After attachment to cell receptors, virus entry is achieved by fusion of the virus envelope with the cell surface membrane. This can occur at neutral pH. Virus replication occurs in the cell cytoplasm and is thought to be independent of host nuclear functions. The genome is transcribed processively from the 3′ end by virion-associated enzymes into 6–10 separate, subgenomic, positive sense mRNAs. Transcription is guided by short (10–13 nt) conserved transcription start and termination/polyadenylation signals flanking each transcriptional element. The mRNAs are capped and possess 3′-poly(A) tracts synthesized by reiterative copying of the polyadenylation site. Intergenic regions are either highly conserved in sequence and length (*Respirovirus*, *Henipavirus*, *Morbillivirus* and all of the newly discovered viruses in the unassigned group; Figure 2 and list on p. 683 below) or are not conserved in sequence and length (*Rubulavirus*, *Avulavirus*, *Pneumovirinae*). RNA replication occurs through an intermediate, the antigenome, that is a complete exact positive sense copy of the genome.

Nucleocapsid assembly occurs in the cytoplasm and is tightly linked to RNA synthesis. Nucleocapsids are enveloped by budding at the cell surface plasma membrane at sites containing virus envelope proteins. Members of the subfamily *Paramyxovirinae* contain 6–7 transcriptional elements that encode 7–11 proteins. Each element encodes a single mRNA with the sole exception of the P/V element. This element is transcribed into an exact-copy mRNA (P or V mRNA, depending on the genus) as well as into an alternative version in which the RNA transcriptase stutters on the template at an editing motif midway down the element. This results in the insertion of one or more pseudo-templated nucleotides ("RNA editing") and shifts the reading frame to access an alternative ORF. The exact-copy and edited mRNAs synthesize two alternative proteins, P and V, which have identical amino-terminal domains but have different carboxy-terminal domains due to the frameshift. Other truncated, or chimeric, proteins (called I, W, or D, depending on the virus) can be produced by shifting into the third reading frame. The C ORF present in respiroviruses, henipaviruses, and morbilliviruses overlaps the P ORF and can initiate synthesis at a non-AUG codon that is accessed by ribosomal choice or at alternative start codons in the same ORF.

Members of the subfamily *Pneumovirinae* have eight (*Metapneumovirus*) or 10 (*Pneumovirus*) transcriptional elements, each of which encodes one mRNA. Each mRNA has a single ORF, except for the M2 mRNA, which encodes two proteins from separate ORFs. There is overlap between the M2 and L transcriptional elements in some pneumoviruses (Figure 2), but these elements nonetheless give rise to separate mRNAs.

## Antigenic properties

The attachment (HN, or H, or G) and fusion (F) proteins are of primary importance in inducing virus-neutralizing antibodies and immunity against reinfection. Antibodies to N and, variably, to other viral proteins also are induced by infection. Various proteins of members of the subfamily *Paramyxovirinae* have been reported to be broken into specific peptides that, when complexed to major histocompatibility glycoproteins, serve as recognition molecules for cytotoxic or helper T cells.

## Biological properties

Paramyxoviruses have been conclusively identified only in vertebrates and mostly in mammals and birds, although they have recently also been detected in reptiles and fish. Most viruses have a narrow host range in nature, but in cultured cells they display a broad host range. Infection of cultured cells generally is lytic, but temperate or persistent infections *in vitro* are common. Other features of infection include the formation of inclusion bodies and syncytia. Cell surface molecules reported

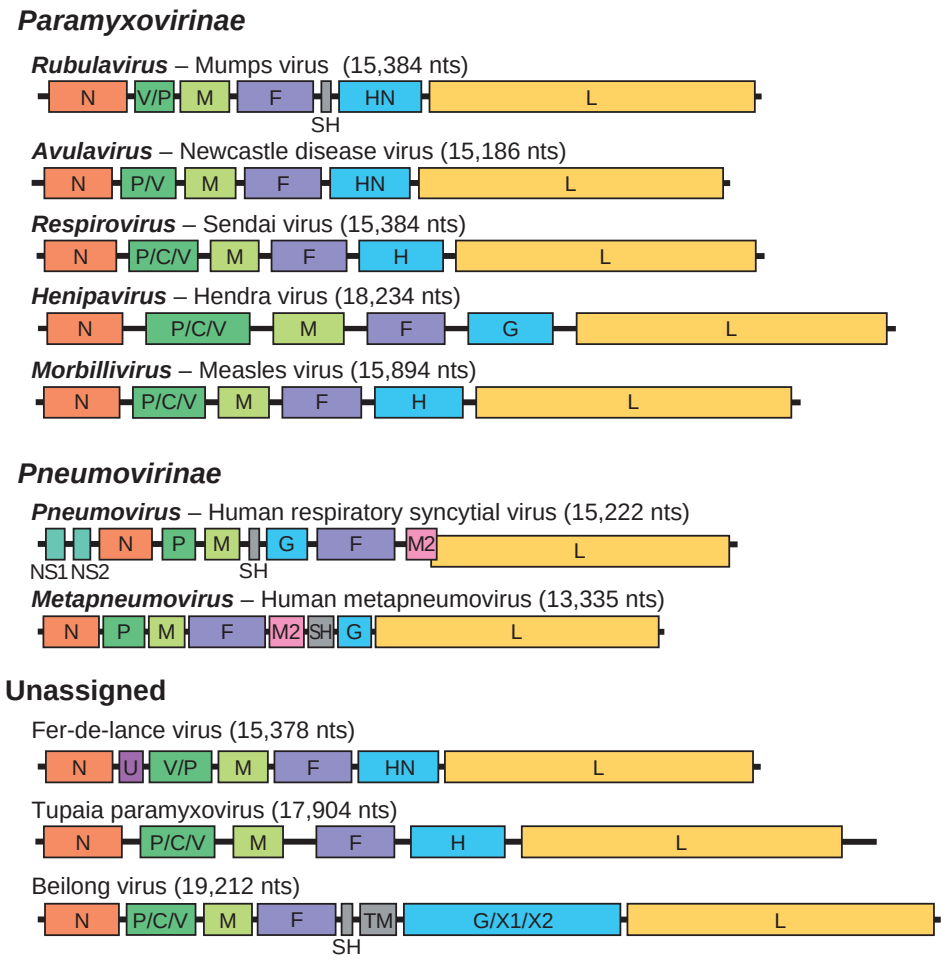


**Figure 2:** Maps of genomic RNAs (3′-to-5′) of viruses in the family *Paramyxoviridae*. Viruses were selected from the seven established genera as well as a group of unassigned viruses, which significantly increased the genetic diversity of the family. Each box represents a separately encoded mRNA; multiple distinct ORFs within a single mRNA are indicated by slashes. The M2 mRNA of members of subfamily *Pneumovirinae* has two overlapping ORFs, M2-1 and M2-2 (not shown). The lengths of the boxes are approximately to scale although the intervening or preceding sequences are not to scale. The D ORF present in the respirovirus HPIV3 is not shown. Certain viruses give rise to additional proteins by the utilization of secondary translational start sites within some of the ORFs: these are not shown. In HPIV1 and HPIV3 of the genus *Respirovirus* the V ORF may be a non-expressed relic. In the genus *Rubulavirus* some species lack the SH gene. In the genus *Pneumovirus*, HRSV has a transcriptional overlap at M2 and L (staggered boxes). There are conserved trinucleotides that serve as intergenic sequences for the respiroviruses, henipaviruses and morbilliviruses. For rubulaviruses, avulaviruses, pneumoviruses and metapneumoviruses, the intergenic sequences are variable (1–190 nt long). In the group of unassigned new viruses, all of them have a 3-nt intergenic region similar to those observed in the genera *Morbillivirus*, *Respirovirus* and *Henipavirus*. However, the genome sizes of these new viruses vary significantly from 15,378 nt to 19,212 nt. There are also novel genes present in these viruses (such as the U gene in FedPV and the TM gene in BeiPV) which have never been seen in previously known paramyxoviruses.

to serve as receptors for the attachment of respiroviruses and rubulaviruses include sialoglycoproteins and glycolipids. The cell surface proteins CD46 and SLAM 150 are major receptors for measles virus. Henipaviruses use ephrin B2 and B3 proteins as entry receptors. HRSV infection *in vitro* involves glucosaminoglycans. Nucleocapsids associate with viral membrane proteins at the plasma membrane and are enveloped by budding. Transmission is horizontal, mainly through airborne routes; no vectors are known. Paramyxovirus infection typically begins in the respiratory tract and may remain at that site (e.g., HRSV and HPIV) or may spread to secondary sites (e.g., lymphoid and endothelial tissues for MeV, the parotid gland, CNS and endothelial tissues for MuV or lung and CNS for HeV and NiV). In general, paramyxovirus infections are limited by, and eliminated by, host immunity. However, virus sometimes can be shed for periods of weeks or months in normal and, especially, immunocompromised individuals. Latent infection is unknown, and long-term

persistent infection is known only for subacute sclerosing panencephalitis, a rare complication that involves defective measles virus and old dog distemper, which can involve persistence of defective or fully infectious virus for weeks or months in normal and, especially, immunocompromised individuals. The recurrence of neurological manifestations has also been noted in NiV patients more than 4 years after recovery from acute encephalitis.

# SUBFAMILY *PARAMYXOVIRINAE*

## Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Paramyxovirinae*** |
| Genus | ***Rubulavirus*** |
| Genus | ***Avulavirus*** |
| Genus | ***Respirovirus*** |
| Genus | ***Henipavirus*** |
| Genus | ***Morbillivirus*** |

## Distinguishing features

Members of the subfamily *Paramyxovirinae* have 6–7 transcriptional elements. Amino acid sequence relatedness is much greater within the subfamily than between subfamilies. Within the subfamily *Paramyxovirinae*, sequence relatedness between corresponding proteins is greater for N, M and L, with F and HN being somewhat less conserved and C and P being poorly conserved although the unique region of V that is not shared with P is highly conserved. The division of this subfamily into the five genera is consistent with phylogenetic grouping based on amino acid sequence relationships. Their nucleocapsids have diameters of 18 nm and a pitch of 5.5 nm; the length of the surface F and H/HN spikes is 8 nm. The genome length must be a multiple of 6 nt for efficient genome replication (the "rule of six"), perhaps reflecting the precise packing of nt by a nucleocapsid protein subunit. RNA editing of the P/V transcriptional element occurs for all members except human parainfluenza virus 1 (HPIV-1). One genus (*Morbillivirus)* lacks a neuraminidase activity and some viruses (canine distemper virus, phocine distemper virus and rinderperst virus) lack a detectable hemagglutinating activity. Viruses from the genus *Henipavirus* lack both neuraminidase and hemagglutinating activities.

# GENUS *RUBULAVIRUS*

Type species ***Mumps virus***

## Distinguishing features

All species of the genus *Rubulavirus* have hemagglutination and neuraminidase activities. They share greater sequence relatedness within the genus than with members of other genera. For example, the N protein of HPIV-2 is 39–74% identical with that of MuV, PIV5, HPIV-4 and SV-41, compared to 18% and 24% identical with HPIV-1 and MeV. Some members (PIV5 and MuV) contain an extra transcriptional element (SH) between the F and HN loci (Figure 2). The unedited and edited versions of the mRNA from the P locus encode the V and P proteins, respectively. The intergenic sequences are of variable length. All members lack a C protein ORF. The rubulavirus P protein is substantially smaller than that of the respiroviruses or morbilliviruses. MuV and HIPV-2 are significant human pathogens.

## Species demarcation criteria in the genus

HPIV-2 and human parainfluenza virus 4 (HPIV-4) represent distinct serotypes that lack significant cross-neutralization and cross-protection. HPIV-2, PIV5, and SV-41 exhibit considerable sequence relatedness and some antigenic relatedness, but these viruses can be distinguished on either basis (for example, the N protein of HPIV-2 is 57% or 74% identical to those of PIV5 or SV-41, respectively)

as well as by host range: HPIV-2, HPIV-4a and HPIV-4b infect humans, PIV5 dogs, monkeys and humans, and SV-41 monkeys. They also have differences in their gene maps: PIV5 and MuV have an additional gene, SH and SV-41 lacks a functional transcription termination signal for the M gene and thus does not express a monocistronic M mRNA. HPIV-4 contains two antigenic subgroups (a and b) that are distinguished by differences in reactivity with monoclonal antibodies but are highly related by sequence – 84% and 95% identity for the HN and F protein, respectively – and should not be considered distinct species. MuV also does not exhibit significant cross-neutralization and cross-protection with other paramyxoviruses, and it is distinguished by its gene map (it contains an SH gene, found within this group only in PIV5), by sequence divergence (the MuV N protein shares 44% or less aa sequence identity with other rubulaviruses), and by its disease.

### List of species in the genus *Rubulavirus*

| | | |
|---|---|---|
| *Human parainfluenza virus 2* | | |
| Human parainfluenza virus 2 V94 | [AF533010] | (HPIV-2-V94) |
| *Human parainfluenza virus 4* | | |
| Human parainfluenza virus 4a M-25 | [AB543336] | (HPIV-4a-M25) |
| Human parainfluenza virus 4b 68-333 | [AB543337] | (HPIV-4b-68-33) |
| *Mapuera virus* | | |
| Mapuera virus BeAnn 370284 | [EF095490] | (MprPV-BeAnn370284) |
| *Mumps virus* | | |
| Mumps virus Miyahara | [AB040874] | (MuV-Miy) |
| *Parainfluenza virus 5* | | |
| Parainfluenza virus 5 W3A | [AF052755] | (PIV5-W3A) |
| (Simian virus 5) | | (SV5) |
| *Porcine rubulavirus* | | |
| Porcine rubulavirus LPMV | [BK005918] | (PorPV-LPMV) |
| (La-Piedad-Michoacan-Mexico virus) | | (LPMV) |
| *Simian virus 41* | | |
| Simian virus 41Toshiba/Chanock | [X64275] | (SV-41-Tos/Ch) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Rubulavirus* but have not been approved as species

| | | |
|---|---|---|
| Tioman virus | [AF298895] | (TioPV) |
| Menangle virus | [AF326114] | (MenPV) |

## GENUS *AVULAVIRUS*

Type species ***Newcastle disease virus***

### Distinguishing features

All species of the genus *Avulavirus* have hemagglutinin and neuraminidase activities. They share greater sequence relatedness within the genus than with members of other genera but are closely related to the rubulaviruses. The major distinguishing feature between avulaviruses and rubulaviruses is that for avulaviruses the exact copy of the P/V mRNA encodes P and the edited form encodes V, and none of the avulaviruses have an SH gene. The intergenic sequences are of variable length. All members lack a C protein ORF. The avulavirus P protein is substantially smaller than that of the respiroviruses or morbilliviruses. These viruses, especially NDV, include significant avian pathogens.

### Species demarcation criteria in the genus

There are many strains of NDV (virulent and avirulent) for chickens that have been extensively analyzed. The many other avian paramyxoviruses are antigenically related and are not well studied.

These are serotypes defined by hemagglutination-inhibition tests, although weak interactions occur between the types. Also, each serotype has a distinct pattern of electrophoretically-separated polypeptides.

### List of species in the genus *Avulavirus*

| | | |
|---|---|---|
| *Avian paramyxovirus 2* | | |
| Avian paramyxovirus 2 (Yucaipa) | [EU338414] | (APMV-2-Yucaipa) |
| *Avian paramyxovirus 3* | | |
| Avian paramyxovirus 3 449/75 | [EU403085] | (APMV-3-449/75) |
| *Avian paramyxovirus 4* | | |
| Avian paramyxovirus 4KR/YJ/06 | [EU877976] | (APMV-4-YJ/06) |
| *Avian paramyxovirus 5* | | |
| Avian paramyxovirus 5 (Kunitachi) | [GU206351] | (APMV-5-Kunitachi) |
| *Avian paramyxovirus 6* | | |
| Avian paramyxovirus 6 4440/2003 | [EF569970] | (APMV-6-4440/2003) |
| *Avian paramyxovirus 7* | | |
| Avian paramyxovirus 7 Tennessee 4/75 | [FJ231524] | (APMV-7-Tennessee) |
| *Avian paramyxovirus 8* | | |
| Avian paramyxovirus 8 Delaware 1053/76 | [FJ215863] | (APMV-8-Delaware) |
| *Avian paramyxovirus 9* | | |
| Avian paramyxovirus 9 New York/22/1978 | [EU910942] | (APMV-9-NY/22/78) |
| *Newcastle disease virus* | | |
| Newcastle disease virus LaSota | [AF077761] | (NDV-La Sota) |
| (Avian parainfluenza virus 1) | | (APMV-1) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Avulavirus* but have not been approved as species

None reported.

## GENUS *RESPIROVIRUS*

Type species ***Sendai virus***

### Distinguishing features

Member viruses of the genus *Respirovirus* possess a hemagglutinin and a neuraminidase. These viruses have six transcriptional elements. Unedited P mRNA encodes the P protein, whereas insertion of a G nucleotide in P mRNA transcripts accesses the V ORF. All members encode a C protein from a separate ORF in the P/V mRNAs. Amino acid sequence relatedness within the genus ranges from low to high, depending on the protein, and always is higher than in comparisons with other genera. For example, within respiroviruses the N protein is 88% identical comparing HPIV-1 to SeV, its "murine counterpart", and 63% identical compared to HPIV-3. Between genera, the N protein is 18% identical between HPIV-1 (*Respirovirus*) and the N protein of MuV or HPIV-2 or HPIV-4 (*Rubulavirus*), and 21% compared with that of MeV (*Morbillivirus*). HPIV-1 and HPIV-3 are significant agents of respiratory tract disease.

### Species demarcation criteria in the genus

HPIV-1 and HPIV-3 represent distinct serotypes defined by a lack of significant cross-neutralization and cross protection. Their gene maps are similar but not identical (editing by HPIV-3 results in a unique D protein not seen elsewhere in *Paramyxoviridae*, but does not access the V coding sequence, whereas HPIV-1 is the only member of the subfamily that lacks editing). They share low to high sequence relatedness among the various proteins (see above). SeV, a close relative of HPIV-1, is found predominantly as a pathogen of laboratory mice. However, this virus was isolated from the

lungs of infants during a fatal epidemic of newborn pneumonitis in Japan in 1952 and this virus is not found in wild mice in either Japan or the USA. SeV is distinguished from HPIV-1 by host range: specifically, HPIV-1 is permissive and pathogenic in humans whereas in mice it grows poorly or not at all and is nonpathogenic. Conversely, SeV is highly permissive, transmissible and pathogenic for mice. The two viruses have considerable sequence relatedness (see above) and antigenic similarity, but also can be clearly distinguished on either basis; also, HPIV-1 lacks editing and a V protein. Bovine parainfluenza virus 3 (BPIV-3) is a close relative of HPIV-3, but differs by their host ranges, which overlap but exhibit specificity. For example, in humans HPIV-3 replicates efficiently, is easily transmissible and causes disease, whereas BPIV-3 is highly attenuated, nonpathogenic and poorly transmissible. Furthermore, compared to HPIV-3, BPIV-3 is restricted 100- to 1000-fold in Old World primates. HPIV-3 and BPIV-3 exhibit considerable genetic and antigenic similarity, but also can clearly be distinguished on either basis. For example, HPIV-3 and BPIV-3 are 25% related antigenically by reciprocal cross-neutralization and hemagglutination inhibition studies. Also, BPIV-3 makes a V protein whereas it is not clear whether HPIV-3 can. Simian virus 10 (SV10; also known as simian agent 10) is a hemagglutinating virus that was recovered from the mouth of a samango monkey (*Cercopithecus mitis*). Sequence analysis indicates that SV10 and HPIV3 are essentially indistinguishable, implying that the species *Simian virus 10* should be abolished and SV10 viewed as being a monkey variant of the species *Human parainfluenza virus 1.* With the exception of Simian virus 10, each species represents a significant pathogen in its respective host.

### List of species in the genus *Respirovirus*

| | | |
|---|---|---|
| *Bovine parainfluenza virus 3* | | |
| Bovine parainfluenza virus 3 Kansas 15626/84 | [AF178654] | (BPIV-3-Kansas) |
| *Human parainfluenza virus 1* | | |
| Human parainfluenza virus 1 Washington/1964 | [AF457102] | (HPIV-1-Washington) |
| *Human parainfluenza virus 3* | | |
| Human parainfluenza virus 3 14702 | [EU424062] | (HPIV-3-14702) |
| *Sendai virus* | | |
| Sendai virus Nagoya (Murine parainfluenza virus 1) | [AB195968] | (SeV-Nagoya) |
| *Simian virus 10* | | |
| Simian virus 10 | [HM583801] | (SA-10 or SV-10) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Respirovirus* but have not been approved as species

None reported.

## GENUS *HENIPAVIRUS*

Type species ***Hendra virus***

### Distinguishing features

The two species of the genus *Henipavirus* have an attachment protein (G) that lacks hemagglutinating and neuraminidase activities. They share greater sequence relatedness within the genus than with members of other genera. The major distinguishing features between henipaviruses and other paramyxoviruses are (i) the long 5′- and 3′-UTRs in the mRNAs and (ii) a genome that as a result is approximately 3000 nt or more longer than other members of the family *Paramyxoviridae*. The unedited P mRNA encodes P protein. The edited P mRNA encodes V protein. The intergenic sequences are three nt at each gene junction. Both members encode a C protein ORF. Both HeV and NiV are indigenous to fruit bats. Each species has been associated with limited outbreaks with high mortality in domesticated animals and humans. Different from most paramyxoviruses,

henipaviruses have a wide host range from bats to pigs, horses and humans. The identification of the highly conserved ephrin B2 as the main functional receptor for both HeV and NiV and the widespread occurrence of the molecule in vertebrates, particularly in arterial, but not venous, endothelial cells, in the smooth muscle of the tunica media and in neurons, provide an explanation for the wide host range of henipaviruses and the systemic nature of the infections they cause.

### Species demarcation criteria in the genus

HeV and NiV are antigenically distinct and are distinct by genome sequence and geographic location. HeV has been detected exclusively in Australian flying foxes whereas NiV or anti-NiV antibodies have been detected in fruit bats from Indonesia, Malaysia, Thailand, Cambodia, India and Bangladesh, to Madagascar and West Africa. The two viruses cross-neutralize and have considerable sequence relatedness, but also can be distinguished on either basis

### List of species in the genus *Henipavirus*

| | | |
|---|---|---|
| *Hendra virus* | | |
| Hendra virus Hendra | [AF017149] | (HeV-Hendra) |
| *Nipah virus* | | |
| Nipah virus Malaysia | [AF212302] | (NiV-Malaysia) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Henipavirus* but have not been approved as species

None reported.

## GENUS *MORBILLIVIRUS*

Type species ***Measles virus***

### Distinguishing features

Members of the genus *Morbillivirus* lack a neuraminidase activity. Member viruses exhibit greater amino acid sequence relatedness within the genus than with other genera. They have an identical gene order, number of transcriptional elements and size of intergenic sequences with members of the genus *Respirovirus* (Figure 2). All morbilliviruses have a P/C/V transcription unit with RNA editing like respiroviruses and henipaviruses, namely the templated exact-copy mRNA encodes P and the predominant edited mRNA form (1 G added) encodes V. All morbilliviruses produce both intracytoplasmic and intranuclear inclusion bodies containing nucleocapsid-like structures. Viruses cross-react in serological tests. Sialic acid does not appear to be a receptor for morbilliviruses. Narrow host-range distribution of receptor defines susceptibility of organisms to infection. For MeV one receptor is CD46 and another CD150. CD150 also appears to be a receptor for CDV and RPV, which have a preference for canine and bovine CD150 respectively. Each species is a significant cause of disease in its respective host.

### Species demarcation criteria in the genus

The morbilliviruses are distinguished by host range, genetic (sequence) and antigenic differences. There is a low to moderate degree of sequence relatedness between members, depending on the protein (for example, the N protein of MeV is 65% related to that of CDV, viruses that represent two branches of the genus). Cross-neutralization and cross-protection also occurs between members of

the genus, although members can also be distinguished on that basis. MeV infects primates, CDV infects members of the order Carnivora, and RPV and peste-des-petits-ruminants virus (PPRV) infect members of the order Artiodactyla (even-toed ungulates), especially ruminants and swine. PPRV is distinguished from RPV by sequence analysis (the N protein of PPRV shares 68–72% identity with that of MeV, RPV or CDV), and because it does not readily infect cattle. Phocine distemper virus (PDV) is most closely related to CDV and is distinguished by host range and sequence divergence. Two cetacean morbilliviruses have been described, dolphin morbillivirus (DMV) and porpoise morbillivirus (PMV), but these are closely related and now are considered to be members of a single species, now named *Cetacean morbillivirus*. Members of this species are most closely related to RPV and MeV: these viruses are distinguished by host range and sequence divergence.

### List of species in the genus *Morbillivirus*

| | | |
|---|---|---|
| *Canine distemper virus* | | |
| Canine distemper virus 007Lm | [AB474397] | (CDV-007Lm) |
| *Cetacean morbillivirus virus* | | |
| Cetacean morbillivirus virus Dolphin | [AJ608288] | (CeMV-dolphin) |
| *Measles virus* | | |
| Measles virus Edmonston | [AF266288] | (MeV-Edmonston) |
| *Peste-des-petits-ruminants virus* | | |
| Peste-des-petits-ruminants virus ICV89 | [EU267273] | (PPRV-ICV89) |
| *Phocine distemper virus* | | |
| Phocine distemper virus Ulster 88 | [D10371*, Y09630*] | (PDV-Ulster88) |
| Seal distemper virus | | |
| *Rinderpest virus* | | |
| Rinderpest virus RBOK | [Z30697] | (RPV-RBOK) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Partial sequences contributing to the complete genome.

### List of other related viruses which may be members of the genus *Morbillivirus* but have not been approved as species

None reported.



## SUBFAMILY *PNEUMOVIRINAE*

### Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Pneumovirinae*** |
| Genus | ***Pneumovirus*** |
| Genus | ***Metapneumovirus*** |

### Distinguishing features

Members differ from those of the subfamily *Paramyxovirinae* in several features: (a) possession of 8–10 separate transcriptional elements; (b) smaller average ORF size; (c) possession of an additional nucleocapsid-associated protein (M2-1, formerly called 22K) and an RNA regulatory protein (M2-2); (d) extensive O-linked glycosylation of the G protein; (e) the P mRNA has a single ORF and does not have RNA editing; (f) a lack of amino acid sequence relatedness with members of the subfamily *Paramyxovirinae* except for a low level in the F and L proteins; and (g) differences in nucleocapsid diameter (13–14 nm compared with 18 nm in the subfamily *Paramyxovirinae*), nucleocapsid pitch (7 nm) and length of glycoprotein spikes (10–14 nm); and (h) lack of a "rule of six" governing the nucleotide length. Species also lack a neuraminidase and a hemagglutinin except in the case of murine pneumonia virus (MPV) (formerly pneumonia virus of mice [PVM]), which has a hemagglutinin. The G attachment protein is structurally unrelated to the HN, H or G proteins of

the subfamily *Paramyxovirinae* and exhibits a high level of inter-strain diversity: only 53% identity among HRSV isolates, 21–30% identity between human and non-human respiratory syncytial viruses, and 37% identity between HMPV strains.

# GENUS *PNEUMOVIRUS*

Type species ***Human respiratory syncytial virus***

## Distinguishing features

Pneumoviruses are distinguished from metapneumoviruses by (i) the presence of the NS1 and NS2 genes, (ii) the SH, G, F and M2 genes being in the order SH-G-F-M2 as opposed to F-M2-SH-G for metapneumoviruses, (iii) a greater genome length (15,190–15,225 nt compared to 13,280–13,378 nt), and (iv) a higher degree of nucleotide and amino acid sequence relatedness within the genus than between genera.

## Species demarcation criteria in the genus *Pneumovirus*

HRSV and MPV are distinguished by host range (humans versus mice) and a lack of cross-neutralization. Amino acid sequence relatedness between these two viruses varies from undetectable to intermediate, depending on the protein (for example, the NS1 and NS2 proteins lack demonstrable relatedness, whereas the N or F proteins share 60% or 40% identity, respectively). Their gene maps differ only in the absence of the M2/L gene overlap in MPV. Bovine respiratory syncytial virus (BRSV) differs from HRSV in host range, specifically cattle versus humans, but the difference is not absolute. For example, both viruses grow efficiently in cultured human or bovine cells, although some specificity may be evident. In chimpanzees, HRSV replicates efficiently, is transmissible and is pathogenic, whereas BRSV is very attenuated and non-pathogenic. The two viruses share considerable sequence and antigenic relatedness, but also can clearly be distinguished on either basis. For example, the N or F proteins are each 81% identical between BRSV and HRSV, compared to 96% or 89% identical, respectively, among different HRSV strains. Antiserum against one virus will cross-neutralize the other with a 6- to 64-fold reduction in efficiency. There are two antigenic subgroups of HRSV, called A and B, which exhibit aa sequence identity ranging from 96% (N) to 53% (G), and which are approximately 50% or 5% related antigenically in the F or G protein, respectively, with the overall difference in reciprocal cross-neutralization being up to four-fold. Comparable antigenic dimorphism also may exist for BRSV.

## List of species in the genus *Pneumovirus*

| | | |
|---|---|---|
| *Bovine respiratory syncytial virus* | | |
| Bovine respiratory syncytial virus ATCC51908 | [AF295543] | (BRSV-ATCC51908) |
| *Human respiratory syncytial virus* | | |
| Human respiratory syncytial virus A2 | [M74568] | (HRSV-A2) |
| Human respiratory syncytial virus B1 | [AF013254] | (HRSV-B1) |
| Human respiratory syncytial virus S2 | [U39662] | (HRSV-S2) |
| *Murine pneumonia virus* | | |
| Murine pneumonia virus 15 (formerly Pneumonia virus of mice [PVM]) | [AY729016] | (MPV-15) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Pneumovirus* but have not been approved as species

Caprine and ovine strains of BRSV also have been described but might represent, with BRSV, a subgroup of ruminant strains rather than different species.

# GENUS *METAPNEUMOVIRUS*

Type species ***Avian metapneumovirus***

## Distinguishing features

The relative placements of SH-G versus F-M2 in the gene order are reversed as compared to pneumoviruses. NS1 and NS2 genes are absent in pneumoviruses, and the genome is nearly 2000 nt shorter. The intergenic regions are longer (up to 190 nt compared with 57 nt). The extent of sequence relatedness is greater within than between genera.

## Species demarcation criteria in the genus

Metapneumovirus species are distinguished on the basis of having an avian or human host. Interestingly, the sequence diversity between avian and human isolates is less than that between certain avian isolates, and thus sequence relatedness is not a reliable distinguishing feature.

## List of species in the genus *Metapneumovirus*

| | | |
|---|---|---|
| *Avian metapneumovirus* | | |
| Avian metapneumovirus Colorado (formerly Turkey rhinotracheitis virus and Avian pneumovirus) | [AY590688] | (AMPV-Colorado) |
| *Human metapneumovirus* | | |
| Human metapneumovirus 00-1 | [AF371337] | (HMPV-00-1) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Metapneumovirus* but have not been approved as species

None reported.

## List of other related viruses which may be members of the family *Paramyxoviridae* but have not been approved as species

| | | |
|---|---|---|
| Fer-de-Lance virus | [AY141760] | (FdlPV) |
| Atlantic salmon paramyxovirus | [EF646380] | (AsaPV) |
| Pacific salmon paramyxovirus | [AY536862] | (PsaPV) |
| Nariva virus | [EF095490] | (NarPV) |
| Mossman virus | [AY286409] | (MosPV) |
| Salem virus | [AF237881] | (SalPV) |
| Tupaia paramyxovius | [AF079780] | (TupPV) |
| J-virus | [AY900001] | (JPV) |
| Beilong virus | [DQ100461] | (BeiPV) |

## Phylogenetic relationships within the family

The literature on the relationships of members of the subfamily *Paramyxovirinae* is consistent with the phylogeny (see Figure 3).

## Similarity with other taxa

The member viruses of the family *Paramyxoviridae* have a similar strategy of gene expression and replication and gene order to those of other families in the order *Mononegavirales*, specifically the families *Rhabdoviridae* and *Filoviridae*.

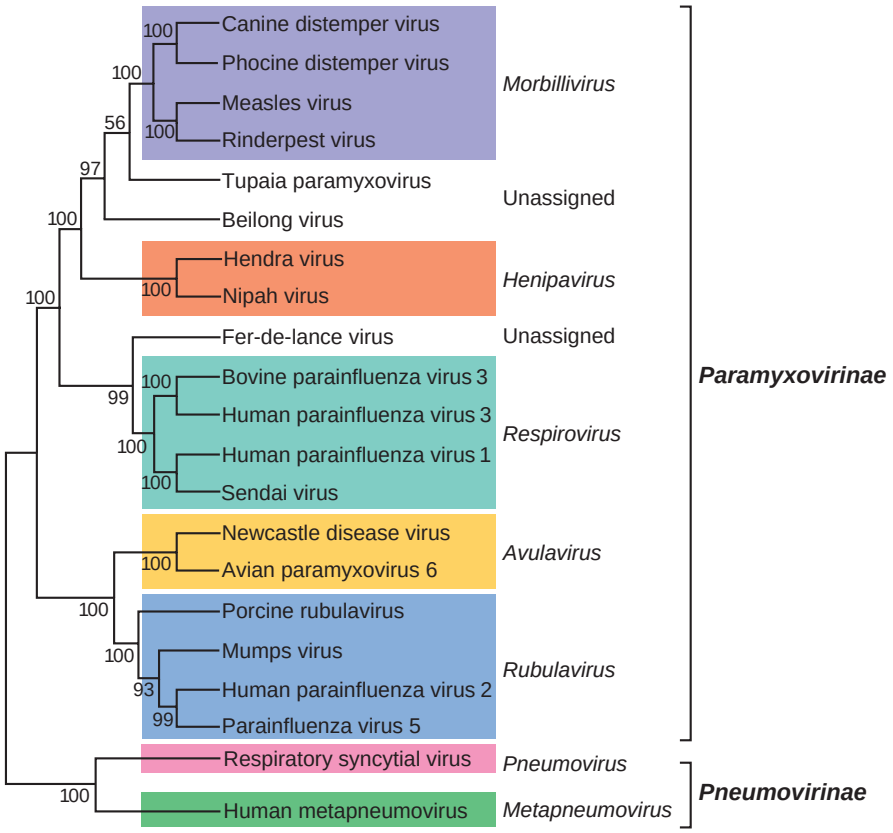


**Figure 3** Phylogenetic analysis of the L proteins of members of the family *Paramyxoviridae*. Phylogenetic analysis using MEGA4.1 was performed on the aa sequence of L proteins from various members of the family *Paramyxoviridae*. The tree shown was based on maximum parsimony; however, analysis of the same data using maximun likelihood produced a tree with nearly identical topology (data not shown).

## Derivation of names

*Avula*: from *av*ian rub*ula* virus.
*Henipa*: from *He*ndra and *Nipa*h viruses.
*Meta*: from Greek *meta*, "after".
*Morbilli*: from Latin *morbillus*, diminutive of *morbus*, "disease".
*Ortho*: from Greek *orthos*, "straight".
*Paramyxo*: from Greek *para*, "by the side of", and *myxa*, "mucus".
*Pneumo*: from Greek *pneuma*, "breath".
*Respiro*: from Latin *respirare*, "respire, breathe".
*Rubula*: *Rubula inflans* – old name for mumps.

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## Contributed by

Wang, L.-F., Collins, P.L., Fouchier, R.A.M., Kurath, G., Lamb, R.A., Randall, R.E. and Rima, B.K.

# Family *Rhabdoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Rhabdoviridae*** |
| Genus | ***Vesiculovirus*** |
| Genus | ***Lyssavirus*** |
| Genus | ***Ephemerovirus*** |
| Genus | ***Novirhabdovirus*** |
| Genus | ***Cytorhabdovirus*** |
| Genus | ***Nucleorhabdovirus*** |

## Virion properties

### Morphology

Virions are 100–430 nm in length and 45-100 nm in diameter. Defective virus particles are proportionally shorter. Viruses infecting vertebrates are bullet-shaped or cone-shaped; viruses infecting plants mostly appear bacilliform when fixed prior to negative staining; in unfixed preparations they may appear bullet-shaped or pleomorphic. The outer surface of virions (except for the quasi-planar end of bullet-shaped viruses) is covered with projections (peplomers) which are 5–10 nm long and about 3 nm in diameter. They consist of trimers of the viral glycoprotein (G). A honeycomb pattern of peplomers is observed on the surface of some viruses. Internally, the nucleocapsid, about 30–70 nm in diameter, exhibits helical symmetry and can be seen as cross-striations (spacing 4.5–5 nm) in negatively stained and thin-sectioned virions. The nucleocapsid consists of a ribonucleoprotein (RNP) complex comprising the genomic RNA and tightly bound nucleoprotein (N) together with an RNA-dependent RNA polymerase (L) and phosphoprotein (P). The RNP complex is active for transcription and replication: the N-RNA template is processed by the L protein, which contains most enzymatic activities, and its cofactor the P protein. In the cytoplasm, the RNP complex is uncoiled and filamentous, about 700 nm in length and 20 nm in diameter (Figure 1). In the virion, the lipid envelope containing the G protein interacts with the coiled RNP complex via the matrix protein (M).

## Physicochemical and physical properties

Virion Mr is $300\times10^6$ – $1000\times10^6$ and $S_{20w}$ is 550–1045S (plant rhabdoviruses have larger $S_{20,w}$ values). Virion buoyant density in CsCl is 1.19–1.20 g cm$^{-3}$; in sucrose it is 1.17–1.19 g cm$^{-3}$. Virus infectivity is rapidly inactivated at 56 °C, or following UV-, gamma- or X-irradiation, or exposure to formalin or to lipid solvents such as detergents.

### Nucleic acid

Virions contain a single molecule of linear, negative sense ssRNA (Mr $4.2\times10^6$–$4.6\times106$, about 11–15 kb in size). The RNA represents about 1–2% of virion weight. The RNA has a 3′-terminal free hydroxyl group and a 5′-triphosphate and is not polyadenylated. The ends have inverted complementary sequences with transcription and replication initiation signals. Defective RNAs, usually significantly shorter than full-length RNA (less than half size), may be identified in RNA recovered from virus populations. They are usually negative sense; however, hairpin RNA forms are also found. Defective RNAs replicate only in the presence of homologous and, occasionally, certain heterologous helper rhabdoviruses which provide the functional genes. Full-length positive sense RNA, which is an intermediate during the replication process, may constitute up to 5% of a viral RNA population. Like the full-length negative sense RNA genome, it is permanently bound to N protein.

### Proteins

Viruses generally have five structural polypeptides (designated L, G, N, P and M; see Table 1 for summary of their location, sizes and functions). The functions of other proteins, including additional non-structural glycoproteins (in ephemeroviruses) or C proteins (in a different ORF of the P mRNA for vesiculoviruses and lyssaviruses) are not known. Plant-adapted viruses have one or more additional non-structural proteins, one of which is thought to facilitate virus movement between plant cells. The structural proteins represent 65–75% of the virus dry weight. For certain viruses, other nomenclature has previously been used for the P protein (NS, M1 or M2) and the M

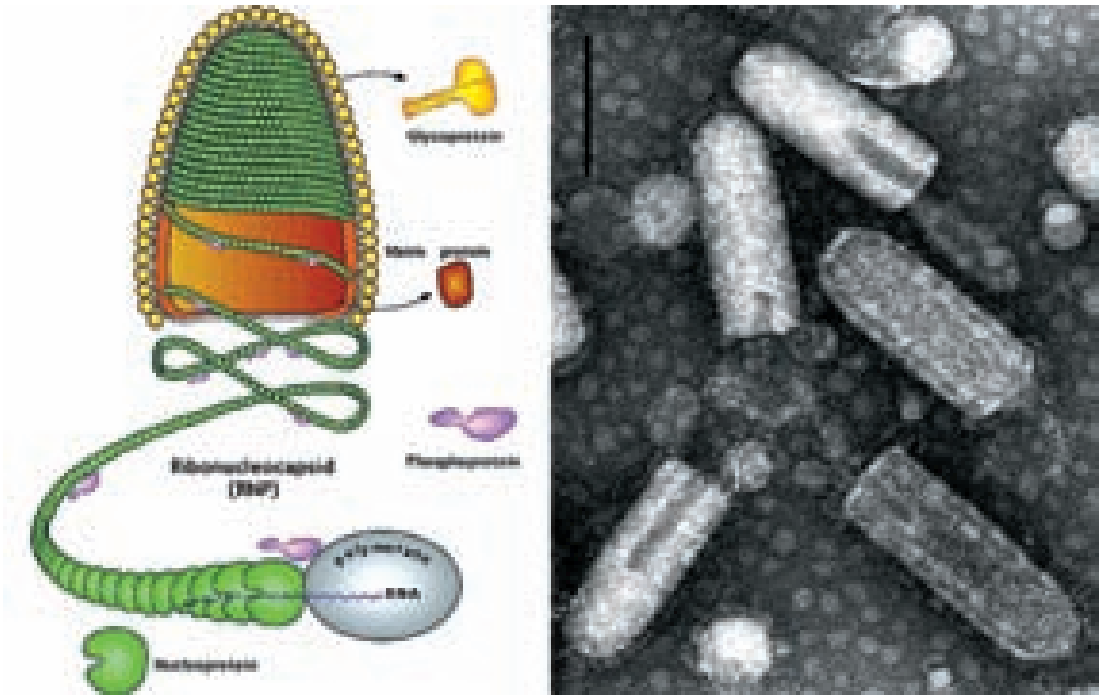


**Figure 1:** (Left) Diagram illustrating a rhabdovirus virion and the nucleocapsid structure (courtesy of P. Le Mercier); (right) negative contrast electron micrograph of virions of an isolate of vesicular stomatitis Indiana virus. The bar represents 100 nm. (Courtesy of P. Perrin.)

**Table 1:** Location and functions of rhabdovirus structural proteins

| Protein | Location, size and function |
|---|---|
| L | A component of the viral nucleocapsid (ca. 220–240 kDa) responsible for most of the functions required for transcription and replication: RdRp, mRNA 5′-capping, 3′-poly(A) synthesis and protein kinase activities. Observed sizes on SDS-PAGE are 150–190 kDa |
| G | Associated into trimers to form the virus surface peplomers (monomer ca. 65–90 kDa). Binds to host cell receptor(s), induces virus endocytosis then mediates fusion of viral and endosomal membranes. G is variously N-glycosylated and palmitoylated; it lacks O-linked glycans and has hemagglutinin activity. Induces and binds virus-neutralizing antibodies and elicits cell-mediated immune responses. G is involved in tropism and pathogenicity |
| N | Major component of the viral nucleocapsid (ca. 47–62 kDa). It associates with full-length negative and positive sense RNAs, or defective RNAs, but not mRNAs. N is not "inert" but an active element of the template, presenting the bases to the polymerase. Newly synthesized N probably modulates the balance between genome transcription and replication by influencing the recognition of the transcription signals. N elicits cell-mediated immune responses and humoral antibodies. In all nucleorhabdoviruses examined, N re-localises to a subnuclear compartment when co-expressed with the cognate P protein |
| P | A cofactor of the viral polymerase (ca. 20–30 kDa). It is variously phosphorylated and migrates on SDS-PAGE as a protein of about 40–50 kDa. The P of the nucleorhabdoviruses migrates faster. P protein is essential for at least two fundamental functions: (1) it mediates the physical link and the correct positioning of the RdRp (L) on the N-RNA template; (2) it acts as a chaperone during the synthesis of N, by forming N–P complexes that prevent N from self-aggregation and binding to cellular RNA. During the genome replication process, N is then transferred from these N–P complexes to the nascent viral RNA in order to ensure its specific encapsidation into new RNP. P elicits cell-mediated immune responses |
| M | A basic protein that is an inner component of the virion (ca. 20–30 kDa). It is believed to regulate genome RNA transcription. M binds to nucleocapsids and the cytoplasmic domain of G, thereby facilitating the process of budding. It is sometimes phosphorylated or palmitoylated. M is found in the nucleus and inhibits host cell transcription. It also mediates other pathological effects (cell rounding for VSIV, apoptosis for lyssaviruses, intracellular accumulation of the inner nuclear membrane for PYDV) |

protein (M1 or M2). RNA-dependent RNA polymerase, 5′ capping, guanosyl transferase, poly(A) polymerase, protein kinase, nucleoside triphosphatase and nucleoside diphosphate kinase activities are harboured by the nucleocapsid. Most catalytic functions have been attributed to L.

### Lipids

Virions are composed of about 15–25% lipids, with their composition reflecting that of the host cell membrane where virions bud. Generally phospholipids represent about 55–60%, and sterols and glycolipids about 35–40% of the total lipids. G protein may have covalently associated fatty acids proximal to the lipid envelope.

### Carbohydrates

Virions are composed of about 3% carbohydrate by weight. The carbohydrates are present as N-linked glycan chains on G protein and as glycolipids. In mammalian cells, the oligosaccharide chains are generally of the complex type; in insect cells they are of non-complex types.

## Genome organization and replication

Viruses contain at least five ORFs in the negative sense genome in the order 3′-N-P-M-G-L-5′ (e.g., for vesicular stomatitis Indiana virus; VSIV), or the equivalent. The corresponding cistrons are flanked by conserved start and stop transcription signals, about 10 nt in length. For certain viruses additional genes are interposed (Figure 2). Genes are transcribed processively (from 3′ to 5′ of the

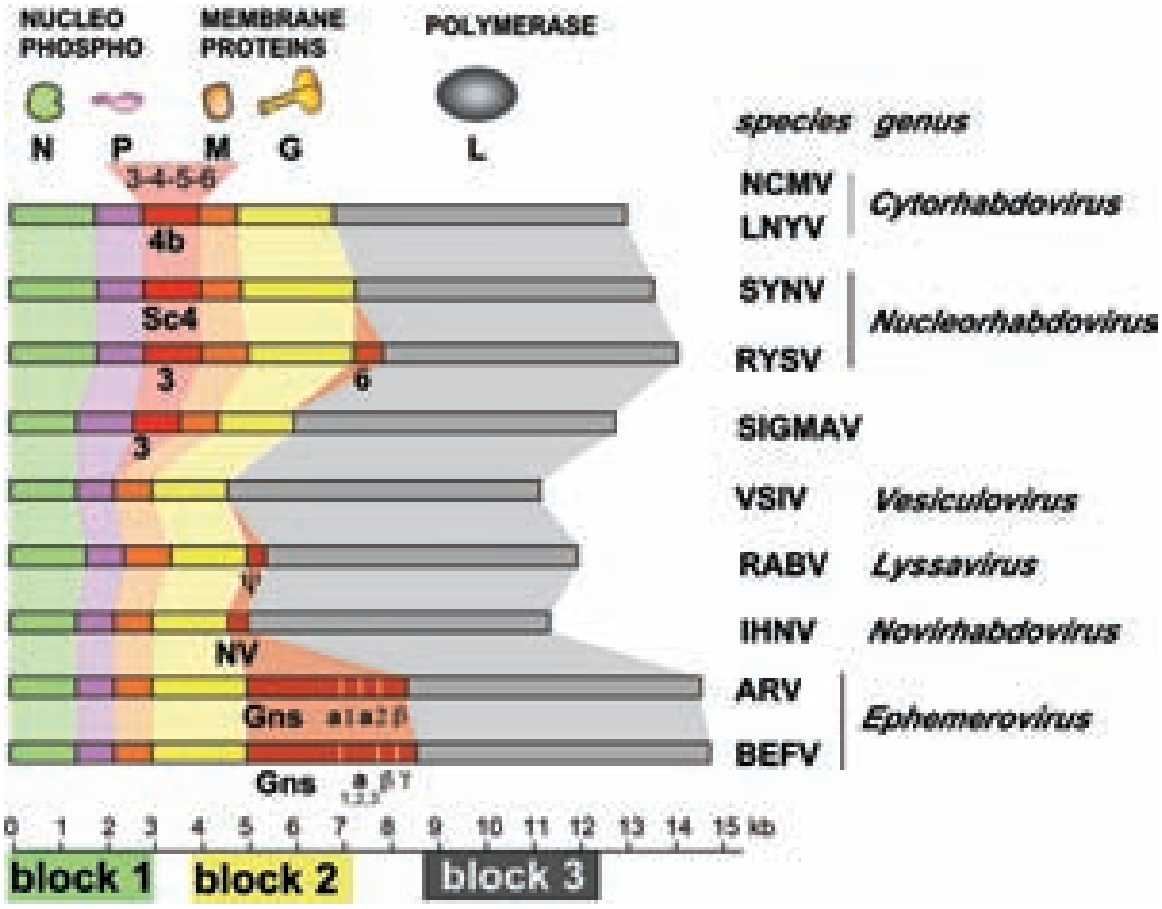



**Figure 2:** Comparison of genome organization of representative members from all genera of the family *Rhabdoviridae*. A modular organization into three blocks has been conserved: the 3′ block #1 encodes N/P proteins required in large amounts; the central block #2 encodes the membrane M/G proteins; the 5′ block #3 encodes the polymerase L required in limited amounts. Between blocks, viruses have inserted typical genes adapted to their particular biology, for example the movement protein (3, 4b) in plant rhabdoviruses. (Courtesy of N. Tordo.)

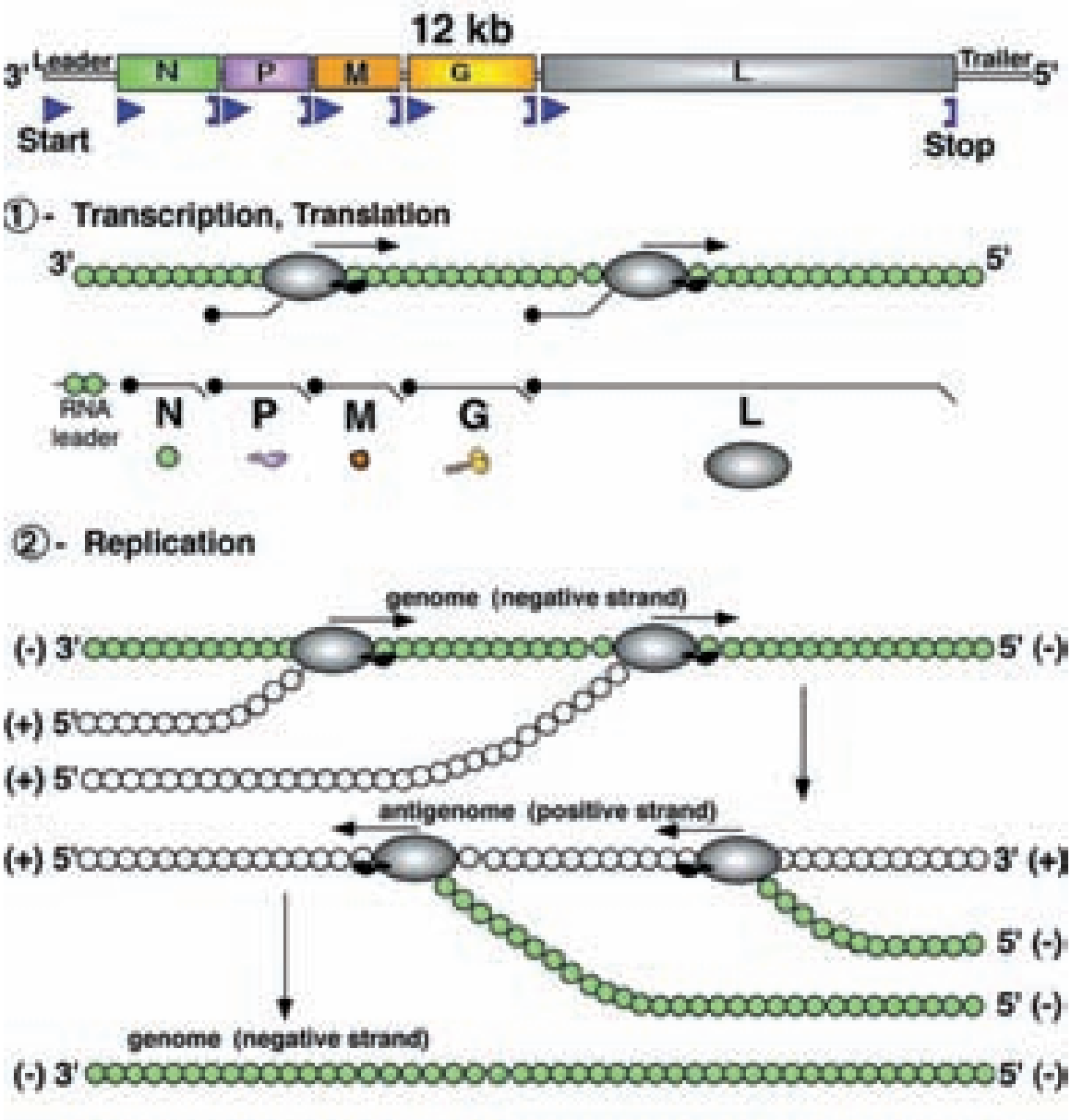



**Figure 3:** Panel (1) shows the genome organization of vesicular stomatitis Indiana virus (VSIV) and the process of consecutive transcription of leader RNA and monocistronic mRNAs. Panel (2) illustrates the replication of the negative sense genome via a positive sense antigenome intermediate. The switch from transcription to replication appears to be regulated by the N protein. (Courtesy of P. Le Mercier.)

template virus RNA and in decreasing molar abundance) as 5′-capped, 3′-polyadenylated and generally monocistronic mRNAs (Figure 3). Polycistronic mRNAs have been identified for viruses in some species; they result from the readthrough or the absence of a stop transcription signal thereby allowing transcription extension across the adjacent 5′-cistron. A short uncapped, unpolyadenylated and untranslated leader RNA, corresponding to the complement of the 3′ terminus of the viral RNA (i.e., preceding the N mRNA), is also transcribed. Unlike mRNAs, leader RNA has a 5′ triphosphate terminus (Figure 3). Leader RNA of some viruses has been identified in the nucleus of infected cells. The mRNAs generally have common 5′-terminal sequences (e.g., $m^7$Gppp(m)AmA(m)CA for vesiculoviruses and lyssaviruses) corresponding to the cap structure fused to the first nucleotides copied from the start transcription signal. The mRNAs also each contain a 3′-poly(A) tail which is produced

by the viral transcriptase upon copying in a reiterative mode the 7 U residues terminating each stop transcription signal. Intergenic sequences are generally short but may be up to about 100 nt in length. In certain cases the 5′ end of an mRNA overlaps the 3′ end of the preceding gene.

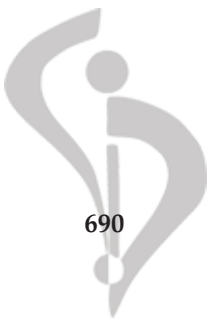
Except for plant rhabdoviruses, which generally penetrate the cell through mechanical damage caused by insect vectors, rhabdovirus adsorption is mediated by G protein attachment to cell surface receptors and penetration of the cell is by endocytosis via coated pits. Various candidate receptors have been postulated for rabies virus (RABV) (nicotinic acetylcholine receptor AChR, neural cell adhesion molecule NCAM, low affinity nerve growth factor receptor p75NTR), vesicular stomatitis viruses (VSV) (phosphatidyl serine), viral hemorrhagic septicemia virus (VHSV) (fibronectin), and others. In addition, carbohydrate moieties, phospholipids and gangliosides may play a complementary role for virus binding. After penetration by endocytosis, low pH within the endosome provokes fusion between endosomal and viral membranes, liberating the RNP complex into the cytoplasm. The pH-induced fusion depends on conformational changes of the glycoprotein, a process that is reversible upon raising the pH. Once the nucleocapsid is released into the cytoplasm, the genome RNA is repetitively transcribed (primary transcription) by the virion transcriptase. N protein removal does not occur since the transcriptase only recognizes the RNA-N protein complex as template. The capped and polyadenylated mRNAs are generally translated in cytoplasmic polysomes except for the G mRNA which is translated on membrane-bound polysomes. Transcription occurs in the presence of protein synthesis inhibitors indicating that it does not depend on *de novo* host protein synthesis. Following translation, RNA replication occurs in the cytoplasm (full-length positive sense and then full-length negative sense RNA synthesis).

Nucleorhabdoviruses replicate in viroplasms in the cell nucleus. Replication again occurs on the RNA-N protein complex and requires the newly synthesized N, P and L protein species to concomitantly encapsidate the nascent RNA into a nucleocapsid structure. Apart from freshly translated N-P-L proteins, replication may require host factors. However, vesiculoviruses can replicate in enucleated cells, indicating that newly synthesized host gene products are not required. It has been proposed that the concomitant binding of N protein to the nascent positive or negative sense viral RNA species may promote replication rather than transcription, by favoring readthrough of transcription termination signals. Replication leads to the synthesis of a full length positive sense antigenome RNA. This, in turn, serves as a replicative intermediate for the synthesis of negative sense genome RNA for the progeny virions. Following replication, further rounds of transcription (secondary transcription), translation and replication ensue. A typical feature of negative sense RNA viruses (shared by all members of the order *Mononegavirales*) is that the RNA genome (or antigenome) is never "naked" in the cell but is always encapsidated by the nucleoprotein. This RNA-N complex is the true template recognized by the viral polymerase (transcriptase or replicase).

Post-translational trafficking and modification of G protein involves translocation across the membrane of the endoplasmic reticulum, removal of the amino-proximal signal sequence and step-wise glycosylation in compartments of the Golgi apparatus. Depending on the cell, the G protein may move to the plasma membrane, particularly to the basolateral surfaces of polarized cells.

Viral nucleocapsid structures are assembled in association with M and lipid envelopes containing viral G protein. The site of formation of particles depends on the virus and host cell. For vesiculoviruses, lyssaviruses, ephemeroviruses and novirhabdoviruses, nucleocapsids are synthesized in the cytoplasm and viruses bud from the plasma membrane in most, but not all cells. Some lyssaviruses bud predominantly from intracytoplasmic membranes and in some cases prominent virus-specific cytoplasmic inclusion bodies containing N protein are observed in infected cells (RABV inclusion bodies are called Negri bodies). Cytorhabdoviruses bud from intracytoplasmic membranes associated with viroplasms; none has been observed to bud from plasma membranes. Nucleorhabdoviruses bud from the inner nuclear membrane and accumulate in the perinuclear space.

Depending on the virus and host cell type, virus infections may inhibit cellular syntheses and cause apoptosis. The mechanisms are under investigation. Complementation between viral mutants of related viruses may occur (e.g., between vesiculoviruses), but not between viruses representing distinct genera. Complementation is also reported to occur by re-utilization of the structural components of UV-irradiated virus (VSIV). Inter-molecular genetic recombination between different virus



isolates is very rare, but intra-molecular recombination may occur during the formation of defective RNAs. Phenotypic mixing occurs between some animal rhabdoviruses and other enveloped animal viruses (e.g., paramyxoviruses, orthomyxoviruses, retroviruses, herpesviruses).

Six genera have been established, on the basis of significant differences in antigenicity, genome organization, replication sites and ecological properties (such as host range, pathobiology and circulation patterns). Phylogenetic relationships based on nucleotide and protein sequences support assignments of species to the identified genera.

## Biological properties

Some rhabdoviruses replicate only in mammals, or birds, or fish, or arthropods, or other invertebrates, many have both arthropod and vertebrate hosts (arboviruses), while some species infect plants and certain plant-feeding arthropods. Some of the vertebrate rhabdoviruses have a wide experimental host range. A diverse range of vertebrate and invertebrate cell lines are susceptible to vertebrate rhabdoviruses *in vitro*. The viruses of plants usually have a narrow host range among higher plants; they replicate in insects and some replicate in insect cell cultures.

Sigma virus was recognized first as a congenital infection in drosophila. No rhabdovirus is known to be transmitted vertically in vertebrates or plants, but transovarial transmission has been documented in insects. Vector transmission may involve mosquitoes, sandflies, culicoids, aphids, leafhoppers or planthoppers. Some viruses are transmitted mechanically in sap or from the body fluids of infected hosts. Mechanical transmission of viruses infecting vertebrates may be by contact, aerosol, bite, or venereal.

# Genus *Vesiculovirus*

Type species ***Vesicular stomatitis Indiana virus***

## Distinguishing features

Vesiculoviruses infect mammals, fish and insects. They have bullet-shaped virions and possess the shortest (11.0–11.3 kb) and simplest basic genome structure of all the rhabdoviruses. Five viral proteins are encoded in the following order from the 3′ genomic end: N, P, M, G and L. There is a small (47–50 nt) leader (l) sequence transcribed from the 3′ end and a 57–60 nucleotide trailer sequence at the 5′ end shown to be involved in viral replication. Most members of the genus encode two small highly basic proteins (C and C′) in a second ORF within the P protein. The function of these proteins remains undefined, as mutant viruses lacking them replicate normally *in vitro*. Interestingly, these ORFs are absent from the genome of Alagoas virus (VSAV). There are approximately 70 non-transcribed nucleotides in the vesiculovirus genome, two nucleotides at each of the four gene junctions, three nucleotides at the l -N junction and the 60 nucleotide trailer sequence at the 5′ terminus. The four non-coding gene junctions separating the five mRNAs contain a 23 nucleotide conserved sequence: 3′-AUACUUUUUUUNNUUGUCNNUAG-5′ which functions as polyadenylation signal terminating each cistron, signals the start of the next mRNA species and templates the capped mRNA 5′ end (m7Gppp(m)Am-A(m)CAGNNAUC ).

## Virion properties

### Morphology

Virions are enveloped and bullet-shaped. Their three-dimensional structures have been recently determined utilizing cryo-electron microscopy (Figure 4). The nucleocapsid forms a conical-shaped tip and a helical structure in the trunk. Each virion contains an outer helix of matrix protein M and an inner helix of N protein and RNA. M has a hub domain with four contact sites which link to neighboring M and N subunits, providing rigidity by clamping adjacent turns of the RNP. Interactions among the N and M proteins provide scaffolding and stability to the complex and are

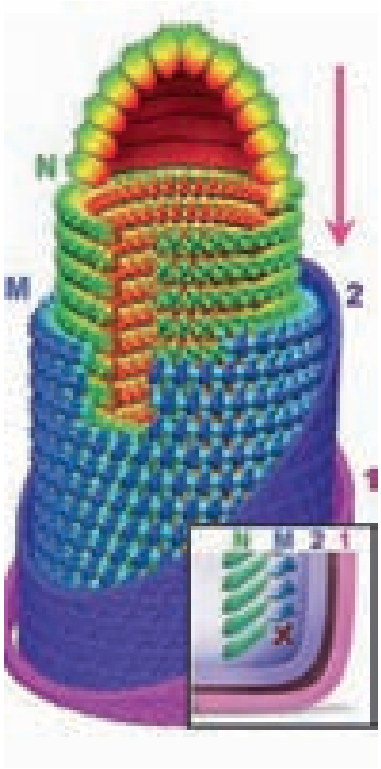



**Figure 4:** Architecture of the vesicular stomatitis virus virion. Montage model of the tip and cryoEM map of the trunk. N is green, M is blue, and the inner (2) and outer (1) leaflets of the membrane are violet and pink. (Inset) illustration of the base region of the VSV virion. The "X" marks the absence of a turn of M helix below the lowest turn of the N helix. (From Ge, P., Tsao, J., Schein, S., Green, T. J., Luo, M. and Zhou, Z. H. (2010). Cryo-EM model of the bullet-shaped vesicular stomatitis virus. *Science*, **327**, 689–93; with permission.)

critical in maintaining the bullet shape. Two layers of lipids obtained from the host cell membrane with embedded trimers of the envelope glycoprotein (G) form the outer layers of the virion and mediate the interaction with cellular receptor(s).

### Nucleic acid

Viruses contain a single molecule of linear, negative sense ssRNA of about 11 kb in size. Defective RNAs, an artifact shown to occur during tissue culture passage of some vesiculoviruses, are usually significantly shorter than full-length RNA and can be found packaged into small virions known as defective interfering (DI) particles.

### Proteins

Vesiculoviruses have five structural proteins (designated N, P, M, G and L). Most vesiculoviruses also have two small, highly basic proteins coded in a second ORF within P (termed C′ and C). The structural proteins represent 65–75% of the virus dry weight. For VSIV the numbers of molecules per infectious virus particle is estimated as: L (20–50); G (500–1,500); N (1,000–2,000); P (100–300); and M (1,500–4,000). Three viral proteins N, P and L combine to form the transcription and replication complex which has RNA-dependent-RNA polymerase (RdRP) activity. The N protein encapsidates the viral RNA and functions in close association with the P protein. The P protein is a highly phosphorylated protein associated with viral polymerase activity. It mediates the binding of the L protein to the nucleocapsid core and facilitates access of the polymerase to the RNA template during transcription and replication. Phosphorylation of P protein seems to be necessary for optimal transcriptase activity. The exact role of the nonstructural C and C′ proteins is unclear. Engineered viruses that do not express C proteins are indistinguishable from wild type virus in protein synthesis, virus production and host-protein synthesis shut off in tissue culture cells. The large L protein is multifunctional and performs most of the polymerase-associated functions including RNA

synthesis, capping, methylation and poly (A) addition. It also has protein kinase activity which preferentially phosphorylates serine residues on the P protein. The matrix protein (M) is the most abundant protein of virions. M binds specifically to G protein monomers and promotes their trimerization. It also associates with N, provides rigidity and stability and is critical in maintaining the bullet shape.

The G protein is a typical class I membrane associated glycoprotein, with approximately 90% of the N-terminal region of the molecule projecting from the surface of the virion or infected cell, a hydrophobic transmembrane domain anchoring the protein in the membrane, and a C-terminal 28 amino acid cytoplasmic domain projecting to the interior of the infected cells. The G protein forms trimers that constitute the approximately 400 spikes that are embedded on the virion bilayer phospholipid envelope. The G protein plays a major role in attachment and penetration of vesiculoviruses into susceptible cells and budding of virions from infected cells. It is the major target of serotype-specific neutralizing antibodies and is capable of inducing cell membrane fusion at low pH.

## Genome organization and replication

Total genomic RNA lengths range from 11,003 nt in Cocal virus (COCV) to 11,336 nt in field isolates of VSIV. The genome organization consists of a 47 nt leader sequence followed by the five structural protein genes in the order 3′ N-P-M-G and L and a 57–59 nt trailer sequence at the 5′ end. Most differences in length are found in non-translated regions, particularly in the M and G mRNAs. Despite the variability in lengths of the five mRNAs of the Indiana subtypes, four of the five predicted structural proteins (N, M, G and L) are within one amino acid residue in length of each other.

**Note:** A supplementary table of genome and gene length variation among vesiculoviruses is available online on Science Direct®, www.sciencedirect.com.

Vesiculovirus replication is well studied and has served as model for replication of the rhabdoviruses. Transcription begins at a single entry site at the 3′ end of the genome with each gene expressed as a capped and polyadenylated monocistronic mRNA. The first transcript is the 3′ leader, which is neither capped nor polyadenylated. It is transported to the nucleus where it inhibits host cell transcription. The leader transcript is followed by the N mRNA, which is capped during synthesis by the virion polymerase complex (composed of N, P and L). The intergenic sequence (5′-AGUUUUUUUCAUA-3′) signals polyadenylation, termination and re-initiation of transcription in decreasing amounts as the polymerase complex moves away from the single entry site. Therefore, the gene order (i.e. 3′ N>P>M>G>L 5′) provides an efficient way of regulating gene expression, by which proteins necessary in larger amounts (such as N) are located near the 3′ end and are transcribed in larger amounts and those needed in smaller amounts are located towards the 5′ end and are transcribed less frequently.

Following translation of the mRNAs to yield the viral proteins, genome replication starts. In the replication process the RdRP initiates at the 3′ end of the genome, ignores all the signals for stop and polyadenylation of individual mRNAs and instead synthesizes a full-length complementary antigenome. This in turn serves as template for synthesis of a 45-nucleotide minus sense leader RNA, and also for synthesis of full-length progeny genomes. Full-length genomes can either serve as templates for secondary transcription, or can be assembled into infectious particles. Factors determining the RdRP functions in transcription or replication modes are not fully understood but the proportion of N protein and available RNP templates are thought to be critical for determining these functions. Following replication, further rounds of transcription (secondary transcription), translation and replication ensue.

## Antigenic properties

Vesiculoviruses have been classified into serotypes based on their neutralization pattern which is determined by epitopes located in the viral G protein. The G protein also determines protection in animals vaccinated either with inactivated whole virus or with subunit vaccines. Furthermore,

recombinant viruses containing the G protein of vesicular stomatitis New Jersey virus (VSNJV), but all other proteins from VSIV, induced protective immune responses against challenge with VSNJV. The N protein is a cross-reactive antigen used in complement fixation tests that help define members of the genus. Weak serological cross-reactions may occur between viruses in different genera. One of the criteria used for vesiculovirus classification is cross-reactivity by complement fixation.

In the case of vesicular stomatitis viruses there are two major serotypes: New Jersey (VSNJV) and Indiana. The serotype Indiana has been subdivided into three distinct serological complexes. Indiana 1 comprises the classical Indiana viruses (VSIV) found from Northern South America to Southern US. The Indiana 2 subtype has (COCV) as the prototype virus which was originally isolated from mites collected from rice rats in Trinidad in 1961. Indiana 2 viruses cause disease in cattle and horses in Brazil and Argentina. The Indiana 3 subtype is represented by (VSAV) which was first isolated from a mule in Alagoas, Brazil, in 1964. This subtype is the most common cause of vesicular stomatitis in livestock in Brazil. There are other vesiculoviruses that infect mammals, some cause disease in humans, e.g. (Piry virus, Isfahan virus and Chandipura virus) and some have been isolated from blood sucking insects but to date are not known to cause disease in vertebrates (e.g., Maraba virus and Carajas virus).

In the case of the fish vesiculovirus Spring viremia of carp virus (SVCV) there is a single serotype. Antibodies directed against SVCV cross-react to various degrees with other fish rhabdoviruses; pike fry rhabdovirus (PFRV), grass carp virus (GrCRV) and tench rhabdovirus (TenRV), indicating that the viruses are closely related. SVCV and PFRV have been shown to share common antigenic determinants on the G, N and M proteins, but can be differentiated by neutralisation assays.

## Biological properties

Vesiculoviruses cause disease in mammals or fish. Those causing disease in mammals are transmitted by insects and therefore are considered arboviruses. The natural cycle of vesiculoviruses infecting mammals remains largely unknown but these viruses are commonly found in insects of a number of species and serological evidence suggests they are capable of infecting not only a number of wild mammals, but also birds and even reptiles living in endemic areas. This wide range of hosts might explain the ability of vesiculoviruses to infect and replicate in a very diverse range of vertebrate and invertebrate cells *in vitro*. In fact, mammalian vesiculoviruses are able to be transmitted both by insects and by contact. Vesicular stomatitis viruses are transmitted to cattle, horses and pigs by various blood-sucking insects found to be infected during epidemics, including sandflies, blackflies and culicoids and also can be transmitted between mammals by direct contact. Experimental mechanical transmission also has been achieved by feeding vesiculovirus laboratory infected grasshoppers to cattle. However, grasshoppers have never been shown to carry vesiculoviruses in nature. Vesiculoviruses have been shown to be transmitted not only transovarially but, interestingly, also horizontally between infected and non-infected black-flies while co-feeding on mammalian hosts. The latter means of transmission might explain the noticeable absence of viremic mammal hosts for vesiculoviruses, an unusual feature for an arbovirus.

In the case of SVCV the hosts are predominantly cyprinid fish. Naturally occurring SVC infections have been recorded from common carp (*Cyprinus carpio carpio*) and koi carp (*Cyprinus carpio koi*), crucian carp (*Carassius carassius*), sheatfish (also known as European catfish or wels) (*Silurus glanis*), silver carp (*Hypophthalmichthys molitrix*), bighead carp (*Aristichthys nobilis*), grass carp (white amur) (*Ctenopharyngodon idella*), goldfish (*Carassius auratus*), orfe (*Leuciscus idus*) and tench (*Tinca tinca*). The virus can be transmitted by ectoparasites such as carp lice (*Argulus foliaceus*) and the leeches (*Pisicola geometra*), but waterborne transmission without any vector organism is also effective.

The replication temperature range of SVCV is typically lower than those of the mammalian rhabdoviruses, reflecting the aquatic poikilothermic nature of the host species, and the viruses are typically isolated on cultured fish cell lines at 15–25 °C. The disease patterns are influenced by water temperature, age and condition of the fish, population density and stress factors. The immune

status of the fish is also an important factor with both non-specific (interferon) and specific immunity (serum antibodies, cellular immunity) having important roles. Clinical disease is usually observed at water temperature between 5–18 °C and is most severe at temperatures below 10 °C, when it is believed the host immune response is suppressed or delayed.

## Species demarcation criteria in the genus

Vesiculovirus species have been defined primarily by serological means coupled with phylogenetic analysis of the genomes. Biological characteristics such as host range and mechanisms of transmission are also used to distinguish viral species within the genus.

## List of species in the genus *Vesiculovirus*

| | | |
|---|---|---|
| *Carajas virus* | | |
| Carajas virus | [FW339542] | (CJSV) |
| *Chandipura virus* | | |
| Chandipura virus CIN0451 | [GU212856] | (CHPV-CIN0451) |
| *Cocal virus* | | |
| Cocal virus Indiana 2 | [EU373657] | (COCV-Ind2) |
| *Isfahan virus* | | |
| Isfahan virus | [AJ810084] | (ISFV) |
| *Maraba virus* | | |
| Maraba virus | | (MARAV) |
| *Piry virus* | | |
| Piry virus | [Z15093*, D26175*] | (PIRYV) |
| *Spring viraemia of carp virus* | | |
| Spring viraemia of carp virus VR-1390 | [AJ318079] | (SVCV-VR1390) |
| *Vesicular stomatitis Alagoas virus* | | |
| Vesicular stomatitis Alagoas virus Indiana 3 | [EU373658] | (VSAV-Ind3) |
| *Vesicular stomatitis Indiana virus* | | |
| Vesicular stomatitis Indiana virus 98COE | [AF473864] | (VSIV-98COE) |
| *Vesicular stomatitis New Jersey virus* | | |
| Vesicular stomatitis New Jersey virus | [K02379*, S61075*, J02433*, M20166*, M14553*, K02747*] | (VSNJV) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.*Sequences do not comprise the complete genome.

## List of other related viruses which may be members of the genus *Vesiculovirus* but have not been approved as species

| | | |
|---|---|---|
| BeAn 157575 virus | | (BeAnV-157575) |
| Boteke virus | [GU816014*] | (BTKV) |
| Calchaqui virus | | (CQIV) |
| Eel virus American | | (EVA) |
| Eel virus European X | [FN557213] | (EXEV) |
| Grass carp rhabdovirus | | (GrCRV) |
| Gray Lodge virus | | (GLOV) |
| Jurona virus | [GU816024*] | (JURV) |
| Klamath virus | | (KLAV) |
| Kwatta virus | | (KWAV) |
| La Joya virus | | (LJV) |
| Malpais Spring virus | | (MSPV) |
| Perinet virus | [AY854652*] | (PERV) |
| Pike fry rhabdovirus | [FJ872827] | (PFRV) |
| Porton virus | [GU816013*] | (PORV) |
| Radi virus | | (RADIV) |
| Tench rhabdovirus | | (TenRV) |
| Ulcerative disease rhabdovirus | | (UDRV) |
| Yug Bogdanovac virus | | (YBV) |

*Sequences do not comprise the complete genome.

# Genus *Lyssavirus*

Type species ***Rabies virus***

## Distinguishing features

Lyssaviruses share common genome organization and constitute a well-delineated monophyletic group within the family *Rhabdoviridae*. Genetic distances between lyssavirus species are significantly shorter than the distances between viruses in other rhabdovirus genera, which has been attributed to evolutionary constraints, possibly imposed by their unique pathobiology or their vectors/reservoirs preference. These viruses cause acute progressive encephalitis (rabies) in mammals, being transmitted between susceptible individuals directly by bites, scratches or contamination of mucous membranes with saliva, without participation of arthropod vectors. Bats (order Chiroptera) are the principal reservoir hosts for the majority of lyssaviruses, whereas "terrestrial" carnivores (order Carnivora), as well as bats, maintain circulation of rabies virus (RABV). Viruses of the genus are distributed worldwide, except Antarctica and several isolated islands, although viruses of different species have different circulation ranges. Based on phylogenetic relationships and antigenic properties, the genus has been subdivided into two phylogroups. Phylogroup I includes RABV, Australian bat lyssavirus (ABLV), Duvenhage virus (DUVV), European bat lyssaviruses 1 and 2 (EBLV1 and 2), Aravan virus (ARAV), Khujand virus (KHUV) and Irkut virus (IRKV), whereas phylogroup II includes Lagos bat virus (LBV) and Mokola virus (MOKV). The most divergent species in the genus, West Caucasian bat virus (WCBV), is not a member of either of these phylogroups.

## Virion properties

### Morphology

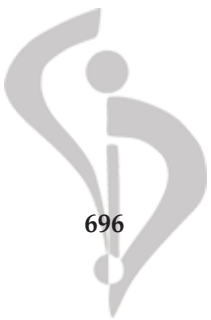
The virions are bullet-shaped, 60–110 × 130–250 nm in size, and composed of two structural units: an internal helical nucleocapsid, about 50 nm in diameter, and a lipid envelope which is derived from the host cytoplasmic membrane during budding. The nucleocapsid is comprised of a ribonucleoprotein (RNP) complex consisting of RNA genome, nucleoprotein (N), phosphoprotein (P) and RNA-dependent RNA polymerase (L). The exact position of the matrix protein (M) remains controversial, and may be either contained in the central channel of the RNP or form a helix between RNP and virion membrane, as has been shown for vesiculoviruses. The glycoprotein (G) is composed of four distinct domains: a signal peptide (non-structural), ectodomain, transmembrane peptide and cytoplasmic domain. Knobbed spikes (10 nm in length), consisting of three glycosylated ectodomains, protrude through the virion membrane.

### Physicochemical and physical properties

Lyssaviruses such as RABV consist of RNA (2–3%), protein (67–74%), lipid (20–26%) and carbohydrate (3%). The five major polypeptides include: N (58–62 kDa), P (35–40 kDa), M (22–25 kDa), G (65–80 kDa), and L (190 kDa). The G protein of RABV is glycosylated and palmitoylated at sites that have been mapped. N and P are both phosphoproteins and in the case of RABV, phosphorylation has been shown to involve several different host protein kinases: for N the cellular casein kinase II is implicated; P is phosphorylated by certain isomers of protein kinase C as well as by an additional kinase, RVPK, yet to be clearly defined. In addition a viral encoded protein kinase activity has been postulated in the L protein. The N:P ratio in the RNP of RABV is 2:1 per virion, which indicates that two molecules of N interact with 1 molecule of P. The M is not phosphorylated, it collaborates with both RNP and the G. The G is the only glycosylated protein, with branched chain oligosaccharides, which account for 10–12% of the total mass of the protein. The lyssavirus virion envelope contains other host-derived minor protein components. A lipoprotein bilayer consists of a mixture of host-derived lipids, including phospholipids, neutral lipids and glycolipids.

### Nucleic acid

The negative sense ssRNA is about 12 kb in length. The RNA is tightly associated with the N protein within the RNP.



## Genome organization and replication

The genome consists of a leader sequence (ca. 50 nt), followed by five structural protein genes in the order 3′-N-P-M-G-L-5′, separated by non-transcribed intergenic regions and followed by an ~70 nt trailer. Transcription initiation signal of each mRNA is conserved (3′-UUGURRNGA-5′), as well as the transcription termination-polyadenylation signal (3′-UCUUUUUUUG-5′). Untranslated regions of mRNAs are relatively short, except the 3′UTR of the G mRNA, which is about 400–700 nucleotides in length. Intergenic regions are variable (2–100 nucleotides), but their lengths increase along the 3′–5′ direction, which has the potential effect of causing a progressive slowing and decreasing efficiency of transcription.

Transcription and replication occur in the cytoplasm of infected cells. After attachment to receptors of the host cell membrane, mediated by the glycoprotein spikes, the virus enters the cell via the endosomal pathway. The pH decrease within the endosome leads to G protein-mediated fusion between endosomal and viral membranes and the RNP is liberated into the cytoplasm. Further, monocistronic positive sense RNAs, corresponding to the leader RNA and the five mRNAs, are transcribed in cascade from the genome encapsidated by the N protein. Transcription is mediated by the viral RNA-dependent RNA polymerase (L) and its co-factor, the P protein. All but one of the monocistronic mRNAs produce a single protein from a single ORF initiated at the first AUG initiation codon. However, the P mRNA yields three or four proteins, initiated from secondary downstream in-frame AUG codons.

As translation products of the most proximal (i.e. abundant) mRNAs during transcription, N and P proteins are produced in greater quantity within the cytoplasm. P acts as a chaperone during the synthesis of N, by forming N–P complexes which keep N soluble while preventing it from binding to cellular RNA. N is then transferred from these N–P complexes to the nascent viral RNA in order to ensure its specific encapsidation. Some of the N protein encapsidates the leader transcript. A switch from transcription to replication produces full-length complementary (positive sense) antigenome copies, which then become the template for progeny negative sense genome RNA. Both antigenome and genome RNAs are co-transcriptionally encapsidated by the soluble N and form RNPs to protect the RNA from enzymatic degradation. Both contain a specific 5′-terminal cis-acting encapsidation signal which is first recognized by N, then encapsidation proceeds in a cooperative way in the 5′–3′ direction.

## Antigenic properties

Relatively short genetic distances between lyssavirus species correlate with their antigenic cross-reactivity. Antigens of RNP, which are most abundant in infected cells, cross-react between all members of the genus described to date. This feature facilitates the use of standardized diagnostic reagents for detection of all lyssaviruses (for example, by direct fluorescent antibody or immunohistochemical assays). In contrast, outer glycoprotein antigens are relatively conserved within phylogroups (ectodomain conservation >75%) but not between phylogroups (ectodomain conservation <65%). As a result, commercially available rabies vaccines and anti-rabies immunoglobulins, that mainly induce or provide neutralizing antibodies targeting the glycoprotein, provide protection against phylogroup I lyssaviruses but not against LBV, MOKV and WCBV.

## Biological properties

Lyssaviruses are essential neurotropic pathogens. Delivered into a wound via a bite or wound contamination, the virus can replicate at the inoculation site, as was shown for skeletal muscle cells. Then the virus reaches the motor or sensory neurons and propagates up to the central nervous system (CNS) by following neuronal connections and using retrograde axonal transport. Neuronal pathways shield the virus from host immune surveillance, resulting in absence of early antibody response. Being delivered to the CNS, the virus disseminates rapidly. Nearly all regions of the CNS may be affected and RABV has been used to map neuronal connections. The duration of the

asymptomatic incubation period might be variable (two months in average), while the symptomatic clinical period is rapid and severe (about one week). Neuropathological changes observed in the infected brain are relatively mild histologically and include gliosis, slight neuronophagia, perivascular infiltration with inflammatory cells, with rare involvement of meninges. Occasionally, more severe brain damage occurs, such as spongiform lesions, extensive neuronal degeneration and widespread inflammation. Functional alteration of the CNS is much more significant than is morphological representation. Reverse dissemination of virus from the CNS during the clinical period of rabies occurs along peripheral nerves. Viral RNA may be detected in a variety of organs and tissues at the end of the clinical period. However, only low titres of infectious virus occasionally can be isolated from extraneural tissues. The exception is the salivary glands, in which virus passes additional replication cycles and is released into the saliva to complete the transmission. Acute generalized CNS dysfunction leads to a lethal outcome of the disease. Very rare cases of survival after manifestation of clinical signs of rabies have occasionally been observed in humans and some animals. However, these sporadic events cannot be taken as support for a theory of lyssavirus persistence.

In nature, lyssaviruses are associated with particular mammalian reservoir hosts. Spill-over infections into vertebrates of other species typically lead to a dead end of the transmission chain in the vast majority of cases. The exception is RABV, which is distributed most broadly, and host shifts of certain variants with the establishment of sustained circulation in a new host of a different species have been well documented. Laboratory rodents (mice, hamsters) are highly susceptible to intracranial inoculation with lyssaviruses, whereas their susceptibility to peripheral inoculation varies, depending on viral species and lineage, inoculation dose and route. Cell cultures derived from the mammalian nervous tissue (such as mouse neuroblastoma, MNA cells) are more susceptible to lyssaviruses than are other mammalian cell cultures (BHK, Vero etc.). Successful propagation in insect cell lines has been described only for MOKV.

## Species demarcation criteria in the genus

Until 1956, RABV was believed to be antigenically unique. The discovery of "rabies-related" viruses in Africa warranted the creation of the genus *Lyssavirus* (Greek *lyssa*: rage). The genus was at first divided into four serotypes by antigenic cross-reactivity with sera and monoclonal antibodies: RABV, LBV, MOKV and DUVV. Further isolations of new lyssaviruses in Europe, then Australia and Asia, and the progress in genetic characterization supported the delineation of lyssavirus genotypes, confirming and expanding antigenic data. Subsequent evaluation of long genome areas in various phylogenetic models, as well as recognition of additional characters outlined below, facilitated the classification of lyssaviruses into species.

In general, demarcation criteria for lyssavirus species include:

1. Genetic distances, with the threshold of 80–82% nucleotide identity for the complete N gene, that provides a better quantitative resolution compared to other genes, or 80–81% nucleotide identity for concatenated coding regions of N+P+M+G+L genes. Globally, all isolates belonging to the same species have higher identity values than the threshold, except the viruses currently included into the LBV species. For that reason some authors suggested that LBV be subdivided into several genotypes. However, as these LBV representatives are segregated into a monophyletic cluster in the majority of phylogenetic reconstructions, in the absence of other sufficient demarcation characters there is currently no possibility to subdivide LBV into several viral species.
2. Topology and consistency of phylogenetic trees, obtained with various evolutionary models.
3. Antigenic patterns in reactions with anti-nucleocapsid monoclonal antibodies (preceded by serologic cross-reactivity and definition of lyssavirus serotypes, using polyclonal antisera).
4. Whenever available, additional characters, such as ecological properties, host and geographic range, pathological features are recruited.

## List of species in the genus *Lyssavirus*

| | | |
|---|---|---|
| *Aravan virus* | | |
| Aravan virus – Kyrgyzstan | [EF614259] | (ARAV-KG) |
| *Australian bat lyssavirus* | | |
| Australian bat lyssavirus (pteropid bat variant) | [AF418014] | (ABLV-pb) |
| Australian bat lyssavirus (insectivorous bat variant) | [AF081020] | (ABLV-ib) |
| *Duvenhage virus* | | |
| Duvenhage virus 86132SA | [EU293119] | (DUVV-86132SA) |
| *European bat lyssavirus 1* | | |
| European bat lyssavirus 1 8918FRA | [EU293112] | (EBLV1-8918FRA) |
| *European bat lyssavirus 2* | | |
| European bat lyssavirus 2 9018HOL | [EU293114] | (EBLV2-9018HOL) |
| *Irkut virus* | | |
| Irkut virus - Russia | [EF614260] | (IRKV-RU) |
| Ozernoe | [FJ905105] | |
| *Khujand virus* | | |
| Khujand virus - Tajikistan | [EF614261] | (KHUV-TJ) |
| *Lagos bat virus* | | |
| Lagos bat virus, lineage A | [EU293108] | (LBVSEN1985; 0406SEN) |
| Lagos bat virus, lineage B | [EU293110] | (LBVNIG1956; 8619NGA) |
| Lagos bat virus, lineage C | [EF547454*, EF547411*, EF547424*, EF547441*] | (LBVSA1980) |
| Lagos bat virus, lineage D | [GU170202] | (KE576) |
| *Mokola virus* | | |
| Mokola virus | [Y09762] | |
| *Rabies virus* | | |
| Pasteur virus | [M13215] | (PV) |
| Street Alabama Dufferin, Bern-19 | [M31046] | (SAD B-19) |
| Silver-haired bat rabies virus | [AY705373] | (SHBRV-18) |
| Rabies virus (European fox) | [EU293115] | (9147FRA) |
| Rabies virus (south-east Asia, dog) | [EU293111] | (8764THA) |
| Rabies virus (North America, raccoon) | [EU311738] | (RRV ON-99-2) |
| Rabies virus (Argentina, free-tailed bat) | [EU293116] | (9704ARG) |
| *West Caucasian bat virus* | | |
| West Caucasian bat virus - Russia | [EF614258] | (WCBV-RU) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

## List of other related viruses which may be members of the genus *Lyssavirus* but have not been approved as species

| | | |
|---|---|---|
| Shimoni bat virus | [GU170201] | (SHIBV) |

# GENUS *EPHEMEROVIRUS*

Type species ***Bovine ephemeral fever virus***

## Distinguishing features

Ephemeroviruses are arthropod-borne animal rhabdoviruses. Bullet-shaped virions have a characteristic tapered appearance at the apical end and a prominent axial channel intruding from the base. The genome is large (>14 kb) and complex, containing a non-structural glycoprotein ($G_{NS}$) gene and several small accessory protein genes. The $G_{NS}$ glycoprotein shares significant amino acid sequence identity with the virion glycoprotein (G) and appears to have arisen by gene duplication. Viruses in the genus cross-react strongly in complement-fixation (CF) and indirect immunofluorescence tests.

## Virion properties

### Morphology

Virions are bullet- or cone-shaped with a length of about 140–200 nm and diameter about 60–80 nm. They display a prominent axial channel intruding from the base and a precisely coiled, helical nucleocapsid with 35 cross-striations at intervals of 4.8 nm.

### Physicochemical and physical properties

Virions have a buoyant density in CsCl of 1.19 g $cm^{-3}$ and sedimentation coefficient of 625S. Viruses are sensitive to acid or alkali and most stable at pH 7.0–8.0. Bovine ephemeral fever virus (BEFV) particles contain at least five structural proteins, designated: L (180 kDa); G (81 kDa); N (52 kDa); P (43 kDa); and M (29 kDa). The G protein is a virus membrane-associated glycoprotein which contains five potential sites for attachment of N-linked glycans. The N protein is phosphorylated. The M protein is also phosphorylated in virions.

### Nucleic acid

The genome comprises a single molecule of negative sense, single stranded RNA >14 kb.

## Genome organization and replication

The 14.9 kb (−)ssRNA genome of BEFV contains 10 genes in the order 3′-N-P-M-G-$G_{NS}$-$\alpha_1$-$\alpha_2$-β-γ-L-5′ and intergenic regions of between 26 and 53 nt. The γ and L genes overlap by 21 nt. Additional small ORFs occur in alternative frames in the P and $\alpha_2$ genes. Each gene, except α1, is initiated from a UUGUCC sequence (mRNA: 5′-cap-AACAGG...) and terminates at a putative polyadenylation site GNAC($U_{6\text{-}7}$)-3′. Each gene is transcribed as a monocistronic mRNA except the $\alpha_1$ and $\alpha_2$ genes which are transcribed in a bicistronic mRNA. The 15.0 kb Berrimah virus (BRMV) genome has the same gene order as BEFV and employs the same consensus transcription initiation and polyadenylation signals. However, unlike BEFV, intergenic regions are up to 100 nt in length, additional small ORFs do not occur in the P or $\alpha_2$ genes, and all genes appear to be transcribed independently. In ARV, the 14.6 kb genome contains nine genes in the order 3′-N-P-M-G-$G_{NS}$-$\alpha_1$-$\alpha_2$-β-L-5′ and intergenic regions of 1–4 nt. The β and L genes overlap by 22 nt. ARV lacks a γ gene comparable to that of BEFV. An additional ORF occurs in an alternative frame in the P gene but not in the $\alpha_2$ gene. Each gene is initiated from a viral 3′-UUGUC sequence (mRNA: 5′-cap-AACAG...). However the putative polyadenylation signals are more variable than those of BEFV and may account for the synthesis of polycistronic mRNAs.

The $G_{NS}$ gene product is a 90 kDa non-virion glycoprotein which has been identified in BEFV-infected mammalian cells. $G_{NS}$ is highly glycosylated (eight potential sites for N-linked glycans). The G and $G_{NS}$ proteins, although not identical, exhibit homologies with each other and to lesser extents with the G proteins of other animal rhabdoviruses. In Adelaide River virus (ARV), the G protein (90 kDa) contains six potential sites for N-linked glycans; the $G_{NS}$ protein contains nine. The products of ephemerovirus $\alpha_1$, $\alpha_2$, β and γ genes have not been identified. The $\alpha_1$ gene product appears to be a viroporin but the functions of other products have not been established.

## Antigenic properties

Ephemeroviruses cross-react strongly in CF or indirect immunofluorescence tests and may show low level cross-reactions by indirect immunofluorescence with viruses of the genus *Lyssavirus*. However, sequence comparisons with other rhabdoviruses indicate that in evolutionary terms the ephemeroviruses are closer to vesiculoviruses than to members of other genera in the family. There is only one known BEFV serotype worldwide, with virus isolates from different regions (Australia, Asia, Africa and the Middle-East) displaying significant cross-neutralization. There is a low level of cross-neutralization between BEFV and BRMV; there is no cross-neutralization between ARV and other ephemeroviruses. The BEFV G protein contains four distinct neutralization sites. The BEFV G protein purified from virions or expressed from recombinant vaccinia virus protects cattle from experimental infection. The $G_{NS}$ glycoprotein does not induce neutralizing antibodies and is not protective.

### Biological properties

Bovine ephemeral fever is an economically important disease of cattle and water buffalo in most tropical and sub-tropical regions of Africa, Australia, the Middle-East and Asia. BEFV infection causes a sudden onset of fever and other clinical signs including lameness, anorexia and ruminal stasis, followed by a sustained drop in milk production. Although the mortality rate is usually low (1–2%), it is highest in well-conditioned beef cattle and high-producing dairy cattle. The virus is transmitted by, and replicates in, hematophagous arthropods and has been isolated from both culicoids and mosquitoes. Other species in the genus are not recognized as animal pathogens, but are known to infect cattle and have been isolated from healthy sentinel cattle.

### Species demarcation criteria in the genus

Species cross-react in complement-fixation and/or indirect immunofluorescence tests but exhibit low to no cross-neutralization. They exhibit similar but distinct genome organizations with the common feature of a non-structural glycoprotein ($G_{NS}$) gene but variations in the number of accessory protein genes and the location of transcriptional control sequences. Different species may share up to 91% identity in N protein amino acid sequence.

### List of species in the genus *Ephemerovirus*

| | | |
|---|---|---|
| *Adelaide River virus* | | |
| Adelaide River virus | [L09206*, L09208*, U05987*, U10363*] | (ARV) |
| *Berrimah virus* | | |
| Berrimah virus | | (BRMV) |
| *Bovine ephemeral fever virus* | | |
| Bovine ephemeral fever virus BB7721 | [AF234533] | (BEFV-BB7721) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

### List of other related viruses which may be members of the genus *Ephemerovirus* but have not been approved as species

| | | |
|---|---|---|
| Kimberley virus | [AY854637*] | (KIMV) |
| Kotonkan virus | [HM474855] | (KOTV) |
| Malakal virus | | (MALV) |
| Obodhiang virus | [HM856902] | (OBOV) |
| Puchong virus | | (PUCV) |

*Sequences do not comprise the complete genome.

## GENUS *NOVIRHABDOVIRUS*

Type species ***Infectious hematopoietic necrosis virus***

### Distinguishing features

This genus comprises one of the two major subgroups of rhabdoviruses known to infect aquatic hosts. Members of the other subgroup include spring viremia of carp virus (SVCV), which is a member of the genus *Vesiculovirus*, and several other viruses that have not been placed taxonomically.

Novirhabdoviruses have five major structural proteins, designated L (150–225 kDa), G (63–80 kDa), N (38–47 kDa), P (22–26 kDa, formerly designated M1), and M (17–22 kDa, formerly designated M2). In addition to the structural proteins, novirhabdoviruses encode a small, sixth, non-virion protein designated NV (12–14 kDa), which is expressed at variable levels in infected cells but is not detectable in purified virions. The NV ORF is preserved in numerous diverse viruses and strains, but the NV protein sequences are significantly less conserved between viruses in different species than

sequences of the other structural proteins, such that there is no significant amino acid sequence similarity between the NV proteins of IHNV and VHSV. The specific function of the NV protein is not yet defined but it is required for efficient virus replication. Results of studies with NV gene deletion mutants generated by reverse genetics are inconsistent in that the NV appears to be required for pathogenicity in IHNV and VHSV but not SHRV.

## Virion properties

### Morphology

Virions are bullet-shaped and measure 45–100 nm in diameter × 100–430 nm in length. Surface projections are densely dispersed, distinctive spikes which cover the whole surface except for the quasi-planar end.

### Physicochemical and physical properties

The replication temperature range and thermal inactivation temperatures for these viruses are typically lower than those of other rhabdoviruses, due to the aquatic poikilotherm nature of their hosts. Optimum virus replication temperatures range from 15–28 °C, depending roughly on the ambient water temperature in the geographic range of each virus.

### Nucleic acid

The genome comprises a single molecule of negative sense. single stranded RNA of about 11 kb.

## Genome organization and replication

The genomic RNA is approximately 11.1 kb, with six genes in the order 3′-N-P-M-G-NV-L-5′. The genome contains a leader region of approximately 60 nt preceding the transcription start of the N gene, and a trailer of about 100 nt following the transcription termination of the L gene. Genes begin with the conserved putative transcription start signal 3′-CCRWG (vRNA sense, most often 3′-CCGUG), and the signal 3′-UUGU is also found upstream of the translation initiation site in many genes. Transcription terminates at the signal 3′-UCURUC$(U)_7$, and non-transcribed intergenic regions are single nucleotides, G or A (vRNA sense).

## Biological properties

Novirhabdoviruses infect fish of numerous species. The natural host ranges of individual viruses vary in breadth, with the type member IHNV limited to salmonid fish, while VHSV infects hosts from a wide range of fish families as diverse as salmonids and herring. In nature and in artificial environments novirhabdoviruses can be transmitted horizontally, from fish to fish, by a waterborne route. Egg-associated transmission has also been clearly demonstrated by several cases in which the spread of virus to new geographic regions has occurred with transport of contaminated eggs. It is increasingly apparent that wild fish can serve as reservoirs of virus. The existence of invertebrate reservoirs or vectors of virus has been postulated but their importance is uncertain. Similarly, the potential for a carrier state in survivors of IHNV infections has been demonstrated, but the frequency and significance of this phenomenon is not well understood.

The geographic distribution of novirhabdoviruses is broad. IHNV is enzootic to western North America, but inadvertent transport of the virus with contaminated eggs and infected fish has resulted in spread and establishment of IHNV in western Europe, Korea, Taiwan, Japan, and mainland China. VHSV is enzootic to cultured rainbow trout in much of western Europe, but more recently several North American and Asian strains have been described, and an extensive VHSV reservoir in marine fish in the northern Atlantic and Pacific Oceans has been demonstrated. Hirame rhabdovirus (HIRRV) is at present only isolated in Japan and Korea, and SHRV occurs in southeast Asia.

Members of the genus *Novirhabdovirus* cause disease in cultured fish hosts, resulting in significant economic losses to aquaculture industries. Both IHNV and VHSV have been well documented as severe pathogens of cultured salmonids since the 1950s, often resulting in losses of 50–100%. Among free-ranging fish IHNV epizootics have been reported in wild salmonids, and VHSV

epizootics have occurred in both marine and freshwater fish of diverse species. IHNV and VHSV both cause hemorrhagic diseases, with petechial hemorrhages evident both externally and internally. Major degenerative changes and necrosis in the kidneys and hematopoietic tissue are evident upon histopathological examination, and are believed to be the actual cause of mortality.

### Species demarcation criteria in the genus

Species within the genus have been distinguished serologically on the basis of cross-neutralization with polyclonal rabbit antisera. In general, strains within a species are neutralized by a single polyclonal antiserum. Thus, IHNV and HIRRV each comprise single serotypes, and VHSV has one major serotype with a small number of associated strains. Viruses from different species do not show cross-neutralization, but in some cases there is a low level of cross-reaction with specific proteins in western blot analyses. Nucleotide sequence data are available for most genes of these viruses, and will undoubtedly contribute to the distinction of viral species in the future. For strains within a virus species the nucleotide sequence divergence values range up to a maximum of 8% for IHNV G and NV genes, and 18% for the G genes of European and North American VHSV. N protein aa sequence identity between IHNV and VHSV is approximately 34%.

### List of species in the genus *Novirhabdovirus*

| | | |
|---|---|---|
| *Hirame rhabdovirus* | | |
| Hirame rhabdovirus CA9703 | [AF104985] | (HIRRV-CA9703) |
| *Infectious hematopoietic necrosis virus* | | |
| Infectious hematopoietic necrosis virus WRAC | [L40883] | (IHNV-WRAC) |
| *Snakehead virus* | | |
| Snakehead virus | [AF147498] | (SHRV) |
| *Viral hemorrhagic septicemia virus* | | |
| Egtved virus | | |
| Viral hemorrhagic septicemia virus Fil3 | [Y18263] | (VHSV-Fil3) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Novirhabdovirus* but have not been approved as species

| | |
|---|---|
| Eel virus B12 | (EEV-B12) |
| Eel virus C26 | (EEV-C26) |

## Plant-adapted rhabdovirus genera, *Cytorhabdovirus* and *Nucleorhabdovirus*

### Distinguishing features

Two genera of plant rhabdoviruses have been established on the basis of the sites of virus replication and morphogenesis (Figure 5) (cytoplasm: *Cytorhabdovirus*; nucleus: *Nucleorhabdovirus*). Moreover, genus classification based on sequence diversity has thus far correlated 100% with classification by intracellular virus maturation. The interrelationships of the different plant viruses within or between the two genera or with the >50 putative plant rhabdoviruses (identified based on their unique particle morphology compared to other plant viruses) have largely yet to be established at the genetic level. There is no significant sequence similarity (>50%) between analogous genes of the species analyzed to date. A wide variety of plants are susceptible to rhabdoviruses although each virus usually has a restricted host range. Plant rhabdoviruses are transmitted by leafhoppers, planthoppers or aphids. Some viruses are also transmitted during vegetative propagation, and some can also be transmitted mechanically from infected sap. In all carefully examined cases, viruses have been shown to replicate in cells of the insect vector as well as in the plant host.

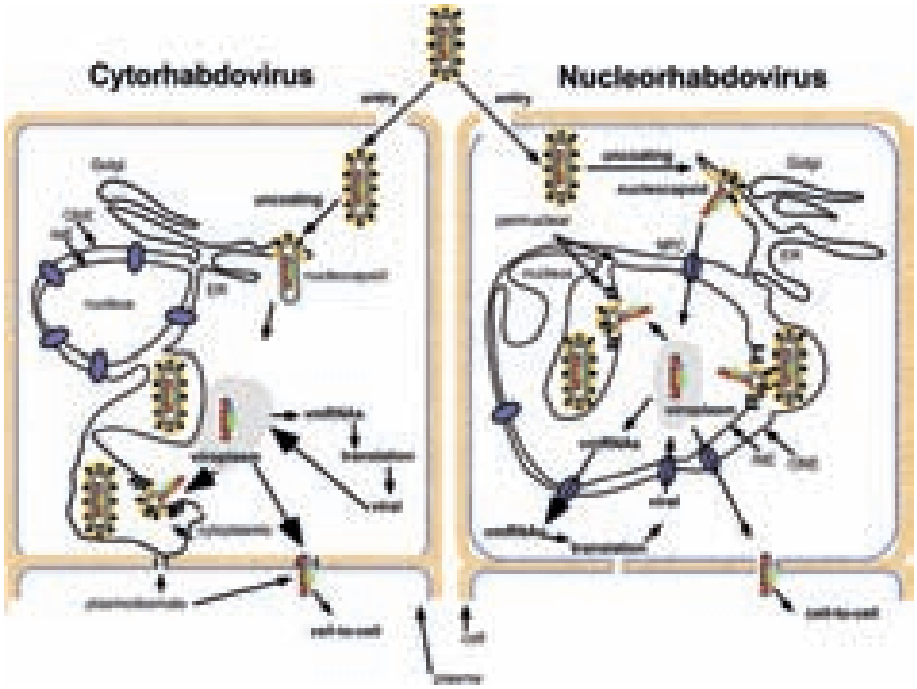



**Figure 5:** Contrasts between the replication cycles of cytorhabdoviruses and nucleorhabdoviruses. Most rhabdoviruses gain entry into host cells during insect vector feeding. Uncoating is believed to take place on ER membranes, followed by release of the nucleocapsid core into the cytoplasm. At this point, the replication cycles of the two genera diverge. In the case of the cytorhabdoviruses, the newly released cores become transcriptionally active and associate with the endoplasmic reticulum to establish viroplasms that function in transcription of viral mRNAs (vmRNAs) and replication of genomic and antigenomic viral RNAs. Following translation of the vmRNAs, viral proteins involved in replication accumulate in the viroplasm. Viral glycoproteins are targeted to cytoplasmic membranes or, possibly, the outer nuclear envelope (ONE). Maturation of cytorhabdoviruses takes place via matrix protein-mediated condensation of cores at sites of G protein accumulation in the endoplasmic reticulum. In the case of the nucleorhabdoviruses, released cores are transported into the nucleus through nuclear pore complexes (NPC). Following transcription and export, vmRNAs are translated and viral proteins are imported into the nucleus, where they participate in replication and formation of large viroplasms. During intermediate stages of infection of plant rhabdoviruses, movement of infectious units from cell to cell occurs. Nucleocapsids most likely are the transported form, and these interact with viral encoded movement proteins that participate in number of activities, including nucleocapsid binding, transport through the NPC to the plasmodesmata, and modifications to the plasmodesmatal size exclusion limits. Morphogenesis occurs near the end of active transcription and replication and involves interactions with the M protein to coil the viral nucleocapsids and form associations with membrane-associated G protein. In the cytorhabdoviruses, electron microscopic observations suggest that budding occurs into proliferated ER associated with the viroplasms. Currently, at least two models can be proposed for morphogenesis of nucleorhabdovirus virions. In recent data outlined in the text, the inner nuclear envelope (INE) proliferates due to redistribution of cytoplasmic membranes and invaginates to form intranuclear spherules, into which viral budding occurs. In the classical model, virus budding occurs through intact INE resulting in an expansion of the outer nuclear envelope. In both models, mature virions accumulate in the perinuclear spaces of infected cells where they may be reacquired during subsequent insect feeding. (Reproduced from Jackson *et al.* (2005); with permission from *Annual Reviews*.)

## GENUS *CYTORHABDOVIRUS*

Type species ***Lettuce necrotic yellows virus***

### Distinguishing features

Cytorhabdoviruses replicate in the cytoplasm of infected cells in association with masses of thread-like structures (viroplasms). Virions bud in association with the endoplasmic reticulum (ER) and accumulate in ER-derived vesicles. A nuclear phase has been suggested but not proven in the replication of some cytorhabdoviruses (e.g. lettuce necrotic yellows virus (LNYV). Evidence of the nuclear involvement in the replication of others is lacking (e.g. barley yellow striate mosaic virus, BYSMV). Endogenous transcriptase activity is readily detectable in cytorhabdovirus preparations where this has been investigated.

## Virion properties

### Morphology
Enveloped, bacilliform virions, 60–75 nm in diameter and 200–350 nm long.

### Physicochemical and physical properties
Buoyant density of 1.19–1.20 g $cm^{-3}$ in sucrose or potassium tartrate. The lipid envelope is derived from the cytoplasmic membranes of plant or insect host cells.

### Nucleic acid
Monopartite, negative sense, single stranded RNA genome, 12.8–14.5 kb in length. Six to ten mRNAs, one for each of the encoded proteins identified in infected plants.

## Genome organization and replication

The genome of LNYV is about 12.8 kb and the genome organization is similar to that of SYNV (see *Nucleorhabdovirus*). Preceded by a non-coding 84 nt leader sequence, the gene order is 3′-N-P-4b-M-G-L-5′. N represents the nucleoprotein, P, M, G and L are the putative phospho-, matrix- and glycoproteins and RNA polymerase, respectively. Protein 4b has been predicted to represent a movement protein. The intergenic regions contain highly conserved consensus sequences. The 5′-non-coding trailer sequence of 187 nt has extensive complementarity to the 3′-leader. The genome of northern cereal mosaic virus (NCMV) is about 13.2 kb with a gene order similar to that of LNYV, except for the presence of three additional small genes of unknown function between P and M and an additional gene between G and L. The genome organization of lettuce yellow mottle virus (LYMoV) (12.9 kb) and strawberry crinkle virus (SCV) (14.5 kb) are similar to that of LNYV, except for the presence in SCV of one additional gene between G and L.

## Species demarcation criteria in the genus

In the genus *Cytorhabdovirus*, species are primarily differentiated by plant host range and vector specificity of the virus. Nucleic acid hybridization has been used to provide confirmation of species and serological criteria have enabled verification of common viruses that infect different hosts. However, no virus strains have been defined unambiguously using serology. The complete nucleotide sequence is available for only four viruses in the genus, LNYV, LYMoV, NCMV and SCV. Thus, this criterion is not presently sufficient for discrimination of species. Hybridization using cloned probes and conserved L gene polymerase motif sequences has been used to differentiate viruses within the genus and to identify some strains. These analyses should be emphasized in future studies.

## List of species in the genus *Cytorhabdovirus*

| | | | |
|---|---|---|---|
| *Barley yellow striate mosaic virus* | | | |
| Barley yellow striate mosaic virus Zanjan-1 | {planthopper} | [FJ665628*] | (BYSMV-Z1) |
| Maize sterile stunt virus | {planthopper} | | (MSSV) |
| Wheat chlorotic streak virus | {planthopper} | | (WCSV) |
| *Broccoli necrotic yellows virus* | | | |
| Broccoli necrotic yellows virus | {aphid} | | (BNYV) |
| *Festuca leaf streak virus* | | | |
| Festuca leaf streak virus | | | (FLSV) |
| *Lettuce necrotic yellows virus* | | | |
| Lettuce necrotic yellows virus 318 | {aphid} | [AJ867584] | (LNYV-318) |
| *Lettuce yellow mottle virus* | | | |
| Lettuce yellow mottle virus - France | | [EF687738] | (LYMoV-FRA) |
| *Northern cereal mosaic virus* | | | |
| Northern cereal mosaic virus - Hebei | {planthopper} | [GU985153] | (NCMV-Hb) |
| *Sonchus virus* | | | |
| Sonchus virus | | | (SonV) |
| *Strawberry crinkle virus* | | | |
| Strawberry crinkle virus - UK | {aphid} | [AY005146*, AY250986*] | (SCV-UK) |
| *Wheat American striate mosaic virus* | | | |

| | | |
|---|---|---|
| Wheat American striate mosaic virus | {leafhopper} | (WASMV) |
| Oat striate mosaic virus | {leafhopper} | (OSMV) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ], natural vector species { } and assigned abbreviations ( ) are also listed.
* Sequences do not comprise the complete genome.

### List of other related viruses which may be members of the genus *Cytorhabdovirus* but have not been approved as species

| | | | |
|---|---|---|---|
| Wheat rosette stunt virus | {planthopper} | [AF059602-04*, AF059677*, AF064784*] | (WRSV) |
| Soybean blotchy mosaic virus | {leafhopper} | [EU877231*] | (SbBMV) |
| Ivy vein banding virus | | [GQ249162*, GQ249163*] | (IVBV) |

*Sequences do not comprise the complete genome.

## Genus *Nucleorhabdovirus*

Type species ***Potato yellow dwarf virus***

### Distinguishing features

Nucleorhabdoviruses replicate in the nuclei of plant cells, which become greatly enlarged and develop large granular nuclear inclusions that are thought to be sites of virus replication. *In situ* hybridization analyses have shown that the viral genomic and antigenomic RNAs are highly expressed in subnuclear foci and immunofluorescence studies have shown that the N, P and L nucleocapsid also accumulate in subnuclear foci. Viral proteins are synthesized from discrete polyadenylated mRNAs and reporter gene analyses have shown that they accumulate in subnuclear foci. Virus morphogenesis occurs at the inner nuclear membrane, and enveloped virus particles accumulate in perinuclear spaces. In protoplasts treated with tunicamycin, morphogenesis is interrupted and nucleocapsids accumulate in the nucleoplasm.

### Virion properties

#### Morphology

Enveloped, bacilliform virions, 45–100 nm in diameter and 130–300 nm long.

#### Physicochemical and physical properties

Virus particles sediment at 800–1000S in sucrose gradients and the buoyant density of virions is 1.18 g $cm^{-3}$ in isopycnic sucrose gradients.

#### Nucleic acid

Monopartite, negative sense, single stranded RNA genome, 12–14 kb in length. Six to seven mRNAs, one for each of the encoded proteins identified in infected plants.

### Genome organization and replication

The genome of potato yellow dwarf virus (PYDV) is about 12.9 kb long and encodes seven ORFs in the order 3′-N-X-P-Y-M-G-L-5′, which likely encode the nucleocapsid (N), phospho- (P), movement (Y), matrix (M), glyco- (G) and RNA-dependent RNA polymerase (L) proteins, respectively. The function of X has not been determined. The ORFs are flanked by a 3′ leader RNA of 149 nt and a 5′ trailer RNA of 97 nt, and are separated by conserved intergenic "gene junction" regions which are similar in length and have sequence relatedness with those of other rhabdoviruses. M protein is able to induce the intranuclear accumulation of the inner nuclear membrane in the absence of any other viral protein. Protein interaction studies in live plants have identified binary interactions between N:N, N:P, M:M, M:Y, M:G, G:G and Y:Y.

The genome of sonchus yellow net virus (SYNV)(ca. 13.7 kb), 3′-N-P-sc4-M-G-L-5′, lacks an X protein. The Y equivalent, named sc4, is believed to be involved in cell-to-cell movement. The 144 nt 3′ leader sequence is transcribed to produce a polyadenylated leader RNA, which localizes in the

cytoplasm. The 5′ trailer RNA is 160 nt long with extensive terminal complementarity with the leader sequence. The N and P proteins contain nuclear localization sequences (NLS) and are independently imported into the nucleus, where they associate and move to a sub-nuclear location. A distinct nuclear polymerase complex composed of N, P and L is present in the nuclei of infected cells.

The genome of rice yellow stunt virus (RYSV) is 14 kb with a gene order similar to that of SYNV, except for the presence of an additional gene between G and L which encodes a virion-associated protein. The genomes of maize mosaic virus (MMV) and taro vein chlorosis virus (TaVCV) are about 12 kb with a gene order similar to that of SYNV. The genome of maize fine streak virus (MFSV) is 13.8 kb with a gene order similar to that of PYDV, with an ORF of unknown function between P and M.

## Species demarcation criteria in the genus

Species are primarily differentiated by plant host range and vector specificity of the virus. Nucleic acid hybridization has been used to provide confirmation of identification and serological criteria have enabled verification of common viruses that infect different hosts. However, no virus strains have been defined unambiguously using serology. The complete nucleotide sequences are available for viruses in six species of the genus (MFSV, MMV, PYDV, RYSV, SYNV, TaVCV) and partial sequences have been determined for eggplant mottled dwarf virus. With additional sequences becoming available in the near future, nucleotide sequences, RT-PCR-based assays and fluorescent viral protein localization may become useful tools for species demarcation. Hybridization using cloned probes has been used to verify viruses within the genus and these and other molecular analyses should be emphasized in future studies.

## List of species in the genus *Nucleorhabdovirus*

| | | | |
|---|---|---|---|
| *Datura yellow vein virus* | | | |
| Datura yellow vein virus | | | (DYVV) |
| *Eggplant mottled dwarf virus* | | | |
| Eggplant mottled dwarf virus-Egg | {leafhopper} | [AM922319*, AM922322*] | (EMDV-Egg) |
| Pittosporum vein yellowing virus | | | (PVYV) |
| Tomato vein yellowing virus | | | (TVYV) |
| Pelargonium vein clearing virus | | | (PVCV) |
| *Maize fine streak virus* | | | |
| Maize fine streak virus - USA | {leafhopper} | [AY618417] | (MFSV-US) |
| *Maize mosaic virus* | | | |
| Maize mosaic virus - USA | {planthopper} | [AY618418*] | (MMV-US) |
| *Potato yellow dwarf virus* | | | |
| Potato yellow dwarf virus - SYDV | {leafhopper} | [GU734660] | (PYDV-SYDV) |
| *Rice yellow stunt virus* | | | |
| Rice yellow stunt virus - China | {leafhopper} | [AB011257] | (RYSV-China) |
| Rice transitory yellowing virus - Ishigaki | {leafhopper} | [AB516283] | (RTYV-Japan) |
| *Sonchus yellow net virus* | | | |
| Sonchus yellow net virus - USA | {aphid} | [L32603] | (SYNV-US) |
| *Sowthistle yellow vein virus* | | | |
| Sowthistle yellow vein virus - USA | {aphid} | | (SYVV-US) |
| *Taro vein chlorosis virus* | | | |
| Taro vein chlorosis virus - Fiji | | [AY674964] | (TaVCV-FJ) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ], natural vector species { } and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

## List of other related viruses which may be members of the genus *Nucleorhabdovirus* but have not been approved as species

| | | | |
|---|---|---|---|
| Cereal chlorotic mottle virus | {leafhopper} | | (CCMoV) |
| Cynodon rhabdovirus | | [EU650683*] | (CRV) |
| Maize Iranian mosaic virus | {planthopper} | [DQ186554] | (MIMV) |
| Sorghum stunt mosaic virus | {leafhopper} | | (SSMV) |

*Sequences do not comprise the complete genome.

## List of unassigned species in the family *Rhabdoviridae*

| | | | |
|---|---|---|---|
| *Flanders virus* | | | |
| Flanders virus - USA | {*Culex* spp. (mosquitoes)} | [AF523194-9] | (FLAV-US) |
| *Ngaingan virus* | | | |
| Ngaingan virus MRM14556 | {*Culicoides brevitarsis*} | [FJ715959] | (NGAV- MRM14556) |
| *Sigma virus* | | | |
| Sigma virus HAP23 | {*Drosophila melanogaster*} | [GQ375258] | (SIGMAV-HAP23) |
| Sigma virus AP30 | {*Drosophila melanogaster*} | [NC_013135] | (SIGMAV-AP30) |
| *Tupaia virus* | | | |
| Tupaia virus - Thailand | {*Tupaia belangeri*} | [AY840978] | (TUPV-TH) |
| *Wongabel virus* | | | |
| Wongabel virus CS264 | {*Culicoides austropalpalis*} | [EF612701] | (WONV-CS264) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ], natural vector species { } and assigned abbreviations ( ) are also listed.

Sigma virus (SIGMAV) naturally infects fruit flies (*Drosophila* spp.) and is transmitted vertically through the germinal cells. Infected flies exposed to carbon dioxide are irreversibly paralyzed. Based on its genome organization, phylogenetic placement and pathobiology SIGMAV appears to be unique and not a member of any previously described genus. Virions are spiked and enveloped bullet-shaped particles of about 75 × 140–200 nm appear to be exclusively cytoplasmic and contain a helical nucleocapsid. The genome contains six genes arranged in the order 3′-N-P-X-M-G-L-5′. There is an additional gene between P and M genes, like viruses in the genera *Cytorhabdovirus* and *Nucleorhabdovirus*; the encoded putative protein is of unknown function but contains conserved domains found in reverse transcriptases. Another unusual feature is that M and G mRNAs overlap by 33 nucleotides.

Flanders virus (FLAV) is associated with mosquitoes and birds in North America. Serological studies demonstrated that FLAV isolates from different states of the USA were closely related to each other, but different from Hart Park virus (HPV). Furthermore, FLAV was found mostly in eastern North America, whereas HPV was found mostly in western North America. Some overlap is found at the geographic extremes but, for the most part, these viruses have been found where their mosquito hosts occur, *Culiseta melanura* in the east (FLAV), *Culex tarsalis* in the west (HPV). FLAV genome is about 13 kb with a gene order of 3′-N-(tc)-P-(tc)-pseudogene1-(tc)-19K-(tc)-pseudogene2-(tt)-M-G-(tt)-L-5′. The unique features include the additional 19K gene, surrounded by two pseudogenes, about 500 nucleotides each, situated between the P and M genes. According to the N and L gene sequences, FLAV demonstrates limited relatedness to WONV and NGAV viruses,which circulate in Australia, and this cluster is distantly related to ephemeroviruses (Figure 6).

Tupaia virus (TUPV) was isolated from spontaneously degenerating hepatocellular carcinoma cells from a tree shrew (*Tupaia belangeri*), imported from Thailand and kept in captivity for about 6 years. The host range of the virus *in vitro* appears to be restricted to tupaia cells. The genome is about 11.5 kb with a gene order 3′-N-P/C-M-SH-G-L-5′. The genome contains a unique small hydrophobic (SH) transcription unit between M and G genes, and the corresponding transcript was found in the infected cells. The combined transmembrane topology and signal peptide prediction algorithms identified the SH as a type I transmembrane protein with a signal peptide, a small extracellular domain, a transmembrane region, and a cytoplasmic tail. Furthermore, the overlapping ORF in the TUPV P mRNA has the potential to code for a 221-amino-acid C protein. Based on the N gene phylogeny, TUPV is mostly related to unassigned viruses African Kolongo virus and Sandjimba virus, both isolated from birds (Figure 6). In other analyses, where limited fragments of the L gene were compared, TUPV demonstrated relatedness to Humpty Doo virus, isolated from gnats in Australia. Therefore, viruses related to TUPV may be distributed quite broadly.

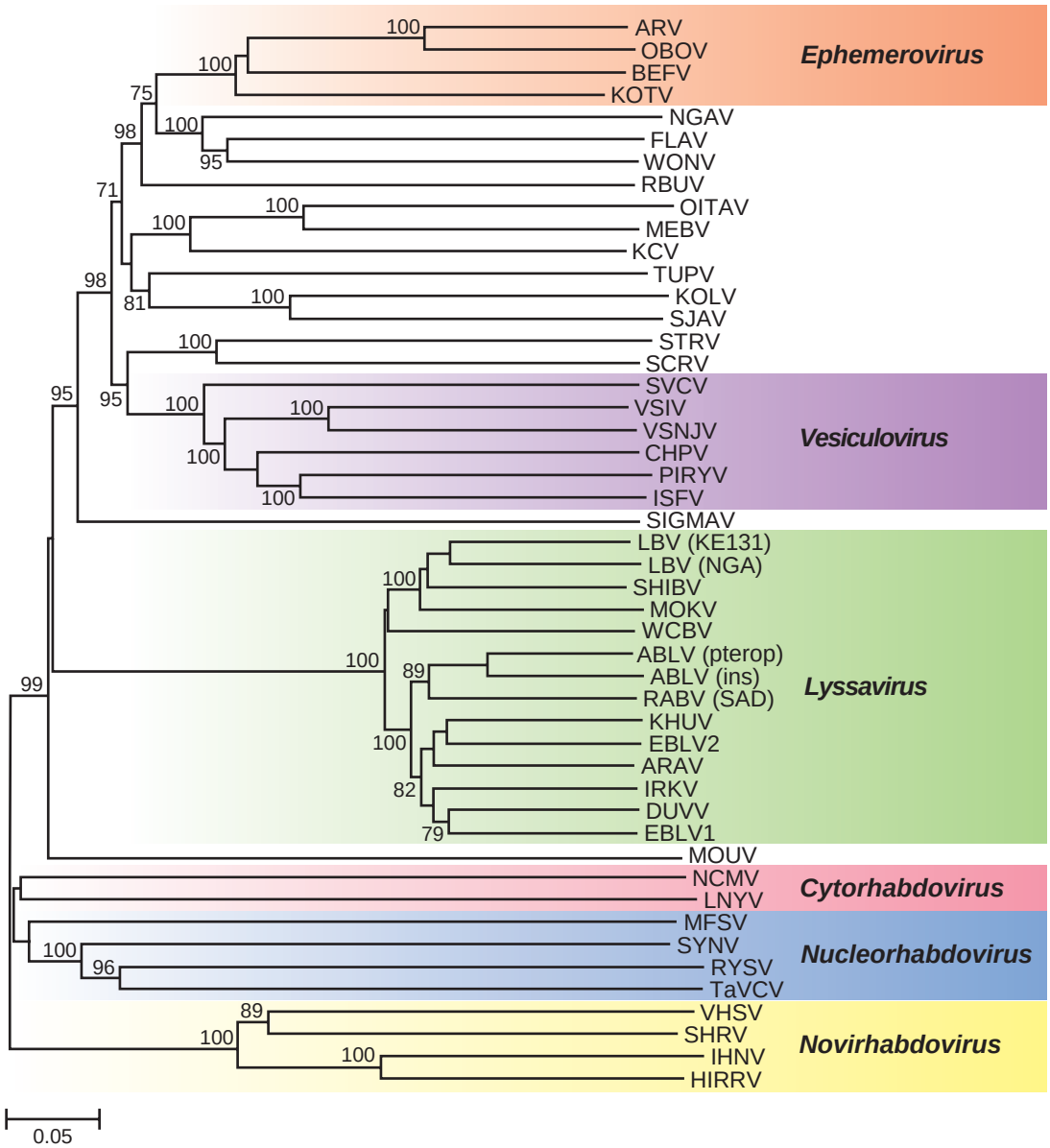



**Figure 6:** Phylogenetic tree of viruses in the *Rhabdoviridae* based on alignment of nucleoprotein gene sequences. The tree was generated by the neighbor-joining method using 1000 bootstrap replicates.

## List of other related animal viruses which may be members of the family *Rhabdoviridae* but have not been approved as species

| | | | |
|---|---|---|---|
| Almpiwar virus | {*Ablepharus boutonii (lizard)*} | [AY854645*] | (ALMV) |
| Aruac virus | {*Trichoprosopon theobaldi* (mosquito)} | | (ARUV) |
| Bahia Grande virus | {*Aedes sollicitans*} | | (BGV) |
| Bangoran virus | {*Culex perfuscus*} | | (BGNV) |
| Barur virus | {*Rattus rattus wroughtoni*} | | (BARV) |
| Bimbo virus | {*Euplectes afra* (bird)} | | (BBOV) |
| Bivens Arm virus | {*Culicoides insignis* (midge)} | | (BAV) |
| Blue crab virus | | | (BCV) |

| | | | |
|---|---|---|---|
| Chaco virus | {*Ameiva ameiva* (lizard)} | | (CHOV) |
| Charleville virus | {*Phlebotomus spp.* (sandfly)} | [AY854644*] | (CHVV) |
| Coastal Plains virus | {*Bos taurus*} | [GQ294473] | (CPV) |
| Connecticut virus | {*Ixodes tentatus* (tick)} | | (CNTV) |
| Cuiaba virus | {*Bufo marinus* (toad)} | | (CUIV) |
| Curionopolis virus | {*Culicoides* spp.} | | (CURV) |
| DakArK 7292 virus | {*Amblyomma variegatum* (tick)} | | (DAKV-7292) |
| Durham virus | {*Fulica americana* (bird)} | | (DURV) |
| Entamoeba virus | | | (ENTV) |
| Farmington virus | {bird} | | (FARV) |
| Fukuoka virus | {*Culicoides punctatus*} | [AY854651*] | (FUKAV) |
| Garba virus | {*Corythornis cristata* (bird)} | | (GARV) |
| Gossas virus | {*Tadarita* sp. (bat)} | | (GOSV) |
| Harlingen virus | {*Culex* spp . (mosquitoes)} | | (HARV) |
| Hart Park virus | {*Culex tarsalis*} | | (HPV) |
| Humpty Doo virus | {*Lasiohelea* sp.(midge)} | [AY854643*] | (HDOOV) |
| Iriri virus | {*Lutzomyia* spp. (sandfly)} | | (IRIV) |
| Inhangapi virus | {*Lutzomyia flaviscutellata*} | | |
| Itacaiunas virus | {*Culicoides* spp. (midge)} | | (ITAV) |
| Joinjakaka virus | {*Mixed Culicine* mosquitoes} | | (JOIV) |
| Kamese virus | {*Culex annulioris*} | | (KAMV) |
| Kannamangalam virus | {*Corvus splendens* (crow)} | | (KANV) |
| Kern Canyon virus | {*Myotis yumanensis* (bat)} | [DQ457101*] | (KCV) |
| Keuraliba virus | {*Tatera kempi* (gerbil)} | | (KEUV) |
| Kolongo virus | {*Euplecies afra* (bird)} | [DQ457100*] | (KOLV) |
| Koolpinyah virus | {*bovine*} | | (KOOLV) |
| Landjia virus | {*Riparia paludicola* (bird)} | | (LJAV) |
| Le Dantec virus | {human} | [AY854650*] | (LDV) |
| Manitoba virus | {*Culex tarsalis*} | | (MNTBV) |
| Marco virus | {*Ameiva ameiva*} | | (MCOV) |
| Morreton virus | {*Lutzomyia* sp.} | | (MRTV) |
| Mosqueiro virus | {*Culex portesi*} | | (MQOV) |
| Mossuril virus | {*Culex sitiens*} | | (MOSV) |
| Mount Elgon bat virus | {*Rhinolophus* sp. (bat)} | [DQ457103*] | (MEBV) |
| Moussa virus | {*Culex decens*} | [FJ985748] | (MOUV) |
| Muir Springs virus | {*Aedes* sp. (mosquitoes)} | | (MSV) |
| Nasoule virus | {*Andropadus virens* (bird)} | | (NASV) |
| Navarro virus | {*Cathartes aura* (bird)} | | (NAVV) |
| New Minto virus | {*Haemaphysalis leporispalustris* (tick)} | | (NMV) |
| Nkolbisson virus | {*Eretmapodites leucopus* (mosquito)} | | (NKOV) |
| Oak-Vale virus | {*Culex* spp.} | [GQ294474*] | (OVRV) |
| Oita virus | {*Rhinolophus cornutus* (bat)} | [AB116386*] | (OITAV) |
| Ouango virus | {*Sitagra melanocephala* (bird)} | | (OUAV) |
| Parry Creek virus | {*Culex annulirostris*} | [AY854647*] | (PCRV) |
| Reed Ranch virus | {*Culex* sp.} | | (RRV) |
| Rio Grande cichlid virus | | | (RGRCV) |
| Rochambeau virus | {*Coquillettidia albicosta*} | [DQ457104*] | (RBUV) |
| Sandjimba virus | {*Acrocephalus schoenobaenus* (bird)} | [DQ457102*] | (SJAV) |
| Sawgrass virus | {*Dermacentor variabilis*} | | (SAWV) |
| Sea trout rhabdovirus | | [AF434992*] | (STRV) |
| Sena Madureira virus | {*Ameiva ameiva*} | | (SMV) |
| Siniperca chuatsi rhabdovirus | | [DQ399789] | (SCRV) |
| Sripur virus | {*Sergentomyia* spp. (sandflies)} | | (SRIV) |
| Sweetwater Branch virus | | | (SWBV) |
| Tibrogargan virus | {*Culicoides brevitarsis*} | [GQ294472] | (TIBV) |
| Timbo virus | {*Ameiva ameiva*} | | (TIMV) |
| Xiburema virus | {*Sabethes intermedius*} | | (XIBV) |
| Yata virus | {*Mansonia uniformis*} | | (YATAV) |

Virus names are in roman script. Sequence accession numbers [ ], natural vector species { } and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome. For most of the listed viruses, no biochemical characterization has been reported. Their listing here is largely based on the distinctive morphology of their virions.

## List of other related plant viruses which may be members of the family *Rhabdoviridae* but have not been approved as species

| | | |
|---|---|---|
| Atropa belladonna virus | | (AtBV) |
| Beet leaf curl virus | {lacebug} | (BLCV) |
| Callistephus chinensis chlorosis virus | | (CCCV) |
| Carnation bacilliform virus | | (CBV) |
| Carrot latent virus | {aphid} | (CtLV) |
| Cassava symptomless virus | | (CsSLV) |
| Chrysanthemum frutescens virus | | (CFV) |
| Chrysanthemum vein chlorosis virus | | (CVCV) |
| Clover enation virus | | (ClEV) |
| Colocasia bobone disease virus | {planthopper} | (CBDV) |
| Coriander feathery red vein virus | {aphid} | (CFRVV) |
| Cow parsnip mosaic virus | | (CPaMV) |
| Cynara virus | | (CraV) |
| Dendrobium leaf streak virus | | (DLSV) |
| Digitaria striate virus | {planthopper} | (DiSV) |
| Euonymus fasciation virus | | (EFV) |
| Finger millet mosaic virus | {planthopper} | (FMMV) |
| Gerbera symptomless virus | | (GeSLV) |
| Gomphrena virus | | (GoV) |
| Holcus lanatus yellowing virus | | (HLYV) |
| Iris germanica leaf stripe virus | | (IGLSV) |
| Ivy vein clearing virus | | (IVCV) |
| Laelia red leafspot virus | | (LRLV) |
| Launea arborescens stunt virus | | (LArSV) |
| Lemon scented thyme leaf chlorosis virus | | (LSTCV) |
| Lolium ryegrass virus | | (LoRV) |
| Lotus stem necrosis | | (LoSNV) |
| Lotus streak virus | {aphid} | (LoSV) |
| Lucerne enation virus | {aphid} | (LEV) |
| Lupin yellow vein virus | | (LYVV) |
| Maize streak dwarf virus | | (MSDV) |
| Malva silvestris virus | | (MaSV) |
| Melilotus latent virus | | (MeLV) |
| Melon variegation virus | | (MVV) |
| Parsley virus | | (PaV) |
| Phalaenopsis chlorotic spot virus | | (PhCSV) |
| Pigeon pea proliferation virus | {leafhopper} | (PPPV) |
| Pineapple chlorotic leaf streak virus | | (PCLSV) |
| Pisum virus | | (PisV) |
| Plantain mottle virus | | (PIMV) |
| Ranunculus repens symptomless virus | | (RaRSV) |
| Raphanus virus | | (RaV) |
| Raspberry vein chlorosis virus | {aphid} | (RVCV) |
| Red clover mosaic virus | | (RCIMV) |
| Saintpaulia leaf necrosis virus | | (SLNV) |
| Sambucus vein clearing virus | | (SVCV) |
| Sarracenia purpurea virus | | (SPV) |
| Soursop yellow blotch virus | | (SYBV) |
| Triticum aestivum chlorotic spot virus | | (TACSV |
| Vigna sinensis mosaic virus | | (VSMV) |
| Winter wheat Russian mosaic virus | {planthopper} | (WWMV) |
| Zea mays virus | | (ZMV) |

Virus names are in roman script. Natural vector species { } and assigned abbreviations ( ) are also listed.

There are many putative plant rhabdoviruses. Many of these agents have not been characterized beyond electron microscopic observations in infected plants and occasionally in their vectors. Hence, their listing here relies on morphological criteria. Some have been transmitted experimentally by mechanical means and by their vectors.

## Phylogenetic relationships within the family

Molecular phylogenies determined by using N, G, or L protein sequences suggest a monophyletic origin and support the assignment of the six established genera and the species within these genera. For P and G proteins, relatively low sequence identities across the family prevent the construction of a universal phylogeny. N and L protein sequences are most highly conserved (see Figure 6). Both phylogenetic analyses indicate that vesiculoviruses and ephemeroviruses are the most closely related of the established genera and together with some currently non-classified viruses may be considered as members of a phylogenetic supergroup named "Dimarhabdovirus" (dipteran-mammal associated rhabdovirus).

## Similarity with other taxa

Rhabdoviruses share several features with viruses of the families *Filoviridae*, *Paramyxoviridae* and *Bornaviridae* in the order *Mononegavirales*. Features they have in common include the non-segmented negative sense, single strand, non-infectious RNA genome, the helical nucleocapsid with the genome template intimately associated with the nucleoprotein (RNase resistant), the initiation of primary transcription by a virion-associated RdRp, similar gene order and single 3′ promoter with short terminal untranscribed regions and intergenic regions. The virions are large enveloped structures with a prominent fringe of spikes. They generally replicate in the cytoplasm (except nucleorhabdoviruses). They mature by budding, predominantly from the plasma membrane with the exception of RABV which buds occasionally from internal membranes and plant rhabdoviruses of the genus *Nucleorhabdovirus* which bud from the inner nuclear membrane. They transcribe discrete unprocessed messenger RNAs for which they ensure 5′-capping and 3′-polyadenylation. Polymerase amino acid sequence similarities with those of plant-infecting viruses outside the *Mononegavirales* include viruses in the genera *Ophiovirus* and *Varicosavirus* and the unclassified orchid fleck virus.

## Derivation of names

*Cyto*: from Greek *kytos*, "cell".
*Ephemero*: from Greek ephemeros, "short-lived".
*Lyssa*: from Greek *lyssa*, "rage, fury, canine madness".
*Novi*: from *non-vi*rion protein.
*Nucleo*: from Latin *nux*, *nucis*, "nut".
*Rhabdo*: from Greek *rhabdos*, "rod".
*Vesiculo*: from Latin *vesicula*, diminutive of *vesica*, "bladder, blister".

## Further reading

### Books and journals

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**Websites**

The Centers for Disease Control and Prevention, Rabies program: http://www.cdc.gov/rabies
IHNV epidemiology and genetic typing database: http://gis.nacse.org/ihnv
North American VHSV epidemiology and genetic typing database: http://gis.nacse.org/vhsv
Fish Pathogens EU database of genetic data on fish viruses IHNV and VHSV: http://www.fish pathogens.eu/vhsv/index.php

## Contributed by

Dietzgen, R.G., Calisher, C.H., Kurath, G., Kuzmin, I.V., Rodriguez, L.L., Stone, D.M., Tesh, R.B., Tordo, N., Walker, P.J., Wetzel, T. and Whitfield, A.E.

# Family *Arenaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Arenaviridae* |
| Genus | *Arenavirus* |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Arenavirus*

Type species *Lymphocytic choriomeningitis virus*

## Virion properties

### Morphology

Virions are spherical to pleomorphic, 50–300 nm in diameter (mean 110–130 nm), with a dense lipid envelope and a surface layer covered by club-shaped projections, 8–10 nm in length. A variable number of 20–25 nm ribosomes are generally present within virus particles. Isolated nucleocapsids, free of contaminating host ribosomes, are organized in closed circles of varying length (450–1300 nm), which have been shown to assume supercoiled forms, and display a linear array of nucleosomal subunits.

### Physicochemical and physical properties

Virion Mr has not been determined. The $S_{20,w}$ is 325–500. The buoyant density in sucrose is about 1.17–1.18 g cm$^{-3}$, in CsCl it is about 1.19–1.20 g cm$^{-3}$, in amidotrizoate compounds it is about 1.14 g cm$^{-3}$. Virions are relatively unstable *in vitro*, and are rapidly inactivated below pH 5.5 and above pH 8.5. Virus infectivity is inactivated at 56 °C, by treatment with organic solvents or detergents, or by exposure to UV- and gamma-irradiation.

### Nucleic acid

The genome consists of two single stranded, ambisense RNA molecules, L and S, of lengths of about 7.5 kb and 3.5 kb, respectively. There are no poly(A) tracts at the 3′ termini. The 3′-terminal sequences (19–30 nucleotides) are similar in the two RNAs and among different arenaviruses. Overall, they are largely complementary to the 5′-end sequences. Although the RNA genomic species are thought to be present in virions in the form of circular nucleocapsids, the genomic RNA is not covalently closed. Variable amounts of full-length viral-complementary RNAs (predominantly S) and viral subgenomic mRNA species have been reported in virus preparations. Preparations of purified virus may also contain RNAs of cellular origin with sedimentation coefficients of 28S, 18S and 4–6S. These include ribosomal RNAs. The viral mRNA species may be associated with encapsidated ribosomes, though another possibility is that the dense inclusion bodies seen in virions are related to self-assembling Z bodies. The RNA species are not present in equimolar amounts, apparently due to the packaging of multiple RNA molecules per virion. For example, virions may package more than one S RNA molecule, as well as RNA molecules with complementary sequences.

### Proteins

The most abundant structural protein is the nucleoprotein (N or NP), a non-glycosylated polypeptide (ca. 63 kDa) found tightly associated with the virus genomic RNA in the form of a ribonucleoprotein complex or nucleocapsid structure. A minor component is the L protein, an RNA polymerase (ca. 200 kDa). A zinc binding protein (Z or p11; 10–14 kDa) is also a structural component of the virus, and functions as a matrix protein (with roles in virus assembly/disassembly and inhibiting transcription). Two glycosylated proteins (GP1 or G1, GP2 or G2; 34–44 kDa) are found in all members of the family and are derived by posttranslational cleavage from an intracellular precursor, GPC (ca. 75–76 kDa). A stable signal peptide (SSP) cleaved during GPC synthesis is also in the virion spike. Other minor proteins and enzymatic activities have been described associated with

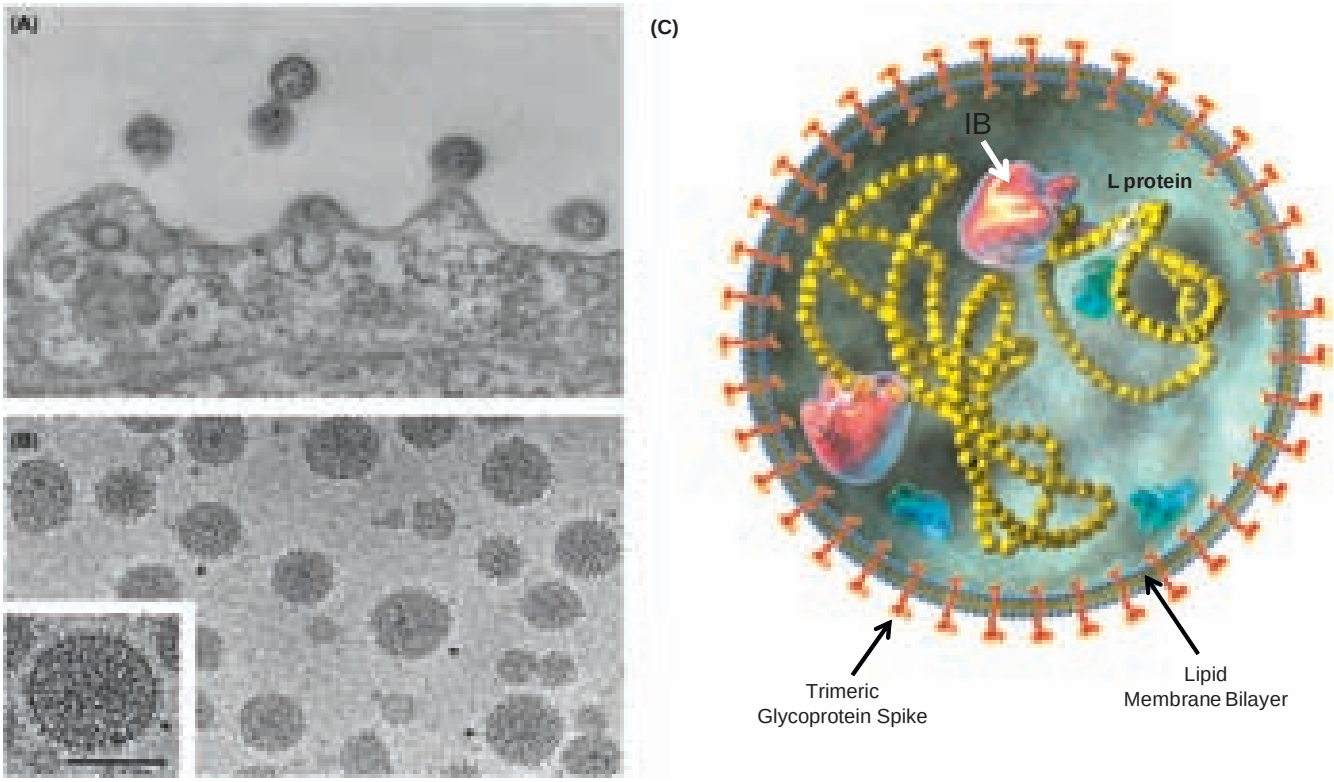


**Figure 1:** (Left) Electron microscopic images of lymphocytic choriomeningitis virus (LCMV). (A) Thin section showing several virions budding from the surface of an infected BHK-21 cell. (B) Cryo-electron microscopic images of purified unstained virions frozen in vitreous ice. Arrowheads indicate glycoprotein spikes which are composed of trans-membrane GP2 and globular heads of GP1. The bar indicates 100 nm. (Courtesy R. Milligan, J. Burns and M. Buchmeier). (C) Diagrammatic representation of virion structure with trimeric spikes (Eschli et al., 2006; Schlie et al., 2010). L protein is the RNA polymerase; IB is inclusion bodies that could be ribosomes or could be related to self-assembling Z bodies (Kentsis *et al.*, 2002). (Courtesy C. Clegg and A. Featherstone, asrf@researchgraphix.co.uk.)

virions including poly (U) and poly (A) polymerases, and a protein kinase that can phosphorylate N. It is thought unlikely that these are virally encoded.

### Lipids

Lipids represent about 20% of virion dry weight and are similar in composition to those of the host plasma membrane.

### Carbohydrates

Carbohydrates in the form of complex glycans on GP1 (five or six sites in lymphocytic choriomeningitis virus [LCMV]) and GP2 (2 sites in LCMV) represent about 8% of virion dry weight.

## Genome organization and replication

The L and S RNAs of arenaviruses each have an ambisense coding arrangement (Figure 2). The L RNA encodes in its viral-complementary sequence the L protein, and in the viral-sense 5′-end sequence the Z protein. The Z mRNA is small (<0.5 kb). The N protein is encoded in the viral-complementary sequence corresponding to the 3′-half of the S RNA, while the viral glycoprotein precursor (GPC) is encoded in the viral-sense sequence corresponding to the 5′-half of the S RNA. The two proteins are made from subgenomic mRNA species transcribed from the viral (for N mRNA) or full-length viral-complementary S RNA species (for GPC mRNA). The intergenic regions of both S and L RNAs contain nt sequences with the potential of forming one or more hairpin configurations. These secondary structural features may function to terminate mRNA transcription from the viral and viral-complementary S RNAs. The mRNAs are capped and contain 1-5

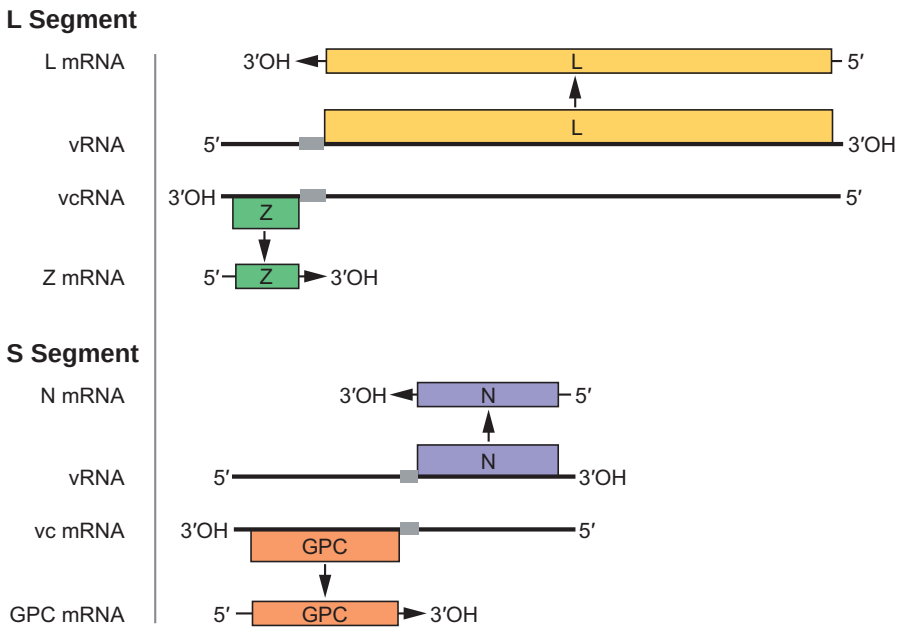


**Figure 2:** Organization, transcription and replication of the arenavirus L and S RNAs. Regions encoding the L, Z, GPC and N proteins are shown as boxes with arrowheads indicating the notional direction of translation. The intergenic regions separating the ORFs are indicated by gray boxes. RNA transcription processes are indicated by solid arrows.

non-templated nt of heterogeneous sequence at their 5′ ends. The mRNAs are not polyadenylated. The transcription mechanism is not fully elucidated. Initiation of transcription may involve cap-snatching. The 3′ termini of the mRNAs have been mapped to locations in the intergenic regions.

The process of infection involves attachment to cell receptors, entry via the endosomal route, uncoating and mRNA transcription in the cytoplasm of infected cells. Because of the ambisense coding arrangement, only N and L mRNAs can be synthesized from the genomic RNAs by the virion polymerase prior to translation. The products of these mRNAs are presumed to be involved in the synthesis of full-length viral complementary species which serve as templates for the synthesis of GPC and Z mRNAs and the synthesis of full-length viral RNAs. The process of RNA replication, which may involve a slippage mechanism during initiation, and readthrough of transcription termination signals, has not been fully elucidated. However, the presence of full-length viral-complementary genomic RNAs and viral sgRNA species in virus preparations may affect this perceived temporal order of RNA and protein synthesis.

The viral envelope glycoproteins are synthesized in cells as a single mannose-rich precursor molecule that is proteolytically cleaved and processed to contain complex glycans during transport to the plasma membrane. First, its signal peptide is co-translationally cleaved and mediates the cleavage of GPC into GP1 and GP2 by SKI protease as well as the assembly of virions. Virions mature by budding at sites on the surface of cells. Ribosomes are also observed at such sites. Interstrain reassortant progeny can be formed, including diploid (or multiploid) species with respect to the genomic RNA segments. Evidence for interspecies reassortment between Lassa virus (LASV) and Mopeia virus has also been obtained. Ribavirin inhibits the replication of several arenaviruses *in vitro* and is effective in the therapy of humans and primates infected with Lassa virus at early disease stage. Several antiviral agents targeting different stages of viral replication are under evaluation.

## Antigenic properties

Viruses possess a number of distinct antigenic determinants as shown by monoclonal and polyclonal antibody analyses. Antigens on the 44 kDa GP1 of lymphocytic choriomeningitis virus

(LCMV) are involved in virus neutralization. These are type-specific, although cross-neutralization tests have demonstrated partially shared antigens between Tacaribe virus and Junín virus. Cross-protection has also been demonstrated against Junín virus following prior infection by Tacaribe virus, or against Lassa virus following infection by Mopeia virus. Major complement-fixing antigens are associated with the viral N proteins, which were used to define the Tacaribe complex of arenaviruses. Monoclonal antibodies react with common epitopes on the N proteins of all arenaviruses and a single highly conserved epitope has also been described in the transmembrane GP2 glycoprotein.

By analyses using monoclonal and polyclonal antibody, the African arenaviruses are distinguishable from the New World arenaviruses. Fluorescent antibody studies show that antisera against New World viruses, as well as those against African viruses, react with LCMV. Cytotoxic T-lymphocyte epitopes have been identified on the nucleoprotein and glycoproteins of LCMV. The number and location of epitopes varies depending on the virus strain and host MHC class I molecules. No hemagglutinin has been identified.

## Biological properties

The reservoir hosts of almost all the arenaviruses are species of rodents. LCMV is found in mouse and the African viruses mainly in the rodents *Mastomys* and *Praomys*, in the sub-family *Murinae*. The New World viruses are mostly found in the Sigmodontine rodents *Calomys*, *Neacomys*, *Neotoma*, *Oryzomys* and *Sigmodon*. Exceptionally, Tacaribe virus was isolated from fruit-eating bats (*Artibeus* spp.), but subsequent attempts to recover it from bats or from other potential hosts have been unsuccessful. It is notable that the geographic range of an arenavirus is generally much more restricted than that of its cognate rodent host. Most of the viruses induce a persistent, frequently asymptomatic infection in their reservoir hosts, in which chronic viremia and viruria occur. Such infections are known or suspected to be caused by a slow and/or insufficient host immune response. Most arenaviruses do not normally infect other mammals or humans. However, Lassa virus is the cause of widespread human infection (Lassa fever) in West Africa (Nigeria, Sierra Leone, Liberia, Guinea), LuJo virus has caused a Lassa fever-like outbreak in South Africa, and Junín virus causes Argentine hemorrhagic fever in agricultural workers in an increasingly large area of that country. Machupo virus (MACV) has caused isolated outbreaks of similar disease in Bolivia, and Guanarito virus (GTOV) is associated with human disease in Venezuela. Sabiá virus was isolated from a fatal human case in Brazil and a related virus, Chapare was isolated in Bolivia. Human infection with LCMV may occur in some rural and urban areas with high rodent populations, and has been acquired from pet hamsters. Organ transplants from LCMV-infected individuals have resulted in at least 10 human deaths since 1998. LCMV acquired from mice has also caused a highly fatal hepatitis in captive Callitrichid primates. Severe laboratory-acquired infections have occurred with LCMV, Lassa, Junín, Machupo, Sabiá and Flexal viruses. Asymptomatic infections with Pichinde virus have been reported.

Success of experimental infection in laboratory animals (mouse, hamster, guinea pig, rhesus monkey, marmoset, rat) varies with the animal species and the virus. In general, New World viruses are pathogenic for suckling but not weaned mice; LCMV and Lassa virus produce the opposite effect. Viruses grow moderately well in many mammalian cells. Receptors mediating host cell entry are thought to be alpha-dystroglycan for Old World viruses (e.g. some strains of LCMV and LASV) and Clade C New World viruses, and transferrin receptor-1 for New World viruses (MACV, GTOV, Junín virus [JUNV]); however virus entry by some strains has been observed in the absence of either receptor. Arenaviruses primarily infect cells of the myeloid and reticuloendothelial lineages but are also found in hepatocytes, lymphocytes and other cells.

Vertical and horizontal (including venereal) transmissions occur in the natural hosts. These include transuterine, transovarian and post-partum transmission and can be via milk-, saliva- or urine-borne routes. Horizontal transmission within and between host species occurs by contamination and aerosol routes. No arthropod vectors are thought to be involved in the normal transmission process.

## Species demarcation criteria in the genus

The parameters used to define a species in the genus are:

- an association with a specific host species or group of species;
- presence in a defined geographical area;
- etiological agent (or not) of disease in humans;
- significant differences in antigenic cross-reactivity, including lack of cross-neutralization activity where applicable;
- significant amino acid sequence difference from other species in the genus (i.e. showing a divergence between species of at least 12% in the nucleoprotein amino acid sequence).

For example, although both Pirital virus and Guanarito virus circulate in the same region of Venezuela, they are distinguished by their isolation from different rodent hosts (*Sigmodon alstoni* and *Zygodontomys brevicauda*, respectively). In addition, in ELISA with hyperimmune mouse ascitic fluids, titers differ by at least 64-fold, and sequence analysis shows less than 55% aa identity between partial nucleocapsid protein sequences. In another example, both Lassa virus and Mopeia virus share a common rodent host (*Mastomys*) at the genus level. However, they are distinguished by their different geographical range, different profiles of reactivity with panels of monoclonal antibodies, and by N protein aa sequence divergences of about 26%. Also, Lassa virus is the cause of hemorrhagic fever in humans and other primates, while Mopeia virus is not associated with human disease and does not cause disease in experimentally infected primates.

## List of species in the genus *Arenavirus*

**Old World arenaviruses**

*Ippy virus*

| | | |
|---|---|---|
| Ippy virus - Dak AN B 188d | [S segment: DQ328877, L segment: DQ328878] | (IPPYV) |
| *Arvicanthis* sp., Central African Republic | | |

*Lassa virus*

| | | |
|---|---|---|
| Lassa virus - GA391 | [S segment: X52400, L segment: U73034] | (LASV-GA391) |
| *Mastomys* sp., West Africa | | |

*Lymphocytic choriomeningitis virus*

| | | |
|---|---|---|
| Lymphocytic choriomeningitis virus - Armstrong 53b | [S segment: AY847350 | (LCMV-Ar53b) |
| *Mus musculus*, Europe, Americas | L segment: AY847351] | |

*Mobala virus*

| | | |
|---|---|---|
| Mobala virus - 3080 | [S segment: AY342390, L segment: DQ328876] | (MOBV-3076) |
| *Praomys* sp., Central African Republic | | |

*Mopeia virus*

| | | |
|---|---|---|
| Mopeia virus - AN 20410 | [S segment: AY772170, L segment: AY772169] | (MOPV-AN20410) |
| *Mastomys natalensis*, Mozambique, Zimbabwe | | |
| Morogoro virus | [S segment: EU914103, L segment: EU914104] | (MORV) |
| *Mastomys natalensis*, Zambia | | |

**New World arenaviruses**

*Allpahuayo virus*

| | | |
|---|---|---|
| Allpahuayo virus - CLHP-2472 | [S segment: AY012687, L segment: AY216502] | (ALLV-CLHP2472) |
| Oecomys bicolor, Oe. paricola | | |

| | | |
|---|---|---|
| *Amapari virus* | | |
| Amapari virus - BeAn 70563 | [S segment: AF485256, L segment: AY216517] | (AMAV-BeAn70563) |
| Oryzomys capito, Neacomys guianae, Brazil | | |
| *Bear Canyon virus* | | |
| Bear Canyon virus - A0070039 | [S segment: AY924391, L segment: AY924390] | (BCNV-A0060209) |
| *Peromyscus californicus* | | |
| *Chapare virus* | | |
| Chapare virus - 810419 | [S segment EU260463, L segment EU260464] | (CHPV-810419) |
| *Homo sapiens*, Bolivia | | |
| *Cupixi virus* | | |
| Cupixi virus - BeAn 119303 | [S segment: AF512832, L segment: AY216519] | (CPXV-BeAn 119303) |
| *Oryzomys* sp. | | |
| *Flexal virus* | | |
| Flexal virus - BeAn 293022 | [S segment: AF512831, L segment: EU627611] | (FLEV-BeAn293022) |
| *Oryzomys* spp., Brazil | | |
| *Guanarito virus* | | |
| Guanarito virus - INH-95551 | [S segment: AY129247, L segment: AY358024] | (GTOV-INH95551) |
| *Zygodontomys brevicauda*, Venezuela | | |
| *Junín virus* | | |
| Junín virus - XJ13 | [S segment: AY358023, L segment: AY358022] | (JUNV-XJ13) |
| *Calomys musculinus*, Argentina | | |
| *Latino virus* | | |
| Latino virus - 10924 | [S segment: AF485259, L segment: EU627612] | (LATV-10924) |
| *Calomys callosus*, Bolivia | | |
| *Machupo virus* | | |
| Machupo virus - Carvallo | [S segment: AY129248, L segment: AY358021] | (MACV-Carvallo) |
| *Calomys callosus*, Bolivia | | |
| *Oliveros virus* | | |
| Oliveros virus - RIID 3229 | [S segment: U34248, L segment: AY216514] | (OLVV-RIID3229) |
| *Bolomys obscurus*, Argentina | | |
| *Paraná virus* | | |
| Paraná virus - 12056 | [S segment: AF485261, L segment: EU627613] | (PARV-12056) |
| *Oryzomys buccinatus*, Paraguay | | |
| *Pichinde virus* | | |
| Pichinde virus - 3739 | [S segment: K02734, L segment: AF427517] | (PICV-3739) |
| *Oryzomys albigularis*, Colombia | | |
| *Pirital virus* | | |
| Pirital virus - VAV488 | [S segment: AF485262, L segment: AY494081] | (PIRV-VAV488) |
| *Sigmodon alstoni*, Venezuela | | |
| *Sabiá virus* | | |
| Sabiá virus - SPH114202 | [S segment: U41071, L segment: AY358026] | (SABV-SPH114202) |
| Natural host unknown, Brazil | | |

| | | |
|---|---|---|
| *Tacaribe virus* | | |
| Tacaribe virus - p2b2<br>*Artibeus* spp., Trinidad | [S segment: M20304 ] | (TCRV-p2b2) |
| Tacaribe virus - TRVLII573<br>*Artibeus* spp., Trinidad | [L segment: J04340] | (TCRV-TRVLII573) |
| *Tamiami virus* | | |
| Tamiami virus - W10777<br>*Sigmodon hispidus*, Florida, USA | [S segment: AF485263, L segment: AY924393] | (TAMV-W10777) |
| *Whitewater Arroyo virus* | | |
| Whitewater Arroyo virus – AV 9310135<br>*Neotoma albigula*, New Mexico, USA | [S segment: AF228063, L segment: AY924395] | (WWAV-AV9310135) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Arenavirus* but have not been approved as species

| | | |
|---|---|---|
| Lujo virus<br>(Lusaka/Johannesburg virus) | [S segment: FJ952384, L segment: FJ952385] | (LUJV) |
| Kodoko virus<br>*Mus minutoides*/Africa | [N gene: EF189586*, L segment: EF179864*] | (KODV-TA777, KD42) |
| Dandenong virus<br>Found in an Australian transplant recipient of an organ from an eastern European donor | [S segment: EU136038, L segment: EU136039] | (DANV) |
| Merino Walk virus<br>*Myotomys unisulcatus*/S Africa | [S segment: GU078660, L segment: GU078661] | (MWV) |

*Partial sequences.

## Phylogenetic relationships within the family *Arenaviridae*

Nucleic acid sequences from the N genes of all the known arenaviruses have provided the basis for phylogenetic analysis that supports previously defined antigenic groupings and further defines virus relationships within them. Sequence data derived from other regions of the genome including the L gene are largely consistent with this analysis (Figure 3). Among the Old World viruses, Lassa virus, Mopeia virus and Mobala virus are monophyletic, while Ippy virus and lymphocytic choriomeningitis virus are more distantly related. One interesting virus, LuJo virus, found in South Africa, is most related to Old World viruses but contains elements of New World sequence in its glycoprotein. The New World viruses can be divided into three groups on the basis of the sequence data. In group A are Pirital virus, Pichinde virus, Paraná virus, Flexal virus, and Allpahuayo (Peru) virus from South America, together with Tamiami virus, Whitewater Arroyo virus and Bear Canyon virus from North America. Group B contains the human pathogenic viruses Machupo virus, Junín virus, Guanarito virus, Sabiá virus and Chapare virus as well as the non-pathogenic Tacaribe virus, Amapari virus, and Cupixi virus (from Brazil). Latino virus and Oliveros virus form a small separate group (group C). The division of the arenaviruses into Old World and New World groups, as well as the subdivision of New World arenaviruses into three groups, is strongly supported by bootstrap resampling analysis. It is important to note that the trait of human pathogenicity appears to have arisen on at least two independent occasions during arenavirus evolution.

Recombination may have influenced the evolution of arenaviruses. The nucleocapsid and glycoprotein genes of Whitewater Arroyo virus, Tamiami virus, and the Bear Canyon virus have divergent phylogenetic histories. Separate analysis of full-length amino acid sequences using maximum parsimony or neighbor-joining methods show that the nucleocapsid protein genes of these three viruses are related to those of Pichinde virus and Pirital virus (New World lineage A), while the

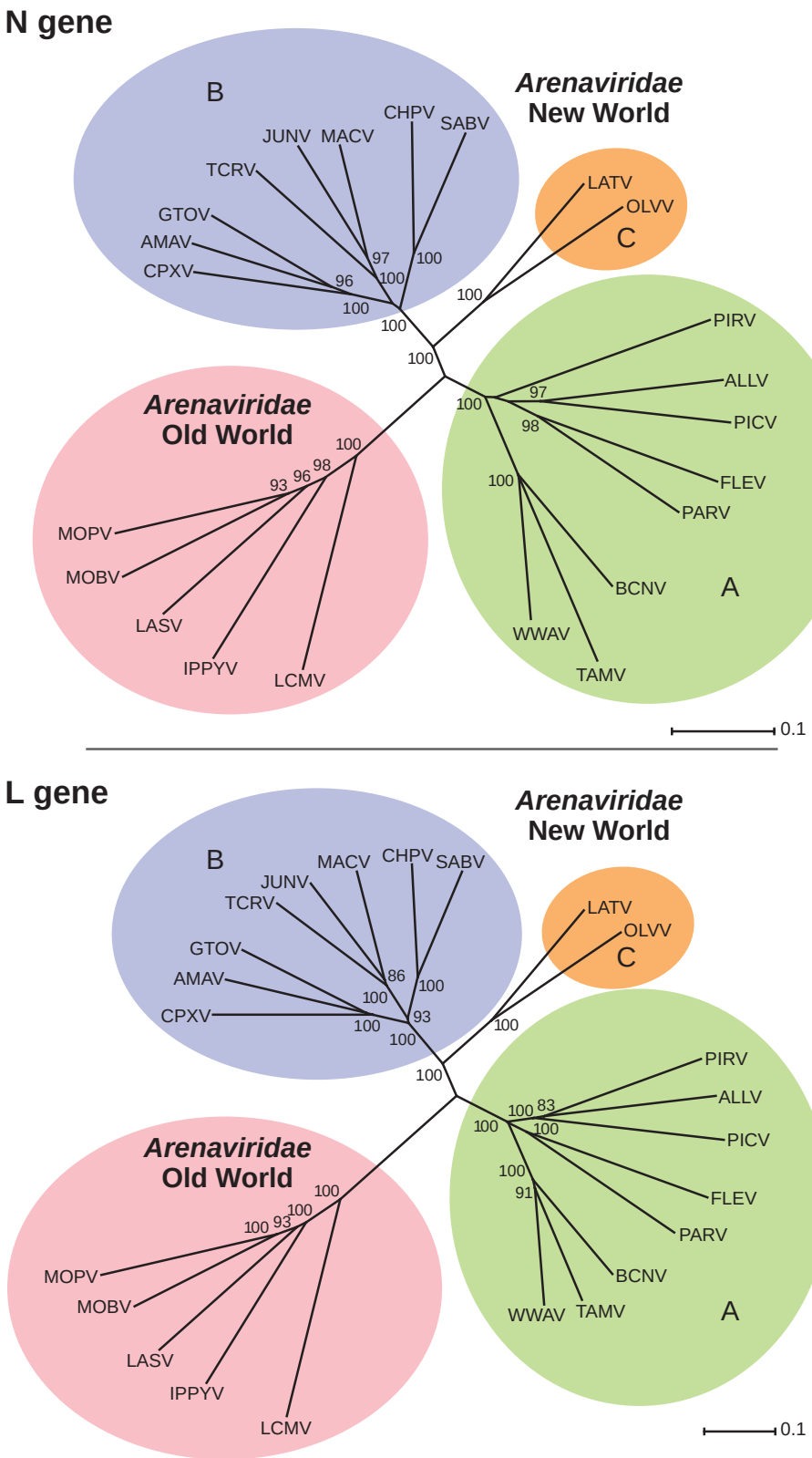


**Figure 3:** Phylogenetic relationships among the *Arenaviridae*. N and L gene codon-aligned nt sequences of an isolate of each species (Table 1) were analyzed in MEGA 4 (maximum composite likelihood distances and 10,000 bootstrap replicates). Bootstrap values are shown at the branches if >80%.

glycoprotein genes are more closely related to those of Junín virus, Tacaribe virus, and Sabia virus (New World lineage B).

## Similarity with other taxa

The *Arenaviridae* are unique amongst the negative strand viruses for their bi-segmented genome with ambisense coding strategy. Arenaviruses are most similar to the *Bunyaviridae*, another segmented negative-strand RNA virus family, whose members have three genome segments, some of which also encode genes in both senses.

## Derivation of name

*Arena*: from Latin *arenosus*, "sandy" and *arena*, "sand", in recognition of the sand-like particles observed in thin section. The name originally proposed was arenovirus, but was subsequently changed to avoid possible confusion with adenovirus.

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## Contributed by

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# Family *Bunyaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Bunyaviridae*** |
| Genus | ***Orthobunyavirus*** |
| Genus | ***Hantavirus*** |
| Genus | ***Nairovirus*** |
| Genus | ***Phlebovirus*** |
| Genus | ***Tospovirus*** |

## Virion properties

### Morphology

Morphological properties vary among viruses in each of the five genera; however, virions generally are spherical or pleomorphic, 80–120 nm in diameter, and display surface glycoprotein projections of 5–10 nm which are embedded in a lipid bilayered envelope approximately 5 nm thick. Virion envelopes are usually derived from cellular Golgi membranes, or on occasion, from cell surface membranes. Viral ribonucleocapsids are 2–2.5 nm in diameter, 200–3000 nm in length, and usually (but not always) display helical symmetry. (See Figure 1.)

### Physicochemical and physical properties

The virion Mr is 300 $\times 10^6$ to 400 $\times 10^6$ and has an $S_{20,W}$ of 350–500. Virion buoyant densities in sucrose and CsCl are 1.16–1.18 and 1.20–1.21 g cm$^{-3}$, respectively. Virions are sensitive to heat, lipid solvents, detergents and formaldehyde.

### Nucleic acid

The viral genome comprises three unique molecules of negative or ambisense ssRNA, designated L (large), M (medium) and S (small), which total 11–19 kb (Table 1). The terminal nucleotides of each genome RNA segment are base-paired forming non-covalently closed, circular RNAs (and ribonucleocapsids). The terminal sequences of genome segments are conserved among viruses in each genus but are different from those of viruses in other genera. The genomic RNAs are not modified



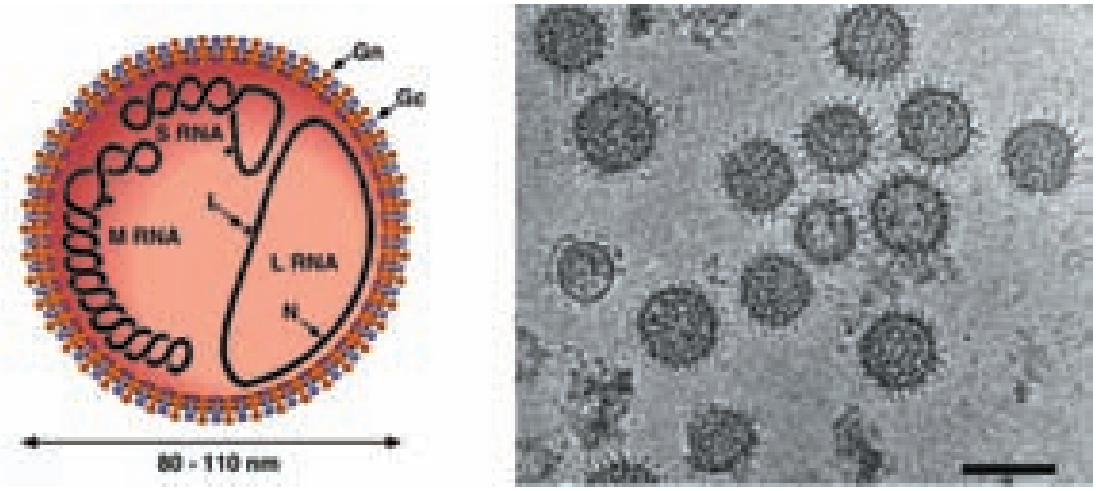


**Figure 1:** (Left) Diagrammatic representation of an orthobunyavirus virion in cross-section. The surface spikes comprise two glycoproteins termed Gn and Gc (previously referred to as G1 and G2). The three helical nucleocapsids are circular and comprise one each of the unique ssRNA segments (L, large; M, medium; S, small) encapsidated by N protein and associated with the L protein (courtesy of R. Pettersson). (Right) Cryo-electron micrograph of particles of California encephalitis virus strain La Crosse virus, taken with large defocus value which demonstrates the glycoprotein spikes (courtesy of B.V.V. Prasad; see Elliott, 1996).

**Table 1:** Nucleotide lengths of selected completely sequenced genomes

| *Genus* | RNA segment | | |
|---|---|---|---|
| **Virus** | **L** | **M** | **S** |
| *Orthobunyavirus* | | | |
| Bunyamwera virus | 6875 | 4458 | 961 |
| California encephalitis virus - La Crosse virus | 6980 | 4526 | 980 |
| *Hantavirus* | | | |
| Hantaan virus - 76-118 | 6533 | 3616 | 1696 |
| Seoul virus - HR80-39 | 6530 | 3651 | 1796 |
| Puumala virus - Sotkamo | 6550 | 3682 | 1830 |
| Sin Nombre virus - NMH10 | 6562 | 3696 | 2059 |
| *Nairovirus* | | | |
| Dugbe virus | 12255 | 4888 | 1712 |
| Crimean-Congo hemorrhagic fever virus (IbAr10200) | 12160 | 5366 | 1672 |
| *Phlebovirus* | | | |
| Rift Valley fever virus | 6404 | 3884 | 1690 |
| Sandfly fever Naples virus - Toscana virus | 6404 | 4215 | 1869 |
| Uukuniemi virus | 6423 | 3231 | 1720 |
| *Tospovirus* | | | |
| Tomato spotted wilt virus | 8897 | 4821 | 2916 |
| Impatiens necrotic spot virus | 8776 | 4972 | 2992 |

at their 5′ ends. The Mr of the genome ranges from $4.8 \times 10^6$ to $8 \times 10^6$ and this constitutes 1–2% of the virion by weight. Viral mRNAs are not polyadenylated and are truncated relative to the genomic RNAs at the 3′ termini. mRNAs have 5′-methylated caps and 10–18 non-templated nt at the 5′ end which are derived from host-cell mRNAs.

### Proteins

All viruses have four structural proteins, two external glycoproteins (Gn, Gc, named in accordance with their relative proximity to the amino or carboxy terminus of the polyprotein encoded by the M segment), a nucleocapsid protein (N) and a large (L) protein, an RNA-dependent RNA polymerase. Non-structural proteins are expressed from the S segments of some bunyaviruses, phleboviruses, tospoviruses and some hantaviruses, and from the M segments of bunyaviruses, nairoviruses, tospoviruses and some phleboviruses. Proteins encoded by each of the genome segments of viruses in each genus of the family are listed in Table 2.

### Lipids

Virions contain 20–30% lipids by weight. Lipids are derived from the membranes where viruses mature and include phopholipids, sterols, fatty acids and glycolipids.

### Carbohydrates

Virions contain 2–7% carbohydrate by weight. Asparagine-linked sugars on the Gn and Gc proteins are largely of the high mannose type when viruses are grown in vertebrate cells.

## Genome organization and replication

The genome organization of the different genera is shown in Figure 2. For all viruses, the L, M and S genome segments encode, respectively, the viral RNA polymerase (L protein), envelope glycoproteins (Gn and Gc) and nucleocapsid protein (N) in the virus-complementary sense RNA. The L protein is encoded in the complementary mRNA. A single, continuous ORF in the M RNA encodes the glycoproteins, and the primary gene product is co-translationally cleaved (except for nairoviruses) to give mature Gn and Gc. Hantaviruses and Uukuniemi-like phleboviruses encode no additional

**Table 2:** Deduced protein sizes (kDa)

| RNA | Genus | | | | |
|---|---|---|---|---|---|
| Protein | *Orthobunyavirus* | *Hantavirus* | *Nairovirus* | *Phlebovirus* | *Tospovirus* |
| L segment | | | | | |
| L | 259–263 | 246–247 | 459 | 238–241 | 330–332 |
| M segment | | | | | |
| Gn | 29–41 | 68–76 | 30–45 | 50–70 | 46–58 |
| Gc | 108–120 | 52–58 | 72–84 | 55–75 | 72–78 |
| NSm | 15–18 | none | 78–85, 92–115 | none or 78 | 34 |
| S segment | | | | | |
| N | 19–26 | 48–54 | 48–54 | 24–30 | 29 |
| NSs | 10–13 | None or 7–12 | none | 29–31 | 52 |

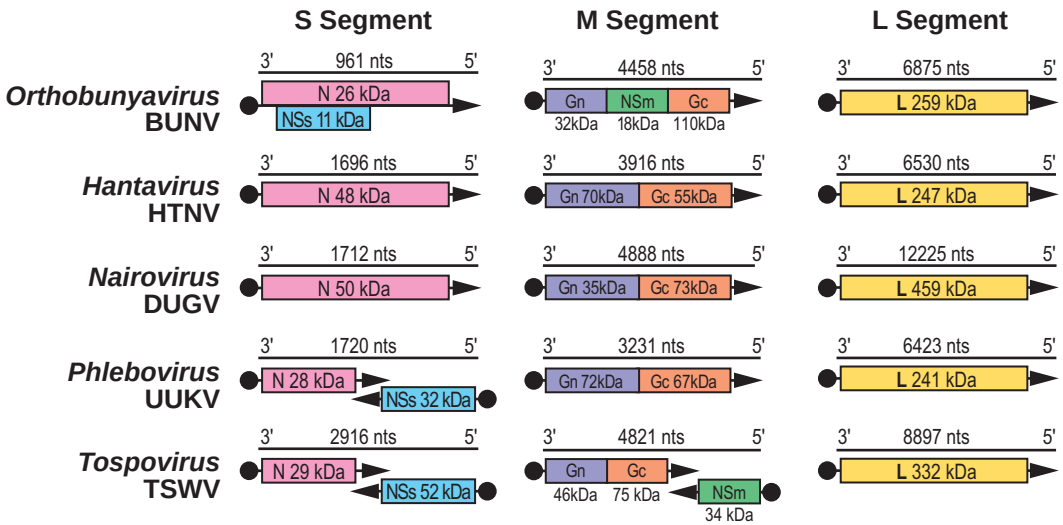


**Figure 2:** Coding strategies of genome segments of members of the family *Bunyaviridae*.

proteins in their M genome segments. Orthobunyaviruses and other phleboviruses encode a nonstructural protein (NSm) in the virion-complementary sense RNA. Nairoviruses encode two proteins of unknown functions: a mucin-rich protein and glycoprotein GP38, which are the products of posttranslational cleavage of preGn. Tospoviruses encode a NSm protein in an ambisense ORF at the 5′ end of virion-sense RNA. Some orthobunyaviruses and some hantaviruses encode a nonstructural protein (NSs) in an overlapping ORF to that encoding N in the 3′-half of the virion-sense S RNA. There is no direct evidence that nairoviruses encode any additional proteins in their S genome segments. Phleboviruses and tospoviruses encode a NSs protein in an ambisense ORF in the 5′-half of virion-sense S RNA. The NSs proteins of orthobunyaviruses, phleboviruses, and hantaviruses have been shown to act as interferon antagonists, NSs of tospoviruses as an RNAi antagonist. The NSm of phleboviruses can act as an apoptosis antagonist.

All stages of replication occur in the cytoplasm. The principal stages of replication are:

- Attachment, mediated by an interaction of one or both of the integral viral envelope proteins with, as yet unidentified, host receptors.
- Entry and uncoating, by endocytosis of virions and fusion of viral membranes with endosomal membranes.
- Primary transcription: i.e., the synthesis of mRNA species complementary to the genome templates by the virion-associated polymerase using host cell-derived capped primers (Figure 3).

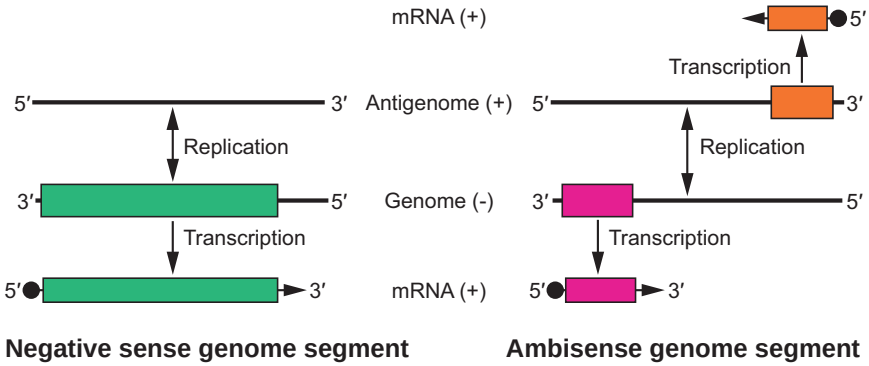


**Figure 3:** Transcription and replication scheme of genome segments of members of the family *Bunyaviridae* for a negative-strand segment (left) and for an ambisense segment (right). The genome RNA and the positive sense viral complementary RNA, known as anti-genome RNA, are only found as ribonucleoprotein complexes and are encapsidated by N protein. The mRNA species contain host-derived primer sequences at their 5′ ends (•) and are truncated at the 3′ end relative to the vRNA template; the mRNAs are not polyadenylated. For the ambisense TSWV M and S RNA-derived subgenomic transcripts, termination occurs in the intergenic region, likely due to folding of a predicted AU-rich hairpin structure in nascent transcripts.

- Translation of primary L and S segment mRNAs by free ribosomes; translation of M segment mRNAs by membrane-bound ribosomes and primary glycosylation of nascent envelope proteins. Co-translational cleavage of a precursor to yield Gn and Gc, and for some viruses, NSm.
- Synthesis and encapsidation of antigenome RNA to serve as templates for genomic RNA or, in some cases, for sgRNA.
- Genome replication (Figure 3).
- Secondary transcription; i.e., the amplified synthesis of the mRNA species and ambisense transcription.
- Morphogenesis, including accumulation of Gn and Gc in the Golgi, terminal glycosylation, acquisition of modified host membranes, generally by budding into the Golgi cisternae; budding at the cell surface has been observed with isolates of Rift Valley fever virus (genus *Phlebovirus*) in rat hepatocytes and Sin Nombre virus (genus *Hantavirus*) in polarized epithelial cells. RNPs of tomato spotted wilt virus (genus *Tospovirus*) are enwrapped by entire Golgi stacks leading to doubly enveloped virus particles. These fuse together and also with ER and lead to the formation of large ER-derived vesicles containing large amounts of mature, singly enveloped tospovirus particles.
- Fusion of cytoplasmic vesicles with the plasma membrane (except for tospoviruses) and release of mature virions.

## Antigenic properties

One or both of the envelope glycoproteins display hemagglutinating and neutralizing antigenic determinants. Complement-fixing antigenic determinants are principally associated with nucleocapsid protein.

## Biological properties

Viruses in the genera *Orthobunyavirus*, *Nairovirus* and *Phlebovirus* are capable of alternately replicating in vertebrates and arthropods, and generally are cytolytic for their vertebrate hosts, but cause little or no cytopathogenicity in their invertebrate hosts. Different viruses are transmitted by mosquitoes, ticks, phlebotomine flies, and other arthropod vectors. Some viruses display a very narrow host range, especially for arthropod vectors. No arthropod vector has been demonstrated for hantaviruses. Tospoviruses are transmitted by thrips and are capable of replicating in both thrips and plants. Transovarial and venereal transmission have been demonstrated for some viruses in their arthropod vectors. Aerosol infection occurs in certain situations or is the principal means of transmission for some viruses, particularly hantaviruses. In some instances, avian host and/or vector movements may result in virus dissemination. Some viruses cause a reduction in host-cell protein

synthesis in vertebrate cells. Hantaviruses cause no detectable reduction in host macromolecular synthesis and routinely establish persistent, non-cytolytic infections in susceptible mammalian host cells, a finding consistent with their non-pathogenic persistence in their natural rodent or insectivore hosts. In natural infections of mammals, viruses are often targeted to a particular organ or cell type. Some viruses induce cell fusion at low pH. Some members have ion-dependent hemagglutinating activity. Genetic reassortment has been demonstrated for certain members both *in vitro* and *in vivo*.

## GENUS *ORTHOBUNYAVIRUS*

Type species ***Bunyamwera virus***

### Distinguishing features

The consensus terminal nucleotide sequences of the L, M and S genome segments are UCAUCACAUG at the 3′ end and AGUAGUGUGC at the 5′ end. The N and NSs proteins are encoded in overlapping reading frames by the S RNA and are translated from the same complementary mRNA as the result of alternate AUG initiation codon usage. Both glycoproteins and an NSm protein of 15–18 kDa are encoded as a precursor polyprotein by the M RNA. Genetic reassortment has been demonstrated between viruses, belonging to the same species but not between viruses from different species.

Viruses are serologically unrelated to members of other genera. Most viruses are mosquito-transmitted though some (e.g. the Tete group) are tick-transmitted. Occasionally alternate arthropods such as culicoid flies and phlebotomines transmit orthobunyaviruses. Some viruses are transmitted transovarially and venereally in arthopods.

### Species demarcation criteria in the genus

The demarcation of orthobunyavirus species has proven difficult due to the lack of biochemical characterization of most of the named virus isolates. Species are thus primarily defined by serological criteria (cross-neutralization and cross-hemagglutination-inhibition tests). The limited available data indicate that one bunyavirus species is unable to form a reassortant with another species. Where known the aa sequences of the N proteins differ by more than 10%.



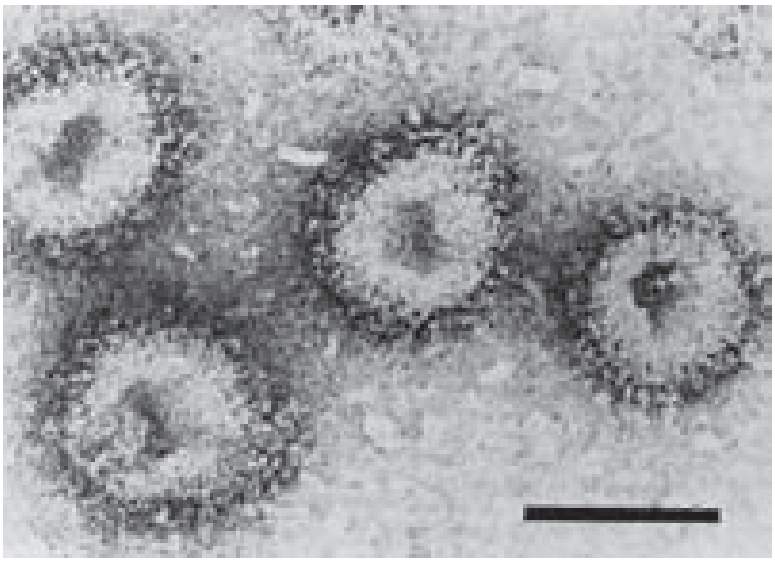

**Figure 4:** Electron micrograph of negatively stained particles of California encephalitis virus strain La Crosse virus. The bar represents 100 nm. (Courtesy of D. H. L. Bishop.)

## List of species in the genus *Orthobunyavirus*

| | | | |
|---|---|---|---|
| *Acara virus* | | | |
| Acara virus - BeAn27639 | {mosquitoes} | | (ACAV) |
| *Akabane virus* | | | |
| Akabane virus - JaGAr39 | {mosquitoes, culicoid flies} | [S: M22011] | (AKAV) |
| *Alajuela virus* | | | |
| Alajuela virus - 75V 2374 | {mosquitoes} | | (ALJV) |
| *Anopheles A virus* | | | |
| Anopheles A virus - 1940 prototype | {mosquitoes} | [S: FJ660415] | (ANAV) |
| *Anopheles B virus* | | | |
| Anopheles B virus - 1940 prototype | {mosquitoes} | [S: FJ660417] | (ANBV) |
| *Bakau virus* | | | |
| Bakau virus - MM2325 | {mosquitoes} | | (BAKV) |
| *Batama virus* | | | |
| Batama virus - AnB1292a | {N.D.} | [S: FJ660420] | (BMAV) |
| *Benevides virus* | | | |
| Benevides virus - BeAn153564 | {mosquitoes} | | (BVSV) |
| *Bertioga virus* | | | |
| Bertioga virus - SPAn1098 | {N.D.} | | (BERV) |
| *Bimiti virus* | | | |
| Bimiti virus - TRVL8362 | {mosquitoes} | | (BIMV) |
| *Botambi virus* | | | |
| Botambi virus - DakArB937 | {mosquitoes} | | (BOTV) |
| *Bunyamwera virus* | | | |
| Bunyamwera virus - 1943 prototype | {mosquitoes} | [L: X14383; M: M11852; S: X73465] | (BUNV) |
| *Bushbush virus* | | | |
| Bushbush virus - TRVL26668 | {mosquitoes} | | (BSBV) |
| *Bwamba virus* | | | |
| Bwamba virus - M459 | {mosquitoes} | | (BWAV) |
| *California encephalitis virus* | | | |
| La Crosse virus | {mosquitoes} | [L: U12396; M: D00202; S: K00610] | (LACV) |
| *Capim virus* | | | |
| Capim virus - BeAn 8582 | {mosquitoes} | | (CAPV) |
| *Caraparu virus* | | | |
| Caraparu virus - BeAn3994 | {mosquitoes} | [S: DQ188948; M: DQ188960; L: EF122411] | (CARV) |
| *Catu virus* | | | |
| Catu virus - BeH151 | {mosquitoes} | | (CATUV) |
| *Estero Real virus* | | | |
| Estero Real virus - K329 | {ticks} | | (ERV) |
| *Gamboa virus* | | | |
| Gamboa virus - 75V 2621 | {mosquitoes} | | (GAMV) |
| *Guajara virus* | | | |
| Guajara virus - BeAn10615 | {mosquitoes} | | (GJAV) |
| *Guama virus* | | | |
| Guama virus - BeAn 277 virus | {mosquitoes} | | (GMAV) |
| *Guaroa virus* | | | |
| Guaroa virus - 352111 | {mosquitoes} | [S: X73466] | (GROV) |
| *Kaeng Khoi virus* | | | |
| Kaeng Khoi virus - S19-8 | {nest bugs} | | (KKV) |
| *Kairi virus* | | | |
| Kairi virus - TRVL8900 | {mosquitoes} | [S: X73467] | (KRIV) |
| *Koongol virus* | | | |
| Koongol virus - MRM31 | {mosquitoes} | | (KOOV) |
| *Madrid virus* | | | |
| Madrid virus - BT4075 | {mosquitoes} | [S: DQ188957] | (MADV) |
| *Main Drain virus* | | | |
| Main Drain virus - BSF5015 | {mosquitoes, culicoid flies} | [S: X73469] | (MDV) |
| *Manzanilla virus* | | | |
| Manzanilla virus - TRVL3587 | {N.D.} | | (MANV) |

| | | | |
|---|---|---|---|
| *Marituba virus* | | | |
| Marituba virus - BeAn15 | {mosquitoes} | [M: DQ188966] | (MTBV) |
| *Minatitlan virus* | | | |
| Minatitlan virus - M67U5 | {N.D.} | | (MNTV) |
| *M'Poko virus* | | | |
| M'Poko virus - BA365 | {mosquitoes} | | (MPOV) |
| *Nyando virus* | | | |
| Nyando virus - MP 401 | {mosquitoes} | | (NDV) |
| *Olifantsvlei virus* | | | |
| Olifantsvlei virus - SAAr5133 | {mosquitoes} | | (OLIV) |
| *Oriboca virus* | | | |
| Oriboca virus - BeAn17 | {mosquitoes} | [S: DQ188946; M: DQ188964] | (ORIV) |
| *Oropouche virus* | | | |
| Oropouche virus - TRVL9760 | {mosquitoes, culicoid flies} | | (OROV) |
| *Patois virus* | | | |
| Patois virus - BT4971 | {mosquitoes} | | (PATV) |
| *Sathuperi virus* | | | |
| Sathuperi virus - IG10310 | {mosquitoes, culicoid flies} | | (SATV) |
| *Shamonda virus* | | | |
| Shamonda virus - I bAn5550 | {culicoid flies} | | (SHAV) |
| *Shuni virus* | | | |
| Shuni virus - IbAn10107 | {mosquitoes, culicoid flies} | | (SHUV) |
| *Simbu virus* | | | |
| Simbu virus - SAAr53 | {mosquitoes, culicoid flies} | | (SIMV) |
| *Tacaiuma virus* | | | |
| Tacaiuma virus - BeAn73 | {mosquitoes} | [S: FJ660416] | (TCMV) |
| *Tete virus* | | | |
| Tete virus - SAAn 3518 | {N.D.} | [S: FJ660419] | (TETEV) |
| *Thimiri virus* | | | |
| Thimiri virus - VRC66414 | {N.D.} | | (THIV) |
| *Timboteua virus* | | | |
| Timboteua virus - BeAn116382 | {mosquitoes} | | (TBTV) |
| *Turlock virus* | | | |
| Turlock virus - S 1954-847-32 | {mosquitoes} | | (TURV) |
| *Wyeomyia virus* | | | |
| Wyeomyia virus | {mosquitoes} | | (WYOV) |
| *Zegla virus* | | | |
| Zegla virus - BT5012 | {N.D.} | | (ZEGV) |

Species names are in italic script; names of isolates are in roman script. Vector type {}, sequence accession numbers [ ] and assigned abbreviations ( ) are also listed. N.D. = no data.
Full table available online on Science Direct ®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Orthobunyavirus* but have not been approved as species

| | | |
|---|---|---|
| Leanyer virus - AusNT16701D | {mosquitoes} | (LEAV) |
| Mojui dos Campos virus BeAn276121 | {N.D.} | (MDCV) |
| Termeil virus - BP8090 | {mosquitoes} | (TERV) |

## GENUS *HANTAVIRUS*

Type species ***Hantaan virus***

### Distinguishing features

The consensus terminal nt sequences of the L, M and S genomic segments are AUCAUCAUCUG at the 3′ end and UAGUAGUAUGC at the 5′ end. Viruses are serologically unrelated to members of other genera. Certain hantaviruses are etiologic agents of hemorrhagic fever with renal syndrome or hantavirus (cardio)pulmonary syndrome (HPS, HCPS). The host range of hantaviruses includes

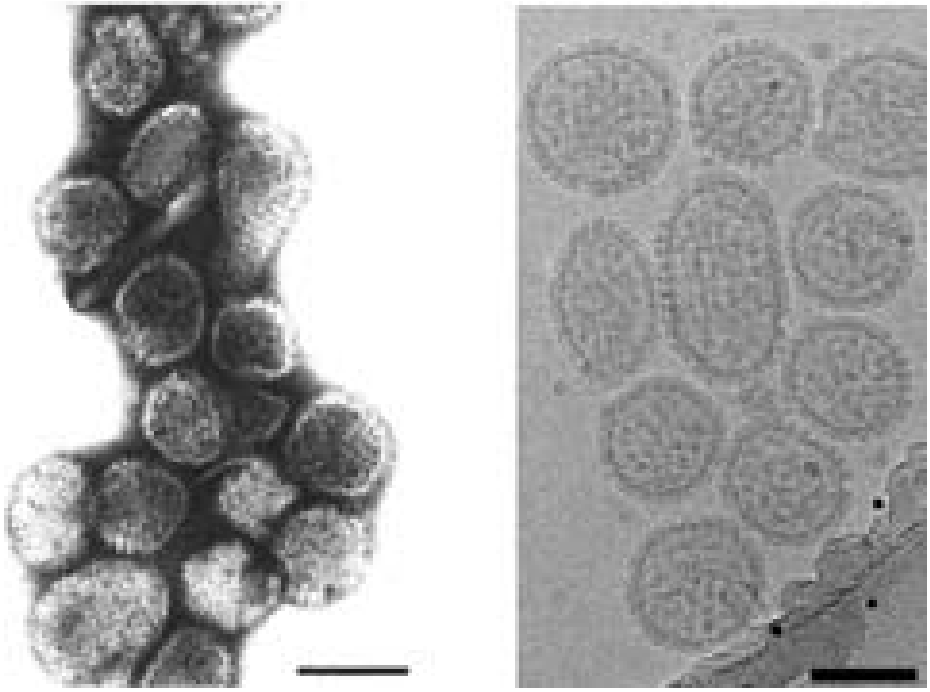

**Figure 5:** Electron micrographs of negatively stained particles of isolates of Hantaan virus (left, courtesy of C.S. Schmaljohn) and Tula virus (right, courtesy of S. Butcher and J. Hepojöki). The bars represent 100 nm.

rodents and insectivores, and genetically distinct hantaviruses are usually associated with a single host species. Human infection is incidental to viral maintenance and is almost always a dead end in the infection chain, with the exception of a human-to-human transmission of Andes virus. In contrast to other viruses in the family, hantaviruses are not transmitted by arthropods, and both rodent and human infections are acquired by aerosol exposure to infectious virus in rodent urine, faeces or saliva, and less frequently by rodent bite. Hantaviruses cause no detectable cytopathology in vertebrate cell cultures and cause persistent, non-pathogenic infections of rodents. Hantavirus infections in insectivores are not systematically studied yet.

## Species demarcation criteria in the genus

Species are usually found in unique ecological niches, i.e. in different primary rodent/insectivore reservoir species. Species exhibit at least 7% difference in aa identity on comparison of the complete glycoprotein precursor and nucleocapsid protein sequences (there are some exceptions presumably caused by historically recent host-switching events). Species show at least four-fold difference in two-way cross neutralization tests. Species do not naturally form reassortants with other species.

## List of species in the genus *Hantavirus*

| | | | |
|---|---|---|---|
| *Andes virus* | | | |
| Andes virus - Chile-9717869 | {*Oligoryzomys longicaudatus*} | [L: NC_003468; M: NC_003467; S: NC_003466] | (ANDV) |
| *Bayou virus* | | | |
| Bayou virus - Louisiana | {*Oryzomys palustris*} | [M: L36930; S: L36929] | (BAYV) |
| *Black Creek Canal virus* | | | |
| Black Creek Canal virus | {*Sigmodon hispidus*} | [M: L39950; S: L39949] | (BCCV) |
| *Cano Delgadito virus* | | | |
| Cano Delgadito virus - VHV-574 | {*Sigmodon alstoni*} | [S: DQ285566] | (CADV) |
| *Dobrava-Belgrade virus* | | | |
| Dobrava virus - Slovenia, prototype | {*Apodemus flavicollis*} | [M: L33685; S: L41916] | (DOBV) |

| | | | |
|---|---|---|---|
| *El Moro Canyon virus* | | | |
| El Moro Canyon virus - RM-97 | {*Reithrodontomys megalotis*} | [M: U26828; S: U11427] | (ELMCV) |
| *Hantaan virus* | | | |
| Hantaan virus - 76-118 prototype | {*Apodemus agrarius coreae*} | [L: X55901; M: M14627; S: M14626] | (HTNV) |
| *Isla Vista virus* | | | |
| Isla Vista virus - MC-SB-47 | {*Microtus californicus*} | [S: U19302] | (ISLAV) |
| *Khabarovsk virus* | | | |
| Khabarovsk virus - MF43 | {*Microtus maximowiczii*, *M. fortis*} | [M: AJ011648; S: U35255] | (KHAV) |
| *Laguna Negra virus* | | | |
| Laguna Negra virus - 510B | {*Calomys laucha*} | [M: AF005728; S: AF005727] | (LANV) |
| *Muleshoe virus* | | | |
| Muleshoe virus - SH-Tx-339 | {*Sigmodon hispidus*} | [S: MHU54575] | (MULV) |
| *New York virus* | | | |
| New York virus - RI-1 | {*Peromyscus leucopus*} | [M: U36801; S: U09488] | (NYV) |
| *Prospect Hill virus* | | | |
| Prospect Hill virus - PH1 | {*Microtus pennsylvanicus*} | [M: X55129; S: X55128] | (PHV) |
| *Puumala virus* | | | |
| Puumala virus - Sotkamo, prototype | {*Myodes glareolus*} | [L: Z66548; M: X61034; S: X61035] | (PUUV) |
| *Rio Mamore virus* | | | |
| Rio Mamore virus - OM556 | {*Oligoryzomys microtis*} | [S: U52136] | (RIOMV) |
| *Rio Segundo virus* | | | |
| Rio Segundo virus | {*Reithrodontomys mexicanus*} | [S: U18100] | (RIOS) (SAAC) |
| *Saaremaa virus* | | | |
| Saaremaa virus - Saaremaa160V prototype | {*Apodemus agrarius agrarius*} | [L: AJ410618; M: AJ009774; S: AJ009773] | (SAAV) |
| *Seoul virus* | | | |
| Seoul virus - HR80-39 | {*Rattus norvegicus*} | [L: X56492; M: S47716; S: NC_005236] | (SEOV) |
| *Sin Nombre virus* | | | |
| Sin Nombre virus - NMH10 virus | {*Peromyscus maniculatus*} | [L: L37901; M: L25783; S: L25784] | (SNV) |
| *Thailand virus* | | | |
| Thailand virus - 741 prototype | {*Bandicota indica*} | [M: L08756; S: AB186420] | (THAIV) |
| *Thottapalayam virus* | | | |
| Thottapalayam virus - VCR66412 | {*Suncus murinus*} | [L: NC_010707; M: NC_010708; S: NC_010704] | (TPMV) |
| *Topografov virus* | | | |
| Topografov virus | {*Lemmus sibiricus*} | [M: AJ011647; S: AJ011646] | (TOPV) |
| *Tula virus* | | | |
| Tula virus - Moravia/Ma5302V | {*Microtus arvalis*, *M. rossiaemeridionalis*} | [L: AJ005637; M: Z69993;S: Z69991] | (TULV) |

Species names are in italic script; names of isolates are in roman script. Vector type {}, sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

## List of other related viruses which may be members of the genus *Hantavirus* but have not been approved as species

| | | | |
|---|---|---|---|
| Altai virus | {*Sorex araneus*} | | (ALTV) |
| Amur/Soochong virus | {*Apodemus peninsulae*} | [L: DQ056292; M: AY675353; S:AY675349] | (ASV) |
| Artybash virus | {*Sorex* spp.} | | (ARTV) |
| Araraquara virus | {*Bolomys lasiuris*} | | (ARAV) |

| | | | |
|---|---|---|---|
| Asama virus | {*Urotrichus talpoides*} | | (ASAV) |
| Ash River virus | {*Sorex cinereus*} | | (ARRV) |
| Calabazo virus | {*Zygodontomys brevicauda*} | | |
| Camp Riley virus | {*Blarina brevicauda*} | | (RPLV) |
| Cao Bang virus | {*Anourosorex squamipes*} | | (CBNV) |
| Castelo dos Sonhos virus | {unknown} | | |
| Choclo virus | {*Oligoryzomys fulvescens*} | | |
| Da Bie Shan virus | {*Niviventer confucianus*} | [L: DQ989237; M: AB027115; S: AB027523] | (DBSV) |
| Fox Creek virus | {*Sorex palustris*} | | (FXCV) |
| Gou virus | {*Rattus rattus*} (?) | [M: AB027521; S: AF184988] | (GOUV) |
| Hokkaido virus | {*Myodes rufocanus*} | [S:AB010730] | (HOKV) |
| Iamonia virus | {*Blarina carolinensis*} | | (IAMV) |
| Imjin virus | {*Crocidura lasiura*} | [L: EF641806; M: EF641798; S: EF641806] | (IMJV) |
| Jemez Springs virus | {*Sorex monticolus*} | | (JMSV) |
| Lena River virus | {*Sorex caecutiens*} | | (LNAV) |
| Limestone Canyon virus | {*Peromyscus boylii*} | [M: AF07323; S: AF07322] | (LSCV) |
| Kenkeme virus | {*Sorex roboratus*} | | (KENV) |
| Muju virus | {*Myodes regulus*} | [M: EF198413; S: DQ138133] | (MUJV) |
| Powell Butte virus | {*Sorex vagrans*} | | (PWBV) |
| Sangassou virus | {*Hylomyscus simus*} | | (SANGV) |
| Seewis virus | {*Sorex araneus*} | | |
| Serang virus | {*Rattus tanezumi*} | | (SERV) |
| Tanganya virus | {*Crocidura theresae*} | | (TGNV) |
| Tualatin River virus | {*Sorex trowbridgii*} | | (TLNV) |
| Vladivostok virus | {*Microtus fortis*} | | (VLAV) |
| Yuanjiang virus | {*Microtus fortis*} | | (YUJV) |

# Genus *Nairovirus*

Type species ***Dugbe virus***

## Distinguishing features

Virions are morphologically similar to other members of the family with very small surface units that appear as a peripheral fringe 7 nm in length (Figure 6). The L RNA segment (12.2 kb) is

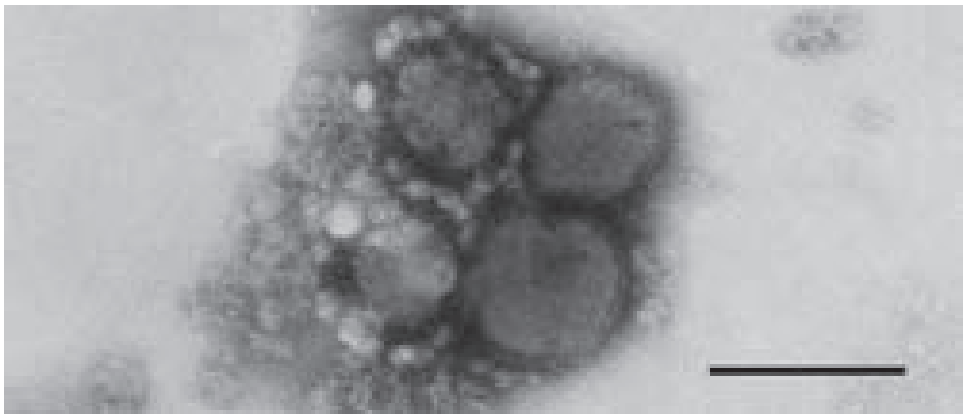

**Figure 6:** Electron micrograph of negatively stained particles of Crimean-Congo hemorrhagic fever virus (CCHFV). The bar represents 100 nm. (Courtesy of C. S. Schmaljohn.)

considerably larger than the L segments of other members of the family. The consensus terminal nt sequences of the L, M and S segments are AGAGUUUCU at the 3′ end and UCUCAAAGA at the 5′ end. The S segment does not encode a nonstructural protein. The M segment encodes a single precursor polyprotein that is processed by cotranslational cleavage into precursors to both Gn and Gc. In addition to Gn, posttranslational cleavage of preGn yields also a mucin-rich product and a glycoprotein GP38. Posttranslational cleavage of preGc removes a polypeptide from its C-terminus and yields a mature Gc.

The L protein is predicted to be much larger than those of other members of the family but has yet to be identified. Viruses are serologically unrelated to members of other genera. Most viruses are transmitted by ticks: CCHFV, DUGV and SAKV species are transmitted mainly by ixodid ticks and DGKV, HUGV and QYBV species are transmitted mainly by argasid ticks. Some viruses are transmitted transovarially in arthropods.

### Species demarcation criteria in the genus

The paucity of biochemical data dictates that nairovirus species are defined by serological reactivities. There are seven species recognized in the genus *Nairovirus*.

### List of species in the genus *Nairovirus*

| | | | |
|---|---|---|---|
| *Crimean-Congo hemorrhagic fever virus* | | | |
| Crimean-Congo hemorrhagic fever virus - IbAr10200 | {ticks} | [S:NC 005302; M: NC 005300; L: NC 005301] | (CCHFV) |
| *Dera Ghazi Khan virus* | | | |
| Dera Ghazi Khan virus - JD254 | {ticks} | | (DGKV) |
| *Dugbe virus* | | | |
| Dugbe virus - IbAr1792 | {ticks} | [S: AF434164; M: M94133; L: U15018] | (DUGV) |
| *Hughes virus* | | | |
| Hughes virus | {ticks} | | (HUGV) |
| *Qalyub virus* | | | |
| Qalyub virus - EgAr37000000 | {ticks} | | (QYBV) |
| *Sakhalin virus* | | | |
| Sakhalin virus - LEIV-71C | {ticks} | | (SAKV) |
| *Thiafora virus* | | | |
| Thiafora virus - AnD11411 | {N.D.} | | (TFAV) |

Species names are in italic script; names of isolates are in roman script. Vector type {}, sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Nairovirus* but have not been approved as species

None reported.

## GENUS *PHLEBOVIRUS*

Type species ***Rift Valley fever virus***

### Distinguishing features

The surface morphology of phleboviruses is distinct in having small round subunits with a central hole (Figure 7). The consensus terminal nucleotide sequences of the L, M and S segments are UGUGUUUC at the 3′ end and ACACAAAG at the 5′ end. The S RNA exhibits an ambisense coding strategy, i.e. it is transcribed by the virion RNA polymerase to a subgenomic virus-complementary sense mRNA that encodes the N protein and, from a full-length antigenome S RNA, to a subgenomic virus-sense mRNA that encodes a nonstructural (NSs) protein. The M segment of viruses in the sandfly fever group but not viruses in the Uukuniemi group has a preglycoprotein

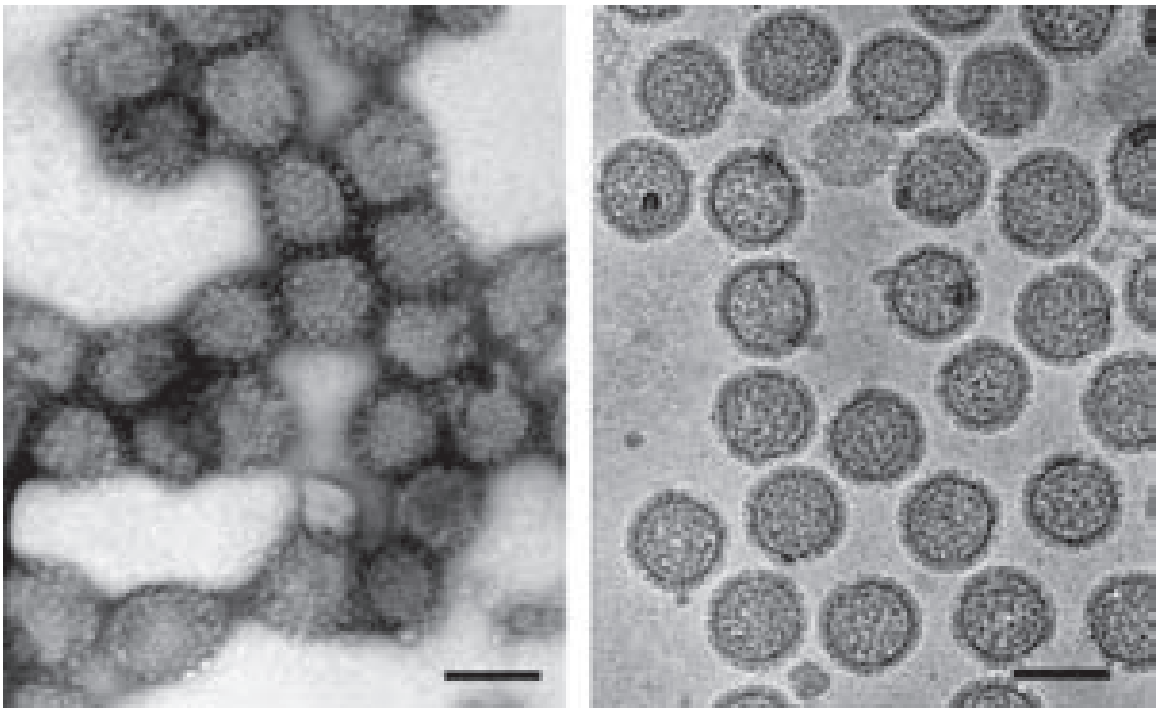

**Figure 7:** (Left) Electron micrograph of negatively stained particles of Rift Valley fever virus. The bar represents 50 nm (courtesy of A. Freiburg and R. Flick). (Right) Cryo-electron micrograph of purified UUKV particles. The bar represents 100 nm. (Courtesy of C-H. von Bornsdorff.)

coding region that codes for a nonstructural protein(s) (NSm). The Gn and Gc glycoproteins were earlier referred to as G1 and G2 based on apparent size on gel electrophoresis. However, the similar sizes of the G1 and G2 proteins resulted in the different G1:G2 order in the M segments of different viruses. The further adoption of the Gn/Gc nomenclature is strongly encouraged so as to achieve more consistency across the *Bunyaviridae* family. Phleboviruses are antigenically unrelated to members of other genera, but cross-react serologically among themselves to different degrees. Sandfly fever group viruses are transmitted by phlebotomines, mosquitoes or ceratopogonids of the genus *Culicoides*; Uukuniemi group viruses are transmitted by ticks.

## Species demarcation criteria in the genus

The lack of biochemical data for most phleboviruses dictates that the species are defined by the serological relationships, and are distinguishable by four-fold differences in two-way neutralization tests.

## List of species in the genus *Phlebovirus*

| | | | |
|---|---|---|---|
| *Bujaru virus* | | | |
| Bujaru virus - BeAn 47693 | {N.D.} | | (BUJV) |
| *Candiru virus* | | | |
| Candiru virus - BeH22511 | {N.D.} | | (CDUV) |
| *Chilibre virus* | | | |
| Chilibre virus VP-118D | {phlebotomines} | | (CHIV) |
| *Frijoles virus* | | | |
| Frijoles virus VP-161A | {phlebotomines} | | (FRIV) |
| *Punta Toro virus* | | | |
| Punta Toro virus - PanD4021A | {phlebotomines} | [M: M11156; S: K02736] | (PTV) |
| *Rift Valley fever virus* | | | |
| Rift Valley fever virus | {mosquitoes} | [L: X56464; M: M11157; S: X53771] | (RVFV) |
| *Salehabad virus* | | | |
| Salehabad virus - I-81 | {phlebotomines} | | (SALV) |
| *Sandfly fever Naples virus* | | | |
| Toscana virus - ISS.Phl.3 | {phlebotomines} | [L: X68414; M: X89628; S: X53794] | (TOSV) |

| | | | |
|---|---|---|---|
| *Uukuniemi virus* | | | |
| Uukuniemi virus - S 23 | {ticks} | [L: D10759; M: M17417; S: M33551] | (UUKV) |

Species names are in italic script; names of isolates are in roman script. Vector type {}, sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Phlebovirus* but have not been approved as species

| | | | |
|---|---|---|---|
| Aguacate virus - VP-175A | {phlebotomines} | | (AGUV) |
| Ambe virus - BeAr407981 | {phlebotomines} | | (AMBEV) |
| Anhanga virus - BeAn46852 | {N.D.} | | (ANHV) |
| Arboledas virus - CoAr170152 | {phlebotomines} | | (ADSV) |
| Ariquemes virus - BeAr485678 | {phlebotomines} | | (ARQV) |
| Armero virus - CoAr171096 | {phlebotomines} | | (ARMV) |
| Arumowot virus - SudAr1284-64 | {mosquitoes} | | (AMTV) |
| Caimito virus - VP-488A | {phlebotomines} | | (CAIV) |
| Chagres virus - JW-10 | {phlebotomines, mosquitoes} | | (CHGV) |
| Corfou virus - PaAr814 | {phlebotomines} | | (CFUV) |
| Durania virus - CoAr171162 | {phlebotomines} | | (DURV) |
| Escharte virus - OBS-6528 | {N.D.} | | (ESCV) |
| Gabek Forest virus - SudAn754-61 | {N.D.} | | (GFV) |
| Gordil virus - DakAnBR496d | {N.D.} | | (GORV) |
| Itaporanga virus | {mosquitoes} | | (ITPV) |
| Ixcanal virus - CA Ar170897 | {phlebotomines} | | (IXCV) |
| Jacunda virus - BeAn428329 | {N.D.} | | (JANV) |
| Leticia virus - CoAr171616 | {phlebotomines} | | (LTCV) |
| Mariquita virus - Mariquita A | {phlebotomines} | | (MRQV) |
| Morolillo virus - HTN351 | {N.D.} | | (MOLV) |
| Morumbi virus - BeH475236 | {N.D.} | | (MRBV) |
| Mucura virus - BeAr455230 | {mosquitoes} | | (MRAV) |
| Odrenisrou virus - ArA1131/80 | {mosquitoes} | | (ODRV) |
| Pacui virus - BeAn27326 | {phlebotomines} | | (PACV) |
| Rio Grande virus - TBM3-24 | {N.D.} | | (RGV) |
| Salobo virus - BeAn578142 | {N.D.} | | (SBOV) |
| Sandfly fever Sicilian virus | {phlebotomines} | [S: J04418] | (SFSV) |
| Saint-Floris virus -DakAnBR512d | {N.D.} | | (SAFV) |
| Serra Norte virus - BeH505240 | {N.D.} | | (SRNV) |
| Tapara virus - BeAr413570 | {phlebotomines} | | (TAPV) |
| Uriurana virus - BeAr479776 | {phlebotomines} | | (URIV) |
| Urucuri virus - BeAn100049 | {N.D.} | | (URUV) |

## GENUS *TOSPOVIRUS*

Type species ***Tomato spotted wilt virus***

### Distinguishing features

Morphogenesis occurs in clusters in the cisternae of the endoplasmic reticulum of host cells. Nucleocapsid material may accumulate in the cytoplasm in dense masses; these masses may be composed of defective particles. The morphology of a tospovirus is shown in Figure 8. The consensus terminal sequences of the L, M and S genomic segments are UCUCGUUA at the 3′ end and AGAGCAAU at the 5′ end. Both the M and S segment RNAs of tospoviruses utilize an ambisense coding strategy. The virion glycoproteins Gn and Gc are encoded in the complementary-sense RNA of the M segment, and a nonstructural protein, NSm, is encoded in the genome-sense RNA. The S segment encodes the nucleocapsid protein in the complementary-sense RNA and a nonstructural protein, NSs, in the genome-sense RNA. The NSm protein represents the viral (cell-to-cell) movement protein, present in all plant-pathogenic viral taxa and essential for systemic infection of a plant. At the front of infection NSm assembles into tubular structures that penetrate through plasmodesmata thus facilitating nucleocapsids to translocate to the next cell. NSs represents the

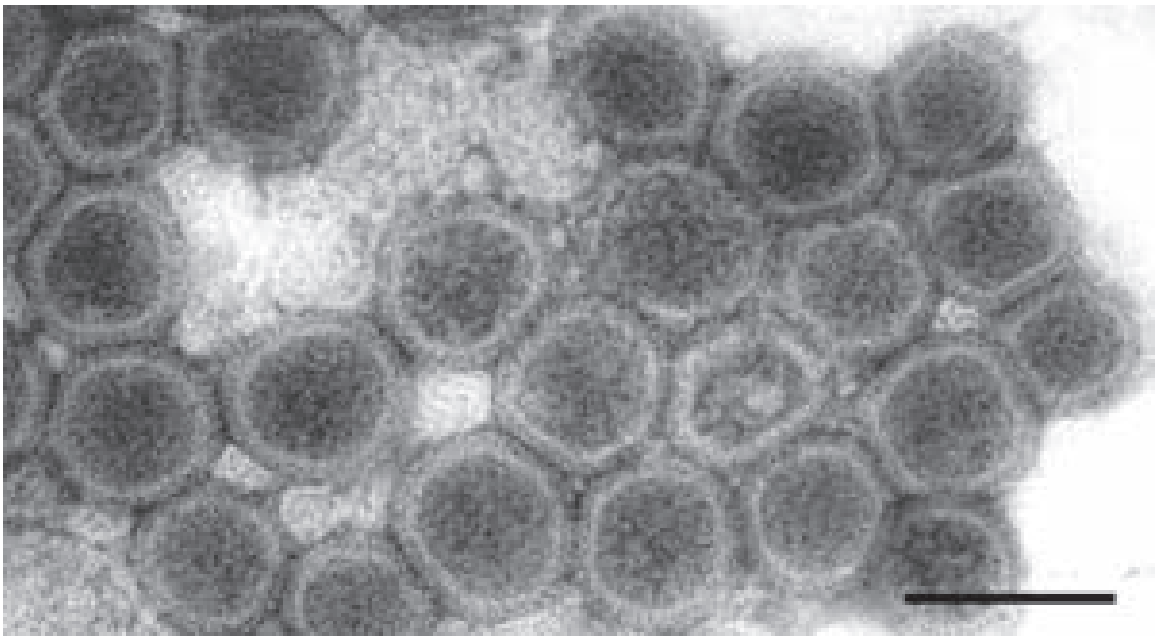

**Figure 8:** Electron micrograph of negatively stained particles of Tomato spotted wilt virus (TSWV). The bar represents 100 nm. (Courtesy of Dr Jan van Lent.)

suppressor of (antiviral) RNAi and can bind both long dsRNA and short dsRNA (i.e. siRNA ans miRNA). In virulent isolates of tospoviruses NSs is highly expressed and may then form paracrystalline or filamentous inclusions in infected plant cells.

At least 13 species of thrips in the genera *Frankliniella* (9), *Thrips* (2), *Scirtothrips* (1) and *Ceratothripoides* (1) have been reported to transmit tospoviruses, and the Gn and/or Gc glycoproteins are involved in virus–vector interactions. Transmission can also be achieved through infected plant sap. For isolates of the type species *Tomato spotted wilt virus*, more than 925 plant species belonging to 70 botanical families are known to be susceptible whereas the other tospoviruses have much narrower host ranges.

## Species demarcation criteria in the genus

Species are defined on the basis of their vector specificity, their plant host range, serological relationships of the N protein and on the criterion that their N protein sequence should show less than 90% aa identity with that of any other described tospovirus species.

## List of species in the genus *Tospovirus*

| | | | |
|---|---|---|---|
| *Groundnut bud necrosis virus* | | | |
| Groundnut bud necrosis virus (Peanut bud necrosis virus) | {*Frankliniella occidentalis*, *Thrips palmi*} | [L: AF025538; M: U42555; S: U27809] | (GBNV) |
| *Groundnut ringspot virus* | | | |
| Groundnut ringspot virus | {*Frankliniella gemina*, *F. occidentalis*, *F. schultzei*} | | (GRSV) |
| *Groundnut yellow spot virus* | | | |
| Groundnut yellow spot virus (Peanut yellow spot virus) | {N.D.} | [S: AF013994] | (GYSV) |
| *Impatiens necrotic spot virus* | | | |
| Impatiens necrotic spot virus | {*Frankliniella occidentalis*} | [L: X93218; M: M74904; S: X66972] | (INSV) |
| *Tomato chlorotic spot virus* | | | |
| Tomato chlorotic spot virus | {*Frankliniella occidentalis*, *F. schultzei*, *F. intonsa*} | [S(N) :S54325] | (TCSV) |
| *Tomato spotted wilt virus* | | | |

| | | | |
|---|---|---|---|
| Tomato spotted wilt virus | {*Frankliniella bispinosa*, *F. cephalica*, *F. gemina*, *F. fusca*, *F. intonsa*, *F. occidentalis*, *F. schultzei*, *F. setosus*, *Thrips tabaci*} | [L: (BR-01) D10066; M: (BR-01) S48091; S:(BR-01) D00645; S: (B) L12048;S: (BL) L20953;S: (L3) D13926] | (TSWV) |
| *Watermelon silver mottle virus* | | | |
| Watermelon silver mottle virus | {*Thrips palmi*} | [M: U75379;S: Z46419] | (WSMoV) |
| *Zucchini lethal chlorosis virus* | | | |
| Zucchini lethal chlorosis virus | {*Frankliniella zucchini*} | [S(N): AF067069] | (ZLCV) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Vector type {}, sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Tospovirus* but have not been approved as species

| | | | |
|---|---|---|---|
| Alstromeria necrotic streak virus | {*Frankliniella occidentalis*} | [S: GQ478668 (N)] | (ANSV) |
| Calla lily chlorotic spot virus | {N.D.} | [L: FJ822961; M: FJ822962] | (CCSV) |
| Capsicum chlorosis virus (Gloxinia tospovirus) (Thailand tomato tospovirus) | {*Ceratotripoides claratris*} | [L: DQ256124, M: DQ256125;S: DQ256123] | (CaCV) |
| Chrysanthemum stem necrosis virus | {*Frankliniella occidentalis*} | [S(N): AF067068] | (CSNV) |
| Groundnut chlorotic fan-spot virus | {*Scirtothrips dorsalis*} | [ S(N):AF080526] | (GCFSV) |
| Iris yellow spot virus | {N.D.} | [L: FJ623474; M: AF214014; S: AF001387] | (IYSV) |
| Melon severe mosaic virus | {N.D.} | [S: EU275149] | (MSMV) |
| Melon yellow spot virus | {*Thrips palmi*} | [L: AB061774; M: AB061773; S: AB038343] | (MYSV) |
| Physalis severe mottle virus | {N.D.} | [S: AF067151] | (PhySMV) |
| Polygonum ringspot virus | {N.D.} | [M: EU271753; S: EF445397] | (PolRSV) |
| Tomato necrosis virus | {N.D.} | [M: AY647437] | (TNeV) |
| Tomato necrotic ringspot virus | {N.D.} | [M: FJ947152; S: FJ489600] | (TNRV) |
| Tomato yellow (fruit) ring virus | {*Thrips tabaci*} | [S: AY686718] | (TYRV) |
| Tomato zonate spot virus | {N.D.} | [L: EF552435; M: EF552434; S: EF552433] | (TZSV) |
| Watermelon bud necrosis virus | {*Thrips palmi*} | [M: FJ694963; S: EU249351] | (WBNV) |

## List of other related viruses which may be members of the family *Bunyaviridae* but have not been approved as species

There are seven groups (19 viruses) and 21 ungrouped viruses which have not been assigned to a recognized genus in the family *Bunyaviridae*. For most, no biochemical characterization of the viruses has been reported to determine their taxonomic status.

**Grouped viruses:**

| | |
|---|---|
| Bhanja virus - IG690 | (BHAV) |
| Forecariah virus - ArK4927 | (FORV) |
| Kismayo virus - A3641 | (KISV) |
| Kaisodi virus - IG14132 | (KSOV) |
| Lanjan virus - Mal TP94 | (LJNV) |
| Silverwater virus - Can131 | (SILV) |
| Mapputta virus - AusNRM186 | (MAPV) |
| Gan Gan virus - NB6057 | (GGV) |
| Maprik virus - MK7532 | (MPKV) |
| Trubanaman virus - AusMRM3630 | (TRUV) |
| Okola virus - Dak YM50/64 | (OKOV) |
| Tanga virus -TanzMP1329 | (TANV) |
| Resistencia virus - AG80-504 | (RTAV) |
| Antequera virus - AG80-226 | (ANTV) |
| Barranqueras virus -AG80-381 | (BQSV) |
| Yogue virus - DakAnD5634 | (YOGV) |
| Kasokero virus - UGZ52969 | (KASV) |

**Ungrouped viruses:**

| | |
|---|---|
| Bangui virus - DakHB745 | (BGIV) |
| Belem virus - BeAn141106 | (BLMV) |
| Belmont virus - R8659 | (BELV) |
| Bobaya virus - AnB2208d | (BOBV) |
| Caddo Canyon virus | (CDCV) |
| Chim virus - LEIV-858Uz | (CHIMV) |
| Enseada virus - 75V25880 | (ENSV) |
| Issyk-Kul virus - LEIV-315K | (ISKV) |
| Keterah virus - P6-1361 | (KTRV) |
| Kowanyama virus - Aus MRM1178 | (KOWV) |
| Lone Star virus - USA TMA1381 | (LSV) |
| Pacora virus - PanJ19 | (PCAV) |
| Para virus - BeAn280577 | |
| Razdan virus - LEIV-2741Ar | (RAZV) |
| Santarem virus - BeAn238758 | (STMV) |
| Sunday Canyon virus - RML52301-11 | (SCAV) |
| Tai virus - ArA 94/79 | (TAIV) |
| Tamdy virus - LEIV-1308z | (TDYV) |
| Tataguine virus - DaKIPD/A252 | (TATV) |
| Wanowrie virus - IG700 | (WANV) |
| Witwatersrand virus - SAAr1062 | (WITV) |
| Yacaaba virus - NB6028 | (YACV) |

## Phylogenetic relationships within the family

As documented above, the analogous genes and gene products of viruses in the different genera vary widely in size, and there is little obvious global similarity at either nt or aa level. Attempts to produce convincing alignments of either genome segments or structural proteins from which to generate phylogenetic trees have so far proved unsuccessful, with the exception of the putative polymerase domain of the L proteins. Such analysis suggests that viruses in the family *Bunyaviridae* fall into two major lineages, comprising orthobunyaviruses, hantaviruses and tospoviruses on one, and nairoviruses and phleboviruses in the other. The significant point to note is that L protein phylogeny does not segregate with the use of an ambisense coding strategy. (See Figure 9.)

## Similarity with other taxa

The plant-infecting tenuiviruses show some similarities to members of the family *Bunyaviridae*, particularly the genus *Phlebovirus*. Tenuiviruses have a ssRNA genome comprising four or five segments that encode proteins using a negative or ambisense coding strategy. The tenuivirus RNA terminal sequences are conserved and the 3′ and 5′-sequences exhibit inverted complementarity; the conserved 3′-sequence, UGUGUUUCAG , is similar to the consensus phlebovirus sequence. Tenuiviruses employ a cap-snatching mechanism to prime viral mRNA synthesis, similar to members of the family *Bunyaviridae.* Weak sequence homology has been noted between the Rice stripe virus 94 kDa protein and phlebovirus glycoproteins, and between tenuivirus nucleocapsid proteins and those of phleboviruses.

## Derivation of names

*Bunya*: from *Bunya*mwera, place in Uganda, where type virus was isolated.
*Hanta*: from *Hanta*an, river in South Korea near where type virus was isolated.
*Nairo*: from *Nairo*bi sheep disease, first reported disease caused by member virus.
*Phlebo*: refers to *phlebo*tomine vectors of sandfly fever group viruses; Greek *phlebos*, "vein".
*Tospo*: from *to*mato *spo*tted wilt virus.

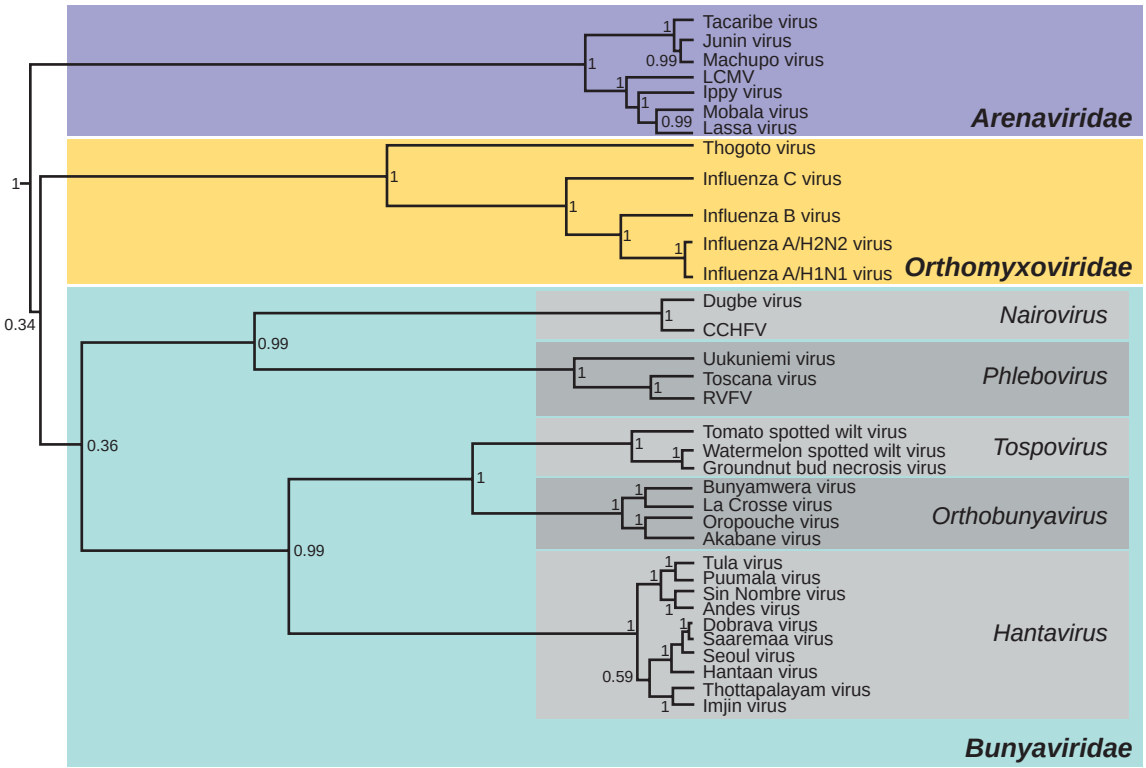


**Figure 9:** Phylogenetic tree of aligned core polymerase domains from the L proteins of members of the family Bunyaviridae and from analogous proteins of other segmented (*Arenaviridae*, *Orthomyxoviridae*) negative strand RNA viruses. The tree was reconstructed using the Bayesian Monte Carlo Markov Chain method in BEAST (http://beast.bio.ed.ac.uk/). A maximum clade credibility tree is shown with mean branch lengths (substitutions per site), and Bayesian posterior probabilities given at the nodes. (Courtesy of T. Sironen.)

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## Contributed by

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# Family *Ophioviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Ophioviridae* |
| Genus | *Ophiovirus* |

## Distinguishing features

Single stranded negative/possibly ambisense RNA genome (11.3–12.5 kb size) divided into 3–4 segments, each encapsidated in a single coat protein (43–50 kDa) forming filamentous virions about 3 nm in diameter, in shape of kinked (probably internally coiled) circles of at least two different contour lengths.

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Ophiovirus*

Type species *Citrus psorosis virus*

## Virion properties

### Morphology

The virions are naked filamentous nucleocapsids about 3 nm in diameter (Figure 1), forming kinked (probably internally coiled) circles of at least two different contour lengths, the shortest length about 760 nm. The circles can collapse to form pseudo-linear duplex structures about 9–10 nm in diameter.

### Physicochemical and physical properties

The Mr and sedimentation coefficients of virions are not known. The particles are unstable in CsCl but the buoyant density in cesium sulfate (for both Ranunculus white mottle virus [RWMV], and Mirafiori lettuce big-vein virus [MiLBVV]) is 1.22 g cm$^{-3}$. The particles have limited stability between pH 6 and 8. Infectivity does not survive in crude sap held at 50 °C for 10 min (40–45 °C for tulip mild mottle mosaic virus, TMMMV). Particle structure survives limited treatment with organic solvents and nonionic or zwitterionic detergents.

### Nucleic acid

The ssRNA genome is 11.3–12.5 kb in size and consists of three or four segments. Viral RNA of both polarities is present in purified virion preparations. As virions appear circularized, the presence of panhandle structures is suggested; however, significant complementation between the 5′- and the 3′-terminal sequences of genomic RNAs has not been demonstrated for either citrus psorosis virus (CPsV) or MiLBVV. At the 5′ and 3′ ends of MiLBVV genomic RNAs there are orthomyxovirus-like palindromic sequences that could fold into a symmetrically hooked conformation. These structures could not be predicted for CPsV. The size of RNA-1 is 8.2 kb for CPsV, about 7.5 kb for RWMV, 7.8 kb for MiLBVV and 7.6 kb for lettuce ring necrosis virus (LRNV). RNA-2 is about 1.8 kb for RWMV, MiLBVV and LNRV, 1.6 kb for CPsV and 1.7 for Freesia sneak virus (FreSV). RNA-3 is about 1.5 kb. For MiLBVV and LRNV a fourth genomic RNA of about 1.4 kb has been reported.

### Proteins

There is one CP, varying in size according to species; 48–50 kDa for CPsV, about 43 kDa for RWMV, about 47 kDa for TMMMV, 48.5 kDa for MiLBVV, 48.4 kDa for FreSV and 48 kDa for LRNV.

### Lipids

Not reported.

### Carbohydrates

Not reported.

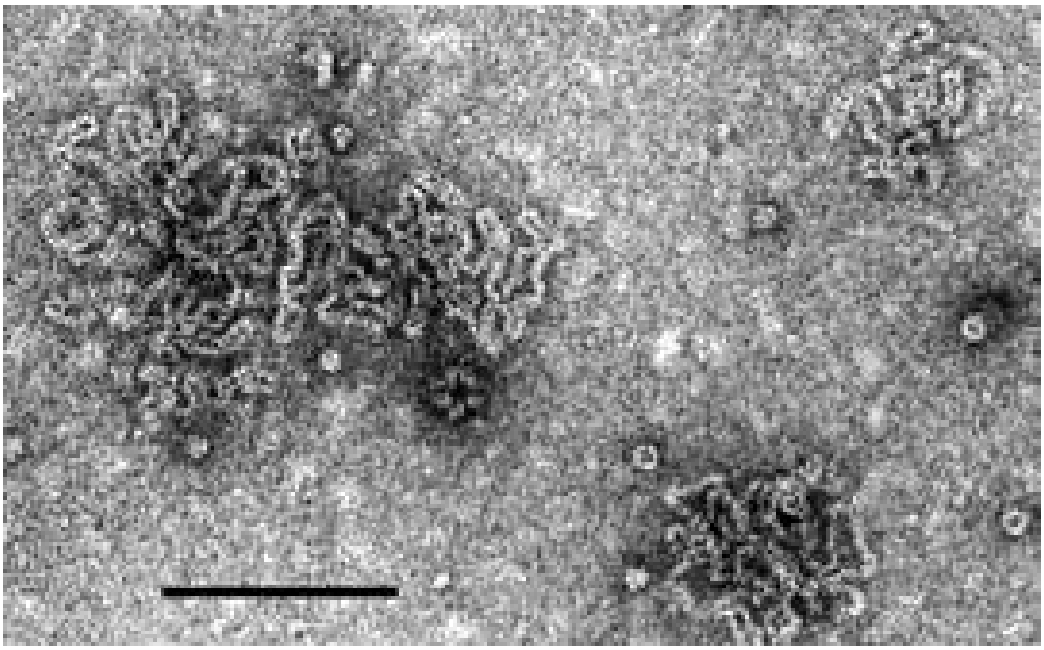

**Figure 1:** Negative contrast electron micrograph (uranyl acetate) of virions of an isolate of Mirafiori lettuce big-vein virus. The bar represents 100 nm. (Courtesy of R. G. Milne.)

## Genome organization and replication

All ophiovirus genome segments appear to be of negative polarity (Figure 2). RNA-1 contains a large ORF coding for a protein that contains the core polymerase module with the five conserved motifs proposed to be part of the RNA-dependent RNA polymerase (RdRp) active site. A paramyxovirus-RdRp domain (pfam 00946) is also present and the SDD sequence, a signature for segmented negative-stranded RNA viruses (*Orthomyxoviridae*, *Arenaviridae* and *Bunyaviridae*), occurs in motif C. A further small ORF has also been reported. RNA-2 contains a single ORF on the complementary strand for CPsV, LRNV and FreSV; for MiLBVV two ORFs are reported: the small second ORF present in the virion sense strand has not yet been confirmed as functional. RNA-3 codes for the CP. RWMV CP antigen accumulates in the cytoplasm of parenchyma cells. RNA-4 (ca. 1.4 kb) has been identified only from MiLBVV and LRNV. While RNA-4 of LRNV potentially encodes only one protein (ca. 38 kDa) of unknown function, that of MiLBVV contains two ORFs (37 and 10.6 kDa) overlapping by 38 nt. The second ORF lacks an initiation codon and is proposed to be expressed by a +1 translational frameshift. Nuclear localization signals (NLS) are reported for the CPsV, MiLBVV and RWMV polymerases and for the RNA-2-encoded proteins of CPsV and MiLBVV.For CPsV the 5′ ends of the complementary strand of RNA-1, RNA-2 and RNA-3 are identical, GATAC$(T)_7$, but the 3′ ends are all different. For MiLBVV, the respective 5′ and 3′ ends are conserved among the four viral RNA segments.

## Antigenic properties

The CP is the only significant antigenic element. In Western blots, CPs of RWMV, TMMMV, MiLBVV and LRNV appear to be slightly to moderately serologically related. CPsV CP is serologically unrelated to the others.

## Biological properties

Ophioviruses can be mechanically transmitted to a limited range of test plants, inducing local lesions and systemic mottle. The natural hosts of CPsV, RWMV, MiLBVV and LRNV are dicotyledonous plants of widely differing taxonomy. In contrast, monocots are naturally infected by TMMMV [tulip (*Tulipa gesneriana* L., *Liliaceae*)] and FreSV [freesia (*Freesia refracta* hybrids, *Iridaceae*) and Lachenalia (*Lachenalia* hybrids, *Hyacinthaceae*)]. CPsV is commonly transmitted by vegetative propagation of the host and no natural vectors have been identified, although natural dispersion of psorosis disease has been observed in a few locations. No vector is known for RWMV. The zoospores of

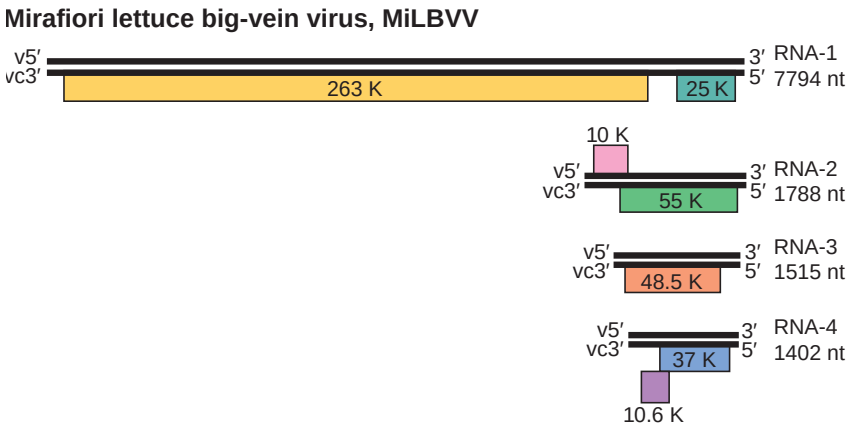


**Figure 2:** Genome organization of genus *Ophiovirus*. Mirafiori lettuce big-vein virus (MiLBVV) is shown here. Boxes represents ORFs. The length of the RNA segments and the predicted sizes of the ORF products are indicated. V, viral RNA; vc, viral complementary RNA. RNA-4 is not reported for all ophioviruses. (Modified from van der Wilk *et al*., 2002.)

*Olpidium brassicae* transmit TMMMV, MiLBVV and LRNV; FreSV is soil-transmitted, presumably by the same vector.

CPsV has a wide geographical distribution in citrus in the Americas, in the Mediterranean and in New Zealand. RWMV has been reported in two species of the family *Ranunculacae* from Northern Italy, and in lettuce in France and Germany. TMMMV has been reported in tulips in Japan. MiLBVV is the causal agent of big-vein symptoms (zones cleared of chlorophyll, parallel to the veins) in lettuce; this widespread and damaging disease probably occurs world-wide. LRNV is closely associated with lettuce ring necrosis disease in The Netherlands, Belgium and France. FreSV has been reported in Europe, Africa, North America and New Zealand.

## Species demarcation criteria in the genus

The different criteria considered for species demarcation in the genus are:

- differences in CP size
- no or distant serological relationship between CPs
- differences in natural host range
- different number, organization and/or size of genome segments.

Alignments between CPsV, FreSV, MiLBVV, LRNV and RWMV (partial cds) CP amino acid sequences show 31–52% identity (53–70% similarity) for isolates belonging to different species, while showing about 92–100% identity for isolates of the same species. Since CPs of MiLBVV and TMMMV (partial cds) share about 80% amino acid sequence identity, this may warrant the establishment of the following molecular criterion for ophiovirus species demarcation:
CP amino acid sequence identity of <85%.

## List of species in the genus *Ophiovirus*

| | | |
|---|---|---|
| *Citrus psorosis virus* | | |
| (Citrus ringspot virus) | | |
| Citrus psorosis virus P-121 | [AY654892-4 = NC_006314-6] | (CPsV-P121) |
| *Freesia sneak virus* | | |
| (Freesia ophiovirus) | | |
| Freesia sneak virus-Fr220205/9 | [DQ885455*] | (FreSV-Fr220205/9) |
| *Lettuce ring necrosis virus* | | |
| Lettuce ring necrosis virus-Belg2 | [AY535016-9 = NC_006051-4] | (LRNV-Belg2) |
| *Mirafiori lettuce big-vein virus* | | |
| (Mirafiori lettuce virus) | | |
| Mirafiori lettuce big-vein virus-LS301-O | [AF525933-6 = NC_004779-82] | (MiLBVV-LS301-O) |

| | | |
|---|---|---|
| *Ranunculus white mottle virus* | | |
| Ranunculus white mottle virus-Rn3 | [AF335429, AY542957*] | (RWMV-Rn3) |
| *Tulip mild mottle mosaic virus* | | |
| Tulip mild mottle mosaic virus-Bas-1 | [AY542958*] | (TMMMV-Bas-1) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

## List of other related viruses which may be members of the genus *Ophiovirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Ophioviridae*

None.

## Phylogenetic relationships within the family

Ophiovirus-specific primers, based on a highly conserved sequence of RNA-1, have been tested in RT-PCR with all ophiovirus species. In all cases, a 136 bp fragment was amplified. Phylogenetic analysis of the deduced 45 aa strings derived from the amplified sequences of available isolates for each species (Figure 3) supported the positions of CPsV, RWMV, LRNV and FreSV as distinct species and indicated a closer relationship between MiLBVV and TMMMV, as already suggested by serological tests and preliminary sequence data. CPsV appears to be more distantly related to the other members, not only from serological and molecular data but also because it infects a woody host and there is no evidence of soil-borne transmission. These differences, if reinforced, may also lead to the re-assignment of the existing species into two separate genera in the family.

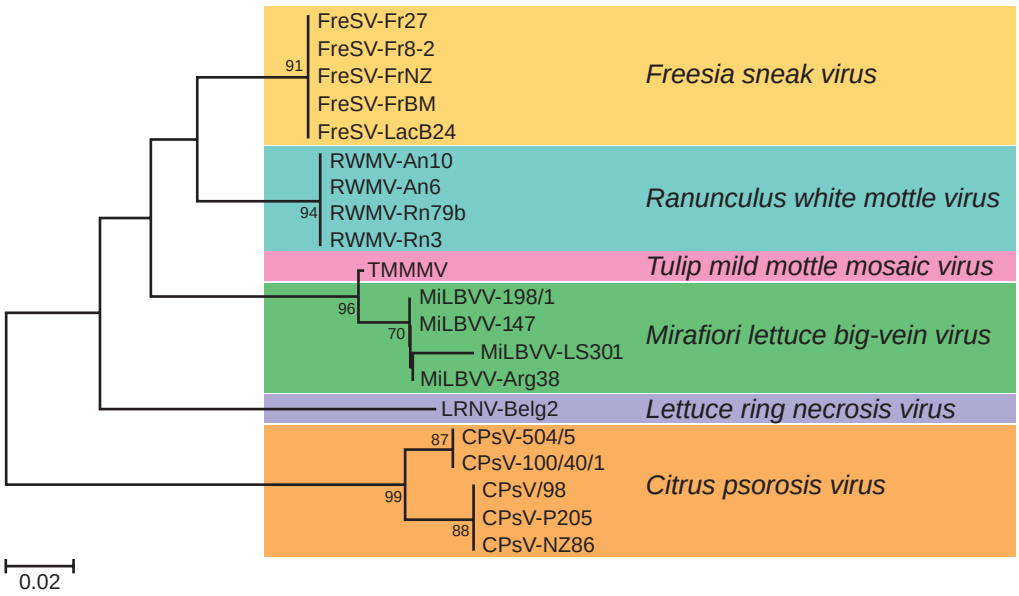


**Figure 3:** Unrooted phylogenetic tree based on the predicted amino acid sequences (45aa) encoded by the conserved 136 nt fragment of the RdRp gene, obtained from 20 ophiovirus isolates belonging to the six species. Alignment was obtained using ClustalW, and analyzed by the neighbor-joining method, with 10 000 bootstrap replications. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches (when >50%). The evolutionary distances were computed using the Poisson correction method. (Courtesy of A. M. Vaira.)

## Similarity with other taxa (Figure 4)

Ophiovirus virion morphology resembles that of the tenuiviruses and the internal nucleocapsid component of members of the family *Bunyaviridae*. Unlike tenuiviruses, ophioviruses do not infect plants in the *Gramineae*, and unlike members of the family *Bunyaviridae* they do not have enveloped virions. Moreover, ophioviruses do not have the conserved identical nucleotides at the genomic RNA termini that are typical of tenui- and phleboviruses (family *Bunyaviridae*). MiLBVV appears to have a terminal "corkscrew"-like conformation similar to that in *Orthomyxoviridae*. RdRp aa sequences show ophioviruses to be similar to members of the families *Paramyxoviridae*,

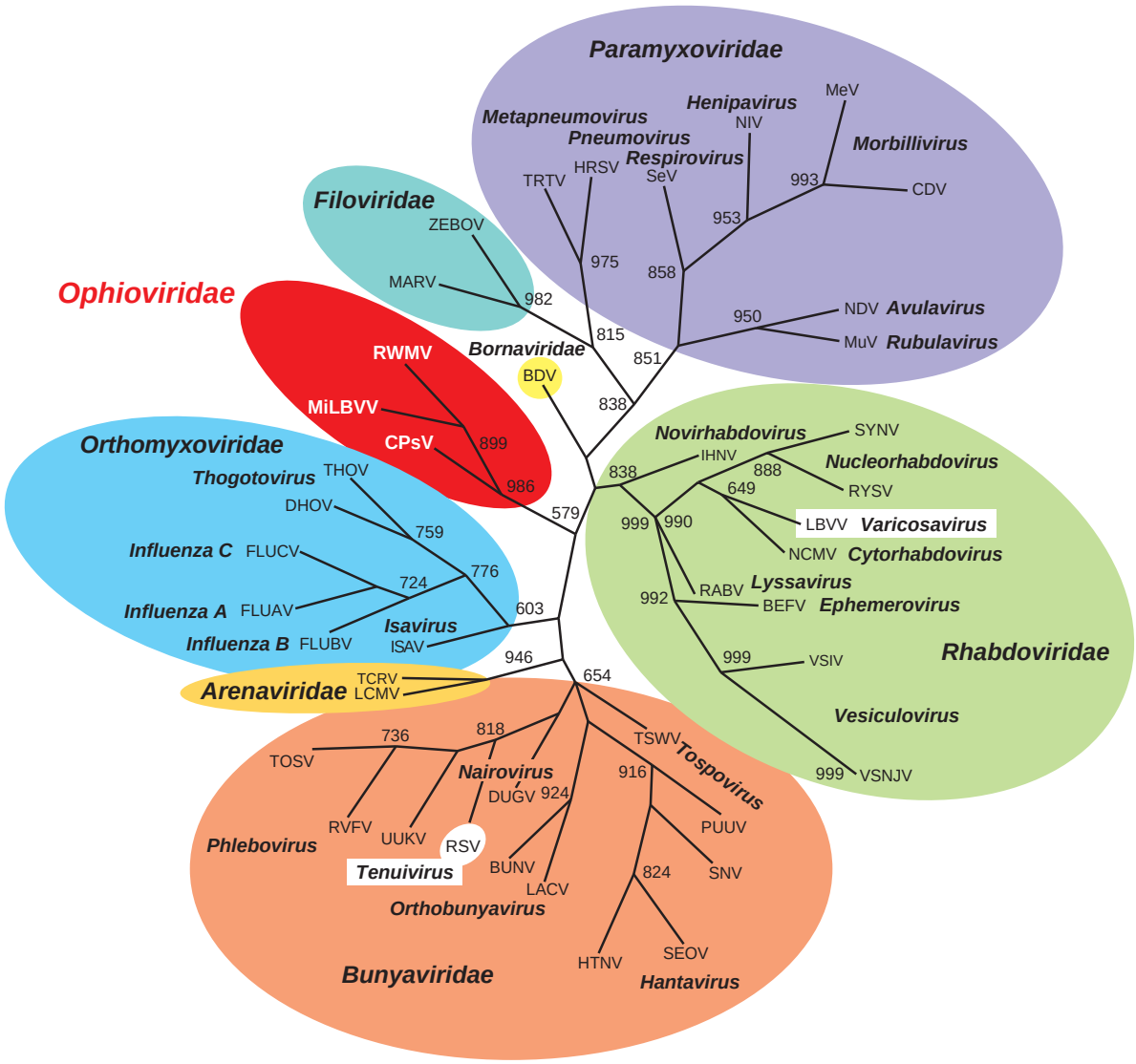


**Figure 4:** Unrooted phylogenetic tree of members of family *Ophioviridae* and other negative stranded RNA viruses based on their conserved RdRp modules. The tree was generated by the neighbor-joining method and bootstrap values (indicated for each branch node when >50%) were estimated using 1000 replicates. Viruses included in the analysis and the accession numbers used [ ] are: BDV [L27077], BEFV [AF234533], BUNV [X14383], CDV [NC_001921], CPsV [AY224663], DHOV [M65866], DUGV [U15018], FLUAV [J02151], FLUBV [M20170], FLUCV [M28060], HRSV [NC_001781], HTNV [X55901], IHNV [L40883], ISAV [AJ002475], LACV [U12396], LBVV [AB075039], LCMV [J04331], MARV [M92834], MEV [K01711], MiLV (MiLBVV) [AF525933], MuV [D10575], NCMV [NC_002251], NDV [X05399], NIV [AF212302], PUUV [M63194], RABV [M13215], RSV [D31879], RVFV [X56464], RWMV [AF335429], RYSV [NC_003746], SEOV [X56492], SeV [M19661], SNV [L37901], SYNV [L32603], TCRV [J04340], THOV [AF004985], TOSV [X68414], TRTV [U65312], TSWV [D10066], UUKV [D10759], VSIV [J02428], VSNJV [M20166], and ZEBOV [AF499101]. (Courtesy of S. Gago-Zachert and M. L. Garcia.)

*Rhabdoviridae*, *Bornaviridae* and *Filoviridae*, and the (unassigned) genus *Varicosavirus* (Figure 4). The RdRp also contains the SDD sequence in motif C, a signature for segmented negative stranded RNA viruses (families *Orthomyxoviridae*, *Arenaviridae* and *Bunyaviridae*). However, phylogenetic reconstructions using the sequences of the conserved RdRp motifs of representative negative stranded RNA viruses reinforce the taxonomic relatedness of the ophioviruses and suggest their separation as a monophyletic group.

## Derivation of name

*Ophio*: from the Greek *ophis*, "serpent", referring to the snaky appearance of the virions.

## Further reading

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Vaira, A.M., Gago-Zachert, S., Garcia, M.L., Guerri, J., Hammond, J., Milne, R.G., Moreno, P., Morikawa, T., Natsuaki, T., Navarro, J.A., Pallas, V., Torok, V., Verbeek, M. and Vetten, H.J.

# Family *Orthomyxoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Orthomyxoviridae*** |
| Genus | ***Influenzavirus A*** |
| Genus | ***Influenzavirus B*** |
| Genus | ***Influenzavirus C*** |
| Genus | ***Thogotovirus*** |
| Genus | ***Isavirus*** |

## Virion properties

### Morphology

Virions are spherical or pleomorphic, 80–120 nm in diameter. Newly isolated influenza viruses contain a significant proportion of filamentous forms, sometimes up to several micrometers in length, whereas laboratory strains with a long passage history in eggs or cell culture are represented mainly by spherical particles (Figure 1). The virion envelope is derived from the cell membrane, incorporating virus glycoproteins (one to three in number) and non-glycosylated proteins (one or two in number). Virion surface glycoprotein projections are 10–14 nm in length and 4–6 nm in diameter. The virus genome is segmented, has helical symmetry, and consists of different size ribonucleoproteins (RNP), 50–150 nm in length.

### Physicochemical and physical properties

The virion molecular mass (Mr) is 250 $10^6$. Virion buoyant density in aqueous sucrose is 1.19 g $cm^{-3}$. $S_{20,w}$ of non-filamentous particles is 700–800S. Virions are sensitive to heat, lipid solvents, non-ionic detergents, formaldehyde, irradiation and oxidizing agents.

### Nucleic acid

Depending on the genus, virions contain different numbers of segments of linear, negative sense ssRNA: eight segments: influenza A virus (FLUAV), influenza B virus (FLUBV) and infectious salmon anemia virus (ISAV); seven segments: influenza C virus (FLUCV) and Dhori virus (DHOV); six segments: Thogoto virus (THOV). Segment lengths range from 736 to 2396 nt. Genome size ranges from 10.0 to 14.6 kb. RNA segments possess conserved and partially complementary 5′- and 3′-end sequences with promoter activity. Shorter viral RNA segments may occur in defective particles.

### Proteins

Structural proteins common to all genera include: three polypeptides that form the viral RdRp (e.g., PA, PB1, PB2 in FLUAV); a nucleoprotein (NP), which is associated with each genome ssRNA segment to form the RNP; a hemagglutinin (HA, HE [HEF] or GP), which is an integral, type I membrane glycoprotein involved in virus attachment, envelope fusion and neutralization; and a non-glycosylated matrix protein (M1 or M). The HA of FLUAV is acylated at the membrane-spanning region and has N-linked glycans at a number of sites. In addition to its hemagglutinating and fusion properties, the HE (HEF) protein of FLUCV has esterase activity that functions as a receptor-destroying enzyme. In contrast, the GP of THOV is unrelated to influenzavirus proteins, but shows sequence homology to a baculovirus surface glycoprotein. Members of the genera *Influenzavirus A* and *Influenzavirus B* have an integral, type II envelope glycoprotein (neuraminidase, NA), which contains sialidase activity. Depending on the genus, viruses possess small integral membrane proteins (M2, NB, BM2, or CM2) that may be glycosylated. M2 and BM2 function as proton-selective ion channels in mammalian cells, acidifying the virion interior during uncoating and fusion and equilibrating the intralumenal pH of the trans-Golgi apparatus with that of the cytoplasm. The ion-channel activity of only the former is inhibited by the adamantane anti-influenza A drugs, amantadine and rimantadine. In addition to the structural proteins and depending on the genus, viruses may code for two nonstructural proteins (NS1, NS2 [NEP]) although NS2 is also found in the virus particle. Virion enzymes (variously represented and reported among genera) include a transcriptase (PB1 in influenzaviruses A, B, C and thogotoviruses), an endonuclease (PA in influenzaviruses A, B, C), and a receptor-destroying enzyme (neuraminidase (NA) for FLUAV and FLUBV, or 9-0-acetylneuraminyl esterase in the case of the FLUCV HE [HEF] protein).

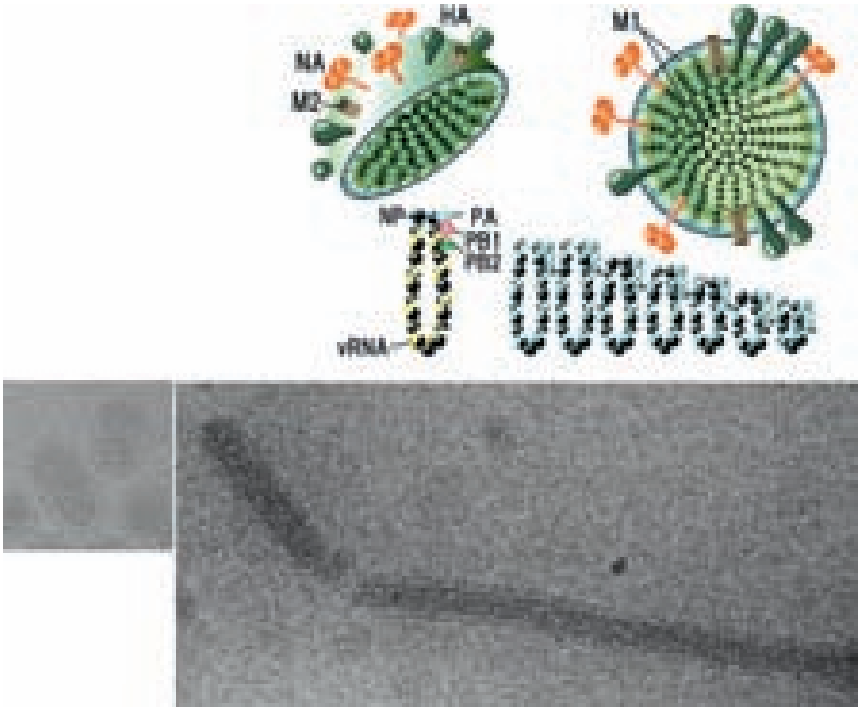


**Figure 1:** (Top) Diagram of an influenza A virus (FLUA) virion in section. The indicated glycoproteins embedded in the lipid membrane are the trimeric hemagglutinin (HA), which predominates, and the tetrameric neuraminidase (NA). The envelope also contains a small number of M2 ion channel proteins. The internal components are the M1 (matrix) protein and the viral ribonucleoprotein (RNP) consisting of RNA segments, associated nucleocapsid protein (NP), and the PA, PB1 and PB2 polymerase proteins. NS2 (NEP), also a virion protein, is not shown. (Bottom) Frozen-hydrated images of a spherical A/Aichi/2/68 X-31 virion (left) and a filamentous A/Udorn/72 virion (right) (image courtesy of Peter Rosenthal, NIMR, London).

### Lipids

Lipids in the virion envelope constitute about 18–37% of the particle weight. They resemble lipids of the host cell plasma membrane.

### Carbohydrates

Carbohydrates in the form of glycoproteins and glycolipids constitute about 5% of the particle weight. They are present as N-glycosidic side chains of glycoproteins, as glycolipids, and as mucopolysaccharides. Their composition is host- and virus-dependent.

## Genome organization and replication

The genome codes for up to 12 proteins of 14–96 kDa. The five largest genome segments encode one protein each (with the exception of the PB1 segment, which for most (but not all) strains, encodes PB1 and PB1-F2 proteins and many also encode a polypeptide PB1 N40 translated in the same open reading frame as PB1 but initiating at a second AUG codon.). By contrast, smaller segments often code for additional proteins from spliced or bicistronic mRNAs. Generally the three largest RNAs encode the P proteins, and the 4th and 5th the viral HA (HE [HEF], GP) and NP proteins. Depending on the virus, the smaller RNA species encode the NA protein (FLUAV NA and FLUBV NA, NB: 6th RNA), the membrane proteins (FLUAV M1, M2 and FLUBV M1, BM2: 7th RNA; FLUCV M1, CM2 and THOV M, ML and DHOV M1: 6th RNA, ISAV: 8th RNA) and NS proteins (FLUAV and FLUBV NS1, NS2 [NEP]: 8th RNA; FLUCV NS1, NS2 [NEP]: 7th RNA; ISAV 7th RNA). Gene reassortment occurs during mixed infections involving viruses of the same genus, but not between viruses of different genera (e.g., FLUAV and FLUBV).

Virus entry involves the HA (HE [HEF], GP) and occurs by receptor-mediated endocytosis. The receptor determinant of influenzaviruses consists of sialic acid bound to glycoproteins or glycolipids. In endosomes, low pH-dependent fusion occurs between viral and cell membranes. For

influenzaviruses, infectivity and fusion depend on the post-translational cleavage of the virion HA (FLUAV and FLUBV: $HA_1$, $HA_2$; FLUCV: $HE_1$ $HE_2$ [$HEF_1$, $HEF_2$]) protein to result in the production of a hydrophobic group of amino acids at the amino terminal of the $HA_2$ molecule. Cleavability depends, among other factors, on the number of basic amino acids at the cleavage site. No requirement for glycoprotein cleavage has been demonstrated for the GP species of thogotoviruses. Integral membrane proteins migrate through the Golgi apparatus to localized regions of the plasma membrane. New virions form by budding, thereby incorporating matrix protein and the viral RNPs which align below regions of the plasma membrane containing viral envelope proteins. Budding is from the apical surface in polarized cells.

Viral RNPs are transported to the cell nucleus where the virion transcriptase complex synthesizes mRNA species. For influenzaviruses, mRNA synthesis is primed by capped RNA fragments 10–13 nt in length that are generated from host heterogeneous nuclear RNA species by viral endonuclease activity associated with the PB1 and PA proteins, after cap recognition by PB2. Thogotoviruses differ from influenzaviruses in having capped viral mRNA without host-derived sequences at the 5′ end. Virus-specific mRNA synthesis is inhibited by actinomycin D or α-amanitin due to inhibition of host DNA-dependent RNA transcription and a (presumed) lack of newly synthesized substrates that allow the viral endonuclease to generate the required capped primers. Virus-specific mRNA species are polyadenylated at the 3′ termini through a mechanism of iterative copying of an oligoU tract in the vRNA template. The mRNAs lack sequences complementary to the 5′-terminal (ca. 16) nucleotides of the viral RNA segment. Certain mRNAs are spliced to provide alternative products (Figure 2).

Protein synthesis occurs in the cytoplasm. However, NP, M1 and NS1 proteins accumulate in the cell nucleus during the first few hours of replication, then migrate to the cytoplasm. Cytoplasmic inclusions of NS1 may be formed.

Complementary RNA molecules which act as templates for new viral RNA synthesis are full-length transcripts and are neither capped nor polyadenylated. These RNAs exist as RNPs in infected cells.

Reverse genetics systems (technologies that allow one to genetically engineer viruses) have been established for FLUAV, FLUBV, FLUCV and THOV, allowing their generation entirely from cloned cDNA.



## Antigenic properties

The best studied antigens are the NP, HA, NA, M1 and NS1 proteins of FLUAV and FLUBV. Considerable variation occurs among the FLUAV HA and NA antigens, less for FLUBV or the HE (HEF) surface antigens of FLUCV. THOV and DHOV do not cross-react in standard serologic tests, while DHOV and Batken virus do. Antibodies to HA, HE (HEF), or GP neutralize virus infectivity. Two major antigenic groups based on the properties of the HA have been identified in ISAV.

Influenza viruses agglutinate erythrocytes of many species. Serotype-specific antibodies may block agglutination. The NA or HE (HEF) of attached influenza virions may destroy sialic acid on the erythrocyte surface and the virus receptors, resulting in the elution of virus. Hemolysis of erythrocytes may be produced by HA at acid pH. In comparison to the influenzaviruses, thogotoviruses and isaviruses exhibit limited hemagglutination with certain erythrocyte species.

## Biological properties

Certain influenzaviruses A naturally infect humans and cause respiratory disease. Particular influenzaviruses A infect other mammalian species and a variety of avian species. Interspecies transmission, though rare, is well documented. Influenza B virus strains appear to naturally infect mainly humans and cause epidemics every few years. Influenzaviruses C cause more limited outbreaks in humans and may also infect pigs. Human influenzaviruses A and B replicate in the amniotic cavity of embryonated hen eggs, and after adaptation they can also be propagated in the allantoic cavity. Influenzaviruses C replicate only in the amniotic cavity. Primary kidney cells from monkeys, humans, calves, pigs and chickens support replication of many FLUAV and FLUBV strains. The majority of these viruses require the addition of trypsin to the growth medium, so that proteolytic HA activation and multiple cycles of replication can occur in some continuous cell lines.

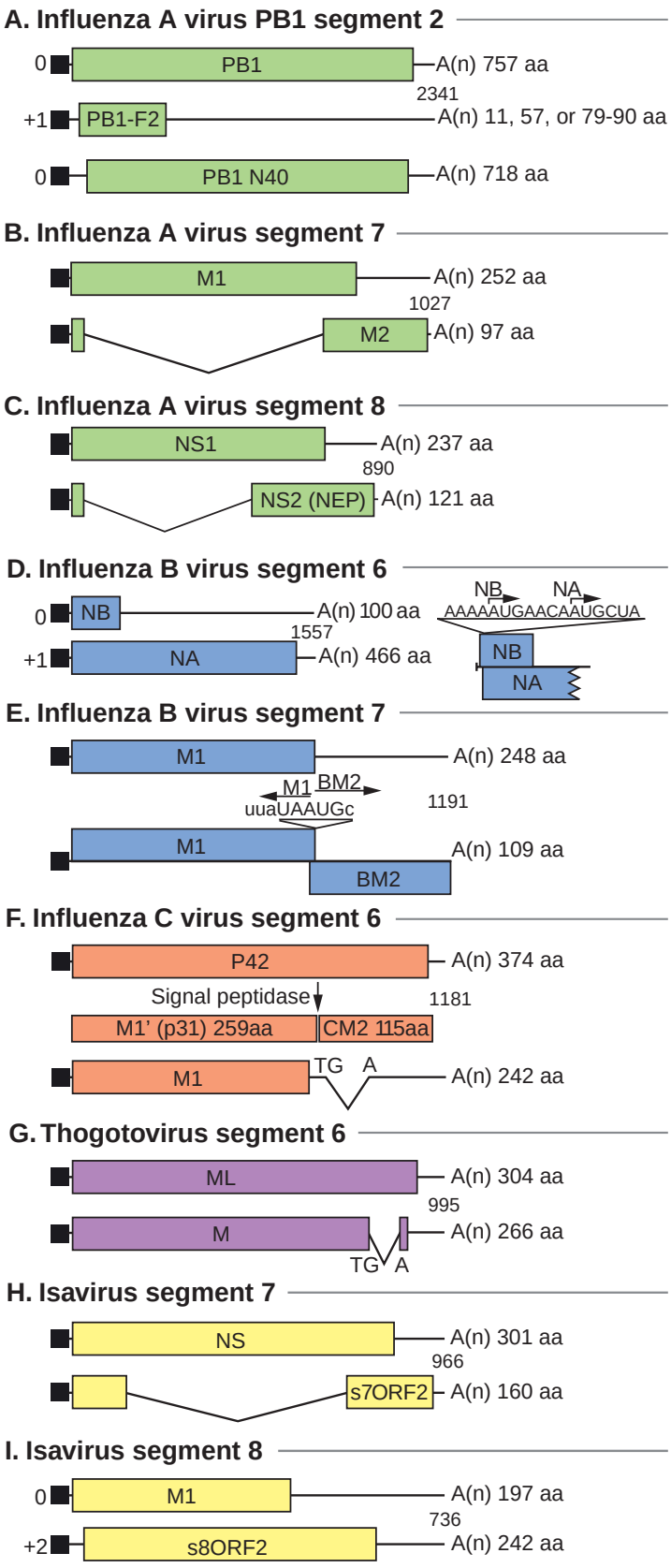

**Figure 2:** Orthomyxovirus genome organization. The genomic organization and ORFs are shown for genes that encode multiple proteins. Segments encoding the polymerase, hemagglutinin and nucleoprotein genes are not depicted as each encodes a single protein. (A) Influenza A virus PB1 segment ORFs. Initiation of PB1 translation is thought to be relatively inefficient based on Kozak's rule, likely allowing initiation of PB1-F2 translation by ribosomal scanning and results in PB1-F2 proteins of different size. In addition, the use of a second AUG, present in many but not all viruses, in frame in the PB1 ORF as the initiation codon encodes the polypeptide PB1 N40, the C terminal 718 amino acids of PB1. (B) Influenza A virus segment 7 showing M1 and M2 mRNAs and their coding regions. M1 and M2 share 9 amino-terminal residues, including the initiating methionine; however, the ORF of M2 mRNA (nt 740–1004) differs from that of M1. (C) Influenza A virus segment 8 showing NS1 and NS2 (NEP) mRNAs and their coding regions. NS1 and NS2 (NEP) share 10 amino-terminal residues, including the initiating methionine. The ORF of NS2 (NEP) mRNA (nt 529-861) differs from that of NS1. (D) ORFs in Influenza B virus RNA segment 6, illustrating the overlapping reading frames of NB and NA. Nucleotide sequence surrounding the 2 AUG initiation codons, in the mRNA sense, is shown. (E) Influenza B virus RNA segment 7 ORFs and the organization of the ORFs used to translate the M1 and BM2 proteins. A stop–start pentanucleotide, thought to couple translation between the two ORFs, is illustrated. (F) Influenza C virus mRNAs derived from RNA segment 6. The unspliced and spliced mRNAs encode P42 and M1, respectively. The cleavage of P42 by a signal peptidase produces M1′(p31) and CM2. (G) Thogoto virus segment 6 showing M and ML. M is translated from a spliced mRNA with a stop codon that is generated by the splicing process itself, as in Influenza C virus M1 mRNA. ML is translated from the unspliced transcript and represents an elongated form of M with a C-terminal extension of 38 aa. (H) Isavirus mRNAs derived from segment 7. The unspliced mRNA encodes the NS protein and the spliced mRNA encodes a polypeptide of unknown function. (I) Isavirus mRNAs derived from segment 8. ORF1 starts at nucleotide 22 and encodes the M1 protein, ORF2 starts at nucleotide 36, in the + 2 reading frame relative to ORF1 and encodes a polypeptide of unknown function. For all panels, the boxes represent different coding regions. Introns in the mRNAs are shown by the V-shaped lines; filled rectangles at the 5′ ends of mRNAs represent heterogeneous nucleotides derived from cellular RNAs that are covalently linked to viral sequences. Lines at the 5′ and 3′ termini of the mRNAs represent untranslated regions. (Modified from Lamb and Horvath (1991). *Trends Genet.*, **7**, 261-266 and Garcia-Rosado et al. (2008). *Virus Res.*, **133**, 228-238.)

Natural transmission of influenzaviruses is by aerosol (human and most non-aquatic hosts) or is water-borne (waterfowl) but direct contact may also be important. Thogoto and Dhori viruses are transmitted by ticks and replicate in both ticks and a variety of tissues and organs in mammalian species as well as in mammalian cell cultures. In some laboratory species (e.g., hamsters for THOV) these infections have a fatal outcome. Unlike influenzaviruses, these viruses do not cause respiratory disease and do not replicate in embryonated hens' eggs. Transmission of isaviruses is via water. Orthomyxoviruses have an Mx1-sensitive step in their multiplication cycle.

## GENUS *INFLUENZAVIRUS A*

Type species ***Influenza A virus***

### Distinguishing features

Member viruses of the genus *Influenzavirus A* all have eight genome segments. The hemagglutinin and neuraminidase receptor-destroying enzyme are different glycoproteins. The conserved end sequences of the viral RNAs are 5′-AGUAGAAACAAGG and 3′-UCG(U/C)UUUCGUCC. The exact order of electrophoretic migration of the RNA segments varies with strain and electrophoretic conditions. On the basis of the gene sequences, for influenza A virus the segments 1–3 encoded PB2, PB1 and PA proteins are estimated to be 84 kDa (observed: 87 kDa), 87 kDa (observed: 96 kDa), and 83 kDa (observed: 85 kDa), respectively. RNA segment 2, the segment that encodes PB1 also encodes a second polypeptide read in an alternative reading frame, PB1-F2, which varies in length between viruses, the full length protein being in the order of 90 amino acids in length, some strains of virus encode a PB1-F2 of around 55 amino acids, the vast majority of the pandemic A(H1N1) 2009 viruses encode a truncated 11-amino acid PB1-F2. The same RNA segment of some viruses encodes a third polypeptide, PB1 N40, 718 amino acids in length. RNA segment 4 encoded (unglycosylated) HA is 63 kDa (glycosylated $HA_1$ is 48 kDa, $HA_2$ is 29 kDa). The segment 5 encoded NP is 56 kDa (observed: 50–60 kDa). The segment 6 encoded NA is 50 kDa (observed: 48–63 kDa). The segment 7 encoded M1 and M2 proteins are 28 kDa (observed: 25 kDa) and 11 kDa (observed: 15 kDa), respectively. The segment 8 encoded NS1 and NS2 (NEP) are 27 kDa (observed: 25 kDa) and 14 kDa (observed: 12 kDa), respectively.

### Antigenic properties

Antigenic variation occurring within the HA and NA antigens of influenzaviruses A has been analyzed in detail. Based on antigenicity, 16 subtypes of HA and nine subtypes of NA are recognized for influenzaviruses A. Additional variation occurs within subtypes. By convention, new isolates

are designated by their serotype/host species/site of origin/strain designation/year of origin and (HA [H] and NA [N] subtype); e.g. A/chicken/Novosibirsk/65/2005 (H5N1). In humans, continual evolution of new strains occurs, and older strains apparently disappear from circulation. The majority of neutralizing antibodies are directed to the HA. If NA antibody is present during multicycle replication, it inhibits virus release and reduces virus yield. Antibody to the amino terminus of M2 reduces virus yield in tissue culture.

## Biological properties

Epidemics of respiratory disease in humans during the 20th–21st century have been caused by influenzaviruses A having the antigenic composition H1N1, H2N2 and H3N2. The pandemics of 1918, 1977 and 2009 were caused by H1N1 viruses, H2N2 caused "Asian influenza" in 1957 and in 1968 "Hong Kong influenza" was caused by an H3N2 virus. H1N2 reassortant viruses between H1N1 and H3N2 human viruses appeared in 2001 and became established, circulating viruses until 2004. Limited outbreaks of respiratory disease in humans caused by antigenically novel viruses occurred in 1976 in Fort Dix, New Jersey, when classical swine H1N1 viruses infected military recruits; and sporadic infections with swine H1N1 viruses have occurred in the intervening years. In 1997 and 2003 in Hong Kong H5N1 viruses caused outbreaks in poultry and contemporary illnesses and deaths in humans. The continued circulation of H5N1 viruses in birds has been associated with zoonotic infections of humans with H5N1 viruses, with a large proportion proving fatal. H9N2 viruses present in poultry have caused occasional illness in humans in China, first observed in 1998, and zoonotic infections have continued to be documented. Influenzaviruses A of subtype H7N7 and H3N8 (previously designated equine 1 and equine 2 viruses, respectively) cause outbreaks of respiratory disease in horses; but H7N7 virus has not been isolated from horses since the late 1970s. Influenzaviruses A (H1N1) and (H3N2) have been isolated frequently from pigs. The H1N1 viruses isolated from swine in recent years appear to be of three general categories: those closely related to classical "swine influenza" and which cause occasional human cases; those first characterized in samples collected from swine in 1979 and genetically more closely related in all gene segments to H1N1 viruses isolated in birds which have become established and cause infection among pigs in Europe and Asia; and those resembling viruses isolated from epidemics in humans since 1977. Swine H1N1 viruses have also been observed as triple reassortants with genes originating from the swine pool, the avian pool and the human pool; the "triple" reassortants with genes form three distinct gene pools were first observed in swine H3N2 viruses. H3N2 viruses from swine appear to contain HA and NA genes closely related to those from human epidemic strains. Triple gene reassortant viruses possessing the H3 HA and N2 NA from a recent human virus and other genes from a swine and/or avian virus were first identified in the North American pig population in 1998 and have been circulating since then. Infections of pigs with the A(H1N1) 2009 pandemic have been documented associated with reverse zoonosis; H1N2 viruses, distinct from those in humans, have been isolated from pigs in UK, France, Japan and the US. Influenzaviruses A (H7N7 and H4N5) have caused outbreaks in seals, with virus spread to non-respiratory tissues in this host. H7N7 viruses have been isolated from conjunctival infections of a laboratory worker and a farm worker in 1980 and 1996, respectively and from humans involved in disease control in 2003. In addition, in 2003, a human was fatally infected with a highly pathogenic avian influenza H7N7 virus. Pacific Ocean whales have reportedly been infected with type A (H1N3) virus. Other influenza subtypes have also been isolated from lungs of Atlantic Ocean whales off North America. FLUAV (H10N4 and H3N2) has caused outbreaks in mink. All subtypes of HA and NA, in many different combinations, have been identified in isolates from avian species, particularly wild aquatic birds, chickens, turkeys and ducks. Pathology in avian species varies from non-apparent infection (often involving replication in, and probable transmission via, the intestinal tract), to more severe infections (observed with subtypes H5 and H7) with spread to many tissues and high mortality rates. The structure of the HA glycoprotein, in particular the specificity of its receptor binding site and its cleavability by host protease(s), appears to be critical in determining the host range and organ tropisms of influenza viruses. The NS1 also contributes to the outcome of infection by mitigating host defense mechanisms; e.g., through anti-interferon activity. In addition, interactions between gene products determine the outcome of infection. Interspecies transmission apparently occurs in some instances without genetic reassortment (e.g., the direct transmission of H1N1 virus from swine to humans and vice versa, H3N2 virus

from humans to swine, and the recent transmissions of H5N1 and H9N2 viruses from poultry to humans). In other cases, interspecies transmission may involve RNA segment reassortment in hosts infected with more than one strain of virus, each with distinct host ranges, or epidemic properties (e.g., 1968 isolates of H3N2 viruses were derived by reassortment between a human H2N2 virus and a virus containing an H3 HA). Laboratory animals that may be infected with influenzaviruses A include ferrets, mice, hamsters and guinea pigs, as well as some small primates such as squirrel monkeys.

### Species demarcation criteria in the genus

Only a single species is currently recognized in the genus *Influenzavirus A*. The species is comprised of a cluster of strains that replicate as a continuous lineage and can genetically reassort with each other. Therefore, although 16 different HA subtypes and nine different NA subtypes are recognized among influenzaviruses A replicating in birds, separate species designations have not been accorded to these subtypes. All isolates are capable of exchanging of RNA segments (reassortment).

### List of species in the genus *Influenzavirus A*

| | | |
|---|---|---|
| *Influenza A virus* | | |
| Influenza A virus A/PR/8/34 (H1N1) | [J02144, J02146, J02148, J02151, V00603, V01099, V01104, V01106] | (FLUAV- A/PR/8/34 (H1N1)) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Influenzavirus A* but have not been approved as species

None reported

## GENUS *INFLUENZAVIRUS B*

Type species ***Influenza B virus***

### Distinguishing features

Member viruses of the genus *Influenzavirus B* all have eight genome segments. As for members of the genus *Influenzavirus A*, hemagglutinin and the neuraminidase receptor-destroying enzyme are different glycoproteins. The conserved end sequences of the viral RNAs of the influenzaviruses B are 5′-AGUAG(A/U)AACAA and 3′-UCGUCUUCGC. Influenza B virus proteins have sizes similar to those for influenza A virus. NB: the second product of FLUBV segment 6 is 11 kDa (glycosylated 18 kDa).

### Antigenic properties

Antigenic variation within the HA and NA antigens of influenzaviruses B has also been analyzed in detail. In contrast to influenzaviruses A, no distinct antigenic subtypes are recognized for members of the species *Influenza B virus*, however, viruses with antigenically and genetically distinguishable lineages of HA and NA (e.g., the B/Victoria/2/87-like and the B/Yamagata/16/88-like viruses) have co-circulated in humans for over two decades. Influenzaviruses B infect humans and they are designated by their serotype/site of origin/strain designation/year of origin (as above B/Victoria/2/87 and B/Yamagata/16/88). There is a report of influenza B infection of marine mammals (seals) and a single virus isolate has been reported; like for influenza A viruses, the species of origin is included for virus strains collected from animals (e.g. B/Seal/Netherlands/1/99). Most neutralizing antibodies bind the HA protein.

### Biological properties

Influenzaviruses B, first isolated in 1940, have been circulating continuously in humans and causing recurrent epidemics of respiratory disease. Antigenic change (antigenic drift) occurs more slowly among influenzaviruses B than influenzaviruses A.

### Species demarcation criteria in the genus

Only a single species is currently recognized in the genus *Influenzavirus B*. The species is comprised of a cluster of strains that replicate as a continuous lineage and can reassort genetically with each other. Although considerable antigenic and sequence differences exist among viruses in this genus, these differences are not sufficient for designation of separate species.

### List of species in the genus *Influenzavirus B*

| | | |
|---|---|---|
| *Influenza B virus* | | |
| Influenza B virus B/Lee/40 | [J02094, J02095, J02096, K00423, K01395, M20168, M20170, M20172] | (FLUBV- B/Lee/40)) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Influenzavirus B* but have not been approved as species

None reported.

## GENUS *INFLUENZAVIRUS C*

Type species ***Influenza C virus***

### Distinguishing features

Member viruses of the genus *Influenzavirus C* naturally infect humans. Viruses have seven genome segments. They lack neuraminidase. The HE (HEF) protein contains the receptor binding and fusion activities and also functions as the receptor-destroying enzyme, 9-0-acetylneuraminyl esterase. The conserved end sequences of the viral RNAs of the influenzaviruses C are 5′-AGCAG(U/G)AGCAAG and 3′-UCGUCUUCGUC. RNA segments 1–3 encode the P proteins (87.8 kDa, 86.0 kDa, and 81.9 kDa, respectively). Segment 4 encodes HE (HEF) (unglycosylated: 72.1 kDa, glycosylated: 88.0 kDa), segment 5 NP (63.5 kDa), segment 6 M1 and P42 (27.0 kDa and 42.0 kDa, respectively) and segment 7 NS1 (27.7 kDa) and NS2 (NEP) (21.0 kDa). Proteolytic cleavage of P42 at an internal signal peptidase cleavage site gives rise to M1′ (p31) and CM2 proteins (31.0 kDa and 18.0 kDa, respectively).

### Antigenic properties

Antigenic drift characterized by the emergence of successive antigenic variants which have descended from those that circulated previously apparently does not occur among influenzaviruses C; however, antigenic variation between distinct co-circulating lineages has been detected in HI tests with both anti-HE (HEF) Mabs and polyclonal antisera. Viruses exhibit no cross-reactivity with influenzaviruses A and B, although homologies of HE (HEF) to influenzavirus A and B HA were identified near the amino and carboxy termini and several of the cysteines co-aligned in the sequences. Antibody to HE (HEF) neutralizes infectivity. Influenzaviruses C are designated by their serotype/site of origin/strain designation/year of origin, e.g. C/Catalonia/1457/2009, and the host from which the viruses was isolated when not human, e.g. C/swine/Beijing/32/81.

### Biological properties

Infection in humans is common in childhood. Occasional outbreaks, but not epidemics, have been detected. Swine in China have been reported to be infected by viruses similar to human Influenza C virus strains.

### Species demarcation criteria in the genus

Only a single species is currently recognized in the genus *Influenzavirus C*. The species is comprised of a cluster of strains that replicate as a continuous lineage and can reassort genetically with each other. Although detectable antigenic and sequence differences exist among this genus, these differences are not sufficient for separate species designation.

### List of species in the genus *Influenzavirus C*

| | | |
|---|---|---|
| *Influenza C virus* | | |
| Influenza C virus C/Ann Arbor/1/50 | [AB126191, AB126192,AB126193, AB126194,AB126195, AB126196, AB283001] | (FLUCV- C/Ann Arbor/1/50) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Influenzavirus C* but have not been approved as species

None reported.

## GENUS *THOGOTOVIRUS*

Type species ***Thogoto virus***

### Distinguishing features

Morphology and morphogenesis of these viruses show similarities with the influenzaviruses. Virions contain segments of linear, negative sense ssRNA. Total genomic size is about 10 kb. Sequences of the ends of vRNA are partially complementary and resemble those of influenzaviruses. The conserved end sequences of THOV viral RNAs are 5′-AGAGA(U/A)AUCAA(G/A)GC and 3′-UCGUUUUUGU(C/U)CG (segments 1-5) or 3′-UCACCUUUGUCCG (segment 6). Intrastrand base-pairings are favored over interstrand base-pairings, leading to a "hook-like" or cork-screw structure. THOV RNA segments 1-3 encode PB2, PB1 and PA proteins (88, 81 and 71.5 kDa, respectively) that exhibit homology to the respective influenzavirus proteins. The single glycoprotein GP (THOV: 75 kDa; DHOV: 65 kDa) is encoded by the fourth segment. It is unrelated to any influenzavirus protein but shows amino acid sequence similarity with the glycoprotein gp64 of baculoviruses. The fifth segment encodes the NP (THOV: 52 kDa; DHOV: 54 kDa), which is related to influenzavirus NP. The sixth segment of THOV encodes the matrix protein M (29 kDa, translated from a spliced mRNA) and a second protein ML (32 kDa, translated from the unspliced mRNA). ML represents an elongated version of M with a C-terminal extension of 38 aa. The sixth segment of DHOV encodes the M1 protein (30 kDa) and may encode another protein, M2 (15 kDa, but not detected in virions) of unknown function. The coding of a putative seventh segment of DHOV is not known.

### Antigenic properties

Antigenic relationships between THOV and DHOV viruses are not apparent and none of the virus proteins are related antigenically to those of influenzaviruses; however, serological cross-reactivity between DHOV and Batken virus has been demonstrated. For THOV and DHOV, several viruses have been isolated; however, the relationships of these isolates to the prototype viruses are not known.

### Biological properties

THOV and DHOV are transmitted between vertebrates by ticks. Comparatively low levels of hemagglutination occur at acidic pH and not at physiological pH. No receptor-destroying enzyme

has been observed. Fusion of infected cells occurs at acidic pH indicating that cell entry is via the endocytic pathway as for the influenzaviruses. Fusion is inhibited by neutralizing monoclonal antibodies directed against GP. Replication is inhibited by actinomycin-D or α-amanitin. Nucleoprotein accumulates early in replication within the nucleus. M of THOV is required for the generation of virus-like particles and infectious recombinant viruses by reverse genetics. In contrast, ML is dispensable for virus growth in cell culture, but appears to be a virulence factor with interferon antagonistic function. THOV is inhibited by the interferon-induced Mx GTPases at an early step in the virus multiplication cycle. Reassortment between THOV temperature-sensitive mutants has been demonstrated experimentally in co-infected ticks and in vertebrates.

### Species demarcation criteria in the genus

THOV has been isolated from *Boophilis* sp. and *Rhipicephalus* sp. ticks in Kenya and Sicily, from *Amblyomma variegatum* ticks in Nigeria, and from *Hyalomma* sp. ticks in Nigeria and Egypt. THOV is known to infect humans in natural settings, and serological evidence suggests that other animals (including cattle, sheep, donkeys, camels, buffaloes and rats) are also susceptible to this virus. THOV has been isolated in the Central African Republic, Cameroon, Uganda, and Ethiopia as well as in southern Europe. DHOV has a somewhat different, but overlapping geographic distribution that includes India, eastern Russia, Egypt and southern Portugal. DHOV has been isolated from *Hyalomma* sp. ticks. As demonstrated by the accidental infection of laboratory workers, DHOV is able to infect humans, causing a febrile illness and encephalitis. Serologic evidence suggests that cattle, goats, camel and waterfowl are also susceptible to this virus. There is no detectable serological reactivity between THOV and DHOV and the sequence diversity of 37% and 31% in the nucleoprotein and the envelope protein, respectively, argues for separate species status. Batken virus isolated from mosquitoes and ticks from Russia cross-reacts serologically with DHOV and shares 98% identity in a portion of the nucleoprotein and 90% identity in a portion of the envelope protein. These data suggest that Batken virus, although isolated from both mosquitoes and ticks, is closely related to DHOV.

### List of species in the genus *Thogotovirus*

| | | |
|---|---|---|
| *Thogoto virus* | | |
| Thogoto virus strain SiAr 126 | [NC_006508, NC_006495, NC_006496, NC_006506, NC_006507, NC_006504] | (THOV) |
| *Dhori virus* | | |
| Dhori virus isolate Dhori/1313/61 | [GU969308, GU969313, GU969309, GU969310, GU969311, GU969312] | (DHOV) |
| Batken virus strain LEIV306K | [X97340, X97341] | (BATV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Thogotovirus* but have not been approved as species

Araguari virus (ARAV)

## GENUS *ISAVIRUS*

Type species ***Infectious salmon anemia virus***

### Distinguishing features

Isaviruses are similar in morphology to influenzaviruses. Surface projections are 10 nm in length. Virion surface glycoproteins are the hemagglutinating and receptor-destroying glycoprotein (HE) and the fusion glycoprotein (F). The receptor-destroying function is an acetylesterase. Isaviruses have eight linear genome segments of negative sense ssRNA. The vRNA 5′-AGUAAAAA(A/U) and 3′-UCG(U/A)UUCUA terminal sequences are conserved among isaviruses and partially

complementary, with some sequence resemblance to those of influenzaviruses. Total genome size is about13.5 kb. Each of the two smallest segments encodes two proteins. Segment 7 gives a spliced mRNA product. The synthesis of mRNA is primed by RNA fragments 8–18 nt in length. The genes of segments 1, 2 and 4 are thought to encode the P proteins based on limited homologies to other RdRp; estimated sizes are 79.9, 80.5, and 65.3 kDa, respectively. Segment 3 encodes NP, which is phosphorylated (68 kDa). Segment 5-encodes F (48.8 kDa), a type 1 membrane glycoprotein. F is proteolytically processed into two disulfide-linked subunits $F_1$ and $F_2$. Segment 6 encodes HE, a type 1 membrane glycoprotein (42.7 kDa). Segment 7 encodes a non-structural protein (NS) with interferon antagonistic function and a protein of unknown function, with estimated sizes of 34.2 and 17.6 kDa, respectively. Segment 8 encodes the matrix protein (M1) and a RNA binding protein with estimated sizes of 22 and 27.6 kDa, respectively.

## Antigenic properties

There is no known antigenic relationship between isavirus proteins and those of influenzaviruses. Neutralizing antibodies are mainly directed against the HE (HEF) protein. The humoral immune response of the host recognizes mainly the HE and NP. There are many isolates of ISAV, and they have been divided into two major antigenic groups based on properties of the HE.

## Biological properties

ISAV is transmitted through water. It agglutinates erythrocytes of many fish species, but not avian or mammalian erythrocytes. Fusion of the virus with infected cells occurs at low pH, suggesting endocytic cell entry. The maximum rate of virus replication in the salmon head kidney cell line (SHK-1) is in the temperature range 10–15 °C; at 20 °C the production of infectious virus is reduced by more than 99% and no replication is observed at 25 °C. Replication is inhibited by actinomycin D and α-amanitin.

## Species demarcation criteria in the genus

Only a single species is currently recognized in the genus *Isavirus*. The species is comprised of a cluster of strains that replicate as a continuous lineage and can reassort genetically with each other. Although detectable antigenic and sequence differences exist among this genus, these differences are not sufficient for separate species designation.

## List of species in the genus *Isavirus*

| | | |
|---|---|---|
| *Infectious salmon anemia virus* | | |
| Infectious salmon anemia virus | [AF404341, AF404340, AF404346, AF404345, AF404344, AF404343, AY373381, AF404342] | (ISAV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Isavirus* but have not been approved as species

None reported.

## List of other related viruses which may be members of the family *Orthomyxoviridae* but have not been approved as species

| | | |
|---|---|---|
| Quaranfil virus | [FJ861695, FJ861694, GQ499304, GQ499303, GQ499302] | (QRFV) |
| Johnston Atoll Virus | [FJ861697, FJ861696] | |
| Lake Chad Virus | [FJ861698] | |

It has been suggested that Quaranfil virus might represent a novel genus in the family *Orthomyxoviridae*. The virus was originally isolated from two children with febrile illness from the villages of Quaranfil and Sindbis in Egypt in 1953. Several strains of Quaranfil virus have been isolated from ticks and seabirds in multiple countries throughout Africa and the Middle East. Johnston Atoll virus is serologically related to Quaranfil. It was originally isolated from ticks (*Ornithodoros capensis*) collected in 1964 from a Noddy Tern (*Anous stolidus*) nest, Sand Island, Johnston Atoll in the central Pacific. Morphology and morphogenesis of these viruses show similarities with the influenzaviruses. Quaranfil virions are reported to contain at least six segments of linear, negative sense ssRNA which have been completely sequenced. Sequences of the ends of vRNA are partially complementary and resemble those of influenzaviruses. The conserved end sequences of both Quaranfil and Johnston Atoll viral RNAs are 5′-AGCAAUCACAA and 3′-UCGUUAGUGU(A/U)(A/G). Quaranfil RNA segments 1–3 (2421 nt, 2404 nt and 2386 nt) encode single ORFs exhibit protein domain homology to the respective influenzavirus polymerase proteins PB2, PA and PB1. The fifth segment (1616 nt) is unrelated to any influenzavirus protein but shows aa sequence similarity with the glycoprotein (gp64) of baculoviruses and the GP of the thogotoviruses. The fourth segment (1726 nt) contains one single predicted ORF of 527 aa which does not share significant sequence homology with any protein currently in the GenBank database. Similarly, the sixth segment of 898 nt contains one predicted ORF of 266 aa which does not share sequence homology with any known protein. It is unclear which segments encode the nucleoprotein or matrix protein of the Quaranfil viruses. There is no significant antigenic relationship between Quaranfil and either the thogotoviruses or the influenzaviruses. Quaranfil and Johnston Atoll are transmitted between vertebrates by ticks. Comparatively low levels of hemagglutination occur at acidic pH and not at physiological pH for Quaranfil viruses against goose red blood cells. Johnston Atoll virus does not agglutinate goose red blood cells across pH 5.75–7.0.

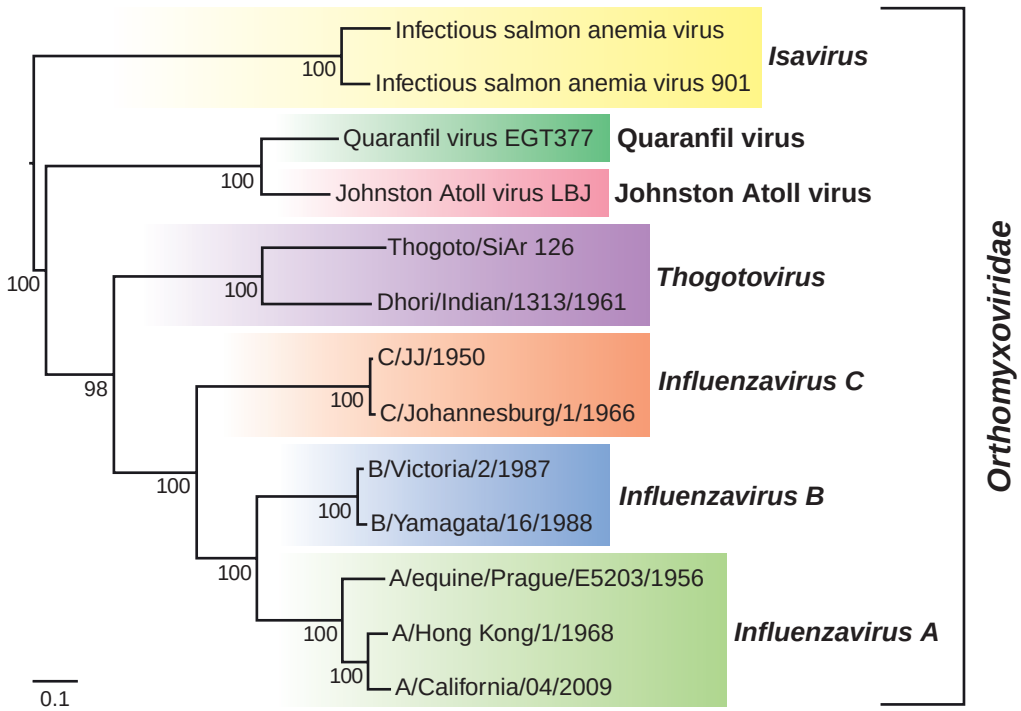


**Figure 3:** Phylogenetic relationships within the family *Orthomyxoviridae*. Nucleotide sequences of the polymerase basic 1 proteins (PB1) were aligned using transAlign and CLUSTAL W, and their phylogenetic relationships were determined by the neighbor-joining method (HKY model) using PAUP* (version 4.0b). The tree was mid-point rooted and bootstrap values (1000 replicates) are indicated on the branches. The GenBank accession numbers for the sequences used for comparison were (top to bottom) AF404346, GU830904, FJ861695, FJ861697, AF004985, M65866, M28060, AF170575, CY018763, CY018771, GU053121, CY044267 and FJ966080.

## Phylogenetic relationships within the family

Phylogenetic relationships within the family are illustrated in Figure 3.

## Similarity with other taxa

Not reported.

## Derivation of names

*Influenza*: Italian form of Latin *influentia*, "epidemic", originally used because epidemics were thought to be due to astrological or other occult "influences".
*Isavirus*: from *i*nfection *s*almon *a*nemia virus
*Myxo*: from Greek *myxa*, "mucus".
*Ortho*: from Greek *orthos*, "straight".
*Thogoto*: from Thogoto Forest near Nairobi, Kenya, where Thogoto virus was first isolated from ticks.

## Further reading

### Journals and books

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Rimstad, E., Mjaaland, S., Snow, M., Mikalsen, A.B. and Cunningham, C. O. (2001). Characterisation of the genomic segment of infectious salmon anemia virus that encodes the putative hemagglutinin. *J. Virol.*, **75**, 5352–5356.
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### Websites

NCBI Influenza Virus Resource: http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html
Influenza Research Database: http://www.fludb.org/brc/home.do?decorator=influenza
Global Initiative on Sharing All Influenza Data (GISAID): http://platform.gisaid.org/

## Contributed by

McCauley J.W., Hongo S., Kaverin N.V., Kochs G., Lamb R.A., Matrosovich M.N., Perez D.R., Palese P., Presti R.M., Rimstad E. and Smith, G.J.D.

# Genus *Deltavirus*

Type species *Hepatitis delta virus*

## Virion properties

### Morphology

Virions of hepatitis delta virus (HDV) are approximately spherical, with an average diameter of 36–43 nm and no visible surface projections (Figure 1). They consist of an outer envelope containing lipid and all three envelope proteins of the co-infecting helper hepadnavirus (human hepatitis B virus (HBV) in nature, though woodchuck hepatitis virus can also act as a helper in the laboratory) (see below), and an inner nucleocapsid of 19 nm comprising the RNA genome of HDV and approximately 70 copies of the only HDV-encoded protein, known as hepatitis delta antigen (HDAg). HDAg exists in two forms (large HDAg, L-HDAg or p27; and small HDAg, S-HDAg or p24), which differ only by a 19 amino acid residue C-terminal extension. Virions contain variable amounts of L-HDAg and S-HDAg in close association with virion RNA. Nucleocapsid symmetry has not been confirmed. Nucleocapsids can be released by treatment of virions with non-ionic detergent and dithiothreitol.

### Physicochemical and physical properties

Virions have a buoyant density in CsCl of about 1.24 g cm$^{-3}$. In rate zonal sedimentation they behave as smaller than HBV.

### Nucleic acid

The genome consists of a single molecule of circular, negative sense ssRNA about 1.7 kb in length. With a high degree (ca. 70%) of intramolecular base pairing, it has the potential to fold on itself, forming an unbranched rod-like structure. Both genomic and antigenomic RNA species can function as a ribozyme to carry out self-cleavage and possibly self-ligation. The above properties make this genome unique and distinct from all other known animal viruses.

### Proteins

HDV RNA encodes one known protein S-HDAg (see above). A second species, L-HDAg, arises via an RNA editing event mediated by a host double stranded RNA adenosine deaminase, ADAR1. As a result of its action, the UAG stop codon for S-HDAg is converted to UGG, thereby allowing readthrough translation giving rise to L-HDAg. Both HDAg species are multifunctional with domains identified that result in (from the N-terminus): (i) dimerization via a coiled coil structure; (ii) nuclear localization via a bipartite signal; (iii) RNA binding via two arginine-rich motifs. In addition, L-HDAg has a domain that includes a prenylation site required for packaging. The two

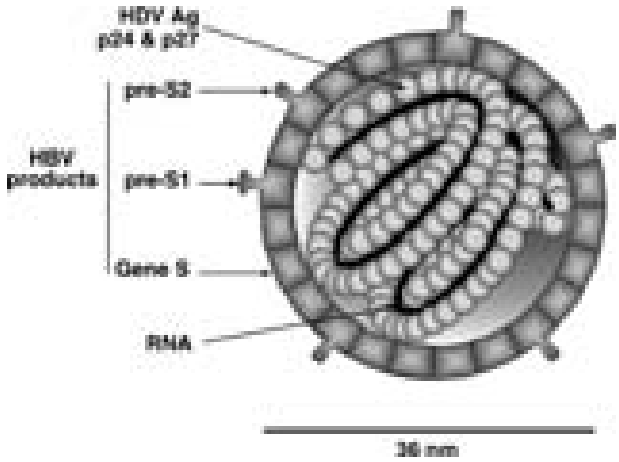


**Figure 1:** Schematic representation of a particle of hepatitis delta virus.

HDAg species play distinct roles in replication; S-HDAg is essential for HDV replication while L-HDAg is essential for packaging and in some situations, may inhibit replication. HDAg can be phosphorylated at serine residues.

The remaining structural proteins of the HDV virion consist of the surface proteins and glycoproteins of the helper hepadnavirus located in the HDV envelope.

### Lipids
These are present but have not been characterized.

### Carbohydrates
Presumably these are only present on the envelope proteins of HBV, the natural helper virus.

## Genome organization and replication

Attachment, entry and uncoating of HDV may be similar to the steps that occur with the helper hepadnavirus. Both viruses require the preS1 domain of the hepatitis B virus large envelope protein for attachment. Genome replication involves RNA-directed RNA synthesis carried out by host cell RNA polymerase II in the nucleus. An additional host polymerase might also be involved. Transcription is thought to occur by a double rolling circle mechanism that generates oligomeric forms of each complementarity, which then undergo site-specific autocatalytic cleavage and ligation to generate circular genomic and antigenomic monomers (Figure 2).

Only one HDV mRNA species has been identified, that coding for HDAg. In transfected cells, only S-HDAg is made initially and L-HDAg appears subsequently as a result of the RNA editing event described above. Such editing occurs during replication in tissue culture as well as in infected chimpanzees and woodchucks.

As HDV assembly requires the envelope proteins of a helper hepadnavirus, its assembly pathway is likely to overlap with that of the helper virus. In dually transfected cells, L-HDAg must be present for delta antigen-containing particles to be released, while S-HDAg is packaged if present in the cell, but is not sufficient for particle formation. Full size, or deleted, HDV RNA molecules are incorporated if present in the cell, as long as they are capable of folding into rod-like structures and binding with HDAgs. In cells undergoing HDV RNA replication, this process is highly specific

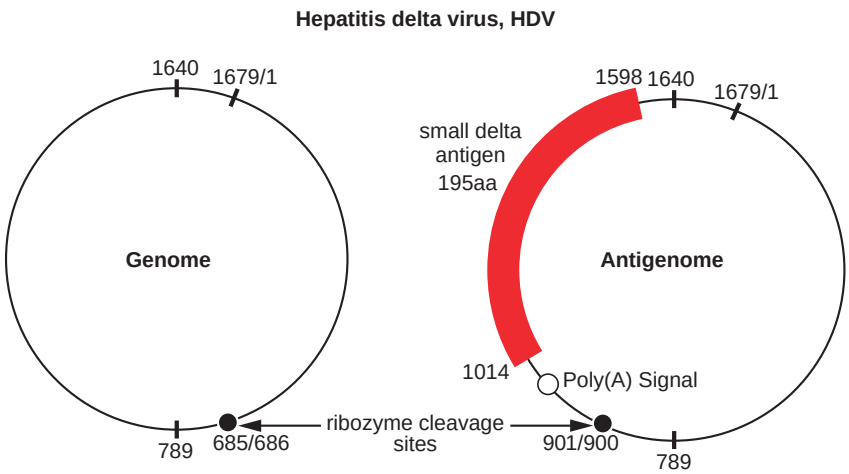


**Figure 2:** Organization of the genome and antigenome of hepatitis delta virus. The genome, by definition, is the 1679-nucleotide RNA that is assembled into virions. During replication one also detects the antigenome, a species that is an exact complement of the genome. Both RNAs are circular in conformation and have the ability to fold into an unbranched rodlike structure via intra-molecular base pairing, with the locations of the ends as indicated. Each has a ribozyme, with the cleavage sites as indicated. The antigenome contains the ORF for the 195 amino acid small delta antigen. However, this protein is actually translated from a third RNA, one that is only about 900 nt long, linear in conformation, 5′-capped and 3′-polyadenylated.

for genomic RNA, while in cells expressing but not replicating HDV RNA, either sense can be assembled.

## Antigenic properties

HDAg molecules have a unique antigenicity. Antibodies to these epitopes are diagnostic of current or past infections.

## Biological properties

Full replication of HDV requires the presence of a helper hepadnavirus to provide envelope proteins, and HDV can therefore be considered as a subviral satellite virus. Natural HDV infection is found only in humans with HBV as helper virus. However, it can be transmitted to chimpanzees if accompanied by HBV, and experimental transmission of HDV to woodchucks has also been achieved using woodchuck hepatitis virus as helper virus.

Transmission of HDV to laboratory mice has been reported, leading to a single round of HDV genome replication in hepatocytes but no further replication, presumably due to the absence of helper virus.

Transmission of HDV in humans occurs by similar routes to those utilized by HBV, although, in many parts of the world, transmission by parenteral contact (e.g. sharing of intravenous needles) is more prominent than sexual or vertical routes. If transmission occurs to an individual with chronic HBV infection, this situation is termed super-infection and HDV infection usually then persists. On the other hand, if both HDV and HBV are simultaneously transmitted to a naïve host, the situation is termed co-infection and both infections are usually transient. HDV distribution is world-wide, but the proportion of HBV carriers who also have chronic HDV infection varies greatly between 0% and 60% in different geographical areas.

Clinical sequelae of acute and chronic HDV infection are variable and cover a similar spectrum to those of HBV alone. They include acute hepatitis, chronic active hepatitis, cirrhosis, fulminant acute hepatitis and hepatocellular carcinoma. However, the frequency of severe sequelae and their rates of progression are significantly higher in chronic HDV infection than in chronic HBV infection alone. A subacute, rapidly progressive form of HDV super-infection has been seen in HBV carriers in Venezuela, and other forms of severe acute and chronic hepatitis D, often fatal, occur in indigenous populations of Venezuela, Colombia, Brazil and Peru.



## Species demarcation criteria in the genus

Humans are the only known natural host for HDV. Only one species is identified in the genus. Sequencing independent isolates of HDV revealed up to 40% variation in nucleotide sequence with up to 25 nucleotides variation in length. Studies of nucleotide homologies have distinguished eight major monophyletic groups or genotypes, HDV1 to HDV8. There is some geographical clustering, with HDV-1 found in USA, Europe and China, HDV-2 and HDV-4 in Japan and Taiwan, HDV-3 in South America predominantly associated with HBV/F, and HDV-5 to HDV-8 in Africa.

## List of species in the genus *Deltavirus*

*Hepatitis delta virus*

| | | |
|---|---|---|
| Hepatitis delta virus - 1 (USA, Europe, China) | [X04451] | (HDV-1) |
| Hepatitis delta virus - 2 (Japan) | [X60193] | (HDV-2) |
| Hepatitis delta virus - 3 (South America) | [L22063] | (HDV-3) |
| Hepatitis delta virus - 4 (Taiwan, Japan) | [AF018077] | (HDV-4) |
| Hepatitis delta virus - 5 (Africa) | [AJ584848] | (HDV-5) |
| Hepatitis delta virus - 6 (Africa) | [AJ584847] | (HDV-6) |
| Hepatitis delta virus - 7 (Africa) | [AJ584844] | (HDV-7) |
| Hepatitis delta virus - 8 (Africa) | [AJ584849] | (HDV-8) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses that may be members of the genus *Deltavirus* but have not been approved as species

None reported.

## Similarity with other taxa

Several features of HDV (genome structure, RNA-RNA transcription using RNA polymerase II, and autocatalytic RNA sites) are similar to properties of some viroids. However, unlike viroids, HDV possesses a larger genome, encodes a functional protein and requires a specific hepadnavirus helper function.

HDV also possesses some features in common with ssRNA satellites of plants, including both the large and circular ssRNA satellites and also (in terms of the satellite-helper relationship) with ssRNA satellite viruses such as chronic bee-paralysis virus-associated satellite virus and tobacco necrosis satellite virus.

However, on the basis of genome size and structure, mode of replication, protein coding strategy, structure of virion and satellite-helper virus relationship, none of the above examples warrants inclusion in a distinct family together with HDV.

## Derivation of name

*Delta*: A novel antigen in HBV infected tissue, unrelated to previously described HBV antigens, was named delta antigen (δAg) by Rizzetto *et al*. in 1977.

## Further reading

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## Contributed by

Mason, W.S., Gerlich, W.H., Taylor, J.M., Kann, M., Mizokami, M., Loeb, D., Sureau, C., Magnius, L. and Norder, H.

# Genus *Emaravirus*

Type species *European mountain ash ringspot-associated virus*

## Distinguishing features

Plant viruses with four components of negative sense ssRNA and enveloped spherical virions. They are only distantly related to other ssRNA viruses.

## Virion properties

### Morphology

Virions are approximately spherical and enveloped with diameter 80–100 nm (Figure 1).

## Physicochemical and physical properties

### Nucleic acid

Four segments of negative sense ssRNA of approximately 7.0, 2.3, 1.6 and 1.4 kb. All four RNA ends are fully conserved in a stretch of 13 nucleotides (nt). The 5′ and 3′ ends of each RNA are almost fully complementary over a sequence of between 19 and 23 nt.

### Proteins

There is a nucelocapsid protein of 35.1 kDa and it is likely that a glycoprotein is integrated into the viral envelope.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

Each segment of the genome encodes a single protein translated from the complementary strand. The largest segment (RNA1) encodes an RNA polymerase (266 kDa). RNA 2 encodes a glycoprotein precursor (75 kDa) that is predicted to be cleaved into products of 52 and 23 kDa. The nucleocapsid protein (35 kDa) is translated from RNA3 and an unkown protein of 27 kDa is the predicted product of the ORF on RNA4 (Figure 2).

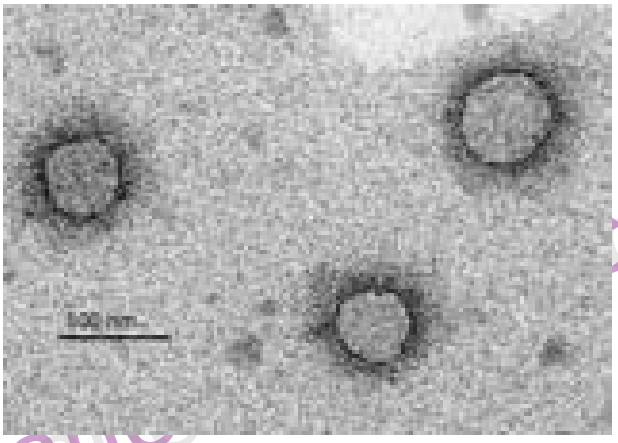


**Figure 1:** Immunosorbent electron micrograph of virions of European mountain ash ringspot-associated virus. The bar represents 100 nm. (Courtesy Inga Ludenberg, University of Hamburg.)

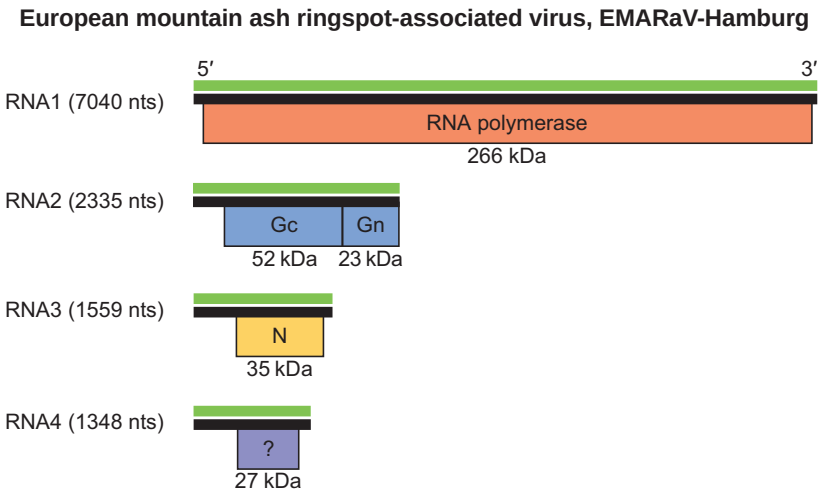


**Figure 2:** Genome organization of the Hamburg isolate of the species European mountain ash ringspot-associated virus. Green lines represent the virion-sense RNA and black lines the virion-complementary sense RNA, from which the proteins shown are translated. Gn and Gc are the two putative glycoproteins cleaved from the precursor molecule. N is the nucleocapsid protein.

## Antigenic properties

No information available.

## Biological properties

The virus is associated with, and the probable cause of, a leaf mottling and ringspot disease of European mountain ash (*Sorbus aucuparia*). It can be transmitted by grafting and there is some evidence that it may be naturally transmitted by mites.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Emaravirus*

| | | |
|---|---|---|
| *European mountain ash ringspot-associated virus* | | |
| European mountain ash ringspot-associated virus-Hamburg | RNA1: [ AY563040 = NC_013105]<br>RNA2: [ AY563041 = NC_013106]<br>RNA3: [ DQ831831 = NC_013108]<br>RNA4: [ DQ831828 = NC_013107] | (EMARaV-Hamburg) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Emaravirus* but have not been approved as species

| | | |
|---|---|---|
| Fig mosaic virus | RNA1: [AM941711]<br>RNA2: [FM864225]<br>RNA3: [FM992851]<br>RNA4: [FM991954] | (FMV) |
| Maize red stripe virus (High Plains virus) | RNA3: [DQ324466] | (MRSV) |
| Pigeon pea sterility mosaic virus | [AJ439561]* | (PPSMV) |

* Probably an incomplete RNA3 sequence containing the C-terminus of the nucleocapsid protein.

## Similarity with other taxa

The genus *Emaravirus* shares some similarities with viruses belonging to the family *Bunyaviridae* and the floating genus *Tenuivirus*. The segmented genome of emaraviruses is of negative polarity. Based on a comparison of the amino acid sequences of the conserved RdRp motifs, highest similarity can be found with the plant-infecting genus *Tospovirus* and the orthobunyaviruses within the family *Bunyaviridae*. In addition, the 3′ and 5′ ends of the genomic RNAs are complementary and their conserved sequence resembles that of the genera *Orthobunyavirus* and *Hantavirus* (*Bunyaviridae*). However, the number of genome segments (at least four) distinguishes the genus *Emaravirus* from bunyaviruses and sequence analyses do not allow classification within either the family *Bunyaviridae* or the genus *Tenuivirus*.

## Derivation of name

*Emara*: from *E*uropean *m*ountain *a*sh *r*ingspot-*a*ssociated virus.

## Further reading

Benthack, W., Mielke, N., Büttner, C. and Mühlbach, H.-P. (2005). Double-stranded RNA pattern and partial sequence data indicate plant virus infection associated with ringspot disease of European mountain ash (*Sorbus aucuparia* L.). *Arch. Virol.*, **150**, 37–52.

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Elbeaino, T., Digiaro, M. and Martelli, G.P. (2009). Complete nucleotide sequence of four viral RNA segments of fig mosaic virus. *Arch. Virol.*, **154**, 1719–1727.

Mielke, N. and Muehlbach, H.-P. (2007). A novel, multipartite, negative-strand RNA virus is associated with the ringspot disease of European mountain ash (*Sorbus aucuparia* L.). *J. Gen. Virol.*, **88**, 1337–1346.

Mielke, N., Weber, M., Khan, S. and Muehlbach, H.-P. (2008). Detection of European mountain ash ringspot associated virus (EMARAV) in *Sorbus aucuparia* L. by a specific antiserum and reverse transcription PCR. *For. Path.*, **38**, 371–380.

Mielke-Ehret, N.,Thoma, J., Schlatermund, N. and Mühlbach, H.-P. (2010). Detection of European mountain ash ringspot-associated virus-specific RNA and protein P3 in the pear leaf blister mite *Phytoptus pyri* (Eriophyidae). *Arch. Virol.*, **155,** 987-991.

## Contributed by

Mühlbach, H.-P. and Mielke-Ehret, N.

# GENUS TENUIVIRUS

Type species *Rice stripe virus*

## Virion properties

### MORPHOLOGY

The ribonucleoproteins (RNPs) have a thin filamentous shape; they consist of nucleocapsids, 3–10 nm in diameter, with lengths proportional to the sizes of the RNAs they contain. The filamentous particles may appear to be spiral-shaped, branched or circular (Figure 1). No envelope has been observed.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

RNP preparations can be separated into four or five components by sucrose density gradient centrifugation, but form one component with a buoyant density 1.282–1.288 g $cm^{-3}$ when centrifuged to equilibrium in CsCl solutions.

### NUCLEIC ACID

The ssRNA genome consists of four or more segments. The sizes are about 9 kb (RNA-1, generally of negative polarity), 3.3–3.6 kb (RNA-2, ambisense), 2.2–2.5 kb (RNA-3, ambisense), and 1.9–2.2 kb (RNA-4, ambisense). RNP preparations of maize stripe virus (MSpV) and Echinochloa hoja blanca virus (EHBV) contain a fifth RNA of negative polarity and with a size of 1.3 kb. A fifth RNA segment has also been reported for some isolates of rice stripe virus (RSV). Rice grassy stunt virus (RGSV) preparations contain six segments, all of which are ambisense. RGSV RNAs-1, -2, -5 and -6 are homologous to RNA-1, -2, -3 and -4 respectively of other tenuiviruses, whereas RNA-3 (3.1 kb) and RNA-4 (2.9 kb) are unique to RGSV.

### PROTEINS

The nucleocapsid proteins are of 34–35 kDa. Small amounts of a minor 230 kDa protein co-purify with RNPs of RSV, rice hoja blanca virus (RHBV) and RGSV. This protein may be an RdRp polymerase, as this activity is associated with filamentous nucleoprotein particles.

### LIPIDS

None reported.



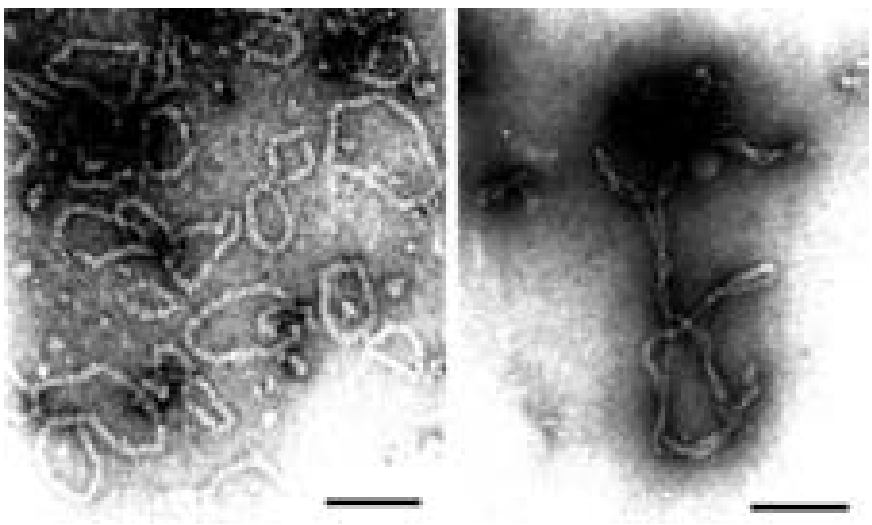

**Figure 1:** Electron micrographs of sucrose density gradient purified RNPs of rice hoja blanca virus (RHBV). (Left) Small circular RNPs from the slowest sedimenting RHBV RNP. (Right) Larger, circular RNPs from the fastest sedimenting RHBV RNP. The bar represents 100 nm. (Courtesy of A.M. Espinoza.)

### Carbohydrates

None reported.

## Genome organization and replication

The 3′- and 5′-terminal sequences of each ssRNA are almost complementary for about 20 bases. Several RNA segments encode two proteins in an ambisense arrangement (Figure 2). In most tenuiviruses, the nucleocapsid protein (pC3; N) is encoded by the 5′-proximal region of the virion-complementary sense strand of RNA-3. Virion-sense RNA-4 encodes in its 5′-proximal region a major non-structural protein (p4; NCP) that accumulates in infected plants. Some of the intergenic non-coding regions (NCRs) between the ORFs can adopt hairpin structures. Some segments (e.g. RNA-1 of RSV and RNA-5 of MSpV) are of negative polarity. RNA-1 encodes the RdRp (pC1; RdRp). Some proteins are translated from sgRNAs (Figure 3). For MSpV, RHBV and RSV mRNAs, the production of mRNAs involves a cap-snatching mechanism. An RNA polymerase has been found associated with purified preparations of RSV, RHBV and RGSV. The RNA polymerase

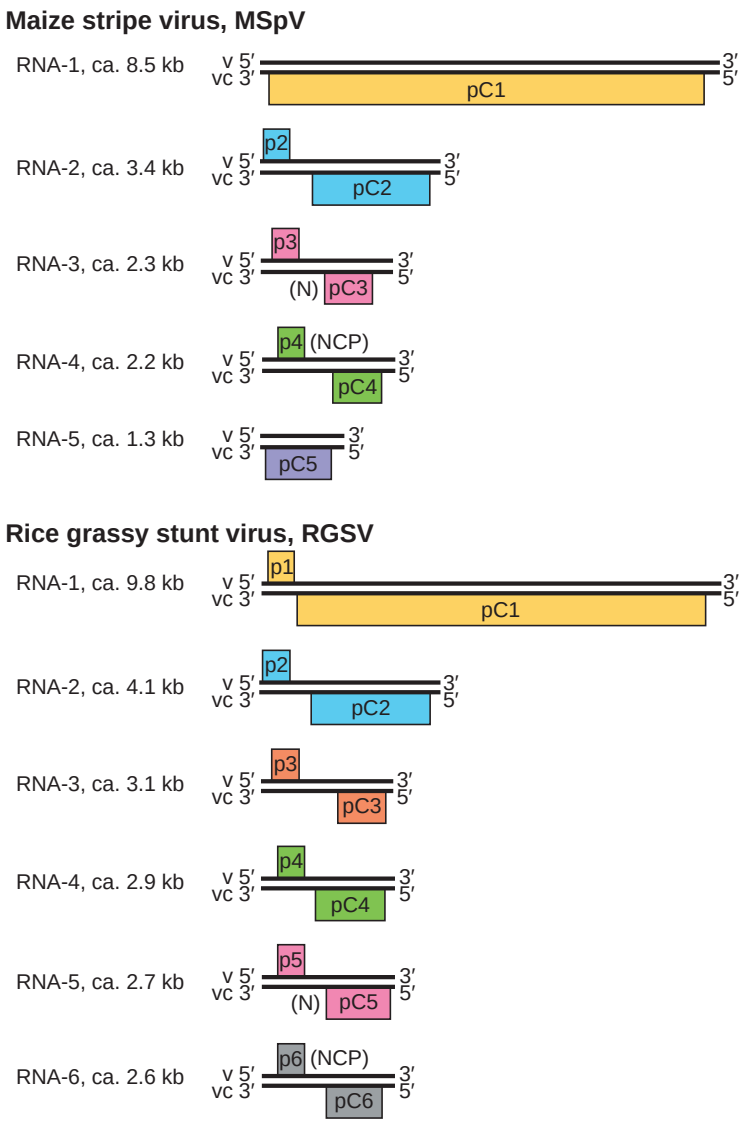


**Figure 2:** Genome organization characteristic of (top) maize stripe virus (MSpV) and (bottom) rice grassy stunt virus (RGSV). Boxes indicate the positions and designations of the ORF translation products. V signifies virion-sense RNA and VC signifies virion-complementary sense RNA.

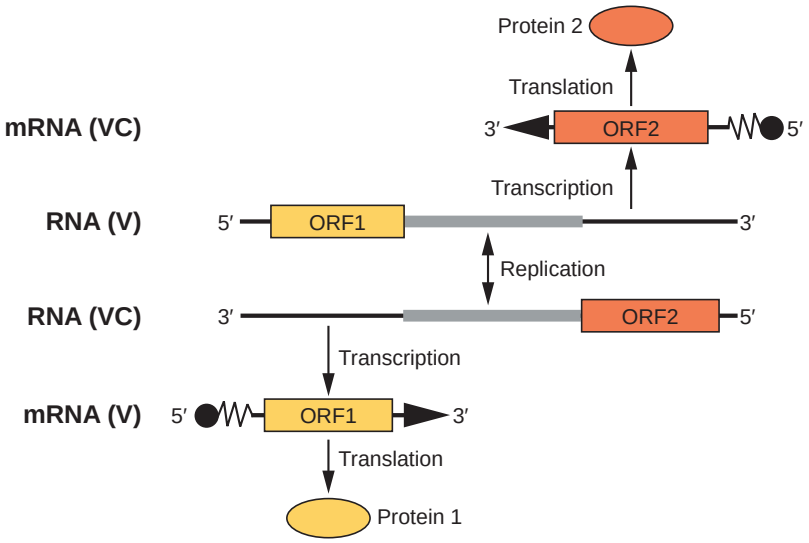


**Figure 3:** Diagram of the expression of the ambisense RNA of tenuiviruses. The black circle signifies the Cap, the broken line signifies non-viral nucleotides, V signifies virion-sense RNA and VC signifies virion-complementary sense RNA.

activity of RHBV is capable of replicating and transcribing the RNA segments *in vitro*. In RHBV and RSV, p3 is a suppressor of RNA silencing. In RSV, pC4 is a movement protein.

## Antigenic properties

The N proteins of RSV and MSpV are serologically related, and both the N and NCP proteins of RSV and RGSV are related. Likewise, the N proteins of RHBV, EHBV and Urochloa hoja blanca virus (UHBV) are serologically related. The RSV N protein reacts weakly with antibodies made to virion preparations of RGSV or RHBV.

## Biological properties

### Host range

Plant hosts of tenuiviruses are all in the family *Gramineae*.

### Transmission

Each species is transmitted by a particular species of planthopper in a circulative, propagative manner. The major vectors are *Laodelphax striatellus* (RSV), *Peregrinus maidis* (MSpV), *Tagosodes orizicolus* (RHBV), *T. cubanus* (EHBV), *Nilaparvata lugens* (RGSV), *Caenodelphax teapae* (UHBV), *Ukanodes tanasijevici* (Iranian wheat stripe virus; IWSV), *Javesella pellucida* (European wheat striate mosaic virus; EWSMV) and *Sogatella kolophon* (Brazilian wheat spike virus; BWSV).

Tenuiviruses can be transmitted transovarially by viruliferous female planthoppers to their offspring, and through sperm from viruliferous males. Mechanical transmission using sap extracts is difficult.

### Cytopathic effects

Characteristic inclusion bodies, consisting almost entirely of NCP are formed in cells of infected plants. The protein p5 of RGSV accumulates in large amounts in both infected plants and vector insects.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Vector specificity, i.e. transmission by different species of vector
- Host range, i.e. different abilities to infect key plant species

- Different sizes and/or numbers of RNA components
- <85% aa sequence identity between any corresponding gene products
- <60% nt sequence identity between corresponding non-coding intergenic regions

An example of species discrimination is that between RSV and MSpV. RSV is transmitted by *L. striatellus* and infects 37 species in the *Gramineae* including wheat and rice. MSpV is transmitted by *P. maidis* and infects maize, occasionally sorghum and a few other graminaceous plants but not wheat or rice. RSV isolates have genomes of four RNA segments of 9090, 3514, 2475 to 2504 and 2137 to 2157 nt; the MSpV genome has five segments (ca. 9000, 3575, 2357, 2227 and 1317 nt). Also, the differences in sequence among the components of these viruses all fall outside the limits set by the Species Demarcation Criteria.

It is difficult to decide if RHBV, EHBV and UHBV are the same or different species. They have different vectors, different hosts, different sizes and numbers of RNA segments and the nt identity of their intergenic regions is less than 60%. However, the aa sequences of the four proteins on RNA-3 and RNA-4 are about 90% identical between RHBV, EHBV and UHBV (although the nt identities of these same coding regions are about 81% identical among them). So four out of five criteria are met, and therefore they could be considered distinct species, possibly only recently separated and now diverging, with little contact in the field between them.

## List of species in the genus *Tenuivirus*

| | | |
|---|---|---|
| *Echinochloa hoja blanca virus* | | |
| Echinochloa hoja blanca virus - cr | [RNA3, L75930; RNA4, L48441; RNA5, L47430] | (EHBV-cr) |
| *Maize stripe virus* | | |
| Maize stripe virus - us | [RNA2, U53224; RNA3, M57426; RNA4, L13438; RNA5, L13446] | (MSpV-us) |
| *Rice grassy stunt virus* | | |
| Rice grassy stunt virus - ph(Laguna) | [RNA1, AB009656; RNA2, AB010376; RNA3, AB010377, RNA4, AB010378; RNA5, AB000403; RNA6, AB000404] | (RGSV-phl) |
| *Rice hoja blanca virus* | | |
| Rice hoja blanca virus - cr | [RNA1, AF009569*; RNA2, L54073; RNA3, L07940; RNA4, AF004657] | (RHBV-cr) |
| *Rice stripe virus* | | |
| Rice stripe virus - jp (t) | [RNA1, D31879; RNA2, D13176; RNA3, X53563; RNA4, D10979] | (RSV-jpt) |
| *Urochloa hoja blanca virus* | | |
| Urochloa hoja blanca virus - cr | [RNA1, U82448*; RNA3, U82447; RNA4, U82446] | (UHBV-cr) |

Species names are in italic script; names of isolates, are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
* Sequences do not comprise the complete genome segment.

## List of other related viruses which may be members of the genus *Tenuivirus* but have not been approved as species

| | | |
|---|---|---|
| Brazilian wheat spike virus | | (BWSpV) |
| European wheat striate mosaic virus | | (EWSMV) |
| Iranian wheat stripe virus | [RNA2, AY312434; RNA3, AY312435; RNA4, AY312436] | (IWSV) |
| Maize yellow stripe virus | [RNA1, AJ969412*; RNA2, AJ696413*; RNA3, AJ969414*; RNA4, AJ969415*; RNA5, AJ969416] | (MYSV) |
| Rice wilted stunt virus | | (RWSV) |
| Winter wheat mosaic virus | | (WWMV) |

* Sequences do not comprise the complete genome segment.

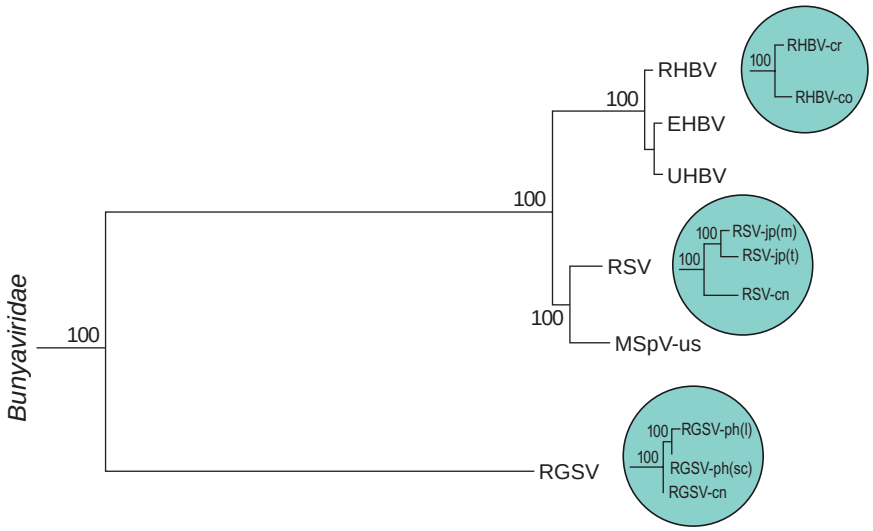


**Figure 4:** Phylogenetic tree showing the relationships among tenuiviruses. Input data were nt sequences of the coding regions of RNA-3 and -4 (RNA-5 and -6 respectively for RGSV) as aligned by PileUp (GCG-Wisconsin 9.0). A total of 3020 characters with clear positional homology across the alignment were used for the phylogenetic analysis. The phylogenetic tree was generated using Maximum Likelihood criteria as implemented by PAUP 4.0(b10), allowing for variable nucleotide substitution rates, rate heterogeneity between characters and rate heterogeneity between lineages. The tree was significantly superior to alternative trees, as determined by likelihood analysis. Bootstrap probabilities were calculated separately for the main tree and for the resolution among the strains of RHBV, RSV and RGSV. The following sequences were used: RHBV-co (AF004658, L14952); RHBV-cr (L07940, AF004657); EHBV (L75930, L48441); UHBV (U82447, U82446); RSV-jp(t) (X53563, D10979); RSV-jp(m) (D01094, D01039); RSV-cn (Y11095,Y11096); MSpV-us (M57426, L13438); RGSV-ph(l) (AB000403, AB000404); RGSV-ph(sc) (AB023779, AB023780); RGSV-cn (AF290947, AF287949).

## Phylogenetic relationships within the genus

Phylogenetic relationships within the genus are illustrated in Figure 4.

## Similarity with other taxa

Tenuiviruses have several similarities with viruses classified in the family *Bunyaviridae*, particularly those in the genus *Phlebovirus*. The multipartite genomes of tenuiviruses contain negative sense and ambisense components. RNPs containing the genomic RNAs can be purified from infected plants. The genomic RNA 5′ and 3′ ends can base-pair, and probably give rise to circular RNPs. Generation of mRNA involves a cap-snatching mechanism. Like viruses in most genera in the family *Bunyaviridae*, tenuiviruses infect their insect vectors as well as their primary hosts, plants. The number of genome components (four to six) and the apparent lack of a membrane-bound virus particle distinguish tenuiviruses from viruses in the family *Bunyaviridae*. Recent data raise the possibility that RGSV be classified in a separate genus. It has six RNA segments that in total encode four or five proteins in addition to those characteristic of the expression of a tenuivirus genome. Moreover, the sequence relatedness of the RGSV gene products with those of other tenuiviruses are all unusually low. Viruses of the recently established genus *Emaravirus* also have properties similar to tenuiviruses and members of the *Bunyaviridae*.

## Derivation of names

*Tenui*: from Latin *tenuis*, “thin, fine, weak”.

## Further reading

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## Contributed by

Shirako, Y., Falk, B.W. and Haenni, A.-L.

# GENUS *VARICOSAVIRUS*

Type species ***Lettuce big-vein associated virus***

## Distinguishing features

Plant viruses with two components of negative sense ssRNA and non-enveloped flexuous rod-shaped virions. They are only distantly related to other ssRNA viruses such as the *Rhabdoviridae*.

## Virion properties

### MORPHOLOGY

Virions are fragile non-enveloped rods mostly measuring 320–360 × about 18 nm; each has a central canal about 3 nm in diameter and an obvious helix with a pitch of about 5 nm (Figure 1). As virions are very unstable *in vitro*, their detection and visualization may be facilitated by prior fixation with glutaraldehyde. The helix of particles, especially those in purified preparations, tends to loosen and particles are then seen as partially uncoiled filaments.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virus particles have a density in $Cs_2SO_4$ of about 1.27 g cm$^{-3}$.

### NUCLEIC ACID

The genome of lettuce big-vein associated virus (LBVaV) consists of ssRNA, but after deproteinization of purified virions, both ds- and ssRNA are detected. The genome is 12.9 kb in size, divided into two components of 6.8 kb (RNA-1) and 6.1 kb (RNA-2); their 3′-termini have no poly(A) tracts. The 3′- and 5′-terminal sequences of the two RNAs are similar, but do not exhibit inverse complementarities. Conserved motifs in the transcription start and end signals resemble those of viruses in the order *Mononegavirales*.

### PROTEINS

The size of the coat protein, when estimated by PAGE, is about 48 kDa. The CP cistron, located on RNA-2, encodes a protein of 397 aa with a predicted size of 44.5 kDa.

### LIPIDS

None reported.

### CARBOHYDRATES

None reported.

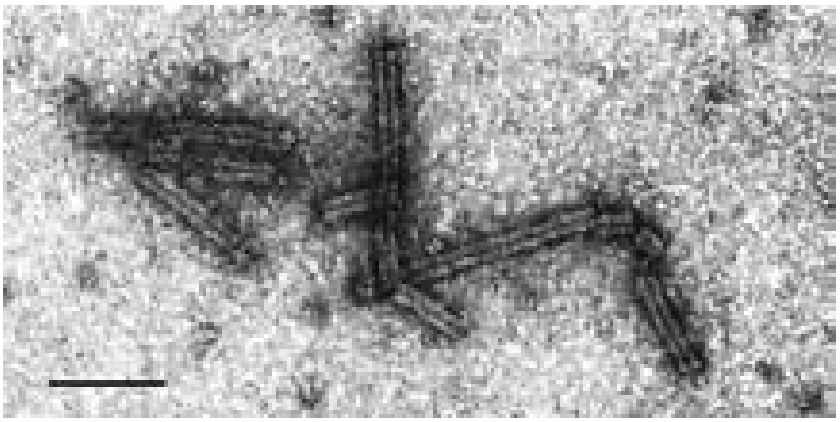

**Figure 1:** Negative contrast electron micrograph of virions of an isolate of lettuce big-vein associated virus. The bar represents 100 nm. (Courtesy J.A. Walsh and C.M. Clay.)

NEG. ssRNA

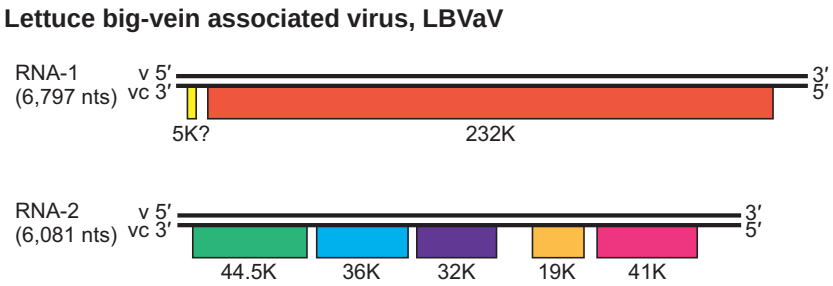


**Figure 2:** Diagram of the genome organization of the two genomic RNAs of lettuce big-vein associated virus. Solid lines represent RNA; boxes represent ORFs. ORFs are translated from the complementary strand vc.

## Genome organization and replication

The first genomic segment (RNA-1) contains one small putative ORF possibly coding for a 5 kDa protein and one large ORF that encodes a protein of 232 kDa (designated L protein by analogy with the L polymerase of rhabdoviruses) (Figure 2). The second genomic segment (RNA-2) contains five ORFs. The first ORF encodes the coat protein (44.5 kDa) and the second to the fifth ORFs code for proteins with unknown functions (36, 32, 19 and 41 kDa respectively) (Figure 2). Although the genome organization of LBVaV is similar to that of rhabdoviruses, LBVaV does not have a non-coding leader sequence. The aa sequence of the L protein is homologous with the L polymerases of some negative sense RNA viruses, especially those of rhabdoviruses.

## Antigenic properties

The CP is a rather poor antigen. LBVaV and tobacco stunt virus (TStV) are serologically closely related.

## Biological properties

### Host range

Varicosaviruses occur naturally in two families of plant species (*Compositae* and *Solanaceae*). LBVaV and TStV infect some common experimental host species.

### Transmission

Both LBVaV and TStV are transmitted in soil and in hydroponic systems by zoospores of the chytrid fungus *Olpidium brassicae*. The non-crucifer strain of *O. brassicae* transmitting Mirafiori lettuce big-vein virus (MLBVV; genus *Ophiovirus*) and TStV in Japan has recently been referred to as *Olpidium virulentus* and the new name was also used for other *O. brassicae* isolates infecting lettuce and shown to be associated with the presence of LBVaV and MLBVV in Australia. A further proposal based on genetic and host range differences of *Olpidium* spp. from many countries suggested that lettuce-infecting isolates could be named *Olpidium compositae*.

Due to the longevity of infectious LBVaV from stored *O. brassicae* resting spores, it is assumed that the virus is carried internally by the fungus.

The viruses are also transmitted experimentally, sometimes with difficulty, by mechanical inoculation. Neither of the viruses is reported to be seed-transmitted.

### Cytopathic effects

None reported.

## Species demarcation criteria in the genus

Not yet definable.

## List of species in the genus *Varicosavirus*

*Lettuce big-vein associated virus*

| | | |
|---|---|---|
| Lettuce big-vein associated virus-Japan:Kagawa | [AB075039=NC_0011558+AB114138=NC_011568] | (LBVaV-JP) |
| Tobacco stunt virus | [AB190521*] | (TStV) |

Species names are in italic script; isolate names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequence does not comprise the complete genome.

## List of other related viruses which may be members of the genus *Varicosavirus* but have not been approved as species

TStV was considered to be distinct from LBVaV. However, based on nucleotide and amino acid sequence identities between the two, the former was later considered to be a tobacco-infecting strain of the latter.

## Phylogenetic relationships within the genus

Comparison of the CP coding regions of eight LBVaV isolates from Japan and Spain and five TStV isolates from Japan showed they were identical in size and had nucleotide and amino acid sequence identities of 95.6–96.5% and 97.2–98.7%, respectively. This and a comparison of sequences of the other genes of one TStV isolate with those of LBVaV led to the conclusion that TStV was a strain of LBVaV. A later, larger analysis of the CP coding regions of 29 LBVaV isolates (from Europe, Australia and Japan) and the five TStV isolates from Japan, revealed sequence identities of 93.6–99.7% (nucleotides) and 94.3–100% (amino acids); the nucleotide and amino acid sequence identities of the 29 LBVaV isolates alone had the same range. The identities of the five TStV were 95.7–99.7% (nucleotides) and 97.2–100% (amino acids). This was taken to support TStV being a strain of LBVaV. In both analyses, all five TStV isolates grouped together in a clade of their own and there were four amino acid differences unique to the TStV isolates. This and the fact LBVaV isolates do not infect tobacco and TStV isolates do not infect lettuce might suggest some merit in keeping an open mind on the strain/species issue until further evidence is available. (See Figure 3.)

## Similarity with other taxa

The genome structure and probable transcription mechanism of LBVaV indicates that it has a close relationship with rhabdoviruses (Figure 3). The aa sequences of both the CP and the L protein of LBVaV have significant similarities with those of rhabdoviruses. LBVaV also resembles rhabdoviruses in possessing conserved transcription termination/polyadenylation signal-like poly(U) tracts and in transcribing monocistronic RNAs. The presence of poly(U) tracts in the NCRs of LBVaV RNA-1 and RNA-2 suggest that transcription of LBVaV is regulated by a mechanism similar to that of rhabdoviruses (order *Mononegavirales*; family *Rhabdoviridae*). However, whereas rhabdoviruses contain a single negative sense ssRNA, LBVaV has two such RNAs.

## Derivation of name

*Vari*: from Latin *varix*, meaning abnormal dilation or enlargement of a vein or artery and referring to the symptom previously thought to be induced by the type species. However, lettuce big-vein disease, although long thought to be induced by a virus previously designated lettuce big-vein virus, is now considered to be caused by isolates of the species *Mirafiori lettuce big-vein virus* (genus *Ophiovirus*). Viruses of this species are soil-borne and often occur in lettuce together with isolates of LBVaV.

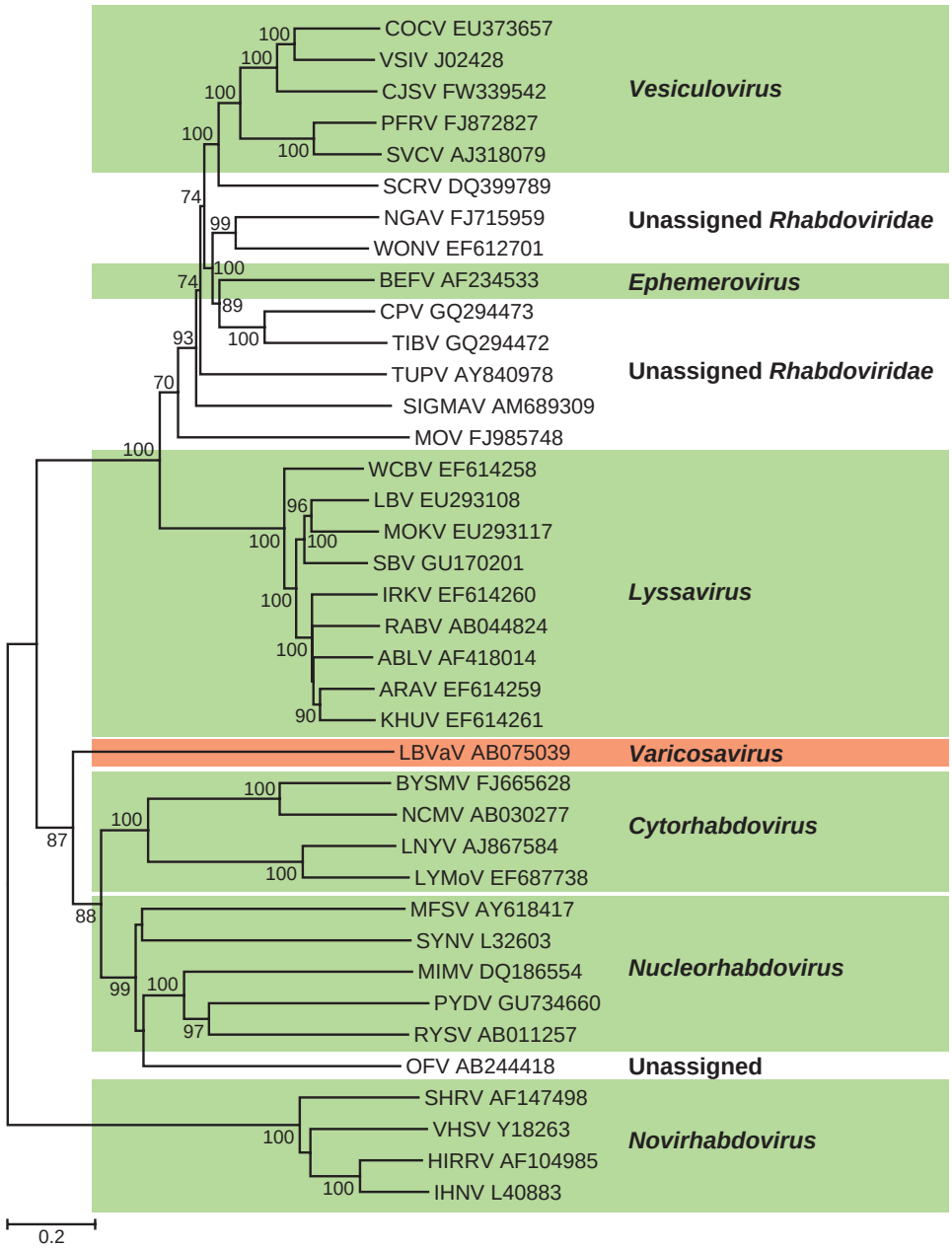


**Figure 3:** Phylogenetic tree based on the codon-aligned nucleotide sequences of the polymerase (L) genes of isolates of Lettuce big-vein associated virus, orchid fleck virus and members of the family *Rhabdoviridae*. The tree was prepared in MEGA4 using maximum composite likelihood distances and 10,000 bootstrap replicates (values shown where >60%). ABLV, Australian bat lyssavirus; ARAV, Aravan virus; BEFV, bovine ephemeral fever virus; BYSMV, barley yellow striate mosaic virus; CJSV, Carajas virus; COCV, Cocal virus; CPV, coastal plains virus; HIRRV, Hirame rhabdovirus; IHNV, infectious hematopoietic necrosis virus; IRKV, Irkut virus; KHUV, Khujand virus; LBV, Lagos bat virus; LBVaV, lettuce big-vein associated virus; LNYV, lettuce necrotic yellows virus; LYMoV, lettuce yellow mottle virus; MFSV, maize fine streak virus; MIMV, maize Iranian mosaic virus; MOKV, Mokola virus; MOV, Moussa virus; NCMV, northern cereal mosaic virus; NGAV, Ngaingan virus; OFV, orchid fleck virus; PFRV, pike fry rhabdovirus; PYDV, potato yellow dwarf virus; RABV, rabies virus; RYSV, rice yellow stunt virus; SBV, Shimoni bat virus; SCRV, Siniperca chuatsi rhabdovirus; SHRV, snakehead virus; SIGMAV, sigma virus; SVCV, spring viraemia of carp virus; SYNV, sonchus yellow net virus; TIBV, Tibrogargan virus; TUPV, Tupaia virus; VHSV, viral hemorrhagic septicemia virus; VSIV, vesicular stomatitis Indiana virus; WCBV, West Caucasian bat virus; WONV, Wongabel virus. (Courtesy of Dr M.J. Adams; used by permission.)

## Further reading

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## Contributed by

Walsh, J.A. and Verbeek, M.

# ORDER *NIDOVIRALES*

## Taxonomic structure of the order

| | |
|---|---|
| Order | *Nidovirales* |
| Family | *Arteriviridae* |
| Genus | *Arterivirus* |
| Family | *Coronaviridae* |
| Subfamily | *Coronavirinae* |
| Genus | *Alphacoronavirus* |
| Genus | *Betacoronavirus* |
| Genus | *Gammacoronavirus* |
| Subfamily | *Torovirinae* |
| Genus | *Torovirus* |
| Genus | *Bafinivirus* |
| Family | *Roniviridae* |
| Genus | *Okavirus* |

## Virion properties

### MORPHOLOGY

The members of the order *Nidovirales* are enveloped, positive-strand RNA viruses of widely different architecture. Depending on whether the external appearance of the virion or the nucleocapsid is considered, similarities and differences can be discerned (Figure 1).

***Coronavirinae*:** As seen in conventional electron micrographs, coronaviruses are roughly spherical enveloped particles, 120–160 nm in diameter, with a characteristic fringe of 15–20 nm petal-shaped surface projections (peplomers). In a subset of betacoronaviruses a second, inner fringe of 5–7 nm surface projections is also seen. Coronavirus (CoV) particles as studied by cryo-electron tomography are homogeneous in size and distinctively spherical (envelope outer diameter 85 ± 5 nm). The envelope exhibits an unusual thickness (7.8 ± 0.7 nm), almost twice that of a typical biological membrane. The nucleocapsid is helical and tightly folded to form a compact structure that tends to closely follow the envelope.

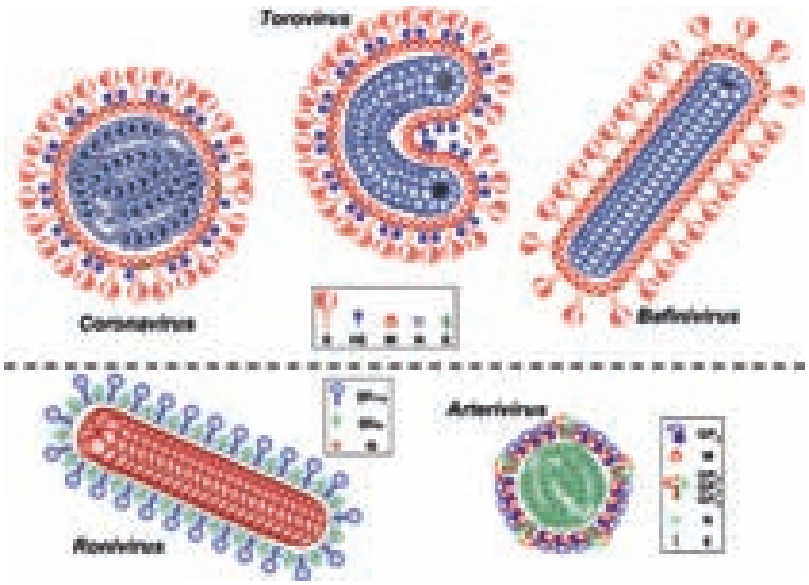


**Figure 1:** Schematic structure of particles of members of the order *Nidovirales*.

***Torovirinae*, *Torovirus*:** Toroviruses appear as a mixture of rod-shaped, kidney-shaped and spherical particles. This is, however, most likely an EM artifact. Native torovirus particles are presumably bacilliform with rounded ends, measuring 100–140 nm in length and 35–42 nm in width (envelope outer dimensions). Virions carry two types of surface projections that in size and shape closely resemble those of (beta)coronaviruses. The most distinctive virion element, the core, is a flexible and seemingly hollow tube of helical symmetry (periodicity ca. 4.5 nm), about 100 nm in length and about 23 nm across with a central channel of about 10 nm in diameter.

***Torovirinae*, *Bafinivirus*:** Bafiniviruses (*ba*cilliform *fi*sh *ni*doviruses) are 130–160 × 37–45 nm in dimension (excluding spikes) with a rod-like nucleocapsid in the form of a rigid cylinder (120–150 × 19–22 nm with a central channel of 2–5 nm). The virion envelope is studded with 20–25 nm coronavirus-like peplomers.

***Roniviridae*:** Roniviruses (*ro*d-shaped *ni*doviruses) are also bacilliform in shape, 150–200 nm in length and about 45 nm in diameter, and contain a tightly coiled nucleocapsid with a diameter of about 25 nm and a 5–7 nm helical periodicity. The ronivirus envelope bears spikes, but smaller in size than those of coronaviruses, projecting approximately 11 nm from the surface.

***Arteriviridae*:** Arterivirus virions are significantly smaller than those of the other nidoviruses, spherical or egg-shaped and with a seemingly isometric core that contains the genome. Complete particles and nucleocapsids, as measured by cryo-EM, average 54 nm and 39 nm in diameter, respectively, with core and envelope separated by a 2–3 nm gap. Three-dimensional reconstructions, based on cryo-EM tomography, suggests that the core might consist of a helical nucleocapsid wrapped into a hollow ball. No spikes are obvious on the arterivirus surface, but a surface pattern of relatively small and indistinct projections has been observed.

### Physicochemical and physical properties

The coronavirus virion Mr is 400 $\times 10^6$, the buoyant density in sucrose is 1.15–1.20 g cm$^{-3}$, the density in CsCl is 1.23–1.24 g cm$^{-3}$, and the virion $S_{20,W}$ is 300–500S. Torovirus and bafinivirus virions have buoyant densities in sucrose of 1.14–1.18 and 1.17–1.19 g cm$^{-3}$, respectively. Arterivirus virion buoyant density is 1.13–1.17 g cm$^{-3}$ in sucrose and 1.17–1.20 g cm$^{-3}$ in CsCl; virion $S_{20,W}$ is 200 to 300S. Ronivirus virion buoyant density in sucrose is 1.18–1.20 g cm$^{-3}$. Nidovirus virions are sensitive to heat, lipid solvents, non-ionic detergents, formaldehyde, oxidizing agents and UV irradiation.

### Nucleic acid

The nidoviruses genome is an infectious, linear, positive sense RNA molecule, which is capped and polyadenylated. Based on the genome size, two groups – large and small nidoviruses – can be distinguished. The genomes of the large nidoviruses are well over 25 kb in length with size differences in the 5 kb range: 26.4–31.7 kb (*Coronavirus*), 28–28.5 kb (*Torovirus*), about 26.6 (*Bafinivirus*), and 26.2–26.6 kb (*Okavirus*). The small nidoviruses include a single family (*Arteriviridae*) with genomes from 12.7– 15.7 kb in length. Members of the families *Corona-* and *Roniviridae* are the largest RNA viruses known to date. Complete genome sequences are available for representatives of all seven nidovirus genera.

### Proteins

Although the structural proteins of the nidoviruses are generally functionally equivalent, there is no firm indication that any single protein species is evolutionary conserved across all of the families. The virion proteins typical for each of the five main nidovirus taxa are listed in Table 1.

Members of the family *Coronaviridae* generally possess three or four envelope proteins. The most abundant one (at least in corona- and toroviruses) is the membrane (M) protein. Though different in sequence, the M proteins of corona-, toro- and bafiniviruses are alike in size, structure and presumably also in function. They have a similar triple-spanning membrane topology with a short amino terminus located on the outside of the virion, and a long C-terminal endodomain, comprising an amphiphilic region and a hydrophilic tail. The amphiphilic segment is believed to associate with the inner leaflet of the membrane to form a matrix-like lattice, which would explain the remarkable thickness of the coronavirus envelope as observed by cryo-electron tomography. Of note, in transmissible gasteroenteritis virus of swine (*Alphacoronavirus 1*), a second population of M proteins adopting an $N^{exo}$-$C^{exo}$ topology in the viral envelope has been described.

**Table 1:** Structural proteins of nidoviruses: acronyms and sizes (in amino acid residues). Boxed proteins are believed to be evolutionarily related

| Protein[a] | | *Coronavirus* | *Torovirus* | *Bafinivirus* | *Okavirus* | *Arterivirus* |
|---|---|---|---|---|---|---|
| Spike glycoprotein | S | 1128–1472 | 1562–1584 | 1220 | - | - |
| Large spike glycoprotein | gp116 | - | - | - | 873[c]–899 | - |
| Small spike glycoprotein | gp64 | - | - | - | 539 | - |
| Minor surface glycoprotein | GP2 | - | - | - | - | 227–249 |
| | GP3 | - | - | - | - | 163–256 |
| | GP4 | - | - | - | - | 152–183 |
| Major surface glycoprotein | GP5 | - | - | - | - | 199–278 |
| Membrane protein | M | 218–263 | 233 | 227 | - | 162–174 |
| Nucleocapsid protein | N | 349–470 | 159–167 | 161 | 144–146 | 110–128 |
| Envelope protein | E | 74–109 | - | - | - | 67–80 |
| Hemagglutinin-esterase protein | HE | 386–440[b] | 416–430 | - | - | - |

[a]Only proteins typical for each lineage are listed; for some CoVs additional, virus species-specific accessory envelope proteins have been described
[b]Only found in a cluster of betacoronaviruses ("phylogroup A", *Betacoronavirus 1*, *Murine coronavirus*, *Human coronavirus HKU-1*).
[c]Size predicted for gill-associated virus gp116 protein.

The spike (S) proteins of corona-, toro- and bafiniviruses are exceptionally large type I membrane glycoproteins (1200–1600 aa residues), heavily *N*-glycosylated and with features characteristic of class I fusion proteins. Remote but significant sequence similarity among S proteins of toro-, bafini- and (to lesser extent) coronaviruses suggests common ancestry and similarity in structure and function, i.e. receptor-binding and membrane fusion. With few exceptions, the S proteins become proteolytically cleaved during virion biogenesis into subunits S1 and S2 that remain associated. Coronavirus S proteins assemble into homotrimers and this most likely also occurs with the S proteins of toro- and bafiniviruses. The bulbous membrane-distal part of the peplomers, comprising the receptor-binding domains, are largely composed of S1 subunits, whereas the C-terminal S2 subunits form a membrane-anchored stalk. Heptad repeat regions in S2 are assumed to drive membrane fusion during entry by undergoing a series of conformational changes culminating in a six-helical bundle. In the primary structure, the N-terminal repeat, HR1, is located immediately downstream of a predicted internal fusion peptide and the other repeat, HR2, immediately upstream of the transmembrane domain.

Coronaviruses code for a small envelope protein (E), a pentameric integral membrane protein exhibiting ion channel and/or membrane permeabilizing (viroporin) activities. With around 20 copies per particle, the E protein is only a minor structural component. Although its precise function remains to be defined, the E protein has been implicated in virion morphogenesis and identified as a virulence factor for Severe Acute Respiratory Syndrome (SARS)-CoV. So far, no E homologs have been identied in toro- and bafiniviruses.

A subset of betacoronaviruses (*Betacoronavirus* 1, *Murine coronavirus* and *Human coronavirus HKU1*), and all toroviruses known to date, code for an additional homodimeric type I membrane glycoprotein, the hemagglutinin-esterase (HE), that mediates reversible virion attachment to *O*-acetylated sialic acids by acting both as a lectin and as a sialate-*O*-acetylesterase. Corona- and torovirus HEs share 30% sequence identity and thus are far more closely related to each other than are the S and M proteins of these nidovirus lineages. While the latter two protein species might have been encoded in the last common ancestor of the *Corona-* and *Torovirinae* lineages, the HE proteins must have been acquired relative recently. Originating from a hemagglutinin-esterase fusion protein resembling that of influenza C virus, the HEs appear to have been introduced into the betacorona- and torovirus proteomes independently (i.e. through two separate horizontal gene transfer events) well after the *Corona–Torovirinae* split, and in the case of the coronaviruses even after their separation into alpha-, beta- and gammacoronaviruses.

The nucleocapsid (N) proteins of corona- and toroviruses are highly basic, RNA-binding phosphoproteins, involved in encapsidation and packaging of the genome. However, as demonstrated for

coronaviruses, N proteins might also play essential roles in RNA synthesis and translation, exhibit RNA chaperone activity and act as antagonists of interferon type I. With molecular masses of about 18 kDa, the torovirus N and proposed bafinivirus N proteins are less than half the size of their coronavirus equivalents. The structural, functional and evolutionary relationships between these protein species remain to be established.

The structural proteins of arteriviruses are apparently unrelated to those of the other members of the order *Nidovirales*. The nucleocapsid contains a single protein species, N. In equine arteritis virus (EAV) and porcine reproductive and respiratory syndrome virus (PRRSV), six envelope proteins have been identified, each essential for virion infectivity. The non-glycosylated membrane protein (M) is thought to span the membrane three times and thus to structurally resemble the M protein of corona- and toroviruses. It forms a disulfide-linked heterodimer with the major glycoprotein (GP5 for EAV, PRRSV and lactate dehydrogenase-elevating virus, LDV; GP7 in simian hemorrhagic fever virus, SHFV), which is also a putative triple-spanning membrane protein. Viral glycoproteins GP2, GP3 and GP4 are minor virion components and form heterotrimers. The remaining envelope protein, E (for envelope), is small, hydrophobic and non-glycosylated, and believed to function as an ion-channel protein. LDV virion composition has been studied in less detail, but is likely similar to that of EAV and PRRSV. Remarkably, SHFV may possess up to three additional envelope proteins.

Ronivirus structural proteins have been studied only for yellow head virus (YHV). Virions contain a highly basic nucleoprotein species (p20) and two envelope glycoprotein species (gp116 and gp64) that form the prominent peplomers on the virion surface. Both gp116 and gp64 are encoded by the ORF3 gene and generated from a long (1640–1666 aa residues) precursor glycopolyprotein (pp3) by post-translational processing at two internal signal peptidase type-1 sites (Figure 2). They are not

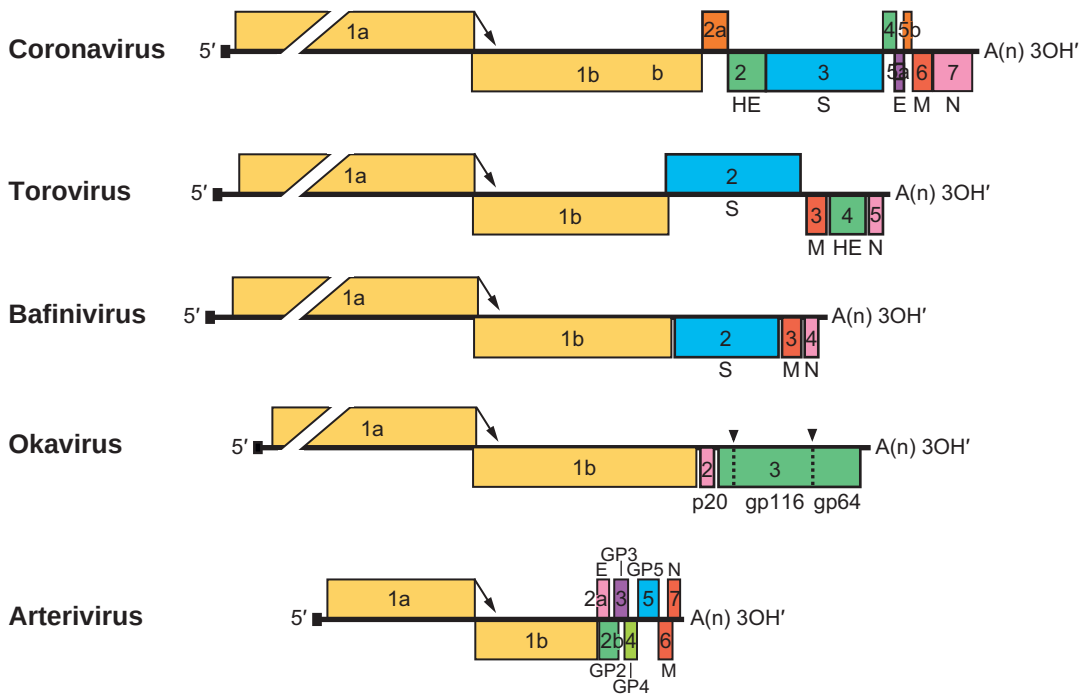


**Figure 2:** Schematic representation of the genome structure of members of the order *Nidovirales* (from top to bottom: murine coronavirus, bovine torovirus, white bream virus, gill-associated virus, equine arteritis virus). Note that, in coronaviruses, the 3′ genome organization and the complement of accessory genes can differ even among members of the same genus. ORFs are represented by boxes. Untranslated sequences are indicated by solid lines. The ribosomal frameshift sites in ORF1 (located at the 1a-1b junction and indicated by arrows) are aligned. Numbers refer to the mRNA species from which the ORFs are expressed. The proteins encoded by the ORFs are indicated above or below. Signal peptidase type-1 cleavage sites in okavirus precursor glycopolyprotein pp3 are indicated by dashed lines and arrowheads. The 5′ leader sequences are depicted by a small black box. Poly(A) tails are indicated by A(n). S, spike protein; M, membrane protein; E, envelope protein; N, nucleocapsid protein; HE, hemagglutinin-esterase protein.

linked by intramolecular disulfide bonds and are anchored in the envelope by either one (gp64) or two (gp116) hydrophobic C-terminal transmembrane domains. Processing of pp3 would also yield an N-terminal product of about 25 kDa, a putative triple-spanning membrane protein, the fate and function of which are not known.

### Lipids

Nidoviruses have lipid envelopes, which are commonly acquired by budding at membranes of the endoplasmic reticulum, intermediate compartment and/or Golgi complex. Coronavirus S and E proteins are palmitoylated; the arterivirus E protein is myristoylated.

### Carbohydrates

Coronavirus S and HE proteins are heavily glycosylated and contain multiple *N*-linked glycans (20–35 and 5–11, respectively). The M protein of coronaviruses contains a small number of either *N*- or *O*-linked glycans, depending on the virus species, located near the amino-terminus. Coronavirus E proteins are not glycosylated. Torovirus S and HE proteins are also heavily *N*-glycosylated (19–25 and 7–13 glycans, respectively); the M protein is not glycosylated, however. Bafinivirus structural proteins have not been characterized in great detail. The S and M proteins appear to be glycosylated. The S protein binds lectins and likely contains $\alpha$-mannose. The gp116 and gp64 proteins of ronivirus YHV contain 6 and 3 *N*-linked glycans, respectively. In arteriviruses, GP2, GP3, GP4 and GP5 contain *N*-linked glycans. GP5 of EAV, LDV, and PRRSV are modified by heterogeneous *N*-acetyl lactosamine addition. Due to extensive and heterogeneous glycosylation, GP5 is of highly variable size (between 26 and 42 kDa). The M and E proteins are not glycosylated.

## Genome organization and replication

Nidovirus replication takes place in the cytoplasm of infected cells and proceeds through the synthesis of minus-strand intermediates. RNA synthesis is catalyzed by an as yet poorly characterized replication–transcription complex, composed of viral and host proteins and presumably associated (at least in corona- and arteriviruses) with a network of modified intracellular membranes, which is derived from the ER and includes unusual double-membrane vesicles.

### Genome organization

Despite considerable differences in genome size and gene composition, nidoviruses are remarkably similar in their genome organization (Figure 2). The 5′-most two-thirds of the genome characteristically comprises two large, partially overlapping ORFs, designated 1a and 1b, that constitute the replicase gene and together encode a collection of enzymes that are part of the replication complex *per se* or control its composition and functioning (see section on *replicase*). The virion RNA functions as mRNA (mRNA1) for ORFs 1a and 1b, but the expression of the latter requires a programmed ribosomal frameshift. Translation of ORF1a yields polyprotein pp1a. In 20–30% of the cases, ribosomes do not reach the ORF1a termination codon, but slip at the ORF1a/1b overlap and shift register to the −1 reading frame to continue translation into ORF1b. The ribosomal frameshift occurs within a specific seven-nucleotide "slippery" sequence, upstream of a pseudoknot structure, and gives rise to a 3′-extended fusion polyprotein, pp1ab. The replicase polyproteins are processed by several virus-encoded proteases to more than a dozen mature products, including the key replicative enzymes/proteins of the virus (further detailed below).

Downstream of the replicase gene there are from three (*Okavirus*) to up to 12 (*Coronavirinae*) ORFs that encode a set of structural proteins typical for the subfamily and/or genus, and, at least for coronaviruses, a variety of "accessory" proteins that may be virus species or even subspecies-specific. These 3′-proximal ORFs are expressed from a 3′-coterminal nested set of dedicated subgenomic (sg) mRNAs, the number of which ranges from two in okaviruses to up to at least eight in certain coronaviruses. All mRNA species, except the smallest ones, are structurally polycistronic. As a rule, however, translation is restricted to the 5′-most ORF(s) not present in the next smaller mRNA of the set; downstream ORF(s) remain translationally silent.

Nidovirus transcription units (i.e one or more ORFs expressed from a single mRNA species) are generally preceded in the genome by short conserved sequence elements commonly termed "transcription-regulating sequences" (TRSs) in corona-, arteri- and bafiniviruses and putative

terminator/promoter elements (TPs) in toroviruses. As toro- and roniviruses differ in their transcription mechanism from the other nidoviruses (see below), TRSs and TPs are not functionally equivalent.

### The replicase gene

Expression of the replicase ORF1a/1b gene yields two huge polyproteins, pp1a and pp1ab. These giant proteins (ranging in size from the approximately 2000-aa pp1a of arteriviruses to the > 7000-aa pp1ab of coronaviruses) have not been observed in infected cells. Their processing by viral proteinases is believed to occur both cotranslationally and posttranslationally, yielding more than a dozen mature proteins (13 in arteriviruses and 15 or 16 in coronaviruses) and an as-yet unknown number of functional intermediates. In arteriviruses and coronaviruses, from one to three (and possibly four) papain-like cysteine proteases ($PL^{pro}$ in coronaviruses, PCP and CP in arteriviruses) control the proteolytic processing of the N-terminal part of pp1a/pp1ab at 2-4 sites. A protease with a chymotrypsin-like fold (known as $3CL^{pro}$ or "main" protease, $M^{pro}$; also designated as serine protease SP in arteriviruses) is responsible for the processing of the remaining largest part of pp1a/pp1ab at 8-11 conserved cleavage sites. Nidovirus pp1a/pp1ab processing products, generally referred to as the nonstructural proteins (nsp's), are numbered according to their position (from N- to C-terminus) in the viral polyproteins (nsp1 to nsp12 in arteriviruses and nsp1 to 16 in coronaviruses; Figure 3). In some cases, alternative names are used to refer to functional domain(s) present in these nsp's, especially in cases where the domains are conserved across nidovirus (sub)families and mediate specific functions and/or enzymatic activities.

Despite a more than two-fold difference in size between the replicase genes of arteriviruses and other nidoviruses, a common backbone of conserved domains can be discerned. Sequence alignments and phylogenetic analyses suggest that the conservation of functional domains in the replicase polyproteins is the result of a continuous evolution from a common nidovirus ancestor. Activities and functions have been identified for many of the conserved replicase domains and the corresponding cleavage products. Replicase subunits conserved across all nidoviruses include (from N- to C-terminus): (i) a chymotrypsin-like protease (3C-like or main protease; $3CL^{pro}$, $M^{pro}$) that is flanked by two hydrophobic transmembrane domains (*tm*-$M^{pro}$-*tm*) in the viral polyprotein and has a substrate specificity resembling that of picornavirus 3C proteases, (ii) a large RdRp, (iii) a 5′-to-3′ helicase domain containing a putative multinuclear Zn-finger-like domain at its N-terminus (Zn-HEL). Some replicase subunits are present only in a subset of nidoviruses. A *n*idoviral *endo*ribonuclease specific for *u*ridylate (NendoU) was long considered to be shared by all nidoviruses and to represent a unique diagnostic molecular marker that would distinguish the members of this order from all other RNA viruses known to date. However, a very recent study shows roniviruses to lack this domain. A 3′-to-5′ exoribonuclease (ExoN) and ribose-2′-*O*-methyltransferase (O-MT) are conserved in the large nidoviruses, but not in arteriviruses. An ADP-ribose-1″-phosphatase (ADRP, also called macrodomain) and a noncanonical "secondary" RdRp with possible primase activity (coronavirus nsp8), have been (tentatively) mapped only in members of the family *Coronaviridae*. A guanine N7 methyltransferase, recently identified in coronaviruses, appears to be conserved in roniviruses. The conservation of key proteolytic and RNA-processing enzymes in the main nidovirus lineages is summarized in Figure 3.

The N-terminal half of pp1ab is quite variable among nidoviruses, even among members of the same genus. This variability contributes significantly to the major size differences between the genomes of large and small nidoviruses. A comparison between the coronavirus and arterivirus N-terminal pp1a/pp1ab sequences does not yield significant sequence similarities beyond the conservation of active sites of papain-like proteases.

### Synthesis of genomic and subgenomic RNAs

Genome replication and sg mRNA synthesis ("transcription") proceed through minus-strand intermediates. The genome serves as a template for the synthesis of full-length minus-strand RNA, from which in turn new genome copies are produced, but it is also believed to be the template for the synthesis of sg minus-strand RNA species (*vide infra*). The synthesis of viral RNAs is highly asymmetrical as plus-strand RNAs are produced in fast excess.

A hallmark of nidovirus transcription is the production of a 3′-coterminal nested set of sg mRNAs. The sg mRNAs of corona-, arteri- and bafiniviruses are chimeric, that is, comprised of sequences

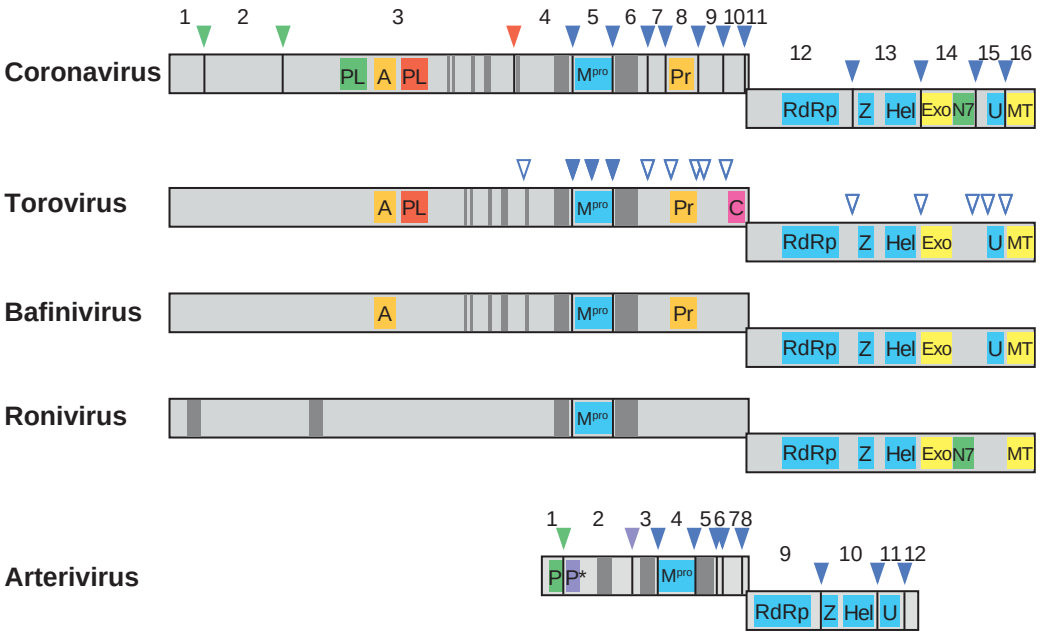


**Figure 3:** Schematic representation of the domain organization of the replicase polyproteins pp1a and pp1ab of representative viruses from the five main nidovirus taxa (from top to bottom: murine coronavirus, bovine torovirus, white bream virus, gill-associated virus, equine arteritis virus). The position of the ribosomal frameshift site was used to align the polyprotein representations. Cleavage sites in pp1a and pp1ab of papain-like proteinases (PL, P) or of the 3C-like main protease ($M^{pro}$) are indicated by color-coded arrowheads; open arrowheads indicate predicted $M^{pro}$ cleavage sites in the torovirus replicase polyproteins. The processing end-products (nonstructural proteins) of corona- and arterivirus polyproteins are numbered; conserved domains are highlighted as follows: PL, coronavirus papain-like proteinase; P and P*, arterivirus papain-like cysteine proteinases PCP and CP, respectively; A, ADP-ribose-1″-phosphatase (macrodomain); $M^{pro}$, 3C-like main protease; Pr, noncanonical RNA-dependent RNA polymerase, putative primase; C, cyclic nucleotide phosphodiesterase domain; RdRp, RNA-dependent RNA polymerase; Z, zinc-binding domain; Hel, helicase domain; Exo, 3′-to 5′ exoribonuclease domain; N7, guanine-N7-methyltransferase; U, nidoviral uridylate-specific endoribonuclease (NendoU); MT, ribose-2′-*O*-methyltransferase domain.

that are non-contiguous in the viral genome. Each carries a short 5′ leader sequence of 55–92, 170–210 nt, and 42 nucleotides, respectively, which is identical to the 5′ end of the viral genome. It was established early on that leader and "body" sequences are not joined through splicing, but via a process of discontinuous RNA synthesis. A key observation was the presence of mirror-copy nested sets of sg minus-strand RNAs in corona- and arterivirus-infected cells. Combined experimental evidence from biochemical and reverse genetics analyses indicates that these sg minus-strand RNAs are in fact the templates for sg mRNA synthesis. Replicative intermediates (RI)/replicative forms with sizes corresponding to the different sg mRNAs were shown to be actively involved in transcription. According to the prevailing 3′-discontinuous extension model, the discontinuous step occurs during the production of sg minus-strand RNAs and entails attenuation of RNA synthesis at the TRSs, followed by a similarity-assisted copy choice RNA recombination event. In corona-, arteri- and bafiniviruses, a TRS is present immediately downstream of the genomic leader sequence. It is believed that, during minus-strand RNA synthesis, the replicase complex upon encounter of an internal TRS dissociates from the template and is transferred to the 5′ end of the genome, guided by sequence complementarity between the anti-TRS on the nascent strand and the genomic TRS. Reinitiation and completion of RNA synthesis would then result in a chimeric minus-strand that in turn would serve as a template for uninterrupted (continuous) synthesis of 5′ leader-containing sg mRNAs.

Discontinuous sg RNA synthesis is not a trait of all nidoviruses. Ronivirus sg mRNAs lack a common 5′ leader and thus apparently arise from non-discontinuous RNA synthesis. Toroviruses employ a mixed transcription strategy; of the four sg RNAs, only RNA 2 carries a 15–18 nt 5′ leader derived from the 5′ end of the genome, whereas the others do not. It is likely that sg mRNAs are

transcribed from sg minus-strand templates also in toro- and in roniviruses. Here, the conserved sequence elements (TPs) preceding the 3′-proximal genes might serve dual roles as signals for premature termination of minus-strand synthesis and as promoters for plus-strand production. The torovirus S gene, expressed from mRNA 2, lacks a TP. Apparently, transcription-competent minus-strand sg RNAs are produced by inclusion of a complementary copy of the 5′-terminal genomic TP via a similarity-assisted RNA recombination process analogous to that seen in corona- and arteriviruses.

## Antigenic properties

In coronaviruses, the S protein is an important target for T cell responses and is the major inducer of virus-neutralizing antibodies, which are elicited by epitopes located mostly in the N-terminal half of the molecule. The surface-exposed N-terminus of the M protein induces antibodies that neutralize virus infectivity in the presence of complement. The N protein is a dominant antigen during the natural infection and, like the S protein, might evoke protective T cell responses. HE induces antibodies that prevent binding to *O*-acetylated sialic acids or inhibit sialate-*O*-acetylesterase activity. The ectodomains of the S and HE proteins are highly variable, suggestive of extensive antigenic drift. In addition, there are several examples of intergenotypic exchange of coding sequences of S (for *Avian coronavirus*, *Murine coronavirus* and for the feline and canine coronaviruses belonging to *Alphacoronavirus 1*) and HE (*Murine coronavirus*) ectodomains through homologous RNA recombination, consistent with the occurrence of antigenic shifts.

All toroviruses described so far are serologically related. During natural infection, antibodies are raised against each of the four structural proteins (S, HE, M and N). The spike (S) protein induces virus-neutralizing antibodies; sera from BToV- or PToV-infected animals cross-neutralize EToV. Comparative sequence analysis of bovine and porcine torovirus field variants revealed several instances in which coding sequences for the HE ectodomain had been exchanged through intergenotypic homologous RNA recombination. Bovine torovirus variants currently prevalent in the field (genotypes II and III) have apparently arisen from a recombination event during which the ancestral BToV (genotype I) swapped its N gene for that of porcine torovirus.

Antibodies against the known arteriviruses (EAV, LDV, PRRSV, SHFV) do not cross-react and there is considerable antigenic variation among different strains of EAV, LDV and PRRSV. Major glycoprotein GP5 (designated GP7 in SHFV) is the main determinant of virus-neutralization. In some arteriviruses (PRRSV type I), minor glycoprotein GP4 also induces neutralizing antibodies. A number of arterivirus proteins have been reported to evoke T cell responses, including GP5 and M.

At present, there are no data available about the antigenic properties of bafinivirus proteins or about the innate defense responses mounted against roniviruses in their invertebrate hosts. Serological interfamily or intergenus cross-reactivity has not been demonstrated.

## Biological properties

Coronaviruses infect birds and mammals, including humans, livestock and companion animals. Bats are believed to play a pivotal role in CoV ecology and evolution as they appear to harbor an exceptionally wide diversity of CoVs. It has even been proposed that bats may be the original hosts from which many if not all alpha- and betacoronavirus lineages are derived.

CoVs predominantly target the epithelia and, consequently, infections are mostly associated with respiratory and gastrointestinal disease. Biological vectors are not known. Depending on the virus species, coronaviruses are transmitted via aerosols, fomites or the fecal–oral route. In many instances a persistent chronic infection develops with prolonged shedding of virus from the enteric tract. Coronavirus infections are often mild. However, in 2002–2003, a novel coronavirus, SARS-CoV, caused an epidemic in human populations of a severe pulmonary disease with a mortality rate of 10%. For other CoVs, hepatitis and infection of the central nervous system (MHV), heart and eye (RbCoV) have been described. Variants of *Alphacoronavirus 1* (feline, canine and ferret coronaviruses) may infect cells of the monocyte/macrophage lineage and cause fatal systemic infections characterized by wide-spread granulomatous lesions in multiple organs.

Toroviruses infect ungulates: horses (EToV, Berne virus), bovines (BToV, Breda virus) and swine (PToV). Humans (HToV) and probably carnivores (mustellids) have also been proposed as hosts for toroviruses. Transmission is probably by the fecal–oral route.

The bafinivirus white bream virus is the only known teleost nidovirus and so far was isolated from one species of fresh water fish (*Blicca bjoerkna L.*). At present no further information is available on its ecology, biology and pathogenic properties.

Arteriviruses infect horses (EAV), mice (LDV), monkeys (SHFV) and swine (PRRSV). Primary host cells for all arteriviruses are macrophages. EAV causes inflammation of small arteries and EAV infection can lead to a wide range of clinical manifestations. A fatal outcome of the disease has been reported in both natural and experimental infections, but most natural infections are either mild or subclinical. In pregnant animals, arteriviruses can cause abortions (PRRSV and EAV) or *in utero* fetal death (PRRSV). Persistent infections – lifelong in the case of LDV – are frequently established. Virus may be shed in saliva and respiratory secretions, feces, urine and milk. Persistently-infected males may shed virus in the semen (EAV, PRRSV). Spread is in general horizontal, via direct contact, aerogenic, fecal–oral and/or venereal transmission routes.

Roniviruses are the only known invertebrate nidoviruses and have been detected exclusively in crustaceans. The black tiger prawn (*Penaeus monodon*) appears to be the natural host of YHV and gill-associated virus (GAV), but other prawn species are susceptible to experimental infection. Infections may be chronic or acute and transmission can occur horizontally and vertically. During acute infections, mortality is usually high and virus occurs in most tissues of ectodermal and mesodermal origin, and particularly in the "Oka" or lymphoid organ. Necrotic cells display intensely basophilic cytoplasmic inclusions. The geographic range of infection encompasses the natural Indo-Pacific distribution of *P. monodon*, in which the prevalence of subclinical infection is commonly high, and there is recent evidence of infection occurring in shrimp species farmed in the Americas.

## Phylogenetic relationships within the order

In rooted and unrooted phylogenetic trees constructed for the main replicative enzymes, members of the families *Corona-*, *Arteri- and Roniviridae* consistently form distinct, well-separated monophyletic clusters. Viruses in the subfamily *Torovirinae* (genera *Bafini-* and *Torovirus*) are phylogenetically more related to each other than to those in the subfamily *Coronavirinae*. The evolutionary relationships between nidovirus (sub)families and genera are illustrated in Figure 4.

## Similarity with other taxa

Nidoviruses can be uniquely distinguished from other RNA viruses on the basis of their replicase polyproteins that comprise a number of characteristic domains arranged in a conserved order. Key diagnostic molecular markers are:

- An ORF1a-encoded protease with a chymotrypsin-like fold and substrate specificity resembling that of picornavirus 3C protease (3C-like protease, also called main protease, $M^{pro}$), flanked by two hydrophobic transmembrane domains (*tm*-$M^{pro}$-*tm*).
- An ORF1b-encoded putative multinuclear Zn-finger-like domain associated with a nucleoside triphosphate (NTP)-binding/5′-to-3′-helicase domain (Zn-HEL).
- The replicase gene constellation separated by a ribosomal frameshifting signal (*fs*): *tm*-$M^{pro}$-*tm*_*fs*_RdRp_Zn-HEL.

Homologs of several (putative) enzymes encoded by viruses of the order *Nidovirales* have been found in non-nidoviruses. The proteolytic enzymes and RdRps cluster together with homologs of viruses of the "Picornavirus-like" supergroup, and RdRps also with homologs in double stranded RNA *Birnaviridae* family members and a subset of members of the family *Tetraviridae*. The nidovirus helicase and ADRP have counterparts in viruses of the "Alphavirus-like" supergroup. The organization of the replicase ORFs, including the $M^{pro}$_FS_RdRp constellation, is also conserved in the family *Astroviridae* and in some viruses in the Sobemo-like supergroup. Parallels in the genome

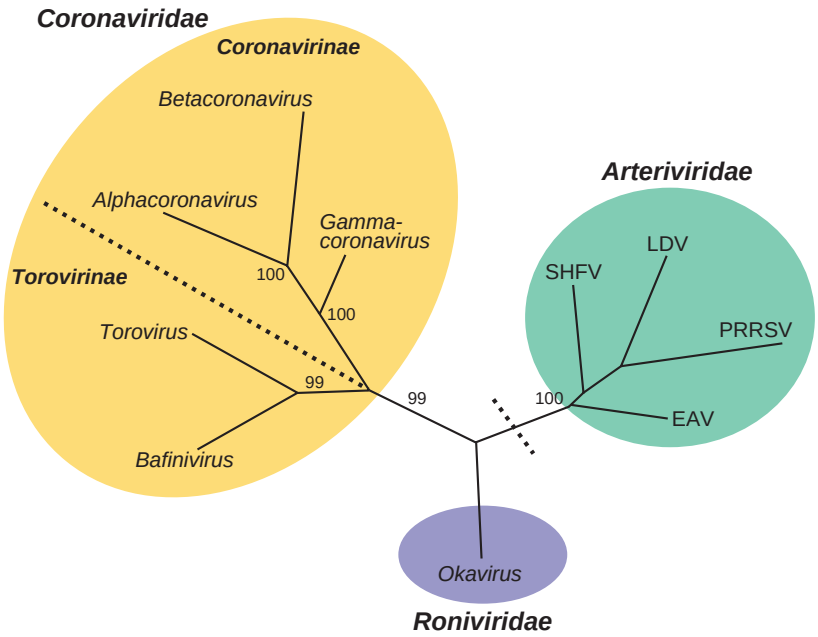


**Figure 4:** Nidovirus phylogeny. The evolutionary relationships between the five major nidovirus lineages are depicted by an unrooted maximum parsimonious tree, inferred by using multiple nucleotide sequence alignments of the RdRp-Hel region of representative members of nidovirus (sub)families and genera. For the main bifurcations, support from 100 bootstraps is given. (Sub)families and genera are highlighted and/or labeled. For arteriviruses, the four main clusters, prototyped by EAV, SHFV, LDV and PRRSV (only one of two currently recognized genotypes shown), are indicated. The divisions between large (*Coronaviridae*, *Roniviridae*) and small (*Arteriviridae*) nidoviruses and the one between *Corona-* and *Torovirinae* are indicated by black dotted lines. (Modified from Gorbalenya, A.E. (2008). Genomics and evolution of the Nidovirales. In: Perlman, Gallagher and Snijder (Eds.), *Nidovirales*. ASM Press, Washington DC, pp.15–28.)

organization and expression strategy are also evident between members of the order *Nidovirales* and the family *Closteroviridae*.

## Derivation of names

*Nido*: from Latin *nidus*, "nest", refers to the synthesis of a 3′-coterminal, nested set of mRNAs, hallmark of nidovirus transcription.
*Arteri*: from equine *arteri*tis, the disease caused by the reference virus.
*Corona*: from Latin *corona*, "halo"; refers to the characteristic appearance of surface projections that create an image reminiscent of the solar corona.
*Toro*: from Latin *torus*, a term used in architecture for the convex molding at the base of a column and in geometry for a three-dimensional structure in the shape of a hollow donut; refers to the nucleocapsid morphology in a subset of particles.
*Bafini*: from *ba*cilliform *fi*sh *ni*doviruses, refers to the virion morphology and host tropism.
*Roni*: from *ro*d-shaped *ni*doviruses, refers to the virion morphology.

## Note added in proof

During the completion of this manuscript, two papers appeared reporting the discovery of insect nidoviruses. These mosquito-associated nidoviruses are likely representatives of a novel family within the order *Nidovirales*.

## Further reading

### Journals and books

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### Websites

VIPR Virus Pathogen Resource: http://www.viprbrc.org/brc/

### Contributed by

de Groot, R.J., Cowley, J.A, Enjuanes, L., Faaberg, K.S., Perlman, S., Rottier, P.J.M., Snijder, E.J., Ziebuhr, J. and Gorbalenya, A.E.

# FAMILY *ARTERIVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Arteriviridae* |
| Genus | *Arterivirus* |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# GENUS *ARTERIVIRUS*

Type species *Equine arteritis virus*

## Virion properties

### MORPHOLOGY

Arteriviruses are pleomorphic but roughly spherical particles. By cryo-electron microscopy porcine reproductive and respiratory syndrome virus (PRRSV) particle diameters were found to range from 50 to 74 nm, with a median value of 54 nm and only few particles larger than 60 nm. Using the same approach the average diameter of the isometric nucleocapsid, which is probably not icosahedrally ordered, was found to be 39 nm. The nucleocapsid is surrounded by a lipid envelope with small surface projections that cover the entire virion surface (Figure 1).

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

The buoyant density of arterivirus particles has been estimated to be 1.13 to 1.17 g cm$^{-3}$ in sucrose. Reported sedimentation coefficients for arteriviruses range from 200S to 300S. Virions are stable when stored at −70 °C. The half-life of arteriviruses progressively decreases with increasing temperature. Virions are stable between pH 6.0 and 7.5, but are inactivated at high or low pH. Arteriviruses are also inactivated by lipid solvents, such as ether, butanol and chloroform and are extremely sensitive to detergent treatment. A brief incubation with a nonionic detergent such as 0.01% NP40 or Triton X-100 efficiently disrupts the viral envelope.

### NUCLEIC ACID

Virions contain a single molecule of linear, positive sense, single stranded RNA that ranges in length from 12.7 to 15.7 kb (Figure 2). The naked RNA, when transfected into permissive cells, is itself infectious. The genomic RNA contains a 5′ type I cap structure (simian hemorrhagic fever virus, SHFV) and a 3′-terminal poly(A) tract. Full-length sequences are available in the GenBank database for representatives of all currently known arterivirus species.

### PROTEINS

Seven structural proteins have been identified in equine arteritis virus (EAV) and PRRSV virions (Table 1). In addition to the nucleocapsid protein (N), there are two major (GP5 and M) and four minor (E, GP2, GP3, GP4) envelope proteins (Figures 1–3). By reverse genetics (EAV and PRRSV), each of these proteins was shown to be required for the production of infectious progeny. The major glycoprotein, GP5, spans the membrane three times and forms a disulfide-linked heterodimer with triple membrane spanning M protein; the heterodimer between conserved cysteine residues is essential for virus infectivity (Figure 3A). The GP5 proteins of EAV and SHFV are predicted to possess 98 residues on the outside of the virion, while both genotypes of PRRSV and lactate dehydrogenase-elevating virus (LDV) are predicted to have ectodomains of approximately 30 amino acids. The predicted orientation of the minor envelope proteins in the viral membrane is also shown in Figure 3B. The E protein of EAV and PRRSV is fatty-acid acylated, and the myristoylation of E has been shown to be non-essential for virus infectivity in both viruses. The E protein appears to be an ion-channel protein that may function in the uncoating process during virus entry and penetration. GP2, GP3 and GP4 form heterotrimers on the surface of the virus particle (EAV and PRRSV). The proteins encoded by ORFs 2a/b, 3 and 4 have not been confirmed as structural components of LDV,

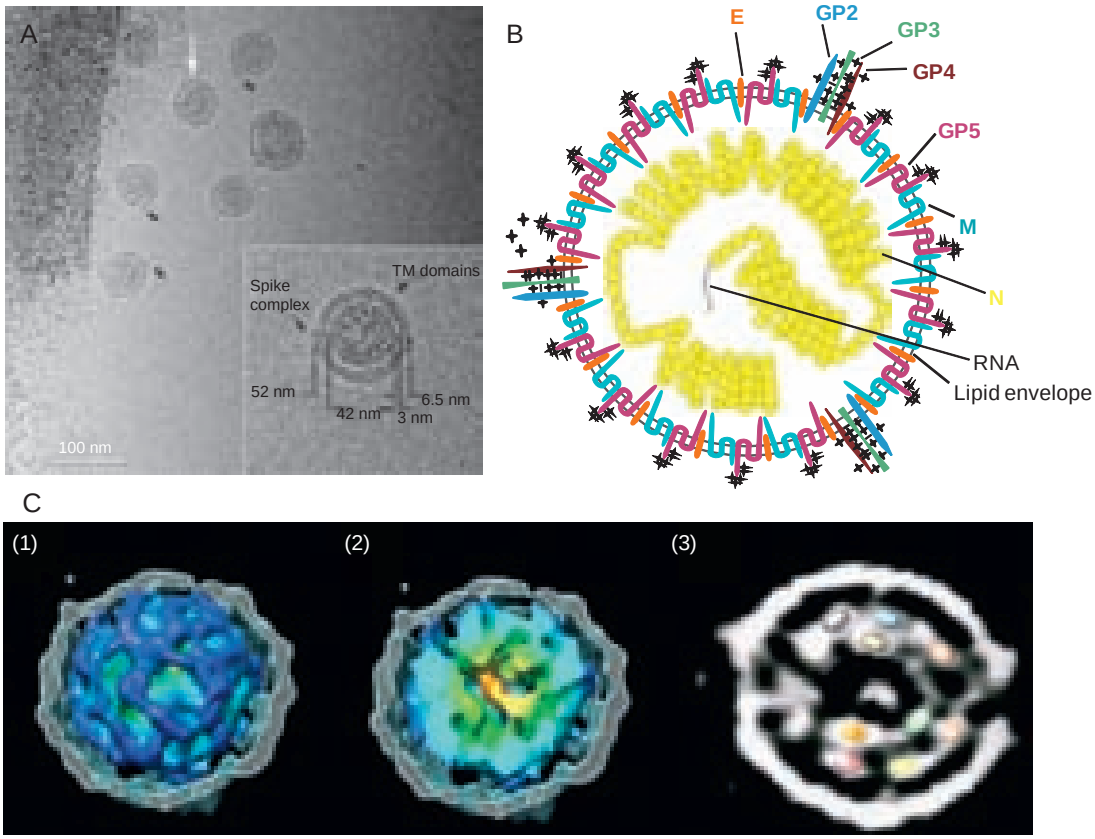


**Figure 1:** Structure of arterivirus virions. (A) Cryo-EM of PRRSV particles (strain SD-23983) in vitreous ice. The bar represents 100 nm. The white arrow points to a particle with a rectangular core. Black arrows indicate protruding features thought to correspond to complexes of the minor envelope proteins. Inset, magnified (×2) view of a single, typical PRRSV particle with dimensions indicated. A presumed envelope spike complex is indicated, as is the striated appearance most likely corresponding to transmembrane domains. The dark area on the left is part of the carbon support film (from Spilman *et al.* (2009). *J. Gen. Virol.*, **90**, 527-535; with permission from the *Journal of General Virology*. (B) Schematic representation of the arterivirus particle. N-glycosylation sites are indicated by stars. (C) Structure of the core. (1) Cutaway view of one PRRSV virion. The envelope, shown in mesh representation, was peeled away to reveal the internal core. The core is shown as an isosurface, coloured by the radius from the centre of the particle (from red to blue). (2) The core has been cut open to show the internal structure and the characteristic central density (red-orange). (3) A 63 nm thick slab through the centre of one particle tomogram, with several copies of the crystal structure of the dimer of the C-terminal domain of N rendered at a comparable resolution to the tomogram and superimposed on the oblong densities in the core (from Spilman *et al.* (2009). *J. Gen. Virol.*, **90**, 527-535; with permission from the *Journal of General Virology*).



nor has the trimerization of these proteins been assessed. A soluble, non-virion associated form of the ORF3 glycoprotein is also released from infected cells (LDV and PRRSV Type 2). In addition, the N proteins of EAV and PRRSV Type 2 have been shown to dimerize. Crystal structures of the putative dimerization domain of arterivirus N proteins (PRRSV Type 2 and EAV) have been determined and indicate that these proteins represent a new class of viral capsid-forming proteins. Virus mutants (EAV) lacking expression of E, GP2, GP3 or GP4 produce non-infectious particles. The virion proteins of SHFV include the two major envelope proteins (GP5 and M) and the N protein; the genome possesses four additional 3′ ORFs (2a′, 2b′, 3′ and 4′), encoding GP2′, E′, GP3′ and GP4′, which may be duplications of ORFs 2 a/b to 4 (Figure 2).

### Lipids

The virion lipids are cell derived, with virus budding occurring on membranes of the ER and the Golgi part of the exocytic pathway.

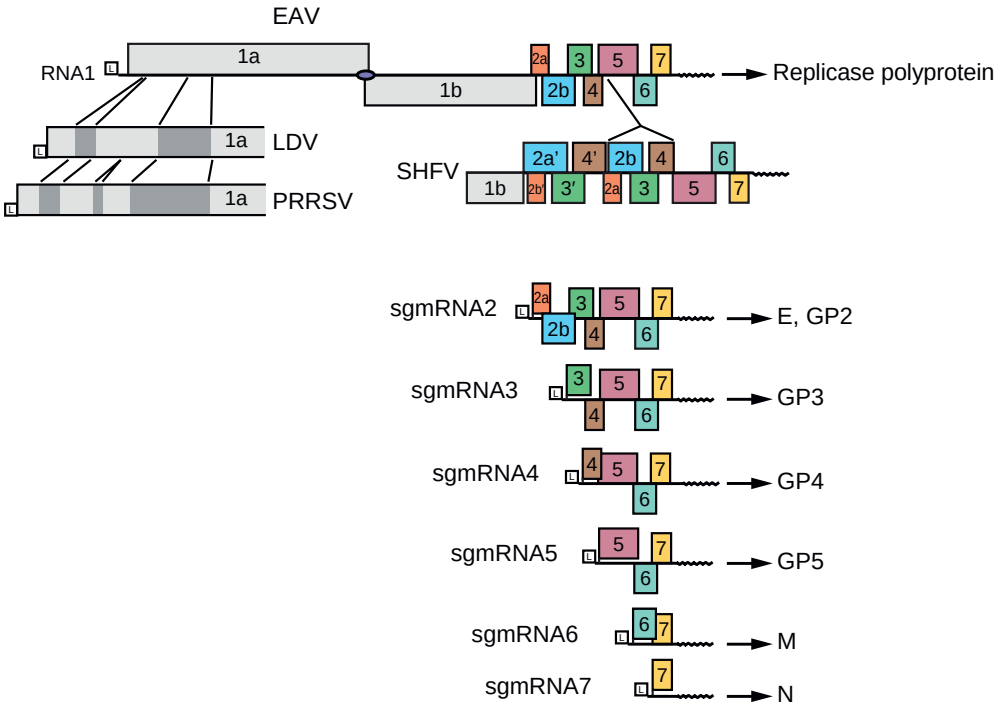


**Figure 2:** Arterivirus genome organization and expression. The general genome organization is shown at the top of the figure with the ORFs represented. The proteins encoded by the ORFs are indicated, while ORF colors for virion-associated proteins match those used in Figure 1B. Other domains: L, 5′ leader sequence or 5′ UTR; 3′ UTR and a 3′ poly(A) tail (zigzag line); filled circle, ORF 1a and 1b ribosomal frameshift site. The grey boxes immediately below represent the regions where PRRSV, LDV, and SHFV contain major insertions compared to EAV. In the infected cell, the full-length genome uses discontinuous transcription during minus strand synthesis to eventually produce a nested set of subgenomic messages (sgRNA), shown below. The proteins produced are listed to the right of the respective RNA. The structural protein composition of SHFV – except for GP5, M, and N – is unknown at present.

**Table 1:** Virion-associated proteins of arteriviruses[a]

| Protein | aa[b] | ORF | mRNA | (Putative) Function(s) |
|---|---|---|---|---|
| E[d] | 67–80 | 2a/2b[c] | 2[d] | Small integral envelope protein, myristoylated, postulated ion channel protein |
| GP2a/b | 227–256 | 2b/2a[c] | 2[d] | Minor glycoprotein, part of GP2/GP3/GP4 heterotrimer |
| GP3 | 163–265 | 3 | 3 | Minor glycoprotein, part of GP2/GP3/GP4 heterotrimer |
| GP4 | 152–183 | 4 | 4 | Minor glycoprotein, part of GP2/GP3/GP4 heterotrimer |
| GP5 | 199–278 | 5 | 5 | Major glycoprotein, carries main determinants for neutralization, part of GP5/M heterodimer |
| M | 162–174 | 6 | 6 | Integral membrane protein, part of GP5/M heterodimer |
| N[e,f] | 110–128 | 7 | 7 | Nucleocapsid protein, partially localizes to the nucleus of infected cells, phosphoprotein |

[a]Based on the genome organization of EAV, PRRSV and LDV, adapted from the Eighth ICTV Report; SHFV ORFs 2a-7 were used to predict structural protein amino acid length.
[b]aa = amino acids.
[c]In PRRSV.
[d]Subgenomic mRNA2 is assumed to be functionally bicistronic.
[e]Partial crystal structures have been determined for EAV and PRRSV N (Protein Data Bank IDs 2I9F and 1P65, respectively).
[f]ORF7 polymorphism in Type 1 (125, 126, 129, 131 AA) and Type 2 (124, 125 AA) PRRSV.

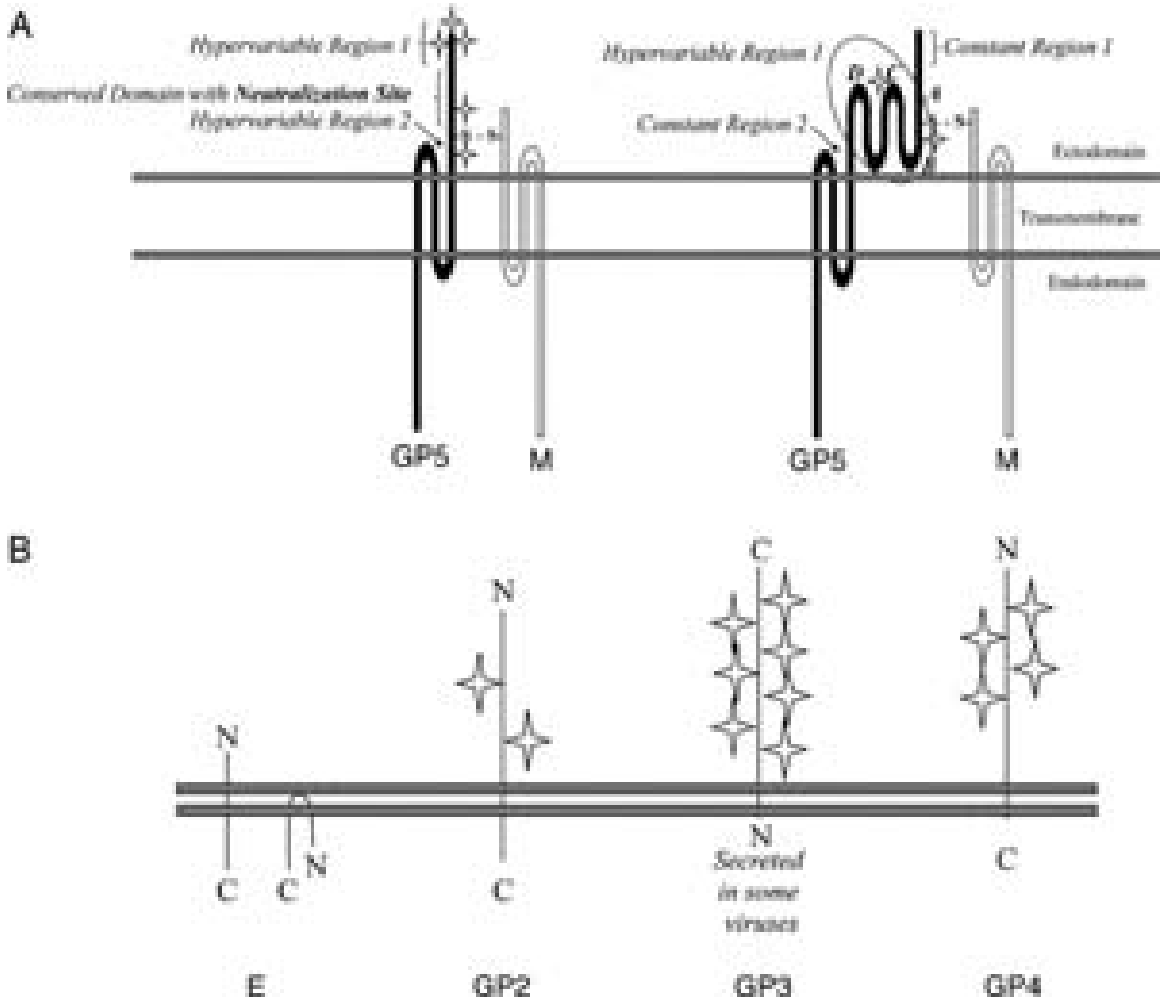


**Figure 3:** (A) Schematic of the major envelope proteins. The GP5 glycoproteins of LDV and PRRSV (left) and EAV (right) are disulfide bonded to the M protein. One neutralization domain has been identified on LDV and PRRSV, and 4 (A-D) on EAV. Hypervariable regions surround the neutralization domains. N-glycan addition (star) varies among arteriviruses, but the N-glycan residues closest to the disulfide bond between GP5 and M (S-S) are mostly conserved. (B) Predicted conformation of the minor envelope proteins in eukaryotic membranes. (Reproduced with modifications from Perlman *et al.* (2008). ASM Press, Washington, DC with permission.)

Pos. ssRNA

## Carbohydrates

Although the number of predicted N-linked glycosylation sites in GP2-5 of EAV, PRRSV and LDV strains differ, the large majority of these sites are highly conserved and present in almost all natural isolates of the three viruses. Specifically, it has been demonstrated that conserved N-linked glycosylation sites in GP5 are essential for virus infectivity and antigenicity (EAV and PRRSV). Recent reverse genetics studies have revealed that PRRSV and EAV mutants void of GP5 N-linked glycosylation are nonviable or readily revert to restore at least one N-linked glycosylation site. Since stable virus mutants lacking these carbohydrates cannot be generated, further investigation of the specific role of glycans is difficult. However, a viable pseudorevertant (deletion mutant) of an EAV mutant with a completely unglycosylated GP5 ectodomain was isolated. This pseudorevertant grew to a lower titer and produced smaller plaques compared to the parental virus with a single conserved GP5 glycosylation site. The sugar composition of the N-linked glycans of GP2-5 of these arteriviruses has not been investigated in detail. Preliminary studies indicate that the EAV GP5

carbohydrate is modified with N-acetyllactosamine, while PRRSV GP5 is thought to contain complex sugars other than polylactosaminoglycans.

## Antigenic properties

Each of the four arteriviruses is antigenically distinct and arteriviruses show no serological cross-reactivity. Furthermore, there is significant antigenic variation among different strains of EAV, LDV and PRRSV, but the antigenic properties of SHFV have not been evaluated. The major neutralization epitopes of arteriviruses have been mapped to the GP5 protein of EAV, PRRSV (both Type 1 and 2) and LDV and SHFV 5. It has been shown that GP5 and M heterodimerization is critical for the expression of neutralization epitopes on GP5 proteins of EAV and PRRSV (Figure 3A). In contrast to Type 2 PRRSV, neutralizing antibodies against GP4 have also been shown to neutralize the Type 1 PRRSV prototype (Lelystad). In addition to GP5, the M protein is the most frequently recognized EAV structural protein by convalescent sera from non-persistently infected horses, whereas sera from carrier stallions also recognize the GP3 and N proteins. The antigenicity of and humoral antibody response to EAV nsps have yet to be determined. Antibodies to several nonstructural proteins (nsps; nsp1, nsp2 and nsp7), N and M proteins and GP5 appear within 7 to 14 days of PRRSV infection. However, the neutralizing antibody response against GP5 is delayed. A number of B cell epitopes have been identified in the nsp2 protein of PRRSV. Similarly, a number of T-cell epitopes have been identified on GP4, GP5, M and N proteins of PRRSV. Infection with PRRSV also leads to suppression of T lymphocytes, and a number of nsps have been implicated in countering host immune responses. Infection with some PRRSV strains result in a transient induction of high levels of serum interferon gamma. Mice infected with LDV develop a strong neutralizing antibody response to GP5 early in the infection that effectively neutralizes the virus. Nevertheless, virus isolated from persistently infected mice is neutralization-resistant, suggesting selection of neutralization escape variants. The activation of T lymphocytes by LDV infection is limited to the first day postinfection and is triggered by the large amounts of interferon alpha produced by the initial productively-infected macrophages. This T-lymphocyte activation is rapidly followed by a transient suppression of T cell responses (cytotoxic and helper) that is maximal at about 3 days postinfection. LDV and PRRSV (in gnotobiotic pigs) also trigger polyclonal B-cell activation.

## Arterivirus entry

The arterivirus cell tropism has been attributed to the presence or absence of specific entry mediators. The entry of PRRSV into the porcine macrophage has been intensively studied but little is yet know about the entry of EAV, LDV and SHFV into their respective target cells. PRRSV virions adhere to macrophages through interactions with heparan sulphate glycosaminoglycans that line the cell surface. Subsequently, the virus binds to the macrophage-specific lectin sialoadhesin. Sialic acids on the virion surface are crucial for this interaction and the M/GP5 glycoprotein complex was identified as a ligand for this lectin receptor. Binding of PRRSV to sialoadhesin triggers uptake of the virus-receptor complex via clathrin-mediated endocytosis and subsequent release of the viral genome into the cytoplasm of the target cell initiates the translational and transcriptional processes that lead to productive infection. PRRSV genome release is dependent on acidification of the virus-containing endosome, and both cellular CD163 and proteases (cathepsin E) appear to play a crucial role in this step. GP2 and GP4 were identified as binding partners for CD163 by co-precipitation experiments, indicating a potential function for these molecules in genome release.

## Genome organization and replication

For EAV, PRRSV and LDV, the genome contains nine functional ORFs, whereas the single reported SHFV sequence contains 13 ORFs, due to an apparent 4-gene duplication (Figure 2). The viral genes for EAV, PRRSV and LDV are arranged in the order 5′-replicase-E/GP2-GP3-GP4-GP5-M-N-3′, and mostly overlap. A schematic of the arterivirus replication cycle, based on studies with EAV, is shown in Figure 4. Following genome translation, dedicated nonstructural proteins (nsps) are thought to induce double-membrane vesicles (DMVs) with which the replication/transcription complexes of arteriviruses become associated in order to engage in RNA-dependent RNA synthesis (Figures 2 and 4). In addition to a full-length minus strand, infected cells also contain a nested set

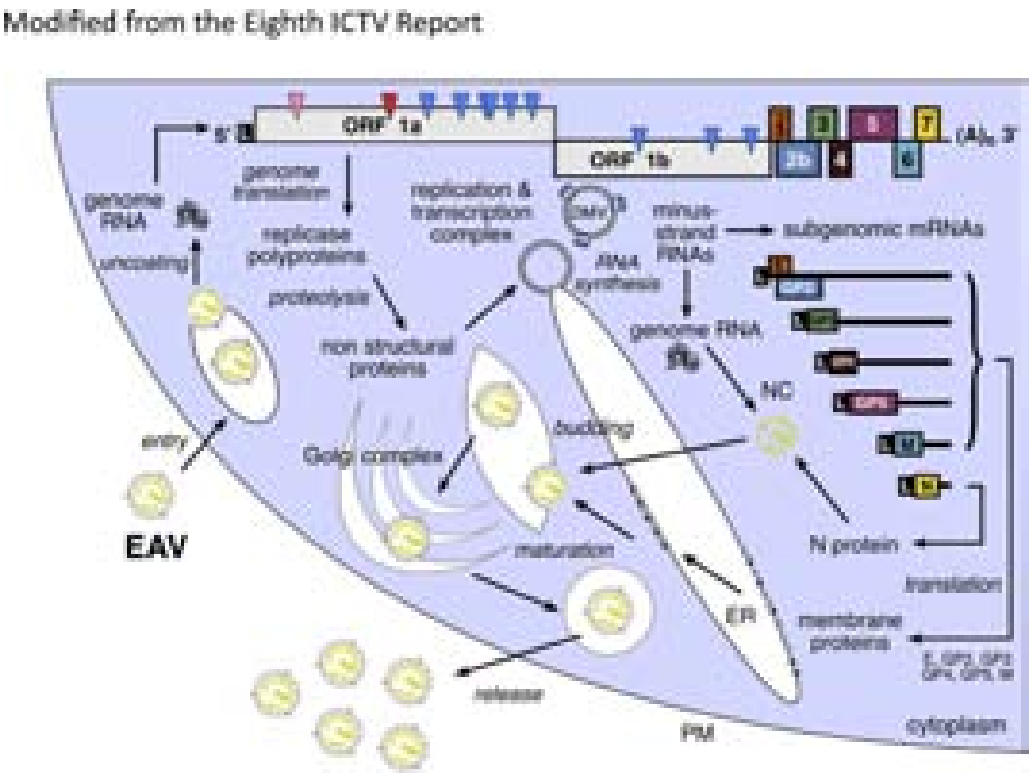


**Figure 4:** Overview of the replication cycle of the arterivirus prototype (EAV). The genome organization, including replicase cleavage sites (arrowheads; see also Figure 5), is shown at the top of the figure. Abbreviations: ER, endoplasmic reticulum; PM, plasma membrane; DMV, double membrane vesicle; NC, nucleocapsid. (Modified from the Eighth ICTV Report.)

of subgenomic minus strand RNAs that are the complements of the subgenomic mRNAs (sgmRNAs) and are believed to function as templates for their transcription. The 5′-proximal 156–211 nt of the sgmRNAs ("leader" or 5′ UTR) are derived from the 5′ end of the genome, whereas the coding region of the sgmRNA (the mRNA "body") is colinear with the 3′-proximal region of the genome (Figure 2). The synthesis of the subgenomic minus strands, which involves the fusion of sequences that are noncontiguous in the genome, is currently thought to occur by discontinuous extension during minus strand synthesis. This is a process in which transcription-regulating sequences (TRSs) direct attenuation of minus strand synthesis, translocate the nascent strand to the leader region in the 5′ end of the genomic template, and (guided by a base-pairing interaction) reinitiate minus strand synthesis to add the leader complement to the nascent subgenomic minus strand RNA. High frequency intraspecies RNA recombination between divergent strains of EAV, LDV and PRRSV has been conclusively shown. For PRRSV, only intragenotype (Type 1 or 2) recombination has been detected to date.

Four cell proteins (36, 55, 86 and 103 kDa) have been identified that bind to a *cis*-acting region required for plus-strand RNA synthesis from the minus-strand template, and two cell proteins (polypyrimidine tract-binding protein and fructose 1,6 bisphosphate aldolase) have been shown to bind to the 3′ UTR of the positive strand, required for negative strand synthesis of SHFV and LDV-C. Other cell proteins have been identified that bind to the 5′ UTRs of EAV positive and negative strand RNAs.

### Nonstructural proteins

The arterivirus genome is polycistronic and contains 9 to 13 ORFs. Three-quarters of the 5′-proximal length of the genome is occupied by two large ORFs that together encode all viral enzyme functions (collectively referred to as the viral "replicase", Figure 2) required for genome replication and

subgenomic mRNA production. Replicase ORFs 1a and 1b are translated into polyproteins pp1a (187–260 kDa) and pp1ab (345–421 kDa), with the latter being a C-terminally extended version of the former. ORF1b translation depends on a -1 ribosomal frameshift just before termination of ORF1a translation. A number of conserved domains are present in the replicases of all arteriviruses (from N-terminus to C-terminus; Figure 5): a zinc finger (ZF in nsp1), a papain-like cysteine proteinase (PLP1α and/or β [and PLP1γ in SHFV) in nsp1], an unusual papain-like cysteine proteinase (PLP2 or CP in nsp2), a chymotrypsin-like serine proteinase (SP in nsp4; alternatively known as serine Main protease, $M^{pro}$), a RNA-dependent RNA polymerase (RdRp in nsp9), a zinc binding domain (Z in nsp 10), a NTPase/RNA helicase (HEL in nsp10), and a nidovirus uridylate-specific endoribonuclease (NendoU; U in nsp11). The RNA polymerase (EAV), helicase (EAV and PRRSV Type 2), and endoribonuclease activities (EAV and PRRSV Type 2) of nsp9, nsp10 and nsp11, respectively, have been corroborated with biochemical assays using purified recombinant proteins. Furthermore, EAV nsp1 has been identified as a critical regulator of the accumulation levels of genomic and subgenomic mRNAs, most likely by modulating the synthesis of their corresponding minus strand templates. ORF1a-encoded subunits with hydrophobic domains, in particular nsp2 and nsp3, have been implicated in the formation of the endoplasmic reticulum-derived DMVs with which the viral replication and transcription complexes are associated. Detailed analysis has shown that the EAV replicase polyproteins pp1a and pp1ab are cleaved into 13 mature nsps by the three viral proteinases (PLP1β, PLP2 and SP) (Table 2), whereas LDV and PRRSV produce at least one additional cleavage product due to the fact that their nsp1 equivalent contains a second internal proteinase that cleaves the nsp1 region into nsp1α and nsp1β. PLP2 (CP) has been shown to cleave nsp2 from nsp3 in EAV and PRRSV, and to assist the SP in cleavage of the nsp4/5 junction in EAV (Table 2). The EAV nsp4 SP is responsible for nine proteolytic cleavages in the C-terminal half of pp1a and

**Table 2:** Nonstructural proteins of EAV[a]

| Protein | aa[b] | Mode of expression[c] | (Putative) Function(s) |
|---|---|---|---|
| nsp1[d] | 260 | TI + nsp1 PLP1β | Zinc finger, proteinase (PLP1), replicase polyprotein processing, transcription, and virion biogenesis (dispensable for genome replication) |
| nsp2 | 571 | TI + nsp1 PLP1β + nsp2 PLP2 | Proteinase (PLP2) with deubiquitinating activity (DUB), integral membrane protein, replication complex (DMV) formation |
| nsp3 | 233 | TI + nsp2 PLP2 + nsp4 SP | Integral membrane protein, replication complex (DMV) formation |
| nsp4[e] | 204 | TI + nsp4 SP | Main proteinase (SP) |
| nsp5 | 162 | TI + nsp4 SP | Integral membrane protein, replication complex (DMV) formation |
| nsp6 | 22 | TI + nsp4 SP | ? |
| nsp7α, β | 225 | TI + nsp4 SP | ? |
| nsp8[f] | 50 | TI + nsp4 SP + TT | ? |
| nsp9 | 693 | TI + RFS + nsp4 SP | RNA-dependent RNA polymerase |
| nsp10 | 467 | TI + RFS + nsp4 SP | RNA helicase/NTPase, putative zinc binding domain, role in subgenomic mRNA synthesis |
| nsp11 | 219 | TI + RFS + nsp4 SP | Nidovirus uridylate-specific endoribonuclease |
| nsp12 | 119 | TI + RFS + nsp4 SP + TT | ? |

[a]Based on the currently known replicase processing scheme of EAV, adapted from the Eighth ICTV Report.
[b]aa = amino acids.
[c]TI, translation initiation; RFS, ORF1a/ORF1b ribosomal frameshifting; TT, translation termination; PLP1, papain-like cysteine proteinase; PLP2, papain-like, cysteine proteinase; SP, serine proteinase.
[d]Nsp1 of LDV and PRRSV is cleaved internally by an additional papain-like proteinase to yield nsp1α and nsp1β. The nsp1α subunit of PRRSV contains two distinct zinc finger configurations, the second of which has been shown to be functional. A crystal structure has been determined for PRRSV nsp1α (Protein Data Bank ID 3IFU).
[e]Crystal structures have been determined for EAV and PRRSV nsp4 (Protein Data Bank IDs 1MBM and 3FAO, respectively).
[f]Due to ribosomal frameshifting, nsp8 is identical to the N-terminal 50 aa of nsp9.

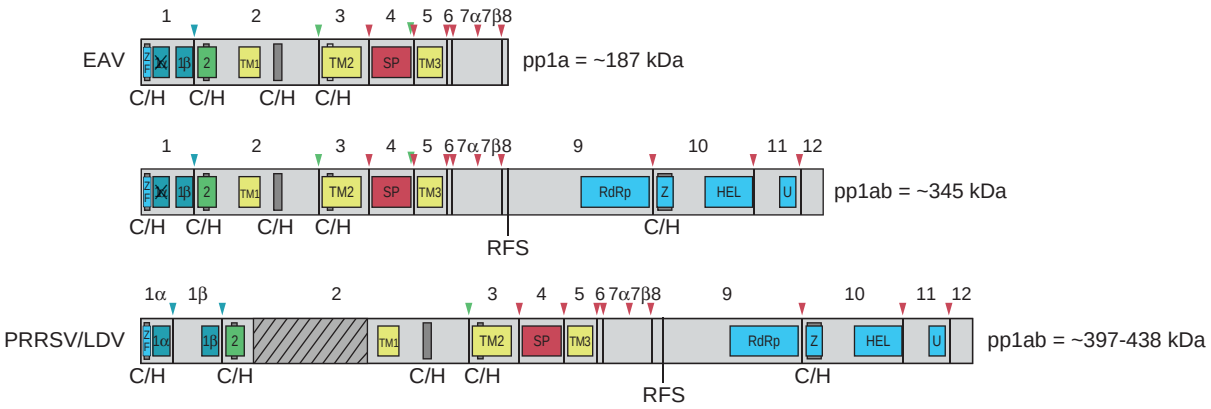


**Figure 5:** Overview of proteolytic processing of the EAV replicase polyproteins, with examined or potential similarities with PRRSV and LDV indicated. The domains for SHFV have not been described. Polyprotein cleavage sites are depicted with arrowheads matching the colour of the proteinase involved. Abbreviations: ZF, zinc-finger; 1 (PLP1), papain-like cysteine proteinase; 2 (PLP2), papain-like, cysteine proteinase; SP (also called Mpro), chymotrypsin-like serine proteinase; TM, transmembrane domain; RdRp, RNA-dependent RNA polymerase; Z, zinc binding; HEL, NTPase/helicase; U, nidoviral endonuclease specific for U (NendoU). In addition, several cysteine/histidine (C/H) recognized motifs, as well as the ribosomal frameshift site (RFS), are indicated below the figures. In EAV, PLP1α has become inactivated although its remnants were detected in the EAV nsp1 region. The large hypervariable region in PRRSV nsp2 that is characterized by insertions and deletions among different strains is shown by the hatched gray bar.

the ORF1b-encoded part of pp1ab. Several cysteine/histidine (C/H) motifs have been identified, and two (putative) zinc binding domains (in nsp1 and nsp 10) have been shown to be critical for the EAV replication cycle (Table 2). By sequence comparison, similar cleavage sites and C/H motifs have been predicted in all other arteriviruses, but most are not confirmed. The PLP1β cleavage site for PRRSV has been shown to be different from what was originally proposed.

## Biological properties

All known arteriviruses infect a single type of vertebrate host in the domain Eucarya and are not transmitted by a vector. Macrophages are the primary host cell. Arteriviruses are cytocidal and usually cause both acute and chronic, persistent disease in their hosts. EAV is the agent of equine viral arteritis (inflammation of small arteries) and infection can lead to extremely variable clinical signs. Viral arteritis has not been a reported characteristic of infection with LDV and PRRSV. LDV causes a lifelong persistent infection of mice, but CNS disease resulting in paralysis is seen in Fv-1n/n mice. Infection of swine with PRRSV results in reproductive failure in sows, respiratory illness in growing swine, and is usually asymptomatic in boars. For PRRSV, extraordinary strain diversity has been detected and is due in part to high levels of viral recombination (Type 1 and 2) that leads to variation in clinical symptoms from mild to severe (especially in Type 2). SHFV causes acute or persistent infections in African monkeys, such as patas (*Erythrocebus patas*), with no overt disease symptoms, but induces a fatal hemorrhagic fever in monkeys of the genus *Macaca*.

## Species demarcation criteria in the genus

Each of the four currently recognized species in the genus *Arterivirus* constitutes a major phylogenetic branch within the genus (Figure 6). For each species except SHFV, multiple full-length sequences were determined and indicate that the species consist of clusters of sometimes widely divergent strains. LDV and PRRSV are most closely related to each other. There are two subspecific types of PRRSV, European (Type 1) and North American (Type 2). Members of the four virus species are antigenically distinct. The viruses of each species have a restricted host range and each species infects different hosts. The size variation of the N-terminal half of ORF1 suggests that this arterivirus region may encode species-specific functions.

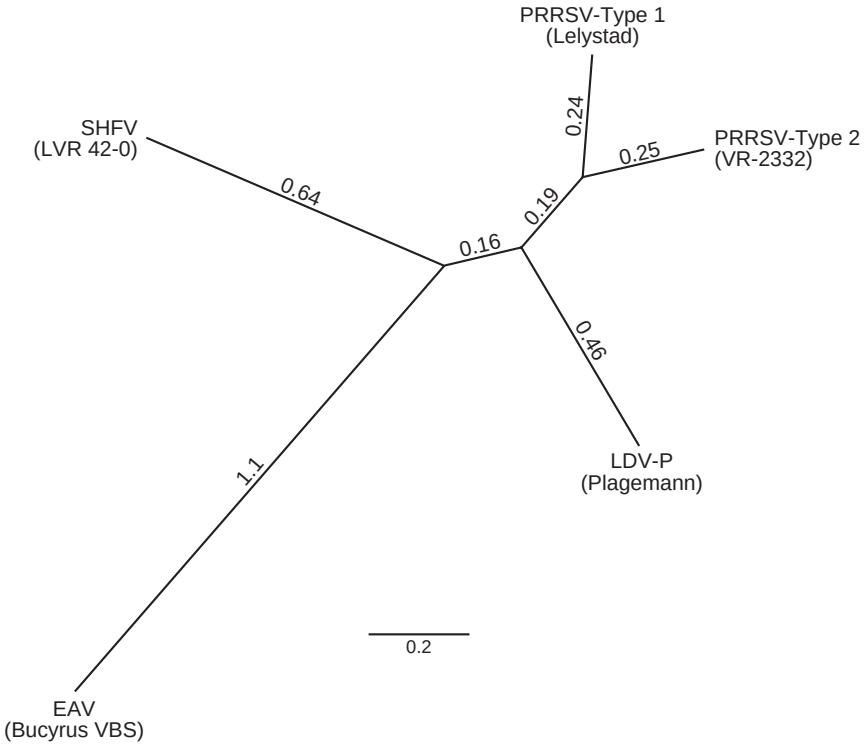


**Figure 6:** Phylogenetic analysis of the *Arteriviridae* was completed using the nucleotide sequences of SHFV (strain LVR 42-0; NC_003092), EAV (strain Bucyrus VBS; DQ846750), LDV-P (Plagemann strain; NC_001639), Type 1 PRRSV (strain Lelystad; M96262) and Type 2 PRRSV (strain VR-2332; U87392). Unrooted phylogenetic analysis of the full-length ORF1b replicase polyprotein. The tree was generated using the Dayhoff substitution model in PHYML, after alignment with the MUSCLE Alignment algorithm (default parameters, 8 iterations) in Geneious Pro 4.8.3 (Biomatters Ltd). Distance lengths represent substitutions per site. (K.S. Faaberg, unpublished data.)

## List of species in the genus *Arterivirus*

| | | |
|---|---|---|
| *Equine arteritis virus* | | |
| Equine arteritis virus - virulent Bucyrus strain (VBS) | [DQ846750] | (EAV-VBS) |
| Equine arteritis virus - cell-culture adapted Bucyrus | [X53459 = NC_002532] | (EAV) |
| *Lactate dehydrogenase-elevating virus* | | |
| Lactate dehydrogenase-elevating virus- Plagemann | [U15146 = NC_001639] | (LDV-P) |
| Lactate dehydrogenase-elevating virus-C | [L13298] | (LDV-C) |
| *Porcine respiratory and reproductive syndrome virus* | | |
| Porcine respiratory and reproductive syndrome virus-Type 1 | [M96262] | (PRRSV-1) |
| Porcine respiratory and reproductive syndrome virus-Type 2 | [U87392] | (PRRSV-2) |
| *Simian hemorrhagic fever virus* | | |
| Simian hemorrhagic fever virus | [AF180391 = NC_003092] | (SHFV) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Arterivirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Arteriviridae*

None reported.

## Phylogenetic relationships within the family

Phylogenetic analyses show that the family includes five major lineages, indicating that lineages represented by Type 1 and Type 2 PRRSV may be recognized as separate species in a future revision of arterivirus taxonomy (Figure 6).

## Similarity with other taxa

The family *Arteriviridae* together with the family *Coronaviridae* and the family *Roniviridae* form the order *Nidovirales*. These viruses have important common features at the level of genome organization, genome expression strategy, phylogeny and internal organization of their large replicase gene. Despite these overall similarities, arterivirus genomes are substantially smaller, and the size, structure and composition of their virions do not resemble those of other members of the order *Nidovirales*. Consequently, arteriviruses may be called small-genome nidoviruses, while the others are recognized as large-genome nidoviruses. Various non-structural proteins contain arterivirus- and/or nidovirus-specific domains or signatures, including an SDD signature (instead of the canonical GDD) in the RdRp (nsp9), a complex N-terminal (putative) zinc binding domain in the helicase (nsp10), and a putative endonuclease domain (NendoU) in nsp11. Non-nidovirus homologs of several (putative) enzymes encoded by viruses of the family *Arteriviridae* have been found in other RNA viruses. The main proteinase and RdRp cluster together with homologs of viruses in the "picornavirus-like" and "sobemovirus-like" supergroups. The helicase has counterparts in viruses of the "alphavirus-like" supergroup. Also, some parallels are evident between the genome organization and expression strategy of members of the family *Arteriviridae* (and other members of the order *Nidovirales*) and those of the plant virus family *Closteroviridae*.

## Derivation of name

*Arteri:* derived from the disease caused by the type member, equine *arteri*tis virus.

## Further reading

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## Contributed by

Faaberg, K.S., Balasuriya, U.B., Brinton, M.A., Gorbalenya, A.E., Leung, F.C-C., Nauwynck, H., Snijder, E.J., Stadejek, T., Yang, H. and Yoo, D.

# FAMILY *CORONAVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Coronaviridae*** |
| Subfamily | ***Coronavirinae*** |
| Genus | ***Alphacoronavirus*** |
| Genus | ***Betacoronavirus*** |
| Genus | ***Gammacoronavirus*** |
| Subfamily | ***Torovirinae*** |
| Genus | ***Torovirus*** |
| Genus | ***Bafinivirus*** |

## Distinguishing features

The members of the family *Coronaviridae*, a monophyletic cluster in the order *Nidovirales*, are enveloped, positive stranded RNA viruses of three classes of vertebrates: mammals (corona -and toroviruses), birds (coronaviruses) and fish (bafiniviruses). Virions are spherical, 120–160 nm across (*Coronavirinae*), bacilliform, 170–200 × 75–88 nm (*Bafinivirus*) or found as a mixture of both, with bacilliform particles characteristically bent into crescents (*Torovirus*). The particles are typically decorated with large, club- or petal-shaped surface projections (the "peplomers" or "spikes"), which in electron micrographs of spherical particles create an image reminiscent of the solar corona. This inspired the name of the "true" coronaviruses (now grouped in the subfamily *Coronavirinae*), which was later adopted for the whole family. Nucleocapsids are helical and can be released from the virion by treatment with detergents. Whereas the coronavirus nucleocapsid appears to be loosely-wound, those of the *Torovirinae* are distinctively tubular.

In terms of genome size and genetic complexity, the *Coronaviridae* are the largest RNA viruses identified so far, rivaled only by the okaviruses, large nidoviruses of invertebrates assigned to the family *Roniviridae*. Replication has been studied in detail only for coronaviruses, but the limited data available for toro- and bafiniviruses suggest that the latter viruses use essentially similar strategies. Virions attach to dedicated host cell surface receptors via their spikes (Table 1) and release their genome into the target cell via fusion of the viral envelope with the plasma membrane and/or the limiting membrane of an endocytic vesicle. The entire replication cycle takes place in the cytoplasm and involves the production of full-length and subgenome-sized (sg) minus-strand RNA intermediates with the viral genome serving both as mRNA for the replicase polyproteins and as a template for minus-strand synthesis. RNA synthesis is catalyzed by an as yet poorly characterized replication–transcription complex, composed of viral and host proteins and associated (at least in coronaviruses) with an interconnected network of modified intracellular membranes and double-membrane vesicles that are presumably endoplasmic reticulum (ER)-derived.

The genome contains multiple ORFs. Its 5′-most two-thirds are occupied by the replicase gene, which is comprised of two overlapping ORFs called 1a and 1b (Figure 1). The replicase gene is translated to produce polyprotein pp1a and, subject to programmed −1 ribosomal frameshifting, a C-terminally extended product, pp1ab. The polyproteins are co- and post-translationally processed by a set of virus-encoded proteinases and, thus, are not detectable as full-length proteins in virus-infected cells. The N-termini of pp1a and pp1ab are processed by one or two papain-like proteinases, whereas the C-terminal half of coronavirus pp1a and the ORF1b-encoded part of pp1ab are cleaved at 11 well-conserved sites by the main proteinase ($M^{pro}$ or $3CL^{pro}$), a nidovirus-wide conserved enzyme with a chymotrypsin-like fold, a poliovirus 3C proteinase-like substrate specificity and either a serine (torovirus, bafinivirus) or a cysteine (coronavirus) as active site nucleophile. In coronaviruses, proteolytic processing results in the production of 15 (in viruses belonging to the species *Avian coronavirus*) or 16 mature products, commonly referred to as non-structural proteins (nsp's) and numbered according to their position – from N- to C-terminus – in the viral polyproteins (Figure 1). Many nsp's are unique enzymes involved in one or more essential step(s) in viral replication. Others appear to be exclusively involved in virus–host interactions (including immune evasion) and are dispensable for virus propagation *in vitro* (Table 2). Polyprotein processing in toro- and bafiniviruses has not been studied in detail.


Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses

**Table 1:** Coronavirus primary attachment factors and receptors

| Virus species | Host | Attachment factor | Main receptor |
|---|---|---|---|
| *Alphacoronavirus 1* | | | |
| Canine coronavirus type I | Dog | | ? |
| Canine coronavirus type II | Dog | | APN |
| Feline coronavirus type I | Cat | | ? |
| Feline coronavirus type II | Cat | | APN |
| Transmissible gastroenteritis virus | Pig | Sialic acid | APN |
| *Human coronavirus 229E* | Human | | APN |
| *Human coronavirus NL63* | Human | | ACE2 |
| *Betacoronavirus* 1 | | | |
| Bovine coronavirus | Cow | | 9-O-Ac Sia? |
| Equine coronavirus | Horse | | 9-O-Ac Sia? |
| Human coronavirus OC43 | Human | | 9-O-Ac Sia? |
| Porcine hemagglutinating encephalomyelitis virus | Pig | | 9-O-Ac Sia? |
| *Murine coronavirus** | Mouse | 4-O- or 9-O-Ac Sia | CEACAM1a |
| *SARS-related coronavirus* | Human | | ACE2 |

Abbreviations: APN, aminopeptidase N; ACE2, angiotensin-converting enzyme 2; CEACAM1a, carcinoembryonic antigen adhesion molecule 1.
*Murine coronaviruses occur in two types that use either 4- or 9-*O*-acetylated sialic acid (*O*-Ac Sia) as primary attachment factor and CEACAM1a as main receptor.

The 3′-proximal genes (3 in bafiniviruses and up to at least 12 in some coronaviruses) code for the structural proteins and, in the case of coronaviruses, a variable number of "accessory" or "niche-specific" proteins. These genes are expressed – as is typical for nidoviruses – from a 3′-coterminal nested set of sg mRNAs that are thought to be transcribed not from the full-length minus-strand anti-genome, but from a mirror copy set of sg minus-strand templates.

Members of the family *Coronaviridae* all seem to share two envelope protein species, the membrane (M) and spike (S) proteins. Similarities in size, predicted structures and presumed function(s) suggest a common ancestry, and the remote, but significant sequence similarities observed for toro-, bafini- and (to lesser extent) coronavirus S proteins lend further support to this view. Presumably, progenitors of the S and M proteins were encoded in the last common ancestor of the *Corona-* and *Torovirinae* lineages. Virus assembly involves budding of preformed nucleocapsids at membranes of the endoplasmic reticulum and early Golgi compartment and the completed virions are released via the exocytotic pathway. Nidovirus replication is discussed in more detail in paragraphs below and also in Chapter *Nidovirales*.

All members of the *Coronaviridae* family share the following characteristics:

- Virions: enveloped and decorated with large (15–20 nm) surface projections.
- Nucleocapsid: helical, comprised of genome and multiple copies of a single basic phosphoprotein species (N).
- Envelope: contains a variable number of viral membrane protein species, two of which seem to be conserved family-wide and are essential for virion morphogenesis and/or infectivity (at least in coronaviruses):
  - a 200- to 250-aa triple-spanning $N^{exo}C^{endo}$ integral membrane protein M
  - an extensively N-glycosylated, 1100- to 1600-aa class I fusion protein S which forms peplomers.
- Genome: positive sense RNA, linear, unimolecular, infectious, 26–32 kb in length, capped, polyadenylated and structurally polycistronic.
- General genome organization: 5′-UTR-replicase-S-M-N-UTR-3′ (genes named after their product), with the genome functioning as mRNA for the replicase gene.

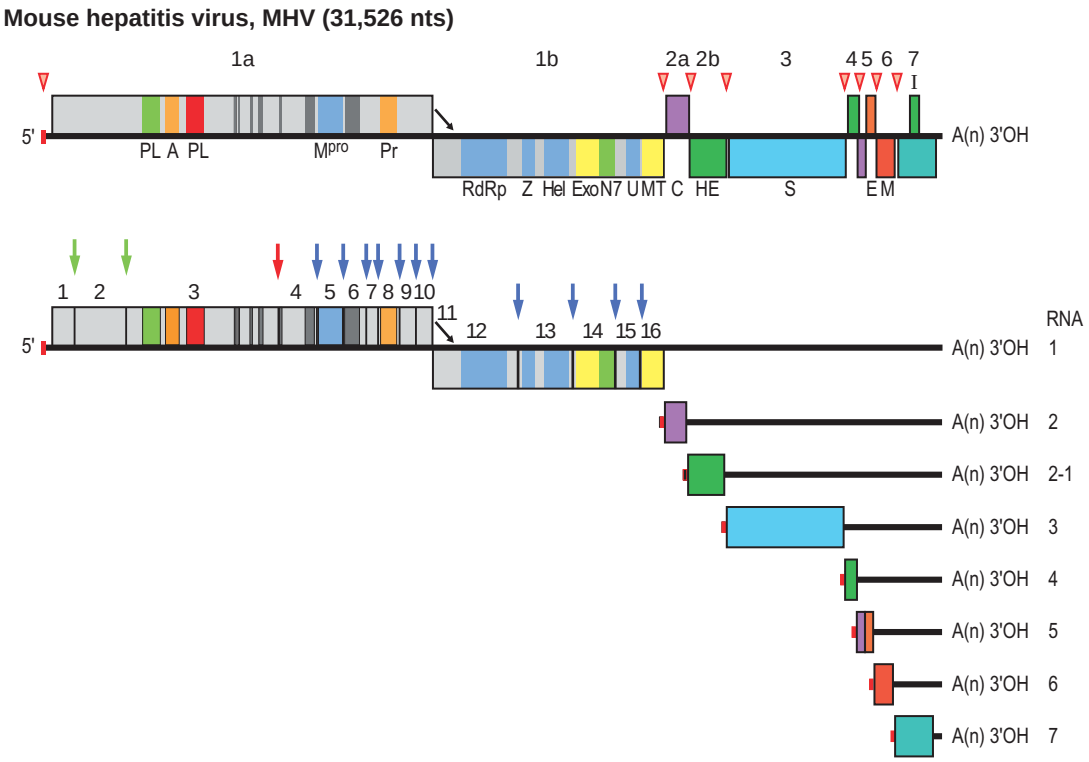


**Figure 1:** Coronavirus genome organization and expression. (Upper panel) Schematic representation of the genome of mouse hepatitis virus (MHV) shown as an example. ORFs are represented by boxes, indicated by number (above) and encoded protein (acronyms below). Regions encoding key domains in replicase polyproteins pp1a and pp1ab are colour-coded with hydrophobic segments shown in dark grey. The 5′-leader sequence is depicted by a small red box. The arrow between ORF 1a and 1b represents the ribosomal frameshifting site. The poly(A) tail is indicated by "A(n)". Red arrowheads indicate the locations of transcription-regulating sequences (TRSs). PL (green) papain-like proteinase 1 ($PL1^{pro}$); PL (red), papain-like proteinase 2 ($PL2^{pro}$); A, ADP-ribose-1″phosphatase (macrodomain); $M^{pro}$, 3C-like main protease; Pr, noncanonical RNA-dependent RNA polymerase, putative primase; RdRp, RNA-dependent RNA polymerase; Z, zinc-binding domain; Hel, helicase domain; Exo, 3′ to-5′ exoribonuclease domain; N7, guanine-N7-methyltransferase; U, nidoviral uridylate-specific endoribonuclease (NendoU); MT, ribose-2′-*O*-methyltransferase domain; HE, hemagglutinin-esterase; S, spike protein; E, envelope protein; M, membrane protein, N, nucleocapsid protein; I, internal ORF. (Lower panel) Processing of the replicase polyproteins and structural relationship between the genomic RNA and subgenomic mRNAs of coronaviruses. Arrows indicate cleavage sites for $PL1^{pro}$ (green), $PL2^{pro}$ (red) and $M^{pro}$ (blue). The locations of the non-structural proteins (nsp's) are indicated by their number (see also Table 2). mRNA species are numbered as by convention on the basis of their size, from large to small, with the genome designated as RNA1. For the sg mRNAs only ORF(s) that are translated are shown.

- Replicase gene: comprised of overlapping ORFs 1a and 1b that code for two huge polyproteins, pp1a and pp1ab, production of the latter requiring a programmed −1 ribosomal frameshift; pp1a and pp1ab are processed autoproteolytically.
- ORFs downstream of the replicase gene: expression from a 3′ co-terminal nested set of two or more subgenomic mRNAs that are capped and polyadenylated.
- Morphogenesis: virion assembly through budding of preformed nucleocapsids at smooth intracellular membranes of endoplasmic reticulum/early Golgi compartments.

The replicase polyproteins of the *Coronaviridae* comprise a number of characteristic domains arranged in a conserved order (see Chapter *Nidovirales*; see also this Chapter Figures 1, 9 and 12 and Table 2). Two ORF1a-encoded replicase domains, an ADP-ribose-1″-phosphatase (ADRP, also called macrodomain; located in coronavirus nsp3) and a noncanonical "secondary" RdRp with possible primase activity (coronavirus nsp8) may represent diagnostic markers that distinguish members of the family *Coronaviridae* from viruses in other nidovirus taxa.

## Demarcation criteria for genera and species

Only viruses for which a complete genome sequence is available (see Supplementary Table 1 available online on Science Direct®, www.sciencedirect.com) are to be considered for taxonomy and the following demarcation criteria are used.

- Established and newly identified members of the family *Coronaviridae* are assigned to a subfamily and genus on the basis of rooted phylogeny and calculation of pair-wise evolutionary distances for the following *Coronaviridae*-wide conserved domains in replicase polyprotein pp1ab: ADRP, nsp5 (3CL$^{pro}$), nsp12 (RdRp), nsp13 (Hel), nsp14 (ExoN), nsp15 (NendoU) and nsp16 (O-MT). This procedure, developed by Lauber and Gorbalenya (*in preparation*), at present unambiguously identifies 20 distinct non-overlapping clusters (with the largest intra-cluster distance being smaller than the smallest inter-cluster distance): 17 coronaviruses, 2 toroviruses, 1 bafinivirus). Likewise, the higher-rank clusters corresponding to genus and subfamily levels are recognized.
- Phylogenetic outliers assigned to the family *Coronaviridae* may be considered representatives of a new genus when they do not cluster with any of the current genera and share less

**Table 2:** Cleavage products of coronavirus replicase polyproteins pp1a and pp1ab: names, assigned functions and structure

| Protein | Assigned function | E/N* | MMDB ID |
|---|---|---|---|
| nsp1† | IFN antagonist | N | ND |
| | Degradation of host mRNAs | | |
| | Inhibition of translation | | |
| | Cell cycle arrest | | |
| nsp2 | Unknown; associates with RTCs | N | ND |
| nsp3 | Papain-like proteinase PL1$^{pro}$; polyprotein processing | N | 37505; 42180 |
| | Papain-like proteinase PL2$^{pro}$; polyprotein processing, DUB | E | |
| | ADP-ribose-1″phosphatase (macrodomain); RNA-binding | N | |
| | IFN antagonist | | |
| | DMV formation? | | |
| nsp4 | Unknown; DMV formation? | | 76092 |
| nsp5 | Main proteinase M$^{pro}$; polyprotein processing | E | 20276; 23158 |
| nsp6 | Unknown; DMV formation? | | ND |
| nsp7 | ssRNA binding | | 36090 |
| nsp8 | Noncanonical "secondary" RdRp with putative primase activity; forms hexadecameric supercomplex with nsp7 | | 36090 |
| nsp9 | ssRNA binding; associates with RTCs | | 26498; 60895 |
| nsp10 | Dodecameric zinc finger protein; associates with RTCs, stimulates nsp16 methyltransferase activity | | 40869; 40904 |
| nsp11 | Unknown | | ND |
| nsp12 | RdRp | E | ND |
| nsp13 | Helicase | E | ND |
| | RNA 5′-triphosphatase | | |
| nsp14 | 3′→5′exoribonuclease (required for RdRp fidelity) | N | ND |
| | Guanine-N7-methyltransferase (RNA cap formation) | N | |
| nsp15 | Hexameric uridylate-specific endoribonuclease | N | 40936 |
| nsp16 | Ribose-2′-*O*-methyltransferase (RNA cap formation) | N | ND |

MMDB ID numbers are listed for replicase proteins for which crystal structures are available. IFN, interferon; RTC, replicase/transcriptase complex; DUB, deubiquitinating enzyme; DMV, double-membrane vesicles.
*Essential (E) or non-essential (N) for replication in cultured cells.
†Absent in gammacoronaviruses.

Pos. ssRNA

than 46% sequence identity in the aforementioned conserved replicase domains with any other established member of the family.

- Viruses that share more than 90% aa sequence identity in the conserved replicase domains are considered to belong to the same species. This 90% identity threshold serves as the sole species demarcation criterion.

# SUBFAMILY *CORONAVIRINAE*

## Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Coronavirinae*** |
| Genus | ***Alphacoronavirus*** |
| Genus | ***Betacoronavirus*** |
| Genus | ***Gammacoronavirus*** |

On the basis of rooted and unrooted phylogenetic trees estimated for different regions of the genome, four coronavirus (CoV) clusters can be distinguished, three of which (corresponding to the former nonofficial "groups" 1, 2 and 3) have been recognized and classified as genera (*Alpha-*, *Beta-* and *Gammacoronavirus*, respectively). The fourth cluster comprises a number of recently identified coronaviruses of birds and by all standards appears to represent a novel (but yet to be approved) genus, provisionally named *Deltacoronavirus*. In the genus *Betacoronavirus*, four separate lineages can be discerned, designated A through D, that correspond to former subgroups 2A through D, respectively (Figure 2).

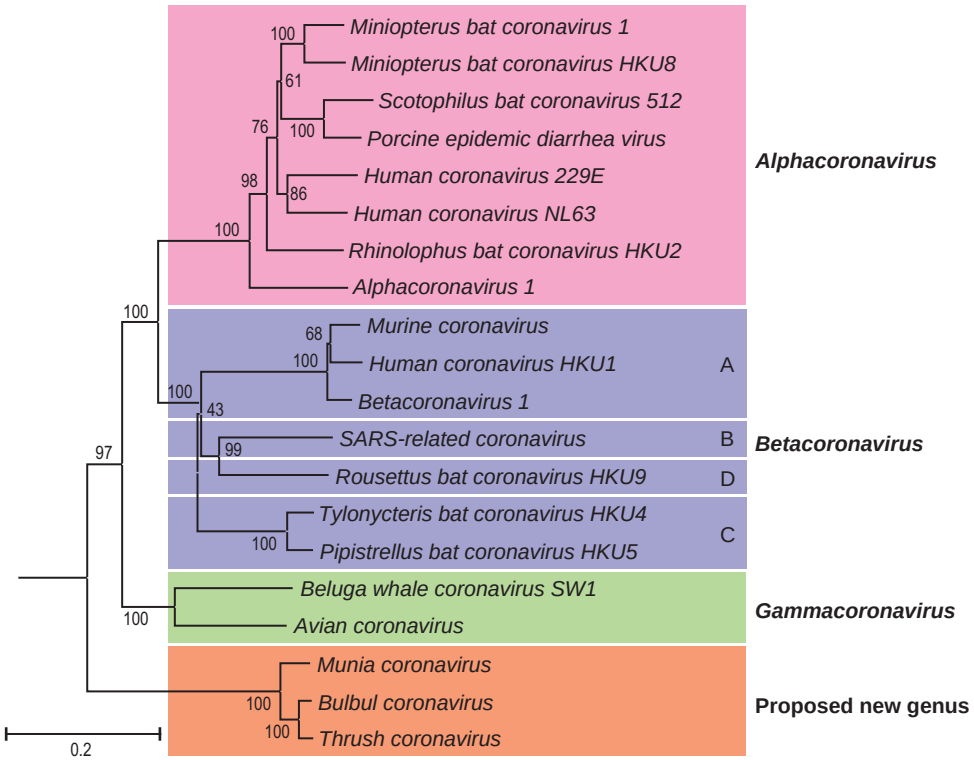


**Figure 2:** Phylogenetic relationships among the members of the subfamily *Coronavirinae*. A rooted neighbor-joining tree was generated from amino acid sequence alignments of RdRp and helicase domains with equine torovirus Berne as outgroup. The tree reveals four main monophyletic clusters corresponding to genera *Alpha-*, *Beta-* and *Gammacoronavirus* and an envisaged new genus (color-coded), and also shows the distinct betacoronavirus lineages A through D.

## Virion properties

### Morphology

By conventional negative-staining electron microscopy, virions appear pleiomorphic, roughly spherical, 120–160 nm in diameter, with a characteristic fringe of large (ca. 20 nm), petal-shaped surface projections that are comprised of trimers of the spike (S) glycoprotein (Figure 3). Group A betacoronaviruses (Figure 2) display a second type of surface projection, 5–7 nm in length, comprised of the homodimeric hemagglutinin-esterase (HE) glycoprotein. Coronavirions as studied by cryo-electron tomography are homogeneous in size and spherical (envelope outer diameter 85 ± 5 nm). The envelope is exceptionally thick (7.8 ± 0.7 nm) in comparison to typical biological membranes (average thickness ca. 4 nm). The nucleocapsid, a loosely-wound helix, seems to be tightly folded to form a compact core that appears to be separated from the envelope by a gap of about 4 nm (Figure 3).

### Physicochemical and physical properties

The estimated Mr of the virion is $400 \times 10^6$, its buoyant density in sucrose and CsCl is 1.15–1.20 g cm$^{-3}$ and 1.23–1.24 g cm$^{-3}$, respectively, and its $S_{20,W}$ is 300 to 500S. Particles are sensitive to heat, lipid solvents, non-ionic detergents, formaldehyde, oxidizing agents and UV irradiation.

### Nucleic acid

Members of the subfamily *Coronavirinae* possess a unimolecular, positive stranded RNA genome, which is capped, polyadenylated and infectious. Genome lengths range from 26.4 in the non-assigned Thrush coronavirus to 31.7 kb for the gammacoronavirus Beluga whale coronavirus, the largest RNA virus known to date. At present, complete genomes are available for more than 300 naturally-occurring coronaviruses. (For a complete list refer to Supplementary Table 1 available online on Science Direct®, www.sciencedirect.com.)

### Proteins

Coronaviruses all share the following structural protein species:

- the spike protein S, a large (1128–1472 aa), homo-trimeric type I membrane glycoprotein. S is a class I fusion protein that mediates receptor-binding and membrane fusion;
- the membrane glycoprotein M, a 218 to 263-aa integral type III membrane protein with predicted triple-spanning $N^{exo}C^{endo}$ topology. Depending on the virus species, the amino-terminal

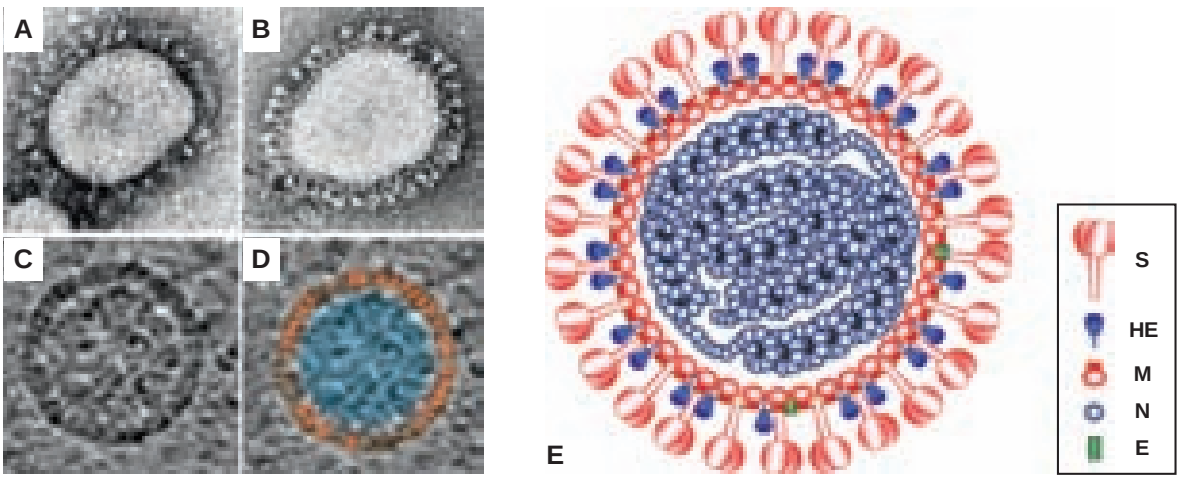


**Figure 3:** Coronavirus virion morphology. (A,B) Negative staining (2% phosphotungstic acid) electron micrographs of murine coronavirus particles. Shown are (A) a virion of murine coronavirus laboratory strain A59 that lacks HE expression and (B) one of a recombinant MHV-59 virus in which HE expression was restored (B) (courtesy Jean Lepault, Laboratory of Molecular and Structural Virology, Gif-sur-Yvette Cedex, France). (C,D) Cryo-electron tomographs of mouse hepatitis virus. A virtual slice (7.5 nm thick) through a reconstructed MHV particle (left) with highlighted features superimposed (right). The envelope is colored in orange with conspicuous striations highlighted; the nucleocapsid region is colored in blue. Note low-density region (ca. 4 nm) between envelope and nucleocapsid (reprinted with permission from Barcéna *et al.* (2008) *Proc. Natl Acad. Sci., U S A*, **106**, 582-587, © 2008 National Academy of Sciences, USA). (E) Schematic representation of a (lineage A) betacoronavirus virion.

ectodomain is decorated with *N*- or *O*-linked glycans. The long C-terminal endodomain, comprising an amphiphilic region and a hydrophilic tail, is believed to associate with the inner leaflet of the membrane to form a matrix-like lattice, which would explain the remarkable thickness of the coronavirus envelope (Figure 3). In transmissible gasteroenteritis virus of swine (TGEV, sp. *Alphacoronavirus 1*), a second population of tetra-spanning M proteins, adopting an $N^{exo}$-$C^{exo}$ topology in the viral envelope, has been described;

- the envelope protein (E), a small (74–109 aa) pentameric integral membrane protein with ion channel and/or membrane permeabilizing (viroporin) activities. With around 20 copies per particle, the E protein is only a minor structural component. Although its precise function remains to be defined, the E protein plays a role in virion assembly and morphogenesis and has been identified as a virulence factor for the severe acute respiratory syndrome-coronavirus (SARS-CoV);
- the nucleocapsid protein N, a 349 to 470 aa RNA-binding phosphoprotein. Besides its obvious function in genome encapsidation, the N protein also is involved in RNA synthesis and translation, displays RNA chaperone activity, and acts as a type I interferon antagonist.

Depending on the coronavirus species, additional accessory proteins may be incorporated into the virion. Group A betacoronaviruses (*Betacoronavirus* 1, *Murine coronavirus* and *Human coronavirus HKU1*) code for an accessory homo-dimeric type I envelope glycoprotein, the hemagglutinin-esterase (HE). It mediates reversible virion attachment to *O*-acetylated sialic acids by acting both as a lectin and as a sialate-*O*-acetylesterase. The coronavirus HE shares about 30% aa sequence identity with the torovirus HE protein and is equally related to subunit 1 of the influenza C virus hemagglutinin-esterase fusion protein (HEF). In SARS-CoV, proteins 3a, 6 and 7 have been described as structural proteins and nsp2 through 5 and nsp9 were all detected in purified virion preparations.

In virions of murine coronavirus, the stoichiometric ratio of N, M and HE proteins is approximately 1:2.6:0.4; in TGEV, N and M occur at a ratio of 1:3. There are no reliable estimates for the S protein as it is present in small quantities in virus particles, may occur both in cleaved and uncleaved forms, and is easily lost during virus purification.

#### Lipids

Coronaviruses acquire their lipid envelopes by budding at membranes of the endoplasmic reticulum, intermediate compartment and/or Golgi complex. The S and E proteins are palmitoylated.

#### Carbohydrates

Coronavirus S and HE proteins are heavily glycosylated and contain multiple *N*-linked glycans (20–35 and 5–11, respectively). The M protein of coronaviruses contains a small number of either *N*- or *O*-linked glycans, depending on the virus species, located near the amino-terminus. Coronavirus E proteins are not glycosylated.

## Genome organization and replication

Coronavirus genomes contain 5′ and 3′ UTRs ranging in size from 200 to 600 and from 200 to 500 nt, respectively. Signals for genome replication and encapsidation reside not only in these UTRs, but also in adjacent and more internal coding regions. Six ORFs are conserved subfamily-wide and arranged in a fixed order: (as listed in the 5′ to-3′ direction) ORFs 1a and 1b, together comprising the replicase gene, and the ORFs for the structural proteins S, E, M and N. Downstream of ORF1b and interspersed between the structural protein genes, there may be up to at least eight accessory (also called "group" or "niche-specific") genes, the products of which are generally dispensable for replication *in vitro*, but key to efficient replication during natural infection (Figure 1).

Apparently, these accessory genes were acquired through horizontal gene transfer and occasionally also lost again as the different coronaviruses evolved and diverged while adapting to new hosts and niches. The diversity of accessory genes, most of which are specific only to a distinct CoV lineage species or strain (see also Figures 5–7), attest to the plasticity and highly dynamic nature of the coronavirus genome.

While the genome serves as an mRNA for the replicase polyproteins, the 3′ proximal genes are expressed from a nested set of sg mRNAs the coding regions of which (the "body" sequences) are

3′-coterminal with the genome. Each of these mRNAs is provided with a short 5′ leader sequence identical to the 5′-terminal end of the genome. Leader and body sequences are not contiguous on the genome (they may in fact be separated by more than 20,000 nts), but become joined in a process of discontinuous minus-strand RNA synthesis (detailed below). Although all except the smallest mRNAs are structurally polycistronic, translation is restricted to the 5′-proximal ORF(s) not present in the next smaller mRNA of the set (Figure 1).

On the genome, each transcription unit (one or more ORFs expressed from a single RNA species) is preceded by a short conserved sequence element, commonly called the transcription-regulating sequence (TRS). A TRS copy is also found at the 5′ end of the genome, immediately downstream of the leader sequence. According to the prevailing model for transcription, leader–body fusion occurs during the synthesis of genome-templated sg minus-strand RNAs by 3′-discontinuous extension via a mechanism resembling homology-assisted RNA recombination. This process apparently is driven by sequence complementarity between the anti-TRS at the 3′ end of the nascent minus-strand and the 5′ genomic TRS (Figure 4). In support of this model, the production of a 5′-terminal nested set of transcriptionally-active sg minus-strand RNAs with a 3′-terminal anti-leader sequence (in effect a mirror copy set of the mRNAs) has been demonstrated in coronavirus-infected cells. It is believed that each mRNA is transcribed from its corresponding sg minus-strand RNA template via a process of "continuous" RNA synthesis. For more information about other aspects of coronavirus replication, please see the preceding paragraphs and Chapter *Nidovirales*.

## Antigenic properties

Cross-reactivity among coronaviruses is limited to (closely-related) species within the same genus. The S protein is the major inducer of virus-neutralizing antibodies that are elicited mainly by epitopes in the amino terminal half of the molecule. The surface-exposed amino-terminus of the M protein induces antibodies that neutralize virus infectivity in the presence of complement, while the HE protein of group A betacoronaviruses induces antibodies that prevent virion binding

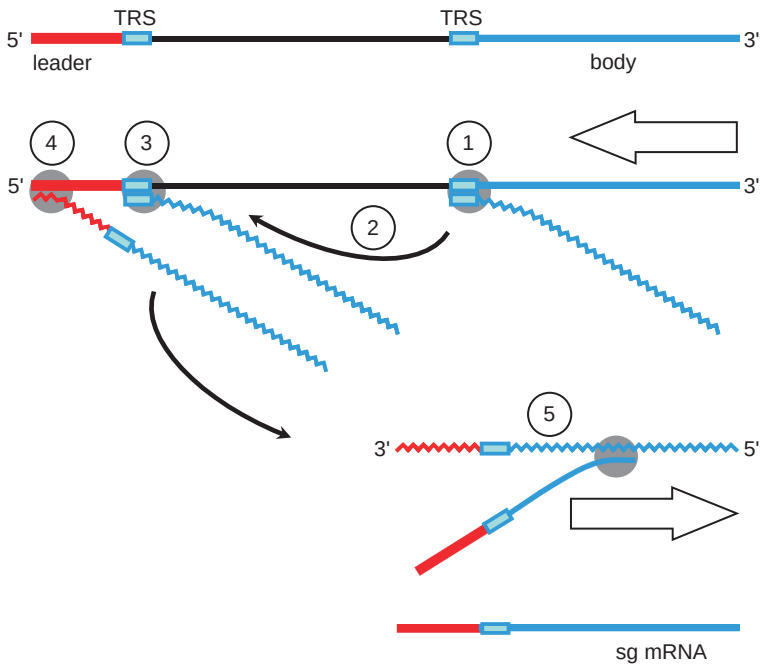


**Figure 4:** Coronavirus mRNA synthesis: the discontinuous 3′-extension model. Minus-strand synthesis initiates at the 3′ end of the genome and proceeds until a TRS is copied (1). The nascent minus-strand RNA may then be transferred to the 5′ end of the genome (2). Base complementarity allows the minus-strand RNA to anneal to the leader TRS (3) after which RNA synthesis resumes and body (in blue) and leader sequences (in red) become fused (4). The chimeric sg minus-strand RNA in turn serves as a template for "continuous" synthesis of sg mRNAs (5).

to *O*-acetylated sialic acids or inhibit sialate-*O*-acetylesterase activity. The N protein is a dominant antigen during the natural infection and while N-specific antibodies may provide little immune protection, they are of serodiagnostic relevance.

The ectodomains of the S and HE proteins are highly variable, suggestive of extensive antigenic drift. There are also indications for the occurrence of antigenic shifts as there are several examples of intra- and possibly interspecies exchange through RNA recombination of coding sequences of S (for *Avian coronavirus*, *Murine coronavirus* and the *Alphacoronavirus 1* subspecies feline and canine coronavirus) and HE ectodomains (*Murine coronavirus*) sometimes with as yet unidentified coronaviruses serving as donors. Studies performed with murine and feline coronaviruses indicate that both structural and non-structural (replicase) proteins serve as $CD4^+$ and $CD8^+$ T cell antigens.

There is no serologic cross-reactivity between corona-, toro- and bafiniviruses.

## Biological properties

Coronaviruses infect birds and mammals and include several pathogens of clinical, veterinary and economic interest. Transmission is not by biological vectors, but – depending on the virus species – via fomites or via aerogenic and/or fecal–oral routes. As CoVs primarily target epithelial cells, they are generally associated with gastrointestinal and respiratory infections that may be acute or become chronic with prolonged shedding of virus. In general, these infections are mild and often asymptomatic. Some coronaviruses, however, cause severe, even lethal disease. Murine coronavirus (genus *Betacoronavirus*) may cause hepatitis and severe neurologic infection, resulting in paralysis and demyelination, providing a rodent model for the study of the neuropathogenesis of human multiple sclerosis. Some members of the species *Alphacoronavirus 1* (feline, canine and ferret coronavirus) cause fatal immune-mediated systemic infections in their respective hosts, presumably through the infection of cells of the macrophage/monocyte lineage, with widespread inflammatory lesions in multiple organs. The human coronaviruses that were identified early on (*Betacoronavirus-1* subspecies HCoV-OC43 and *Alphacoronavirus* HCoV-229E) mostly cause common colds and have long been considered of modest clinical importance. It is now recognized that these viruses may also cause severe lower respiratory tract infections (LRTI) in infants and elderly, and apparently are responsible for about 5% of infant hospitalizations from LRTI, globally.

In 2002–2003, a previously unknown coronavirus, SARS-CoV, caused an epidemic in human populations of a severe pulmonary disease with a mortality rate of 10% that rapidly spread to four continents, infecting 8,096 individuals and claiming 774 victims before it was contained. Epidemiological evidence indicates that this novel human virus originated in bats, spread to Himalayan palm civets, Chinese ferret badgers and raccoon dogs at the wet markets of Guangdong, China, to enter the human population through handling or consumption of these exotic species. Although SARS has since vanished, the episode does underline the pathogenic potential of coronaviruses and the possibility of novel emerging coronavirus infections arising from cross-species transmissions. Similar incidents, though with a less dramatic outcome, seem to have given rise to human coronavirus OC43 (a single cross-species transmission of bovine coronavirus from cattle to humans), to human coronavirus 229E (transmitted from bats?) and, more recently, to canine respiratory coronavirus (transmission of bovine coronavirus to dogs). In the wake of the SARS epidemic, molecular surveillance and virus discovery studies have yielded evidence for at least 60 novel coronaviruses among which are two new human respiratory coronaviruses, HCoV-HKU1 and HCoV-NL63. The latter is considered an important cause of (pseudo)croup and bronchiolitis in children. These studies also revealed a new lineage of predominantly avian viruses (Thrush, Bulbul and Munia coronavirus), with possible relatives in mammals (Asian leopard cat, Chinese ferret badger), that on the basis of rooted phylogeny appear to belong to a new genus (Figure 2). Bats harbor an exceptionally wide diversity of coronaviruses and have been proposed to play a vital role in coronavirus ecology and evolution, maybe even as the original hosts from which many if not all alpha- and betacoronavirus lineages were derived. Bat population densities and their roosting and migration habits would all favor such a role. Although this hypothesis has its merits and the recent virus discovery studies that prompted this view have been of truly Herculean proportions, it is of note that the actual coronavirus sampling size remains in fact limited and as efforts so far focused mainly on bats, our present perceptions may be biased. Further surveillance studies of similar extent must be performed in other host species (rodents, birds) before final conclusions can be drawn.

# Genus *Alphacoronavirus*

Type species ***Alphacoronavirus 1***

## Distinguishing features

The viruses in this genus form a distinct monophyletic group within the *Coronavirinae* subfamily. Apart from their relatively close phylogenetic relationship, the only general characteristics that would set them apart from other coronaviruses are (i) a unique type of nsp1, distinct in size and sequence from betacoronavirus nsp1 and without apparent counterpart in the gammacoronaviruses, and (ii) the presence of a commonly-shared accessory gene (designated ORF3 in most alphacoronavirus species, ORF3b and 3c in TGEV and in FCoV/CCoV, respectively) for a dispensable multi-spanning alphacoronavirus membrane protein (αmp). While for some alphacoronaviruses, αmp is the only accessory protein, others may carry up to at least six accessory genes (e.g. members of the subspecies canine coronavirus in the species *Alphacoronavirus 1*; note that "subspecies" is not an officially recognized level in virus taxonomy; the term is used here and throughout this chapter to indicate well-defined monophyletic groups of viruses within a coronavirus species that are genetically and biologically distinct from other members of the same species). A comparison of the genome organization of alphacoronaviruses is presented in Figure 5.

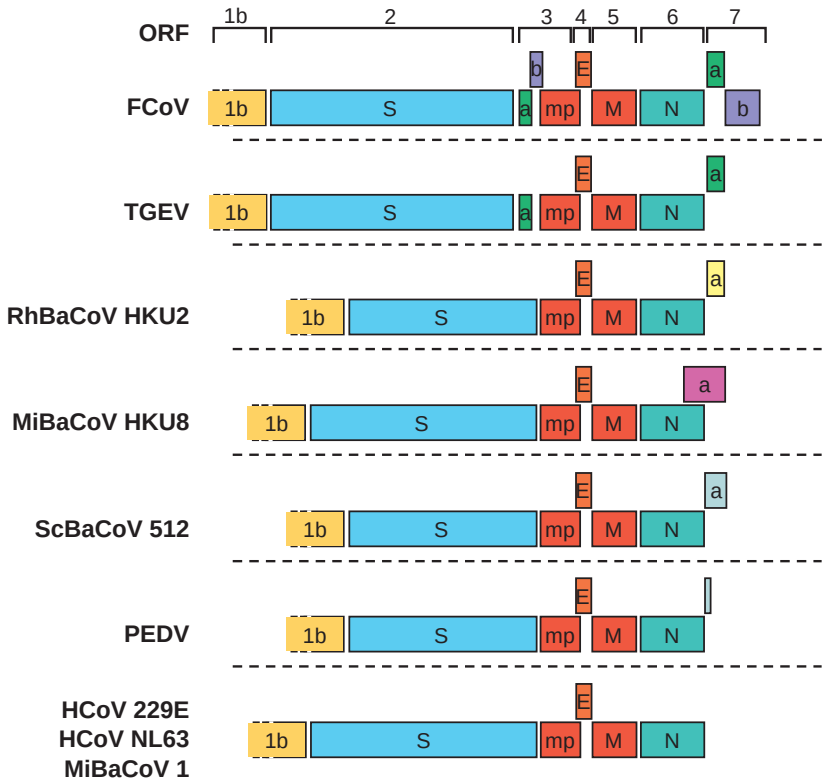


**Figure 5:** Alphacoronavirus genome organization. Comparison of the 3′-terminal genomic regions downstream of ORF1b of alphacoronaviruses representative of the different species and subspecies. ORFs are depicted as coloured boxes and indicated by number (above) and encoded protein. ORFs for accessory proteins are named as by convention according to number (referring to the mRNA species from which they are expressed) and, in the case of multiple ORFs in one transcription unit, alphabetically. Conservation of genes is indicated by identical colouring. Accessory genes of different viruses that are located in the same genomic location but believed to encode non-related products are coloured differently. For the abbreviations of virus names, please see list of species in the genus *Alphacoronavirus* below. 1b, ORF1b; mp, alphacoronavirus-specific accessory membrane protein αmp; all other acronyms as in Figure 1.

Pos. ssRNA

## List of species in the genus *Alphacoronavirus*

| | | |
|---|---|---|
| *Alphacoronavirus 1* | | |
| Canine coronavirus | | |
| Canine coronavirus type I | | |
| Canine coronavirus strain Elmo/02 | [AY426983] | (CCoV Elmo/02) |
| Canine coronavirus type II | | |
| Canine coronavirus strain NTU336/F/2008 | [GQ477367] | (CCoV NTU336/F/2008) |
| Feline coronavirus | | |
| Feline coronavirus type I | | |
| Feline coronavirus C1Je | [DQ848678] | (FCoV C1Je) |
| Feline coronavirus type II | | |
| Feline infectious peritonitis virus WSU 79-1146 | [AY994055] | (FIPV 79-1146) |
| Porcine respiratory coronavirus | | |
| Porcine respiratory coronavirus ISU-1 | [DQ811787] | (PRCV ISU-1) |
| Transmissible gastroenteritis virus | | |
| Transmissible gastroenteritis virus virulent Purdue | [AJ271965] | (TGEV virulent Purdue) |
| *Human coronavirus 229E* | | |
| Human coronavirus 229E | [AF304460 = NC_002645] | (HCoV 229E) |
| *Human coronavirus NL63* | | |
| Human coronavirus NL63 Amsterdam 1 | [AY567487] | (HCoV NL63) |
| *Miniopterus bat coronavirus 1* | | |
| Miniopterus bat coronavirus 1A | | |
| Miniopterus bat coronavirus 1A AFCD62 | [EU420138 = NC_010437] | (Mi-BatCoV 1A AFCD62) |
| Miniopterus bat coronavirus 1B | | |
| Miniopterus bat coronavirus 1B AFCD307 | [EU420137 = NC_010436] | (Mi-BatCoV 1B AFCD307) |
| *Miniopterus bat coronavirus HKU8* | | |
| Miniopterus bat coronavirus HKU8 AFCD77/08/05 Mm | [EU420139 = NC_010438] | (Mi-BatCoV HKU8 AFCD77/08/05 Mm) |
| *Porcine epidemic diarrhea virus* | | |
| Porcine epidemic diarrhea virus CV777 | [AF353511 = NC_003436] | (PEDV CV777) |
| *Rhinolophus bat coronavirus HKU2* | | |
| Rhinolophus bat coronavirus HKU2/HK/46/2006 | [EF203065] | (Rh-BatCoV HKU2/HK/46/2006) |
| *Scotophilus bat coronavirus 512* | | |
| Scotophilus bat coronavirus 512/2005 | [DQ648858 = NC_009657] | (Sc-BatCoV 512/2005) |

Species names are in italic script; names of subspecies and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Alphacoronavirus* but have not been approved as species

| | | |
|---|---|---|
| Carollia bat coronavirus 1FY2BA/Trinidad/2007 | | (Ca-BatCoV 1FY2BA/Trinidad/2007) |
| Chaerophon bat coronavirus 40/Kenya/2006 | | (Ch-BatCoV 40/Kenya/2006) |
| Chaerophon bat coronavirus 22/Kenya/2006 | | (Ch-BatCoV 22/Kenya/2006) |
| Chinese ferret badger coronavirus DM95/03 | [EU769560] | (CFBCoV DM95/03) |
| Eptesicus bat coronavirus 65/RM/2006 | [EF544566] | (Ep-BatCoV 65/RM/2006) |
| Ferret coronavirus | [GU338456; GU338457] | (FerCoV) |
| Glossophaga bat coronavirus 1CO7BA/Trinidad/2007 | | (Gl-BatCoV 1CO7BA/Trinidad/2007) |
| Kenya bat coronavirus BtKY12 | [GQ920811] | (BatCoV BtKY12) |
| Kenya bat coronavirus BtKY21 | [GQ920819] | (BatCoV BtKY21) |
| Myotis bat coronavirus HKU6/HK/21/2005 | [DQ249224; DQ249247] | (My-BatCoV HKU6/HK/21/2005) |
| Myotis bat coronavirus D2.2/Germany/2007 | | (My-BatCoV D2.2/Germany/2007) |
| Myotis bat coronavirus D8.38/Germany/2007 | | (My-BatCoV D8.38/Germany/2007) |
| Myotis bat coronavirus 3/RM/2006 | [EF544567] | (My-BatCoV 3/RM/2006) |
| Myotis bat coronavirus 48/RM/2006 | [EF544565] | (My-BatCoV 48/RM/2006) |

| | | |
|---|---|---|
| Myotis Bat coronavirus M.mac/Australia/CoV034/2008 | [EU834951] | (My-BatCoV M.mac/Aus/CoV034/2008) |
| Miniopterus bat coronavirus 088/Australia/2007 | [EU834952] | (Mi-BatCoV 088/Australia/2007) |
| Miniopterus bat coronavirus HKU7/HK/13/2005 | [DQ249226; DQ249249] | (Mi-BatCoV HKU7/HK/13/2005) |
| Nyctalus bat coronavirus VM366/NLD/2008 | | (Ny-BatCoV VM366/NLD/2008) |
| Pipistrellus bat coronavirus D5.16/Germany/2007 | | (Pi-BatCoV D5.16/Germany/2007) |
| Pipistrellus bat coronavirus D5.71/Germany/2007 | | (Pi-BatCoV D5.71/Germany/2007) |
| Pipistrellus bat coronavirus VM312/NLD/2008 | | (Pi-BatCoV VM312/NLD/2008) |
| Raccoon dog coronavirus GZ43/03 | [EU769559; EF192159] | (RDCoV GZ43/03) |
| Rhinolophus bat coronavirus A970/SD/2005 | | Rh-BatCoV A970/SD/2005 |
| Rousettus bat coronavirus HKU10/GD/183/2005 | | (Ro-BatCoV HKU10/GD/183/2005) |
| Yellow-bellied weasel coronavirus GX/D726/2005 | [ABQ39953.1 \| ] | (YWCoV GX/D726/05) |

# Genus *Betacoronavirus*

Type species ***Murine coronavirus***

## Distinguishing features

Betacoronaviruses form a distinct monophyletic group in the *Coronavirinae* subfamily. Except for their relatively close phylogenetic relationship, the only known general characteristic that would set them apart from other coronaviruses is their unique nsp1, distinct in size and sequence from alphacoronavirus nsp1 and without obvious counterpart in the gammacoronaviruses. Four betacoronavirus lineages can be distinguished (A through D; Figure 2) each with a unique set of accessory genes (Figure 6).

## List of species in the genus *Betacoronavirus*

| | | |
|---|---|---|
| *Betacoronavirus 1* | | |
| Bovine coronavirus | | |
| Bovine coronavirus Mebus | [U00735] | (BCoV Mebus) |
| Equine coronavirus | | |
| Equine coronavirus NC99 | [EF446615 = NC_010327] | (ECoV NC99) |
| Human coronavirus OC43 | | |
| Human coronavirus OC43 ATCC VR-759 | [AY585228] | (HCoV OC43 ATCC VR-759) |
| Porcine hemagglutinating encephalomyelitis virus | | |
| Porcine hemagglutinating encephalomyelitis virus VW572 | [DQ011855 = NC_007732] | (PHEV VW572) |
| *Human coronavirus HKU1* | | |
| Human coronavirus HKU1 N1 | [AY597011 = NC_006577] | (HCoV HKU1 N1) |
| *Murine coronavirus* | | |
| Mouse hepatitis virus | | |
| Murine hepatitis virus JHM | [NC_006852] | (MHV JHM) |
| Rat coronavirus (Rat sialodacryoadenitis coronavirus) | | |
| Rat coronavirus Parker | [FJ938068] | (RCoV Parker) |
| *Pipistrellus bat coronavirus HKU5* | | |
| Pipistrellus bat coronavirus HKU5/HK/03/2005 | [EF065509 = NC_009020] | (Pi-BatCoV HKU5/HK/03/2005) |
| *Rousettus bat coronavirus HKU9* | | |
| Rousettus bat coronavirus HKU9/GD/005/2005 | [EF065513 = NC_009021] | (Ro-BatCoV HKU9/GD/005/2005) |
| *Severe acute respiratory syndrome-related coronavirus* | | |
| SARS-related human coronavirus | | |
| SARS-related human coronavirus Urbani | [AY278741] | (SARS CoV Urbani) |
| SARS-related Rhinolophus bat coronavirus RF1 | | |

Pos. ssRNA

| | | |
|---|---|---|
| SARS-related Rhinolophus bat coronavirus Rf1/2004 | [DQ412042 = NC_009695] | (SARSr-Rh-BatCoV RF1/2004) |
| SARS-related Rhinolophus bat coronavirus Rm1 | | |
| SARS-related Rhinolophus bat coronavirus Rm1/2005 | [DQ412043 = NC_009696] | (SARSr-Rh-BatCoV Rm1/2005) |
| SARS-related Rhinolophus bat coronavirus Rp3 | | |
| SARS-related Rhinolophus bat coronavirus Rp3/2004 | [DQ071615 = NC_009693] | (SARSr-Rh-BatCoV Rp3/2004) |
| SARS-related Rhinolophus bat coronavirus HKU3 | | |
| SARS-related Rhinolophus bat coronavirus HKU3/HK/24/2005 | [DQ022305 = NC_009694] | (SARSr-Rh-BatCoV HKU3/HK/24/2005) |
| SARS-related palm civet coronavirus | | |
| SARS-related palm civet coronavirus SZ3/2003 | [AY304486] | (SARSr-CiCoV SZ3/2003) |
| SARS-related chinese ferret badger coronavirus | | |
| SARS-related chinese ferret badger coronavirus CFB/SZ/94/03 | [AY545919] | (SARSr CoV CFB/SZ/94/03) |
| *Tylonycteris bat coronavirus HKU4* | | |
| Tylonycteris bat coronavirus HKU4/HK/04/2005 | [EF065505 = NC_009019] | (Ty-BatCoV HKU4/HK/04/2005) |

Species names are in italic script; names of subspecies and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

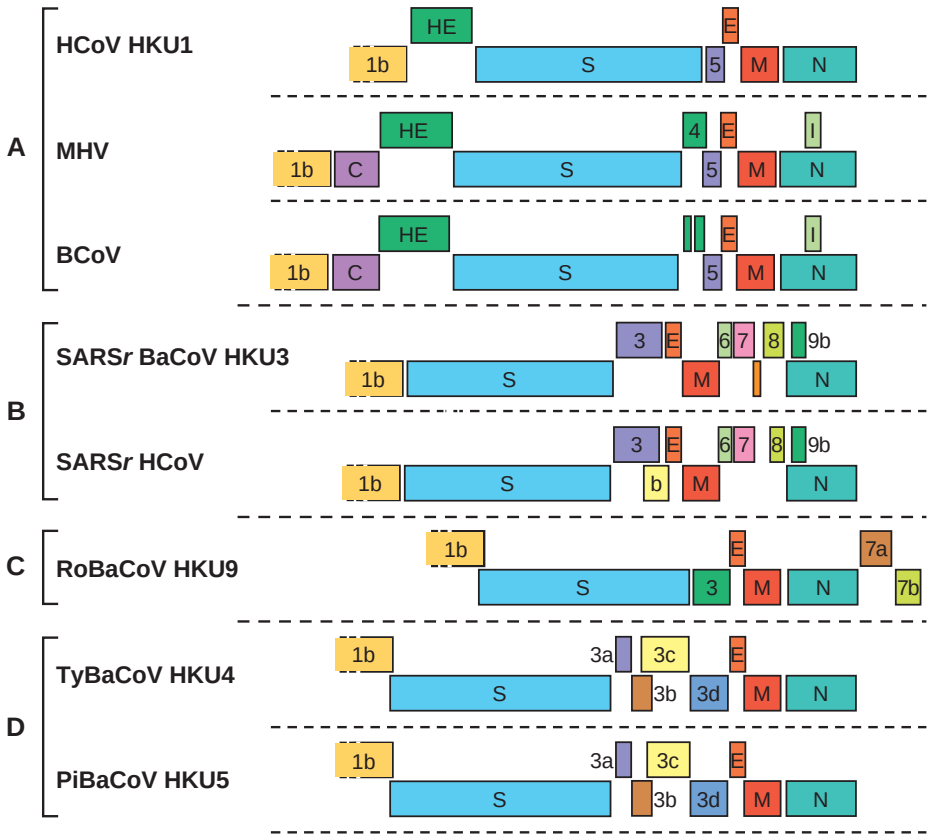


**Figure 6:** Betacoronavirus genome organization. Comparison of the 3′-terminal genomic regions downstream of ORF1b of betacoronaviruses representative for the different species and subspecies. ORFs are depicted as boxes, color-coded and indicated by number and gene product as in Figure 5. For the abbreviations of virus names, please see the list of species in the genus *Betacoronavirus*.

### List of other related viruses which may be members of the genus *Betacoronavirus* but have not been approved as species

| | | |
|---|---|---|
| Rhinonicteris bat coronavirus 000/Australia/2006 | [EU834950] | (Rh-BatCoV 000/Australia/2006) |

## GENUS *GAMMACORONAVIRUS*

Type species *Avian coronavirus*

### Distinguishing features

Gammacoronaviruses form a distinct monophyletic group in the *Coronavirinae* subfamily. Except for their relatively close phylogenetic relationship, there are no known common characteristics in terms of virion morphology, genome organization and gene composition, replication or biology that would set them apart from other coronaviruses. Viruses of the species *Avian coronavirus* lack an nsp1 moiety. Whether this is also the case for members of the other gammacoronavirus species, *Beluga whale coronavirus*, remains to be determined. For the genome organization of gammacoronaviruses see Figure 7.

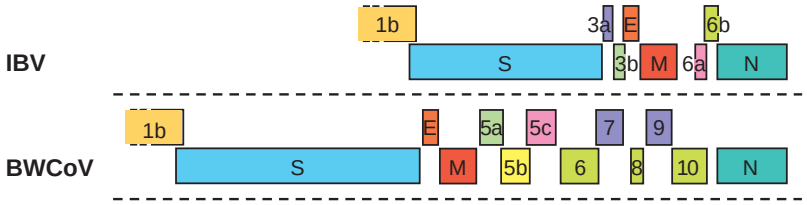


**Figure 7:** Gammacoronavirus genome organization. Comparison of the 3′-terminal genomic regions downstream of ORF1b of infectious bronchitis virus (IBV; sp. *Avian coronavirus*) and Beluga whale coronavirus SW1 (BWCoV). ORFs are depicted as boxes, colour-coded and indicated by number and gene product as in Figures 5 and 6.

### List of species in the genus *Gammacoronavirus*

| | | |
|---|---|---|
| *Avian coronavirus* | | |
| Infectious bronchitis virus | | |
| Infectious bronchitis virus Beaudette | [M95169 = NC_001451] | (IBV Beaudette) |
| Turkey coronavirus | | |
| Turkey coronavirus ATCC | [EU022526] | (TCoV ATCC) |
| *Beluga whale coronavirus SW1* | | |
| Beluga whale coronavirus SW1 | [EU111742 = NC_010646] | (BWCoV SW1) |

Species names are in italic script; names of subspecies and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Gammacoronavirus* but have not been approved as species

| | | |
|---|---|---|
| Duck coronavirus | [AJ854130] | (DCoV) |
| Goose coronavirus | [AJ854145-AJ854159] | (GCoV) |
| Pigeon coronavirus | [AJ854131] | (PCoV) |

### List of unassigned species in the subfamily *Coronavirinae*

None reported.

### List of other related viruses which may be members of the subfamily *Coronavirinae* but have not been approved as species

| | | |
|---|---|---|
| Asian leopard cat coronavirus | [EF584908] | (ALCCoV) |
| Bulbul coronavirus HKU11 | [FJ376619] | (BuCoV HKU11) |

Pos. ssRNA

| | | |
|---|---|---|
| Chinese ferret badger coronavirus GX/247/06 | [ABQ39964] | (CFBCoV GX/247/06) |
| Munia coronavirus HKU13 | [FJ376622 = NC_011550] | (MunCoV HKU13) |
| Parrot coronavirus AV71/99 | [ABB45386] | (PaCoV) |
| Thrush coronavirus HKU12 | [FJ376621 = NC_011549] | (ThCoV HKU12) |

# Subfamily *Torovirinae*

## Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Torovirinae*** |
| Genus | ***Torovirus*** |
| Genus | ***Bafinivirus*** |

## Distinguishing features

The members of the bigeneric subfamily *Torovirinae* (family *Coronaviridae*) form a distinct monophyletic cluster that is well-separated from the "true" coronaviruses united in the subfamily *Coronavirinae* (for a phylogram depicting the relationships among the *Coronaviridae*, see Chapter *Nidovirales*, Figure 4). Apart from their relatively close phylogenetic relationship, bafini- and toroviruses can be distinguished from their closest relatives, the true coronaviruses, by the following common features:

- Bacilliform virion particles
- Tubular nucleocapsids of presumably helical symmetry
- Small nucleocapsid proteins (ca. 160 aa), less than half the size of that of the coronavirus N protein
- The apparent lack of an equivalent of coronavirus envelope protein E
- A relatively simple genome organization (no accessory genes with the debatable exception of the torovirus HE gene) and exceptionally long (>800 nt) 5′-terminal UTRs.

Toro- and bafiniviruses can be distinguished from each other by:

- their transcription mechanism (detailed below)
- an ORF1a-encoded cyclic nucleotide phosphodiesterase domain (CPD) unique to toroviruses
- the HE gene, present only in toroviruses.

Remarkably, in coronaviruses, related CPD and HE proteins have been identified, but exclusively in one subset of betacoronaviruses (group A) and in completely different genome locations; here, the CPD protein is encoded by an accessory gene, located immediately downstream of ORF1b (see Figure 6). The torovirus CPD and HE coding sequences are believed to have been acquired by horizontal gene transfer from as yet unknown donors presumably after the toro-bafinivirus split.

# Genus *Torovirus*

Type species ***Equine torovirus***

## Virion properties

### Morphology

In conventional negative-staining electron micrographs, toroviruses appear as a mixture of spherical, rod- and kidney-shaped particles (Figure 8A). Native torovirus particles presumably are bacilliform with rounded ends, measuring 100–140 nm in length and 35–42 nm in width (envelope outer rim). Virions carry two types of surface projections that in size and shape resemble those of the (beta) coronaviruses: multimers of the S protein comprising large 15-20 nm peplomers and homo-dimers of the HE protein forming an inner ring of smaller (5-7 nm) spikes. The most distinctive virion element, the core, is a flexible and seemingly hollow tube of helical symmetry (periodicity ca. 4.5 nm),

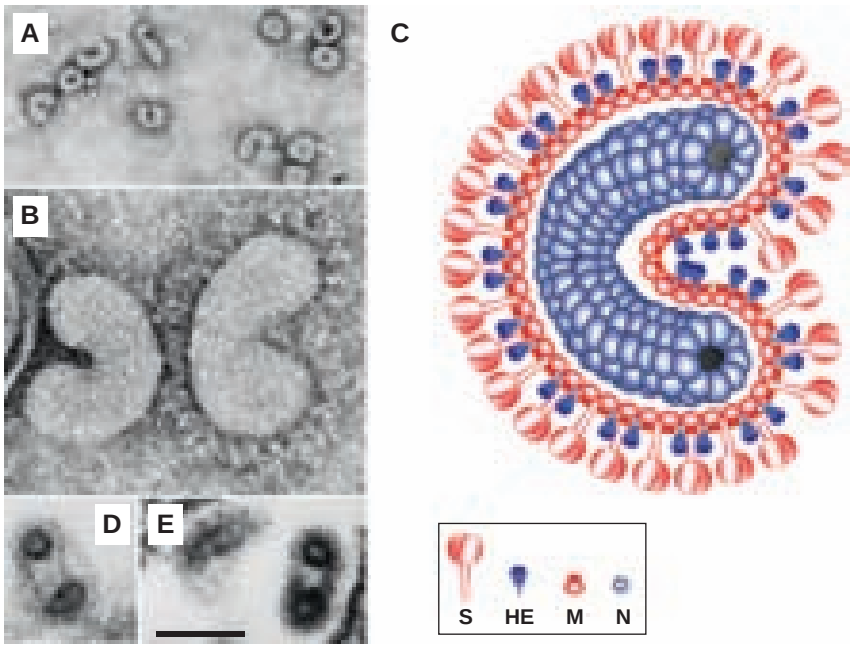


**Figure 8:** Virion morphology of equine torovirus Berne. (A) Negative staining electron micrograph of extracellular EToV particles (2% phosphotungstic acid). (B) Close-up of negatively-stained EToV virions. (Courtesy of Dolores Rodriguez Aguirre, Department of Molecular and Cell Biology, National Centre of Biotechnology, Madrid, Spain.) Note that in EToV strain Berne, the HE gene is inactivated and that virions consequently display only one type of spike, the peplomers comprised of the S protein. (C) Schematic representation of a torovirus virion. (D and E) Cross-sections of intracellular EToV virions showing the tubular nucleocapsid with central cavity and the viral envelope. The bar represents 100 nm.

about 100 nm in length and about 23 nm across with a central channel of about 10 nm in diameter. In crescent-shaped and spherical (disk-shaped?) particles, the nucleocapsid is bent into an open toroid, from which the name "torovirus" was derived (Figure 8).

### Physicochemical and physical properties

Virions have a buoyant density in sucrose of 1.14–1.18 g cm$^{-3}$. Particles are sensitive to heat, lipid solvents, non-ionic detergents, formaldehyde, oxidizing agents and UV irradiation, but highly resistant to bile salts (0.1% deoxycholate) and extreme pH conditions (infectivity not affected by exposure to pH values between 2.5 and 10.3).

### Nucleic acid

The torovirus genome is a positive-stranded, capped and polyadenylated RNA molecule of about 28 kb in length that is infectious when transfected into mammalian cells. At present, complete genomes are available only for bovine torovirus (BToV) strain Breda and equine torovirus (EToV) strain Berne. Partial sequences, mostly for the genes for the structural proteins, are available for various Eurasian BToV and porcine torovirus (PToV) field strains.

### Proteins

Virions of torovirus field strains contain the following protein species: (i) the spike protein S, a large (1562-1584 aa), presumably trimeric envelope glycoprotein with features typical for class I fusion proteins (bioinformatical analysis revealed heptad repeat regions and a putative fusion peptide); (ii) the hemagglutinin-esterase protein HE, a homo-dimeric 416 to 430 aa type I membrane glycoprotein that mediates reversible virion attachment to *O*-acetylated sialic acids by acting both as a lectin and as a receptor-destroying enzyme; (iii) the membrane protein M, a highly conserved 233-aa nonglycosylated integral membrane protein with three predicted transmembrane regions and a $N^{exo}C^{endo}$ topology; (iv) the nucleocapsid protein N, a 159 to 167-aa basic RNA-binding phosphoprotein. The HE protein is dispensable for replication *in vitro* and its expression has been lost in EToV strain Berne presumably as a result of adaptation to replication in cultured cells.

### Lipids

Torovirus virions acquire their lipid envelope by budding at smooth intracellular membranes of ER and Golgi complex.

### Carbohydrates

Torovirus S and HE proteins carry multiple *N*-glycans (19–25 and 7–13, respectively).

## Genome organization and replication

During natural infection, toroviruses presumably attach to their host cells by binding to 9-mono-*O*- (PToV) or 7,9-di-*O*-acetylated sialic acids (BToV) via their HE protein. Entry, however, would require the S protein to bind to a main receptor, most likely a specific glycoprotein, and to mediate fusion between the viral envelope and a cellular membrane. Whether entry occurs at the plasma membrane or via endocytosis is not known nor has any torovirus main receptor been identified so far.

The viral genome contains 5′ and 3′ UTRs of 821–857 and 200 nt, respectively, and six ORFs called ORF1a and 1b (together comprising the replicase gene), -2, -3, -4 and -5, the latter four encoding the structural proteins (S, M, HE and N, respectively; Figure 9). Signals for EToV genome replication and possibly also for encapsidation apparently reside in the 5′-terminal 604 and 3′-terminal 200 residues as suggested by studies with defective interfering RNAs.

Replication is believed to occur largely as described for coronaviruses with the genes for the structural proteins being expressed from a nested set of four sg mRNAs (designated (m)RNAs 2 through 5) that are 3′-coterminal with the genome (RNA 1). However, in striking contrast to corona- and bafiniviruses, toroviruses employ a mixed transcription strategy and combine discontinuous and continuous RNA synthesis to produce their complement of mRNAs. mRNAs 3, 4 and 5 lack a common leader sequence and are fully co-linear with the viral genome. The genes for M, HE and N, expressed from these RNAs, are each preceded by a conserved 13–14 nt sequence element, conforming to consensus (C)ACN$_{3-4}$CUUUAGA, a copy of which (but without the 5′-terminal C residue) is also found at the extreme 5′ end of the genome. This sequence element is thought to act as a premature termination signal of genome-templated minus-strand synthesis and, in the resulting sg minus-strand RNAs, as a promoter for mRNA synthesis with transcription initiating at the 5′-most adenine residue. The S gene lacks such an internal putative terminator/promoter (TP) element and apparently is expressed via a process similar to, yet distinct from, discontinuous RNA synthesis in coronaviruses. Its mRNA (mRNA 2) is the only sg mRNA species to carry a short 15–18 nt leader identical to the genomic 5′ end (i.e. the genomic TP). A conserved hairpin structure in ORF1b is believed to attenuate minus-strand synthesis to allow a subsequent similarity-assisted template switching event facilitated by sequence complementarity between the 3′ end of the nascent minus strand RNA and residues 16 through 38 of the genome that are located immediately downstream of the 5′-terminal genomic TP copy. This would result in a chimeric sg minus-strand RNA that, because of its acquisition of a complementary copy of the genomic TP, can serve as template to direct 'continuous' synthesis of mRNA 2 (Figure 9).

Limited information is available about torovirus morphogenesis. The M and S proteins are produced in the endoplasmic reticulum and largely retained in premedial-Golgi compartments. Assembly of torovirus virions occurs through budding of preformed nucleocapsids at smooth membranes consistent with the ER-to-Golgi intermediate compartment and/or Golgi complex. Mature particles egress by exocytosis.

## Antigenic properties

EToV, PToV and BToV are serologically related. During natural infection, antibodies arise against each of the four structural proteins (S, HE, M and N). The spike (S) protein induces virus-neutralizing antibodies; sera from BToV- or PToV-infected animals cross-neutralize EToV.

## Biological properties

So far, torovirus infection has been conclusively demonstrated only in ungulates: horse (EToV), bovine (BToV) and swine (PToV). Evidence is based on classical virological studies (isolation and

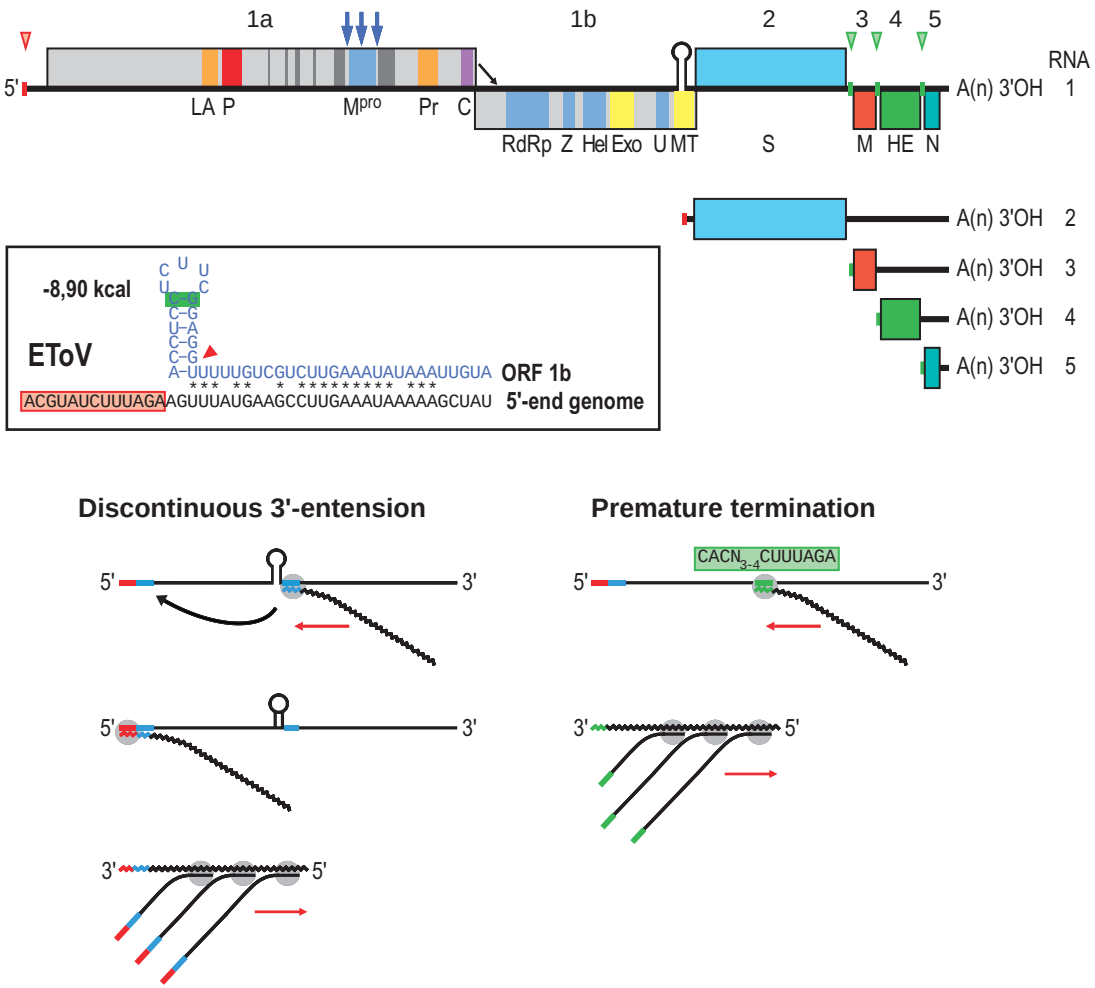


**Figure 9:** Organization and expression of the torovirus genome. (Upper panel) Schematic representation of the equine torovirus genome. ORFs and other genome elements are indicated as in Figure 1. The 5′-leader sequence present in the genome and sg mRNA 2 is depicted by a small red box. Green and red arrowheads/boxes indicate the locations of the internal and the 5′-terminal putative terminator/promoter (TP) elements, respectively. Blue arrows indicate established $M^{pro}$ cleavage sites. The location of the discontinuous transcription element (DTE) driving mRNA 2 synthesis is shown by a hairpin. PL, papain-like proteinase; C, torovirus-specific ORF1a-encoded cyclic nucleotide phosphodiesterase domain. All other acronyms as in Figure 1. (inset) Structure of the mRNA 2 discontinuous transcription element, showing the hairpin structure and downstream "homology region" with sequence identity to the 5′ end of the genome indicated by asterisks. A hairpin residue-pair displaying co-variation in BToV and PToV is highlighted in green. The site of mRNA 2 leader–body fusion is indicated by an arrowhead. The 5′-terminal genomic TP copy is highlighted by a red box. (Lower panel) Models for discontinuous (left) and non-discontinuous sg RNA synthesis (right) in toroviruses. The hairpin indicates the mRNA 2 DTE. Red boxes correspond to the 5′-terminal genomic TP copy and complementary sequences, blue boxes to the DTE homology region and the corresponding 5′ genomic acceptor sequence. The TP consensus sequence is shown and highlighted by a green box. Internal TPs and complementary sequences are shown in green. The models show (from top to bottom) synthesis of genome-templated minus-strand RNA (minus-strand RNAs indicated by a wiggly line), attenuation and 3′-discontinuous extension directed by the DTE element and premature termination directed by internal TPs, and subsequent mRNA synthesis from sg minus-strand templates. Details are described in the text.

propagation *in vitro* of EToV and BToV strains; experimental infection of cattle with BToV Breda), serology and molecular genetic analysis (RT-PCR amplification of torovirus sequences from fecal samples of infected animals). There is serological evidence for torovirus infections also in goats and sheep. Among the non-ungulate species that have been proposed as potential hosts for toroviruses are human (HToV), turkey and carnivores, including dog, cat, mustelids, but these claims are supported only by EM detection of torovirus-like particles and/or limited genetic analysis and would require further experimental confirmation.

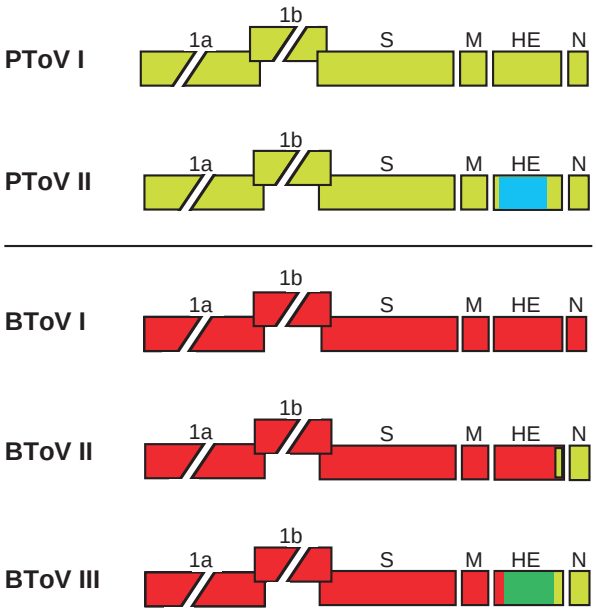


**Figure 10:** Genome organization of torovirus genotypes reveals evidence for multiple interspecies RNA recombination events. The genes for the replicase polyproteins (1a, 1b) and for the structural proteins S, M, HE, and N are depicted as boxes. For PToV, HE coding sequences are shown in different colors to indicate that one of the genotypes exchanged part of its HE gene through homologous RNA recombination with an as yet unknown torovirus. For BToV, sequences acquired from PToV (in yellow) and from a hitherto unidentified torovirus donor (in green) are also indicated.

Toroviruses of horses, swine and cattle have a world-wide distribution and are evidently ubiquitous as seroprevalence in host populations may exceed 80%. Transmission is probably via the oral/nasal route through contact with contaminated feces or nasopharyngeal secretions. Infected animals shed virus in the feces; in the case of BToV, nasal shedding has also been reported. Toroviruses are likely to cause both acute and chonic infections. Equine and porcine toroviruses are associated with asymptomatic enteric infections and remain viruses in search of a disease. Bovine torovirus, an established respiratory and enteric pathogen of cattle, may cause mild to profuse diarrhoea. The virus, originally designated Breda virus, was first isolated during an outbreak of severe neonatal gastroenteritis with 56.5% morbidity and 8.7% mortality in cattle from dairy farms round the township Breda, Iowa, and duly identified as the etiological agent. In experimentally-infected animals, BToV infects the epithelial cells lining the small and large intestine, with progression from areas of the mid jejunum down to the ileum and colon. Within the small intestine, cells of the upper third of the crypt and the epithelium overlying the Peyer's patches, including M cells, also become infected. Neonatal calves appear to be most susceptible to clinical infection. Maternal antibodies do not prevent infection, but modify the outcome of the disease as colostrum-deprived animals are more prone to develop severe diarrhoea.

Bovine and porcine toroviruses display host species preference at least to a certain degree. In phylogenetic analyses, all PToVs cluster, while extant BToVs mostly resemble the New World BToV isolate Breda, identified 30 years ago. However, there is evidence for recurring intergenotypic/interspecies recombination, suggesting that cross-species transmission may occur at least incidentally. Currently circulating Eurasian BToVs seem to have arisen from a genetic exchange, during which the 3′ end of the HE gene, the N gene, and the 3′ UTR of a Breda virus-like parent were exchanged for those of PToV. Moreover, some PToV and BToV variants carry chimeric HE genes, which apparently resulted from recombination events involving hitherto unknown toroviruses as donors. For the provisional nomenclature of BToV and PToV genotypes and a comparison of their genome organization, see Figure 10.

### Species demarcation criteria in the genus

Only a modest number of toroviruses has been characterized and complete genome sequences are available solely for BToV strain Breda and EToV strain Berne. Thus far, host preference has been the main criterion for torovirus species demarcation, but future taxonomic classifications should follow the general criteria as outlined at the beginning of this chapter. According to these criteria, bovine and equine toroviruses justify their current status as distinct species.

For PToV, sequences are available only for the 3′-terminal region of the genome, containing the genes for the structural proteins. In phylogenetic trees, constructed for S, HE and M genes, all PToV field strains cluster and appear to be separated from EToV and BToV by an evolutionary distance larger than that between the latter two viruses, supporting the notion that PToV represents a distinct species. The limitations of taxonomy based solely upon phylogenetic analysis of genes for the structural proteins, however, are poignantly illustrated by the occurrence of toroviruses that have acquired novel HE and N genes via interspecies recombination.

Although HToV is designated as a species, evidence for the existence of the virus remains tenuous. Available sequence data are open to varying interpretation and, in any case, are insufficient to justify classification. Future taxonomic proposals are planned to resolve the issue.

### List of species in the genus *Torovirus*

| | | |
|---|---|---|
| *Bovine torovirus* | | |
| Bovine torovirus Breda I | [AY427798] | (BToV Breda) |
| *Equine torovirus* | | |
| Equine torovirus strain Berne | [DQ310701; X52374] | (EToV Berne) |
| *Human torovirus* | | |
| Human torovirus | | (HToV) |
| *Porcine torovirus* | | |
| Porcine torovirus Markelo | | (PToV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Torovirus* but have not been approved as species

None reported.

## GENUS *BAFINIVIRUS*

Type species ***White bream virus***

### Virion properties

#### MORPHOLOGY

Virions are enveloped and bacilliform in shape (130–160 × 37–45 nm, excluding the surface projections; Figure 11). The most conspicuous virion elements are the large coronavirus-like spikes (20–25 nm) and a seemingly rigid tubular nucleocapsid of presumably helical symmetry (120–150 × 19–22 nm, with a central channel of 2–5 nm in diameter).

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virions have a buoyant density in sucrose of 1.17–1.19 g cm$^{-3}$ and are sensitive to lipid solvents.

#### NUCLEIC ACID

White bream virus DF24/00, the sole bafinivirus described to date, possesses a 26.6 kb RNA genome, which is capped, polyadenylated and infectious.

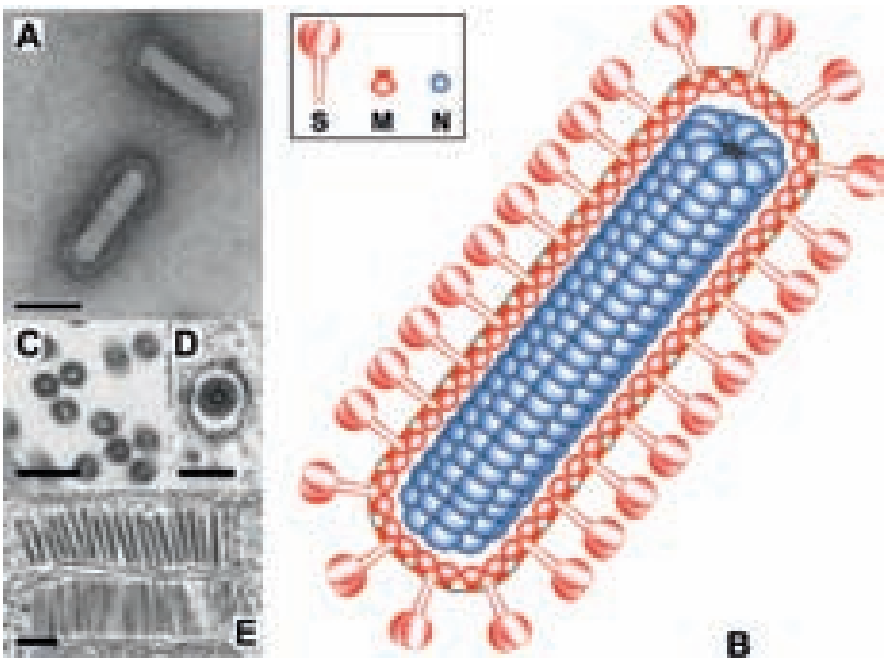


**Figure 11:** Virion morphology and morphogenesis of white bream virus. (A) Negative staining electron micrograph of extracellular WBV particles (2% phosphotungstic acid, pH 7.4) (courtesy Harald Granzow and Thomas C. Mettenleiter, Friedrich Loeffler Institut, Bundesforschungsinstitut für Tiergesundheit, Greifswald Insel Riems, Germany). (B) Schematic representation of the WBV virion. (C and D) Cross-sections of (C) intracytoplasmic nucleocapsids and (D) a virion, with the nucleocapsid seemingly organized by subunits arranged in helical symmetry. (E) Preformed WBV nucleocapsids in the cytoplasm arranged side by side at smooth membranes. All bars represent 100 nm. (C, D and E from Granzow *et al.* (2001). Identification and ultrastructural characterization of a novel virus from fish. *J. Gen. Virol.*, **82**, 2849-2859; with permission.)

### Proteins

Virions contain the following protein species: (i) the spike protein S, a 1220-aa type I membrane glycoprotein, with features typical for class I fusion proteins (bioinformatical analysis revealed heptad repeat regions and a putative fusion peptide); (ii) a 227-aa integral membrane protein M with three predicted transmembrane regions; (iii) a 161-aa basic nucleocapsid protein.

### Lipids

WBV acquires its lipid envelope primarily by budding at intracellular membranes and, only rarely, at the plasma membrane.

### Carbohydrates

From carbohydrate-specific labelling experiments, the S and M proteins appear to be glycosylated. Glycans on the S protein are recognized by the lectin concanavalin-A and thus are likely to contain $\alpha$-mannose.

## Genome organization and replication

The WBV genome contains five ORFs called ORF 1a, 1b (together comprising the replicase gene) and -2, -3 and -4 (for the spike (S), membrane (M) and nucleocapsid protein (N), respectively). The structural proteins are expressed from three sg mRNA species that are 3′ co-terminal with the genome and believed to be produced via a process of discontinuous minus-strand RNA synthesis similar to that of coronaviruses (Figure 12). Each sg mRNA carries a 42-nt leader sequence identical to the 5′-terminal end of the genome. A conserved nonanucleotide sequence, CA(G/A)CACUAC, located upstream of each structural protein gene and immediately downstream of the genomic 5′-leader sequence, presumably represents the WBV equivalent of the coronavirus TRS core element. Bafinivirus virions assemble through the budding of preformed nucleocapsids at membranes of the ER and/or Golgi complex (Figure 11E) and subsequently egress via exocytosis.

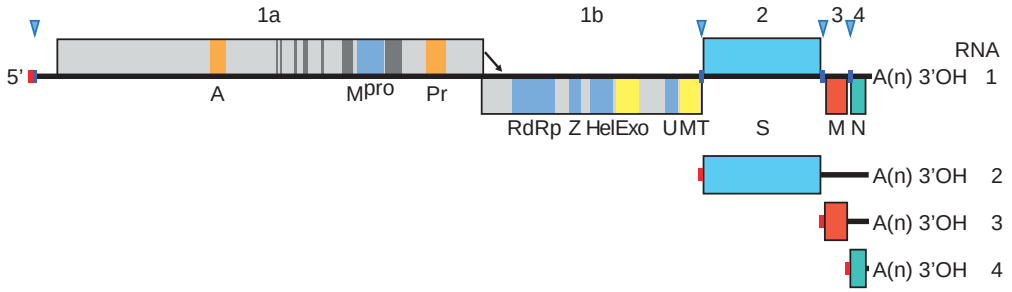


**Figure 12:** Organization and expression of the WBV genome. Schematic representation of the genome of white bream virus and structural relationship between the genomic RNA and subgenomic mRNAs. 5′ leader sequences are indicated by red boxes, TRSs by blue arrowheads and boxes. Other genome elements and acronyms as in Figure 1.

## Antigenic properties

None reported.

## Biological properties

White bream virus is the first nidovirus to be isolated from a teleost, white bream (*Blicca bjoerkna L.*), a species of fresh water fish (family *Cyprinidae)*. No information is available on its ecology, biology and pathogenic properties.

## Species demarcation criteria in the genus

Newly identified viruses are to be assigned to (or excluded from) the genus *Bafinivirus* on the basis of rooted phylogeny and pair-wise comparisons of *Coronaviridae*-wide conserved domains in replicase polyprotein pp1ab as outlined at the beginning of this chapter.

## List of species in the genus *Bafinivirus*

*White bream virus*
White bream virus DF 24/00 [DQ898157] (WBV DF24/00)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Bafinivirus* but have not been approved as species

None reported.

## List of unassigned species in the subfamily *Torovirinae*

None reported.

## List of other related viruses which may be members of the subfamily *Torovirinae* but have not been approved as species

None reported.

## Phylogenetic relationships within the family *Coronoviridae*

In rooted and unrooted phylogenetic trees constructed for the main replicative enzymes, members of the family *Coronaviridae* consistently form a monophyletic cluster that is separate from the *Arteri-* and *Roniviridae* (see Chapter *Nidovirales*, Figure 4). The relatively close relationship between members of the family *Coronaviridae* is supported (i) by the presence of unique ORF1a-encoded enzyme

domains in the replicase polyproteins that might be considered diagnostic molecular markers, i.e. the ADRP and the non-canonical RdRp/putative primase (coronavirus nsp8), and (ii) by similarities in their structural proteins (S and M for all *Coronaviridae*, S, M and N in the *Torovirinae*). On the basis of rooted phylogeny and pair-wise comparisons of *Coronaviridae*-wide conserved replicase domains, four well-separated monophyletic clusters can be distinguished within the subfamily *Coronavirinae*, three of which are established genera (*Alpha-*, *Beta-* and *Gammacoronavirus*). It is anticipated that the remaining cluster, comprised of recently identified avian coronaviruses (Thrush, Bulbul and Munia coronavirus) and related viruses in mammals, will be classified as a new genus (Figure 2). Viruses in the subfamily *Torovirinae* (genera *Bafini-* and *Torovirus*) are phylogenetically more related to each other than to those in the subfamily *Coronavirinae.*

## Similarity with other taxa

For features shared with other members of the order *Nidovirales* and with non-nidovirus taxa, please see Chapter *Nidovirales*.

## Derivation of names

*Corona*: from Latin *corona*, "halo"; refers to the characteristic appearance of surface projections that create an image reminiscent of the solar corona.
*Toro*: from Latin *torus*, a term used in architecture for the convex molding at the base of a column and in geometry for a three-dimensional structure in the shape of a hollow donut; refers to the nucleocapsid morphology in a subset of particles.
*Bafini*: from *ba*cilliform *fi*sh *ni*doviruses, refers to the virion morphology and host tropism.

## Further reading

### Journals and books

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### Websites

http://www.viprbrc.org/brc/ (VIPR Virus Pathogen Resource)
http://veb.lumc.nl/SNAD/
http://veb.lumc.nl/SARGENS/

## Contributed by

de Groot, R.J., Baker, S.C., Baric, R., Enjuanes, L., Gorbalenya, A.E., Holmes, K.V., Perlman, S., Poon, L., Rottier, P.J.M., Talbot, P.J., Woo, P.C.Y. and Ziebuhr, J.

# Family *Roniviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Roniviridae*** |
| Genus | ***Okavirus*** |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Okavirus*

Type species ***Gill-associated virus***

## Virion properties

### Morphology

Virions are enveloped, bacilliform in shape with rounded ends, and 40–60 nm × 150–200 nm in dimensions (Figure 1). The envelope is studded with prominent peplomers projecting approximately 11 nm from the surface. Nucleocapsids are 20–30 nm in diameter and have a helical symmetry with coil periodicity of 5–7 nm. Long filamentous nucleocapsid precursors (approximately 15 nm in diameter and 80–450 nm in length) occur in the cytoplasm of infected cells and acquire envelopes by budding at endoplasmic reticulum membranes. Newly budded mature enveloped virions often appear as multimers butted end-to-end and paracrystalline arrays of virions can occur within vesicles.

### Physicochemical and physical properties

Virion buoyant density in sucrose is 1.18–1.20 g $cm^{-3}$. Yellow head virus (YHV) is inactivated by heating at 60 °C for 15–30 minutes but has been reported to survive in 25 °C–28 °C seawater for at least four days. Virions are sensitive to calcium hypochlorite and sodium dodecyl-sulfate but sensitivity to other treatments is not known.

### Nucleic acid

Virions contain a single linear segment of positive sense ssRNA varying in length from 26,235 nt for gill-associated virus (GAV) to 26,662 nt for YHV. The genome contains a 5′-terminal 7-methylguanosine cap and a 3′-polyadenylated tail.

### Proteins

YHV virions contain a 20–22 kDa nucleoprotein (p20) that forms the nucleocapsid and two envelope glycoproteins of 110–135 kDa (gp116) and 63–67 kDa (gp64) that form the prominent peplomers on the virion surface. The mature gp116 and gp64 glycoproteins originate by post-translational cleavage of a precursor polyprotein (pp3) encoded by the ORF3 gene. Cleavage occurs at the C-terminal side of the third and fifth of six hydrophobic transmembrane (TM) domains, which display characteristics of signal peptidase type 1-like sites. The glycoproteins gp116 and gp64 are anchored to the virion envelope by either two (TM4 and TM5) or one (TM6) transmembrane domain, respectively. There is no evidence that gp116 and gp64 are linked by intermolecular disulfide bonds.

### Lipids

Virions possess a lipid envelope derived from endoplasmic membranes of host cells.

### Carbohydrates

*N*-linked glycosylation sites are present in the amino acid sequences of gp116 (7–8 sites) and gp64 (four sites), and in virions, both are glycosylated extensively. In YHV, all but one of the seven sites in gp116 has been shown to be occupied, and three of four sites in gp64 are occupied. Carbohydrates attached to gp64 and gp116 comprise primarily mannose-type glycans, and those on gp116 also comprise N-acetyl-galactosamine and N-acetyl-glucosamine in lesser abundance. Few if any of the multiple *O*-linked glycosylation sites in each glycoprotein appear to be utilized.

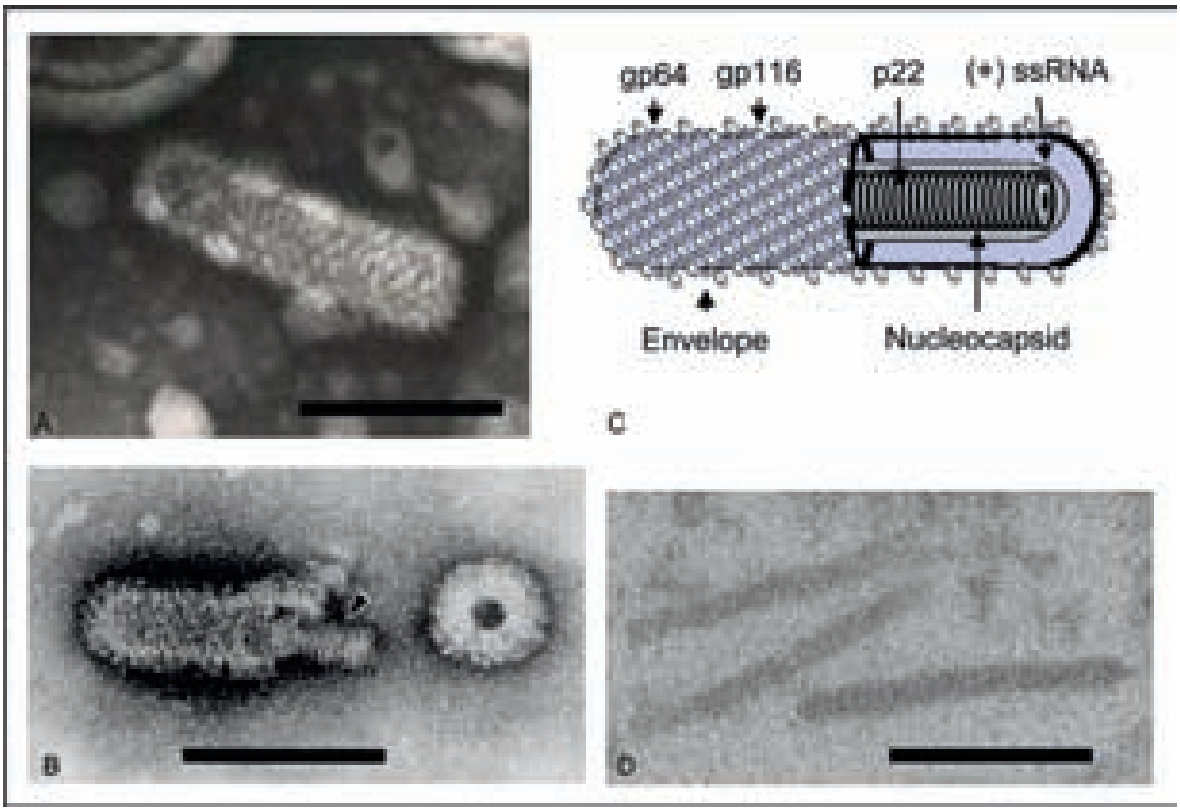


**Figure 1:** (A) Transmission electron micrograph of negative-stained virion of gill-associated virus (GAV). (B) Transmission electron micrograph of partially disrupted yellow head virus (YHV) virion displaying the internal nucleocapsid and a ring-like structure which appears to be a disrupted virion in cross-section. (C) Schematic illustration of an okavirus virion. (D) Transmission electron micrograph of unenveloped cytoplasmic nucleocapsids in a thin section of GAV-infected shrimp cells. The bars represent 100 nm. (Electron micrographs provided by K.M. Spann, P. Loh, J.A. Cowley and R.J. McCulloch and reproduced with permission.)

## Genome organization and replication

The positive sense ssRNA genome contains, in order from the 5′ terminus, a large replicase gene (ORF1a/ORF1b) followed by genes encoding a p20 nucleoprotein (ORF2), a pp3 precursor polyprotein (ORF3), from which the gp116 and gp64 envelope glycoproteins are derived, and a small putative protein (ORF4) of unknown function that is not translated in abundance (Figure 2). The overlap between ORF1a and ORF1b contains a slippery sequence (AAAUUUU) followed by a complex RNA pseudoknot structure causing a −1 ribosomal frameshift, with an estimated efficiency of about 23%, that allows read-through translation of the pp1a polyprotein encoded by ORF1a to generate a pp1ab polyprotein encoded by ORF1a and ORF1b. The ORF1a coding sequence contains four regions containing multiple hydrophobic domains (HD1-HD4) a chymotrypsin-like cysteine (3C-like) proteinase (3CL$^{pro}$ or "main" protease, M$^{pro}$) flanked by HD3 and HD4 that is involved in autolytic processing of the pp1ab polyprotein. The 3CL$^{pro}$ of GAV has a tentative consensus VxHE↓(L,V) cleavage site specificity that distinguishes it from the corresponding M$^{pro}$ enzymes in vertebrate nidoviruses, and taken together with sequence differences, it appears to combine the Cys-His catalytic dyad of the coronavirus M$^{pro}$ with a potyvirus-like substrate binding pocket. ORF1a also contains two putative papain-like proteinase domains (PLP1 and PLPx) that share remote structural similarity, but very little direct sequence identity, with the PLP domains of coronaviruses. Like the corresponding papain-like proteases (PL$^{pro}$) of toroviruses, both PLP1 and PLPx lack a $Zn^{2+}$-binding finger. In vertebrate nidoviruses, the 3CL$^{pro}$ and PL$^{pro}$s process the pp1a/pp1ab polyproteins into functional nonstructural proteins (nsp's) numbered from the N-terminus

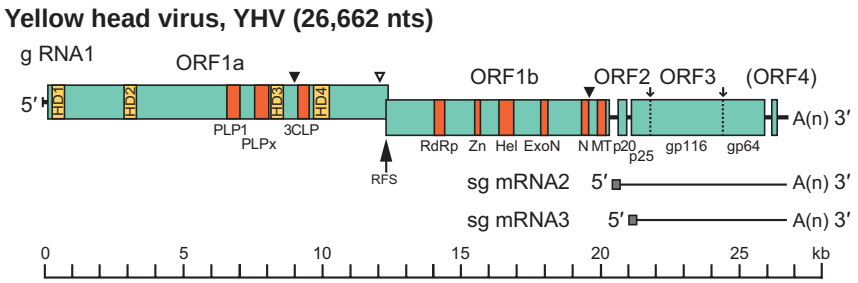


**Figure 2:** Schematic representation of the 26,662 nt polyadenylated (+) ssRNA genome of YHV (g RNA1) and the two 3′-coterminal sub-genomic RNAs sg mRNA2 and sg mRNA3. Functional domains in ORF1a: hydrophobic regions (HD1-HD4), 3C-like protease (3CLP), papain-like protease (PLP1) and a domain with homology to PLP1 but lacking the canonical α + β fold of papain-like proteases (PLPx). Functional domains in ORF1b: RNA polymerase (RdRp), cysteine- and histidine-rich zinc finger and helicase (Zn-Hel), exoribonuclease (ExoN), uridylate-specific endoribonuclease (N) and ribose-O-methyl transferase (MT). ORF2 encodes the nucleoprotein (p20). ORF3 encodes a precursor polyprotein (pp3) that undergoes post-translation processing to generate envelope glycoproteins (gp116 and gp64) and an N-terminal triple-membrane-spanning fragment of unknown function (p25). The ribosomal frameshift site (RFS) allows read-through translation of polyprotein pp1ab from ORF1a and ORF1b. Known (▼) and likely (∇) sites of proteolytic cleavage of expressed polyproteins pp1a and pp1ab are indicated together with the two signal peptidase type 1 cleavage sites in pp3 (↓).

as nsp1 to nsp12 in arteriviruses and nsp1 to nsp16 in coronaviruses. Whilst the $3CL^{pro}$ of GAV has been shown to cleave at an immediate upstream site and at a site toward the pp1ab C-terminus, the number and composition of nsps generated from pp1ab by the $3CL^{pro}$ in combination with the putative $PL^{pro}$s is not yet known. ORF1b also contains multiple sequence motifs with homologues in vertebrate nidoviruses. These include a large RdRp polymerase domain with an "SDD" active site motif and putative multinuclear Zn-finger-like domains preceding a 5′-to-3′ helicase domain (Zn-HEL), as well as homologs of the 3′-to-5′exoribonuclease (ExoN), *n*idoviral *endo*ribonuclease specific for *u*ridylate (NendoU) and ribose-2′-O-methyltransferase (O-MT) domains that exist in the C-terminal region of pp1ab replicase polyproteins of the large vertebrate nidoviruses.

Compared to the genomes of vertebrate nidoviruses, the okavirus genome is unique in that the nucleoprotein gene (ORF2) is located upstream of the glycoprotein gene (ORF3), there is no discrete gene encoding a structural membrane (M) protein, and the virion envelope glycoproteins gp116 and gp64 are generated by proteolysis of a pp3 precursor polyprotein encoded by the ORF3 gene. The approximately 25.2 kDa polypeptide cleaved from the pp3 N-terminus contains three predicted TM domains and *N*-linked glycosylation sites in its predicted ectodomain. Whilst this triple-membrane-spanning glycoprotein is similar in size to the M protein of some vertebrate nidoviruses, it is not abundantly present in infected cells and evidence of its association with virions in very low amounts is tenuous. Compared to the 249-nt ORF4 in GAV, ORF4 in other okavirus genotypes studied so far is truncated substantially due to deletions or sequence changes interrupting the reading frame. There is evidence of ORF4 being expressed at low levels in tissues of GAV-infected shrimp, but whether the ORF4 protein has a specific function remains unknown.

In okavirus-infected cells, two subgenome-sized (sg) mRNAs are produced, sg mRNA2 and sg mRNA3, from which ORF2 and ORF3 are translated, respectively. The genome length RNA (g RNA1) and the two sg mRNAs are 3′-coterminal and each possesses a 5′-7-methylguanosine cap structure and a 3′-poly(A) tail (Figure 2). It is likely that okavirus sg mRNAs are transcribed from complementary sg(-) strand templates as is the case in other nidoviruses. In support of this, double strand (ds) RNAs equivalent in size to the genomic and two sg mRNAs have been detected in shrimp cells infected with GAV, and likely represent replicative-intermediate RNAs. Unlike the sg mRNAs of corona- and arteriviruses, however, the okavirus sg mRNAs do not possess a common leader sequence identical to the genome 5′-terminal sequence. In contrast, g RNA1, sg mRNA2 and sg mRNA3 each initiate with a 5′AC-dinucleotide. For the sg mRNAs, this 5′-terminal AC-dinucleotide maps precisely to a central position of a sequence (GGUCAAUUACAACCUA) that is conserved in the intergenic regions (IGRs) preceding the ORF2 and ORF3 genes. Presumably, this conserved element serves a dual function, in the genome as a terminator of minus-strand RNA

synthesis and in the resulting minus-strand sg RNA templates, as a transcriptional promoter for sg mRNA synthesis. Thus, okaviruses would differ from corona- and arteriviruses by not using a discontinuous transcription process to produce their mRNAs, but rather a "continuous" transcription strategy similar to that utilized by members of the subfamily *Torovinae* (see family *Coronaviridae* chapter). Interestingly, a putative terminator/promoter element is also present in the IGR preceding ORF4. However, in all genotypes studied so far, the putative transcription initiation site nucleotide is either a G or U rather than an A, which based on the absence of a sg mRNA initiating from this element, appears to destroy its functioning.

## Antigenic properties

Not known.

## Biological properties

Okaviruses have been detected only in crustaceans. The giant tiger shrimp (*Penaeus monodon*) appears to be the primary natural host of GAV and YHV but other penaeid and crustacean species are susceptible to natural and experimental infection. The geographic range of okaviruses mirrors that of its primary penaeid host and extends across the Indo-Pacific from Eastern Africa, Southern and Southeast Asia, Australia to islands in the South Pacific. Infections have also been detected recently in blue shrimp (*P. stylirostris*) from the Gulf of California. Infections may be chronic or acute and transmitted horizontally or vertically. The prevalence of subclinical infection in *P. monodon* can be high in some regions, with pathology limited to the lymphoid organ. In acute infections associated with disease and mortalities, virus invades most tissues of ectodermal and mesodermal origin. Only genotypes 1 (YHV) and 2 (GAV) have been associated with disease. High virulence is retained by a variant of genotype 1 (genotype 1b) with a 54 amino acid (aa) deletion at the gp116 N-terminus, although its $LD_{50}$ is somewhat lower than the highly-virulent genotype 1a virus.

## Species demarcation criteria in the genus

In the genus ***Okavirus***, differences in genome organization and biological properties of the six assigned genotypes detected in *P. monodon* shrimp appear insufficient to warrant their demarcation into separate species. Evidence of genetic recombination between genotypes also indicates that all should be classified as a single species.

## List of species in the genus *Okavirus*

| | | |
|---|---|---|
| *Gill-associated virus* | | |
| Gill-associated virus - Australia | [AF227196] | (GAV-AUS) |
| Yellow head virus | [FJ848673] | (YHV) |

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Okavirus* but have not been approved as species

None reported.

## Phylogenetic relationships within the family

Phylogenetic analyses of a partial ORF1b gene sequence has clustered okaviruses into six distinct genotypes numbered 1 (YHV), 2 (GAV), 3, 4, 5 and 6 (Figure 3). Genotype 1, 2, 3 and 5 viruses have been detected in *P. monodon* from various locations in Southeast Asia, but genotypes 2, 4 and 6 are the sole genotypes thus far detected in *P. monodon* from Australia, India and eastern Africa, respectively. There is evidence of naturally occurring genetic recombination involving genotypes 1 (YHV), 2 (GAV), 3 and 5.

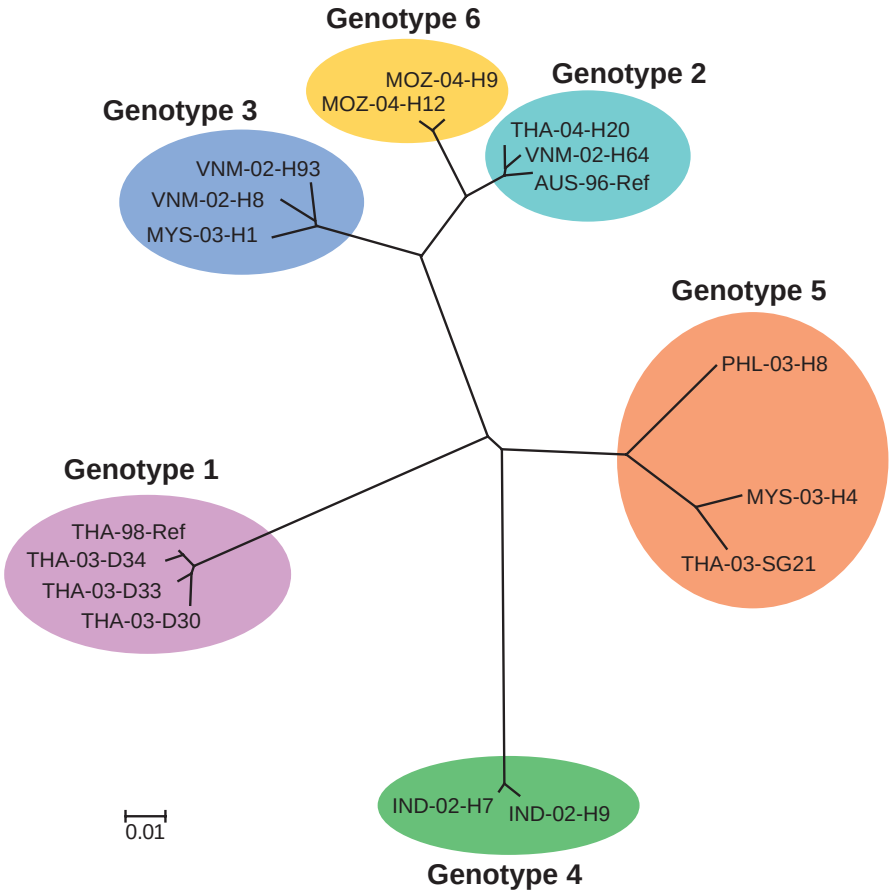


**Figure 3:** Unrooted phylogenetic tree of representatives of each of the six okavirus genotypes based on a 781 nucleotide region of the ORF1b gene [see Wijegoonawardane *et al*. (2008) for strain details and expanded phylogenetic analyses]. The sequences were aligned using Clustal W, phylogenetic inference was determined by the neighbor-joining method and the tree was constructed using Treeview software. Bootstrap values obtained for a 1000 replicate analyses were 1000 at each genotype branching node. Branch lengths are proportional to phylogenetic distance and the bar represents a sequence divergence of 1.0%.

## Similarity with other taxa

Similarities in the genome size and organization, sequences of core replicase proteins and the sgmRNA transcription strategy used by roniviruses clearly link them to other members of the order *Nidovirales*. The RNA pseudoknot component of the −1 ribosomal frameshift in the ORF1a/1b gene overlap resembles the gag/pol pseudoknots of some retroviruses. The structure and substrate specificity of the pp1ab chymotrypsin-like cysteine proteinase ($3CL^{pro}$) bridges phylogenetic distinctions between the corresponding proteinases of coronaviruses and plant potyviruses.

## Derivation of names

*Roni*: from *ro*d-shaped *ni*dovirus referring to the virion morphology of viruses in the family.
*Oka*: refers to the lymphoid or so named "oka" organ, an anatomical structure common to penaeid shrimp, and which is a site of virus replication and pathology in both subclinical and acute infections.

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## Contributed by

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# ORDER PICORNAVIRALES

## Taxonomic structure of the order

| | |
|---|---|
| Order | *Picornavirales* |
| Family | *Dicistroviridae* |
| Genus | *Cripavirus* |
| Genus | *Aparavirus* |
| Family | *Iflaviridae* |
| Genus | *Iflavirus* |
| Family | *Marnaviridae* |
| Genus | *Marnavirus* |
| Family | *Picornaviridae* |
| Genus | *Enterovirus* |
| Genus | *Cardiovirus* |
| Genus | *Aphthovirus* |
| Genus | *Hepatovirus* |
| Genus | *Parechovirus* |
| Genus | *Erbovirus* |
| Genus | *Kobuvirus* |
| Genus | *Teschovirus* |
| Genus | *Sapelovirus* |
| Genus | *Senecavirus* |
| Genus | *Tremovirus* |
| Genus | *Avihepatovirus* |
| Family | *Secoviridae* |
| Subfamily | *Comovirinae* |
| Genus | *Comovirus* |
| Genus | *Fabavirus* |
| Genus | *Nepovirus* |
| Subfamily | (Unassigned) |
| Genus | *Cheravirus* |
| Genus | *Sadwavirus* |
| Genus | *Sequivirus* |
| Genus | *Torradovirus* |
| Genus | *Waikavirus* |

## Introduction

The order *Picornavirales* contains viruses with a monopartitite or bipartite positive-strand RNA genome that share the following properties: auto-proteolytically processed polyprotein(s), a common three-domain replication block (Hel-Pro-Pol domain consisting of a superfamily III helicase, a proteinase with a chymotrypsin-like structure and a superfamily I RNA-dependent RNA polymerase) and non-enveloped icosahedral virions approximately 30 nm in diameter with a pseudo-T = 3 symmetry. The RNAs are usually characterized by the presence of a small VPg protein (typical 3–4 kDa) linked to their 5′ end and a poly(A) tail at their 3′ end. Members of the family *Picornaviridae* (genus *Enterovirus*) and of the family *Secoviridae* (genus *Comovirus*) were the first characterized members of the order and infect vertebrates and plants, respectively. The order also includes viruses infecting invertebrates (families *Dicistroviridae* and *Iflaviridae*) or algae (family *Marnaviridae*). Large-scale environmental genomic studies suggest the presence of a large number of uncharacterized picorna-like viruses in the ocean.

## Virion properties

### MORPHOLOGY

Virions are non-enveloped icosahedral particles approximately 30 nm in diameter with a pseudo-T = 3 symmetry. Virus preparations often contain empty virus particles.

### Physicochemical and physical properties

Empty virus particles sediment with $S_{20,W}$ values between 50 and 80S. Viruses with a single large genomic RNA (families *Picornaviridae*, *Iflaviridae* and *Dicistroviridae* and genera *Sequivirus* and *Waikavirus*) sediment with $S_{20,W}$ values of 140–190S. Viruses with a bipartite genome (family *Secoviridae* with the exception of the genera *Sequivirus* and *Waikavirus*) encapsidate each RNA separately. Virions containing RNA1 sediment at 110–135S and virions containing RNA2 sediment at 84–128S.

### Nucleic acid

Most members of the order have a single molecule of positive sense RNA ranging between 7,000 and 12,500 nt in length. Some members of the order (genera *Comovirus*, *Fabavirus*, *Nepovirus*, *Sadwavirus*, *Cheravirus* and *Torradovirus* all in the family *Secoviridae*) have a bipartite genome with an RNA1 ranging in size between 5800 and 8400 nt and an RNA2 between 3200 and 7300 nts. The presence of a small (3–5 kDa) VPg linked to the 5′ end of the RNAs has been confirmed for most members of the order; comparative genomics strongly suggests that this property is universally conserved. Viral RNAs are normally polyadenylated at their 3′ end. The only known exception is the genomic RNA of parsnip yellow fleck virus (genus *Sequivirus*, family *Secoviridae*) which is apparently not polyadenylated.

### Proteins

The capsid contains 60 units, each consisting of three paralogous jelly-roll domains. Each jelly-roll domain is unique in sequence but folds in a similar 8-stranded beta-barrel structure. In most members of the order, the three jelly-roll domains are contained in three separate coat proteins (CP) of approximately 25 kDa each. However, in some members of the family *Secoviridae*, the three jelly-roll domains can be contained in a single large CP of approximately 60 kDa (genus *Nepovirus*) or in two CPs, a 40–45 kDa CP containing two jelly-roll domains and a 21–29 kDa CP containing one jelly-roll domain (genera *Comovirus, Fabavirus* and *Sadwavirus*). An additional unrelated small structural protein, termed VP4, is encoded by the genome of some members of the families *Picornaviridae* (upstream of VP2), *Dicistroviridae* and *Iflaviridae* (upstream of VP3). This small protein is encapsidated within the virion.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

The viral RNA is infectious and serves as a template for replication and as mRNA. In the case of viruses with a bipartite genome, RNA1 can replicate independently of RNA2 within the first infected cell but RNA2 is required for encapsidation and for cell-to-cell movement. The combined size of the 5′ and 3′ UTRs is <15% of the genome. They contain sequence motifs and secondary structures necessary for replication/translation. The 5′-UTR is larger than 3′-UTR in animal viruses. The RNAs of viruses in the family *Secoviridae* often contain a much larger 3′-UTR (Figure 1).

The RNA genomes of viruses in the order are generally monocistronic with a single ORF that encodes a single large polyprotein. For many members of the order, translation of the unique ORF has been shown to be directed by an internal ribosome entry site (IRES) located in the 5′ UTR. In viruses with a monopartite genome, the replication proteins (Hel, Pro, Pol) are generally present in the C-terminal region of the unique polyprotein and structural proteins are present in the N-terminal region of the polyprotein (family *Picornaviridae* and *Iflaviridae*, genera *Sequivirus* and *Waikavirus*). However, in the families *Dicistroviridae* and *Marnaviridae*, the structural proteins are located downstream of the replication proteins either within a single polyprotein (*Marnaviridae*) or as a separate polyprotein (*Dicistroviridae*). In bipartite members of the order (genera *Comovirus*, *Fabavirus*, *Nepovirus*, *Sadwavirus*, *Cheravirus* and *Torradovirus*), the RNA1-encoded polyprotein contains the domains for replication proteins and the RNA2-encoded polyprotein includes the domains for structural proteins and the movement protein (located upstream of the structural proteins).

There are some exceptions to the general rule that one polyprotein is encoded by each viral RNA. In the family *Dicistroviridae*, the RNA genome is dicistronic with two non-overlapping ORFs that are

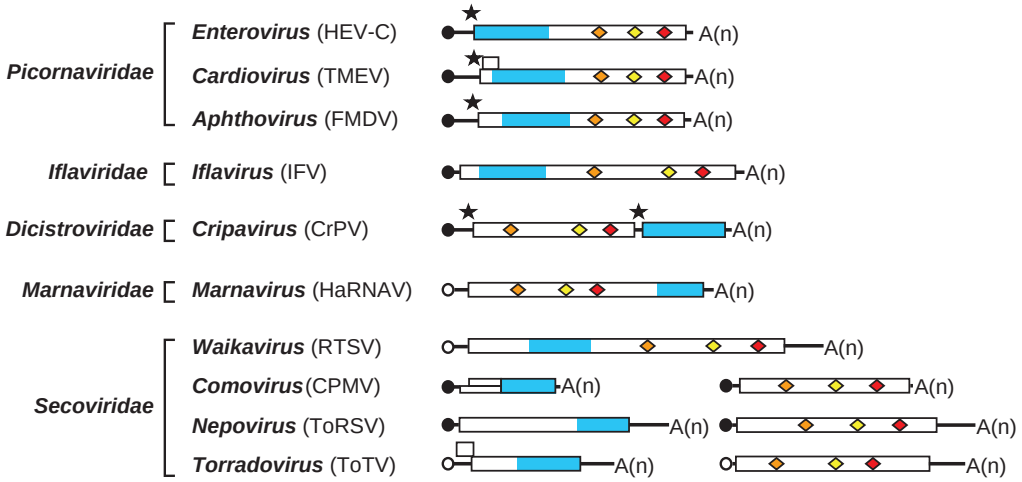


**Figure 1:** Genome organization of representative members of the order *Picornavirales*. Each RNA is shown with the ORF(s) represented with the boxes. Circles depict VPg molecules covalently attached at the 5′ end of the RNAs. Black circles represent VPg confirmed experimentally and open circles represent putative VPgs. Poly(A) tails are represented at the 3′ end of the RNAs [$A_{(n)}$]. Conserved motifs for the helicase (orange diamonds), 3C or 3C-like proteinase (yellow diamonds), RNA-dependent RNA polymerase (red diamonds) and the domains for coat protein(s) (blue rectangles) are shown. Please refer to chapters describing each family for further details (presence and function of additional protein domains, position of cleavage sites, etc.). When characterized, internal ribosome entry sites are shown with the black stars (families *Picornaviridae* and *Dicistroviridae*). This has not been studied for other members of the order. Abbreviation for virus species are as follows: human enterovirus C (HEV-C), Theiler's murine encephyalomyocarditis virus (TMEV), foot-and-mouth disease virus (FMDV), infectious flacherie virus (IFV), cricket paralysis virus (CrPV), Heterosigma akashiwo RNA virus (HaRNAV), rice tungro spherical virus (RTSV), cowpea mosaic virus (CPMV), tomato ringspot virus (ToRSV), tomato torrado virus (ToTV).

separated by an intergenic untranslated region (IGR). The first ORF encodes the replication proteins and the second ORF codes for structural proteins. Translation of the second ORF is directed by an IRES located in the IGR. In the genus *Comovirus*, two polyproteins are synthesized from the RNA2 single long ORF. The second shorter polyprotein is produced by translation initiation at an internal AUG. In the genus *Torradovirus*, two overlapping ORFs are found on RNA2. The function of the putative protein encoded by the first ORF and the mode of translation of the two ORFs have not been investigated. In a virus of the genus *Cardiovirus*, a small ORF overlaps with the beginning of the major ORF.

Some viruses within the order possess multiple VPgs encoded in tandem (three in the species *Foot-and-mouth disease virus*, two to six copies in most members of the family *Dicistroviridae*). It has been suggested that the unclassified seal picornavirus 1 has two VPgs. The VPg ORF(s) lie(s) between the $2C^{hel}$ and $3C^{pro}$ genome regions. In picornaviruses, VPg is covalently attached, via a tyrosine at position 3 to the 5′ uracil of the genome. In comoviruses it is the amino-terminal serine which is used.

Most of the polyprotein cleavage sites are processed by a cysteine proteinase with a chymotrypsin-like structure. This proteinase is termed 3C proteinase for members of the family *Picornaviridae* and 3C-like proteinase in other members of the order. In the case of Heterosigma akashiwo RNA virus (the only characterized member of the family *Marnaviridae*), the cysteine in the catalytic triad of the proteinase is replaced by a serine. 3C and 3C-like proteinases usually cut after a glutamine or glutamate residue, although there are notable exceptions in the families *Secoviridae* and *Marnaviridae*. In the case of viruses with a bipartite genome, the 3C-like proteinase is encoded by RNA1 and the proteinase cleaves *in trans* the RNA2-encoded polyprotein. Members of the genus *Enterovirus* (family *Picornaviridae*) encode an additional proteinase, 2A, that cleaves at its own N-terminus *in cis*. The leader protein of members of the genera *Aphthovirus* and *Erbovirus* (family *Picornaviridae*) also has proteolytic activity.

For several members of the families *Picornaviridae* and *Secoviridae*, replication has been shown to occur in the cytoplasm in association with intracellular membranes. Several viral proteins interact

directly with these membranes and anchor the replication complex to the membranes. Mode of replication of other members of the order has not been investigated.

## Antigenic properties

These vary depending on the genus.

## Biological properties

Members of the order infect a wide range of hosts including vertebrates (family *Picornaviridae*), plants (family *Secoviridae*), arthropods (family *Dicistroviridae* and *Iflaviridae*) and unicellular organisms (family *Marnaviridae*). Specific host range and transmission properties differ among individual genera.

## Phylogenetic relationships within the order

In an unrooted maximum-likelihood tree built for representatives of the order using the amino acid sequence contained in the proteinase-polymerase region, all families except the *Iflaviridae* form monophyletic branches (Figure 2).

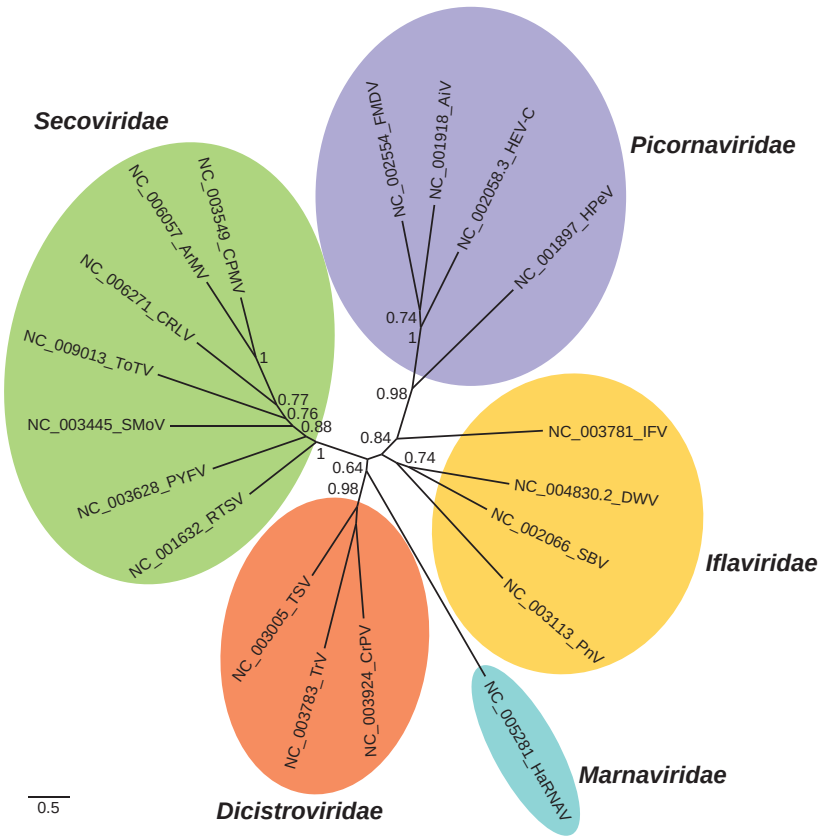


**Figure 2:** Phylogeny of the order *Picornavirales*. Unrooted phylogenetic analysis was conducted for 19 viruses representing the diversity of the order (A.E. Gorbalenya and C. Lauber, unpublished data). A multiple alignment of the Pro-Pol portion of polyproteins flanked by the CG/SG protease active site from the N-terminus and extended to cover (almost) the entire RNA-dependent RNA polymerase was produced using HMMER, Clustal and Muscle programs with the Viralis software platform. The alignment was submitted to the PhyML server to produce a maximum-likelihood tree. The support for internal branching was generated using approximate likelihood-ratio test (aLRT) values. Branch lengths indicate number of substitutions per residue. Abbreviated virus names and reference to the sequence RefSeq accession numbers are shown on the branches of the tree. Families are shown with distinct colors. Viruses represent species that are either defined in Figure 1 or as follows: arabis mosaic virus (ArMV), cherry rasp leaf virus (CRLV), strawberry mottle virus (SMoV), parsnip yellow fleck virus (PYFV), Taura syndrome virus (TSV), Triatoma virus (TrV), Perina nuda virus (PnV), sacbrood virus (SBV), deformed wing virus (DWV), human paraechovirus (HPeV), Aichi virus (AiV).

## Similarity with other taxa

Presence of the Hel-Pro-Pol domain within polyproteins is a property that is shared with members of the families *Caliciviridae*, *Potyviridae* and some unclassified viruses. However, members of these families differ in several properties including virus particle structure (elongated particles with helical symmetry for potyvirids and icosahedral particles but with a true T = 3 structure for caliciviridids), type of helicase (superfamily II helicase for potyvirids) and size of VPg (25 kDa for potyvirids and 12–15 kDa for caliciviridids). In addition, some members of the family *Caliciviridae* express their structural proteins from a subgenomic RNA. Members of the order *Picornavirales* are not known to produce subgenomic RNAs. There are also other virus families which use either jelly-roll-based capsids and/or 3C-like proteases.

## Derivation of name

The name is derived from the family *Picornaviridae*.

## Further reading

Le Gall, O., Christian, P., Fauquet, C.M., King, A.M.Q., Knowles, N.J., Nakashima, N., Stanway, G. and Gorbalenya, A.E. (2008). *Picornavirales*, a proposed order of positive-sense single-stranded RNA viruses with a pseudo-T = 3 virion architecture. *Arch. Virol.*, **153**, 715–727.

## Contributed by

Sanfaçon, H., Gorbalenya, A.E., Knowles, N.J. and Chen, Y.P.

# Family *Dicistroviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Dicistroviridae*** |
| Genus | ***Cripavirus*** |
| Genus | ***Aparavirus*** |

## Virion properties

### Morphology

Virions are roughly spherical with a particle diameter of approximately 30 nm and no envelope (Figure 1). The virions exhibit icosahedral, pseudo T = 3 symmetry and are composed of 60 protomers, each comprised of a single molecule of each of VP2, VP3 and VP1 (Figure 1). A smaller protein, VP4, is also present in the virions of some members and is located on the internal surface of the 5-fold axis below VP1.

### Physicochemical and physical properties

Virions are stable in acidic conditions (to pH = 3.0). Virions have a buoyant density of 1.34–1.39 g ml$^{-1}$ in CsCl in the pH range of 7 to 9 and sedimentation coefficients of between 153 and 167S. However, physicochemical properties have not been fully established for all members of the family.

### Nucleic acid

Virions contain a single molecule of infectious, linear, positive sense, single stranded RNA (ssRNA) of approximately 8500–10,000 nt in size with a GC content ranging from 35 to 45%. RNA constitutes about 30% of the virion weight. A small genome-linked virus protein (VPg), is covalently attached to the 5′ end of the genome. A 5′ UTR is followed by two open reading frames (ORF 1 and ORF 2) of approximately 5500 and 2500 nt, respectively. The ORFs are separated by an intergenic untranslated region (IGR) of about 170–530 nt. A UTR is also found at the 3′ end of the genome. The 3′ end of the viral RNA genome is polyadenylated. The length of the UTRs at each end of the genome is variable.

### Proteins

Proteins account for 70% of the virion weight. The approximately 200 kDa nonstructural polyprotein and 100 kDa structural polyprotein are encoded by ORF 1 and ORF 2, respectively. Virions contain three major structural (capsid) viral proteins, VP1, VP2 and VP3. The size of these capsid proteins ranges from 24 to 40 kDa; an exception is Taura syndrome virus (TSV) in which VP1 is

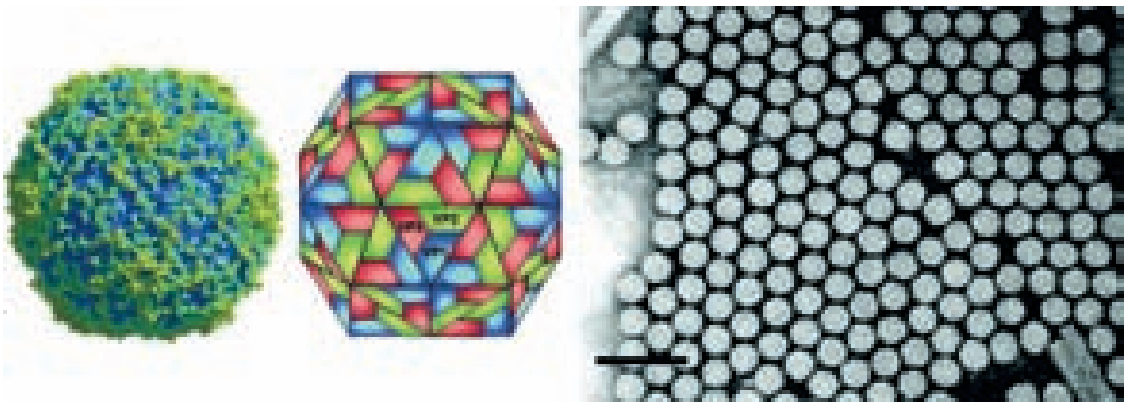

**Figure 1:** (Left) Rendering of a particle of an isolate of cricket paralysis virus (CrPV) at 2.4 Å resolution (Courtesy of Reddy *et al.*). (Center) Diagram showing the packing of surface proteins of cricket paralysis virus (CrPV). (Right) Negative contrast electron micrograph of isometric particles of CrPV. The bar represents 100 nm (Courtesy of C. Reinganum).

55 kDa. A fourth smaller capsid protein (VP4) of around 4.5–9 kDa also has been reported in some species. In most species a protein precursor (VP0) is present which is cleaved to yield capsid proteins VP3 and VP4.

### Lipids
None reported.

### Carbohydrates
None reported.

## Genome organization and replication

The RNA genome is monopartite and dicistronic with two non-overlapping ORFs that are separated and flanked by UTRs (Figure 2). The 5′-proximal and 3′-proximal ORFs encode non-structural and structural protein precursors, respectively. Components of the non-structural polyprotein include an RNA-dependent RNA polymerase (RdRp), cysteine protease and RNA helicase. The VPg attached covalently at the 5′ end of the genome plays an important role in RNA replication. The VPg sequence is repeated in most dicistrovirus genomes with the number of repeats being species-dependent. The presence of multiple VPgs is predicted to allow for multiple progeny RNA molecules to be produced per template.

Replication occurs exclusively in the cytoplasm of infected cells. Translation of the dicistronic RNA genome of dicistroviruses proceeds directly from two distinct Internal Ribosome Entry Sites (IRESs) located within the 5′-UTR and the IGR. The IGR IRES can initiate translation without codon-anticodon base pairing between the initiation AUG codon and the initiator Met-tRNA. Moreover, the IGR IRES can directly bind and manipulates the ribosome in the absence of canonical initiation factors. The activities of the IGR IRES depend on conserved RNA sequences and structures. The predicted pseudo-knot and multiple stem-loop structures in the IGR IRES are highly conserved across all members of the family and appear to be necessary for interactions with the ribosome. Conversely, the 5′-UTR IRES is not well conserved within the group and there are no clear structural homologies between the 5′-UTR IRES and IGR IRES. Translation activity of the IGR IRES is comparatively greater than that of the 5′-UTR IRES.

## Antigenic properties

Many members of the family are serologically distinguishable.

## Biological properties

All members of the family infect invertebrates. Most members of the family are widely distributed in nature. Aggregates and crystalline arrays of virus particles have been found in the cytoplasm of infected cells. Dicistrovirus infection is not usually associated with overt disease although infection commonly leads to reduced life expectancy. Different virus isolates vary considerably in virulence and pathogenicity; the severity of disease can range from lethal through acute to chronic and inapparent.

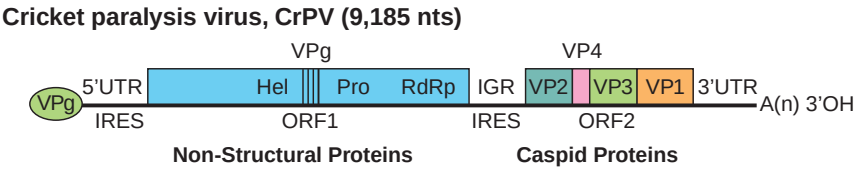


**Figure 2:** Genome structure of cricket paralysis virus (CrPV). The RNA genome contains two non-overlapping ORFs separated by an intergenic region (IGR). The 5′ proximal ORF encodes the nonstructural proteins: RNA helicase (Hel), cysteine protease (Pro) and RNA-dependent RNA polymerase (RdRp). The structural proteins are encoded by the 3′ proximal ORF and are expressed as a polyprotein that is subsequently processed to yield three major structural proteins (VP1, VP2 and VP3). VP4 is presumed to be an N-terminal extension of VP3 which is cleaved from the precursor; VP4 is considered a minor structural component of the virion. Distinct internal ribosome entry sites (IRES) are located in the 5′ UTR and IGR. The genome has a small peptide covalently linked to the 5′ end (genome-linked virus protein, VPg) and a 3′ polyadenylated terminus.

### Species demarcation criteria in the family

The species demarcation criteria are:

- Natural host range: species can be differentiated on the basis of their natural host range and their relative ability to replicate in a range of cultured insect cells.
- Serology: species are serologically distinct.
- Sequence identity between the capsid proteins of isolates and strains of a species is above 90%.

## GENUS *CRIPAVIRUS*

Type species ***Cricket paralysis virus***

### Distinguishing features

The typical IGR-IRES elements of cripaviruses possess a conserved bulge sequence (UGAUCU and UGC) in the 5′ region (Figure 3). There is no additional stem loop presented in the 3′ region of the IGR IRES, as seen in aparaviruses.

### Biological properties

Cricket paralysis virus has the widest host range among members of the genus and can infect insect species classified in the orders *Diptera*, *Lepidoptera*, *Orthoptera*, *Hemiptera* and *Hymenoptera*, as well as a diverse range of cultured insect cells. Other viruses have host ranges restricted to families of only one insect order. Drosophila C virus has been isolated from dipteran species. Black queen cell virus has been isolated from hymenopteran species. Aphid lethal paralysis virus, Himetobi P virus, Homalodisca coagulata virus-1, Plautia stali intestine virus, Rhopalosiphum padi virus (RhPV) and Triatoma virus (TrV) have been isolated from different families within the *Hemiptera*. TrV infects *Triatoma infestans*, a major vector of Chagas' disease of humans. RhPV can be transmitted vertically between aphids and horizontally via the plant, using the plant as a passive reservoir. RhPV does not replicate in the plant.

### List of species in the genus *Cripavirus*

| | | |
|---|---|---|
| *Aphid lethal paralysis virus* | | |
| Aphid lethal paralysis virus | [AF536531] | (ALPV) |
| *Black queen cell virus* | | |
| Black queen cell virus | [AF183905] | (BQCV) |
| *Cricket paralysis virus* | | |
| Cricket paralysis virus | [AF218039] | (CrPV) |
| *Drosophila C virus* | | |
| Drosophila C virus | [AF014388] | (DCV) |
| *Himetobi P virus* | | |
| Himetobi P virus | [AB017037] | (HiPV) |
| *Homalodisca coagulata virus-1* | | |
| Homalodisca coagulata virus-1 | [DQ288865] | (HoCV-1) |
| *Plautia stali intestine virus* | | |
| Plautia stali intestine virus | [AB006531] | (PSIV) |
| *Rhopalosiphum padi virus* | | |
| Rhopalosiphum padi virus | [AF022937] | (RhPV) |
| *Triatoma virus* | | |
| Triatoma virus | [AF178440] | (TrV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Cripavirus* but have not been approved as species

None reported.

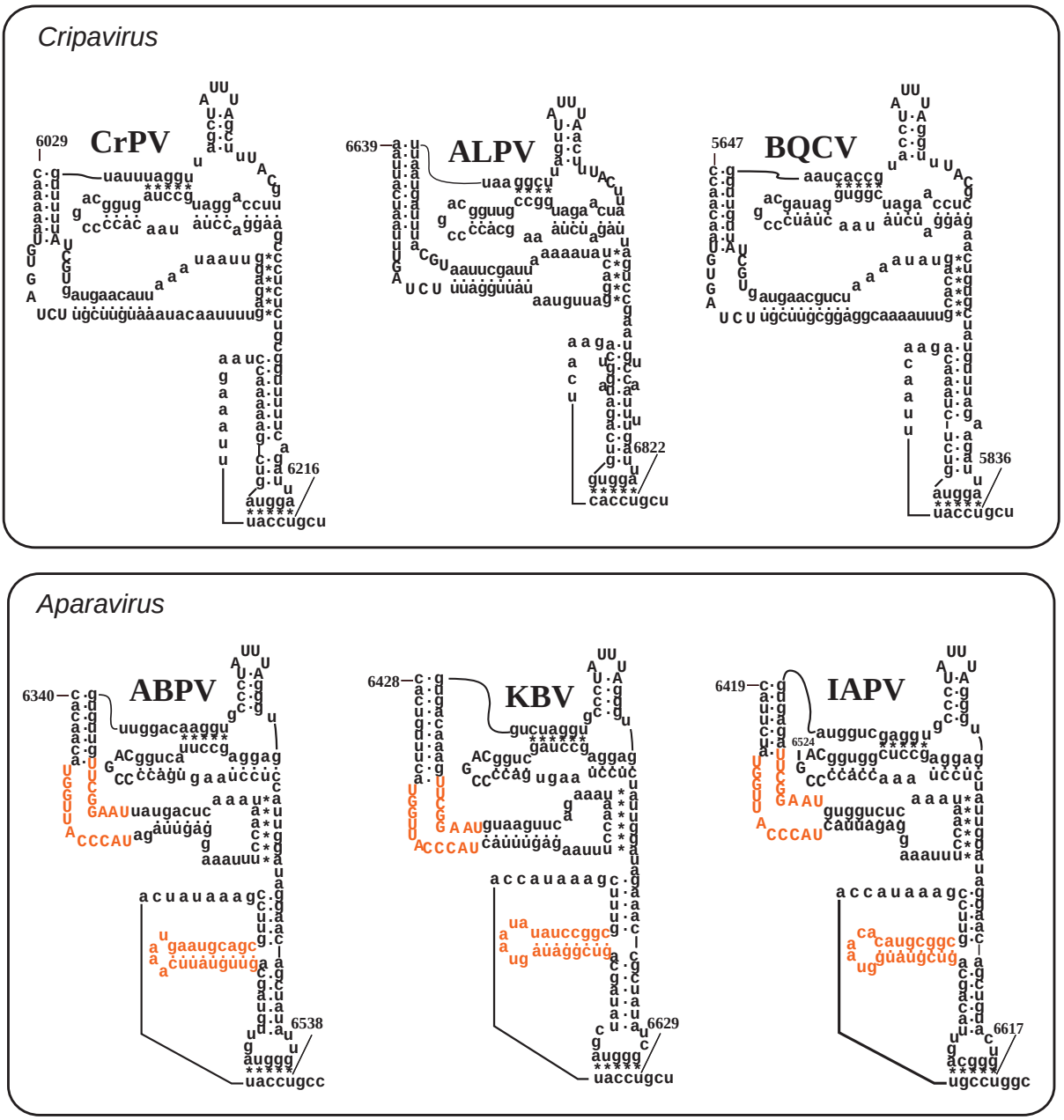


**Figure 3:** Comparison of IGR-IRES structural elements between cripaviruses and aparaviruses. The cripaviruses possess a conserved bulge sequence (UGAUCU and UGC) in the IGR IRES while the aparaviruses possess a bulge sequence (UGGUUACCCAU and UAAGGCUU). Additional stem loop structures and nucleotide sequences in the bulge regions are present in aparaviruses (indicated in orange).

## Genus *Aparavirus*

Type species *Acute bee paralysis virus*

### Distinguishing features

The members of the genus have distinguishing bulge sequence (UGGUUACCCAU and UAAGGCUU) in the region of the IGR IRES (Figure 3) and an additional stem loop in the 3′ region.

## Biological properties

Except for TSV isolated from penaeid shrimps, all members of the genus infect hymenopteran species. Acute bee paralysis virus (ABPV), Israeli acute paralysis virus (IAPV) and Kashmir bee virus (KBV) are pathogens of honey bees. Consequently, these viruses can have significant detrimental effects on agricultural production by reducing pollination by honey bees. As a result of intensive international trade of honey bees and hive-associated products, these viruses have a global distribution. The parasitic mite *Varroa destructor*, is an effective vector for transmission of ABPV, IAPV and KBV to honey bees. Solenopsis invicta virus-1 was isolated from the red imported fire ant.

## List of species in the genus *Aparavirus*

| | | |
|---|---|---|
| *Acute bee paralysis virus* | | |
| Acute bee paralysis virus | [AF150629] | (ABPV) |
| *Israeli acute paralysis virus* | | |
| Israeli acute paralysis virus | [EF219380] | (IAPV) |
| *Kashmir bee virus* | | |
| Kashmir bee virus | [AY275710] | (KBV) |
| *Solenopsis invicta virus-1* | | |
| Solenopsis invicta virus-1 | [AY634314] | (SINV-1) |
| *Taura syndrome virus* | | |
| Taura syndrome virus | [AF277675] | (TSV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

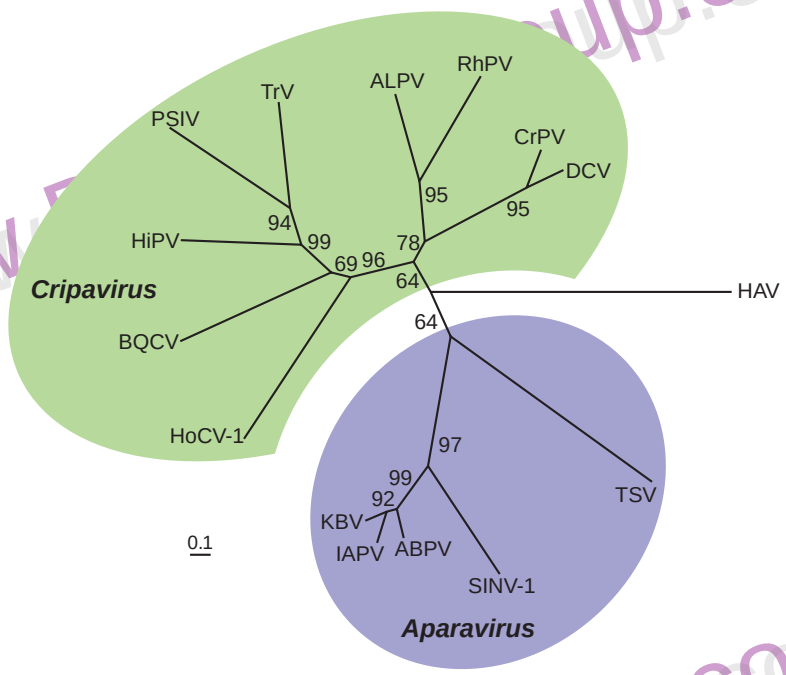


**Figure 4:** Neighbor-joining tree constructed from an alignment of the deduced amino acid sequence of structural proteins of dicistroviruses. Deduced amino acid sequence for capsid protein precursor of Hepatitis A virus (HAV) was used as an outgroup. Cripaviruses: ALPV, aphid lethal paralysis virus, AF536531; BQCV, black queen-cell virus, AF183905; CrPV, cricket paralysis virus, AF218039; DCV, Drosophila C virus, AF014388; HiPV, Himetobi P virus, AB017037; HoCV-1, Homalodisca coagulata virus-1, DQ288865; PSIV, Plautia stali intestine virus, AB006531; RhPV, Rhopalosiphum padi virus, AF022937; TrV, Triatoma virus, AF178440. Aparaviruses; ABPV, acute bee paralysis virus, AF150629; IAPV, Israeli acute paralysis virus, EF219380; KBV, Kashmir bee virus, AY275710; SINV-1, Solenopsis invicta virus-1, AY634314; TSV, Taura syndrome virus, AF277675.

## List of other related viruses which may be members of the genus *Aparavirus* but have not been approved as species

None reported.

## Phylogenetic relationships within the family *Dicistroviridae*

Phylogenetic analysis of deduced amino acid sequences of capsid protein precursors indicates that cripaviruses and aparaviruses form distinct groups in the family (Figure 4).

## Similarity with other taxa

Members in the family *Dicistroviridae* have similarity to viruses in the *Iflaviridae*, *Picornaviridae*, *Marnaviridae* and *Secoviridae*. The genomes of viruses of these taxa are positive sense ssRNAs with a VPg and a poly (A) tail, and are translated into autoproteolytically processed polyprotein(s). Nonstructural proteins contain sequence motifs for helicase (Hel), 3C-like cysteine proteinase (Pro) and RNA-dependent RNA polymerase (RdRp) with the characteristic gene order: (Hel)-(Pro)-(RdRp). Virions contain capsid proteins organized in a module containing three related jelly-roll domains which form non-enveloped, isometric particles of pseudo T = 3 symmetry and 30 nm diameter.

## Derivation of names

*Dicistro*: from the characteristic di-cistronic arrangement of the genome.
*Cripa*: from the name of the type member of the genus, *Cricket paralysis virus*
*Apara*: from the type member of the genus *Acute bee paralysis virus*.

## Further reading

Bonning, B.C. and Miller, W.A. (2010). Dicistroviruses. *Annu. Rev. Entomol.*, **55**, 129–150.

Gall, O.L., Christian, P., Fauquet, C.M., King, A.M.Q., Knowles, N.J., Nakashima, N., Stanway, G. and Gorbalenya, A.E. (2008). *Picornavirales*, a proposed order of positive-sense single-stranded RNA viruses with a pseudo-T = 3 virion architecture. *Arch. Virol.*, **153**, 715–727.

## Contributed by

Chen, Y.P., Nakashima, N., Christian, P.D., Bakonyi, T., Bonning, B.C., Valles, S.M. and Lightner, D.

# FAMILY *IFLAVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Iflaviridae* |
| Genus | *Iflavirus* |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# GENUS *IFLAVIRUS*

Type species *Infectious flacherie virus*

## Virion properties

### MORPHOLOGY

Virions are roughly spherical and exhibit icosahedral symmetry with a diameter of 26–30 nm. Virions have no envelope and no distinctive surface structures (Figure 1).

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virions have a buoyant density of between 1.33 and 1.38 g cm$^{-3}$.

### NUCLEIC ACID

Virions contain one molecule of linear, positive sense, single stranded RNA. The genome has a size ranging from 8800–10,100 nt and contains a single large ORF encoding a polyprotein of 2858–3085 amino acids. A small genome-linked virus protein, VPg is linked covalently to the 5′ end of the genomic RNA. The untranslated regions (UTRs) flanking both ends of the ORF vary in size by species. The 5′-UTR of infectious flacherie virus (IFV) and sacbrood virus (SBV) (130–180 nt) is significantly shorter than that of the other iflaviruses (400–1200 nt).

### PROTEINS

All proteins arise by proteolytic cleavage of a single polyprotein. Mature virions contain three major structural proteins (VP1, VP2 and VP3) generally between 28 and 44 kDa. A fourth smaller capsid protein (VP4) of around 4–12 kDa has been reported in some species and is located in the second

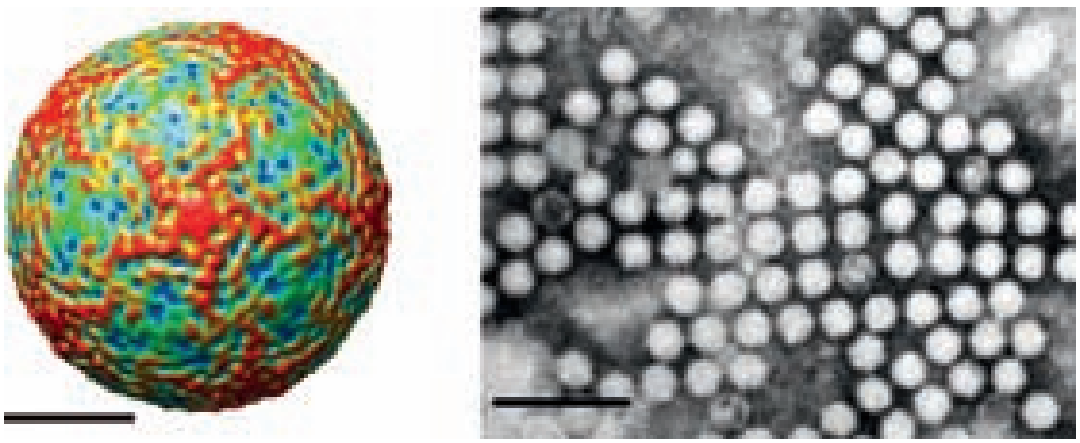

**Figure 1:** (Left) The outer surface view of the virion of infectious flacherie virus (IFV) along a five-fold axis reconstructed by cryo-electron microscopy. The bar represents 10 nm (courtesy of J. Hong). (Right) Negative contrast electron micrograph of isometric particles of an isolate of IFV. The bar represents 100 nm (courtesy of H. Bando).

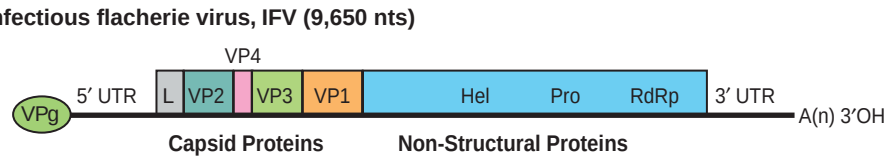


**Figure 2:** Genome structure of infectious flacherie virus (IFV). The genome encodes a single polyprotein that is autocatalytically cleaved into three major structural proteins (VP2, VP3 and VP1) and the non-structural proteins. The 5′ end of the genome carries a covalently linked protein, VPg, which plays an important role in RNA replication and the 3′ end of the genome is polyadenylated. The structural proteins are encoded at the 5′ end of the polyprotein and the non-structural proteins at the 3′ end. The capsid proteins, arranged in the order VP2-VP4-VP3-VP1, are preceded by a short leader protein (L). The approximate positions of the helicase (Hel), protease (Pro) and RNA-dependent RNA polymerase (RdRp) domains in the non-structural protein are shown.

position of the capsid precursor coding region. Minor quantities of the uncleaved precursors have been reported in some species.

### Lipids
None reported.

### Carbohydrates
None reported.

## Genome organization and replication

The genome is monopartite and contains a large ORF with genes encoding capsid proteins at the 5′ end and genes encoding the non-structural proteins at the 3′ end of the genome. The capsid proteins, arranged in the order of VP2-VP4-VP3-VP1, are preceded by a short leader protein (L) that is removed from VP2 before capsid assembly and has no known function. VP4 is analogous to VP4 present in some dicistroviruses and in the case of IFV is present as a minor structural component of the capsid. The non-structural proteins include a RNA helicase, a 3C-like cysteine protease, and a RNA-dependent RNA polymerase (5′ to 3′ orientation as illustrated in Figure 2). It is unclear if there are any conserved RNA secondary structures in the UTRs.

The viral RNA is infectious and serves as both genomic and viral mRNA. The replication of viruses occurs in the cytoplasm of infected cells but the mechanism of viral genomic RNA entry into the host cell cytoplasm is unknown. The genomic RNA is translated into a polyprotein which is autocatalytically cleaved into structural and non-structural component peptides. For three members in the family (Ectropis obliqua virus (EoV), Perina nuda virus (PnV) and Varroa destructor virus-1 (VDV-1)), there is evidence to suggest that translation of the polyprotein is mediated by an IRES. Mechanisms of polyprotein processing and the effects on host cell macromolecular synthesis during infection have not been well studied for the members of this family.

## Antigenic properties

Honeybee viruses in the genus are serologically distinct. There are no known serological relationships between other members of the genus.

## Biological properties

All member viruses have been isolated from arthropod species and appear to have restricted host ranges. IFV is known from the lepidopteran species *Bombyx mori* and *Glyphodes pyloalis*. PnV is known only from the lepidopteran *Perina nuda*. EoV infects the lepidopteran *Ectropis obliqua*. Deformed wing virus (DWV) and SBV are common viruses of the honey bee, *Apis mellifera*. VDV-1 was isolated from the honey bee parasitic mite, *Varroa destructor*. SBV and DWV are distributed globally likely from intensive international trade and transportation of bees and bee products. With the exception of PnV, which replicates in a cell line established from *Perina nuda*, there are no cell culture systems available for the propagation of other viruses in the group.

## Species demarcation criteria in the genus

The species demarcation criteria are:

- Natural host range: species can be differentiated on the basis of their natural host range.
- Sequence identity at the amino acid level between the CPs of isolates and strains of a species is above 90%.

## List of species in the genus *Iflavirus*

| | | |
|---|---|---|
| *Deformed wing virus* | | |
| Deformed wing virus-Italy | [AJ489744] | (DWV-IT) |
| Kakugo virus | [AB070959] | |
| *Ectropis obliqua virus* | | |
| (Ectropis obliqua picorna-like virus) | | |
| Ectropis obliqua virus | [AY365064] | (EoV) |
| *Infectious flacherie virus* | | |
| Infectious flacherie virus | [AB000906] | (IFV) |
| *Perina nuda virus* | | |
| (Perina nuda picorna-like virus) | | |
| Perina nuda virus | [AF323747] | (PnV) |
| *Sacbrood virus* | | |
| Sacbrood virus-Rothamsted | [AF092924] | (SBV-Roth) |
| *Varroa destructor virus-1* | | |
| Varroa destructor virus-1-Netherlands | [AY251269] | (VDV-1-NL) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Iflavirus* but have not been approved as species

| | | |
|---|---|---|
| Bee slow paralysis virus | [EU035616] | (BsPV) |
| Brevicoryne brassicae virus 1 | [EF517277] | (BrBV-1) |

## Phylogenetic relationships within the family

Phylogenetic analysis of the complete genomes and deduced amino acid sequence of capsid protein precursors indicate close genetic relationships between DWV and VDV-1 (nucleotide identities 79–89% and amino acid identities 89–98% in different regions of the genomes), reflecting a short evolutionary distance after the separation from a common ancestor. EoV and PnV also show a close genetic relatedness to each other. By contrast, the much larger genetic distances dividing these two pairs of species from each other, and from SBV and IFV, are more typical of the distances between different genera that are seen in some members of the order *Picornavirales*, e.g. the *Picornaviridae* and *Secoviridae* (Figure 3).

## Similarity with other taxa

The viruses in this family have a structure and genome arrangement similar to the viruses in the *Dicistroviridae*, *Picornaviridae*, *Marnaviridae* and *Secoviridae*. The RNA genome contains a single large ORF encoding the capsid proteins at the 5′ end and the non-structural proteins at the 3′ end. The 5′ end of the genome is covalently linked to a small peptide, VPg, which plays an important role in RNA replication. The replicases of iflaviruses resemble those of the dicistroviruses, picornaviruses, marnaviruses and secoviruses by containing sequence motifs typical of RNA helicase (Hel), a chymotrypsin-like 3C protease (Pro), and an RNA-dependent RNA polymerase (RdRp) in the 5′ to 3′ orientation. The RdRp domains are phylogenetically related (Figure 3). The virions are icosahedral with pseudo-T = 3 symmetry and a diameter about 30 nm.

## Derivation of name

*Ifla*: from the type species of the genus, *Infectious flacherie virus*.

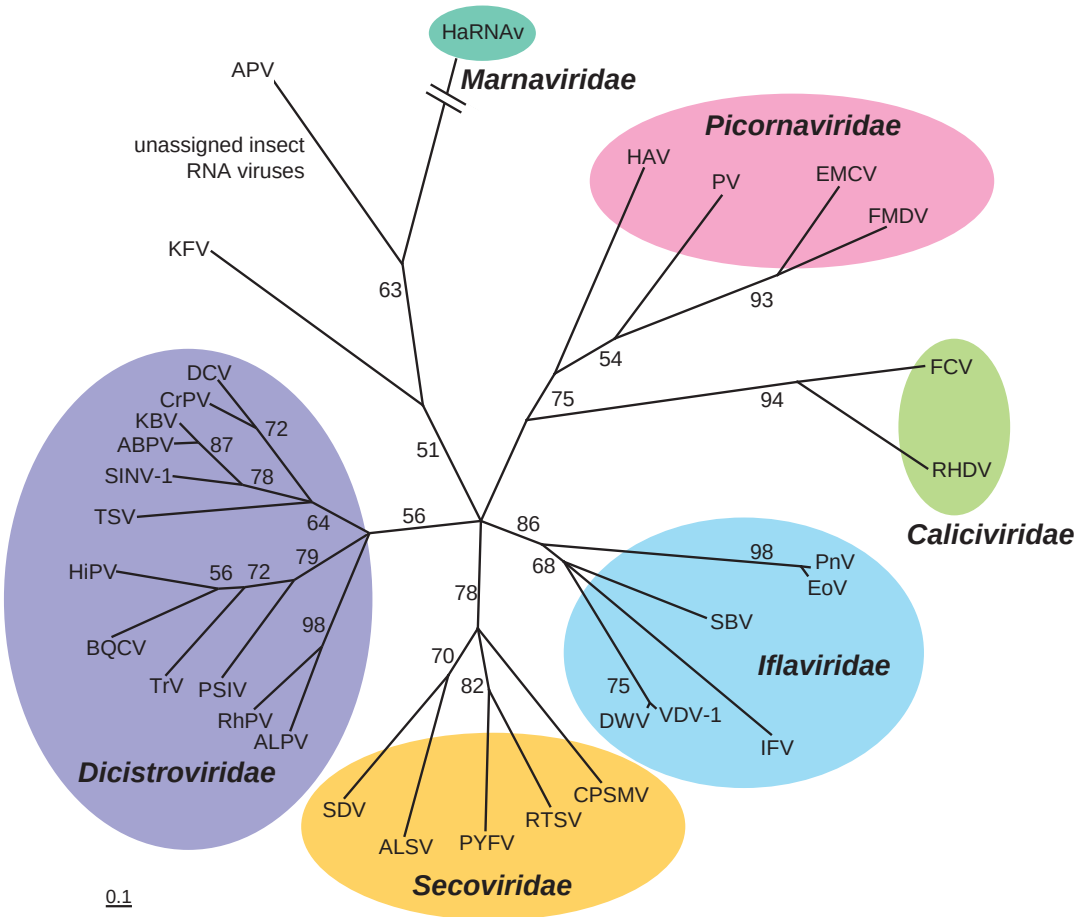


**Figure 3:** Unrooted phenogram derived from the RdRp domain of the viral non-structural proteins showing the relationships of viruses in the family *Iflaviridae* and other families of the order *Picornavirales* (*Picornaviridae*, *Dicistroviridae*, *Marnaviridae* and *Secoviridae*) and in the closely related family *Caliciviridae*. The branch length for HaRNAv is shortened to one-fourth because of the distant relationship of this virus. Taxa used (with virus name (abbr.) and accession number) were as follows. *Dicistroviridae*: acute bee paralysis virus (ABPV) AF150629, aphid lethal paralysis virus (ALPV) AF536531, black queen cell virus (BQCV) AF183905, cricket paralysis virus (CrPV) AF218039, Drosophila C virus (DCV) AF014388, Himetobi P virus (HiPV) AB017037, Kashmir bee virus (KBV) AY27571O, Plautia stali intestine virus (PSIV) AB006531, Rhopalosiphum padi virus (RhPV) AF022937, Solenopsis invicta virus-l (SINV-l) AY6343 14, Taura syndrome virus (TSV) AF277675, Triatoma virus (TrV) AFl78440. *Iflaviridae*: deformed wing virus (DWV) AY292384, ectropis obliqua virus (EoV) AY365064, infectious flacherie virus (IFV) AB000906, perina nuda virus (PnV) AF323747, sacbrood virus (SBV) AF092924, Varroa destructor virus 1 (VDV-1) AY251269. *Secoviridae*: parsnip yellow fleck virus (PYFV) D14066, rice tungro spherical virus (RTSV) M95497, cowpea severe mosaic virus (CPSMV) M83830, satsuma dwarf virus (SDV) AB009958, apple latent spherical virus (ALSV) AB030940. *Picornaviridae*: poliovirus (PV) VOl149, foot-and-mouth disease virus (FMDV) X00871, encephalomyocarditis virus (EMCV) M81861, hepatitis A virus (HAV) M14707. *Caliciviridae*: rabbit hemorrhagic disease virus (RHDV) M67473, feline calicivirus (FCV) M86379. *Marnaviridae*: Heterosigma akashiwo RNA virus (HaRNAv) AY337486. Unassigned insect RNA viruses; kelp fly virus (KFV) DQ112227, Acyrthosiphon pisum virus (APV) AF0245 14.

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## Contributed by

Chen, Y.P., Nakashima, N., Christian, P.D., Bakonyi, T., Bonning, B.C., Valles, S.M. and Lightner, D.

# Family *Marnaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Marnaviridae* |
| Genus | *Marnavirus* |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Marnavirus*

Type species ***Heterosigma akashiwo RNA virus***

## Virion properties

### Morphology

At present, the only characterized representative of the family *Marnaviridae* is Heterosigma akashiwo RNA virus (HaRNAV). Based on electron micrographs, HaRNAV virions are approximately 25 nm in diameter, polyhedral in shape, do not appear to have an envelope, and have no discernible projections (Figure 1).

### Physicochemical and physical properties

HaRNAV is not sensitive to chloroform.

### Nucleic acid

HaRNAV has an 8.6 kb ssRNA genome containing a single ORF. The genome has a poly(A) tail at the 3′ terminus. The 5′- and 3′-UTRs are 483 and 361 nt long, respectively, accounting for a total of 9.8% of the genome. Computer predictions (mfold 3.0) of secondary structure of the 5′-UTR and a notable pyrimidine-rich stretch of sequence upstream of the predicted start codon, suggest the presence of an IRES, a feature observed in many picorna-like viruses. The genome sequence has two large pseudo repeats in the 5′- and 3′-UTRs. A 136 nt sequence in the 5′-UTR shares 123 exact bases with a 137 nt sequence in the 3′ end. These repeated sequences may have some function in replication and/or translation.

### Proteins

The major structural proteins of HaRNAV are characterized in Table 1. The 33 and 29 kDa protein sequence revealed similarities to the VP3 proteins from members of the families *Dicistroviridae* and *Picornaviridae* and the VP1 proteins from the *Dicistroviridae*, respectively. Cleavage sites delineating the structural protein domains lack a recognizable pattern raising the possibility that there may be more than one protease involved in polyprotein processing.

### Lipids

Undetermined.

### Carbohydrates

Undetermined.

## Genome organization and replication

The map of protein domains within the predicted HaRNAV polyprotein sequence is shown in Figure 2. Domains were identified on the basis of similarities with conserved domains for other members of the order *Picornavirales* including helicases, RdRps, and CPs. HaRNAV also encodes an amino acid sequence that resembles the 3C cysteine proteinases of members of the family *Picornaviridae* and that is also related to the chymotrypsin-related serine protease catalytic domain. A VPg-like protein, characteristic of most picorna-like viruses, has not been identified.

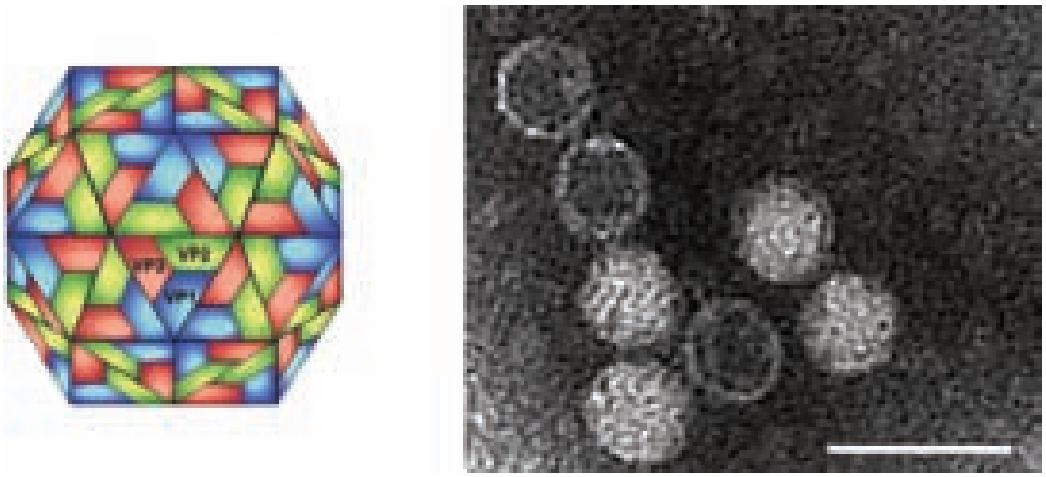

**Figure 1:** (Left) Diagrammatic representation of the possible structure of Heterosigma akashiwo RNA virus (HaRNAV) particles. (Right) Electron micrograph of HaRNAV particles stained with phosphotungstic acid. The bar represents 50 nm.

**Table 1:** Characteristics of major HaRNAV structural proteins

| Protein (kDa)[a] | Position of N-teminus in polyprotein[b] | Putative sequence at cleavage site[b] | Location on genome map (Figure 2) |
|---|---|---|---|
| 39 | 1990 | PTST-SEIV | 3 |
| 33 | 2318 | FVST-SEII | 5 |
| 29 | 2060 | LFGY-SRPP | 4 |
| 26 | 1776 | EKLL-TETL | 1 |
| 24 | 1810 | RPGE-VDGD | 2 |

[a]Based on SDS-PAGE.
[b]Based on N-terminal sequencing and genome sequence analysis.

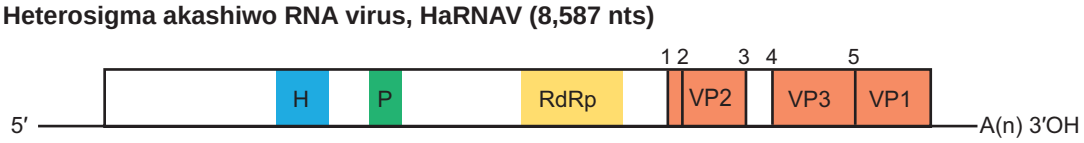


**Figure 2:** Representation of the genome organization of HaRNAV. The location of conserved picorna-like protein domains are indicated within the polyprotein box: H, helicase; P, protease; RdRp, RNA-dependent RNA polymerase; VP2, VP3 and VP1, structural proteins. The locations of N-termini found by sequencing the HaRNAV structural proteins are shown by black lines in the box and numbered to indicate the sequences at these processing sites, given in Table 1.

## Antigenic properties

Undetermined.

## Biological properties

The host range of HaRNAV is restricted to specific strains of *Heterosigma akashiwo*. Of 15 host strains isolated from the Northwest Pacific, Western Atlantic Ocean and Japanese coastal waters, five were permissive to HaRNAV infection. HaRNAV replication appears to be cytolytic. Cytopathic effects begin approximately 48 h after infection. Ultrastructural changes include swelling of the endoplasmic reticulum, vacuolation and disintegration of the cytoplasm, and the appearance of fibrous

material in vacuolated areas. Particles of HaRNAV are distributed in the cytoplasm in crystalline arrays or as individuals.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Marnavirus*

*Heterosigma akashiwo RNA virus*
Heterosigma akashiwo RNA virus SOG263 [AY337486] (HaRNAV-SOG263)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Marnavirus* but have not been approved as species

Closely related viruses have been identified in marine environments, although these were not isolated and studied directly. A degenerate RT-PCR approach targeting the RdRp gene found a number of related RdRp sequences that were different from the genome-sequenced strain of HaRNAV, SOG263. These RdRp sequences did have a high level of identity with HaRNAV and likely represented different strains of the virus.

## List of unassigned species in the family *Marnaviridae*

None.

## List of other related viruses which may be members of the family *Marnaviridae* but have not been approved as species

| | | |
|---|---|---|
| Chaetoceros socialis f. radians RNA virus | [AB469874] | (CsfrRNAV) |
| Chaetoceros tenuissimus RNA virus | [AB375474] | (CtenRNAV) |
| Rhizosolenia setigera RNA virus | [AB243297] | (RsRNAV) |

## Phylogenetic relationships within the family

Not applicable.

## Similarity with other taxa

The HaRNAV genome is composed of one molecule of positive sense ssRNA that exhibits the 2C-3C$^{pro}$-3D$^{pol}$ gene order, and the particles are icosahedral with a diameter of about 25 nm, criteria that are consistent with placing the family *Marnaviridae* within the order *Picornavirales*. However, the structure of the viral genome and the patterns of sequence relationships of HaRNAV proteins to those of other viruses within the *Picornavirales* clearly show that HaRNAV does not belong within any of the other established families. The HaRNAV genome structure is most like the potyviruses (e.g., tobacco etch virus) in that the non-structural protein domains are located at the N-terminus and the structural proteins are at the C-terminus in a single large polyprotein encoded on a monopartite genome. However, potyvirus capsids are filamentous and phylogenetic analyses demonstrated no significant relationship with this family (Figure 3). Moreover, a phylogenetic analysis of picorna-like RdRps does not place the HaRNAV sequence within any established family of picorna-like viruses (Figure 3).

HaRNAV appears to be most closely related to three unclassified viruses that infect species of marine diatoms, Chaetoceros socialis f. radians RNA virus (CsfrRNAV), Chaetoceros tenuissimus RNA virus (CtenRNAV) and Rhizosolenia setigera RNA virus (RsRNAV). HaRNAV, CsfrRNAV, CtenRNAV and RsRNAV have several characteristics in common including icosahedral

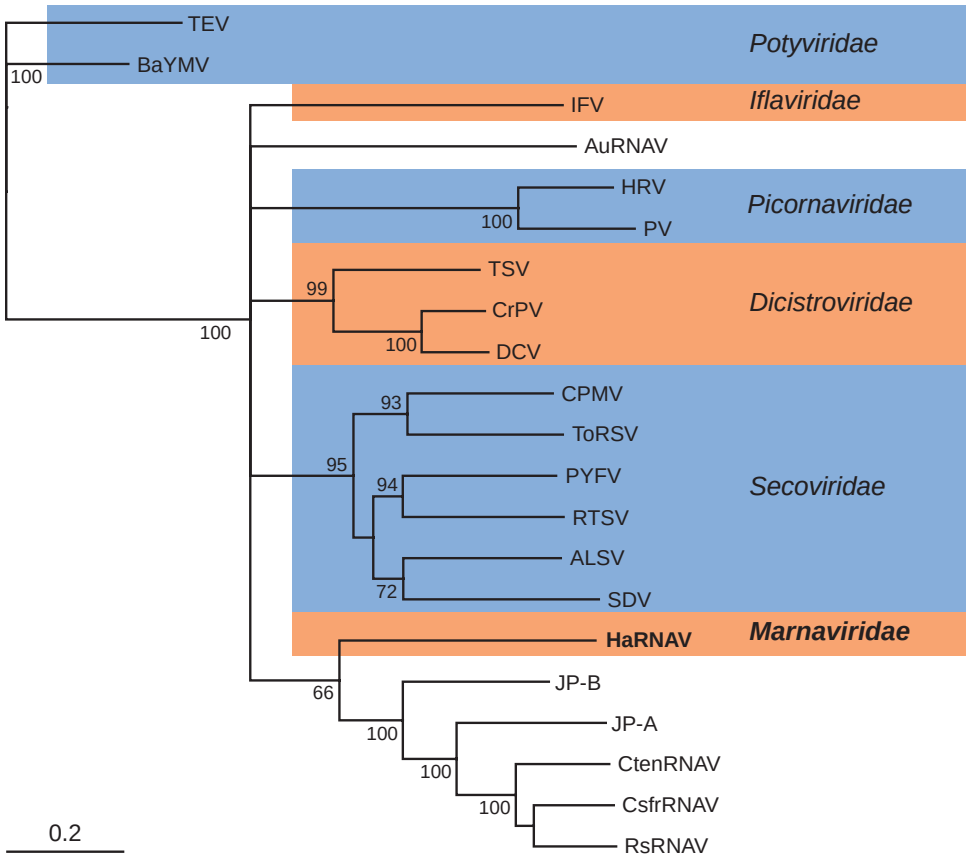


**Figure 3:** Bayesian maximum likelihood phylogenetic tree of RdRp sequences from the order *Picornavirales*. Residues 1362–1619 of the HaRNAV polyprotein that represent the conserved regions I-VIII and the corresponding regions from the other viruses included were aligned with DIALIGN-TX. Amino acid alignments were transformed into likelihood distances with Mr Bayes v3.1.2 using 1,100,000 generations. Bayesian clade credibility values are shown for relevant nodes. The Bayesian scale bar indicates a distance of 0.2. Taxonomic groups are indicated where known. TEV, tobacco etch virus; BaYMV, barley yellow mosaic virus; IFV, infectious flacherie virus; AuRNAV, Aurantiochytrium single stranded RNA virus; HRV, human rhinovirus; PV, poliovirus; TSV, taura syndrome virus; CrPV, cricket paralysis virus; DCV, Drosophila C virus; CPMV, cowpea mosaic virus; ToRSV, tomato ringspot virus; PYFV, parsnip yellow fleck virus; RTSV, rice tungro spherical virus; ALSV, apple latent spherical virus; SDV, satsuma dwarf virus; CtenRNAV, Chaetoceros tenuissimus RNA virus; CsfrRNAV, Chaetoceros socialis f. radians RNA virus; RsetRNAV, Rhizosolenia setigera RNA virus. JP-A and JP-B are environmental sequences.

morphology, a positive sense single stranded genome with a poly(A) tail, the non-structural ORF gene order, the organization of the non-structural protein domains relative to the structural proteins, and all infect protistan hosts. However, whereas the three diatom virus genomes are dicistronic, the HaRNAV genome encodes a single polyprotein. Phylogenetic analysis based on an alignment of the RdRp suggests that HaRNAV clusters with, but is distantly related to, CsfrRNAV, CtenRNAV and RsRNAV (Figure 3). There is a proposal under consideration to create a new genus, named *Bacillarnavirus*, for these three diatom viruses. The genus would be unassigned in the order *Picornavirales*.

Metagenomic libraries and single-gene surveys targeting marine RNA viruses have uncovered a diverse group of RNA virus phylotypes that appear to be from viruses related to *Picornavirales*. The genomes JP-A and JP-B were assembled from reverse-transcribed whole-genome shotgun libraries constructed from virus communities harvested from the Strait of Georgia, British Columbia. The genomes of JP-A and JP-B are 9.2 and 8.8 kb in length, respectively, have poly(A) tails, are dicistronic, and have a similar organization to the genomes of RsRNAV, CsfrRNAV and CtenRNAV. JP-A and JP-B appear to be most closely related to the diatom viruses discussed above, nevertheless,

they belong to a clade that includes HaRNAV (Figure 3). Phylogenetic analysis shows that the vast majority of RNA virus RdRp sequences amplified from marine virus communities form a well-supported clade with homologous sequences from the *Picornavirales* that infect marine protists, including HaRNAV. However, with the exception of the nearly identical HaRNAV-like sequences discussed above, these environmental viral phylotypes appear to be most closely related to CsfrRNAV, CtenRNAV and RsRNAV. In general, the taxonomy of *Picornavirales* reflects the taxonomy of their hosts, therefore it is likely that close relatives of HaRNAV are absent because an RNA virus infecting a close relative of *Heterosigma akashiwo* has yet to be isolated. Nevertheless, the diverse number of RNA virus phylotypes with unknown hosts recovered from a limited number of samples intimates that the discovery of new members of the *Marnaviridae* is just a matter of time.

## Derivation of name

*Marna*: from Latin *mare*, "sea", and *RNA*.

## Further reading

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## Contributed by

Culley, A.I., Lang, A.S. and Suttle, C.A.

# Family *Picornaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Picornaviridae*** |
| Genus | ***Enterovirus*** |
| Genus | ***Cardiovirus*** |
| Genus | ***Aphthovirus*** |
| Genus | ***Hepatovirus*** |
| Genus | ***Parechovirus*** |
| Genus | ***Erbovirus*** |
| Genus | ***Kobuvirus*** |
| Genus | ***Teschovirus*** |
| Genus | ***Sapelovirus*** |
| Genus | ***Senecavirus*** |
| Genus | ***Tremovirus*** |
| Genus | ***Avihepatovirus*** |

## Virion properties

### Morphology

Virions consist of a capsid, with no envelope, surrounding a core of ssRNA. Hydrated native particles are 30 nm in diameter, but vary from 22 to 30 nm in electron micrographs due to drying and flattening during preparation. Electron micrographs reveal no projections on most picornaviruses, the virion appearing as an almost featureless sphere; however, kobuviruses, and possibly parechoviruses, show a surface structure that is distinct from small round structured viruses (astroviruses and caliciviruses) (Figure 1). The capsid is composed of 60 identical units (protomers), each consisting of three surface proteins, 1B, 1C and 1D, of 24–41 kDa, and, in most picornaviruses, an internal protein, 1A of 5.5–13.5 kDa; however, in some viruses 1AB (VP0) remains uncleaved. Total protomer is 80–97 kDa. Proteins 1A, 1B, 1C and 1D are also commonly named VP4, VP2, VP3 and VP1, respectively. Proteins 1B, 1C and 1D each possess a core structure comprising an eight-stranded β-sandwich ("β-barrel"). The β-barrels pack together in the capsid with T = l, pseudo T = 3, icosahedral symmetry. These structural features are shared by the other members of the order *Picornavirales*. Genera differ in the external loops that interconnect the β strands. These loops account for differences in surface relief of each genus (Figure 1) and in thickness of the capsid wall. Assembly occurs via pentameric intermediates (pentamer = five protomers). Proteins within each pentamer are held together by an internal network formed from the N-termini of the three major capsid proteins (CPs), the C-termini lying on the outer capsid surface. Empty capsids, which are produced by some picornaviruses, are very similar to virions, except that 1A and 1B are normally replaced by the uncleaved precursor, 1AB.

### Physicochemical and physical properties

Virion Mr is $8 \times 10^6$ to $9 \times 10^6$, $S_{20,w}$ is 140–165S (empty particle $S_{20,w}$ is 70–80S). Buoyant density in CsCl is 1.33–1.45 g cm$^{-3}$, depending on the genus. Some species are unstable below pH 7; many are less stable at low ionic strength than at high ionic strength. Virions are insensitive to ether, chloroform, or non-ionic detergents. Viruses are inactivated by light when grown with, or in the presence of photodynamic dyes such as neutral red or proflavin. Thermal stability varies with viruses as does stabilization by divalent cations.

### Nucleic acid

Virions contain one molecule of positive sense, ssRNA, 7–8.8 kb in size, and possessing a single long ORF. A poly(A) tail, heterogeneous in length, is located after the 3′-terminal heteropolymeric sequence. A small protein, VPg (ca. 2.2–3.9 kDa), is linked covalently to the 5′ terminus. The UTRs at both termini contain regions of secondary structure which are essential to genome function. The long 5′-UTR (0.5–1.5 kb) includes a 5′-terminal domain involved in replication (e.g. the poliovirus "clover-leaf") and an IRES of 220–450 nt upstream of the translational start site; most picornaviral IRES elements can be assigned to one of four types (I to IV), according to their secondary structure. Between the 5′-terminal domain and the IRES there may be one, or more, pseudoknots and/or a

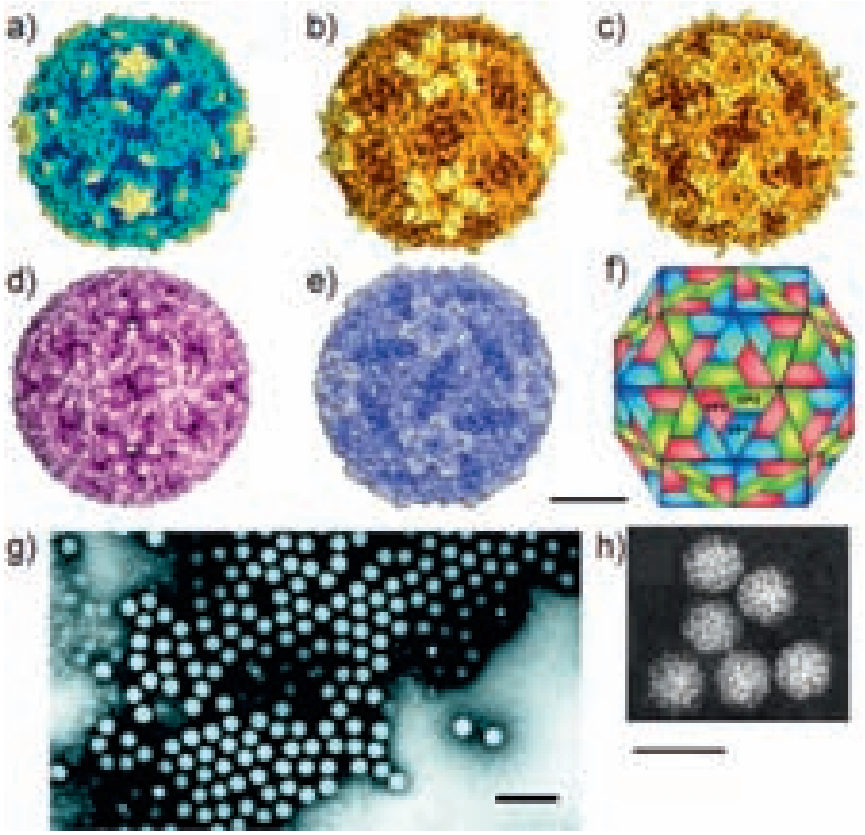


**Figure 1:** Pictures of picornavirus structures; (a) poliovirus 1 (2PLV); (b) Mengo (2MEV); (c) TMEV (1TME); (d) FMDV O (1FOD); (e) SVV (3CJI); the bar represents 10 nm (images courtesy of Jean-Yves Sgro, with permission). (f) Diagram of a picornavirus particle. The surface shows proteins VP1, VP2 and VP3. The fourth capsid protein, VP4, is located about the internal surface of the pentameric apex of the icosahedron. (g) Negative contrast electron micrograph of poliovirus (PV) particles; the bar represents 100 nm (courtesy of Ann C. Palmenberg). (h) Negative contrast electron micrograph of Aichi virus (genus *Kobuvirus*) showing surface structure; the bar represents 50 nm (courtesy of Teruo Yamashita).

poly(C) tract (Figure 2). The 3′-UTR, which may also contain a pseudoknot, ranges from 40 to 330 nt in length. The overall sequence identity between the genomes of viruses of different genera is typically less than 40%. The G + C content of picornavirus genomes ranges from 35 to almost 60%.

### Proteins

In addition to the major CPs, 1A, 1B, 1C and 1D, and 3B (VPg), described above, small amounts of 1AB (VP0) are commonly seen in lieu of one or more copies of 1A and 1B. Protein 1A is small in hepatoviruses, and 1AB is uncleaved in avihepatoviruses, kobuviruses, parechoviruses and a number of unclassified picornaviruses. Traces of other proteins, including the viral RdRp, $3D^{pol}$, may also be present in purified virus preparations.

### Lipids

Some picornaviruses carry a sphingosine-like molecule (“pocket factor”) in a cavity (“pocket”) located inside 1D. Protein 1A, where present, generally has a molecule of myristic acid covalently attached to the amino terminal glycine.

### Carbohydrates

None of the viral proteins is glycosylated.

## Genome organization and replication

The virion RNA is infectious and serves as both the genome and the viral mRNA. Gene maps are shown in Figure 3. Initiation of protein synthesis is stimulated by the IRES. Translation of the single ORF produces the polyprotein precursor 240–250 kDa to the structural proteins (derived from the

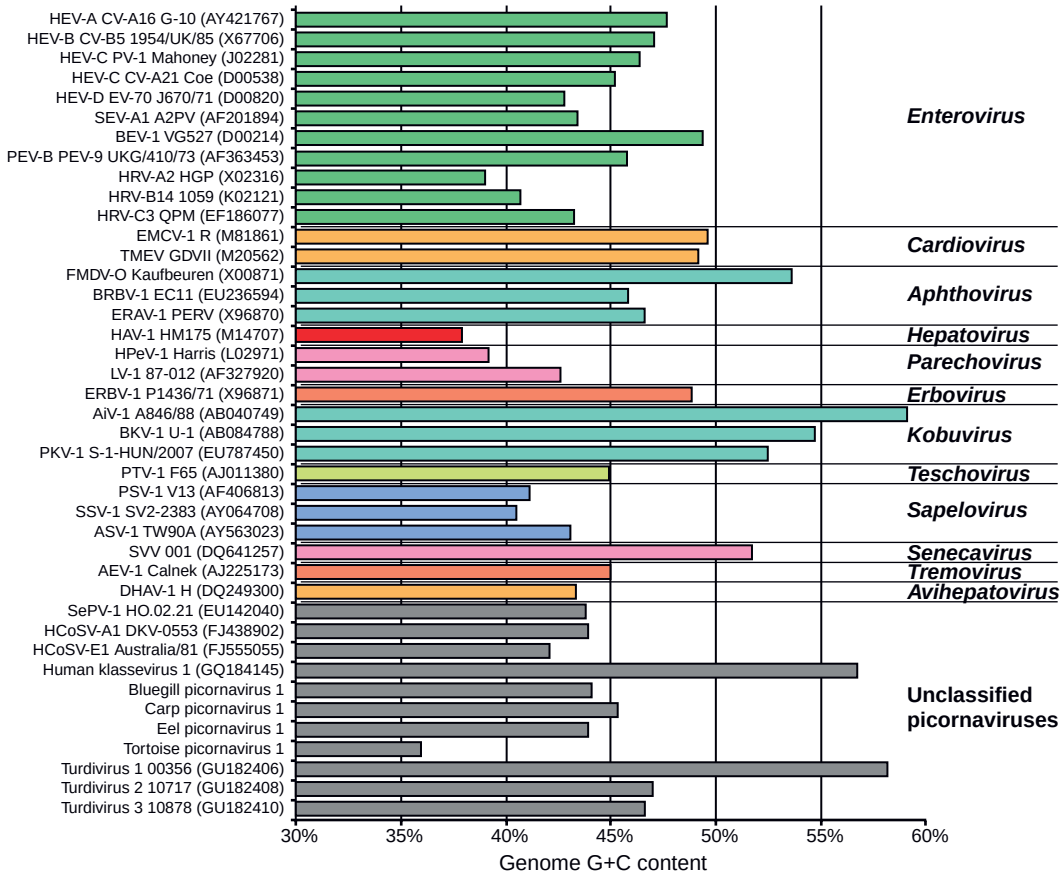


**Figure 2:** Percentage G+C content of picornavirus genomes.

P1 region of the genome) and the non-structural proteins (from the P2 and P3 regions). In some viruses P1 is preceded by a leader protein (L). The polyprotein is cleaved to functional proteins by specific proteases contained within it. Intermediates are denoted by letter combinations (e.g. 3CD, the uncleaved precursor of 3C and 3D). The viral proteases are as follows: $3C^{pro}$, a chymotrypsin-like cysteine protease encoded by all picornaviruses, performs most of the cleavages. In enteroviruses, and possibly sapeloviruses, 2A is also associated with proteolytic activity ($2A^{pro}$); the 2A of aphthoviruses, avihepatoviruses, cardioviruses, erboviruses, senecaviruses, teschoviruses and Ljungan virus (genus *Parechovirus*) contains a NPG↓P motif (↓ = cleavage site) and acts only *in cis*. The leader proteins of aphthoviruses and erboviruses have proteolytic activity ($L^{pro}$). Some intermediates are stable and serve functions distinct from those of their cleavage products (e.g. cleavage of poliovirus P1 by $3CD^{pro}$, not by $3C^{pro}$). Where it occurs, the cleavage of 1AB, which accompanies RNA encapsidation, is thought to be autocatalytic, but the precise mechanism is unknown.

A typical picornavirus genome layout may be represented by the following:

$$VPg{+}5'UTR[1A{-}1B{-}1C{-}1D\,/\,2A{-}2B{-}2C\,/\,3A{-}3B{-}3C{-}3D]3'UTR{-}poly(A)$$

Where "[" and "]" define the extent of the polyprotein-coding region, "/" represents primary cleavages and "−" represents the final cleavages. Where a particular polypeptide is present only in some members of the genus it can be shown between parentheses. This schema can also be used to indicate some protein functions or amino acid motifs where they differ between viruses (e.g. $2A^{pro}$ or $2A^{npgp}$ or $2A^{H\text{-}box/NC}$). There may be multiple copies of a particular genomic regions in the picornavirus genome, including repeated copies of one particular region (e.g. three 3Bs in the FMDV genome) or different types of a particular region (e.g. two different 2A motifs in Ljungan virus of the genus *Parechovirus* and three different 2A motifs in the duck hepatitis A virus genome of the genus *Avihepatovirus*).

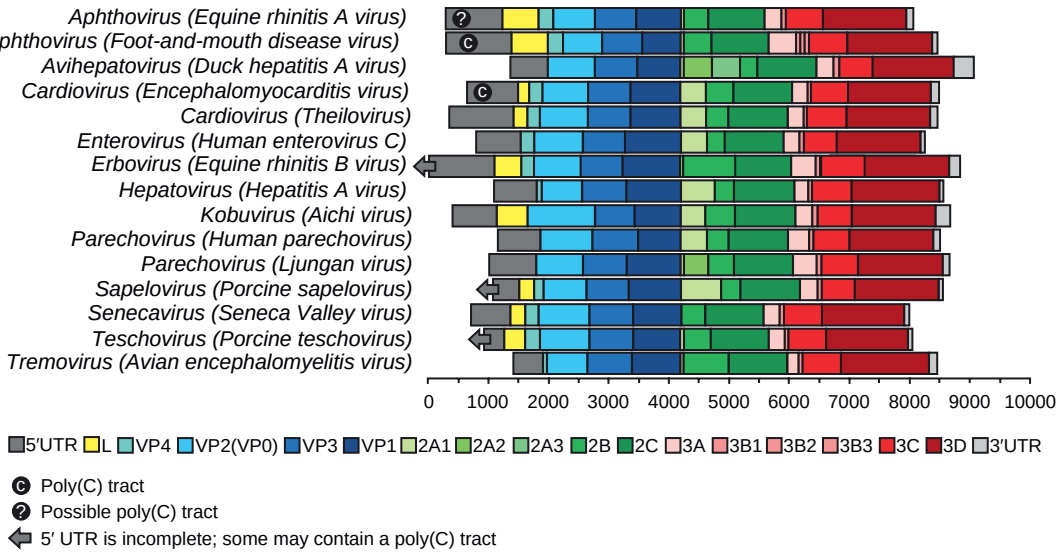


**Figure 3:** Genome structure and gene organization of members of the family *Picornaviridae*. Each of the 12 genera is represented, as are species where there is a significant difference within a genus. Circles within the 5′ UTR indicate poly(C) tracts that are present in some members. The 1A gene products of many members are myristylated at the amino terminal glycine. The 5′ UTR is followed by a long ORF encoding the polyprotein, that is in turn followed by the 3′ UTR and a poly(A) tail. The eventual cleavage products of the polyprotein are indicated by vertical lines and different shading. The nomenclature of the polypeptides follows an L:4:3:4 scheme corresponding to the genes (numbers) encoded by the L, P1, P2, P3 regions. The P1 region encodes the structural proteins 1A, 1B, 1C and 1D, also referred to as VP4, VP2, VP3 and VP1, respectively. VP0 (1AB) is the intermediate precursor for VP4 and VP2 and in avihepato-, kobu- and parechoviruses it remains uncleaved. In all viruses 3C is a protease, in enteroviruses 2A is a protease, while in all viruses 3D is considered to be a component of the RNA replicase. Only foot-and-mouth disease virus encodes 3 VPg proteins that map in tandem.

Replication of viral RNA occurs in complexes associated with cytoplasmic membranes. These complexes contain proteins derived from the whole of the 2BC-P3 region of the polyprotein, including the polymerase ($3D^{pol}$, an RNA chain-elongating enzyme), and 2C (an ATPase containing a nucleotide binding sequence motif). The poliovirus $3C^{pro}$ component has been shown to be required for binding to the 5′-terminal RNA cloverleaf. The short virus-encoded protein, VPg, acts as a transcription primer for both positive and negative strand RNA synthesis. Prior to transcription two uridine residues are covalently linked to the conserved tyrosine at position 3 in VPg to form $VPgpUpU_{OH}$ via a templating mechanism involving a *cis*-acting replication element (*cre*) and the virus 3D polymerase. The *cre* is a stem loop containing the sequence "AAAC" in the loop and is found at various places in the genome depending on virus species/genus. Many compounds that specifically inhibit replication have been described. Mutants resistant to, or dependent on, drugs have been reported. Genetic recombination, complementation, and phenotypic mixing occur. Defective particles, carrying deletions in the CPs or L, have been produced experimentally but have not been observed in natural virus populations.

## Antigenic properties

Serotypes are classified, depending on genus, by cross-protection, neutralization of infectivity, complement-fixation, specific ELISA using a capture format or immunodiffusion. Some serotypes can be identified using hemagglutination-inhibition. Antigenic sites, defined by mutations that confer resistance to neutralization by monoclonal antibodies, typically number 3 or 4 per protomer.

## Biological properties

Most picornaviruses are specific for one, or a very few host species [exceptions are foot-and-mouth disease virus (FMDV) and encephalomyocarditis virus (EMCV)]. Members of most species can be grown in cell culture. Resistant host cells (e.g., mouse cells in the case of the primate-specific polioviruses) can often be infected (for a single round) by transfection with naked, infectious RNA.

Transmission is horizontal, mainly by fecal–oral, fomite or airborne routes. Transmission by arthropod vectors is not known, although EMCV has been isolated from mosquitoes and ticks and poliovirus from flies; therefore mechanical transmission may be possible.

Infection is generally cytolytic, but persistent infections are common with some species and reported with others. Poliovirus infected cells undergo extensive vacuolation as membranes are reorganized into viral replication complexes. Infection may be accompanied by rapid inhibition of cap-dependent translation of cellular mRNAs ($2A^{pro}$ of poliovirus and $L^{pro}$ of aphthovirus are each powerful inhibitors), mRNA synthesis, and the cellular secretory pathway (poliovirus 2B and 3A have been implicated).

### Species demarcation criteria in the family

A picornavirus species is a class of phylogenetically-related serotypes or strains which would normally be expected to share (i) a limited range of hosts and cellular receptors, (ii) a significant degree of compatibility in proteolytic processing, replication, encapsidation and genetic recombination, and (iii) essentially identical genome maps. The polyprotein sequences of viruses in different genera differ by at least 58% aa identity. To distinguish virus names from species names where they have been the same the *Picornaviridae* Study Group recommends that viruses be assigned a type number, e.g. encephalomyocarditis virus will now be known as encephalomyocarditis virus 1 (EMCV-1); this currently affects some virus names in the following genera: *Cardiovirus*, *Aphthovirus*, *Hepatovirus*, *Kobuvirus*, *Sapelovirus*, *Senecavirus* and *Tremovirus*.

## GENUS *ENTEROVIRUS*

Type species ***Human enterovirus C***

### Virion properties

#### MORPHOLOGY

CPs 1B, 1C and 1D of the human enteroviruses and rhinoviruses are among the largest in the family (VP1-3 chain lengths, 238–302 aa), and this is reflected in the typically long inter-β-strand loops, the larger than average thickness of the capsid wall (46 Å), and a surface relief that is strongly pronounced compared to most other picornaviruses. Encircling a raised area at the five-fold axis is a 25 Å deep groove, or "canyon", into which the cellular receptor for poliovirus binds. The binding site for the pocket factor lies beneath the floor of this canyon within the 1D β-barrel. Virions can be converted by a variety of treatments (gentle heating, binding to receptor, or some neutralizing antibodies) to altered ("A") particles of 135S which lack 1A (VP4) and possess altered antigenicity.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Acid stability is variable. The virions of most enterovirus species are stable at pH 3.0, while those of the rhinovirus species are unstable below pH 5–6. Similarly, the buoyant density in CsCl of the enterovirus virions is 1.30–1.34 g cm$^{-3}$, while the rhinoviruses range from 1.38 to 1.42 g cm$^{-3}$. Sometimes a small proportion (about 1% of the population) of heavy particles (density: 1.43 g cm$^{-3}$) can be observed in the enteroviruses. Empty capsids are often observed in virus preparations.

#### NUCLEIC ACID

The genome contains a type I IRES and no poly(C) tract. The *cre* is located in 2C (HEV-A, HEV-B, HEV-C and HEV-D) or 2A (HRV-A) or 1D (HRV-B) or 1B (HEV-C). Sequence identities for different enteroviruses, or between enteroviruses and rhinoviruses, are more than 50% over the genome as a whole although it may be greater or less than this for particular genomic regions. The 5′-UTR of human rhinoviruses is shorter (ca. 650 nt) than that of enteroviruses, due to a deletion of approximately 100 nt between the IRES and the translation start site. Some members of HEV-C and HEV-D also have smaller deletions in this region. Bovine enteroviruses have a non-perfect duplication of the first ~100 nucleotides, allowing the formation of a second clover-leaf-like RNA structure. Porcine enteroviruses have an insertion of about 30 nt approximately 65 nt from the 5′ end of the genome resulting in a longer stem-loop D in the cloverleaf structure. Varying size deletions in the same region have been observed in some of the human enteroviruses.

## Genome organization and replication

Genome layout:

$$VPg{+}5'UTR[1A{-}1B{-}1C{-}1D/2A^{pro}{-}2B{-}2C/3A{-}3B{-}3C{-}3D]3'UTR{-}poly(A)$$

Genomes encode a single VPg and no L protein. Protease $2A^{pro}$, which is related to the family of small bacterial serine proteases, cleaves the polyprotein at its own N-terminus. Certain hydrophobic molecules that bind to the capsid in competition with pocket factor exert a powerful antiviral action by interfering with receptor binding and/or uncoating. Antiviral, pocket-binding drugs have been described.

## Antigenic properties

Native virions are antigenically serotype-specific (designated "N" or "D" for poliovirus), whereas "A" particles exhibit group specificity (designated "H" or "C" for poliovirus).

## Biological properties

Viruses multiply primarily in the gastrointestinal tract or the upper respiratory tract or sometimes both, but they can also multiply in other tissues, e.g., nerve, muscle, etc. Infection may frequently be asymptomatic. Clinical manifestations include common cold, mild meningitis, encephalitis, myelitis, myocarditis and conjunctivitis. Swine vesicular disease virus is a variant of coxsackievirus B5 and causes a vesicular disease in pigs clinically indistinguishable from foot-and-mouth disease (genus *Aphthovirus*). Cap-dependent translation of host mRNA is inhibited by $2A^{pro}$, which cleaves the host eukaryotic initiation factor 4G (eIF-4G). Many different cell surface molecules, many of them uncharacterized, serve as viral receptors. Well characterized receptor/virus interactions include poliovirus receptor (PVR) / polioviruses), coxsackievirus-adenovirus receptor (CAR)/coxsackie B viruses, intercellular adhesion molecule 1 (ICAM-l)/major-group rhinoviruses and some members of the *Human enterovirus C* species, low-density lipoprotein receptor (LDLR)/minor-group rhinoviruses, decay-accelerating factor (DAF)/various enteroviruses, integrin VLA-2/echovirus 1, and sialic acid/enterovirus D70.

## Species demarcation criteria in the genus

Members of a species of the genus *Enterovirus*:

- share greater than 70% aa identity in the polyprotein
- share greater than 60% aa identity in P1
- share greater than 70% aa identity in the non-structural proteins 2C + 3CD
- share a limited range of host cell receptors
- share a limited natural host range
- have a genome base composition (G+C) which varies by no more than 2.5%
- share a significant degree of compatibility in proteolytic processing, replication, encapsidation and genetic recombination.

## List of species in the genus *Enterovirus*

Certain viruses initially reported as novel echoviruses were later shown to have been misidentified. Thus E-8 is the same serotype as E-1, E-10 is now reovirus 1, E-28 is now human rhinovirus 1A, E-22 is now human parechovirus 1, E-23 is now human parechovirus 2. Similarly CV-A23 is the same serotype as E-9, and CV-A15 is the same serotype as CV-A11 and CV-A18 is the same as CV-A13. Hepatitis A virus 1 (HAV-1; genus *Hepatovirus*) was previously assigned the name enterovirus 72. Human rhinovirus 87 has been found to be a strain of EV-D68. A number of simian viruses (SV), previously listed as tentative members of the genus, have been moved to the genus *Sapelovirus*, species *Simian sapelovirus* and renamed simian sapelovirus (SSV) 1 (SV2), SSV-2 (SV 49) and SSV-3 (SV16, SV-18, SV42, SV44 and SV45). Simian agent 4 (SA4), SV4, SV28 and A2-plaque virus have been assigned to the species *Simian enterovirus A*. Simian enteroviruses N125 and N203

have been placed as a new type, EV-108, which is not yet assigned to a species. Similarly EV-103, SV6 and SV-47 also remain types unassigned to a species. Porcine enteroviruses (PEV) belonging to CPE group I (types 1–7 and 11–13) have been moved to the genus *Teschovirus* and renamed porcine teschovirus (PTV) 1–10. The species *Porcine enterovirus A* (PEV type 8; CPE group II) has been moved to the genus *Sapelovirus* and renamed *Porcine sapelovirus* (porcine sapelovirus 1).

| | |
|---|---|
| *Bovine enterovirus* | |
| Bovine enterovirus 1 [VG(5)27] | [D00214] |
| Bovine enterovirus 2 [BEV-261] | [DQ092770] |
| *Human enterovirus A* | |
| Coxsackievirus A2 [Fleetwood] | [AY421760] |
| Other types: CV-A3 to A8, CV-A10, CV-A12, CV-A14, CV-A16, enterovirus (EV) A71, EV-A76, EV-A89 to EV-A92, EV-A114, SV19, SV43, SV46, baboon enterovirus A13 | |
| *Human enterovirus B* | |
| Coxsackievirus B1 [Conn-5] [AJ295196] | |
| Other types: CV-B2 to CV-B6, CV-A9, echovirus (E) 1, E-2 to E7, E-9, E-11 to E-21, E-24 to E-27, E-29 to E-33, EV-B69, EV-B73 to EV-B74, EV-B75, EV-B77 to EV-B88, EV-B93, EV-B97, EV-B98, EV-B100, EV-B101, EV-B106, EV-B107, EV-B110, SA5 | |
| *Human enterovirus C* | |
| Poliovirus 1 [Mahoney] | [J02281] |
| Other types: PV-2, PV-3, CV-A1, CV-A11, CV-A13, CV-A17, CV-A19, CV-A20, CV-A21, CV-A22, CV-A24, EV-C95, EV-C96, EV-C99, EV-C102, EV-C104, EV-C105, EV-C109, EV-C113, EV-C116 | |
| *Human enterovirus D* | |
| Enterovirus D68 [Fermon] | [AY426531] |
| Other types: EV-D70, EV-D94, EV-D111 | |
| *Porcine enterovirus B* | |
| Porcine enterovirus 9 [UKG/410/73] | [AF363453] |
| Porcine enterovirus 10 [LP54/England/75] | [AF363455] |
| *Simian enterovirus A* | |
| Simian enterovirus A1 [SV4-1715 UWB] (SV4, SV28, SA4) | [AF326759] |
| *Human rhinovirus A* | |
| Human rhinovirus A1 [2060] | [FJ445111] |
| Other types: HRV-A2, HRV-A7, HRV-A8 to HRV-A13, HRV-A15, HRV-A16, HRV-A18 to HRV-A25, HRV-A28 to HRV-A34, HRV-A36, HRV-A38 to HRV-A41, HRV-A43 to HRV-A47, HRV-A49 to HRV-A51, HRV-A53 to HRV-A68, HRV-A71, HRV-A73 to HRV-A78, HRV-A80 to HRV-A82, HRV-A85, HRV-A88 to HRV-A90, HRV-A94 to HRV-A96, HRV-A98, HRV-A100 to HRV-A103 | |
| *Human rhinovirus B* | |
| Human rhinovirus B3 [FEB] | [DQ473485; |
| Other types: HRV-B4 to HRV-B6, HRV-B14, HRV-B17, HRV-B26, HRV-B27, HRV-B35, HRV-B37, HRV-B42, HRV-B48, HRV-B52, HRV-B69, HRV-B70, HRV-B72, HRV-B79, HRV-B83, HRV-B84, HRV-B86, HRV-B91 to HRV-B93, HRV-B97, HRV-B99 | EF173422] |
| *Human rhinovirus C* | |
| Human rhinovirus C1 [NAT001 ] | [EF077279] |
| Other types: HRV-C2 to HRV-C49 | |

Species names are in italic script. Beneath each species name is listed a representative isolate in roman script, with sequence accession number [ ]. The names of any additional strains are also listed, abbreviated as follows: bovine enterovirus (BEV); coxsackievirus (CV); echovirus (E); enterovirus (EV); human rhinovirus (HRV); poliovirus (PV); porcine enterovirus (PEV); simian agent (SA); simian virus (SV); simian enterovirus (SEV).
Full table available online on Science Direct®, www.sciencedirect.com.

## List of other related viruses which may be members of the genus *Enterovirus* but have not been approved as species

| | | |
|---|---|---|
| Enterovirus 103 [USA/GA99-POo-1] | [FJ007373] | (EV-103) |
| Enterovirus 108 [N125] | [AF414372] | (EV-108) |
| Enterovirus 112 [BAN-11217] | | (EV-112) |
| Enterovirus 115 [BAN-11617] | | (EV-115) |
| Simian virus 6 [1631] | [AF326766] | (SV6) |
| Simian virus 47 [OM107] | | (SV47) |

# Genus *Cardiovirus*

Type species ***Encephalomyocarditis virus***

## Virion properties

### Morphology

Empty capsids are seen only rarely, if ever. When compared by mean wall thickness, surface unevenness and chain length of the major proteins, the cardiovirus capsid is intermediate between the enteroviruses and aphthoviruses. In place of a continuous, circular, canyon, seen in enteroviruses, is a five-fold repeated pit. There is no pocket factor.

### Physicochemical and physical properties

Virion buoyant density in CsCl is 1.33–1.34 g cm$^{-3}$. Virions are moderately stable to acidic pH.

### Nucleic acid

EMCV has a poly(C) tract of variable length (usually 80–250 nt) about 150 nt from the 5′-terminus of the viral RNA, while theilovirus isolates lack this feature. All EMCV members have two pseudoknots 5′ to their poly(C) tracts. The IRES is of type II. The *cre* is located in the 1B region of both cardiovirus species. The nt sequence identity over the entire genome for different species of the genus *Cardiovirus* is more than 50% (e.g. TMEV has 54% nt sequence identity to EMCV).

## Genome organization and replication

Genome layout:

$$\mathrm{VPg{+}5'UTR[L/1A{-}1B{-}1C{-}1D{-}2A^{npgp}/2B{-}2C/3A{-}3B{-}3C{-}3D]3'UTR{-}poly(A)}$$

The viral genome encodes a leader (L) protein which lacks proteolytic activity, unlike the L of aphthoviruses; thus L is cleaved from P1 by the virus encoded protease 3C. The 1D/2A junction is also cleaved by 3C$^{pro}$, rather than by 2A. The 2A protein causes polypeptide chain interruption, between P1-2A and downstream sequences at an essential sequence, NPG↓P.

## Antigenic properties

Four independent antigenic sites have been described. There is no evidence of an N-D conversion, nor of "A" particles. The species *Encephalomyocarditis virus* consists of a single serotype. However, the species *Theilovirus* consists of 12 genetic types, Theiler's murine encephalomyelitis virus (TMEV), Vilyuisk human encephalomyelitis virus (VHEV), thera virus (formerly named Theiler-like virus of rats), and Saffold virus (SAFV) types 1 to 9. There is no cross-neutralization between TMEV and VHEV; however, the antigenic relationships between these and thera and saffold viruses is presently not known.

## Biological properties

Encephalomyocarditis viruses have been isolated from over 30 host species, including mammals, birds and invertebrates. Clinical manifestations include encephalitis and myocarditis in mice and many other animals. TMEV can be divided into two biological subgroups which both infect mice; one causes an acute and fatal polioencephalomyelitis and the other causes a chronic persistent demyelinating infection of the white matter. VHEV was isolated (in mice) from a person suffering from a degenerative neurological disease (Vilyuisk encephalitis), however, since the virus was extensively passaged in mice during the 1950s it is not clear if it is of human or mouse origin. Thera virus has been isolated from clinically normal rats. Saffold viruses have been isolated from humans, especially children, and have been associated with both respiratory disease and gastroenteritis. Cardiovirus infection does not cause cleavage of the host eIF-4G. The cellular receptor used by EMCV to attach to murine vascular endothelial cells has been identified as VCAM-1. However, in human cell lines an as yet unidentified sialoglycoprotein(s) has been found. EMCV binds to human erythrocytes via

glycophorin A. Low-neurovirulence TMEVs use sialic acid to attach to mammalian cells, while glycosaminoglycan heparan sulphate is involved in the attachment of high-neurovirulence TMEVs.

### Species demarcation criteria in the genus

Members of a species of the genus *Cardiovirus*:

- share greater than 70% aa identity in the polyprotein
- share greater than 60% aa identity in P1
- share greater than 70% aa identity in 2C + 3CD
- share a natural host range
- share a common genome organization.

### List of species in the genus *Cardiovirus*

| | | |
|---|---|---|
| *Encephalomyocarditis virus* | | |
| Encephalomyocarditis virus 1 [R] | [M81861] | (EMCV-1) |
| *Theilovirus* | | |
| Theiler's murine encephalomyelitis virus [GD VII] | [M20562, X56019] | (TMEV) |
| Other types: Vilyuisk human encephalomyelitis, thera virus, Saffold virus 1 to 9 | | |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Cardiovirus* but have not been approved as species

None reported.

## Genus *Aphthovirus*

Type species ***Foot-and-mouth disease virus***

### Virion properties

#### Morphology

The capsid of FMDV is thin-walled (mean thickness ca. 33 Å) and has an unusually smooth surface. A long (17–23 aa), mobile loop, the G-H loop, projects from the surface of 1D. There is a pore at the five-fold axis, where part of the underlying 1C is exposed. Some serotypes of FMDV accumulate empty capsids.

#### Physicochemical and physical properties

Virions are acid-labile; FMDV particles are unstable below pH 6.8; equine rhinitis A virus (ERAV) particles are unstable below pH 5.5. The buoyant density in CsCl is 1.43–1.45 g cm$^{-3}$. Virions of FMDV sediment at 146S, empty capsids at 75S.

#### Nucleic acid

There is a poly(C) tract close to the 5′ terminus of the genome. In FMDV it is located about 360 nt from the end, and varies in length from 100 to more than 400 nt. Current data suggest that the poly(C) tract in ERAV is shorter (ca. 40 nt) and closer to the 5′ end. In the RNA of FMDV there is a series of 3–4 pseudoknots on the 3′-side of the poly(C); in ERAV these pseudoknots are formed by perfectly repeated sequences each consisting of 21 bases; the total 5′-UTR is thus extremely long (1.1–1.5 kb). No pseudoknots have so far been identified in the bovine rhinitis viruses. The IRES is of type II. The FMDV *cre* is located in the 5′ UTR between the repeated pseudoknots and the IRES, but has not been identified for the other aphthovirus species. ERAV and FMDV differ by approximately 50% in nt sequence across the entire genome.

## Genome organization and replication

Genome layout:

$$VPg{+}5'UTR[L^{pro}/1A{-}1B{-}1C{-}1D{-}2A^{npgp}/2B{-}2C/3A{-}3B1{-}(3B2{-}3B3){-}3C{-}3D]3'UTR{-}poly(A)$$

Translation starts at two alternative in-frame initiation sites, resulting in two forms of the L protein (Lab and Lb). L is a papain-like cysteine protease which cleaves itself from the virus polyprotein. The 2A polypeptide is very short (chain length = 18 aa in FMDV), and is involved in NPGP-dependent polypeptide chain interruption at its C-terminus as in cardioviruses. The genome of FMDV encodes three species of VPg while those of ERAV, BRAV and BRBV encode only one.

## Antigenic properties

Five independent antigenic sites have been reported in FMDV type O, two of which have determinants in the G-H loop of 1D. There is no evidence of N-D conversion, nor "A" particles.

## Biological properties

This genus is comprised of viruses which primarily infect via the upper respiratory tract. FMDV infects mainly cloven-hoofed animals, but has been isolated from at least 70 species of mammals. Clinical manifestations of FMDV infections include foot-and-mouth disease (vesicular lesions), sometimes with associated acute fatal myocarditis in young animals. ERAV causes upper respiratory tract infections of horses, but may infect a number of other species including man. Bovine rhinitis A viruses (BRAV) and bovine rhinitis B virus (BRBV) infect the respiratory tract of cattle. FMDV and ERAV may produce persistent upper respiratory tract infections. FMDV infects cells by binding to integral membrane proteins of the integrin family through its 1D G-H loop; the principle integrin used is $\alpha_v\beta_6$. Heparan sulphate proteoglycans may also serve as receptors in cell cultures and at least one other unidentified receptor has been proposed. ERAV can use sialic acid to bind to cells. Cap-dependent translation of host mRNA is inhibited by $L^{pro}$, which cleaves the host eIF-4G.

## Species demarcation criteria in the genus

Members of a species of the genus *Aphthovirus*:

- share greater than 70% aa identity in the polyprotein
- share greater than 60% aa identity in P1
- share greater than 80% aa identity in 2C + 3CD
- share a natural host range
- have a genome base composition which varies by no more than 1%
- share a common genome organization.

## List of species in the genus *Aphthovirus*

| | | |
|---|---|---|
| *Bovine rhinitis B virus* | | |
| Bovine rhinitis B virus 1 [EC 11] (Bovine rhinovirus 2) | [EU236594] | (BRBV-1) |
| *Equine rhinitis A virus* | | |
| Equine rhinitis A virus 1 [PERV] (Equine rhinovirus 1) | [DQ272578, X96870] | (ERAV-1) |
| *Foot-and-mouth disease virus* | | |
| Foot-and-mouth disease virus O [UK/1/24 (OV1)] Other types: FMDV-A, FMDV-C, FMDV-SAT1, FMDV-SAT2, FMDV-SAT3, FMDV-Asia 1 | [AY593829] | (FMDV-O) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Aphthovirus* but have not been approved as species

| | |
|---|---|
| Bovine rhinitis A virus 1 [RS 3x] (Bovine rhinovirus 1) | (BRAV-1) |
| Bovine rhinitis A virus 2 [H-1] (Bovine rhinovirus 3) | (BRAV-2) |

## GENUS *HEPATOVIRUS*

Type species ***Hepatitis A virus***

### Distinguishing features

In contrast to those of other picornaviruses, protein 1A of hepatoviruses is extremely small, does not appear to be myristoylated at its N-terminus, and may not be a component of the mature virus particle. Immature HAV may contain uncleaved 1D2A (PX) precursor protein.

### Virion properties

#### MORPHOLOGY

No surface morphology is visible by EM.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Viruses are very stable, resistant to acid pH and elevated temperatures (60 °C for 10 min). Buoyant density in CsCl is 1.32–1.34 g cm$^{-3}$.

#### NUCLEIC ACID

There is little similarity between the genome sequences of hepatoviruses and those of other picornaviruses. Although the IRES is distantly related to the type II IRES, it has been designated as type III. The 5′-UTR contains a 5′-terminal hairpin, two putative pseudoknots, and a short (ca. 40 nt) pyrimidine-rich (i.e. not a pure poly-C) tract upstream of the IRES. The *cre* is located in the 3D region. Nucleotide sequence identity between different hepatitis A virus (HAV) strains is generally greater than 80%. The G+C content of HAV genomes is unusually low at about 38%.

### Genome organization and replication

Genome layout:

VPg+5′UTR[1A–1B–1C–1D–2A / 2B–2C / 3A–3B–3C–3D]3′UTR–poly(A)

The polyprotein contains only a single protease (3C$^{pro}$). There is no clearly defined L protein, and 2A has no proteolytic activity. The primary cleavage of the polyprotein occurs at the 2A/2B junction, and is catalyzed by 3C$^{pro}$. The 1D/2A cleavage may be directed by an unknown cellular protease, or the VP1 protein may be subject to C-terminal trimming as in cardioviruses. Replication in cell culture occurs slowly, with little CPE, and with low yields of virus compared to most other picornaviruses. The IRES differs from those of other picornaviruses in that its activity is dependent on intact eIF-4G.

### Antigenic properties

Hepatitis A viruses belong to a single serotype and are highly conserved in their antigenic properties. Most antibodies are directed against a single, conformationally defined immunodominant antigenic site that is comprised of aa residues of the VP3 and VP1 proteins on the surface of the virion.

### Biological properties

HAV infects epithelial cells of the small intestine and hepatocytes of primates. Virus is predominantly replicated within the liver, excreted via the bile and present in feces in high titer. Viral shedding is maximal shortly before the onset of clinical signs of hepatitis, which probably represents

immunopathologically-mediated liver injury. Clinical manifestations are fever, jaundice, light stools, abdominal pain, and occasionally diarrhea. HAV generally establishes a persistent infection when inoculated on to any of a wide range of primate cells *in vitro*, but persistent infection does not occur *in vivo*, and the viruses are not associated with chronic hepatitis. HAVs can be divided into two distinct biotypes that are phylogenetically distinct and have different preferred hosts (all species of primates: humans, chimpanzees, owl monkeys and marmosets, for one biotype, versus green monkeys and cynomolgus monkeys for the other). These two biotypes share cross-reacting antigens, but have biotype-specific epitopes that can be distinguished by monoclonal antibodies.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Hepatovirus*

*Hepatitis A virus*
Hepatitis A virus 1 [HM-175] [M14707] (HAV-1)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Hepatovirus* but have not been approved as species

None reported.

## Genus *Parechovirus*

Type species ***Human parechovirus***

### Distinguishing features

Predicted protein sequences of parechoviruses are highly divergent from other picornaviruses, no protein having a greater than 30% level of identity when compared with corresponding proteins of other genera. In contrast to most other picornaviruses, protein 1AB of parechoviruses appears not to be cleaved, and its N-terminus, also unusually, is not myristoylated. The mature capsid therefore appears to comprise only three proteins, 1AB, 1C and 1D.

### Virion properties

#### Morphology

No surface morphology is visible by EM.

#### Physicochemical and physical properties

Virions are acid-stable. The buoyant density in CsCl is 1.36 g cm$^{-3}$.

#### Nucleic acid

The 5′-UTR is 710–730 nt and contains a typical type II IRES. The *cre* has been identified in the 1AB region for human parechoviruses and is thought to lie within the 3B region of Ljungan viruses. The ORF is 2180/2250 codons and the 3′-UTR 87 and 111 nt in human parechovirus and Ljungan virus, respectively.

### Genome organization and replication

Genome layout:

$$VPg{+}5'UTR[1AB{-}1C{-}1D{-}(2A^{npgp})/2A^{H\text{-}box/NC}{-}2B{-}2C/3A{-}3B{-}3C{-}3D]3'UTR{-}poly(A)$$

The polyprotein contains only a single protease ($3C^{pro}$). The 2A protein is believed to lack protease activity and is related distantly to a family of cellular proteins involved in the control of cell proliferation, as well as to that of kobuviruses and tremoviruses. Ljungan virus possesses an NPGP motif following the predicted 1D polypeptide, suggesting the possible presence of a second 2A; however, it is believed that this short 2A-like sequence may form part of 1D.

## Antigenic properties

Human parechoviruses are divided into 14 genetic types and there is no cross-neutralization between types 1, 2 and 3. There may be a cross-reaction between types 2 and 5, however, the remaining 10 types have not been tested. Ljungan viruses are divided into four genetic types, but antigenic relationships have not been studied.

## Biological properties

Human parechoviruses replicate in the respiratory and gastrointestinal tract. Infection is particularly prevalent in young children but it is probably often asymptomatic. In addition to respiratory infections and diarrhea, infections of the central nervous system have occasionally been reported. HPeV types 1 and 6 have been found in monkeys with diarrhea, although disease association was not proven. The cytopathology may be unusual in including changes in granularity and chromatin distribution in the nucleus, when viewed in the electron microscope. Ljungan viruses appear to infect predominantly rodents (voles) and have been proposed to infect humans; however, conclusive data are awaited. Some human parechoviruses (types 1, 2, 4, 5 and 6) possess an RGD tri-peptide (towards the carboxy-terminus of 1D) which is involved in integrin receptor-binding. The integrins $\alpha_v\beta_3$ and $\alpha_v\beta_6$ have been shown to be the primary receptors for HPeV-1 in A549 cells. Receptor usage for the remaining human parechoviruses and Ljungan viruses is not known.

## Species demarcation criteria in the genus

Members of a species of the genus *Parechovirus*:

- share greater than 70% aa identity in the polyprotein
- share greater than 70% aa identity in P1
- share greater than 80% aa identity in 2C + 3CD
- share a natural host range
- share a common genome organization
- have a similar genome base composition which varies by no more than 1%.

## List of species in the genus *Parechovirus*

| | | |
|---|---|---|
| *Human parechovirus* | | |
| Human parechovirus 1 [Harris] | [L02971] | (HPeV-1) |
| (Human echovirus 22) | | |
| Other types: HPeV-2 to HPeV-16 | | |
| *Ljungan virus* | | |
| Ljungan virus 1 [87-012] | [AF327920] | (LV-1) |
| Other types: LV-2 to LV-4 | | |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

## List of other related viruses which may be members of the genus *Parechovirus* but have not been approved as species

None reported.

# Genus *Erbovirus*

Type species ***Equine rhinitis B virus***

## Virion properties

### Morphology

No surface morphology is visible by EM.

### Physicochemical and physical properties

Equine rhinitis B virus (ERBV) has a buoyant density in CsCl of 1.41–1.45 g cm$^{-3}$. pH stability is variable; ERBV-1 and ERBV-2 are labile below pH 5.0 while ERBV-3 is stable over a wide pH range (2.2–8.0). ERBV is rapidly inactivated by heating at 50 °C but divalent cations stabilize against thermal inactivation.

### Nucleic acid

ERBV possesses possibly the longest picornavirus genome, approaching 9 kb (the 5′ end has not been sequenced). The IRES is of type II, and a poly(C) tract is thought to be present. No pseudoknots have yet been identified within the 5′ UTR. The location of the *cre* has not been identified.

### Proteins

The CPs have between 25% and 47% aa sequence identity to those of ERAV, FMDV and EMCV, although protein modelling studies indicate that the capsid of ERBV more closely resembles that of EMCV.

## Genome organization and replication

Genome layout:

$$VPg{+}5'UTR[L^{pro}/1A{-}1B{-}1C{-}1D{-}2A^{npgp}/2B{-}2C/3A{-}3B{-}3C{-}3D]3'UTR{-}poly(A)$$

No evidence for alternative sites of initiation of protein synthesis is available, as is found in the aphthoviruses. The L protein appears to be a protease, but has only 23% and 18% aa sequence identity to the L proteins of FMDV and ERAV, respectively. The 2B and 3C proteins have exceptionally large chain lengths (283 and 251 aa). The 2A protein has a chain length of 18 aa, ending in NPG↓P, and there is only one VPg. The 3′-UTR is relatively long at 167 nt.

## Antigenic properties

ERBV consists of three serotypes, ERBV-1, -2 and -3.

## Biological properties

ERBV causes upper respiratory tract disease in horses, with a viremia and fecal shedding. Infections may be persistent.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Erbovirus*

*Equine rhinitis B virus*
Equine rhinitis B virus 1 [P1436/71] [X96871] (ERBV-1)
(Equine rhinovirus 2)
Other types: ERBV-2, ERBV-3

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Erbovirus* but have not been approved as species

None reported.

## GENUS *KOBUVIRUS*

Type species *Aichi virus*

### Distinguishing features

Protein 1AB appears not to be cleaved; however, a myristoylation signal (GxxxT) is present at the amino terminus of the polypeptide.

### Virion properties

#### MORPHOLOGY

Unlike other picornaviruses, kobuvirus capsids show a distinctive surface morphology when observed by electron microscopy (Figure 1h).

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virions are stable at pH 3.5.

#### NUCLEIC ACID

The genome of Aichi virus (AiV) has a high G+C base composition (59%), and a very long 3′-UTR (240 nt), however, the 3′-UTRs of bovine kobuvirus (BKV) and porcine kobuvirus (PKV) are shorter at 177 and 170 nt, respectively. In AiV-1, there is a 5′-proximal stem-loop involved in RNA replication and encapsidation. The IRES of AiV-1 and BKV-1 is of type II, however, PKV (which has not yet been assigned to a species) possesses a type IV IRES. The location of the *cre* has not been identified.

### Genome organization and replication

Genome layout:

$$VPg{+}5'UTR[L/1AB{-}1C{-}1D/2A^{H\text{-}box/NC}{-}2B{-}2C/3A{-}3B{-}3C{-}3D]3'UTR{-}poly(A)$$

There is a leader polypeptide of unknown function, and distinctive length (170–195 aa rather than 67 aa or 217 aa in EMCV and FMDV, respectively). The 2A protein contains an H-Box/NC motif and is distantly related to that of parechoviruses and tremoviruses.

### Antigenic properties

The two kobuvirus species, *Aichi virus* and *Bovine kobuvirus*, each consists of a single serotype.

### Biological properties

AiV grows in cell cultures (BSC-1, Vero). AiV is thought to be a cause of human gastroenteritis. BKV has been isolated from cattle and sheep. PKV has been isolated from pigs.

### Species demarcation criteria in the genus

Members of a species of the genus *Kobuvirus*:

- share greater than 70% aa identity in the polyprotein
- share greater than 70% aa identity in P1
- share greater than 80% aa identity in 2C + 3CD
- share a common genome organization.

### List of species in the genus *Kobuvirus*

| | | |
|---|---|---|
| *Aichi virus* | | |
| Aichi virus 1 [A846/88] | [AB040749] | (AiV-1) |
| *Bovine kobuvirus* | | |
| Bovine kobuvirus 1 [U-1] | [AB084788] | (BKV-1) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Kobuvirus* but have not been approved as species

| | | |
|---|---|---|
| Porcine kobuvirus 1 [swine/S-1-HUN/2007/Hungary] | [EU787450] | (PKV-1) |

## GENUS *TESCHOVIRUS*

Type species ***Porcine teschovirus***

### Virion properties

#### MORPHOLOGY

No surface morphology is visible by EM.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virions are stable at acid pH. Buoyant density in CsCl is 1.33 g cm$^{-3}$. Empty capsids are often observed in virus preparations.

#### NUCLEIC ACID

Teschoviruses have a type IV IRES about 290 nt in length which is functional in the absence of eIF-4G. In both these properties the IRES resembles that of hepatitis C virus (family *Flaviviridae*); sequence similarity has also been observed. The location of the *cre* is thought to be within the 2C region.

#### PROTEINS

Genomes encode a single VPg and a leader (L) protein. The 2A polypeptide is very short and ends in NPG↓P, indicative of an aphthovirus 2A-like molecule.

### Genome organization and replication

Genome layout:

$$\text{VPg}+5'\text{UTR}[\text{L}/1\text{A}-1\text{B}-1\text{C}-1\text{D}-2\text{A}^{\text{npgp}}/2\text{B}-2\text{C}/3\text{A}-3\text{B}-3\text{C}-3\text{D}]3'\text{UTR}-\text{poly(A)}$$

The genome layout is similar to that of the aphthoviruses, except that only a single VPg is present. The function of the leader polypeptide is unknown and is not predicted to have proteolytic activity.

### Antigenic properties

Porcine teschoviruses are divided into 11 serotypes (PTV-1 to -11) which are distinct in cross-neutralization tests and can be differentiated using their 1D (VP1) sequences.

### Biological properties

Clinical manifestations may include a polioencephalomyelitis ("Teschen/Talfan disease", also known as teschovirus encephalomyelitis), which may vary in severity. The viruses have been associated with a number of disease syndromes, including reproductive and gastrointestinal disorders. The pig is the only known host.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Teschovirus*

*Porcine teschovirus*
Porcine teschovirus 1 [Talfan] [AF231769, AB038528] (PTV-1)
(Porcine enterovirus 1)
Other types: PTV-2 to PTV-11

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Teschovirus* but have not been approved as species

None reported.

## GENUS *SAPELOVIRUS*

Type species ***Porcine sapelovirus***

### Distinguishing features

Sapeloviruses are most closely related to members of the *Enterovirus* genus, but possess a leader polypeptide of unknown function. The 2A polypeptide may be a cysteine protease, but it is distinct from that of the enteroviruses. The 2B and 3A proteins are also very different from the enteroviruses. In all three sapelovirus species the IRES is type IV, whereas in all enteroviruses species it is type I.

### Virion properties

#### MORPHOLOGY

No surface morphology is visible by EM.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Viruses are very stable, resistant to acid pH and elevated temperatures (60 °C for 10 min). Buoyant density in CsCl is 1.32–1.34 g $cm^{-3}$.

#### NUCLEIC ACID

The IRES of all three sapelovirus species is type IV. The location of the *cre* has not been identified.

### Genome organization and replication

Genome layout:

VPg+5′UTR[L/1A−1B−1C−1D/2A−2B−2C/3A−3B−3C−3D]3′UTR−poly(A)

The predicted leader polypeptide of avian sapelovirus (ASV) is the longest known at 451 aa and suspected to be a trypsin-like protease. The leader polypeptide porcine sapelovirus (PSV) and simian sapelovirus (SSV) are much shorter at 84 aa and 88 aa, respectively. The 2A region of ASV is predicted to be mostly deleted with a residual 12 aa remaining. The 2A polypeptides of PSV and SSV are longer at 226 aa and 302 aa, respectively; these are both suspected to be a protease. The L/VP0 cleavage and presence of a potential myristoylation site on VP0 is predicted for ASV (kQ/GqvqS). However, the precise L/VP0 cleavage for PSV or SSV is not clear. The sequences in this region are quite well conserved between the three viruses but it would require unusual cleavages in PSV (qL/GqvhS) and SSV (qC/GqvqS) to generate a myristoylation signal. VP0 is cleaved to VP4 and VP2 as shown by N-terminal sequencing of the VP2 polypeptide for ASV and SSV.

### Antigenic properties

Porcine sapelovirus consists of a single antigenically diverse serotype, PSV-1. Avian sapelovirus also consists of a single serotype, ASV-1. Simian sapelovirus consists of three genetically-defined types, SSV-1 to SSV-3.

### Biological properties

Pigs, monkeys and ducks are the only known hosts for porcine, simian and avian sapeloviruses, respectively.

### Species demarcation criteria in the genus

Members of a species of the genus *Sapelovirus* have:

- share greater than 70% aa identity in the polyprotein
- share greater than 64% aa identity in P1
- share greater than 70% aa identity in 2C + 3CD
- a defined tissue tropism and host range
- a similar genome base composition which varies by no more than 1%
- a common genome organization.

### List of species in the genus *Sapelovirus*

| | | |
|---|---|---|
| *Porcine sapelovirus* | | |
| Porcine sapelovirus 1 [V13] (Porcine enterovirus 8) | [AF406813] | (PSV-1) |
| *Simian sapelovirus* | | |
| Simian sapelovirus 1 [SV2-2383] (Simian virus 2) | [AY064708] | (SSV-1) |
| Other types: SSV-2, SSV-3 | | |
| *Avian sapelovirus* | | |
| Avian sapelovirus 1 [TW90A] (Duck picornavirus TW90A) | [AY563023] | (ASV-1) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Sapelovirus* but have not been approved as species

None reported.

## GENUS *SENECAVIRUS*

Type species ***Seneca Valley virus***

### Virion properties

#### MORPHOLOGY

No surface morphology is visible by EM. The X-ray crystallographic structure of Seneca Valley virus (SVV) has been determined at 2.3 Å resolution. The overall folds of the CPs are very similar to the corresponding proteins in other picornaviruses. Similar to cardioviruses, VP1 of SVV possesses a hydrophobic pocket without a pocket factor. However, the entrance to the hydrophobic cleft in SVV is almost completely sealed off by a number of VP1 residues. In cardioviruses the entrance is narrow while in the human rhinoviruses it is wide.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

SVV is stable at pH 3.0.

### Nucleic acid

The 5′ UTR is 666 nt long and contains a type IV IRES. The 3′ UTR is 71 nt long and the predicted folding of this region reveals two stem-loops with the potential to form a kissing-loop structure. This type of structure has been shown to be important in enterovirus replication. The location of the *cre* has not been identified.

## Genome organization and replication

Genome layout:

$$VPg{+}5'UTR[L/1A{-}1B{-}1C{-}1D{-}2A^{npgp}/2B{-}2C/3A{-}3B{-}3C{-}3D]3'UTR{-}poly(A)$$

The SVV genome encodes a leader polypeptide of unknown function. It lacks both catalytic residues, present in the aphtho- and erboviruses, which are necessary for proteolytic activity and zinc finger/tyrosine-phosphorylation motifs present in the cardioviruses. 1AB possesses a myristoylation signal at its amino-terminus and is cleaved to VP4 and VP2. VP1 is followed by a short FMDV-like (NPG↓P) 2A.

## Antigenic properties

Only a single serotype, Seneca Valley virus 1 (SVV-1), has been recognized.

## Biological properties

The only known natural host is the pig but SVV can replicate and cause CPE in a wide range of cell cultures including those derived from pigs, sheep, rabbits, hamsters, monkeys and humans. There is no known association with disease. SVV has potent cytolytic activity and high selectivity for human tumour cell lines having neuroendocrine properties versus adult normal cells. Its use for treatment of human metastatic neuroendocrine cancers has been investigated.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Senecavirus*

*Seneca Valley virus*

| | | |
|---|---|---|
| Seneca Valley virus 1 [SVV-001] | [DQ641257] | (SVV-1) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## Other related viruses which may be members of the genus *Senecavirus* but have not been approved as species

None reported.

# Genus *Tremovirus*

Type species *Avian encephalomyelitis virus*

## Distinguishing features

Avian encephalomyelitis virus (AEV) is most similar to hepatitis A virus (genus *Hepatovirus*), but differs by possessing (i) a type IV IRES (HAV is type III), (ii) 2A with a H-box/NC motif and (iii) 2B and 3A polypeptides with little sequence identity to the HAV counterparts.

## Virion properties

### Morphology
No surface morphology is visible by EM.

### Physicochemical and physical properties
AEV is stable at pH 3.0 and has a buoyant density of 1.31 to 1.33 g cm$^{-3}$.

### Nucleic acid
There is little similarity between the genome sequences of tremoviruses and those of other picornaviruses. The 5′-UTR contains a 5′-terminal hairpin, two putative pseudoknots, and a short (ca. 40 nt) pyrimidine-rich (i.e. not pure poly-C) tract upstream of the type IV IRES. Nucleotide sequence identity between different strains is generally greater than 80%. AEV RNA contains the shortest of all picornavirus 5′-UTRs, at 494 nt. The location of the *cre* is thought to possibly lie within the 3D region.

### Proteins
Similar to hepatoviruses, protein lA of AEV is predicted to be extremely small, does not appear to be myristoylated at its N-terminus, and therefore may not be a component of the mature virus particle.

## Genome organization and replication
Genome layout:

$$VPg{+}5'UTR[1A{-}1B{-}1C{-}1D?2A^{H\text{-}box/NC}?2B{-}2C/3A{-}3B{-}3C{-}3D]3'UTR{-}poly(A)$$

The polyprotein contains only a single protease ($3C^{pro}$). There is no L protein, and 2A probably has no proteolytic activity. It is not known if the first primary cleavage occurs between 1D and 2A or between 2A and 2B, nor if one or both of these cleavages are mediated by $3C^{pro}$. The IRES is type IV, similar to avihepatoviruses, sapeloviruses, senecaviruses and teschoviruses. The 2A protein of AEV contains H-box/NC motifs and is distantly related to the 2A of avihepatoviruses, kobuviruses and parechoviruses.

## Antigenic properties
Only a single serotype, avian encephalomyelitis virus 1 (AEV-1), has been recognized.

## Biological properties
AEV causes encephalomyelitis in young chickens, pheasants, quail and turkeys. It can be transmitted both vertically and by the fecal–oral route; field strains are enterotropic. A live, highly enterotropic AEV vaccine is widely used to control the disease.

## Species demarcation criteria in the genus
Not applicable.

## List of species in the genus *Tremovirus*

*Avian encephalomyelitis virus*
Avian encephalomyelitis virus 1 [Calnek] [AJ225173] (AEV-1)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Tremovirus* but have not been approved as species
None reported.

# GENUS *AVIHEPATOVIRUS*

Type species ***Duck hepatitis A virus***

## Virion properties

### MORPHOLOGY

No surface morphology is visible by EM.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

DHAV-1 is both heat- and acid-stable.

### NUCLEIC ACID

Duck hepatitis A viruses have a 5′ UTR of 625–655 nt which contains a type IV IRES. The location of the *cre* has not been identified. The 3′ UTR is particularly long, at around 318 nt.

## Genome organization and replication

Genome layout:

$$VPg{+}5'UTR[1AB{-}1C{-}1D{-}2A1^{npgp}/2A2{-}2A3^{H\text{-}box/NC}{-}2B{-}2C/3A{-}3B{-}3C{-}3D]3'UTR{-}poly(A)$$

The 1AB polypeptide lacks a myristoylation signal. The genome sequences of all three DHAV types have three 2A motifs: (i) NPGP; (ii) AIG1-like protein containing a GxxGxGKS NTP-binding motif; and (iii) a H-box/NC motif (similar to the 2A of parecho-, kobu- and tremoviruses). However, it is not clear if this genome region encodes one, two or three mature polypeptides.

## Antigenic properties

Duck hepatitis A virus is divided into three genetic types: DHAV-1 to -3. DHAV-2 and DHAV-3 are not neutralized by DHAV-1 antiserum; however, the relationship between types 2 and 3 remains unstudied.

## Biological properties

DHAV causes a highly fatal contagious disease of young ducklings, 1–28 days of age. The onset of the disease is very rapid, it spreads quickly through the flock and may cause up to 90% mortality. Sick ducklings develop spasmodic contractions of their legs and die within an hour in a typical "arched-backward" position. The liver is enlarged and shows hemorrhagic spots. A DHAV-1 live-attenuated vaccine is widely used.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Avihepatovirus*

| | | |
|---|---|---|
| *Duck hepatitis A virus* | | |
| Duck hepatitis A virus 1 [R85952] | [DQ226541] | (DHAV-1) |
| (Duck hepatitis virus 1) | | |
| Other types: DHAV-2, DHAV-3 | | |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table available online on Science Direct®, www.sciencedirect.com.

## List of other related viruses which may be members of the genus *Avihepatovirus* but have not been approved as species

None reported.

## List of other related viruses which may be members of the family *Picornaviridae* but have not been approved as species

A number of picornaviruses have recently been sequenced, but not formally classified. They include the first confirmed picornaviruses of wild birds, reptiles and fish.

**Bat kobu-like virus** Nucleotide sequence analysis of about 60% of the genome of a picornavirus detected in bat guano suggests a distant relationship with the kobuviruses.

**Bluegill picornavirus** Genome layout:

$$\text{VPg}+5'\text{UTR}[1\text{AB}-1\text{C}-1\text{D}-2\text{A1}^{\text{npgp}}/2\text{A2}^{\text{npgp}}/2\text{B}-2\text{C}/3\text{A}-3\text{B}-3\text{C}-3\text{D}]3'\text{UTR}-\text{poly(A)}$$

Bluegill picornavirus (BGPV) infects the bluegill (*Lepomis macrochirus*) freshwater fish in North American lakes. BGPV particles are icosahedral and average 30 nm in diameter. The virus can be grown in a cell line, BF-2, derived from bluegill fry. The genome layout is typical of a picornavirus except that there appears to be at least two 2A polypeptides each having an NPG↓P motif. The first is predicted to be 17 aa analogous to the FMDV 2A, while the second is 136 aa in length. BGPV may represent a novel picornavirus genus. Within the 3′ UTR, there is a poly(C) tract of 21 residues preceding the poly(A) tail.

**Carp picornavirus** Genome layout:

$$\text{VPg}+5'\text{UTR}[1\text{AB}-1\text{C}-1\text{D}-2\text{A1}^{\text{npgp}}/2\text{A2}^{\text{npgp}}/2\text{B}-2\text{C}/3\text{A}-3\text{B}-3\text{C}-3\text{D}]3'\text{UTR}-\text{poly(A)}$$

Carp picornavirus (CPV) infects the common carp (*Cyprinus carpio*), a freshwater fish. 7634 nt of the genome including 524 nt of the 5′ UTR and 284 nt of the unusually long 3′ UTR have been sequenced. Like BGPV, CPV is predicted to possess two 2A polypeptides both with NPG↓P motifs, the second of which is 133 aa in length. CPV may represent a novel picornavirus genus or may belong in the same genus as BGPV.

**Eel picornavirus** Genome layout:

$$\text{VPg}+5'\text{UTR}[1\text{AB}-1\text{C}-1\text{D}-2\text{A1}^{\text{npgp}}/2\text{A2}^{\text{h-box/NC}}-2\text{B}-2\text{C}/3\text{A}-3\text{B}-3\text{C}-3\text{D}]3'\text{UTR}-\text{poly(A)}$$

Eel picornavirus (EPV) was isolated from a common eel (*Anguilla anguilla*) in Lake Constance on the river Rhine. EPV can be propagated in eel kidney 1 (EK-1) cells. It is pathogenic in experimentally infected glass eels and induces a high mortality. 7501 nucleotides of the genome were sequenced. The 3′ UTR comprises 235 nt, part of the 5′ UTR has to be determined. The 3D sequence shows closest similarity to parechoviruses, tremovirus and seal picornavirus. The polyprotein has no leader peptide, a 2A1 protein with NPG↓P motif and a 2A2 protein with the H-box/NC sequence motives. Protein 1AB appears not to be cleaved. EPV may represent a novel picornavirus genus.

**Human cosaviruses** Genome layout:

$$\text{VPg}+5'\text{UTR}[1\text{A}-1\text{B}-1\text{C}-1\text{D}-2\text{A}^{\text{npgp}}/2\text{B}-2\text{C}/3\text{A}-3\text{B}-3\text{C}-3\text{D}]3'\text{UTR}-\text{poly(A)}$$

Cosaviruses have been detected in human stools, but have not yet been cultivated. It is proposed that they belong to five different species, based on the same sequence distance criteria used to distinguish human enterovirus species. They are most closely related to cardio- and senecaviruses, but lack a leader polypeptide. The 2A/2B junction is predicted based on a NPG↓P sequence motif, however, evidence for a conserved cleavage site between VP1 and 2A poor. The 5′ UTR is long (>1100 nt) and is predicted to contain a type II IRES. It has been suggested that human cosaviruses may represent a novel picornavirus genus.

**Human klassevirus/salivirus** Genome layout:

$$\text{VPg}+5'\text{UTR}[\text{L}/1\text{AB}-1\text{C}-1\text{D}/2\text{A}-2\text{B}-2\text{C}/3\text{A}-3\text{B}-3\text{C}-3\text{D}]3'\text{UTR}-\text{poly(A)}$$

Two very closely related (with a polyprotein aa identity of >95%) viruses have recently been described and named klassevirus (from *k*obu-*l*ike viruses *a*ssociated with stool and *se*wage) and salivirus (from stool *A*ichi-*li*ke viruses). The IRES possibly belongs to type II. These viruses possess a leader polypeptide of unknown function. 1AB may not be cleaved to give 1A and 1B, but like kobuviruses 1AB does have a myristoylation signal GxxxT). Kobuviruses possess H-Box/NC motifs in their predicted 2A proteins, however, these are absent in klasse/saliviruses. It has been suggested that the 2A protein is a trypsin-like protease based on the detection of H, C, and D catalytic triad residues at approximately the same location as in the 2A of rhinoviruses and enteroviruses.

**Seal picornavirus** Genome layout:

$$\text{VPg}+5'\text{UTR}[1\text{AB}-1\text{C}-1\text{D}-2\text{A}1^{\text{npgp}}/2\text{A}2-2\text{B}-2\text{C}/3\text{A}-3\text{B}1-3\text{B}2-3\text{C}-3\text{D}]3'\text{UTR}-\text{poly(A)}$$

Seal picornavirus 1 (SePV-1) has been isolated from Arctic ringed seals (*Pusa hispida*) in northern Canada and common (harbour) seals (*Phoca vitulina*) in the North Sea. Any role in disease is unclear. SePV-1 is predicted to possess two tandemly-repeated VPgs, an uncleaved 1AB (which also lacks a myristoylation motif) and two 2A polypeptides. The IRES is type IV. It has been suggested that SePV-1 may represent a novel picornavirus genus.

**Tortoise picornavirus (aka virus "X")** Genome layout:

$$\text{VPg}+5'\text{UTR}[\text{L}/1\text{A}-1\text{B}-1\text{C}-1\text{D}-2\text{A}^{\text{npgp}}/2\text{B}-2\text{C}/3\text{A}-3\text{B}-3\text{C}-3\text{D}]3'\text{UTR}-\text{poly(A)}$$

A virus isolated from a spur-thighed tortoise (*Testudo graeca*) has been cultivated in Terrapene heart cells causing a lytic infection. Approximately 7 kb of the genome has been sequenced (although an unknown length of the 5′ UTR remains to be determined). The predicted polyprotein has a typical picornavirus layout. The short leader polypeptide (52 aa) contains seven cysteine residues and has some similarity to metallothionein-like proteins. The capsid region is most closely related to the erboviruses. The 2B polypeptide is distantly related to that of TMEV, while 2C, 3C and 3D are most closely related to human cosavirus A, FMDV and Aichi virus, respectively. The predicted junction between 2A and 2B is NPG↓P, as it is in the most closely related picornaviruses. The 3′ UTR is 232 nt long and extremely A + T rich (87%). It has been suggested that TPV may represent a novel picornavirus genus.

**Turdiviruses:** Genome layout:

$$\text{Pg}+5'\text{UTR}[\text{L}/1\text{AB}-1\text{C}-1\text{D}/2\text{A}^{(\text{H-box/NC})}-2\text{B}-2\text{C}/3\text{A}-3\text{B}-3\text{C}-3\text{D}]3'\text{UTR}-\text{poly(A)}$$

Three novel picornaviruses, named turdiviruses 1, 2 and 3 (TV-1, TV-2 and TV-3), have been identified in birds of different genera in the family *Turdidae*. Regions P1, P2 and P3 of the three turdiviruses possess, respectively, <40, <40 and <50% amino acid identities with those of other picornaviruses. Moreover, P1, P2 and P3 of TV-1 also possessed, respectively, <40, <40 and <50% amino acid identities with those of TV-2 and TV-3. Phylogenetic analysis revealed that TV-1, TV-2 and TV-3 were distantly related to members of the genus *Kobuvirus*. The genomic features of TV-2 and TV-3 were also distinct from TV-1, including a lower G + C content and a shorter predicted leader polypeptide. The 2A of TV-1, but not TV-2 or TV-3, appears to be of the H-box/NC type. It has been suggested that the turdiviruses may fall into two novel picornavirus genera.

**Turkey hepatitis virus:** Genome layout:

$$\text{VPg}+5'\text{UTR}[1\text{AB}-1\text{C}-1\text{D}/2\text{A}^{?}-2\text{A}^{?}-2\text{A}^{(\text{H-box/NC})}-2\text{B}-2\text{C}/3\text{A}-3\text{B}-3\text{C}-3\text{D}]3'\text{UTR}-\text{poly(A)}$$

Turkey hepatitis virus (THV) has been identified in the liver, bile, intestine, serum and cloacal swabs of poults with disease, but not in clinically normal birds. The genome sequence has been determined for two related viruses and each was over 9 kb, the longest amongst the picornaviruses. The 5′ UTR IRES shares some similarity with that of DHAV and therefore may be of type IV. Comparative predictions suggest that 1AB (VP0) is neither cleaved nor myristoylated. There is a large insertion of about 1.2 kb between VP1 and a recognizable 2A-like region containing Hbox-NC motifs (also present in hepato-, kobu-, parecho- and tremoviruses). Prediction of cleavage sites within this region suggests that an additional two distinct 2A polypeptides of unknown function may be present. THV is most closely related to the kobuviruses and the unclassified human klasse/saliviruses and avian turdiviruses. The presence of a poly(A) tail is presumed, but not yet proven.

| | | |
|---|---|---|
| Bat kobu-like virus | [HM228880 to HM228884] | (BKLV) |
| Bluegill picornavirus 1 [04-032] | | (BGPV-1) |
| Carp picornavirus 1 [F37/06] | | (CPV-1) |
| Eel picornavirus 1 [F15/05] | | (EPV-1) |
| Human cosavirus A1 [0553] | [FJ438902] | (HCoSV-A1) |
| Human cosavirus A2 [6344] | [FJ438903] | (HCoSV-A2) |
| Human cosavirus A3 [6572] | [FJ438904] | (HCoSV-A3) |
| Human cosavirus A4 [5006] | [FJ438905, FJ438906] | (HCoSV-A4) |
| Human cosavirus B1 [2263] | [FJ438907] | (HCoSV-B1) |
| Human cosavirus C1 [5152] | [FJ442995] | (HCoSV-C1) |
| Human cosavirus D1 [5004] | [FJ438908] | (HCoSV-D1) |
| Human cosavirus E1 [Australia/81] | [FJ555055] | (HCoSV-E1) |
| Human klassevirus 1 [02394-01] | [GQ184145] | (HKV-1) |
| Salivirus [NG-J1 ] | [GQ179640] | (SaV) |
| Seal picornavirus 1 [HO.02.21] | [EU142040] | (SePV-1) |
| Sheep picornavirus 1 [VS65.60] | | (ShPV-1) |
| Tortoise picornavirus 1 (virus X) [TGT1A/96] | | (TPV-1) |
| Turdivirus 1 [00356 & 00805] | [GU182406, GU182407] | (TV-1) |
| Turdivirus 2 [10717 & 007167] | [GU182408, GU182409] | (TV-2) |
| Turdivirus 3 [10878 & 00742] | [GU182410, GU182411] | (TV-3) |
| Turkey hepatitis virus [2993D & 0091.1] | [HM751199, HQ189775] | (THV) |

Names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

A number of other candidate picornaviruses exist for which no sequence data are yet available. These have been described as picornaviruses or picorna-like viruses based mainly on EM morphology and size.

| | |
|---|---|
| Aesculapian snake picorna-like virus | (ASPLV) |
| Avian entero-like virus 2 [EF84/700] | (AELV-2) |
| Barramundi virus 1 | (BaV) |
| Boa constrictor picorna-like virus | (BCPLV) |
| Cockatoo entero-like virus | (CELV) |
| European smelt picornavirus | (ESV) |
| Grass carp picornavirus | (GCPV) |
| Greasy grouper virus | (GGV) |
| Guineafowl transmissible enteritis virus | (GTEV) |
| Juruaca virus [BeAn 401933] | (JURV) |
| Malabar grouper virus | (MGV) |
| Rainbow smelt picornavirus | (RSPV) |
| Salmonid viruses | |
| Sandbar shiner virus | (SSV) |
| Sea-bass virus 1 | (SBV-1) |
| Sikhote-Alyn virus [LEIV 113P] | (SAV) |
| Smelt virus 1 | (SmV-1) |
| Smelt virus 2 | (SmV-2) |
| Syr-Darya Valley fever virus [Kaz-3] | (SDFV) |
| Turbot virus 1 | (TuV-1) |
| Turkey entero-like virus | (TELV) |
| Turkey pseudo enterovirus 1 | (TPEV-1) |
| Turkey pseudo enterovirus 2 | (TPEV-2) |

## Phylogenetic relationships within the family

Viruses in each genus are phylogenetically distinct from members of other genera in those genome regions which are homologous, i.e. $P1^{cap}$, $2C^{hel}$, $3C^{pro}$ and $3D^{pol}$.

## Similarity with other taxa

The family *Picornaviridae* together with the families *Dicistroviridae*, *Iflaviridae*, *Marnaviridae* and *Secoviridae* form the order *Picornavirales*. There are also similarities to the families *Caliciviridae* and *Potyviridae*, including the order of the Hel-VPg-Pro-Pol non-structural polypeptides.

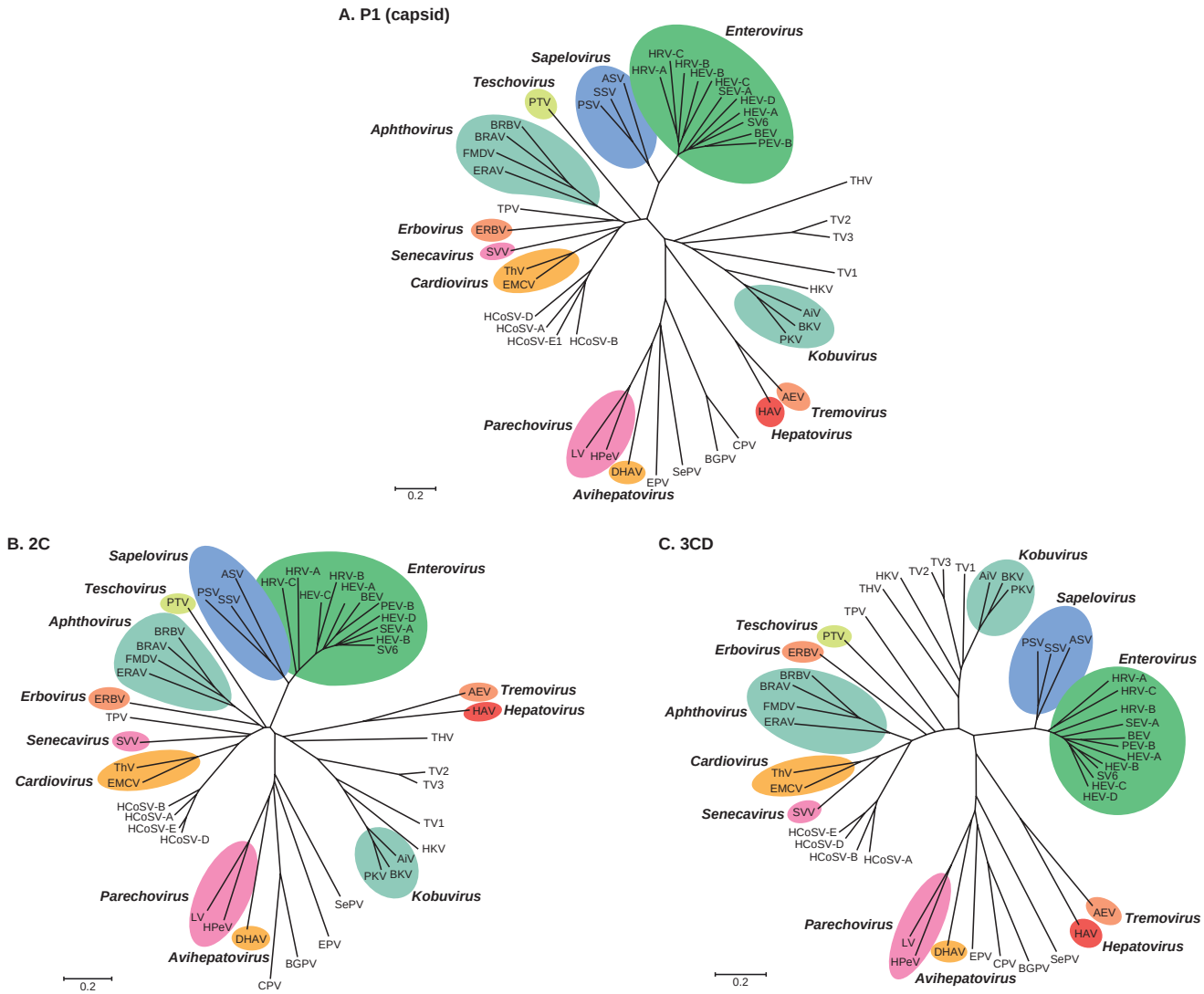


**Figure 4:** Phylogenetic trees showing the relationships between the genera, species and unclassified members of the family *Picornaviridae*: (A.P1) protein P1; (B.2C) protein 2C; and (C.3CD) proteins 3C + 3D. The neighbor-joining trees were produced using MEGA 4.0 and the poisson model for correction of multiple substitutions. Abbreviations: AEV, *Avian encephalomyelitis virus*; AiV, *Aichi virus*; ASV, *Avian sapelovirus*; BEV, *Bovine enterovirus*; BGPV, bluegill picornavirus; BKV, *Bovine kobuvirus*; BRAV, bovine rhinitis A virus; BRBV, *Bovine rhinitis B virus*; CPV, carp picornavirus; DHAV, *Duck hepatitis A virus*; EMCV, *Encephalomyocarditis virus*; EPV, eel picornavirus; ERAV, *Equine rhinitis A virus*; ERBV, *Equine rhinitis B virus*; FMDV, *Foot-and-mouth disease virus*; HAV, *Hepatitis A virus*; HCoSV, human cosavirus; HEV, human enterovirus; HKV, human klassevirus; HPeV, *Human parechovirus*; HRV, human rhinovirus; LV, *Ljungan virus*; PEV-B, *Porcine enterovirus B*; PKV, porcine kobuvirus; PSV, *Porcine sapelovirus*; PTV, *Porcine teschovirus*; SePV, seal picornavirus; SEV-A, *Simian enterovirus A*; SSV, *Simian sapelovirus*; SVV, *Seneca Valley virus*; ThV, *Theilovirus*; THV, turkey hepatitis virus; TPV, tortoise picornavirus; TV, turdivirus.

## Derivation of names

*Aphtho*: from Greek *aphthae*, "vesicles in the mouth"; English: *aphtha*, "thrush"; French: *fièvre aphteuse*.
*Avihepato*: from *avi*an and Greek *hepatos*, "liver".
*Cardio*: from Greek *kardia*, "heart".
*Entero*: from Greek *enteron*, "intestine".
*Erbo*: for *e*quine *r*hinitis *B* virus.
*Hepato*: from Greek *hepatos*, "liver".
*Kobu*: from Japanese *kobu*, "knuckle" (reference to surface structure of virus particle).
*Parecho*: from *par*(a)*echo* (*echo*, the former name of the type species, an acronym for "enteric cytopathic human orphan").
*Picorna*: from the prefix "*pico*" ( = "micro-micro") and RNA.
*Sapelo*: from *s*imian, *a*vian and *p*orcine *e*ntero-*l*ike viruses.
*Seneca*: from Seneca Valley virus.
*Tescho*: from *Tesch*en disease.
*Tremo*: from an alternative name for avian encephalomyelitis, epidemic *tremo*r.

## Further reading

### Journals and books

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### Websites

The Picornaviruses Pages: www.picornaviridae.com/
Picornaviridae Study Group: www.picornastudygroup.com/

## Contributed by

Knowles, N.J., Hovi, T, Hyypiä, T., King, A.M.Q., Lindberg, A.M., Pallansch, M.A., Palmenberg, A.C., Simmonds, P., Skern, T., Stanway, G., Yamashita, T. and Zell, R.

# Family *Secoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Secoviridae*** |
| Subfamily | ***Comovirinae*** |
| Genus | ***Comovirus*** |
| Genus | ***Fabavirus*** |
| Genus | ***Nepovirus*** |
| Genera not assigned to a subfamily | |
| Genus | ***Cheravirus*** |
| Genus | ***Sadwavirus*** |
| Genus | ***Torradovirus*** |
| Genus | ***Sequivirus*** |
| Genus | ***Waikavirus*** |

## Virion properties

### Morphology

Virions are non-enveloped 25–30 nm in diameter and exhibit icosahedral symmetry (T = 1, pseudo T = 3, Figure 1). Many virus preparations contain empty virus particles. In the case of viruses with a bipartite genome, the two RNAs are encapsidated in separate virions.

### Physicochemical and physical properties

Different classes of virions are distinguished according to their buoyant densities (top, middle and bottom components, also termed T, M and B, Figure 1). The main virions (M and B components) contain RNA. Viruses belonging to the genera *Sequivirus* and *Waikavirus*, which have a single large monopartite genome, sediment with $S_{20,W}$ values of 150–190S. For viruses with a bipartite genome, virions containing RNA1 (B component) sediment at 110–135S. Virions containing RNA2 (M component) sediment at 84–128S and contain one or two molecules of RNA2. In cases where the size of RNA1 and RNA2 are similar, the M and B components may be difficult to separate. Empty shells (T component) sediment with $S_{20,W}$ values of 49–63S depending on the virus considered.

### Nucleic acid

The genome consists of one or two molecules of linear positive sense ssRNA. The size of RNA(s) differs among genera (Table 1). The genomic RNA(s) contain a 3′-terminal poly(A) tract of variable length. The only known exception is the genomic RNA of a sequivirus (parsnip yellow fleck virus), which is apparently not polyadenylated. For several comoviruses and nepoviruses and for strawberry latent ringspot virus, an unassigned member of the family, a polypeptide, designated VPg (2–4 kDa) has been shown to be covalently bound at the 5′ end. The presence of a 5′-linked VPg has not been confirmed for other genera but has been suggested because, in many cases, infectivity of the RNA(s) has been shown to be protease-sensitive.

### Proteins

Nepoviruses have a single coat protein (CP) of 52–60 kDa. Comoviruses, fabaviruses, sadwaviruses and strawberry latent ringspot virus have two CPs of 40–45 kDa and 21–29 kDa. Cheraviruses, torradoviruses, sequiviruses and waikaviruses have three CPs of similar sizes (24–35 kDa, 20–26 kDa and 20–25 kDa). The size and number of the CP(s) of two unassigned members of the family (strawberry mottle virus and black raspberry necrosis virus) has not been determined yet. Virions have 60 copies of each CP per particle. For three comoviruses (cowpea mosaic virus, bean pod mottle virus and red clover mottle virus) and one nepovirus (tobacco ringspot virus), the atomic structure has been solved and found to be very similar (pseudo T = 3) to that of viruses belonging to the family *Picornaviridae*. Each capsid subunit is made of three beta-barrels (jelly roll domains) that can be present in one large CP with three jelly roll domains (*Nepovirus*), two CPs (one large CP including two jelly roll domains and one smaller CP with a single jelly roll domain; *Comovirus*, *Fabavirus* and *Sadwavirus*) or three CPs each containing a single jelly roll domain (*Cheravirus*, *Torradovirus*, *Sequivirus*, *Waikavirus*) (Figure 2).

Pos. ssRNA

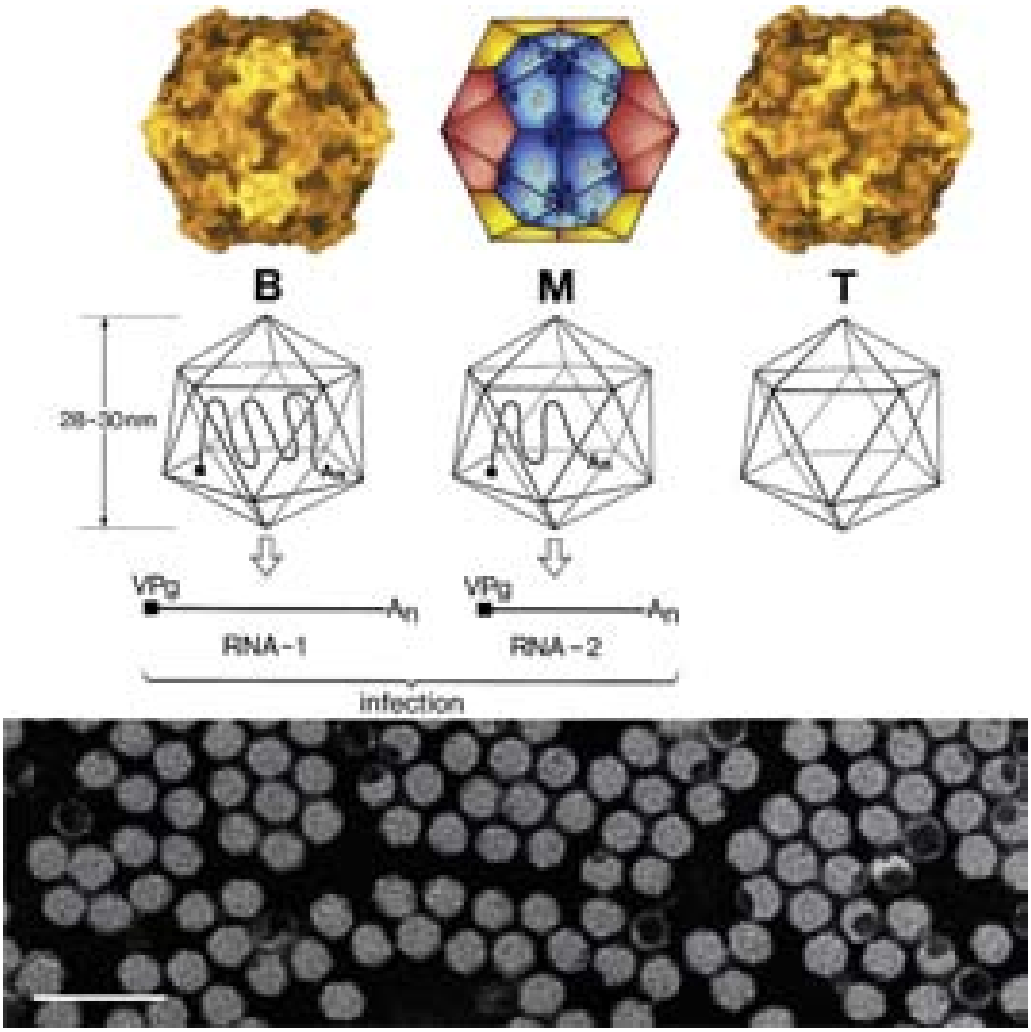


**Figure 1:** (Top left): Molecular rendering of the cowpea mosaic virus particle (Lin *et al.* (1999). *Virology* **265**, 20-34; with permission). (Top central): Diagrammatic representation of a T = 1 lattice. A = Small capsid protein, B = C-terminal domain of the large capsid protein and C = N-terminal domain of the large capsid protein. (Top right): Molecular rendering of the red clover mottle virus particle (Lin *et al.* 2000, with permission). (Center): Diagram of the three types of comovirus particles with the B-particle containing one molecule of RNA-1, the M-particle containing one molecule of RNA-2 and the T-particle being empty. (Bottom): Negative contrast electron micrograph of particles of cowpea mosaic virus. The bar represents 100 nm.

### Lipids
None reported.

### Carbohydrates
None reported. A report that the CPs of comoviruses contain carbohydrates has later been shown to be mistaken.

## Genome organization and replication

Unfractionated viral RNA is highly infective. In the case of viruses with a bipartite genome, neither RNA species alone can infect plants systemically. RNA1 carries all the information required for replication and can replicate in individual cells in the absence of RNA2 although no virus particles are produced (as demonstrated for comoviruses and nepoviruses).

Viral proteins are usually expressed as large polyproteins, which are cleaved by 3C-like proteinases. Each RNA usually encodes a single polyprotein (Figure 3). A notable exception is the RNA2

**Table 1:** Sizes of the genomes (nts) of representative viruses in the family *Secoviridae*

| Genus/virus* | RNA-1 | RNA-2 |
|---|---|---|
| *Comovirus* | 5,850–6,100 | 3,300–4,000 |
| Cowpea mosaic virus-SB | (5,889) | (3,810) |
| *Fabavirus* | 5,800–6,000 | 3,300–4,000 |
| Broad bean wilt virus 2-ME | (5,951) | (3,607) |
| *Nepovirus* | 7,200–8,400 | 3,700–7,300 |
| Grapevine fanleaf virus-F13 | (7,342) | (3,774) |
| Beet ringspot virus-S | (7,356) | (4,662) |
| Tomato ringspot virus-Rasp2 | (8,214) | (7,273) |
| *Cheravirus* | 6,800–7,100 | 3,200–3,700 |
| Cherry rasp leaf virus-USA | (6,992) | (3,274) |
| *Sadwavirus* | 6,800–7,000 | 5,300–5,600 |
| Satsuma dwarf virus-S58 | (6,795) | (5,345) |
| *Torradovirus* | 7,200–7,800 | 5,300–5,900 |
| Tomato torrado virus-PRI-0301 | (7,793) | (5,389) |
| *Sequivirus* | 9,800–10,000 | |
| Parsnip yellow fleck virus-P121 | (9,871) | NA |
| *Waikavirus* | 11,800–12,500 | NA |
| Rice tungro spherical virus-LB | (12,433) | |
| Unassigned species in the family | | |
| Strawberry latent ringspot virus-MEN454 | (7,496) | (3,842) |
| Strawberry mottle virus-1134 | (7,036) | (5,619) |

NA: not applicable.
*Please refer to tables within the text for the sequence accession numbers for the type isolate for each virus.

of torradoviruses, which contains two open reading frames. Another exception is the RNA2 of comoviruses. Although a single large ORF is present, internal initiation at a second AUG allows the formation of two distinct polyproteins. The 3′ UTR of the RNA varies in length, sometimes even within a genus (nepoviruses). In some cases, extensive regions of sequence identity between RNA1 and RNA2 are found in the 5′ and/or 3′ UTRs (Figure 3).

Within the polyproteins, protein domains are organized in a manner that is common to that of other members of the order *Picornavirales* (Figure 3). The replication block contains domains characteristic of NTP-binding proteins (NTB or putative helicase), 3C-like proteinase (Pro) and RNA-dependent RNA polymerase (Pol). In viruses with a monopartite genome, the structural proteins are located upstream of the replication block in the single polyprotein. In viruses with a bipartite genome, structural proteins are contained in the RNA2-encoded polyprotein. In comoviruses, cheraviruses and nepoviruses, the movement protein is located upstream of the CP(s), and enables viral movement to adjacent cells. Both movement protein and CP(s) are required for cell-to-cell movement of the virus. The movement protein of comoviruses and nepoviruses was shown to be a structural component of tubular structures that traverse the cell wall and contain virus-like particles. Putative movement proteins have been suggested to be encoded upstream of the CP(s) coding regions for many other viruses in the family but their biological function has not been confirmed.

The RNA1-encoded 3C proteinase cleaves both RNA1 and RNA2-encoded polyproteins. The cleavage site specificity of the proteinase differs with the specific genera considered (and in the case of nepoviruses it differs with the specific subgroup, Table 2). An amino acid in the substrate-binding pocket of the proteinase interacts directly with the amino acid in the −1 position of the cleavage site and plays a key role in the specificity of the proteinase.

Formation of replication complexes has been studied for comoviruses and nepoviruses. Replication occurs in association with intracellular membranes derived from the endoplasmic reticulum. Two

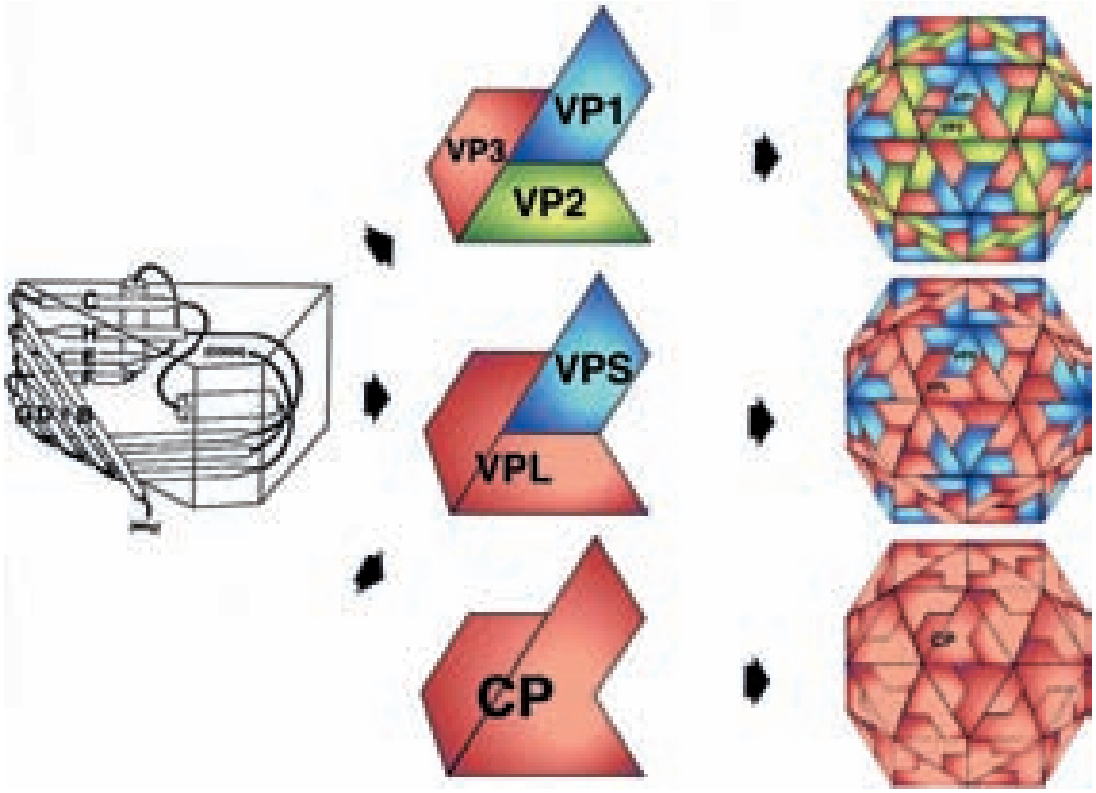


**Figure 2:** Architecture of the capsid of members of the family *Secoviridae*. In sequiviruses, waikaviruses, cheraviruses and torradoviruses, each subunit is composed of three separate small coat proteins (CPs) each containing a single beta-barrel domain (VP1-VP3, top). In comoviruses, fabaviruses and sadwaviruses, the three beta-barrels are present in two CPs (VPL with two barrels and VPS with a single barrel, middle). In nepoviruses, the single CP is folded in three barrels (bottom).

RNA1-encoded proteins (the NTB protein and the protein immediately upstream of NTB) interact directly with ER membranes and have been implicated in the proliferation of membrane vesicles in the cytoplasm of infected cells and in the assembly of the replication complex. This has not been studied for other viruses in the family.

## Antigenic properties

Virus preparations are usually good immunogens and polyclonal antibodies prepared against purified virus particles recognize all CPs. Species belonging to the same genus can be serologically interrelated, but often distantly.

## Biological properties

All members of the family infect plants. Host range and symptoms vary with the genera and viruses considered (Table 3). Many viruses in the family have a known biological vector, although some (sequiviruses) require a helper virus and others do not have a known vector. Most viruses are transmissible experimentally by mechanical inoculation. However, waikaviruses are not sap-transmissible. Many viruses are readily transmissible by seed or pollen.

## Genus demarcation criteria in the family

- Number of genomic RNAs
- Number of protein domains and/or processing sites within the polyprotein(s)
- Number of CPs
- Presence of additional ORFs and/or subgenomic RNAs

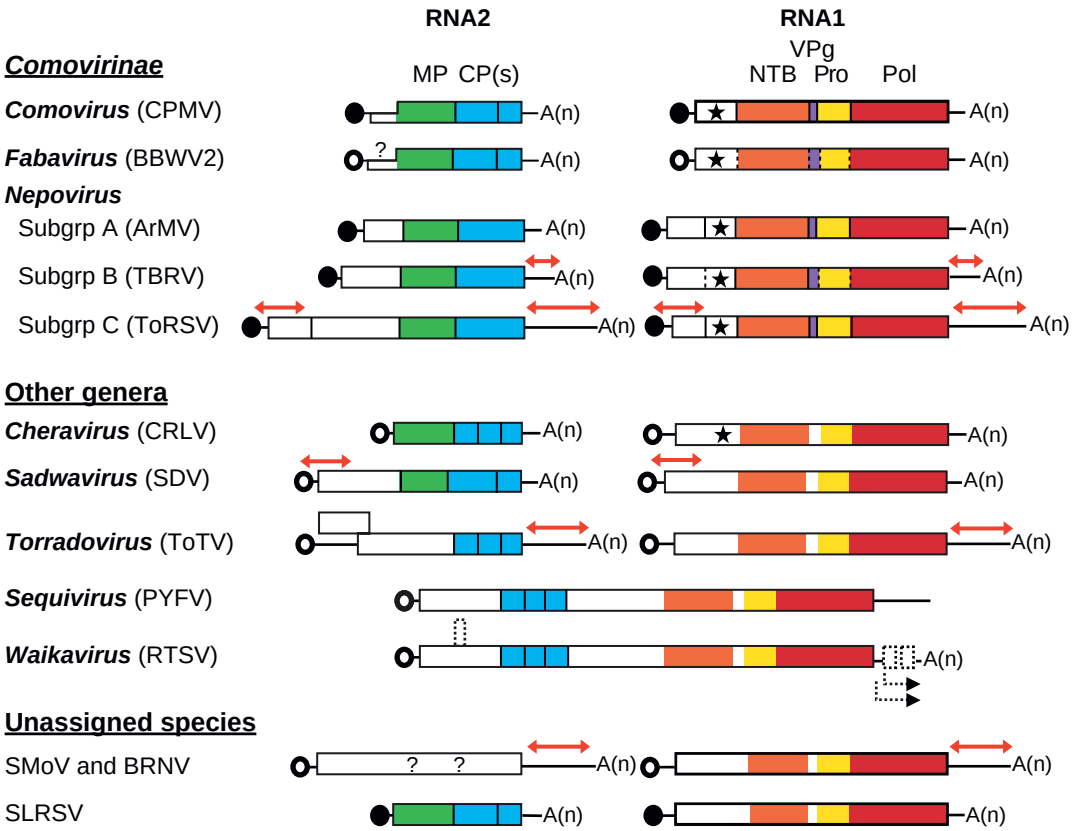


**Figure 3:** Genome organization of representative members of the family *Secoviridae*. Each RNA is shown with the ORFs represented with the boxes. Circles depict VPg molecules covalently attached at the 5′ end of the RNAs. Black circles represent VPg confirmed experimentally and open circles represent putative VPgs. Poly(A) tails are represented at the 3′ end of the RNAs when present [A(n)]. Red arrows above the sequences represent regions of extensive sequence identity between RNAs 1 and 2. Protein domains with conserved motifs for the putative NTP-binding protein (NTB, shown in orange), VPg (purple), proteinase (Pro, yellow), RNA-dependent RNA polymerase (Pol, red), movement protein (MP, green) and coat protein(s) (CP, blue) are shown. The star represents a conserved motif found in the Co-Pro protein of comoviruses and in the equivalent protein of other viruses. Proteinase cleavage sites identified experimentally or deduced by sequence comparisons are shown by the solid or dotted vertical lines, respectively. Possible ORFs in the genome of waikaviruses are shown with the dotted squares and putative subgenomic RNAs are shown by dotted arrows below the waikavirus genome. For each virus, the genomic organization is shown for the type isolate as described in the text.

- Clustering as a single branch in phylogenetic trees derived from amino acid sequence alignments of the conserved Pro-Pol region when compared with other genera of the family *Secoviridae* (see Figure 5 below). The Pro-Pol region is delineated by the "CG" motif of the 3C-like proteinase and the "GDD" motif of the polymerase. Identification of proteinase cleavage sites is not required to delineate the Pro-Pol region.

Not all criteria may need to be met simultaneously.

## Species demarcation criteria

- CP aa sequence with less than 75% identity (for viruses with two or three CPs, combined CP sequences are considered)
- Conserved Pro-Pol region aa sequence (as defined above) with less than 80% identity
- Differences in antigenic reactions
- Distinct host range
- Distinct vector specificity
- Absence of cross-protection
- For viruses with a bipartite genome, absence of re-assortment between RNA1 and RNA2.

**Table 2:** Cleavage site specificity of the 3C-like proteinase of viruses in the family *Secoviridae*

| Genus | Proteinase substrate binding pocket* | Dipeptide at cleavage site† |
|---|---|---|
| *Comovirus* | His | Q/G, Q/M, Q/S, Q/T, Q/A |
| *Fabavirus* | His | Q/S, Q/A, Q/G |
| *Nepovirus* | | |
| Subgroup A | Leu | R/G, C/S, C/A, A/S, G/E, G/V, C/G |
| Subgroup B | Leu | K/S, K/A, R/A, R/S, R/G |
| Subgroup C | His | Q/G, Q/S, D/S |
| *Cheravirus* | His | Q/G, E/G |
| *Sadwavirus* | ? | R/G, T/S, T/N, A/N, A/S, A/A |
| *Torradovirus* | His | ? |
| *Sequivirus* | Leu | ? |
| *Waikavirus* | His | Q/S, Q/M, Q/V, Q/A |
| Unassigned species in the family | | |
| Strawberry latent ringspot virus | His | S/G |
| Strawberry mottle virus | His | Q/G |

*The indicated amino acid in the substrate-binding pocket of the proteinase interacts with the amino acid at the −1 position of the cleavage site and plays an important role in determining the cleavage site specificity of the proteinase. In the case of sadwaviruses, neither a His nor a Leu are present at the equivalent position in the deduced amino acid sequence of the proteinase.
†Cleaved dipeptides at the cleavage sites are shown with the scissile bond indicated with the slanted line. The amino acids are shown using the one-letter code. Proteolytic cleavages at dipeptides shown in red have been confirmed experimentally. Dipeptides shown in black are putative cleavage sites, inferred from sequence alignments.

**Table 3:** Biological properties of viruses in the family *Secoviridae*

| Genus | Host range | Vector | Seed or pollen transmission |
|---|---|---|---|
| *Comovirus* | Narrow (Leguminosae) | Beetle | Rare |
| *Fabavirus* | Wide | Aphid | Rare |
| *Nepovirus* | Wide | Nematode (most) or mite (blackcurrant reversion virus) or unknown | Seed and/or pollen |
| *Cheravirus* | Wide or narrow | Nematode (cherry rasp leaf virus) or unknown | Seed |
| *Sadwavirus* | Wide | Unknown | Seed |
| *Torradovirus* | Narrow | Whitefly | Unlikely |
| *Sequivirus* | Relatively wide | Aphid (requires helper virus) | None |
| *Waikavirus* | Narrow | Aphid or leafhopper | None |
| Unassigned species | | | |
| Strawberry latent ringspot virus | Wide | Nematode | Seed |
| Strawberry mottle virus | Relatively wide | Aphid | None |

Not all criteria need to be met simultaneously. In some cases, sequence information alone can be a good indicator of a distinct species (i.e., when the percentage of sequence identity in both the Pro-Pol and CP(s) regions is well below the proposed cut-off). However, analyzing only one region of the genome is generally not sufficient and both the Pro-Pol and CP(s) regions should be considered. In cases where the percentage of sequence identity in one or both sequences is near the proposed cut-off (e.g., between 75 and 85% in the Pro-Pol region or between 70 and 80% in the CP(s)

region), other criteria should be considered and information on biological properties of the virus (host range, vector specificity, possibility of reassortment between RNAs) is useful. For example, beet ringspot virus (BRSV) and tomato black ring virus (TBRV) (genus *Nepovirus*) are closely related in the Pro-Pol sequence (89% sequence identity) but are much more divergent in the CP sequence (62% sequence identity). They differ in their antigenic reactions and also in the specificity of nematode transmission (BRSV is transmitted more efficiently by *Longidorus elongatus* and TBRV is transmitted more efficiently by *Longidorus attenuatus*).

## SUBFAMILY *COMOVIRINAE*

### Taxonomic structure of the subfamily

| | |
|---|---|
| Subfamily | ***Comovirinae*** |
| Genus | ***Comovirus*** |
| Genus | ***Fabavirus*** |
| Genus | ***Nepovirus*** |

### Distinguishing features

The genome of members of the subfamily *Comovirinae* consists of two ssRNAs with a 5′-bound polypeptide (VPg) and a 3′ poly(A) tail. Members of the subfamily group as a single branch in phylogenetic trees using the conserved Pro-Pol region (see section on phylogenetic relationships in the family and Figure 5 below). Other genera are more distantly related. Within the subfamily, genera are distinguished by their specific genomic organization, biological properties and phylogenetic relations. Each genus within the subfamily *Comovirinae* represents a single sub-branch in the Pro-Pol phylogenetic tree.

## GENUS *COMOVIRUS*

Type species ***Cowpea mosaic virus***

### Distinguishing features

The comovirus capsid is made of two types of polypeptides (large CP: 40–45 kDa and small CP: 21–27 kDa). The small CP suppresses RNA silencing and surface-exposed amino acids are required for this function.

The 5′ and 3′ NTRs of RNA-1 and RNA-2 are similar in sequence but not identical. RNA-2 is translated into two largely overlapping polyproteins that are processed into three domains. Production of the smaller polyprotein is caused by internal initiation at a downstream AUG, which is placed in a more favorable context than the upstream AUG (Figure 4). The 58K protein released from the N-terminus of the larger polyprotein (P2) is necessary for replication of RNA-2. The 48K protein released from the N-terminus of the smaller polyprotein (P2′) is the MP, with a typical "LPL" motif. The CP domains are encoded at the C-terminus of both polyproteins. The MP and the CPs are required for cell-to-cell movement of the virus. The MP is a structural component of tubular structures containing virus-like particles that traverse the cell wall. The C-terminal region of the MP also interacts with the large CP. RNA-1 is translated into a single polyprotein that is processed into five domains, through alternative processing pathways (Figure 4). The N-terminal 32K protein limits the processing of the RNA-1-encoded polyprotein *in cis* and assists the processing of the RNA-2-encoded polyprotein. This protein is often referred to as the protease co-factor or Co-Pro. The replication block on the RNA1-encoded polyprotein includes the 58K protein with sequence motifs characteristic of an NTP-binding helicase, the VPg, the Pro and the Pol. The 32K Co-Pro and 58K NTB proteins are involved in inducing the cytopathic structure through proliferation of ER-derived membranes.

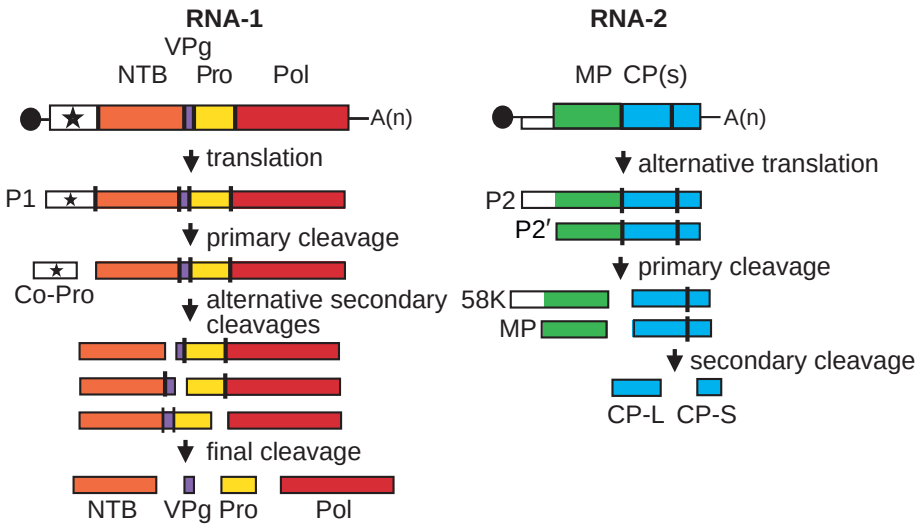


**Figure 4:** Genome organization and polyprotein processing of cowpea mosaic virus. The ORFs are boxed and the function of the proteins is indicated. MP: movement protein; CPL and CPS: large and small coat proteins; Co-Pro: proteinase co-factor; NTB: NTP-binding proteins; Pro: proteinase; Pol: RNA-dependent RNA polymerase. Proteolytic cleavage sites are indicated on the polyproteins with the vertical lines. All intermediate and final cleavage products have been detected in infected cells. The black circles at the 5′ end of the RNA represents the VPg, and A(n) at the 3′ end the poly-A tail.

Comoviruses have narrow host ranges, 11 of the 15 species being restricted to a few species of the family *Leguminosae*. Mosaic and mottle symptoms are characteristic, but usually not ringspots. Transmission in nature is exclusively by beetles, especially members of the family *Chrysomelidae*. Beetles retain their ability to transmit virus for days or weeks.

## List of species in the genus *Comovirus*

| | [RNA1] | [RNA2] | |
|---|---|---|---|
| *Andean potato mottle virus* | | | |
| Andean potato mottle virus-C | | [L16239] | (APMoV-C) |
| *Bean pod mottle virus* | | | |
| Bean pod mottle virus-KYG7 | [U70866 = NC_003496] | [M62738 = NC_003495] | (BPMV-KYG7) |
| *Bean rugose mosaic virus* | | | |
| Bean rugose mosaic virus-Parana | | [AF263548*] | (BRMV-Parana) |
| *Broad bean stain virus* | | | |
| Broad bean stain virus-Loewe-07013PC | | [FJ028650] | (BBSV-07013PC) |
| *Broad bean true mosaic virus* (Echtes Ackerbohnemosaik virus) (Vicia virus 1) | | | |
| Broad bean true mosaic virus-PV-0098 | | [FJ442942*] | (BBTMV-PV-0098) |
| *Cowpea mosaic virus* (Cowpea yellow mosaic virus) | | | |
| Cowpea mosaic virus–SB | [X00206 = NC_003549] | [X00729 = NC_003550] | (CPMV-SB) |
| *Cowpea severe mosaic virus* (Arkansas cowpea mosaic virus) (Cowpea mosaic virus - severe) (Trinidad cowpea mosaic virus) (Puerto Rico cowpea mosaic virus) | | | |
| Cowpea severe mosaic virus-DG | [M83830 = NC_003545] | [M83309 = NC_003544] | (CPSMV-DG) |

| | | | |
|---|---|---|---|
| *Glycine mosaic virus* | | | |
| Glycine mosaic virus-New South Wales | | | (GMV-NSW) |
| *Pea green mottle virus* | | | |
| Pea green mottle virus-Czechoslovakia | | | (PGMV-CZ) |
| *Pea mild mosaic virus* | | | |
| Pea mild mosaic virus-New Zealand | | | (PMiMV-NZ) |
| *Quail pea mosaic virus* (Bean curly dwarf mosaic virus) | | | |
| Quail pea mosaic virus-Arkansas | | | (QPMV-AR) |
| *Radish mosaic virus* (Radish enation mosaic virus) | | | |
| Radish mosaic virus-Japan | [AB295643 = NC_010709] | [AB295644 = NC_010710] | (RaMV-Japan) |
| *Red clover mottle virus* | | | |
| Red clover mottle virus-S | [X64886 = NC_003741] | [M14913 = NC_003738] | (RCMV-S) |
| *Squash mosaic virus* (Cucurbit ring mosaic virus) (Muskmelon mosaic virus) (Pumpkin mosaic virus) | | | |
| Squash mosaic virus-Y | [AB054688 = NC_003799] | [AB054689 = NC_003800] | (SqMV-Y) |
| *Ullucus virus C* | | | |
| Ullucus virus C-Andes | | | (UVC-Andes) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome segment.
Only one type isolate is listed for each species. The type isolate was chosen as the isolate with the most complete nucleotide sequence information. When several isolates with complete sequence information are available, the isolate with the earlier database deposition is provided as the type isolate. A more extensive list of isolates with significant sequence information is available online on Science Direct ®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Comovirus* but have not been approved as species

| | [RNA1] | [RNA2] | |
|---|---|---|---|
| Turnip ringspot virus-Toledo | [FJ712026 = NC_013218] | [FJ712027 = NC_013219] | (TuRSV-Toledo) |

Turnip ringspot virus (TuRSV) is related to radish mosaic virus (RaMV). They infect similar hosts. The degree of aa sequence identity between the two viruses is close to the proposed species demarcation criteria (73% aa sequence identity in the combined CP region and 80% aa sequence identity in the Pro-Pol region). It is not known whether re-assortment between the RNAs of TuRSV and RaMV is possible. Therefore, the taxonomic position of TuRSV as a distinct species in the genus *Comovirus* or as a distant strain of the species *Radish mosaic virus* remains unclear.



## GENUS *FABAVIRUS*

Type species ***Broad bean wilt virus 1***

### Distinguishing features

The genomic organization of fabaviruses is similar to that of comoviruses, although it is not known whether RNA2 encodes two overlapping polyproteins in fabaviruses (Figure 3). The cleavage of polyproteins is presumed to be similar to that of comoviruses but this has not been investigated in detail. Fabaviruses have wide host ranges among dicotyledonous plants and some families of monocotyledonous plants. Symptoms are ringspots, mottling, mosaic, distortion, wilting and apical necrosis. In nature, fabaviruses are transmitted by aphids in a non-persistent manner.

## List of species in the genus *Fabavirus*

| | [RNA1] | [RNA2] | |
|---|---|---|---|
| *Broad bean wilt virus 1* | | | |
| (Nasturtium ringspot virus) | | | |
| Broad bean wilt virus 1-ATCC PV132 | [AB084450 = NC_005289] | [AB084451 = NC_005290] | (BBWV1-PV132) |
| *Broad bean wilt virus 2* | | | |
| (Parsley virus 3) | | | |
| (Petunia ringspot virus) | | | |
| (Plantago II virus) | | | |
| Broad bean wilt virus 2-ME | [AF225953 = NC_003003] | [AF225954 = NC_003004] | (BBWV2-ME) |
| Patchouli mild mosaic virus | [AB050782 = NC_003975] | [AB011007 = NC_003974] | (PatMMV) |
| *Gentian mosaic virus* | | | |
| (Mikania micrantha mosaic virus) | | | |
| Gentian mosaic virus-N-1 | [AB084452] | [AB084453] | (GeMV-N-1) |
| *Lamium mild mosaic virus* | | | |
| Lamium mild mosaic virus-Cambridge | | | (LMMV-Cambridge) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
A more extensive list of isolates is available online on Science Direct ®, www.sciencedirect.com.

## List of other related viruses which may be members of the genus *Fabavirus* but have not been approved as species

None reported.

# Genus *Nepovirus*

Type species ***Tobacco ringspot virus***

## Distinguishing features

Nepoviruses are the only known members of the family that encode a single large CP of 52–60 kDa. Genome organization and expression are similar to those of comoviruses, except that RNA-2 specifies a single polyprotein of 105–207 kDa. Nepoviruses can be divided into three subgroups. Subgroup A has an RNA-2 of 3,700–4,000 nts in length, present in both M and B components. Subgroup B has an RNA-2 of 4,400–4,700 nts in length, present only in the M component. Subgroup C has an RNA-2 of 6,400–7,300 nts in length, present in M component particles that are sometimes barely separable from those of B component. The three subgroups also differ in the cleavage sites recognized by their proteinase (see Table 2).

Additional linear or circular satellite RNAs, which sometimes modulate symptoms, are found associated with several nepoviruses of all three subgroups. They are either linear (1100–1800 nt) with a 5′-linked VPg, a 3′ poly(A) tail and encoding a 36–48 kDa polypeptide, or circular (300–460 nt) and apparently non-coding. They are present in some natural isolates but are not necessary for virus accumulation.

The RNA2-encoded polyprotein of subgroup A and B nepoviruses is processed into three domains. In grapevine fanleaf virus (GFLV), the N-terminal protein of the RNA2-encoded polyprotein (P2A) was shown to be involved in RNA-2 replication. The two other protein domains are the MP and the unique CP. Both are required for cell-to-cell movement of the virus. Similarly to comoviruses, the MP has a LPL motif, interacts with the CP and is a structural component of tubular structures containing virus-like particles and traversing the cell wall. In tomato ringspot virus (ToRSV) (subgroup C), the N-terminal region of the RNA2-encoded polyprotein is cleaved at an additional site, defining two domains (X3 and X4). The X3 protein contains some sequence similarity with the P2A protein of GFLV but the X4 protein is a unique protein of unknown function. The RNA-1 of nepoviruses is translated into a single polyprotein that is processed into six domains. The C-terminal region of the polyprotein contains the replication block, and is similar to that of comoviruses (NTB-VPg-Pro-Pol).

In contrast, the N-terminal region of the polyprotein contains an additional cleavage site defining two protein domains (X1 and X2) instead of the single domain present upstream of NTB in the comovirus genome. Cleavage at this additional site was demonstrated for arabis mosaic virus (subgroup A) and ToRSV (subgroup C). A putative cleavage site at this position has been implied for other nepoviruses. The function of X1 is unknown. X2 contains a sequence motif in common with the comovirus Co-Pro protein but does not seem to modulate the activity of the proteinase. However, similarly to the comovirus Co-Pro, the X2 protein of ToRSV associates with ER-derived membranes and a role in viral replication has been proposed. When comparing RNA-1 and RNA-2, the 5′ and 3′ NTRs are similar in sequence but not identical in subgroup A nepoviruses. In subgroup B nepoviruses, the 5′-NTRs also show sequence similarity between RNA-1 and RNA-2, while the 3′-NTRs are identical in both RNAs. In subgroup C nepoviruses, both NTRs are identical or nearly identical between RNA-1 and RNA-2. The region of sequence similarity extends into part of the coding region of the polyproteins in ToRSV, but not in blackcurrant reversion virus.

Nepoviruses are widely distributed in temperate regions. The natural host range of nepoviruses varies from wide to restricted, depending on the virus. Ringspot symptoms are characteristic, but mottling and spotting are equally frequent. Twelve species are acquired and transmitted persistently by longidorid nematodes (*Xiphinema*, *Longidorus* or *Paralongidorus* spp.), three are transmitted by pollen, one is transmitted by mites (blackcurrant reversion virus) and the others have no known biological vector. Seed and/or pollen transmission is very common. In herbaceous plants, the symptoms induced by nepoviruses are often transient, with newly emerging leaves appearing symptomless a few weeks after infection (the so-called "recovery" phenomenon). Symptom recovery is associated with induction of RNA silencing, an antiviral defence, and is sometimes (but not always) accompanied with reduced concentration of viral RNAs.

## List of species in the genus *Nepovirus*

| | | | |
|---|---|---|---|
| **Subgroup A** | [RNA1] | [RNA2] | |
| *Arabis mosaic virus* (Ash ring and line pattern virus) (Forsythia yellow net virus) (Raspberry yellow dwarf virus) (Rhubarb mosaic virus) | | | |
| Arabis mosaic virus-NW | [AY303786 = NC_006057] | [AY017339 = NC_006056] | (ArMV-NW) |
| *Arracacha virus A* | | | |
| Arracacha virus A-Huanuco | | | (AVA-Huanuco) |
| *Artichoke Aegean ringspot virus* | | | |
| Artichoke Aegean ringspot virus | | | (AARSV) |
| *Cassava American latent virus* | | | |
| Cassava American latent virus-South America | | | (CsALV-SA) |
| *Grapevine deformation virus* | | | |
| Grapevine deformation virus-Turkey | | [AY291208] | (GDeV-Turkey) |
| *Grapevine fanleaf virus* (Grapevine infectious degeneration virus) | | | |
| Grapevine fanleaf virus-F13 | [D00915 = NC_003615] | [X16907 = NC_003623] | (GFLV-F13) |
| *Olive latent ringspot virus* | | | |
| Olive latent ringspot virus-Italy | | [AJ277435] | (OLRSV-Italy) |
| *Potato black ringspot virus* | | | |
| Potato black ringspot virus-Greece | [AJ616715*] | | (PBRSV-Gr) |
| *Raspberry ringspot virus* (Raspberry Scottish leaf curl virus) (Redcurrant ringspot virus) | | | |
| Raspberry ringspot virus-Cherry | [AY303787 = NC_005266] | [AY303788 = NC_005267] | (RpRSV-Che) |
| *Tobacco ringspot virus* | | | |
| Tobacco ringspot virus-Bud blight | [U50869 = NC_005097] | [AY363727 = NC_005096] | (TRSV-Bud blight) |

| | | | |
|---|---|---|---|
| **Subgroup B** | | | |
| *Artichoke Italian latent virus* | | | |
| Artichoke Italian latent virus-Southern Italy | | [X87254*] | (AILV-SI) |
| *Beet ringspot virus* | | | |
| (Tomato black ring virus-Scottish) | | | |
| Beet ringspot virus-S | [D00322 = NC_003693] | [X04062 = NC_003694] | (BRSV-S) |
| *Cocoa necrosis virus* | | | |
| (Cacao swollen shoot virus-S) | | | |
| Cocoa necrosis virus-ATCC PV-283 | | [EU741694*] | (CoNV-PV283) |
| *Crimson clover latent virus* | | | |
| Crimson clover latent virus-UK Hertfordshire | | | (CCLV-UK) |
| *Cycas necrotic stunt virus* | | | |
| Cycas necrotic stunt virus-Japan | [AB073147 = NC_003791] | [AB073148 = NC_003792] | (CNSV-Japan) |
| *Grapevine Anatolian ringspot virus* | | | |
| Grapevine Anatolian ringspot virus-Turkey | | [AY291207] | (GARSV-Turkey) |
| *Grapevine chrome mosaic virus* | | | |
| Grapevine chrome mosaic virus-Hungary | [X15346 = NC_003622] | [X15163 = NC_003621] | (GCMV-Hungary) |
| *Mulberry ringspot virus* | | | |
| Mulberry ringspot virus-Japan | | | (MRSV-Japan) |
| *Tomato black ring virus* | | | |
| (Bean ringspot virus) | | | |
| (Lettuce ringspot virus) | | | |
| (Potato bouquet virus) | | | |
| Tomato black ring virus-MJ | [AY157993 = NC_004439] | [AY157994 = NC_004440] | (TBRV-MJ) |
| **Subgroup C** | | | |
| *Apricot latent ringspot virus* | | | |
| Apricot latent ringspot virus-Modesto | | [AJ278875*] | (ALRSV-Modesto) |
| *Artichoke yellow ringspot virus* | | | |
| (Tomato white ringspot virus) | | | |
| Tomato white ringspot virus-T818 | [EF205130*] | [EF205131*] | (TWRSV-T818) |
| *Blackcurrant reversion virus* | | | |
| Blackcurrant reversion virus-Finland Piikkio | [AF368272 = NC_003509] | [AF020051 = NC_003502] | (BRV-Piikkio) |
| *Blueberry leaf mottle virus* | | | |
| Blueberry leaf mottle virus-Michigan | [U20622*] | [U20621*] | (BLMoV-Michigan) |
| *Cassava green mottle virus* | | | |
| Cassava green mottle virus-Solomon Islands | | | (CsGMV-Solomon) |
| *Cherry leaf roll virus* | | | |
| (Elm mosaic virus) | | | |
| (Golden elderberry virus) | | | |
| (Walnut black line virus) | | | |
| Cherry leaf roll virus-walnut W8 | [Z34265*] | [U24694*] | (CLRV-W8) |
| *Chicory yellow mottle virus* | | | |
| (Parsley carrot leaf virus) | | | |
| Chicory yellow mottle virus-Italy | | | (ChYMV-Italy) |
| *Grapevine Bulgarian latent virus* | | | |
| Grapevine Bulgarian latent virus-Bulgaria | | | (GBLV-Bulgaria) |
| *Grapevine Tunisian ringspot virus* | | | |
| Grapevine Tunisian ringspot virus-Tunisia | | | (GTRSV-Tunisia) |

| | | | |
|---|---|---|---|
| *Hibiscus latent ringspot virus* | | | |
| Hibiscus latent ringspot virus-Ibadan | | | (HLRSV-Ibadan) |
| *Lucerne Australian latent virus* | | | |
| Lucerne Australian latent virus-TN | | | (LALV-TN) |
| *Myrobalan latent ringspot virus* | | | |
| Myrobalan latent ringspot virus-Prunus cerasifera | | | (MLRSV-Pc) |
| *Peach rosette mosaic virus* | | | |
| (Grape decline virus) | | | |
| (Grapevine degeneration virus) | | | |
| Peach rosette mosaic virus-Michigan | [AF016626] | | (PRMV-Michigan) |
| *Potato virus U* | | | |
| Potato virus U-Lichte Industrie | | | (PVU-LI) |
| *Tomato ringspot virus* | | | |
| (Grape yellow vein virus) | | | |
| (Nicotiana virus 13) | | | |
| (Peach yellow bud mosaic virus) | | | |
| (Tobacco ringspot virus n°2) | | | |
| Tomato ringspot virus-raspberry 2 | [L19655 = NC_003840] | [D12477 = NC_003839] | (ToRSV-Rasp2) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome segment.
A more extensive list of isolates is available online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Nepovirus* but have not been approved as species

None reported.

## Other genera in the family *Secoviridae*

## GENUS *CHERAVIRUS*

Type species ***Cherry rasp leaf virus***

### Distinguishing features

Cheraviruses have three CPs of similar sizes. In some cases, these proteins are not fully or reproducibly resolved from each other by electrophoresis. The genome of cheraviruses is bipartite and the genomic organization is similar to that of comoviruses, although RNA2 is thought to encode a single polyprotein (Figure 3). The RNA2-encoded movement protein of apple latent spherical virus (ALSV) is 42 kDa, suggesting that translation initiation occurs at the second AUG, which is in a better context. Tubular structures containing virus-like particles are observed in infected cells and are likely involved in cell-to-cell movement of the virus. The movement protein and all three CPs are necessary for cell-to-cell movement of the virus. The MP binds to VP25, one of the three CPs. VP20 of ALSV, another CP, is a suppressor of silencing that interferes with systemic movement of the silencing signal.

The host range is broad or narrow, depending on viruses, and includes weed plants found in the vicinity of infected crops. Symptoms are usually mild or absent. Cherry rasp leaf virus is transmitted by nematodes (*Xiphinema americanum*) in the field, and is readily seed-transmitted. ALSV is also seed-transmitted through both embryo and pollen in apple.

### List of species in the genus *Cheravirus*

| | [RNA1] | [RNA2] | |
|---|---|---|---|
| *Apple latent spherical virus* | | | |
| Apple latent spherical virus-Fukushima | [AB030940 = NC_003787] | [AB030940 = NC_003788] | (ALSV-Fukushima) |
| *Cherry rasp leaf virus* | | | |
| Cherry rasp leaf virus-USA potato | [AJ621357 = NC_006271] | [AJ621358 = NC_006272] | (CRLV-USA) |
| *Stocky prune virus* | | | |
| Stocky prune virus-Brugères | [DQ143874*] | [DQ143875*] | (StPV-Brugères) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome segment.
A more extensive list of isolates is available online on Science Direct ®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Cheravirus* but have not been approved as species

None reported.

## GENUS *SADWAVIRUS*

Type species ***Satsuma dwarf virus***

### Distinguishing features

Similarly to comoviruses, sadwaviruses have two CPs, one large and one small. The genome of sadwaviruses is bipartite and the genomic organization is similar to that of comoviruses. The proteinase of sadwaviruses is distinct from that of other viruses in the family in that it does not have a conserved His or Leu in the active site. In addition, the cleavage sites recognized by sadwavirus proteinases are unique with an A or a T at the −1 position (Table 2). In contrast to comoviruses, there is no evidence that two overlapping polyproteins are encoded by RNA-2. Similarly to some nepoviruses, extensive sequence identity between RNA-1 and RNA-2 are found in the 5′ NTRs as well as in the 5′end of the putative coding region.

All isolates of satsuma dwarf virus infect citrus trees. There are no known biological vectors.

### List of species in the genus *Sadwavirus*

| | [RNA1] | [RNA2] | |
|---|---|---|---|
| *Satsuma dwarf virus* | | | |
| Satsuma dwarf virus-S58 | [AB009958 = NC_003785] | [AB009959 = NC_003786] | (SDV-S58) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
A more extensive list of isolates is available online on Science Direct ®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Sadwavirus* but have not been approved as species

None reported.

# GENUS *TORRADOVIRUS*

Type species ***Tomato torrado virus***

## Distinguishing features

Similarly to cheraviruses, torradoviruses have a bipartite genome and three capsid proteins. The RNAs are polyadenylated. Presence of a VPg at the 5′ end of the RNAs has not been tested experimentally. The genomic organization is similar to that of other members of the family with a bipartite genome. The replication block is found in the RNA-1 polyprotein while the structural proteins are present in the C-terminal region of the RNA-2 polyprotein. A putative movement protein suggested upstream of the CP domains shares little sequence similarity with that of other bipartite members of the family with the exception of the small LPL motif. A distinguishing feature of the torradovirus genome is the presence of a second open reading frame upstream and partially overlapping with the large ORF in RNA-2 (Figure 3). This reading frame encodes a protein of unknown function, which exhibits a large degree of sequence diversity (61–74%) among torradoviruses. The 3′ NTRs share a large region with near sequence identity (>99%) between the RNA-1 and RNA-2 of a given species but differ substantially between species.

Tomato torrado virus was reported to be transmitted by whiteflies. Information is not available regarding vector transmission of other torradoviruses.

## List of species in the genus *Torradovirus*

| | [RNA1] | [RNA2] | |
|---|---|---|---|
| *Tomato torrado virus* | | | |
| Tomato torrado virus-PRI-0301 | [ DQ388879 = NC_009013] | [DQ388880 = NC_009032] | (ToTV-PRI-0301) |
| *Tomato marchitez virus* (Tomato apex necrosis virus) | | | |
| Tomato marchitez virus-PRI-0601 | [EF681764 = NC_010987] | [EF681765 = NC_010987] | (ToMarV-PRI-0601) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
A more extensive list of isolates is available online on Science Direct ®, www.sciencedirect.com.

## List of other related viruses which may be members of the genus *Torradovirus* but have not been approved as species

| | [RNA1] | [RNA2] | |
|---|---|---|---|
| Tomato chocolate virus-G01 | [FJ7560489] | [FJ560490] | (ToChV-G01) |
| Tomato chocolate spot virus-Guatemala | [GQ305131] | [GQ305132] | (ToChSV-Guatemala) |

Tomato chocolate virus (ToChV) and tomato chocolate spot virus (ToChSV) are related to tomato marchitez virus (ToMarV) and to a lesser degree to tomato torrado virus (ToTV). All viruses infect tomato and cause similar symptoms. Comparison of the aa sequence of the Pro-Pol and combined CP regions would suggest that ToChV and ToChSV are distant strains of ToMarV (82–90% aa sequence identity for the Pro-Pol region and 83–87% aa sequence identity in the combined CP region among the three viruses). However, other regions of the genome (RNA2-encoded ORF1 and the 3′ NTR) show significant sequence variation. In addition the length of the 3′ NTR varies significantly among these viruses. It is not known whether reassortment between the RNAs of ToMarV, ToChV and/or ToChSV is possible. Therefore, the taxonomic position of ToChV and ToChSV as two distinct species in the genus *Torradovirus*, as two strains of a single new species in the genus *Torradovirus* or as distant strains of the species *Tomato marchitez virus* remains unclear.

# Genus *Sequivirus*

Type species ***Parsnip yellow fleck virus***

## Distinguishing features

Virions contain three CPs of about 32–34, 22–26 and 22–24 kDa. The genome consists of a single molecule of ssRNA that encodes a single large polyprotein. The replication block (NTB-Pro-Pol) is contained in the C-terminal region of the polyprotein. The structural protein domains are present in the N-terminal region of the polyprotein but are separated from the N-terminus by a protein domain of about 40–60 kDa. Infectivity of the genome is susceptible to proteinase treatment suggesting the presence of a 5′-linked VPg. The parsnip yellow fleck virus (PYFV) RNA is not polyadenylated. This is a unique property within this family. In contrast, the RNA of carrot necrotic dieback virus is polyadenylated. Tubular structures containing virus-like particles have been observed traversing the cell wall of PYFV-infected cells. However, their role in cell-to-cell movement has not been investigated and the presence of a movement protein in the polyprotein (possibly upstream of the CPs) needs to be confirmed.

The natural host range of sequiviruses includes species in several plant families. Transmission of PYFV is by aphids in a semi-persistent manner. However, it is dependent on the presence of a helper virus in the genus *Waikavirus*.

## List of species in the genus *Sequivirus*

| | | |
|---|---|---|
| *Carrot necrotic dieback virus* | | |
| Carrot necrotic dieback virus-Anthriscus | [EU980442] | (CNDV-Anthriscus) |
| *Dandelion yellow mosaic virus* | | |
| Dandelion yellow mosaic virus-DSM2 | [DQ675189*] | (DaYMV-DSM2) |
| *Parsnip yellow fleck virus* | | |
| (Celery yellow net virus) | | |
| Parsnip yellow fleck virus-P121 | [D14066 = NC_003628] | (PYFV-P121) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

## List of other related viruses which may be members of the genus *Sequivirus* but have not been approved as species

None reported.

# Genus *Waikavirus*

Type species ***Rice tungro spherical virus***

## Distinguishing features

The genomic organization of waikaviruses is similar to that of sequiviruses. However, small ORFs have been identified near the 3′ end of the RNA or overlapping with the main polyprotein but in a different reading frame (Figure 3). Some experimental evidence has been presented suggesting that subgenomic RNAs are produced from the 3′ region of the RNA. The biological significance of the small open reading frames or of the putative subgenomic RNAs is not known. The genomic RNAs are polyadenylated at their 3′ end. The presence of a 5′-linked VPg has not been confirmed experimentally.

The natural host range of waikaviruses is usually restricted to species within a few plant families. Waikaviruses are not sap-transmitted. Field transmission is in the semi-persistent manner by aphids or leafhoppers. A virus-encoded helper protein is probably needed. Some waikaviruses are helper

viruses for the insect transmission of other viruses: PYFV (genus *Sequivirus*) in the case of anthriscus yellows virus and rice tungro bacilliform virus (family *Caulimoviridae*) in the case of rice tungro spherical virus (this association being responsible for the very damaging rice tungro disease).

## List of species in the genus *Waikavirus*

| | | |
|---|---|---|
| *Anthriscus yellows virus* | | |
| Anthriscus yellows virus-Anthriscus sylvestris | | (AYV-As) |
| *Maize chlorotic dwarf virus* | | |
| Maize chlorotic dwarf virus-Tennessee | [U67839 = NC_003626] | (MCDV-Tennessee) |
| *Rice tungro spherical virus* | | |
| Rice tungro spherical virus-Los Banos Phillipine | [M95497 = NC_001632] | (RTSV-LB) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
A more extensive list of isolates is available online on Science Direct ®, www.sciencedirect.

## List of other related viruses which may be members of the genus *Waikavirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Secoviridae*

| | [RNA1] | [RNA2] | |
|---|---|---|---|
| *Black raspberry necrosis virus* | | | |
| Black raspberry necrosis virus-BrdAV-1 | [DQ344639 = NC_008182] | [DQ344640 = NC_008183] | (BRNV-BrDAV1) |
| *Strawberry latent ringspot virus* (Rhubarb virus 5) | | | |
| Strawberry latent ringspot virus-NCGR MEN 454.001 | [AY860978 = NC_006964] | [AY860979 = NC_006965] | (SLRSV-MEN454) |
| *Strawberry mottle virus* | | | |
| Strawberry mottle virus-1134 | [AJ311875 = NC_003445] | [AJ311876 = NC_003446] | (SMoV-1134) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
A more extensive list of isolates is available online on Science Direct ®, www.sciencedirect.com.

Strawberry mottle virus and black raspberry virus are related to satsuma dwarf virus (SDV) in phylogenetic trees using the conserved Pro-Pol region (see Figure 5). They also have a bipartite genome. However, the nature of their capsid protein(s) and their genomic organization are not known. For this reason, they are considered unassigned species in the family *Secoviridae*. Strawberry latent ringspot virus was formerly considered a sadwavirus because it has two CPs and some distant relation with SDV in phylogenetic trees using the Pro-Pol sequence (Figure 5). However, its genomic organization is more related to that of cheraviruses (with the exception of the number of CPs, Figure 3) and it branches more closely with cheraviruses than with sadwaviruses in the phylogenetic trees using the Pro-Pol sequence (Figure 5). For these reasons, it is not considered a sadwavirus any more, and is now an unassigned species in the family *Secoviridae*.

## Phylogenetic relationships within the family

Members of the family *Secoviridae* were previously classified in two different families: *Comoviridae* (including the genera *Comovirus*, *Fabavirus* and *Nepovirus*) and *Sequiviridae* (including the genera *Sequivirus* and *Waikavirus*) and in two unassigned genera: *Cheravirus* and *Sadwavirus*. The families and genera were recently amalgamated to create the new family *Secoviridae*, which regroups all plant picornavirales.

The conserved Pro-Pol region, delineated by the "CG" motif of the 3C-like proteinase and the "GDD" motif of the polymerase, has been used to determine the relationship among picornavirales. Comparison of the Pro-Pol sequence among members of the family *Secoviridae* allows the

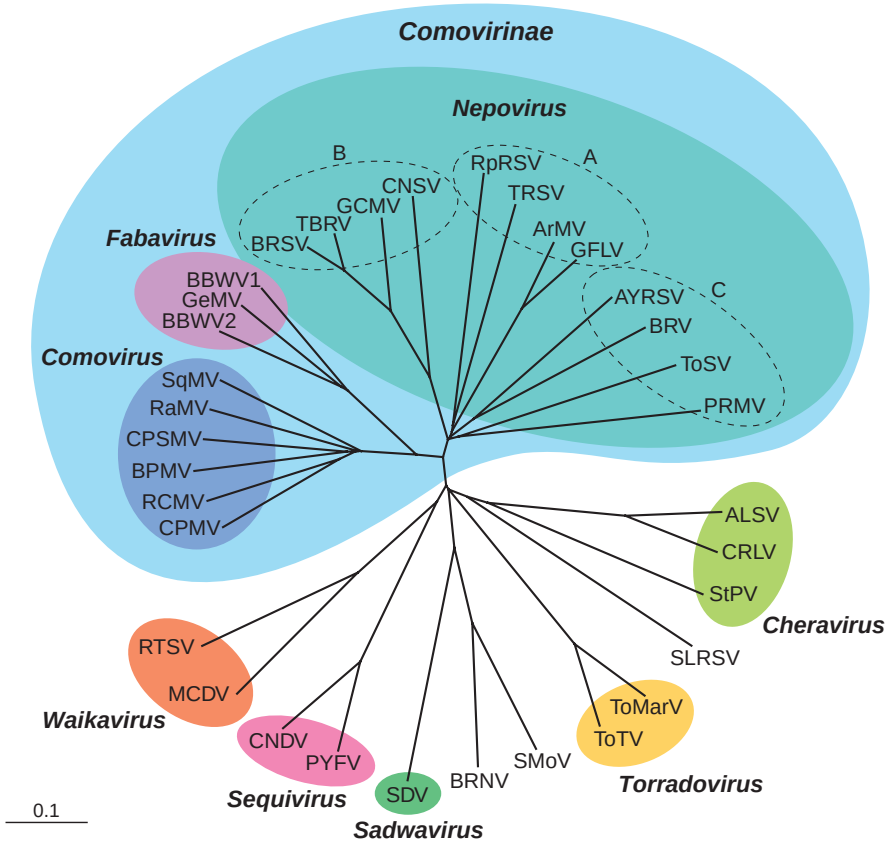


**Figure 5:** Hierarchical clustering of members of the family *Secoviridae* based on the amino acid sequences of the conserved domains between the "CG" motif of the 3C-proteinase and the "GDD" motif of the polymerase (Pro-Pol region). Results are presented as an unrooted radial tree. The bar represents a p-distance of 0.1. Different genera within the family are shown with the color ovals. The subfamily *Comovirinae* is shown with the light blue shading. Unfilled circles with the letters represent the different nepovirus subgroups. For each species, the sequence of the type isolate was used for the alignments (see species tables for the sequence accession numbers).

definition of branches that generally correspond to the distinct genera. Members of the subfamily *Comovirinae* (genera *Comovirus*, *Fabavirus* and *Nepovirus*) are more closely related to each other than to other genera within the family (Figure 5). Within this subfamily, fabaviruses and comoviruses are more closely related to each other than to nepoviruses. Nepovirus subgroups are not clearly separated in the Pro-Pol tree (with the exception of subgroup B which constitutes a separate branch) but are more clearly separated in phylogenetic trees using the CP sequence (not shown).

## Similarity with other taxa

Secovirids are related to members of other families in the order *Picornavirales*. They all share a common virion structure, organization of the replication block within the polyproteins and conserved properties of the replication proteins, including the 3C-like proteinase. Secovirids are also related to members of the families *Potyviridae* and *Caliciviridae* in some aspects (common replication block, polyprotein strategy, VPg bound to the 5′ end of the genome and poly(A) tail at the 3′ end of the genome) but differ in other properties.

## Derivation of names

*Seco*: derived from the amalgamation of the previous families *Se*quiviridae and *Co*moviridae.
*Como*: from *co*wpea *mo*saic virus, the type member.
*Faba*: derived from the Latin *faba*, "bean"; also *Vicia faba*, broad bean.
*Nepo*: from *ne*matode-transmitted, *po*lyhedral particles.
*Chera*: from *che*rry *ra*sp leaf virus, the type member.
*Sadwa*: from *sa*tsuma *dwa*rf virus, the type member.
*Torrado*: derived from tomato *torrado* virus, the type member. In Spanish, *torrado* means "toasted" to refer to the severe necrosis (burnt-like phenotype) observed in the disease induced by ToTV.
*Sequi*: from Latin *sequi*, "follow", "accompany" (in reference to the dependent aphid transmission of parsnip yellow fleck virus).
*Waika*: from Japanese, describing the symptoms induced in rice by infection with rice tungro spherical virus alone (i.e. in the absence of rice tungro bacilliform virus).

## Further reading

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## Contributed by

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# ORDER *TYMOVIRALES*

## Taxonomic structure of the order

| | |
|---|---|
| Order | ***Tymovirales*** |
| Family | ***Alphaflexiviridae*** |
| Genus | ***Allexivirus*** |
| Genus | ***Botrexvirus*** |
| Genus | ***Lolavirus*** |
| Genus | ***Mandarivirus*** |
| Genus | ***Potexvirus*** |
| Genus | ***Sclerodarnavirus*** |
| Family | ***Betaflexiviridae*** |
| Genus | ***Capillovirus*** |
| Genus | ***Carlavirus*** |
| Genus | ***Citrivirus*** |
| Genus | ***Foveavirus*** |
| Genus | ***Trichovirus*** |
| Genus | ***Vitivirus*** |
| Family | ***Gammaflexiviridae*** |
| Genus | ***Mycoflexivirus*** |
| Family | ***Tymoviridae*** |
| Genus | ***Maculavirus*** |
| Genus | ***Marafivirus*** |
| Genus | ***Tymovirus*** |

## Introduction

The order *Tymovirales* contains viruses that mostly infect plants, have a single molecule of positive sense ssRNA and which are united by the similarities in their replication-associated polyproteins (which account for the majority of the coding capacity of the genome). While there are differences in particle morphology between families, phylogenetic analysis requires the family *Tymoviridae* (with isometric virions) to be included within a "flexivirus" grouping of viruses that have filamentous virions. There is therefore a convincing case for a common ancestor for all members of the *Tymovirales* that excludes all other viruses so far characterized.

## Virion properties

### MORPHOLOGY

Virions within the families *Alphaflexiviridae*, *Betaflexiviridae* and *Gammaflexiviridae* are flexuous filaments, usually 12–13 nm in diameter and from about 470 to 1000 nm in length, depending on the genus. They have helical symmetry and in some genera there is clearly visible cross-banding. Members of the family *Tymoviridae* have isometric virions about 30 nm in diameter with a rounded contour and prominent surface structure.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

These vary, depending on the particle morphology. Those with filamentous particles usually sediment as single bands with an $S_{20,w}$ of 92–176S, depending on the genus. In the family *Tymoviridae*, particles sediment as two centrifugal components ($S_{20,w}$ values of 42–55 and 109–125S).

### NUCLEIC ACID

In all members of the order, virions contain a single molecule of positive sense ssRNA between 5.9 and 9.0 kb in length. This constitutes 5–6% by weight of the virion in those with filamentous particles and 25–35% in those with isometric virions (*Tymoviridae*). The genomes are polyadenylated at the 3′ terminus (except in the genus *Tymovirus* where they usually have a tRNA-like structure at the 3′ end) and capped with $m^7G$ at the 5′ end.

Pos. ssRNA

### Proteins

Almost all members have a single coat protein of 18–44 kDa. In a few instances (genus *Lolavirus* and some marafiviruses) there are two structural proteins which are different forms from the same gene. In a single case (genus *Sclerodarnavirus*) no structural protein is known.

### Lipids

None reported.

### Carbohydrates

Usually none but there are reports that the coat protein is glycosylated in Lolium latent virus (genus *Lolavirus*) and in some strains of potato virus X (genus *Potexvirus*).

## Genome organization and replication

The largest protein encoded is a replication-associated polyprotein of about 150–250 kDa close to the 5′ end of the genome and which is translated directly from the genomic RNA. The protein contains a set of functional domains whose amino acid sequences and order are conserved in all viruses of the alphavirus-like superfamily of positive-strand RNA viruses. A methyltransferase type 1 domain (Mtr) is located near the N terminus and an RNA-dependent RNA polymerase domain (RdRp) with a characteristic core motif $S/TGx_3Tx_3NS/Tx_{22}GDD$ occurs near the C terminus. An RNA helicase domain of superfamily 1 (Hel) is localized upstream from the RdRp domain. The Mtr is involved in capping the RNA, the Hel in unwinding RNA and the RdRp in RNA synthesis. Upstream from the Hel in some genera, there is a papain-like cysteine protease domain (P-Pro) that processes the replication polyprotein. Immediately upstream of the P-Pro, some members have a protease of the Ovarian Tumor (OTU) family. Finally, some members have an AlkB (alkylated DNA repair protein) motif between the Mtr and protease or helicase regions. Except in the genus *Sclerodarnavirus*, where the replicase is the only protein, there are 1 to 5 smaller proteins, translated in most cases from subgenomic RNAs. These include the capsid protein and one or more proteins involved in cell-to-cell movement. The exact organization of the genome is usually a characteristic of the individual genus.

## Antigenic properties

These vary depending on the genus.

## Biological properties

Most members of the order infect plants but a few species are from plant pathogenic fungi. Host range and transmission are often characteristic of individual genera.

## Phylogenetic relationships within the order

In phylogenetic analysis of the replication protein, each genus and family forms a distinct, well-supported branch. The viruses with flexuous virions in the families *Alphaflexiviridae* and *Gammaflexiviridae* are more closely related to those with icosohedral particles in the family *Tymoviridae* than they are to members of the *Betaflexiviridae* (Figure 1).

## Similarity with other taxa

In an analysis of the replication protein, the order forms a very distinct group within the alphavirus-like superfamily, with a rather distant relationship to plant virus families such as *Closteroviridae*, *Bromoviridae* and *Virgaviridae*.

## Derivation of name

The name is derived from the genus *Tymovirus* (and family *Tymoviridae*). This was chosen because the other constituent families have names reflecting their flexuous virions (not a characteristic of all members of the order).

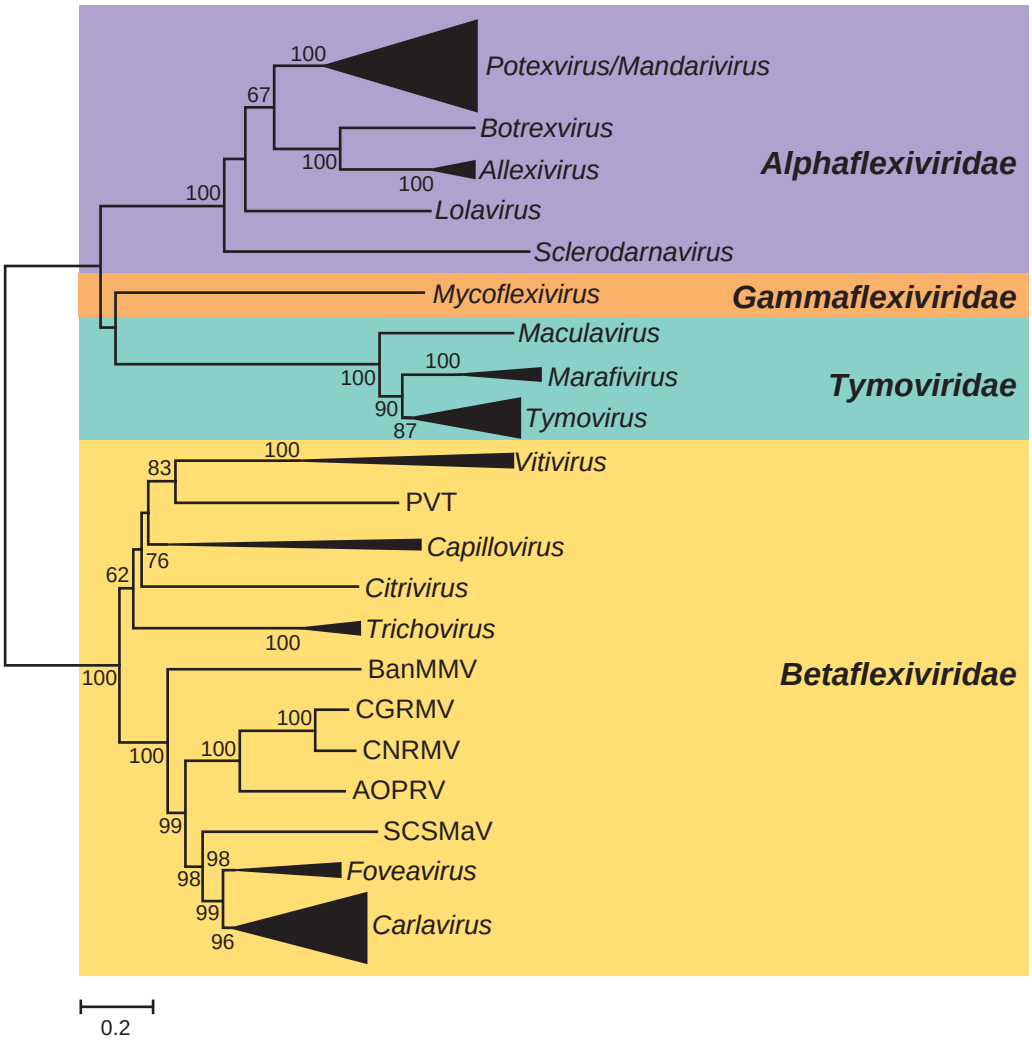


**Figure 1:** Phylogenetic (distance) tree based on the amino acid sequences of the entire replication protein of members of the order *Tymovirales*. A single representative isolate of each sequenced species in the order was included. Genera and families (which are all monophyletic) have been collapsed into a triangle, the size of which corresponds to the variation found within the clade. Numbers on branches indicate percentage of bootstrap support out of 1000 bootstrap replications (when >60%). The scale indicates JTT (Jones-Taylor-Thornton matrix) amino acid distances. Tree produced in MEGA4.



## Further reading

Martelli, G., Adams, M.J., Kreuze, J.F. and Dolja, V. V. (2007). Family *Flexiviridae*: a case study in virion and genome plasticity. *Ann. Rev. Phytopathol*, **45**, 73–100.

## Contributed by

Adams, M.J., Kreuze, J.F. and Martelli, G.P.

# Family *Alphaflexiviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Alphaflexiviridae*** |
| Genus | ***Allexivirus*** |
| Genus | ***Botrexvirus*** |
| Genus | ***Lolavirus*** |
| Genus | ***Mandarivirus*** |
| Genus | ***Potexvirus*** |
| Genus | ***Sclerodarnavirus*** |

## Distinguishing features

The family contains viruses with flexuous filamentous virions that infect plants and a few viruses discovered in plant-infecting fungi. They share a distinct lineage of alphavirus-like replication proteins that is unusual in lacking any recognized protease domain.

## Virion properties

### Morphology

Virions are flexuous filaments, usually 12–13 nm in diameter (range 10–15 nm) and from 470 to about 800 nm in length, depending on the genus. They have helical symmetry with a pitch of about 3.4 nm (range 3.3–3.7 nm) and in some genera there is clearly visible cross-banding.

### Physicochemical and physical properties

Virions sediment as a single band (or occasionally two very close bands) with an $S_{20,w}$ of 92–176*S*, depending on the genus.

### Nucleic acid

Virions contain a single molecule of linear ssRNA of about 5.9–9.0 kb which is 5–6% by weight of the virion. The RNA is capped (or probably capped) at the 5′ terminus with $m^7G$ and has a polyadenylated tract at the 3′ terminus. Smaller 3′-co-terminal sgRNAs are encapsidated in some, but not all, members of the genus *Potexvirus.*

### Proteins

The viral capsid of all members of the family (except in the genus *Lolavirus*) is composed of a single polypeptide ranging in size from 18 to 43 kDa. In allexiviruses, a 42 kDa polypeptide was also detected as a minor component of virions. In lolaviruses a shorter (ca. 28 kDa) carboxy co-terminal polypeptide forms an equimolar fraction of the virion with the polypeptide originating from the first AUG (ca. 32 kDa).

### Lipids

None reported.

### Carbohydrates

Usually none, but the coat protein of some strains of the species *Potato virus X* (genus *Potexvirus*) and both forms of the lolavirus coat protein are reported to be glycosylated.

## Genome organization and replication

There are five or six genes depending upon the genus (except in the genus *Sclerodarnavirus*). The ORF1-encoded product, which follows a short 5′-UTR sequence, has homologies with polymerase proteins of the "alphavirus-like" supergroup of RNA viruses. This protein (150–195 kDa) contains conserved methyltransferase, helicase and RNA-dependent RNA polymerase (RdRp) motifs; some also include an AlkB domain (alkylated DNA repair protein). In all plant-infecting members, ORFs 2–4 encode the "triple gene block" (TGB) proteins involved in cell-to-cell movement and ORF5 is the viral coat protein. In some genera (*Allexivirus*, *Lolavirus* and *Mandarivirus*) a final ORF encodes a protein with a zinc binding finger motif and the ability to bind nucleic acids. ORFs downstream of



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the polymerase are translated from 3′-terminal sgRNAs that can often be found in infected tissue. Replication is (or is presumed to be) cytoplasmic and the product of ORF1 is the only virus-encoded protein known to be involved.

## Antigenic properties

Virions are usually highly immunogenic. Within (but not usually between) genera, some viruses are serologically related.

## Biological properties

Members have been reported from a wide range of mono- and dicotyledonous plant species but the host range of individual members is usually limited. Many of the viruses have relatively mild effects on their host. All species can be transmitted by mechanical inoculation, often readily. Many of the viruses have no known invertebrate or fungus vectors; however, allexiviruses are thought to be mite-borne. Aggregates of virus particles accumulate in the cytoplasm but there are usually no specific cytopathic structures.

## Species and genus demarcation criteria in the family

Genera are distinguished by various features of genome organization and host. These are summarized in Table 1. Throughout the family, isolates of different species should have less than about 72% nt identity (or 80% aa identity) between their respective CP or polymerase genes. Viruses from different genera usually have less than about 45% nt identity in these genes.

**Table 1:** Distinguishing properties of genera in the family *Alphaflexiviridae*

| Genus | Host | Virion length (nm) | ORFs | Rep[a] (kDa) | CP[b] |
|---|---|---|---|---|---|
| *Allexivirus* | plants | ca. 800 | 6 | 170–195 | 26–29 |
| *Botrexvirus* | fungi | ca.720 | 5 | 158 | 43 |
| *Lolavirus* | plants | 640 | 6 | 196 | 32 |
| *Mandarivirus* | plants | 650 | 6 | 187 | 34 |
| *Potexvirus* | plants | 470–580 | 5 | 150–195 | 18–27 |
| *Sclerodarnavirus* | fungi | n/a[c] | 1 | 193 | n/a[c] |

[a]Rep, replication protein size (kDa).
[b]CP, coat protein size (kDa).
[c]No virions found.

Pos. ssRNA

# Genus *Allexivirus*

Type species ***Shallot virus X***

## Distinguishing features

Allexiviruses are distinguished by mite transmission and by the presence of a large ORF4 in the position where the third, and smallest, of the TGB proteins is found in the other plant-infecting members of the family.

## Virion properties

### Morphology

Virions are highly flexible filamentous particles, about 800 nm in length and 12 nm in diameter. They resemble potyviruses in their length, but closteroviruses in their flexibility and cross-banded substructure (Figure 1).

### Physicochemical and physical properties

Virions of shallot virus X sediment with an $S_{20,w}$ of about 170S in 0.1 M tris-HC1, pH 7.5 at 20 °C and have a buoyant density in CsCl of 1.33 g cm$^{-3}$.

### Nucleic acid

Virions contain a single molecule of linear ssRNA, about 9.0 kb in size, with a 3′-poly(A) tract.

### Proteins

Virions are composed of a 28–36 kDa polypeptide as a major CP. A 42 kDa polypeptide is a minor component of virions.

## Genome organization and replication

The genomic RNA contains six large ORFs and short UTRs at the 5′ and 3′ termini (Figure 2). The ORFs code for polypeptides of about 195, 26, 11, 42, 28 and 15 kDa, respectively from 5′ end to 3′ end. The 195 kDa polypeptide is the polymerase. The 26 and 11 kDa proteins are similar to the first two proteins encoded by the TGB of related plant viruses and are probably involved in cell-to-cell movement of the virus. There is a coding sequence for a third small (7–8 kDa) TGB protein but it lacks the initiation AUG-codon. The 42 kDa polypeptide (ORF4) has no significant homology with any known proteins but, in plants infected with an isolate of the type species, it was expressed in relatively large amounts and was shown to be involved in virion assembly. The 28 kDa polypeptide is the CP. In SDS-polyacrylamide gel electrophoresis it migrates as an apparently 32–36 kDa protein, which could be due to its high hydrophilicity. The 15 kDa protein has a zinc binding finger motif and an ability to bind nucleic acids. The function of this polypeptide is not known.

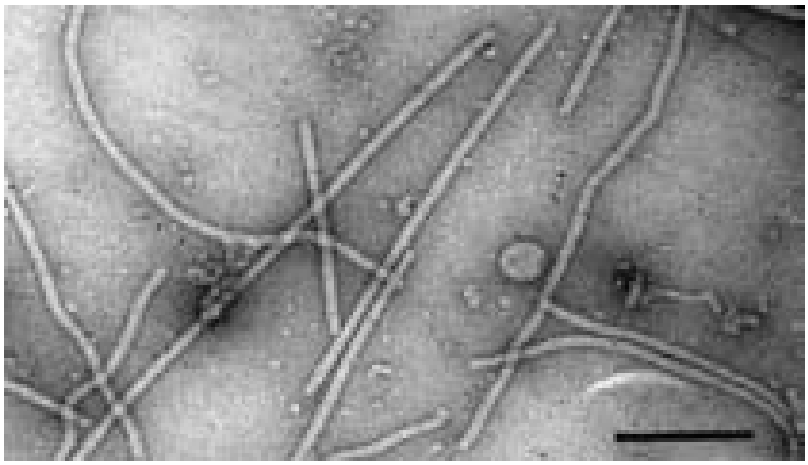

**Figure 1:** Electron micrograph of negatively-stained virions of an isolate of shallot virus X. The bar = 200 nm.

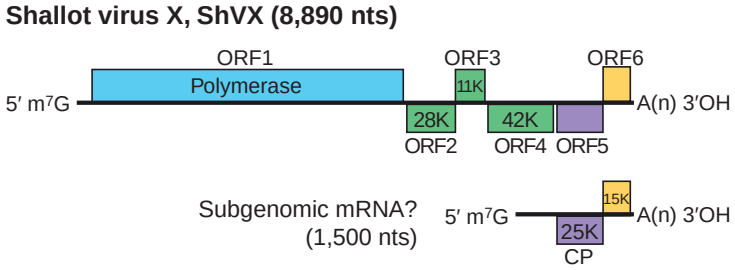


**Figure 2:** Genome organization and translation strategy of shallot virus X.

## Antigenic properties

Allexivirus particles are good immunogens. Some members of the genus are serologically interrelated. Specific antisera and monoclonal antibodies against pure virus particles as well as antisera against recombinant CPs have been used for differentiation purposes.

## Biological properties

### Host range

Host range is extremely restricted. Some isolates from shallot, onion, garlic and sand leek have been experimentally transmitted to *Chenopodium murale*, in which they induced local lesions.

### Transmission

Allexiviruses are thought to be mite-borne. Garlic viruses C and D have been shown to be transmitted by the eriophyd mite, *Aceria tulipae*. All are manually transmissible by sap inoculation to healthy host plants. None could be transmitted by aphids.

### Geographical distribution

Allexiviruses are widely distributed and probably occur wherever the host plants are grown.

### Cytopathic effects

Most induce no visible or only very mild symptoms in many species, although certain isolates can cause severe damage to crops. In infected tissue allexiviruses can induce formation of granular inclusion bodies and small bundles of flexible particles.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Members of distinct species have less than about 72% nt identity (or 80% aa identity) between their CP or polymerase genes.
- Different reactions with antisera.

## List of species in the genus *Allexivirus*

The available information about the identity of the members in the genus *Allexivirus* is still fragmentary. They are almost always found in mixed infections of vegetatively propagated species and it is difficult or impossible to isolate and/or separate the viruses.

| | | |
|---|---|---|
| *Garlic mite-borne filamentous virus* | | |
| Garlic mite-borne filamentous virus-South Korea | | (GarMbFV-KO) |
| *Garlic virus A* | | |
| Garlic virus A-Japan | [AB010300 = NC_003375] | (GarV-A-JA) |
| *Garlic virus B* | | |
| Garlic virus B-Japan | [AB010301*] | (GarV-B-JA) |
| *Garlic virus C* | | |
| Garlic virus C-Japan | [AB010302 = NC_003376] | (GarV-C-JA) |
| *Garlic virus D* | | |
| Garlic virus D-Japan | [AB010303*] | (GarV-D-JA) |
| *Garlic virus E* | | |
| Garlic virus E-China:Yuhang | [AJ292230 = NC_004012] | (GarV-E-YH) |
| *Garlic virus X* | | |
| Garlic virus X-Korea | [U89243 = NC_001800] | (GarV-X-KO) |
| *Shallot virus X* | | |
| Shallot virus X-Russia | [M97264 = NC_003795] | (ShVX-RU) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

## List of other related viruses which may be members of the genus *Allexivirus* but have not been approved as species

None reported.

# Genus *Botrexvirus*

Type species ***Botrytis virus X***

## Distinguishing features

The single member of the genus infects a filamentous fungus. The genome lacks the TGB characteristic of plant-infecting members of the family.

## Virion properties

### Morphology

Virions are flexuous filaments of 720 nm modal length and about 13 nm in diameter.

### Physicochemical and physical properties

No information.

### Nucleic acid

Virions contain a single molecule of linear ssRNA, 6966 nt in length, excluding the 3′-poly(A) tail.

### Proteins

The only structural protein is the coat protein composed of 400 aa (43 kDa).

## Genome organization and replication

The genomic RNA comprises five putative ORFs on the positive strand, a 5′-UTR of 95 nt and a 3′-UTR of 149 nt, followed by a poly(A) tail (Figure 3). ORF1 is the 158 kDa polymerase, ORF3 encodes the 44 kDa coat protein and the functions of the other predicted proteins (ORF2, 30 kDa; ORFs 4 and 5 both 14 kDa) are unknown.

## Antigenic properties

No information.

## Biological properties

The virus was discovered infecting an isolate of the plant pathogenic fungus *Botrytis cinerea*. Its mode of transmission is unknown. The same fungal isolate was also infected with a virus now classified as *Botrytis virus F* (genus *Mycoflexivirus*, family *Gammaflexiviridae*).

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Botrexvirus*

*Botrytis virus X*
Botrytis virus X-New Zealand:Auckland [AY055762 = NC_005132] (BotVX-AUK)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

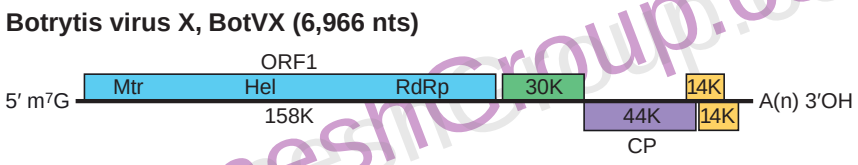


**Figure 3:** Genome organization of Botrytis virus X showing the relative positions of the ORFs and their expression products. Mtr, methyltransferase; Hel, helicase; RdRp, RNA-dependent RNA polymerase; CP, capsid protein.

### List of other related viruses which may be members of the genus *Botrexvirus* but have not been approved as species

None reported.

## GENUS *LOLAVIRUS*

Type species ***Lolium latent virus***

### Distinguishing features

This genus consists of a single species. There are probably 6 ORFs (although the putative ORF6 is smaller than in other genera). The coat protein is larger and the virions are longer than the potexviruses, which it otherwise resembles. Notably two carboxy co-terminal forms of the coat protein are found in essentially equimolar amounts in both extracts of infected plants and in purified virions.

### Virion properties

#### MORPHOLOGY

Virions are slightly flexuous filaments of 640 nm modal length and about 13 nm diameter (Figure 4).

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virions have a buoyant density in CsCl of about 1.33 g $cm^{-3}$.

#### NUCLEIC ACID

Virions contain a single molecule of linear ssRNA, 7674 nt in length, excluding the poly(A) tail. Infectious clones have been produced.

#### PROTEINS

The only structural proteins are two carboxy co-terminal forms of the coat protein, composed of 293 amino acids (32 kDa) and about 28 kDa. The two protein forms occur in virions in equimolar amounts. Both forms are glycosylated at one or more of several potential sites.

### Genome organization and replication

The genomic RNA is comprised of six putative ORFs on the positive strand, a 5′-UTR of 87 nt and a 3′-UTR of 97 nt, followed by a poly(A) tail (Figure 5). ORF 1 (196 kDa) contains conserved methyl transferase, AlkB, helicase and RdRp motifs. ORFs 2–4 encode the TGB proteins of 30.5, 13 and 7.5 kDa respectively. ORF 5 encodes the viral coat protein of 31.6 kDa; the smaller carboxy

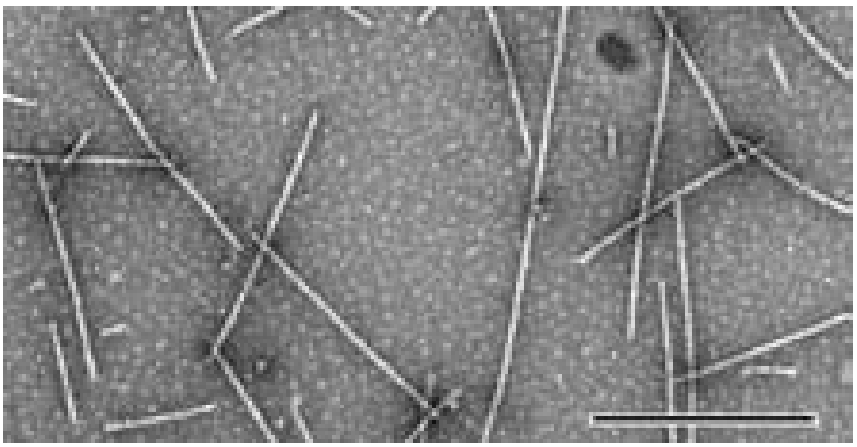

**Figure 4:** Negative contrast electron micrograph of particles of an isolate of Lolium latent virus stained in phosphotungstic acid. The bar represents 500 nm. (Courtesy of M.M. Dienelt.)

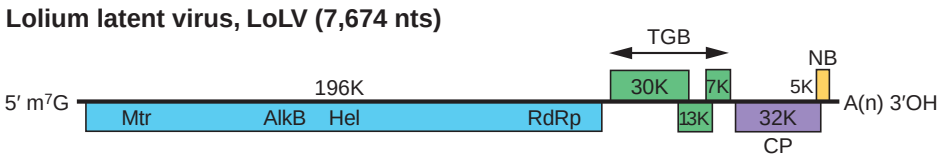


**Figure 5:** Genome organization of Lolium latent virus showing the relative positions of the ORFs and their expression products. Mtr, methyltransferase; Hel, helicase; RdRp, RNA-dependent RNA polymerase; TGB, triple gene block; CP, capsid protein; NB, putative nucleic acid binding protein.

co-terminal coat protein is potentially derived by internal initiation at a second AUG 141 nt downstream from the first (and in a stronger context) but might also arise from proteolytic removal of a non-canonical chloroplast transit peptide. ORF 6 partially overlaps ORF 5, and encodes a predicted 45 amino acid, 5.1 kDa highly basic protein (pI 9.56), which may act as a nucleic acid binding protein (NABP); three Cys and two His residues differ in spacing from those of characterized NABPs (10–23 kDa), and no significant homology is observed to other proteins in the database.

## Antigenic properties

The virus is a good immunogen and its antiserum reacts with both forms of the coat protein. In indirect ELISA, the antiserum reacted to an isolate of *Alternanthera mosaic virus* (genus *Potexvirus*) in infected *Nicotiana benthamiana*. However, the virus was not detected with antiserum specific to this or other viruses in the genera *Potexvirus* and *Carlavirus*.

## Biological properties

### Host range

The natural host range of the virus is restricted to gramineaceous species including ryegrass (*Lolium*). It also readily infects *N. benthamiana* and a few other dicotyledonous species. In *N. benthamiana*, it induces local lesions and systemic mosaic that varies from mildly chlorotic to white.

### Transmission

Readily transmitted to healthy host plants by sap inoculation.

### Geographical distribution

Reported from Europe (Germany, the Netherlands, France and the United Kingdom), and from the United States.

### Cytopathic effects

The virus induces insignificant symptoms or mild chlorotic flecking in its natural hosts. More severe chlorotic to necrotic streaking may occur in mixed infectious with other viruses. In infected tissue masses of flexuous virions may be observed in association with the chloroplasts.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Lolavirus*

*Lolium latent virus*
Lolium latent virus-US1 [EU489641 = NC_010434] (LoLV-US1)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Lolavirus* but have not been approved as species

None reported.

# GENUS *MANDARIVIRUS*

Type species ***Indian citrus ringspot virus***

## Distinguishing features

This genus contains a single virus species infecting a tree host. There are six ORFs in the genome and the coat protein is the largest of any plant-infecting member of the family.

## Virion properties

### MORPHOLOGY

Virions are flexuous filaments of 650 nm modal length, 13 nm in diameter, with clearly visible cross-banding (Figure 6).

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

The virus forms a single band in caesium sulphate density gradients. Purified preparations show maximum absorption at 260 nm with a $A_{260/280}$ ratio of 1.1 (corrected for light scattering).

### NUCLEIC ACID

Virions contain a single molecule of linear ssRNA, 7560 nt in length, excluding the 3′-poly(A) tail.

### PROTEINS

The only structural protein is the CP, composed of 325 aa (34 kDa).

## Genome organization and replication

The genomic RNA comprises six ORFs on the positive strand, a 5′-UTR of 78 nt and a 3′-UTR of 40 nt, followed by a poly(A) tail (Figure 7). No significant ORFs are in the negative strand. ORF1 encodes the viral polymerase. ORFs 2, 3 and 4 form the TGB. ORF5 encodes the CP. ORF6 encodes a putative protein of unknown function that shows limited similarity with nucleic acid-binding proteins encoded by ORF6 of allexi- and carlaviruses.

## Antigenic properties

Particles are good immunogens, rabbit antisera can have titers of 1/128 and 1/2048 in gel diffusion and EM decoration, respectively.

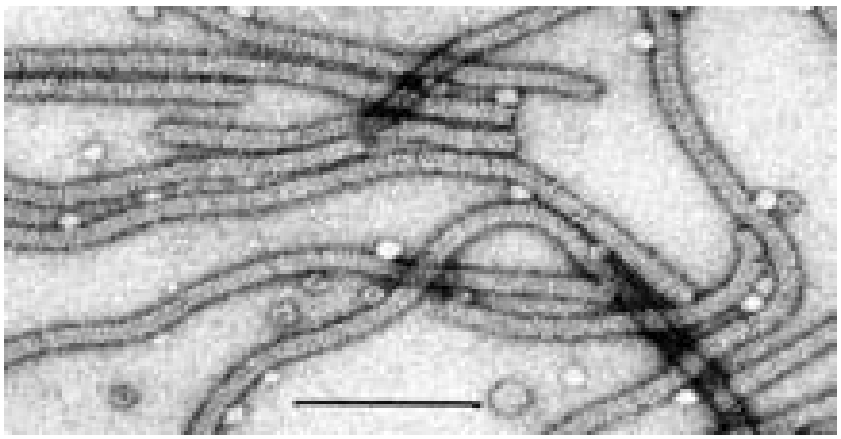

**Figure 6:** Electron micrograph of particles of an isolate of Indian citrus ringspot virus, stained in 1% uranyl acetate. The bar represents 100 nm.

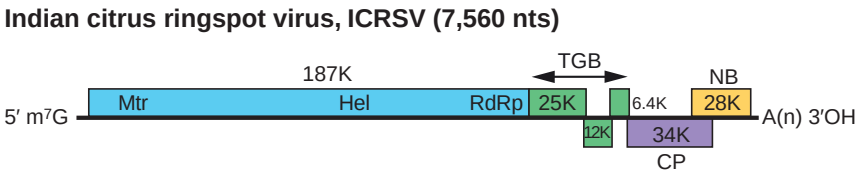


**Figure 7:** Genome organization of Indian citrus ringspot virus (ICRSV) showing the relative positions of the ORFs and their expression products. Mtr, methyltransferase; Hel, helicase; RdRp, RNA-dependent RNA polymerase; CP, capsid protein; NB, nucleic acid binding protein. The 25K, 12K and 6.4K proteins constitute the triple gene block.

## Biological properties

The virus causes a serious disease of citrus, especially Kinnow mandarin, in India, with bright yellow ringspots on mature leaves, followed by rapid decline of the tree. Experimentally the virus can be mechanically inoculated to leaves of *Chenopodium quinoa*, *C. amaranticolor*, *Glycine max*, *Vigna unguiculata* and *Phaseolus vulgaris*, giving local lesions, but systemic infection only in *P. vulgaris*. No natural vector is known, but it is transmitted by grafting and persists in the host.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Mandarivirus*

*Indian citrus ringspot virus*
Indian citrus ringspot virus-K1 [AF406744 = NC_003093] (ICRSV-K1)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Mandarivirus* but have not been approved as species

None reported.

# Genus *Potexvirus*

Type species ***Potato virus X***

## Distinguishing features

Compared to other genera in the family, potexviruses have short virions (<700 nm) and only five ORFs. They infect herbaceous hosts and have no known vectors.

## Virion properties

### Morphology

Virions are flexuous filaments, 470–580 nm in length and 13 nm in diameter, with helical symmetry and a pitch of 3.3–3.7 nm (Figure 8). A central axial canal, about 3 nm in diameter, is discernible only in best preparations. The number of protein subunits per turn of the primary helix is slightly less than 9.0. The RNA backbone is at a radial position of 3.3 nm.

### Physicochemical and physical properties

Virion Mr is about. 3.5 $\times 10^6$; $S_{20,w}$ is 115–130S; buoyant density in CsCl is 1.31 g cm$^{-3}$.

### Nucleic acid

Virions contain a single linear molecule of positive sense ssRNA of about. 5.9–7.0 kb which is approximately 6% by weight of the virion. The RNA is capped at the 5′ terminus with m$^7$G and has a polyadenylated tract at the 3′ terminus.

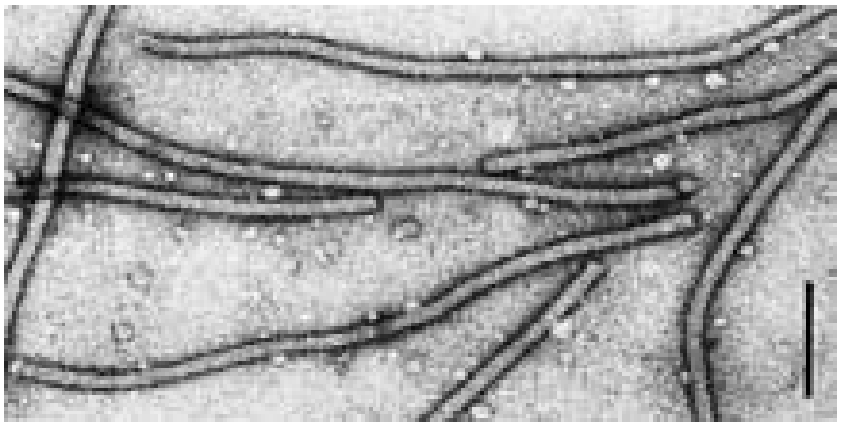

**Figure 8:** Negative contrast electron micrograph of particles of an isolate of potato virus X. The bar represents 100 nm. (Courtesy of D.-E. Lesemann.)

### Proteins

The virus capsid consists of 1000–1500 protein subunits of a single 18–27 kDa polypeptide. Partial proteolytic cleavage of the CP subunits can occur during storage of purified virus.

## Genome organization and replication

Virions of the type member contain only genomic RNA, but other potexviruses also encapsidate the sgRNA for the CP. Genomic RNA is translated as a functionally monocistronic message; only the 5′-proximal RNA-polymerase gene is translated directly by ribosomes, producing the RNA polymerase (150–181 kDa). The 5′-UTR leader sequence of PVX RNA consists of 83 nt (excluding the cap-structure) and efficiently enhances translation. In infected plants, some potexviruses produce sgRNAs including one that acts as messenger RNA for the CP (Figure 9).

The genomic RNA of potexviruses typically has five ORFs. ORF1, at the 5′ terminus, is the polymerase gene and ORF5, located at the 3′ terminus, is the CP gene. Between ORF1 and ORF5 is the TGB of three overlapping ORFs, the products of which (25, 12 and 8 kDa) are involved in cell-to-cell movement of viral RNA. The 25 kDa protein (as well as the 166 kDa replicase) contains an NTPase-helicase domain, but is not involved in RNA replication. It has been shown to have RNA silencing suppressor activity which is necessary for virus movement. The 12 and 8 kDa proteins contain large blocks of uncharged aa and are associated with membrane vesicles derived from the endoplasmic reticulum. TGB3 of AltMV (but not that of PVX) is targeted to the chloroplast and is required for movement from the epidermis to the mesophyll layer. The CP is also involved in cell-to-cell movement. ORFs 2 to 5 are expressed via the production (and subsequent translation) of appropriate sgRNAs. Two or three 3′-co-terminal sgRNAs can be isolated from plants infected with potexviruses (2.1, 1.2 and 1.0 kb); the double stranded counterparts of these sgRNAs have also been detected. The medium-size sgRNA (1.2 kb) is probably functionally bicistronic, its translation yielding the 12 and 8 kDa proteins.

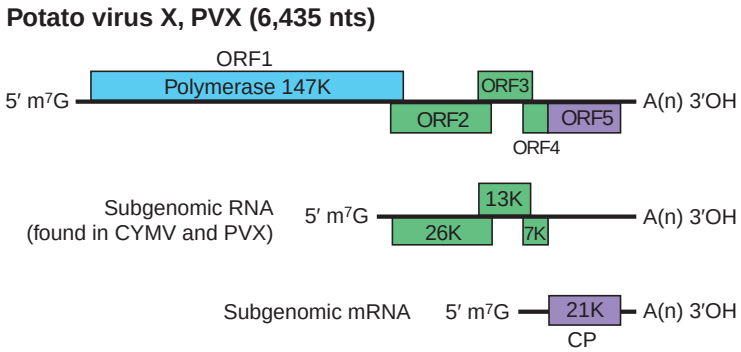


**Figure 9:** Potato virus X genome organization and expression.

## Antigenic properties

Virions are highly immunogenic; some species are antigenically related, but others are serologically distinct.

## Biological properties

### Host range

Some of the viruses are moderately pathogenic, causing mosaic or ringspot symptoms in a wide range of mono- and dicotyledonous plant species, but others alone cause little damage to infected plants. The host range of individual members is limited.

### Transmission

The viruses are transmitted in nature by mechanical contact. Potato aucuba mosaic virus can be vectored by aphids when assisted by a potyvirus providing a helper protein.

### Geographical distribution

As a group, potexviruses occur world-wide. The distribution of some species is very wide but others are apparently more restricted.

### Cytopathic effects

The cytoplasm of infected cells contains fibrous, banded or irregular aggregates of virus particles, and often membrane accumulations. There is no cytopathology specific to potexviruses, although some viruses induce unique structures such as the beaded sheets found in cells infected by the type member.

## Species demarcation criteria in the genus

The list of species demarcation criteria in the genus is:

- Host range: the natural host range is usually specific to different species.
- Distinct species fail to cross-protect in infected plants.
- Serology: species and strains of some species are also readily distinguishable in differential reactions with monoclonal antibodies.
- Sequence: isolates of different species have less than about 72% nt identity (or 80% aa identity) between their CP or polymerase genes.

## List of species in the genus *Potexvirus*

| | | |
|---|---|---|
| *Alstroemeria virus X* | | |
| Alstroemeria virus X-Japan | [AB206396 = NC_007408] | (AlsVX-JA) |
| *Alternanthera mosaic virus* | | |
| Alternanthera mosaic virus-USA:Pennsylvania | [AY863024 = NC_007731] | (AltMV-PA) |
| *Asparagus virus 3* | | |
| Asparagus virus 3-Japan | [AB304848 = NC_010416] | (AV3-J) |
| Scallion virus X-China: Hangzhou | [AJ316085 = NC_003400] | (ScaVX-HZ) |
| *Bamboo mosaic virus* | | |
| Bamboo mosaic virus-O | [D26017 = NC_001642] | (BaMV-O) |
| *Cactus virus X* | | |
| Cactus virus X-Taiwan | [AF308158 = NC_002815] | (CVX-TW) |
| *Cassava common mosaic virus* | | |
| Cassava common mosaic virus-Brazil | [U23414 = NC_001658] | (CsCMV-BR) |
| *Cassava virus X* | | |
| Cassava virus X-Colombia | | (CsVX-COL) |
| *Clover yellow mosaic virus* | | |
| Clover yellow mosaic virus-USA | [D29630 = NC_001753] | (ClYMV-USA) |
| *Commelina virus X* | | |
| Commelina virus X-United Kingdom | | (ComVX-UK) |
| *Cymbidium mosaic virus* | | |
| Cymbidium mosaic virus-Singapore | [U62963 = NC_001812] | (CymMV-SIN) |
| *Daphne virus X* | | |
| Daphne virus X-New Zealand | | (DVX-NZ) |

| | | |
|---|---|---|
| *Foxtail mosaic virus* | | |
| Foxtail mosaic virus-USA | [M62730 = NC_001483] | (FoMV-USA) |
| *Hosta virus X* | | |
| Hosta virus X-type strain: HVX-Kr | [AJ620114 = NC_011544] | (HVX-Kr) |
| *Hydrangea ringspot virus* | | |
| Hydrangea ringspot virus-PD 109 | [AY707100 = NC_006943] | (HdRSV-PD109) |
| *Lettuce virus X* | | |
| Lettuce virus X-Iran:Karaj | [AM745758 = NC_010832] | (LeVX-IR) |
| *Lily virus X* | | |
| Lily virus X-Netherlands | [AJ633822 = NC_007192] | (LVX-NL) |
| *Malva mosaic virus* | | |
| Malva mosaic virus-Chenopodium mosaic virus X | [DQ660333 = NC_008251] | (MalMV-ChMVX) |
| *Mint virus X* | | |
| Mint virus X-USA | [AY789138 = NC_006948] | (MVX-USA) |
| *Narcissus mosaic virus* | | |
| Narcissus mosaic virus-Netherlands | [D13747 = NC_001441] | (NMV-NL) |
| *Nerine virus X* | | |
| Nerine virus X-Japan | [AB219105 = NC_007679] | (NVX-J) |
| *Opuntia virus X* | | |
| Opuntia virus X-CC10 | [AY366209 = NC_006060] | (OpVX-CC10) |
| *Papaya mosaic virus* | | |
| Papaya mosaic virus-USA:Florida | [D13957 = NC_001748] | (PapMV-FLA) |
| *Pepino mosaic virus* | | |
| Pepino mosaic virus-Sp-13 | [AF484251 = NC_004067] | (PepMV-Sp13) |
| *Phaius virus X* | | |
| Phaius virus X-Japan | [AB353071 = NC_010295] | (PhVX-JA) |
| *Plantago asiatica mosaic virus* | | |
| Plantago asiatica mosaic virus-Russia | [Z21647 = NC_003849] | (PlAMV-RU) |
| Nandina mosaic virus-USA | [AY800279] | (NaMV-USA) |
| *Plantago severe mottle virus* | | |
| Plantago severe mottle virus-Canada | | (PlSMoV-CN) |
| *Plantain virus X* | | |
| Plantain virus X-United Kingdom | | (PlVX-UK) |
| *Potato aucuba mosaic virus* | | |
| Potato aucuba mosaic virus-Netherlands | [S73580 = NC_003632] | (PAMV-NL) |
| *Potato virus X* | | |
| Potato virus X-X3 | [D00344 = NC_011620] | (PVX-X3) |
| *Schlumbergera virus X* | | |
| Schlumbergera virus X-K11 | [AY366207 = NC_011659] | (SchVX-K11) |
| *Strawberry mild yellow edge virus* | | |
| Strawberry mild yellow edge virus-MY-18 | [D12517 = NC_003794] | (SMYEV-MY18) |
| *Tamus red mosaic virus* | | |
| Tamus red mosaic virus-Italy | | (TRMV-IT) |
| *Tulip virus X* | | |
| Tulip virus X-J | [AB066288 = NC_004322] | (TVX-J) |
| *White clover mosaic virus* | | |
| White clover mosaic virus-USA | [X06728 = NC_003820] | (WClMV-USA) |
| *Zygocactus virus X* | | |
| Zygocactus virus X-B1 | [AY366208 = NC_006059] | (ZyVX-B1) |

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Potexvirus* but have not been approved as species

| | | |
|---|---|---|
| Allium virus X | [FJ670570 = NC_012211] | (AlVX) |
| Caladium virus X | [AY727533*] | (CalVX) |
| Dioscorea latent virus | | (DLV) |
| Paris polyphylla virus X | [DQ530433*] | (PPVX) |
| Parsnip virus 3 | | (ParV-3) |
| Viola mottle virus | | (VMoV) |
| Wineberry latent virus | | (WLV) |

*Sequences do not comprise the complete genome.

# Genus *Sclerodarnavirus*

Type species ***Sclerotinia sclerotiorum debilitation-associated RNA virus***

## Distinguishing features

The genus consists of a single member, a capsid-less mycovirus. Despite the lack of capsid, phylogenetic analysis of the polymerase places it within this family.

## Virion properties

### Morphology

There are no virions.

### Physicochemical and physical properties

No information.

### Nucleic acid

A single linear molecule of positive sense ssRNA of 5470 nt with a polyadenylated tract at the 3′ terminus.

## Genome organization and replication

There is a single ORF encoding a replication protein of 193 kDa.

## Antigenic properties

Not applicable.

## Biological properties

The single member was discovered in the plant pathogenic fungus *Sclerotinia sclerotiorum* and appears to cause debilitation (hypovirulence).

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Sclerodarnavirus*

| | | |
|---|---|---|
| *Sclerotinia sclerotiorum debilitation-associated RNA virus* | | |
| Sclerotinia sclerotiorum debilitation-associated RNA virus-China | [AY147260 = NC_007415] | (SSDaRV-CN) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Sclerodarnavirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Alphaflexiviridae*

None.

## List of other related viruses which may be members of the family *Alphaflexiviridae* but have not been approved as species

| | | |
|---|---|---|
| Ambrosia asymptomatic virus 1 | [EU362849-50*] | (ASV1) |
| Blackberry virus X | [DQ378057*] | (BlVX) |

*Sequences do not comprise the complete genome.

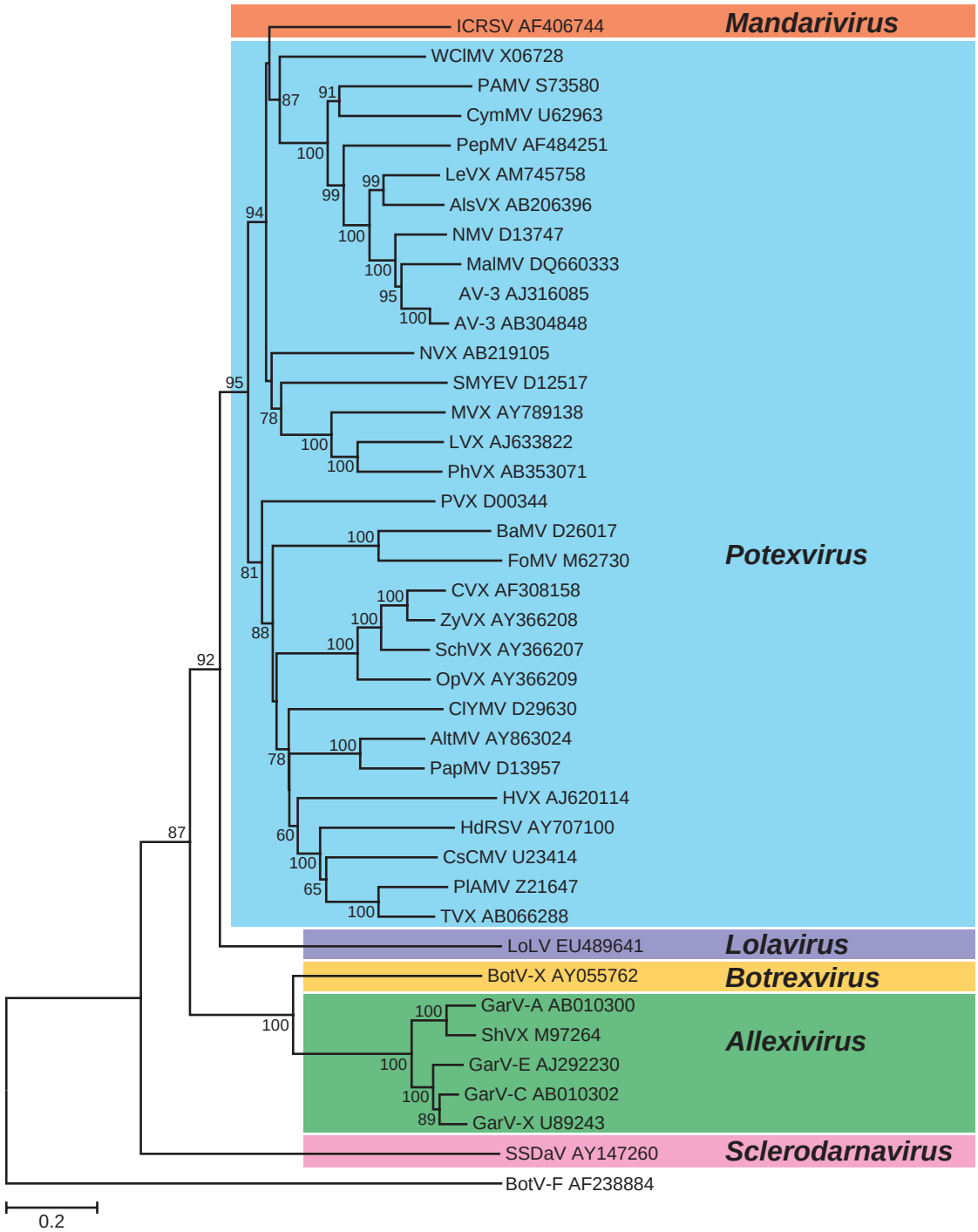


**Figure 10:** Phylogenetic (distance) tree based on the amino acid sequences of the entire replication protein of members of the family *Alphaflexiviridae*. A single representative isolate of each sequenced species in the family was included and the tree is rooted with Botrytis virus F (BotV-F; genus *Mycoflexivirus*, family *Gammaflexiviridae*). Numbers on branches indicate percentage of bootstrap support out of 1000 bootstrap replications (when >60%). The scale indicates JTT amino acid distances. Tree produced in MEGA4.

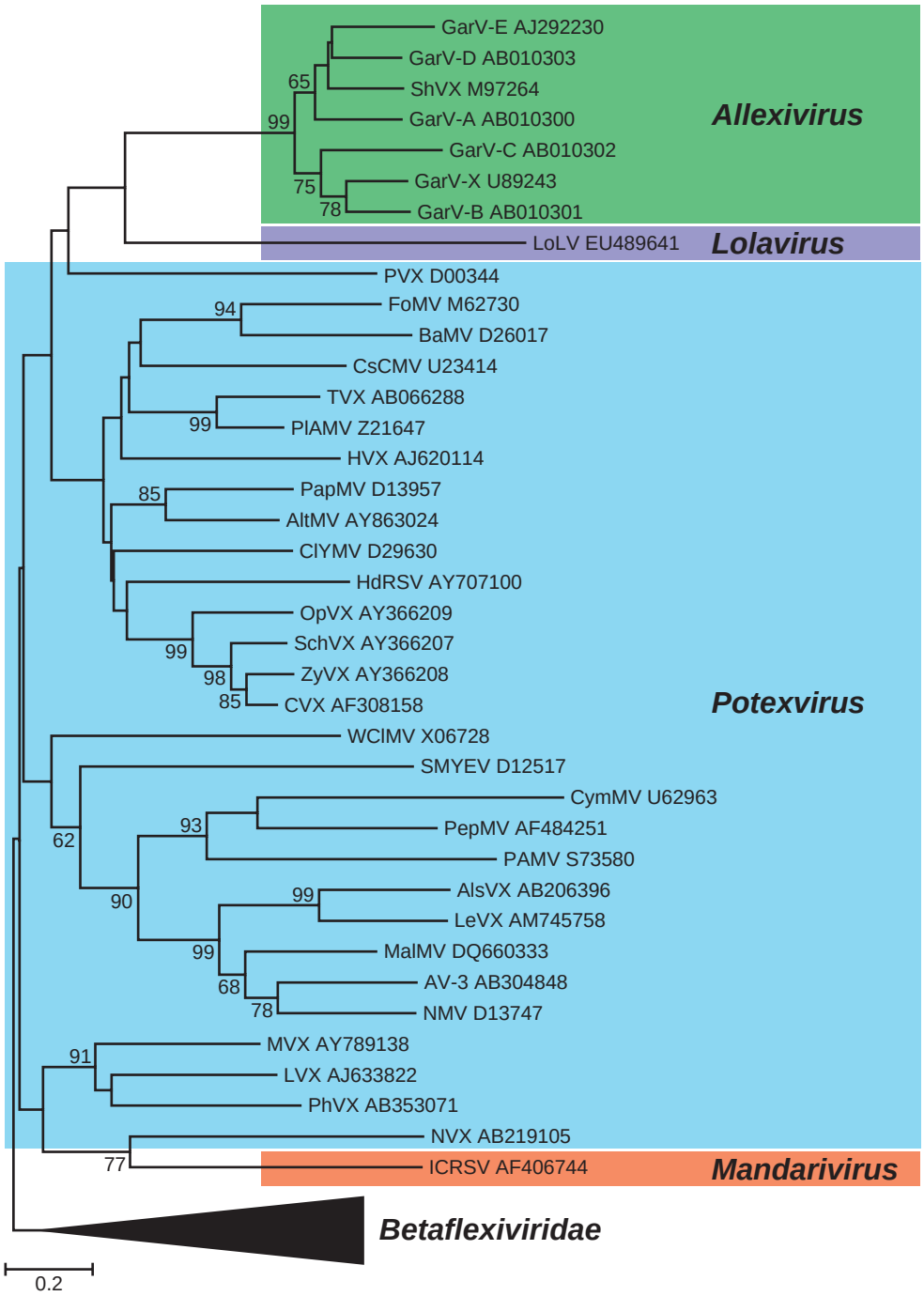


**Figure 11** Phylogenetic (distance) tree based on the amino acid sequences of the first triple gene block protein of members of the family *Alphaflexiviridae*. A single representative isolate of each sequenced species in the family was included; the tree also contains similar sequences from the family *Betaflexiviridae* collapsed into a triangle, the length of which corresponds to the variation found within the clade. Numbers on branches indicate percentage of bootstrap support out of 1000 bootstrap replications (when >60%). The scale indicates JTT amino acid distances. Tree produced in MEGA4.

## Phylogenetic relationships within the family

In a phylogenetic analysis of the replication protein, most genera fall on well-supported branches but the single member of the genus *Mandarivirus* groups with *Potexvirus* (Figure 10, p. 917). Similar results are obtained in analyses of the first (and largest) TGB protein (Figure 11) although members of the genus *Potexvirus* do not form such a coherent group.

## Similarity with other taxa

The polymerase proteins are members of the "alphavirus-like" supergroup of RNA viruses and are most closely related to those of the other families in the order, namely *Betaflexiviridae*, *Gammaflexiviridae* and *Tymoviridae*. The TGB proteins are related to those of some genera in the family *Betaflexiviridae* and, more distantly, to those of rod-shaped viruses in the family *Virgaviridae* (genera *Hordeivirus*, *Pecluvirus* and *Pomovirus*).

## Derivation of names

*Allexi*: from *Allium* (the genus name for the principal host, shallot) + *X*.
*Botrex*: from *Botrytis virus X*.
*Flexi*: from Latin *flexus*, "bent".
*Lola*: from *Lolium latent virus*.
*Mandari*: from mandarin (*Citrus reticulata*), the host of the type species, *Indian citrus ringspot virus*.
*Potex*: from *Potato virus X*.
*Sclerodarna*: from *Sclerotinia sclerotiorum debilitation-associated RNA virus*.

## Further reading

Adams, M.J., Antoniw, J.F., Bar-Joseph, M., Brunt, A.A., Candresse, T., Foster, G.D., Martelli, G.P., Milne, R.G., Zavriev, S.K. and Fauquet, C.M. (2004). The new plant virus family *Flexiviridae* and assessment of molecular criteria for species demarcation. *Arch. Virol.*, **149**, 1045–1060.

Martelli, G., Adams, M.J., Kreuze, J.F. and Dolja, V.V. (2007). Family *Flexiviridae*: a case study in virion and genome plasticity. *Annu. Rev. Phytopathol.*, **45**, 73–100.

Verchot-Lubicz, J., Ye, C.-M. and Bamunusinghe, D. (2007). Molecular biology of potexviruses: Recent advances. *J. Gen. Virol.*, **88**, 1643–1655.

## Contributed by

Adams, M.J., Candresse, T., Hammond, J., Kreuze, J.F., Martelli, G.P., Namba, S., Pearson, M.N., Ryu, K.H. and Vaira, A.M.

# Family *Betaflexiviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Betaflexiviridae*** |
| Genus | ***Capillovirus*** |
| Genus | ***Carlavirus*** |
| Genus | ***Citrivirus*** |
| Genus | ***Foveavirus*** |
| Genus | ***Trichovirus*** |
| Genus | ***Vitivirus*** |

## Distinguishing features

The family contains viruses infecting plants which share a distinct lineage of alphavirus-like replication proteins.

## Virion properties

### Morphology

Virions are flexuous filaments, usually 12–13 nm in diameter (range 10–15 nm) and from 600 to over 1000 nm in length, depending on the genus. They have helical symmetry with a pitch of about 3.4 nm (range 3.3–3.7 nm) and in some genera there is clearly visible cross-banding.

### Physicochemical and physical properties

Virions sediment as single (or occasionally two very close) bands with an $S_{20,w}$ of 92–176S, depending on the genus.

### Nucleic acid

Virions contain a single molecule of linear ssRNA of about 5.9–9.0 kb which is 5–6% by weight of the virion. The RNA is capped (or probably capped) at the 5′ terminus with $m^7G$ and has a polyadenylated tract at the 3′ terminus. In the genus *Carlavirus* some viruses have two subgenomic RNAs (sgRNAs) of 2.1–3.3 kb and 1.3–1.6 kb, which are possibly encapsidated in shorter particles.

### Proteins

The viral capsid of all members is composed of a single polypeptide ranging in size from 18 to 44 kDa.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

The number of genes is between three and six depending upon the genus (Figure 1) but, in all species, the ORF1-encoded product, which follows a short 5′-UTR sequence, has homologies with polymerase proteins of the "alphavirus-like" supergroup of RNA viruses. This protein (190–250 kDa) contains the conserved domains for methyltransferase (Mtr), helicase (Hel) and RNA-dependent RNA polymerase (RdRp) activity. Most members also have AlkB and papain-like protease (P-Pro) domains between the Mtr and Hel. Smaller ORFs encode the proteins involved in cell-to-cell movement, either a single MP of the "30K" superfamily (*Capillovirus*, *Citrivirus*, *Trichovirus*, *Vitivirus*) or a "triple gene block" (TGB) (remaining genera and viruses). These are usually located following (3′-proximal to) the polymerase but in capillovirus genomes the MP ORF2 is nested within the ORF1 and in vitiviruses an extra ORF is present between the polymerase and MP genes. The CP gene always follows the MP(s) and in some genera (*Carlavirus*, *Vitivirus* and some trichoviruses) a final ORF encodes a protein with a zinc binding finger motif and the ability to bind nucleic acids. In vitiviruses, this small protein has been shown to have RNA silencing suppressor


Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses

activity. ORFs downstream of the polymerase are translated from 3′-terminal sgRNAs that can often be found in infected tissue. In some viruses, notably in the genera *Citrivirus*, *Vitivirus* and *Trichovirus*, nested sets of 5′-terminal sgRNAs and their associated dsRNAs can also be detected. Replication is (or is presumed to be) cytoplasmic and the product of ORF1 is the only virus-encoded protein known to be involved.

## Antigenic properties

Virions are highly immunogenic in members of the genus *Carlavirus* but those of other genera are only moderate to poor antigens. Within (but not usually between) genera, some viruses are serologically related.

## Biological properties

Members have been reported from a diverse range of plant species but the host range of individual members is usually limited. With the exception of most members of the genus *Carlavirus*, natural infections are mostly or exclusively of woody hosts. Many of the viruses have relatively mild effects on their host. All species can be transmitted by mechanical inoculation, although some with difficulty. Many of the viruses have no known invertebrate or fungus vectors; however some trichoviruses are known to be mite-borne, most carlaviruses are transmitted naturally by aphids in the non-persistent manner and a range of vectors (pseudococcid mealybugs, scale insects and aphids) have been reported for different vitiviruses. Aggregates of virus particles accumulate in the cytoplasm. Many carlaviruses induce the formation of ovoid or irregularly shaped inclusions but otherwise there are usually no specific cytopathic structures.

**Table 1:** Distinguishing properties of genera in the family *Betaflexiviridae*

| Genus | Virion length (nm) | ORFs | Rep[a] | MP(s)[b] | CP[c] |
|---|---|---|---|---|---|
| *Capillovirus* | 640–700 | 2 | 210–245 | 30K | 25–27 |
| *Carlavirus* | 610–700 | 6 | 215–225 | TGB | 32–36 |
| *Citrivirus* | 960 | 3 | 227 | 30K | 41 |
| *Foveavirus* | 800+ | 5 | 230–250 | TGB | 28–44 |
| *Trichovirus* | 640–890 | 3 or 4 | 215–220 | 30K | 21–24 |
| *Vitivirus* | 725–785 | 5 | 190–200 | 30K | 18–22 |

[a]Rep, Replication protein size (kDa).
[b]MP, Movement protein either of the "30K" superfamily or a triple gene block (TGB).
[c]CP, Coat protein size (kDa).

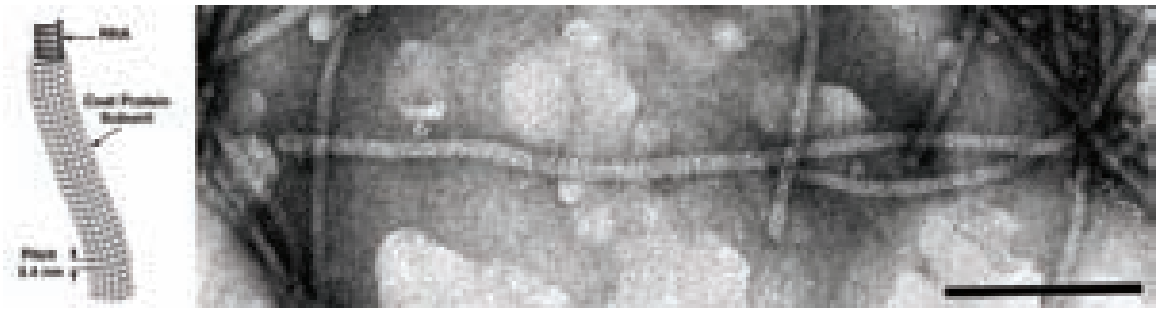

**Figure 1:** (Left) Schematic representation of a fragment of a particle of a capillovirus. (Right) Negative contrast electron micrograph of particles of an isolate of the species *Apple stem grooving virus*. The bar represents 100 nm. (Courtesy of N. Yoshikawa.)

## Species and genus demarcation criteria in the family

Genera are distinguished by various features of genome organization and the natural mode of transmission. These are summarized in Table 1. Throughout the family, isolates of different species should have less than about 72% nt identity (or 80% aa identity) between their respective CP or polymerase genes. Viruses from different genera usually have less than about 45% nt identity in these genes.

# Genus *Capillovirus*

Type species *Apple stem grooving virus*

## Distinguishing features

Capilloviruses have a distinctive genomic organization, with two ORFs encoding a large replication-associated protein fused with the coat protein and (as a nested ORF) a putative movement protein. The MP and CP are expressed from subgenomic RNAs. No vectors are known. Virions have prominent cross-banding.

## Virion properties

### Morphology

Virions are flexuous filaments, 640–700 × 12 nm, constructed from helically arranged protein subunits in a primary helix with a pitch of 3.4 nm and between 9 and 10 subunits per turn with prominent cross-banding (see Figure 1 above).

### Physicochemical and physical properties

The $S_{20,w}$ of particles is about 112S, isoelectric point is about pH 4.3 at ionic strength 0.1 M, and electrophoretic mobility is 10.3 and $6.5 \times 10^{-5}$ $cm^{-2} sec^{-1} volt^{-1}$, at pH 7.0 and 6.0 respectively (ionic strength 0.1 M; data for apple stem grooving virus).

### Nucleic acid

Virions contain linear positive sense ssRNA, 6.5–7.4 kb in size, constituting about 5%, by weight, of virions. The RNA is polyadenylated at its 3′ end. Isolates of the species *Apple stem grooving virus* from different hosts show wide variations in the sequence of a 284 aa region of ORF1-encoded protein, between the polymerase and CP domains.

### Proteins

Virions are composed of a single 24–27 kDa protein.

## Genome organization and replication

The genomic RNA of all sequenced viruses has the same organization, and two ORFs (Figure 2). ORF1 encodes a 240–266 kDa protein followed by a UTR of 140–300 nt upstream of the 3′-poly(A) tail. ORF2 is nested within ORF1 near its 3′ end, and encodes a 36–52 kDa protein. Although the CP cistron is located in the C-terminal end of ORF1, and ORF2 is nested within ORF1, the strategy of expression

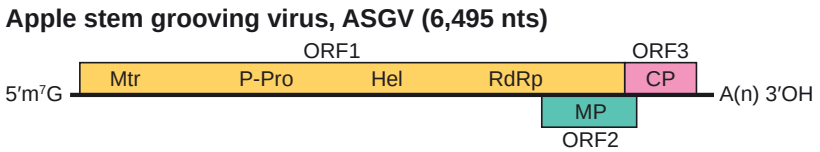


**Figure 2:** Genome organization of apple stem grooving virus showing the relative positions of the ORFs and their expression products. Mtr, methyltransferase; P-Pro, papain-like protease; Hel, helicase; RdRp, RNA-dependent RNA polymerase; MP, putative movement protein; CP, capsid protein.

of both CP and putative MP may be based on sgRNA production, as suggested by the analysis of dsRNA patterns from infected tissues. dsRNAs of the type member consist of five major bands with sizes of approximately 6.5, 5.5, 4.5, 2.0 and 1.0 kbp. The 6.5 kbp species probably represents the double stranded form of the full-length genome, and the 2.0 and the 1.0 kbp species may be the double-stranded forms of sgRNAs that code for the putative MP and the CP, respectively. Replication is likely to occur in the cytoplasm, in which virus particles accumulate in discrete bundles.

## Antigenic properties

Virions are moderately antigenic. There are no serological relationships between species.

## Biological properties

### Host range

Apple stem grooving virus (ASGV) is pathogenic to pome fruits and citrus and induces stock/scion incompatibility, i.e. top-working disease of apple and bud union crease syndrome of citrus. It also infects lily. Cherry virus A (CVA) is frequently found in sweet and sour cherry (and less frequently in other *Prunus* hosts) but no disease has been associated with it.

### Transmission

No vectors are known. ASGV was transmitted through seed to progeny seedlings of *Chenopodium quinoa*, and lily. ASGV, CVA, and Nandina stem pitting virus (NSPV) are transmitted by grafting. NSPV has not been transmitted by sap inoculation, but by slashing stems with a partially purified virus preparation.

### Geographical distribution

Geographical distribution ranges from wide to restricted according to the virus. ASGV has been recorded from most areas where apples are grown, and is widespread in citrus in China, Japan, United States, Australia and South Africa. CVA is widespread and probably occurs worldwide in cherry hosts. NSPV is found only in the United States.

### Cytopathic effects

No distinct cytological alterations have been observed in infected cells. Virus particles occur in bundles in mesophyll and phloem parenchyma cells, but not in the epidermis and sieve elements.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Natural host range.
- Serological specificity (all known species are serologically unrelated).
- Less than about 72% nt identity (or 80% aa identity) between their CP or polymerase genes.

## List of species in the genus *Capillovirus*

| | | |
|---|---|---|
| *Apple stem grooving virus* | | |
| Apple stem grooving virus-P-209 | [D14995 = NC_001749] | (ASGV-P209) |
| Citrus tatter leaf virus-lily | [D16681] | (CTLV-L) |
| *Cherry virus A* | | |
| Cherry virus A-Germany | [X82547 = NC_003689] | (CVA-DE) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accessions [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Capillovirus* but have not been approved as species

| | |
|---|---|
| Nandina stem pitting virus | (NSPV) |

# Genus *Carlavirus*

Type species *Carnation latent virus*

## Distinguishing features

Carlaviruses have six ORFs, including a TGB, and are insect-transmitted.

## Virion properties

### Morphology

Virions are slightly flexuous filaments, 610–700 nm in length and 12–15 nm in diameter (Figure 3). They have helical symmetry with a pitch of about 3.4 nm.

### Physicochemical and physical properties

Virion Mr is about $60 \times 10^6$, with a nucleic acid content of about 6%. Virion $S_{20,w}$ is 147–176S, and the buoyant density in CsCl solutions is 1.3 g cm$^{-3}$.

### Nucleic acid

Virions contain a single molecule of linear ssRNA that has a size range of 7.4–7.7 kb when estimated by agarose gel analysis, although full-length sequence analysis suggests that genome sizes are in the 8.3–8.7 kb range. Some species also have two sgRNAs of 2.1–3.3 kb and 1.3–1.6 kb, which are possibly encapsidated in shorter particles. The genomic RNAs have a 3′-poly(A) tract and a 5′-cap.

## Genome organization and replication

There are typically six ORFs with short UTRs at the 5′ and 3′ termini. In potato virus M (Figure 4), ORF1 encodes a polypeptide of 223 kDa that is the viral replicase; ORFs 2, 3 and 4 form the triple gene block and encode polypeptides of 25, 12 and 7 kDa which facilitate virus movement. ORF5 encodes the 34 kDa CP and overlaps ORF6, which encodes a cysteine-rich protein of 11–16 kDa. The function of the 11–16 kDa polypeptide has yet to be determined, but its ability to bind nucleic acid indicates that it may facilitate aphid transmission or be involved in host gene transcription/gene silencing and/or viral RNA replication.

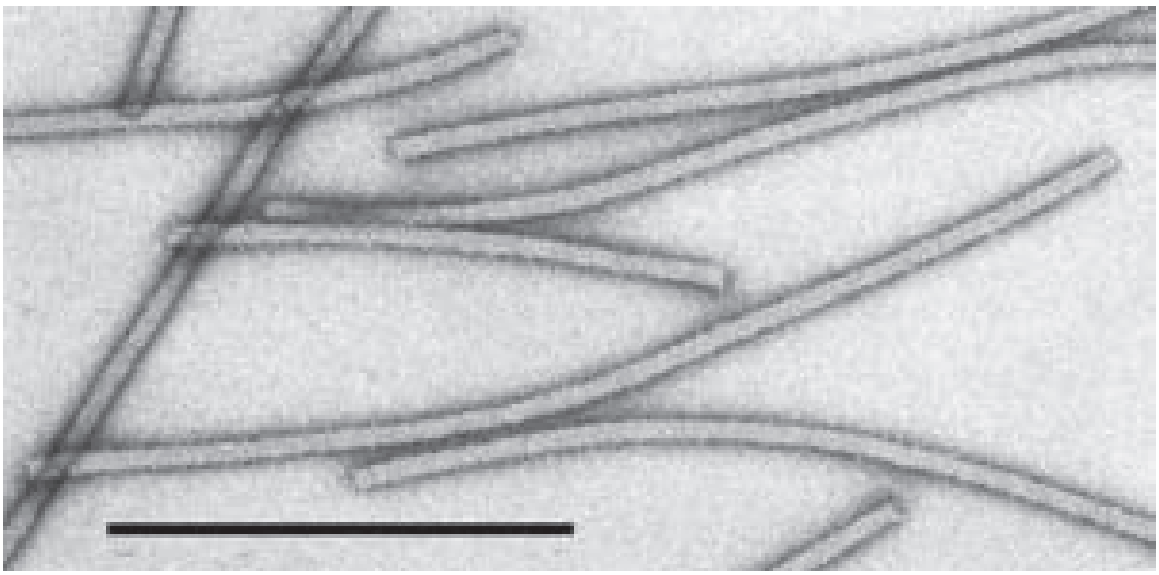

**Figure 3:** Filamentous particles of an isolate of the species *Carnation latent virus*. The bar represents 100 nm. (Courtesy R.G. Milne.)

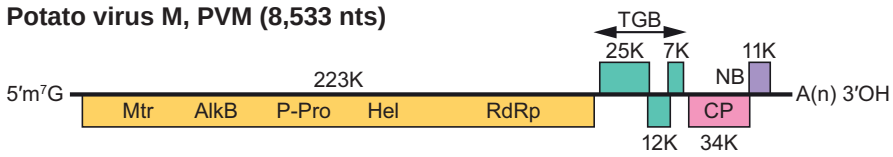


**Figure 4:** Genome organization of potato virus M showing the relative positions of the ORFs and their expression products. Mtr, methyltransferase; P-Pro, papain-like protease; Hel, helicase; RdRp, RNA-dependent RNA polymerase; CP, capsid protein; NB, nucleic acid binding protein. The 25K, 12K and 7K proteins constitute the triple gene block.

Only ORF1 is translated from the full length genomic RNA. With blueberry scorch virus and probably other carlaviruses the product of ORF1 is proteolytically processed by a papain-like proteinase activity, with about 30–40 kDa being removed. The 3′-terminal ORFs appear to be translated from two sgRNAs that can be found in infected tissue, and, for some viruses, can be detected in purified virus preparations. The 5′-untranslated leader sequence of the genomic RNA and the sgRNA for the CP of potato virus S (PVS) have both been shown to act as efficient enhancers of translation.

## Antigenic properties

Carlavirus virions are good immunogens. Some species are serologically interrelated, but others are apparently distinct.

## Biological properties

### Host range

Individual viruses have restricted natural host ranges, but some can infect a wide range of experimental hosts.

### Transmission

Most species are transmitted naturally by aphids in the non-persistent manner; cowpea mild mottle virus (CPMMV) is transmitted by whiteflies (*Bemisia tabaci*), pea streak virus, red clover vein mosaic virus and CPMMV are seedborne in their leguminous hosts. All are mechanically transmissible; some (e.g. carnation latent virus and PVS) are sufficiently infectious to be so transmitted this way in the field.

### Geographical distribution

The geographical distribution of many species is restricted, but those infecting vegetatively-propagated crops are usually widely distributed, presumably due to inadvertent dissemination in vegetative propagules. Most species commonly occur in temperate climates, but CPMMV is restricted to tropical and sub-tropical regions.

### Cytopathic effects

Virions of aphid-borne species are scattered throughout the cytoplasm or occur in membrane-associated bundle-like or plate-like aggregates. Many species also induce the formation of ovoid or irregularly shaped inclusions that appear in the light microscope as vacuolate bodies; these consist of aggregates of virus particles, mitochondria, endoplasmic reticulum and lipid globules. The particles of CPMMV, the whitefly-transmitted carlavirus, also occur in aggregates in cytoplasm; those of most, but not all, strains of CPMMV form brush-like inclusions.

## Species demarcation criteria in the genus

Each distinct species usually has a specific natural host range. Distinct species do not cross-protect in infected common host plant species. Distinct species are readily differentiated by serological procedures; strains of individual species are often distinguishable in reactions with polyclonal antisera, but more readily so with monoclonal antibodies. Distinct species have less than about 72% nt identity (or 80% aa identity) between their CP or polymerase genes.

## List of species in the genus *Carlavirus*

| | | |
|---|---|---|
| *Aconitum latent virus* | | |
| Aconitum latent virus-Japan:D | [AB051848 = NC_002795] | (AcLV-D) |
| *American hop latent virus* | | |
| American hop latent virus-USA:Washington State | | (AHLV-WA) |
| *Blueberry scorch virus* | | |
| Blueberry scorch virus-NJ-2 | [L25658 = NC_003499] | (BlScV-nj2) |
| *Cactus virus 2* | | |
| Cactus virus 2-Germany | | (CV-2-DE) |
| *Caper latent virus* | | |
| Caper latent virus-Italy | | (CapLV-IT) |
| *Carnation latent virus* | | |
| Carnation latent virus-United Kingdom | [AJ010697*] | (CLV-UK) |
| *Chrysanthemum virus B* | | |
| Chrysanthemum virus B-Japan:Showa | [AB245142] | (CVB-S) |
| *Cole latent virus* | | |
| Cole latent virus-Brazil | [AY340584*] | (CoLV-BR) |
| *Coleus vein necrosis virus* | | |
| Coleus vein necrosis virus-USA | [EF527260 = NC_009764] | (CVNV-USA) |
| *Cowpea mild mottle virus* | | |
| Cowpea mild mottle virus-M | [AF024629*] | (CPMMV-M) |
| *Dandelion latent virus* | | |
| Dandelion latent virus-Canada:British Colombia | | (DaLV-BC) |
| *Daphne virus S* | | |
| Daphne virus S-type strain: K | [AJ620300 = NC_008020] | (DVS-K) |
| *Elderberry symptomless virus* | | |
| Elderberry symptomless virus-United Kingdom | | (ElSLV-UK) |
| *Garlic common latent virus* | | |
| Garlic common latent virus-Germany | [AB004805*] | (GarCLV-DE) |
| *Helenium virus S* | | |
| Helenium virus S-Germany | [D10454*] | (HVS-DE) |
| *Helleborus net necrosis virus* | | |
| Helleborus net necrosis virus-G5 | [FJ196835 = NC_012038] | (HNNV-G5) |
| *Honeysuckle latent virus* | | |
| Honeysuckle latent virus-United Kingdom | | (HnLV-UK) |
| *Hop latent virus* | | |
| Hop latent virus-Japan | [AB032469 = NC_002552] | (HpLV-JA) |
| *Hop mosaic virus* | | |
| Hop mosaic virus-Australia | [EU527979 = NC_010538] | (HpMV-AUS) |
| *Hydrangea latent virus* | | |
| Hydrangea latent virus-USA | | (HdLV-USA) |
| *Kalanchoë latent virus* | | |
| Kalanchoë latent virus-PV-0290B | [FJ531634 = NC_013006] | (KLV-PV0290B) |
| *Ligustrum necrotic ringspot virus* | | |
| Ligustrum necrotic ringspot virus-USA | [EU074853 = NC_010305] | (LNRSV-USA) |
| *Lilac mottle virus* | | |
| Lilac mottle virus-USA | | (LiMoV-USA) |
| *Lily symptomless virus* | | |
| Lily symptomless virus-South Korea | [AJ516059 = NC_005138] | (LSV-Kor) |
| *Melon yellowing-associated virus* | | |
| Melon yellowing-associated virus-Bessa | [AB510477*] | (MYaV-Bessa) |
| *Mulberry latent virus* | | |
| Mulberry latent virus-Japan | | (MLV-JP) |
| *Muskmelon vein necrosis virus* | | |
| Muskmelon vein necrosis virus-USA:California | | (MuVNV-CAL) |
| *Narcissus common latent virus* | | |
| Narcissus common latent virus-Zhangzhou | [AM158439 = NC_008266] | (NCLV-ZZ) |
| *Narcissus symptomless virus* | | |
| Narcissus symptomless virus-Hangzhou | [AM182569 = NC_008552] | (NSV-HZ) |
| *Nerine latent virus* (Hippeastrum latent virus) | | |
| Nerine latent virus-Taiwan | [DQ098905 = NC_011540] | (NeLV-TW) |

| | | |
|---|---|---|
| *Passiflora latent virus* | | |
| Passiflora latent virus-Israel | [DQ455582 = NC_008292] | (PLV-IS) |
| *Pea streak virus* | | |
| Pea streak virus-ATCCPV-87 | [AF354652*] | (PeSV-ATCCPV87) |
| *Poplar mosaic virus* | | |
| Poplar mosaic virus-PV-0341 | [AY505475 = NC_005343] | (PopMV-PV0341) |
| *Potato latent virus* | | |
| Potato latent virus-Canada | [EU433397 = NC_011525] | (PotLV-CAN) |
| *Potato virus M* | | |
| Potato virus M-Russian wild type | [D14449 = NC_001361] | (PVM-RU) |
| *Potato virus P* | | |
| Potato virus P-Brazil | [EU338239] | (PVP-BRZ) |
| Potato rough dwarf virus | [EU020009 = NC_009759] | (PRDV) |
| *Potato virus S* | | |
| Potato virus S-Leona | [AJ863509 = NC_007289] | (PVS-Leona) |
| *Red clover vein mosaic virus* | | |
| Red clover vein mosaic virus-Washington | [FJ685618 = NC_012210] | (RCVMV-Washington) |
| *Shallot latent virus* | | |
| Shallot latent virus-YH1 | [AJ292226 = NC_003557] | (SLV-YH1) |
| *Sint-Jan's onion latent virus* | | |
| Sint-Jan's onion latent virus-Netherlands | | (SJOLV-NL) |
| *Strawberry pseudo mild yellow edge virus* | | |
| Strawberry pseudo mild yellow edge virus-USA | | (SPMYEV-USA) |
| *Sweet potato chlorotic fleck virus* | | |
| Sweet potato chlorotic fleck virus-Uganda | [AY461421 = NC_006550] | (SPCFV-UG) |
| *Verbena latent virus* | | |
| Verbena latent virus-Israel | [AF271218*] | (VeLV-IS) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

### List of other related viruses which may be members of the genus *Carlavirus* but have not been approved as species

| | | |
|---|---|---|
| Arracacha latent virus | | (ALV) |
| Artichoke latent virus M | | (ArLVM) |
| Artichoke latent virus S | | (ArLVS) |
| Butterbur mosaic virus | [AB517596 = NC_013527] | (ButMV) |
| Cardamine latent virus | | (CaLV) |
| Carrot virus S | [EU881919*] | (CarVS) |
| Helleborus mosaic virus | [FJ196838*] | (HeMV) |
| Hydrangea chlorotic mottle virus | [EU754720 = NC_012869] | (HCMoV) |
| Phlox virus B | [EU162589 = NC_009991] | (PhlVB) |
| Phlox virus M | [EF507476*] | (PhlVM) |
| Phlox virus S | [EF492068 = NC_009383] | (PhlVS) |
| Sedum latent virus | [FJ560901*] | (SeLV) |

*Sequences do not comprise the complete genome.



## GENUS *CITRIVIRUS*

Type species ***Citrus leaf blotch virus***

### Distinguishing features

This genus consists of a single species. There are three ORFs, similar to trichoviruses, but it is distinct from them in phylogenetic analyses, has longer virions and a much larger coat protein that more closely resembles those of foveaviruses.

## Virion properties

### Morphology
Virions are slightly flexuous filaments, 960 nm in length and 12–15 nm in diameter (Figure 5).

### Physicochemical and physical properties
No information.

### Nucleic acid
Virions contain a single molecule of positive sense ssRNA, about 8.7 kb long. There is a methylated cap at the 5′ terminus and a polyadenylated 3′ terminus.

### Proteins
The viral capsid is composed of a single polypeptide of about 41 kDa.

## Genome organization and replication
The genome contains three ORFs (Figure 6). ORFs 2 and 3 are separated by a short intergenic region and ORFs 1 and 2 overlap by 1 nt. ORF1 is the replication protein and is probably directly expressed from genomic RNA. It is assumed that the two smaller downstream ORFs, which code respectively for the putative "30K" MP and CP, are expressed via sgRNAs.

## Antigenic properties
No information.

## Biological properties

### Host range
The virus causes abnormal bud union and leaf blotching in various citrus varieties. Citrus is the only known natural host and mechanical transmission to a range of herbaceous hosts has been unsuccessful.

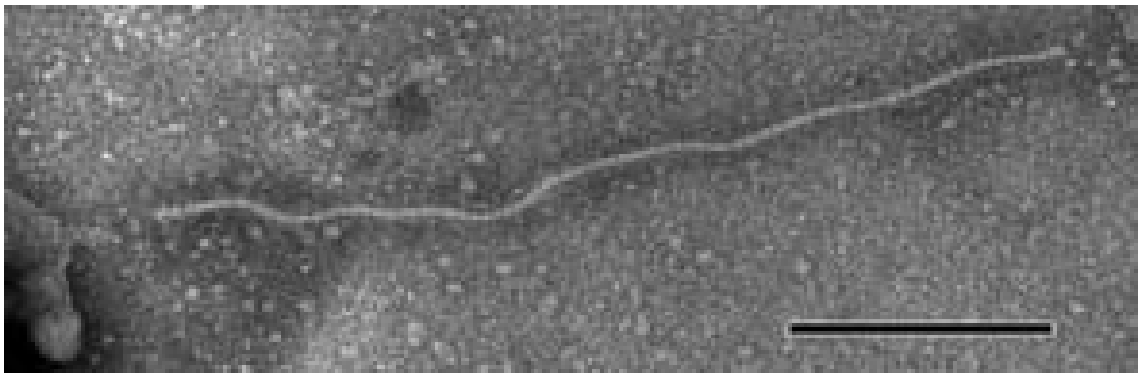

**Figure 5:** Negative contrast electron micrograph of particles of an isolate of the species *Citrus leaf blotch virus*. The bar represents 200 nm.

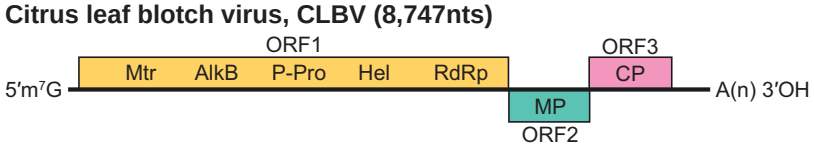


**Figure 6:** Genome organization of citrus leaf blotch virus showing the relative positions of the ORFs and their expression products. Mtr, methyltransferase; P-Pro, papain-like protease; Hel, helicase; RdRp, RNA-dependent RNA polymerase; MP, putative movement protein; CP, capsid protein.

### Transmission
The virus is transmitted to citrus by grafting. There is no known natural vector.

### Geographical distribution
The virus has been reported from citrus germplasm worldwide.

### Cytopathic effects
No information.

## Species demarcation criteria in the genus
Not applicable.

## List of species in the genus *Citrivirus*

*Citrus leaf blotch virus*
(Dweet mottle virus)
Citrus leaf blotch virus-SRA-153 [AJ318061 = NC_003877] (CLBV-SRA153)

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Citrivirus* but have not been approved as species
None reported.

# Genus *Foveavirus*

Type species *Apple stem pitting virus*

## Distinguishing features
Foveaviruses are distinct in having five ORFs and larger CPs than most other members of the family.

## Virion properties

### Morphology
Virions are flexuous filaments, about 800 to over 1000 nm in length and 12–15 nm in diameter with helical symmetry exhibiting a surface pattern with cross-banding and longitudinal lines (Figure 7). Particles of some viruses, including apple stem pitting virus (ASPV), show a tendency for end-to-end aggregation.

### Physicochemical and physical properties
ASPV virions sediment as two or three bands in sucrose density gradients but yield a single band at equilibrium in Omnipaque 350 density gradients. They resist moderately high temperatures (thermal inactivation is around 60 °C) but not organic solvents, and are unstable in cesium chloride and sulfate.

### Nucleic acid
Virions contain a single molecule of positive sense ssRNA, polyadenylated at the 3′ terminus.

### Proteins
The viral capsid of all species is composed of a single polypeptide with a size ranging from 28 kDa (grapevine rupestris stem pitting-associated virus, GRSPaV) to 44 kDa (ASPV and apricot latent virus, ApLV).

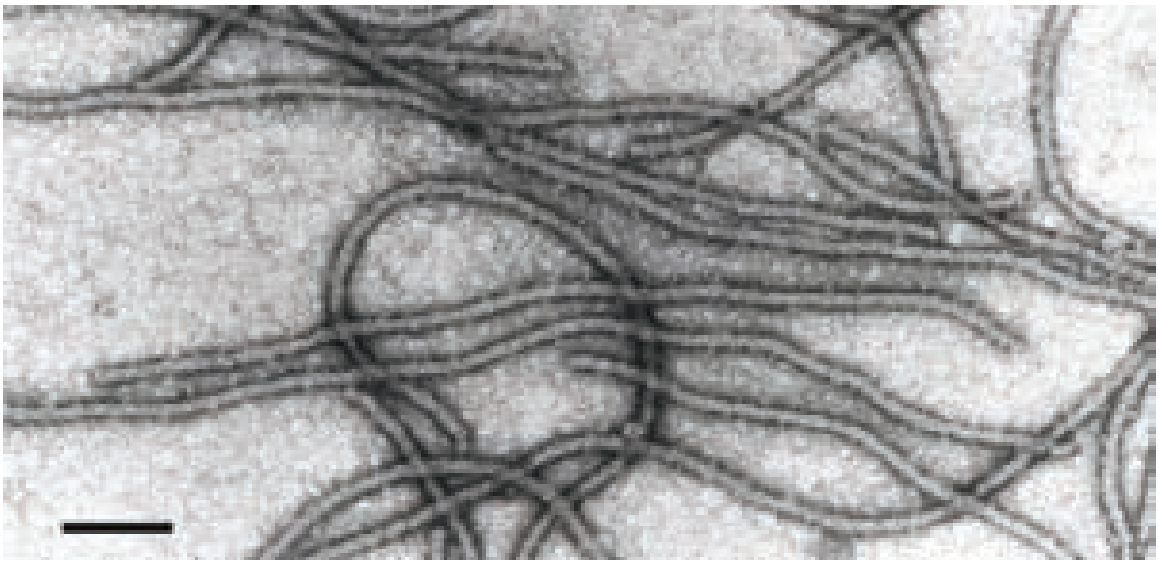

**Figure 7:** Negative contrast electron micrograph of particles of an isolate of the species *Apple stem pitting virus*. The bar represents 100 nm. (Courtesy of H. Koganezawa.)

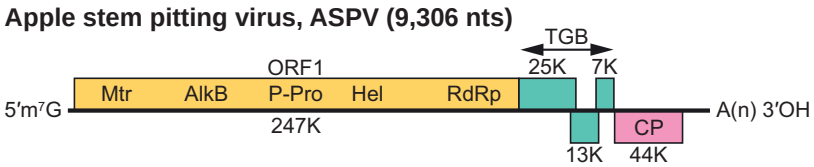


**Figure 8:** Genome organization of apple stem pitting virus showing the relative position of the ORFs and their expression products. Mtr, methytransferase; P-Pro, papain-like protease; Hel, helicase; Pol, polymerase; TGB, triple gene block; CP, capsid protein.

## Genome organization and replication

The genomes of all fully sequenced members contain five ORFs (Figure 8). The 5′ region initiates with a UTR of 33–72 nt, ORF1 codes for the replication-related protein, ORF2, ORF3 and ORF4 constitute the TGB and ORF5 is the CP cistron. A non-coding sequence of 176–312 nt followed by a poly(A) tail terminate the genome. ASPV virions accumulate in the cytoplasm, where multiplication is likely to occur following a strategy comparable to that of other viruses in the family, based on direct expression of the 5′-proximal ORF, and expression of downstream ORFs from sgRNAs. Multiple dsRNAs are found in infected hosts.

## Antigenic properties

Antisera to ASPV that can be used for serological detection tests have been raised from purified virions or chimeric fusion CPs expressed in *E. coli*. ASPV and ApLV are serologically related, but there are no recognized serological relationships among other members of the genus.

## Biological properties

### Host range

The natural host range of individual species is restricted to a single (GRSPaV) or a few hosts (ASPV, ApLV). ASPV infects primarily pome fruits, causing diseases of apple (topworking disease) when

grafted on susceptible rootstocks, of pear (vein yellows and necrotic spot) and quince. ApLV is the putative agent of peach asteroid spot and peach sooty ringspot diseases. GRSPaV is a pathogen of grapevine. Experimental host ranges are also restricted.

**Transmission**
No vector is known for any of the viruses. ASPV is transmitted by grafting and persists in the host propagative material. ASPV is mechanically transmissible, with some difficulty, to *Nicotiana occidentalis* and its subspecies *obliqua.*

**Geographical distribution**
All members have a wide geographical distribution.

**Cytopathic effects**
ASPV elicits a severe derangement of the cytology of infected cells but no specific cytopathic structures or inclusion bodies. Virus particles accumulate in bundles in the cytoplasm.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Natural host range.
- Serological specificity.
- CP size.
- Less than about 72% nt identity (or 80% aa identity) between their CP or polymerase genes.

## List of species in the genus *Foveavirus*

| | | |
|---|---|---|
| *Apple stem pitting virus* | | |
| Apple stem pitting virus-PA66 | [D21829 = NC_003462] | (ASPV-PA66) |
| *Apricot latent virus* | | |
| Apricot latent virus-Caserta12 | [AF318062*] | (ApLV-Caserta12) |
| *Grapevine rupestris stem pitting-associated virus* | | |
| Grapevine rupestris stem pitting-associated virus-USA | [AF057136 = NC_001948] | (GRSPaV-USA ) |
| *Peach chlorotic mottle virus* | | |
| Peach chlorotic mottle virus-Agua-4N6 | [EF693898 = NC_009892] | (PCMoV-Agua4N6 ) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

## List of other related viruses which may be members of the genus *Foveavirus* but have not been approved as species

| | | |
|---|---|---|
| Asian prunus virus 1 | [FJ824737] | (APV1) |
| Asian prunus virus 2 | [DQ205237*] | (APV2) |
| Asian prunus virus 3 | [DQ205238*] | (APV3) |

*Sequences do not comprise the complete genome.

# Genus *Trichovirus*

Type species ***Apple chlorotic leaf spot virus***

## Distinguishing features

Trichoviruses have three (or sometimes four) ORFs including a movement protein of the "30K" superfamily.

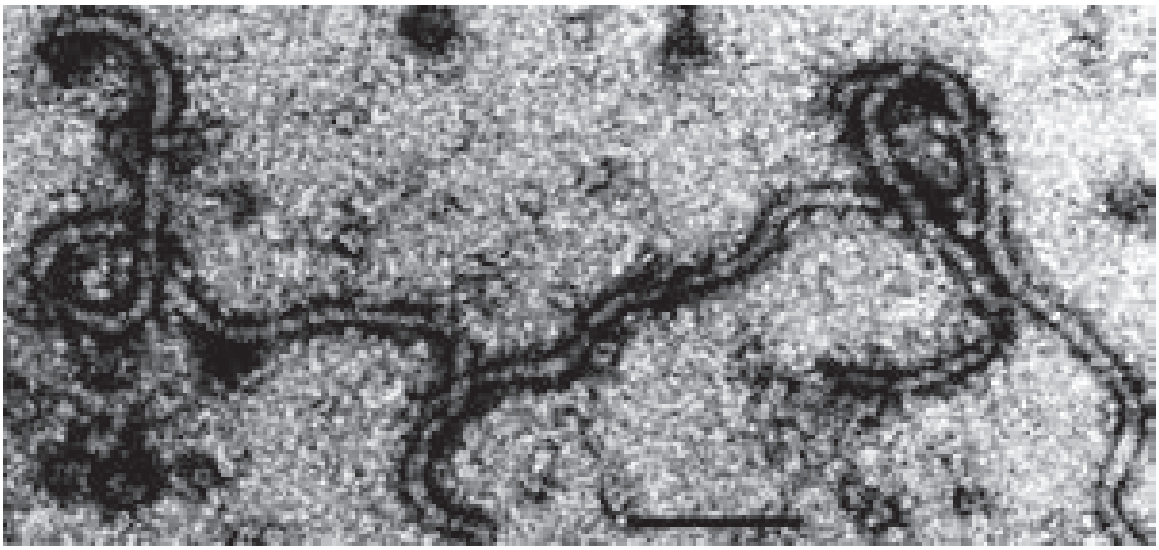

**Figure 9:** Negative contrast electron micrograph of particles of an isolate of the species *Apple chlorotic leaf spot virus*. The bar represents 100 nm. (Courtesy of M.A. Castellano.)

## Virion properties

### Morphology

Virions are very flexuous filaments, 640–890 × 10–12 nm in size, helically constructed with a pitch of 3.3–3.5 nm, and about 10 subunits per turn of the helix. Virions may show cross banding, criss-cross or rope-like features according to the negative contrast material used (Figure 9).

### Physicochemical and physical properties

Virions sediment as single or as two very close bands with an $S_{20,w}$ of about 100S. Apple chlorotic leaf spot virus (ACLSV) virions are sensitive to ribonucleases. Virions of all viruses in the genus resist moderately high temperatures (thermal inactivation is around 55–60 °C) and are moderately resistant to organic solvents.

### Nucleic acid

Virions contain a single molecule of linear, positive sense, ssRNA about 7.5– 8.0 kb in size, with a polyadenylated 3′ terminus, accounting for about 5% of the particle weight. Indirect evidence suggests that the genome RNA of ACLSV is capped at its 5′-end with $m^7G$. An infectious cDNA clone of ACLSV has been produced. ACLSV isolates show a high variability in their nt sequence with an overall identity between 76 and 82%. The CP is the most conserved protein (87–93% identity), whilst the putative MP is the most divergent (77–85% identity).

### Proteins

Virions of all members are composed of a single 20.5–27 kDa polypeptide.

## Genome organization and replication

The genomes of ACLSV and grapevine berry inner necrosis virus (GINV) contain three slightly overlapping ORFs while other members and possible members of the genus have an additional ORF at the 3′ terminus (Figure 10). The large 5′ ORF of ACLSV is directly expressed from genomic RNA, whereas the two smaller downstream ORFs that code, respectively, for the MP and CP, are expressed via sgRNAs. The fourth ORF (where present) has homologies to the vitivirus nucleic acid binding proteins. ACLSV-infected tissues contain six dsRNA species of approximately 7.5, 6.4, 5.4, 2.2, 1.1 and 1.0 kbp. The 7.5 kbp species represents the double-stranded form of the full-length genome, whereas the 2.2 and the 1.1 kbp species are the double-stranded forms of sgRNAs coding for the MP and the CP, respectively. The most abundant dsRNA species, the function of which are unknown, are 5′ co-terminal with genomic RNA, and have sizes of 6.4 and 5.4 kbp, respectively. Replication is

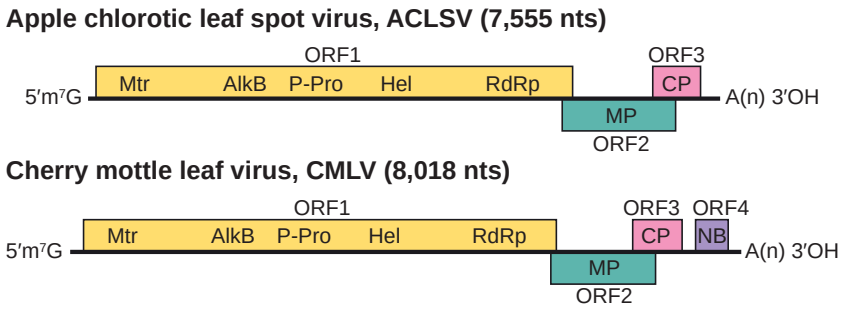


**Figure 10:** Genome organization of apple chlorotic leaf spot virus and cherry mottle leaf virus, showing the relative positions of the ORFs and their expression products. Mtr, methyltransferase; P-Pro, papain-like protease; Hel, helicase; Pol, polymerase; MP, putative movement protein; CP, capsid protein; NB, nucleic acid binding protein.

presumed to be cytoplasmic and to involve the translation product of ORF1. The MP of ACLSV is a suppressor of silencing that interferes with systemic movement of the silencing signal.

## Antigenic properties

Virions are moderate to poor antigens. Cherry mottle leaf virus (CMLV) and peach mosaic virus (PcMV) are serologically related to one another but not to the other members of the genus.

## Biological properties

### Host range

The natural host range of individual species is relatively narrow (ACLSV, PcMV), or restricted to a single host (GINV, CMLV). The experimental host range is somewhat wider, but still limited to a few herbaceous species. In the natural hosts, infections induce few or no symptoms (ACLSV in certain hosts), or mottling, rings, line patterns and fruit injuries (i.e. pseudosharka) (ACLSV), mottling with stunting and internal necrosis of shoots and berries (GINV), mottling and severe distortion of the leaves (CMLV), mottling and deformation of leaves and fruits and color break in the petals (PcMV).

### Transmission

The viruses are readily transmitted by mechanical inoculation, by grafting (ACLSV, GINV, CMLV, PcMV) and through propagating material. GINV is transmitted by the grape erineum mite *Colomerus vitis*, CMLV by the scale mite *Eriophyes inequalis*, and PcMV by the peach bud mite *Eriophyes insidiosus*.

### Geographical distribution

Geographical distribution varies from wide to restricted, according to the virus. ACLSV is ubiquitous, whereas GINV is reported only from Japan, and CMLV and PcMV from North America.

### Cytopathic effects

Infected cells are damaged by ACLSV to varying extents. Virions are found in phloem and parenchyma cells of leaves and roots and accumulate in the cytoplasm, sometimes in the nucleus, in bundles or paracrystalline aggregates. No inclusion bodies are formed.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Natural and experimental host range.
- Serological specificity.
- Less than about 72% nt identity (or 80% aa identity) between their CP or polymerase genes.
- Transmission by a vector.
- Vector specificity.

## List of species in the genus *Trichovirus*

| | | |
|---|---|---|
| *Apple chlorotic leaf spot virus* | | |
| Apple chlorotic leaf spot virus-P863 | [M58152 = NC_001409] | (ACLSV-P863) |
| *Apricot pseudo-chlorotic leaf spot virus* | | |
| Apricot pseudo-chlorotic leaf spot virus-Sus2 | [AY713379 = NC_006946] | (APsCLSV-Sus2) |
| *Cherry mottle leaf virus* | | |
| Cherry mottle leaf virus-SA1162-21 | [AF170028 = NC_002500] | (CMLV-SA116221) |
| *Grapevine berry inner necrosis virus* | | |
| Grapevine berry inner necrosis virus-Japan | [D88448 = NC_015220] | (GINV-JP) |
| *Peach mosaic virus* | | |
| Peach mosaic virus-2022-01 (CA-1) | [DQ117579 = NC_011552] | (PcMV-202201 (CA1)) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Trichovirus* but have not been approved as species

| | | |
|---|---|---|
| Fig latent virus 1 | [FN377573*] | (FLV-1) |
| Phlomis mottle virus | [AM920542*] | (PhMV) |

*Sequences do not comprise the complete genome.

# GENUS *VITIVIRUS*

Type species ***Grapevine virus A***

## Distinguishing features

Vitiviruses have a distinctive genome organization with five ORFs, including a 20K ORF between the polymerase and the movement protein of the "30K" superfamily. Natural transmission is by pseudococcid mealybugs, soft scale insects and aphids.

## Virion properties

### MORPHOLOGY

Virions are flexuous filaments 725–825 × 12 nm in size, showing distinct cross-banding, helically constructed with a pitch of 3.3–3.5 nm and about 10 subunits per turn of the helix (Figure 11).

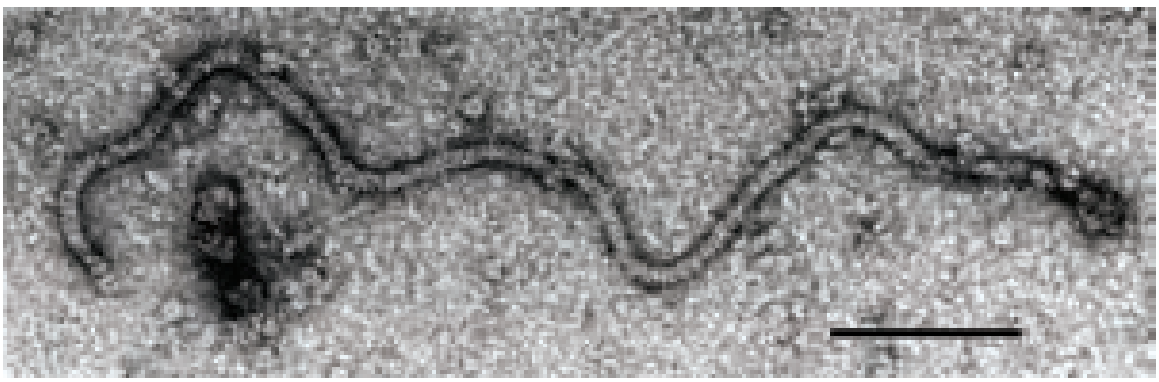

**Figure 11:** Negative contrast electron micrograph of particles of an isolate of the species *Grapevine virus A*. The bar represents 100 nm. (Courtesy of A.A. Castellano.)

### Physicochemical and physical properties

Virions sediment as a single or two very close bands in sucrose or $Cs_2SO_4$ gradients, with an $S_{20,w}$ of about 92S. Virions of Heracleum latent virus (HLV) are sensitive to ribonucleases. Virions of all members of the genus resist moderately high temperatures (thermal inactivation is around 60 °C) and are moderately resistant to organic solvents.

### Nucleic acid

Virions contain a single molecule of positive sense ssRNA, about 7.6 kb in size, capped at the 5′ terminus with $m^7G$ and polyadenylated at the 3′ terminus. The RNA accounts for about 5% of the particle weight. Infectious cDNA clones have been produced for grapevine viruses A and B (GVA and GVB).

### Proteins

The CPs are composed of a single 18–21.5 kDa polypeptide.

## Genome organization and replication

The genomes contain five slightly overlapping ORFs (Figure 12). The 5′ regions of grapevine viruses A and B initiate with an A/T-rich (60–68%) UTR of 47–86 nt. ORF1 is the replication-related protein. ORF2 is a 19–20 kDa polypeptide of unknown function with no significant sequence homology to known proteins, which, in GVB infections, does not accumulate in phase with MPs. ORF3 (31–36.5 kDa) is the movement protein and ORF4 is the CP. The final ORF is a 10–14 kDa polypeptide with weak homologies to proteins with RNA-binding properties and which has been shown (in GVA) to have RNA silencing suppressor activity.

The strategy of expression is based on sgRNA production, as suggested by the analysis of dsRNA patterns from infected tissues. The four dsRNAs have sizes of 7.6, 6.48, 5.68 and 5.1 kbp for GVA and GVD, and 7.6, 6.25, 5.03 and 1.97 kbp for GVB. In GVA there are nested sets of 5′-terminal sgRNAs 5.1, 5.5 and 6.0 kb in size and of 3′-terminal sgRNAs 1.0, 1.8 and 2.2 kb that serve for the expression of all ORFs, except for ORF5, which may be expressed via a bi- or polycistronic mRNA. The generation of these 5′- and 3′-terminal sgRNAs appears to be controlled by internal *cis*-acting elements. Replication occurs in the cytoplasm, possibly in association with membranous vesicles.

## Antigenic properties

Virions are moderate or poor antigens. Most species are very distantly serologically related. Monoclonal antibodies to GVA, GVB and GVD and recombinant protein antibodies to the putative

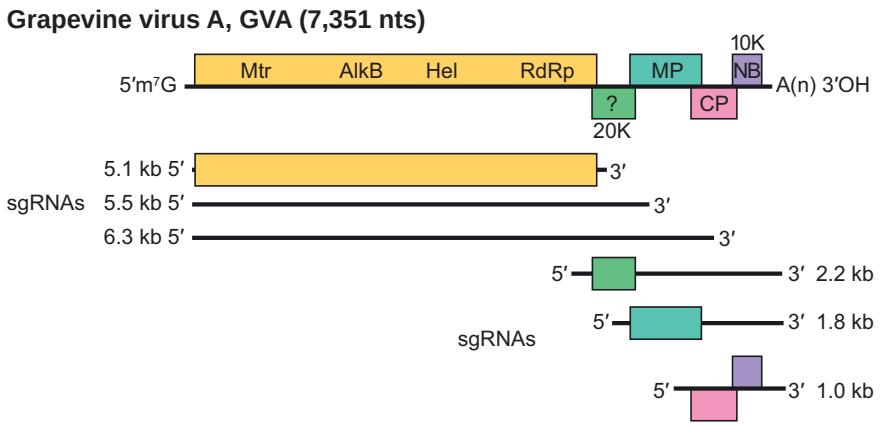


**Figure 12:** Organization and expression of the genome of grapevine virus A (GVA) showing the relative position of the ORFs, their expression products, and the nested sets of 5′- and 3′-terminal sgRNAs. Mtr, methyltransferase; Hel, helicase; Pol, polymerase; MP, putative movement protein; CP, capsid protein.

Pos. ssRNA

MP of GVA have been produced. The relationship between GVA, GVB and GVD is due to a few common internal antigenic determinants (cryptotopes). GVA particles carry a highly structured epitope centered in a common peptide region of the CP sequence.

## Biological properties

### Host range

The natural host range of individual species is restricted to a single host. Infections induce either no symptoms (HLV and mint virus 2 (MV-2)) or severe diseases characterized by pitting and grooving of the wood (GVA, GVB and GVD). The experimental host range varies from wide (HLV) to restricted (GVA, GVB, GVD and GVE).

### Transmission

All species except for MV-2 are transmitted by mechanical inoculation, those infecting grapevines with greater difficulty. Transmission by grafting and dispersal through propagating material is common with grapevine-infecting species. GVA and GVB are transmitted in a semi-persistent manner by different species of pseudococcid mealybugs of the genera *Pseudococcus* and *Planococcus*. GVA is also transmitted by the scale insect *Neopulvinaria innumerabilis*. HLV and MV-2 are transmitted semi-persistently by aphids, in association with a helper virus.

### Geographical distribution

Geographical distribution varies from very wide (GVA, GVB, GVD) to restricted (HLV).

### Cytopathic effects

Infected cells are damaged to a varying extent. All species investigated elicit the formation of vesicular evaginations of the tonoplast containing finely fibrillar material, possibly representing replicative forms of viral RNA. Virions of grapevine-infecting species are strictly phloem-limited, but in herbaceous hosts they also invade the parenchyma. Virus particles accumulate in the cytoplasm in bundles or paracrystalline aggregates.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- The natural host range.
- Serological specificity using discriminatory polyclonal and monoclonal antibodies.
- Epidemiology: individual species or groups of species are transmitted by different types and species of vectors.
- Differences in dsRNA pattern.
- Less than about 72% nt identity (or 80% aa identity) between their CP or polymerase genes.

## List of species in the genus *Vitivirus*

| | | |
|---|---|---|
| *Grapevine virus A* | | |
| Grapevine virus A-Is 151 | [X75433 = NC_003604] | (GVA-Is 151) |
| *Grapevine virus B* | | |
| Grapevine virus B-Italy | [X75448 = NC_003602] | (GVB-IT) |
| *Grapevine virus D* | | |
| Grapevine virus D-Italy | [Y07764*] | (GVD-IT) |
| *Grapevine virus E* | | |
| Grapevine virus E-Japan:TvAQ7 | [AB432910 = NC_011106] | (GVE-TvAQ7) |
| *Heracleum latent virus* | | |
| Heracleum latent virus-Scottish: Murant | [X79270*] | (HLV-MUR) |
| *Mint virus 2* | | |
| Mint virus 2-USA | [AY913795*] | (MV-2-USA) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

## List of other related viruses which may be members of the genus *Vitivirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Betaflexiviridae*

| | | |
|---|---|---|
| *African oil palm ringspot virus* | | |
| African oil palm ringspot virus-Colombia | [AY072921 = NC_012519] | (AOPRV-COL) |
| *Banana mild mosaic virus* | | |
| Banana mild mosaic virus-Australia | [AF314662 = NC_002729] | (BanMMV-AUS) |
| *Cherry green ring mottle virus* | | |
| Cherry green ring mottle virus-USA | [AF017780 = NC_001946] | (CGRMV-USA) |
| *Cherry necrotic rusty mottle virus* | | |
| Cherry necrotic rusty mottle virus-Germany | [AF237816 = NC_002468] | (CNRMV-DE) |
| *Potato virus T* | | |
| Potato virus T-Peru | [EU835937 = NC_011062] | (PVT-Peru) |
| *Sugarcane striate mosaic-associated virus* | | |
| Sugarcane striate mosaic-associated virus-Australia | [AF315308 = NC_003870] | (SCSMaV-AUS) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the family *Betaflexiviridae* but have not been approved as species

| | | |
|---|---|---|
| Banana virus X | [AY710267*] | (BanVX) |
| White ash mosaic virus | [DQ412998 = NC_011533] | (WAMV) |

*Sequences do not comprise the complete genome.

## Phylogenetic relationships within the family

In a phylogenetic analysis of the replication protein, most genera fall on well-supported branches (Figure 13, p. 938). The family falls into two broad parts that correspond with the types of movement protein. *Carlavirus* and *Foveavirus* with a number of unassigned species that have a TGB form one branch, while the remaining genera and viruses (which all have a "30K"-type movement protein) also cluster together. Of the unassigned species, *Potato virus T* is expected to become the type member of a new genus (proposed *Tepovirus*). The remaining unassigned species resemble foveaviruses in their genome organization but do not form a monophyletic group.

## Similarity with other taxa

The polymerase proteins are members of the "alphavirus-like" supergroup of RNA viruses and are most closely related to those of the other families in the order, namely *Alphaflexiviridae*, *Gammaflexiviridae* and *Tymoviridae*. There are also similarities between the TGB-containing genera and members of the family *Alphaflexviridae* in the 3' end of the genome (Figure 14, p. 939). The TGB proteins are also more distantly related to those of rod-shaped viruses in the family *Virgaviridae* (genera *Hordeivirus*, *Pecluvirus* and *Pomovirus*) (Figure 15a, p. 940). The "30K" movement proteins are related to those in the family *Virgaviridae* (genera *Furovirus*, *Tobamovirus* and *Tobravirus*) (Figure 15b, p. 940).

## Derivation of names

*Capillo*: from Latin *capillus*, "a hair".
*Carla*: from *Carnation latent virus*.
*Citri*: from *Citrus leaf blotch virus*.
*Flexi*: from Latin *flexus*, "bent".
*Fovea*: from Latin *fovea*, "pit" or "hole", a type of symptom induced by the type species.
*Tricho*: from Greek *thrix*, "hair".
*Viti*: from *Vitis*, generic name of the grapevine, *Vitis vinifera*, the host of the type species.

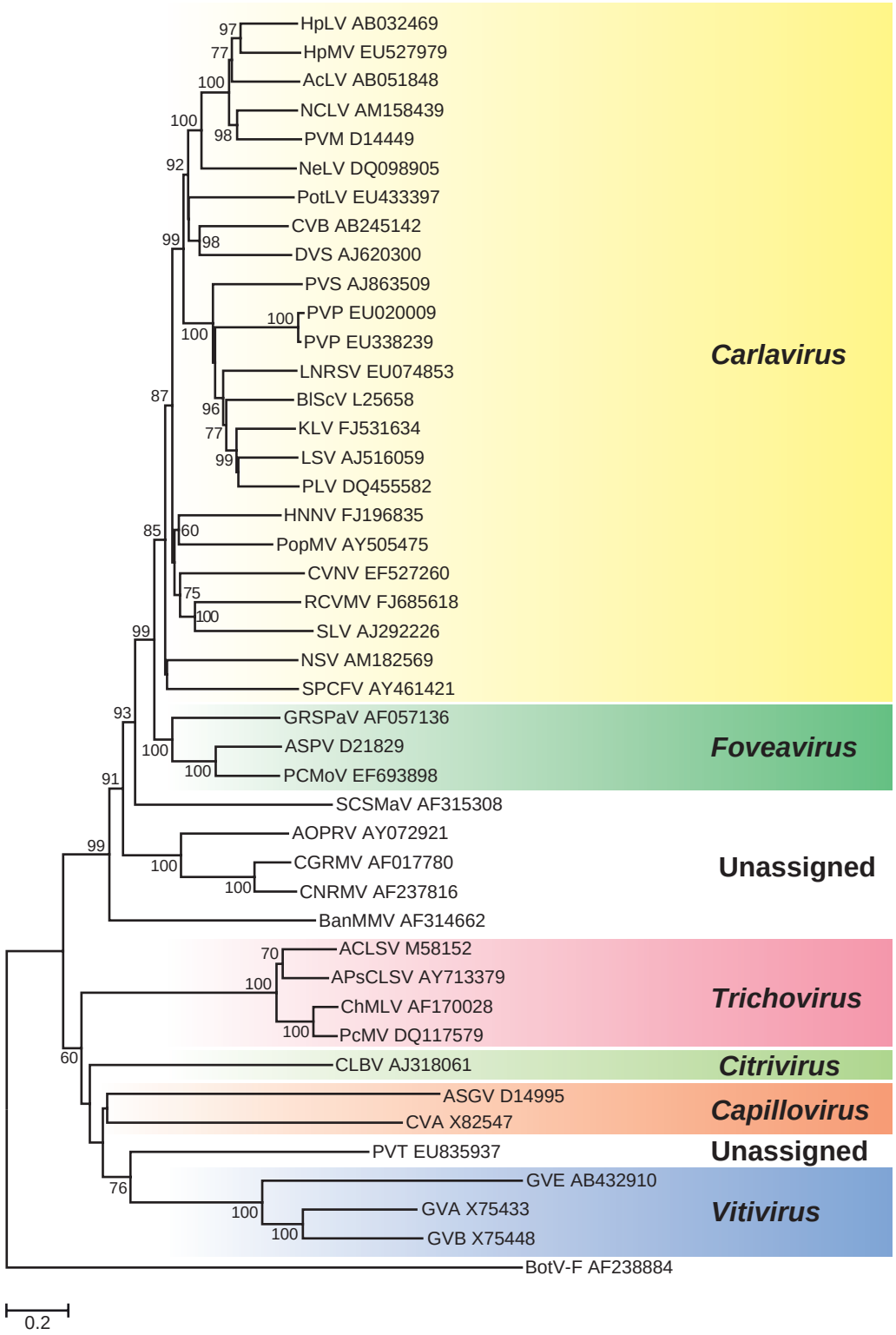


**Figure 13:** Phylogenetic (distance) tree based on the amino acid sequences of the entire replication protein of members of the family *Betaflexiviridae*. A single representative isolate of each sequenced species in the family was included and the tree is rooted with Botrytis virus F (BotV-F; genus *Mycoflexivirus*, family *Gammaflexiviridae*). Numbers on branches indicate percentage of bootstrap support out of 1000 bootstrap replications (when >60%). The scale indicates JTT amino acid distances. Tree produced in MEGA4.

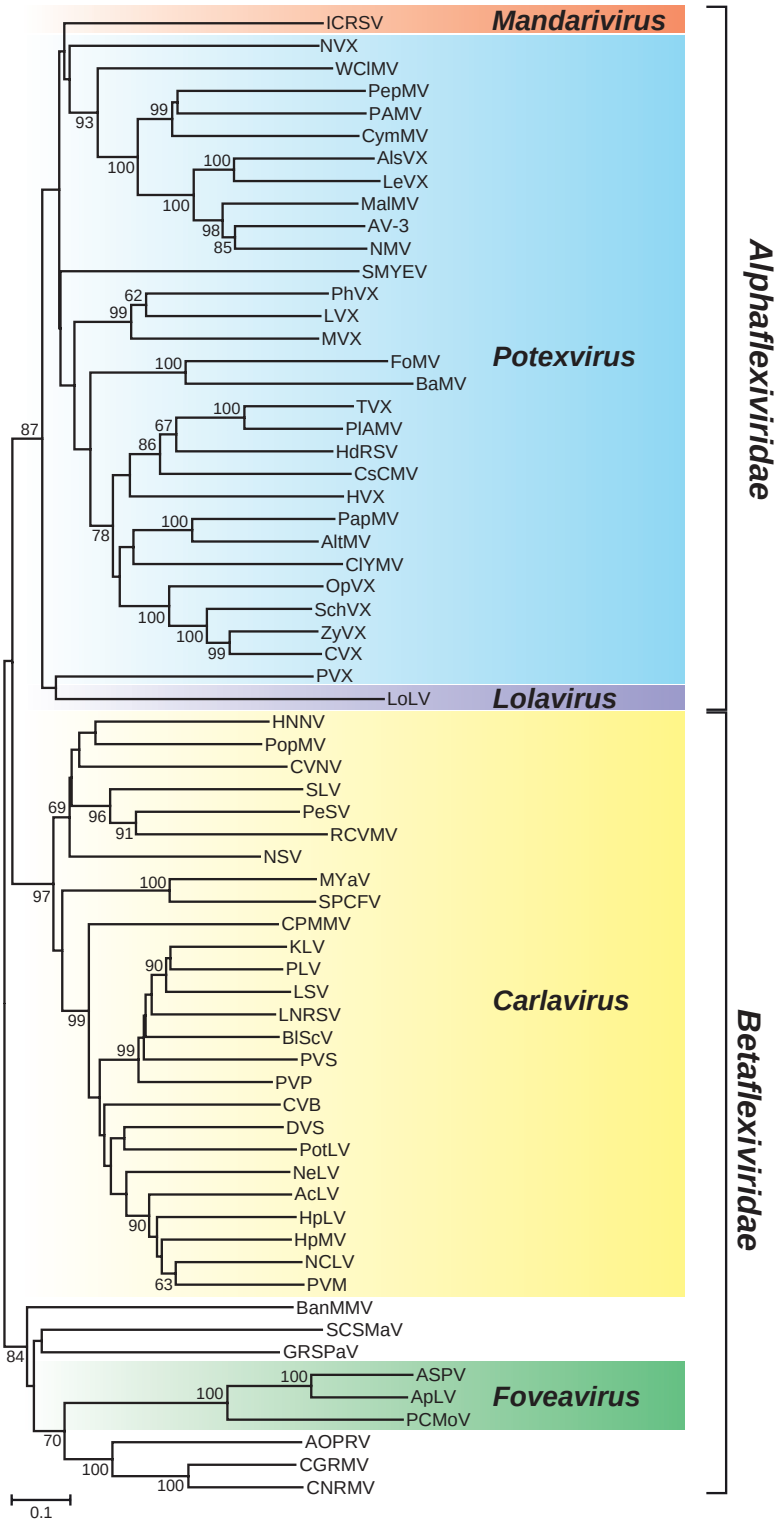


**Figure 14:** Phylogenetic (distance) tree based on the 3′-terminal nucleotide sequences of those members of the families *Betaflexiviridae* and *Alphaflexiviridae* that have a triple gene block (TGB). The sequence used includes the entire TGB and coat protein genes (alignment length 2466 nt). A single representative isolate of each sequenced species in each family was used. Numbers on branches indicate percentage of bootstrap support out of 10,000 bootstrap replications (when >60%). The scale indicates maximum composite likelihood distances. Tree produced in MEGA4.

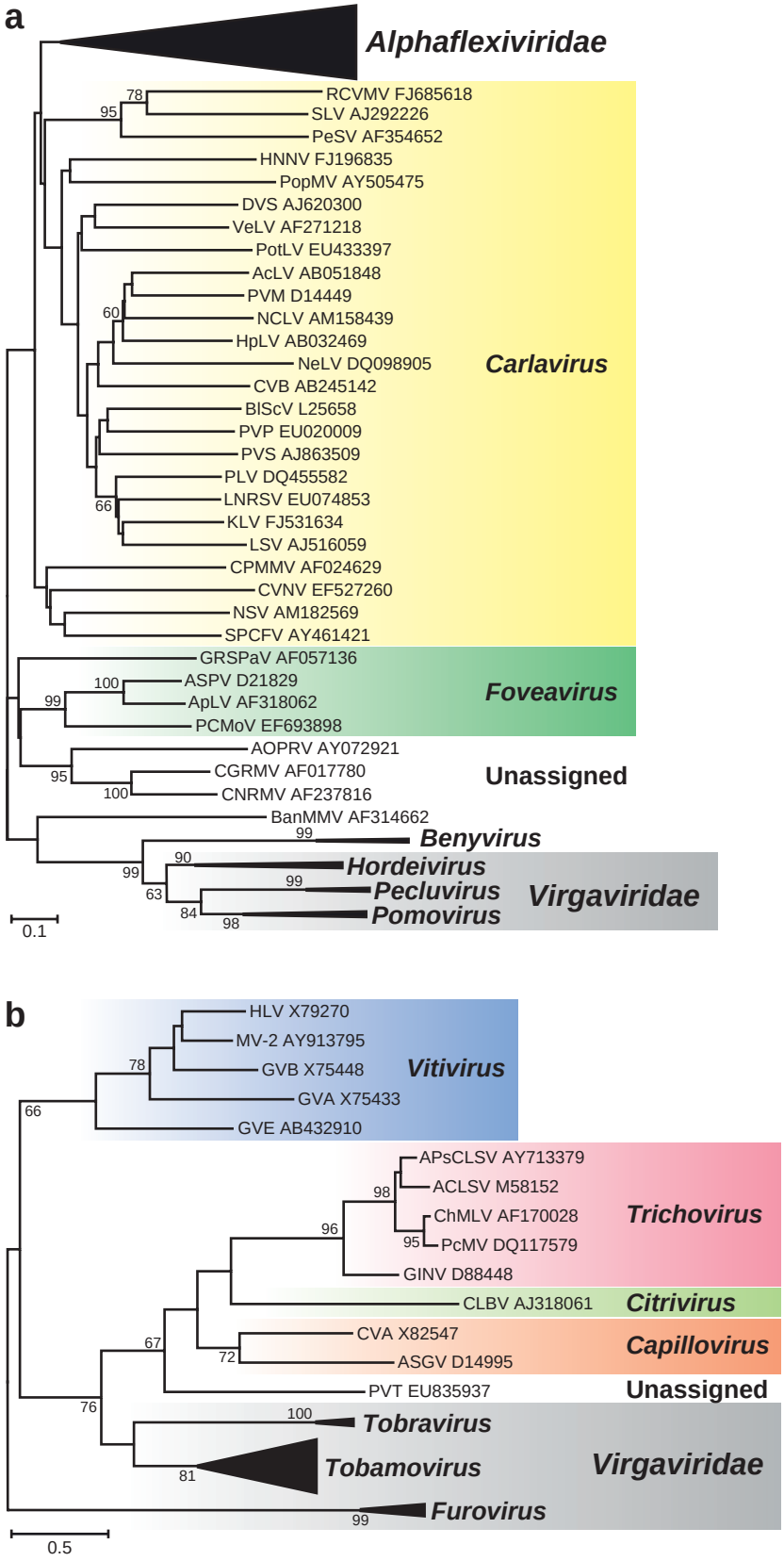


**Figure 15:** Phylogenetic (distance) trees produced in MEGA4 showing how the cell-to-cell movement proteins of viruses in the family *Betaflexiviridae* are related to those of other plant viruses. (a) Tree based on the codon-aligned nucleotide sequences of the first triple gene block protein (TGBp1). A single representative isolate of each sequenced species in the family was included. The tree also contains similar sequences from the family *Alphaflexiviridae* and those of rod-shaped plant viruses collapsed into triangles, the length of which corresponds to the variation found within the clade. Numbers on branches indicate percentage of bootstrap support out of 10,000 bootstrap replications (when >60%). The scale indicates maximum composite likelihood distances. Tree produced in MEGA4. (b) Tree based on the amino acid sequences of the "30K"-like movement protein. A single representative isolate of each sequenced species in the family was included. The tree also contains similar sequences from genera in the family *Virgaviridae* collapsed into triangles, the length of which corresponds to the variation found within the clade. Numbers on branches indicate percentage of bootstrap support out of 1000 bootstrap replications (when >60%). The scale indicates JTT amino acid distances.

## Further reading

### Journals and books

Adams, M.J., Antoniw, J.F., Bar-Joseph, M., Brunt, A.A., Candresse, T., Foster, G.D., Martelli, G.P., Milne, R.G., Zavriev, S.K. and Fauquet, C.M. (2004). The new plant virus family *Flexiviridae* and assessment of molecular criteria for species demarcation. *Arch. Virol.*, **149**, 1045–1060.

Bratlie, M.S. and Drabløs, F. (2005). Bioinformatic mapping of AlkB homology domains in viruses. *BMC Genomics*, **6**, 1–15.

Martelli, G., Adams, M.J., Kreuze, J.F. and Dolja, V.V. (2007). Family *Flexiviridae*: a case study in virion and genome plasticity. *Annu. Rev. Phytopathol.*, **45**, 73–100.

Vives, M.C., Galipienso, L., Navarro, L., Moreno, P. and Guerri, J. (2001). The nucleotide sequence and genomic organization of citrus leaf blotch virus: candidate type species for a new virus genus. *Virology*, **287**, 225–233.

### Websites

International Council for the Study of Virus and Virus-like Diseases of the Grapevine: http://www.icvg.ch.

## Contributed by

Adams, M.J., Candresse, T., Hammond, J., Kreuze, J.F., Martelli, G.P., Namba, S., Pearson, M.N., Ryu, K.H., Saldarelli, P. and Yoshikawa, N.

# Family *Gammaflexiviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Gammaflexiviridae* |
| Genus | *Mycoflexivirus* |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Mycoflexivirus*

Type species *Botrytis virus F*

## Virion properties

### Morphology
Virions are flexuous filaments of 720 nm modal length and about 13 nm in diameter.

### Physicochemical and physical properties
Not known.

### Nucleic acid
Virions contain a single molecule of linear ssRNA, 6827 nt in length, excluding the 3′-poly(A) tail.

### Proteins
The only structural protein is the coat protein composed of 302 aa (32 kDa).

### Lipids
None reported.

### Carbohydrates
None reported.

## Genome organization and replication

The genomic RNA comprises two major ORFs on the positive strand, a 5′-UTR of 63 nt and a 3′-UTR of 71 nt, followed by a poly(A) tail (Figure 1). ORF1 contains conserved methyltransferase and helicase motifs, terminating with an opal stop codon (UGA) and yielding a protein of 153 kDa. Readthrough of this codon is expected to extend the protein to 212 kDa that then also includes an RNA-dependent RNA polymerase (RdRp) domain. ORF2 encodes the 32 kDa coat protein.

## Antigenic properties

Not known.

## Biological properties

The virus was discovered infecting an isolate of the plant pathogenic fungus *Botrytis cinerea*. Its mode of transmission is unknown. The same fungal isolate was also infected with a virus now classified as *Botrytis virus X* (genus *Botrexvirus*, family *Alphaflexiviridae*).

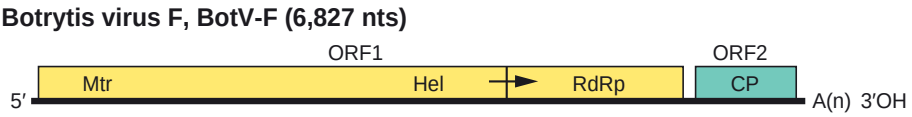


**Figure 1:** Genome organization of Botrytis virus F showing the relative positions of the ORFs and their expression products. Mtr, methyltransferase; Hel, helicase; RdRp, RNA-dependent RNA polymerase; CP, capsid protein. The arrow marks the "leaky" opal stop codon in ORF1.


Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Mycoflexivirus*

*Botrytis virus F*
Botrytis virus F-New Zealand:Auckland [AF238884 = NC_002604] (BotV-F-NZ)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Mycoflexivirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Gammaflexiviridae*

None.

## Similarity with other taxa

There are clear phylogenetic relationships in both ORFs to other viruses in the family *Tymovirales*. Nearly all these are plant-infecting viruses and many of them also have flexuous virions (which is unusual for a mycovirus). This may suggest that the virus was originally acquired by a filamentous fungus from its plant host but has subsequently lost the cell-to-cell movement protein(s) characteristic of plant viruses. A separate family for this genus is justified by phylogenetic analyses of the order and also because of the readthrough translation strategy of the replication protein, a strategy that has not been found elsewhere in the order.

## Derivation of names

*Flexi*: from *flexus*, Latin for "bent".
*Mycoflexi*: from *myco*virus with *flex*uous virions.

## Further reading

Howitt, R.L.J., Beever, R.E., Pearson, M.N. and Forster, R.L.S. (2001). Genome characterization of *Botrytis* virus F, a flexuous rod-shaped mycovirus resembling plant "potex-like" viruses. *J. Gen. Virol.*, **82**, 67–78.

Martelli, G., Adams, M.J., Kreuze, J.F. and Dolja, V.V. (2007). Family *Flexiviridae*: a case study in virion and genome plasticity. *Annu. Rev. Phytopathol.*, **45**, 73–100.

## Contributed by

Adams, M.J., Kreuze, J.F. and Pearson, M.N.

# FAMILY *TYMOVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Tymoviridae*** |
| Genus | ***Tymovirus*** |
| Genus | ***Marafivirus*** |
| Genus | ***Maculavirus*** |

## Distinguishing features

This is the only family within the order *Tymovirales* that has viruses with isometric particles. The replication protein polymerase domain forms a distinctive phylogenetic lineage.

## Virion properties

### MORPHOLOGY

Virions are isometric, non-enveloped, about 30 nm in diameter, with a rounded contour and prominent surface structure, with clustering of CP subunits in pentamers and hexamers. The capsids of tymoviruses are made up of 20 hexameric and 12 pentameric subunits arranged in a T = 3 icosahedron and the RNA appears to be at least partially ordered in an icosahedral arrangement in the center of the protein shell. (See Figure 1.)

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virus particles sediment as two centrifugal components: T, made up of non-infectious protein shells that contain little or no RNA (primarily subgenomic CP mRNA), and B, composed of intact nucleoprotein particles. Sedimentation coefficients ($S_{w,20}$) of component T and B range from 42 to 55 and from 109 to 125, respectively. Buoyant densities in CsCl of components T and B are 1.26–1.28 g cm$^{-3}$ and 1.40–1.46 g cm$^{-3}$ respectively. Virions resist high temperatures (thermal inactivation point is 60–65 °C up to above 80 °C with some tymoviruses) and organic solvents, but are disrupted by SDS.

### NUCLEIC ACID

Virions contain a single molecule of positive sense, ssRNA constituting 25–35% of the particle weight. The RNA has a very high cytidine content (from 32 to about 50%) and ranges from 6.0 to 7.5 kb in length. Tymovirus genomes are capped at the 5′ terminus with m$^7$G and most, though not all, have a tRNA-like structure at the 3′ end, which for turnip yellow mosaic virus (TYMV) and several other members, accepts valine. The genomes of marafiviruses, maculaviruses and of the

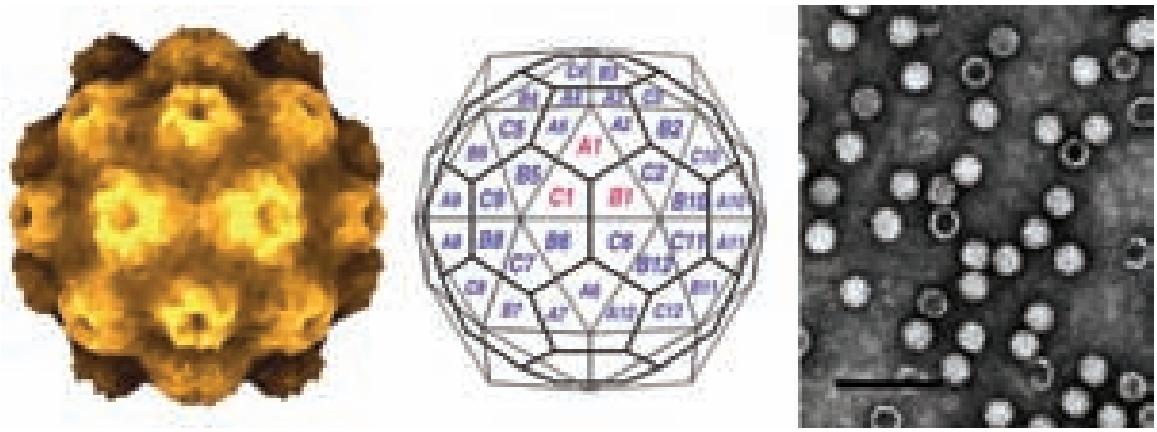

**Figure 1:** (Left) Atomic rendering of a virion of turnip yellow mosaic virus (TYMV) (Canady *et al.*, *Nature Structural Biology*, **3**, 771–781, 1996). (Center) Diagram of a TYMV virion with CP clusters in hexa- and pentamers. (Right) Negative contrast electron micrograph of virions and "empty particles" of Belladonna mottle virus (BeMV), a representative of the genus Tymovirus. Particles of members of the genera Marafivirus and Maculavirus have the same morphology. The bar represents 100 nm.

unassigned Poinsettia mosaic virus (PnMV) are polyadenylated at the 3′ terminus and are thought to be capped at the 5′ end with $m^7G$.

### Proteins

The CP of virus particles consists of either a single protein species with molecular mass of 20 kDa (tymoviruses), 24.5–25 kDa (maculaviruses), or a major protein of about 21 kDa and a minor protein of 22.5–25 kDa (marafiviruses). The capsids of PnMV and a strain of *Bermuda grass etched line virus* are made up of a single protein of about 21 kDa.

## Genome organization and replication

The genomes of tymoviruses contain two extensively overlapping ORFs that begin 7 nt apart, and a third ORF (for CP) that is expressed from a 3′-coterminal subgenomic RNA (sgRNA). The longest ORF encodes a replication polyprotein of about 220 kDa that contains recognized domains for a methyltransferase (Mtr), a papain-like cysteine protease (P-Pro), an RNA helicase (Hel) and an RNA-dependent RNA polymerase (RdRp). The genomes of the marafiviruses, maculaviruses and PnMV possess a similar polyprotein ORF, and a CP ORF in the same reading frame that is either fused to the long ORF (marafiviruses and PnMV) or separated from it by two adjacent stop codons (grapevine fleck virus, GFkV). MRFV is the sole marafivirus with an extensive ORF near the 5′ end of the genome that entirely overlaps the replication protein ORF (see Figure 3 below). The genome of GFkV possesses two additional ORFs towards the 3′ end. Viral RNA of tymoviruses replicates in the cytoplasm in association with the double-membraned invaginations that line the periphery of the chloroplasts. Comparable vesicles occurring at the peripheries of mitochondria or chloroplasts of cells infected with maculaviruses and PnMV may have the same function. Genome expression of the tymoviruses includes post-translational autocatalytic cleavage of the replication polyprotein by the P-Pro, and synthesis and translation of a 3′-coterminal sgRNA for CP expression.

## Antigenic properties

Virions are moderately to highly antigenic. Tymoviruses cluster roughly into two serological groups with cross-reactivity ranging from strong to weak within each group. Cross-reactivities between group members are weak to undetectable. Serological relationships occur between some marafiviruses, but not between individual maculaviruses.

## Biological properties

### Host range

Members of this family infect dicotyledonous plants, with the exception of some marafiviruses that primarily infect plants in the *Gramineae*. Natural and experimental host ranges of individual virus species are narrow, sometimes restricted to a single type of host (e.g., GFkV infects only *Vitis;* MRFV only *Zea*). Disease symptoms are bright yellow mosaic or mottling (tymoviruses and PnMV), chlorotic stripes, vein clearing, etched lines or dwarfing (marafiviruses), and flecking of the leaves (maculaviruses).

### Transmission

Tymoviruses and PnMV, but not other members of the family, are readily transmissible by mechanical inoculation. They replicate to high titres and invade all main tissues of the host. Marafi- and maculaviruses are phloem-limited. Some marafiviruses (MRFV, oat blue dwarf virus (OBDV) and Bermuda grass etched-line virus (BELV)) are distinguished by being vectored in a persistent manner by leafhoppers, in which they replicate. Some tymoviruses are weakly seed-transmissible, and are also spread by beetles, which serve as low-efficiency local vectors. Maculaviruses do not have recognized vectors, and the grapevine-associated marafi- and maculaviruses are disseminated primarily through infected propagating material.

### Geographical distribution

Members of the family have been recorded from most parts of the world. Geographical distribution of individual species varies from restricted to widespread.

### Cytopathic effects

Most species elicit derangement of the internal structure and alteration of the shape of chloroplasts and/or mitochondria, which also show rows of peripheral vesicles derived from localized invaginations of the limiting membrane.

## Genus demarcation criteria in the family

The criteria demarcating genera in the family are:

- Biological criteria: Tymoviruses are mechanically transmissible, invade parenchyma tissues, and infect dicotyledonous plants. Marafiviruses and maculaviruses are both phloem-limited and are not mechanically transmissible.
- Epidemiological criteria: Tymoviruses are transmitted by beetles, some marafiviruses by leafhoppers. Maculaviruses have no known vector.
- Cytopathological criteria: Tymoviruses elicit the formation of double-membraned vesicles at the periphery of chloroplasts while maculaviruses elicit them at the periphery of mitochondria. Chloroplast vesicles induced by PnMV are single-membraned.
- Physicochemical criteria: (a) Genome size: 6.0–6.7 kb (tymoviruses), 6.3–6.5 kb (marafiviruses), 7.5 kb (maculaviruses); (b) number and molecular mass of the CP: one subunit type of 20 kDa (tymoviruses), 25 kDa (maculaviruses) or 21 kDa (PnMV), or two subunit types (21 and 23–25 kDa) (marafiviruses).
- Molecular criteria: Tymovirus genomes have three ORFs and usually a tRNA-like structure at the 3′ terminus. Maculavirus genomes have four ORFs, a 3′-terminal poly(A) tail. Marafivirus genomes have a single large ORF (two ORFs in the case of MRFV) and a 3′-terminal poly(A) tail. Tymovirus genomes possess a conserved sequence that serves as a sgRNA promoter known as the "tymobox". The genomes from marafiviruses and PnMV contain a variant termed the "marafibox", while no such sequence is evident in maculavirus genomes.

# Genus *Tymovirus*

Type species ***Turnip yellow mosaic virus***

## Distinguishing features

The genomic RNA (6.0–6.7 kb in size) contains three ORFs (Figure 2). ORF1 encodes the large replication polyprotein. The 3′ ends of these large ORFs are highly conserved and contain a 16 nt sequence known as the "tymobox" (GAGUCUGAAUUGCUUC), which functions as subgenomic RNA promoter. ORF2 initiates 7 nt upstream of ORF1, almost entirely overlaps ORF1, and encodes a 50–80 kDa proline-rich protein that for TYMV is dispensable for replication but is required for cell-to-cell movement. ORF3 codes for the viral CP (20 kDa), which is expressed via a sgRNA. The narrow host range of tymoviruses makes host susceptibility an important property in distinguishing among species. All members induce vesicles at the periphery of chloroplasts and to a lesser degree at mitochondria, resulting in characteristic aggregates of swollen and modified chloroplasts. All main tissues of the hosts are invaded. Empty virion shells sometimes accumulate in the nuclei. Beetles of the families *Chrysomelidae* and *Curculionidae* serve as local vectors, transmitting the virus in a semi-persistent manner. All members of the genus are mechanically transmissible and a few (TYMV, eggplant mosaic virus and Dulcamara mottle virus) are transmitted through seeds.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Overall genome sequence identity of less than 80%.
- Capsid protein sequences less than 90% identical.
- Differential host range.
- Differences in the 3′-terminal structure.
- Serological specificity.

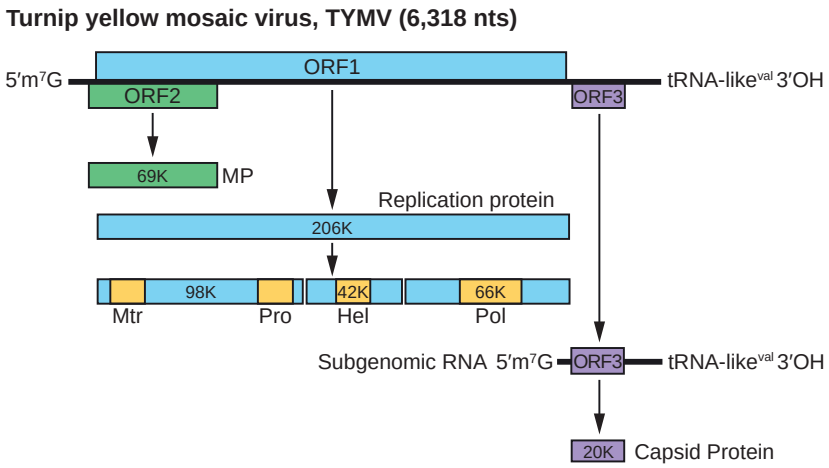


**Figure 2:** Organization and expression of the genome of turnip yellow mosaic virus (TYMV). Mtr, methyltransferase; Pro, papain-like protease; Hel, helicase; Pol, polymerase (RdRp).

## List of species in the genus *Tymovirus*

| | | |
|---|---|---|
| *Anagyris vein yellowing virus* | | |
| Anagyris vein yellowing virus - Apulia, Italy 1983 | [AY751780 = NC_011559] | (AVYV-IT) |
| *Andean potato latent virus* | | |
| Andean potato latent virus-Hu | [AF035402]* | (APLV-Hu) |
| *Belladonna mottle virus* | | |
| Belladonna mottle virus-Eur | [X54529]* | (BeMV-Eur) |
| *Cacao yellow mosaic virus* | | |
| Cacao yellow mosaic virus - West Africa | [X54354]* | (CYMV-WA) |
| *Calopogonium yellow vein virus* | | |
| Calopogonium yellow vein virus - Malaysia | [U91413]* | (CalYVV-MAL) |
| *Chayote mosaic virus* | | |
| Chayote mosaic virus - Costa Rica | [AF195000 = NC_002588] | (ChMV-CR) |
| *Clitoria yellow vein virus* | | |
| Clitoria yellow vein virus - Kenya | [AF035200]* | (CYVV-KE) |
| *Desmodium yellow mottle virus* | | |
| Desmodium yellow mottle virus - USA | [AF035201]* | (DYMoV-USA) |
| *Dulcamara mottle virus* | | |
| Dulcamara mottle virus - Hertfordshire, UK | [AY789137 = NC_007609] | (DuMV-UK) |
| *Eggplant mosaic virus* | | |
| Eggplant mosaic virus - Trinidad | [J04374 = NC_001480] | (EMV-TRD) |
| *Erysimum latent virus* | | |
| Erysimum latent virus - Eastern Germany | [AF098523 = NC_001977] | (ErLV-DE) |
| *Kennedya yellow mosaic virus* | | |
| Kennedya yellow mosaic virus-JB - Australia | [D00637 = NC_001746] | (KYMV-AUS) |
| *Melon rugose mosaic virus* | | |
| Melon rugose mosaic virus - Yemen | | (MRMV-YE) |
| *Nemesia ring necrosis virus* | | |
| Nemesia ring necrosis virus - Bavaria, Germany | [AY751778 = NC_011538] | (NeRNV-DE) |
| Diascia yellow mottle virus DiaYMV-WA | [EU684141 = NC_011086] | (DiaYMV-WA) |
| *Okra mosaic virus* | | |
| Okra mosaic virus - Nigeria | [EF554577 = NC_009532] | (OkMV-NG) |
| *Ononis yellow mosaic virus* | | |
| Ononis yellow mosaic virus - England | [J04375 = NC_001513] | (OYMV-UK) |
| *Passion fruit yellow mosaic virus* | | |
| Passion fruit yellow mosaic virus - Colombia | [AF467107]* | (PFYMV-COL) |
| *Peanut yellow mosaic virus* | | |
| Peanut yellow mosaic virus | | (PeYMV) |
| *Petunia vein banding virus* | | |
| Petunia vein banding virus - Rio Grande do Sol, Brazil | [AF210709]* | (PetVBV-BR) |

| | | |
|---|---|---|
| *Physalis mottle virus* | | |
| Physalis mottle virus – Iowa (Belladonna mottle virus-Iowa) | [Y16104 = NC_003634] | (PhyMV-IO) |
| *Plantago mottle virus* | | |
| Plantago mottle virus - USA | [AY751779 = NC_011539] | (PlMoV-USA) |
| *Scrophularia mottle virus* | | |
| Scrophularia mottle virus - Germany | [AY751777 = NC_011537] | (SrMV-DE) |
| *Turnip yellow mosaic virus* | | |
| Turnip yellow mosaic virus - Europe | [X07441 = NC_004063] | (TYMV-Eur) |
| Turnip yellow mosaic virus-TYMC; infectious clone | [X16378] | (TYMV-C) |
| Turnip yellow mosaic virus-BL - Australia | [AF035403] | (TYMV-BL) |
| *Voandzeia necrotic mosaic virus* | | |
| Voandzeia necrotic mosaic virus - Côte d'Ivoire | | (VNMV-CdI) |
| Wild cucumber mosaic virus | | |
| Wild cucumber mosaic virus - Oregon, USA | [AF035633]* | (WCMV-OR) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

### List of other related viruses which may be members of the genus *Tymovirus* but have not been approved as species

| | | |
|---|---|---|
| Chiltepin yellow mosaic virus | [FN563123 = NC_014127] | (ChiYMV) |

## Genus *Marafivirus*

Type species ***Maize rayado fino virus***

### Distinguishing features

The distinct feature of the marafivirus genome (6.3–6.8 kb in size) is a large ORF encoding a precursor polypeptide consisting of the replication-associated proteins and the larger (23–25 kDa) of the two forms of CP found in the virions. The "marafibox" [CA(G/A)GGUGAAUUGCUUC] is a conserved 16 nt stretch comparable to the "tymobox", from which it differs by three or four residue changes. The 23–25 kDa CP is produced initially as a C-terminal fusion of the replication protein, while the smaller (ca. 21 kDa) CP is produced from a 3′-co-terminal sgRNA. Viruses presently classified as marafiviruses exhibit some diversity in genome design (Figure 3): some marafivirus genomes possess a putative second ORF overlapping the main ORF either towards the 5′ end (MRFV) or 3′ end (citrus sudden death-associated virus). Host ranges are very narrow, making host susceptibility an important identifying criterion. Marafiviruses are strictly confined to the phloem of infected hosts and are not readily transmissible by sap inoculation, although MRFV and OBDV have been transmitted by vascular puncture. For MRFV, OBDV and BELV, transmission is by leafhoppers in a persistent-propagative manner, with virus replication occurring in the insect host. Species-specific virus-vector associations occur: i.e., MRFV is transmitted by *Dalbulus*, OBDV by *Macrosteles*, and BELV by *Aconurella*. The insect-transmitted species infect primarily plants in the *Gramineae*, although a notable exception is the infection of flax by OBDV. None of the species is transmitted through seeds. No insect vectors have been identified for other marafiviruses.

### Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Overall genome sequence identity of less than 80%.
- Capsid protein sequences less than 90% identical.
- Differences in the 3′-terminal structure and in the number of ORFs.
- Differential host range.
- Vector specificity.
- Serological specificity.

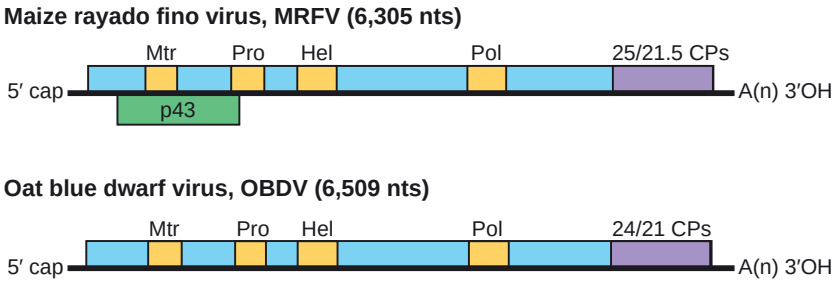


**Figure 3:** The two known types of genome structure in the genus *Marafivirus*, exemplified by maize rayado fino virus (MRFV) and oat blue dwarf virus (OBDV), showing the relative position of the ORFs and their expression products. Mtr, methyltransferase; Pro, papain-like protease; Hel, helicase; Pol, polymerase (RdRp); CPs, capsid proteins; p43, proline-rich protein.

## List of species in the genus *Marafivirus*

| | | |
|---|---|---|
| *Bermuda grass etched-line virus* | | |
| Bermuda grass etched-line virus - Morocco | [AY040531]* | (BELV-MOR) |
| *Citrus sudden death-associated virus* | | |
| Citrus sudden death-associated virus - Brazil | [AY884005 = NC_006950] | (CSDaV-BR) |
| *Maize rayado fino virus* | | |
| Maize rayado fino virus - Costa Rica | [AF265566 = NC_002786] | (MRFV-CR) |
| *Oat blue dwarf virus* | | |
| Oat blue dwarf virus - North Dakota, USA | [U87832 = NC_001793] | (OBDV-ND) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequence does not comprise the complete genome.

## List of other related viruses which may be members of the genus *Marafivirus* but have not been approved as species

| | | |
|---|---|---|
| Blackberry virus S | [FJ915122] | (BlVS) |
| Grapevine asteroid mosaic-associated virus | [AJ249357, AJ249358]* | (GAMaV) |
| Grapevine rupestris vein feathering virus | [AY706994] | (GRVFV) |
| Grapevine Syrah virus-1(Grapevine virus Q) | [FJ436028][FJ977041] | (GSyV-1) |

*Sequences do not comprise the complete genome.

# GENUS *MACULAVIRUS*

Type species ***Grapevine fleck virus***

## Distinguishing features

The genomic RNA of GFkV (7.5 kb in size) is the largest in the family, contains four ORFs and is 3′-polyadenylated (Figure 4). ORF1 encodes the replication polyprotein but does not seem to have a conserved sequence comparable to the "tymobox" or the "marafibox". ORF2 codes for the 24 kDa CP. ORF3 and ORF4, which are located at the extreme 3′ end of the genome, code for proline-rich proteins of 31 and 16 kDa, respectively, which show a distant relationship with the putative movement proteins (ORF2) of tymoviruses. GFkV is restricted to *Vitis* species, which are infected latently, with the exception of *V. rupestris*, which reacts to GFkV with translucent spots (flecks) on the leaves. GFkV is strictly confined to the phloem of infected hosts and is not transmissible by sap inoculation. The cytopathology of GFkV infections is characterized by a severe modification of mitochondria into structures called "multivesiculate bodies". Field spread of GFkV has been reported, but the vector is unknown. GFkV is not transmitted through seeds, thus virus dissemination is primarily through distribution of infected propagative material.

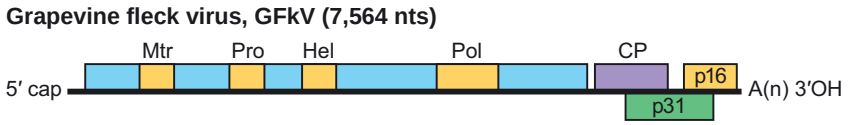


**Figure 4:** Genome structure of an isolate of *Grapevine fleck virus*, the type species of the genus *Maculavirus* showing the relative position of the ORFs and their expression products. Mtr, methyltransferase; Pro, papain-like protease; Hel, helicase; Pol, polymerase (RdRp); CP, capsid protein; p31 and p16, proline-rich proteins.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Overall genome sequence identity of less than 80%.
- Capsid protein sequences identity less than 90%.
- Serological specificity.

## List of species in the genus *Maculavirus*

| | | |
|---|---|---|
| *Grapevine fleck virus* | | |
| Grapevine fleck virus - Italy | [AJ309022 = NC_003347] | (GFkV-IT) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Maculavirus* but have not been approved as species

| | | |
|---|---|---|
| Bombyx mori macula-like latent virus | [AB186123] | (BmMLV) |
| Grapevine red globe virus | [AJ249360, AF521977]* | (GRGV) |

*Sequences do not comprise the complete genome.

## List of unassigned species in the family *Tymoviridae*

None.

## List of other related viruses which may be members of the family *Tymoviridae* but have not been approved as species

| | | |
|---|---|---|
| Olive latent virus 3 | [FJ444852 = NC_013920] | (OLV3) |
| Poinsettia mosaic virus | [AJ271595 = NC_002164] | (PnMV) |

PnMV has characteristics of both the tymoviruses and marafiviruses, and some distinct characteristics. Like tymoviruses, PnMV is mechanically transmissible, infects all main tissues of the host, and amplifies to high titre. Like that of the marafivirus OBDV, the genome contains a single long ORF comprising fused replication and CP domains, a poly(A) tail at the 3′ end and a "marafibox" variant of the "tymobox". Unlike marafiviruses, PnMV is not insect-transmitted, and possesses only a single CP. PnMV infection induces invaginations of the chloroplast membrane, but these are bound by a single membrane, rather than the double membrane, characteristic of tymoviruses. Sequence relationships (Figure 5) support classification distinct from the existing genera of the family *Tymoviridae*.

## Phylogenetic relationships within the family

Phylogenetic relationships within the family are depicted in Figure 5.

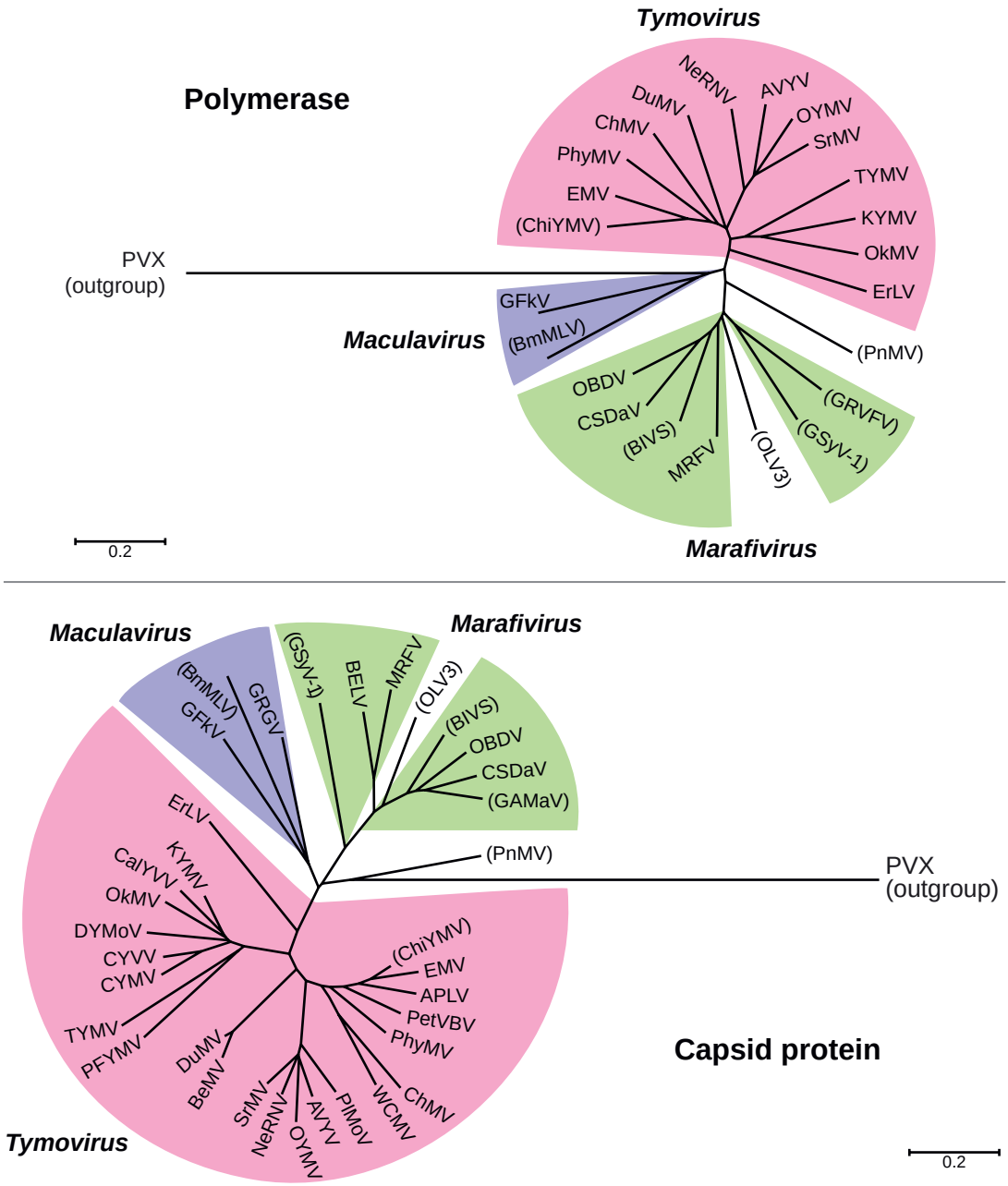


**Figure 5:** Phylogenetic tree showing the relationship between the species and genera of the family *Tymoviridae* based on the RdRp (top) and CP (bottom) sequences. The abbreviations and the sequence accession numbers are indicated in the lists of species. The tree was produced using the neighbor-joining method in MEGA4 and is drawn to scale. Names enclosed in parentheses denote viruses that have not yet been approved as species belonging to the *Tymoviridae*. Note that OLV-3, which has sequences related to the marafiviruses, possesses a maculavirus-like genome organization, and is therefore likely to be proposed as an unassigned species in the family *Tymoviridae*.

## Similarity with other taxa

The replication polyproteins are members of the "alphavirus-like" supergroup of RNA viruses and are most closely related to those of the other families in the order, namely *Alphaflexiviridae*, *Betaflexiviridae* and *Gammaflexiviridae*.

Pos. ssRNA

## Derivation of names

*Macula*: from *macula*, Latin for "fleck".
*Marafi*: from *ma*ize *ra*yado *fi*no virus.
*Tymo*: from *t*urnip *y*ellow *mo*saic virus.

## Further reading

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## Supplementary reading

A supplementary list of notable *Tymoviridae* papers is available online on Science Direct®, www.sciencedirect.com.

## Contributed by

Dreher, T.W., Edwards, M.C., Gibbs, A.J., Haenni, A.-L., Hammond, R.W., Jupin, I., Koenig, R., Sabanadzovic, S. and Martelli, G.P.

# Family *Astroviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Astroviridae* |
| Genus | *Avastrovirus* |
| Genus | *Mamastrovirus* |

## Virion properties

### Morphology

Virions shed in feces are 28–30 nm in diameter, spherical in shape and non-enveloped. A distinctive five- or six-pointed star is discernible on the surface of about 10% of virions. Virions derived from cell culture are up to 41 nm in diameter, with well-defined surface spikes (Figure 1).

### Physicochemical and physical properties

Virion $M_r$ is about $8 \times 10^6$, $S_{20,w}$ is about 160S. Virion buoyant density in CsCl is 1.35–1.39 g cm$^{-3}$. Virions are resistant to pH 3, heating at 50 °C for 1 h or 60 °C for 5 min, chloroform, lipid solvents and non-ionic, anionic, and zwitterionic detergents.

### Nucleic acid

Virions contain one molecule of infectious, positive sense, ssRNA, 6.4–7.7 kb in size. A poly(A) tract is located after the 3′-NTR. The structure of the 5′ end of the genome is not known. A small protein with similarity to calicivirus and picornavirus VPg has been identified by sequence comparisons. No RNA helicase domain has been described. Eighteen to twenty nucleotides preceding the ORF2 initiation codon are highly conserved among most astroviruses. The lengths of the NTRs at both ends of the genome vary by genus, between 11–85 and 59–305 at 5′ and 3′ ends, respectively.

### Proteins

In human astroviruses, intracellular particles are formed from the 87–90 kDa primary polyprotein product of ORF2. Purified particles and extracellular virions of HAstV-8 are assembled from a 70 kDa protein species that results from intracellular processing of the carboxy-terminal region of the 87–90 kDa protein by caspases. Particles composed of the 70 kDa protein are cleaved extracellularly by trypsin through a complex pathway resulting in highly-infectious particles with capsid proteins of 32–34, 27–29 and 25–26 kDa. In most other astroviruses, virion protein composition has not been established, and usually consists of three mature capsid species ranging between 24 and 39 kDa.

### Lipids

Virions do not contain a lipid envelope.

### Carbohydrates

None of the viral proteins are glycosylated.

## Genome organization and replication

The astrovirus genome is arranged in three ORFs: ORF1a and ORF1b at the 5′ end encoding the non-structural proteins, and ORF2 at the 3′ end encoding the structural proteins (Figures 2 and 3). The virion RNA is infectious and serves as a messenger RNA for the non-structural polyproteins, nsp1a and nsp1ab. A frame-shifting mechanism located between ORF1a and ORF1b is employed to translate the RNA-dependent RNA polymerase. A polyadenylated, sgRNA (ca. 2.8 kb) corresponding to ORF2 is detected in the cytoplasm of infected cells. Viral RNA replication is resistant to actinomycin D. For the best characterized human astroviruses, a full-length capsid polyprotein resulting from expression of ORF2 from the sgRNA forms particles in the cytoplasm of infected cells. Extracellular particles exposed to trypsin form three major capsid proteins with enhanced infectivity. There is evidence that astroviruses can undergo recombination.

## Antigenic properties

Eight serotypes of the classical human astroviruses (HAstV) have been defined by immune electron microscopy and neutralization tests and have been confirmed by sequence comparisons, mainly

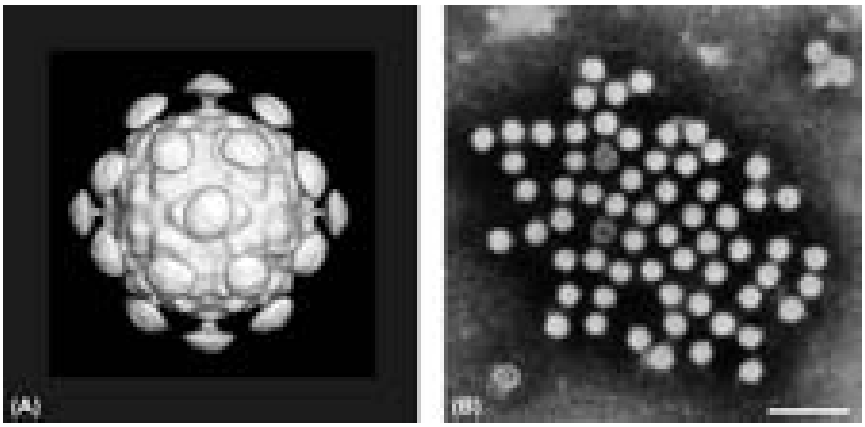

**Figure 1:** (A) Surface-shaded view of human astrovirus 1 (HAstV-1) reconstructed from 367 images recorded by electron cryo-microscopy and calculated at 30 Å resolution. The map displays a diameter of 440 Å and T = 3 icosahedral symmetry (courtesy of Dr M. Yeager). (B) Negative contrast electron micrograph of virions of human astrovirus from a stool specimen. The bar represents 100 nm.

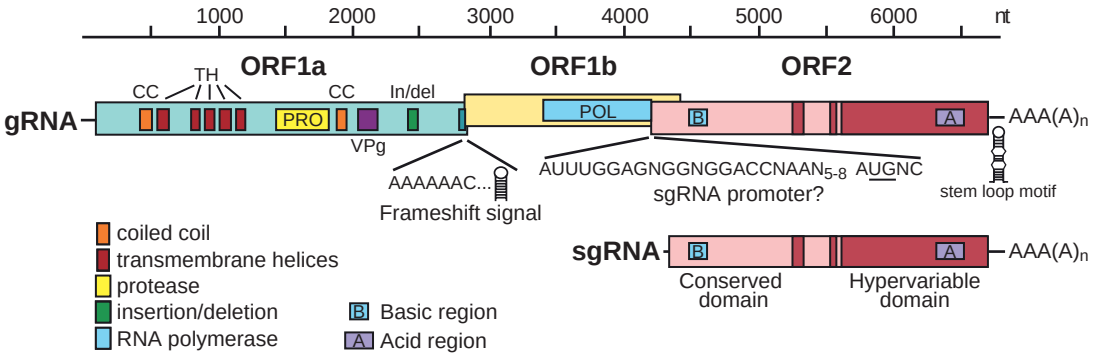


**Figure 2:** Organization of human astrovirus genomic RNA (gRNA) (HAstV-8). ORF 2 is expressed from a sgRNA detected in the cytoplasm of infected cells.

of the carboxy-terminal hypervariable domain of the structural protein encoded by ORF2. They share at least one common epitope recognized by a monoclonal antibody localized at the conserved amino-terminal domain. Neutralization epitopes have been mapped to the 26 and 29 kDa capsid proteins of HAstV-1 and HAstV-2. Two distinct serotypes of bovine astrovirus, two of turkey astrovirus 2 (TAstV-2), two of avian nephritis virus (ANV) and two of chicken astrovirus (CAstV) have been defined by neutralization. Since 2008, several novel human astroviruses have been identified (MLB1 and MLB2; VA1, VA2, and VA3; HMO-A, HMO-B and HMO-C), but there is little information on the antigenic properties of these strains.

## Biological properties

Astroviruses have been isolated from stools from a wide variety of mammals and birds (humans, cats, cattle, deer, dogs, ducks, mice, pigs, sheep, mink, turkeys, chickens, bats, cheetahs, guinea fowl, rats and marine mammals), mostly associated with gastroenteritis in young individuals. Besides enteric disease, astrovirus infections have been associated with fatal hepatitis in ducks, and interstitial nephritis causing growth retardation in young chicken. Transmission is by the fecal–oral route and no intermediate vectors have been described. Astrovirus pathogenesis appears to vary by genus. It has been shown in cell monolayers that astrovirus-induced permeability occurs independently of viral replication and is modulated by the capsid protein. Certain strains of HAstV induce an apoptotic response and activation of caspases, whose activity is required for processing of the capsid precursor, and for virus release. HAstV-1 capsid protein has also been demonstrated to bind

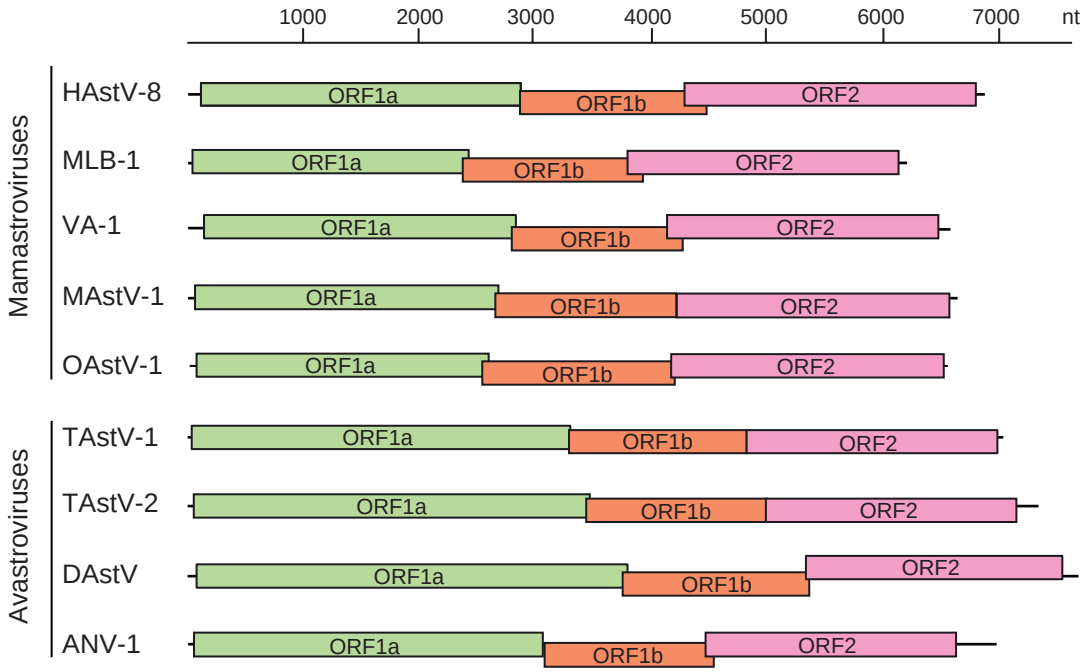


**Figure 3:** Genome organization of selected strains of astroviruses belonging to different host species.

complement proteins C1q and MBL thus inhibiting activation of the classical and lectin pathways of the human complement system, respectively.

## GENUS *AVASTROVIRUS*

Type species ***Turkey astrovirus***

### Distinguishing features

Members of the genus *Avastrovirus* infect birds.

### Biological properties

Infection with *Avastrovirus* species often involves extra-intestinal manifestations (e.g. damage to liver, kidney, or the immune system). Duck astrovirus (DAstV) causes an often-fatal hepatitis in ducklings. Astroviruses infecting turkeys (TAstV-1, TAstV-2 and ANV), guinea fowl (TAstV-2) and chickens (ANV and CAstV) affect multiple organs, including the kidney and thymus.

Duck astrovirus grows in embryonated hens' eggs following blind passage in the amniotic sac. Few infected embryos die in less than 7 days. Infected embryos appear stunted and have greenish, necrotic livers in which astrovirus particles have been identified. Certain strains of chicken astrovirus grow in LMH cells. The turkey astroviruses grow in embryonated turkey eggs following passage in the amniotic sac. Few infected embryos die. Infected embryos range from no signs of infection to severely enlarged, distended and fluid-filled intestines. Both the intestinal tissue and fluid contain infectious viral particles.

### Species demarcation criteria in the genus

The current avastrovirus species are based on the hosts from which they were isolated. As such, the species do not correspond to genetic phylogenies. The classification of avastrovirus species is

currently being redefined. Based on genetic analysis of the complete capsid region at the amino acid level, avian astroviruses will be divided into two main genogroups: genogroup I and genogroup II. Each genogroup includes astroviruses infecting different host species, and can be further subdivided into genotype species based on both genetic and host species criteria. Mean amino acid genetic distances (p-dist) between the two genogroups is 0.704 ± 0.013. Within each genogroup, amino acid genetic distances between genotypes range between 0.576 and 0.741.

### List of species in the genus *Avastrovirus*

| | | |
|---|---|---|
| *Chicken astrovirus* | | |
| Avian nephritis virus 1 | [AB033998] | (ANV-1) |
| Avian nephritis virus 2 | [AB046864*] | (ANV-2) |
| Avian nephritis virus 3 | [FJ940720*] | (ANV-3) |
| *Duck astrovirus* | | |
| Duck astrovirus C-NGB | [FJ434664] | (DAstV-C-NGB) |
| *Turkey astrovirus* | | |
| Turkey astrovirus 1 | [Y15936] | (TAstV-1) |

Species names are in italic script; names of isolates are in roman script. Sequence accessions [ ], and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

### List of other related viruses which may be members of the genus *Avastrovirus* but have not been approved as species

Once the classification of avastrovirus species is redefined based on genetic criteria applied to the complete capsid region, these related viruses may form a new genotype or may be assigned to previously described genotypes.

| | | |
|---|---|---|
| Turkey astrovirus 2 | [AF206663] | (TAstV-2) |
| Turkey astrovirus 3 | [AY769616*] | (TAstV-3) |
| Chicken astrovirus 2 | [DQ324850*] | (CAstV-2) |
| Chicken astrovirus 3 | | (CAstV-3) |
| Duck hepatitis virus 3 | [EU669004*] | (DHV-3) |

*Sequences do not comprise the complete genome.

## GENUS *MAMASTROVIRUS*

Type species ***Human astrovirus***

### Distinguishing features

Members of the genus *Mamastrovirus* infect mammals.

### Biological properties

The predominant feature of infection with mamastroviruses is gastroenteritis. In humans, astrovirus has been detected in duodenal biopsies in epithelial cells located in the lower part of villi. In experimentally infected sheep, astrovirus was found in the small intestine in the apical two-thirds of villi. In calves, astrovirus infection was localized to specialized M cells overlying the Peyer's patches. Human astroviruses are distributed worldwide and have been associated with 2–8% of acute, non-bacterial gastroenteritis in children. Astroviruses have also been associated with gastroenteritis outbreaks and with gastroenteritis in immunocompromised children and adults.

### Species demarcation criteria in the genus

The current mamastrovirus species are based on the hosts from which they were isolated. As such the species do not correspond to genetic phylogenies. The classification of mamastrovirus species is currently being redefined. Based on genetic analysis of the complete capsid region at the amino acid level, mammalian astroviruses will be divided into two main genogroups: genogroup I and genogroup II.

Each genogroup includes astroviruses infecting different host species, and can be further subdivided based on both genetic and host species criteria. Mean amino acid genetic distances (p-dist) between the two genogroups is 0.671 ± 0.016. Within each genogroup, amino acid genetic distances between genotypes range between 0.338 and 0.783. Serotypes within each genotype are defined on the basis of 20-fold, or greater, two-way cross-neutralization titers, and are given consecutive numbers.

## List of species in the genus *Mamastrovirus*

| | | |
|---|---|---|
| *Bovine astrovirus* | | |
| Bovine astrovirus 1 | | (BAstV-1) |
| Bovine astrovirus 2 | | (BAstV-2) |
| *Human astrovirus* | | |
| Human astrovirus 1 | [Z25771] | (HAstV-1) |
| Human astrovirus 2 | [L13745] | (HAstV-2) |
| Human astrovirus 3 | [AF141381] | (HAstV-3) |
| Human astrovirus 4 | [AY720891] | (HAstV-4) |
| Human astrovirus 5 | [DQ028633] | (HAstV-5) |
| Human astrovirus 6 | [GQ495608] | (HAstV-6) |
| Human astrovirus 7 | [Y08632*] | (HAstV-7) |
| Human astrovirus 8 | [AF260508] | (HAstV-8) |
| *Feline astrovirus* | | |
| Feline astrovirus 1 | [AF056197*] | (FAstV-1) |
| *Porcine astrovirus* | | |
| Porcine astrovirus 1 | [Y15938*] | (PAstV-1) |
| *Mink astrovirus* | | |
| Mink astrovirus 1 | [AY179509] | (MAstV-1) |
| *Ovine astrovirus* | | |
| Ovine astrovirus 1 (Sheep astrovirus) | [Y15937] | (OAstV-1) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

## List of other related viruses which may be members of the genus *Mamastrovirus* but have not been approved as species

Once the classification of avastrovirus species is redefined based on genetic criteria applied to the complete capsid region, these related viruses may form a new genotype or may be assigned to previously described genotypes.

| | | |
|---|---|---|
| Bat astrovirus Tm/Guangxi/LD71/2007 | [FJ571067*] | |
| Bat astrovirus MmAstV/HK/AFCD57/05 | [EU847144*] | |
| Bat astrovirus TmAstV/GX/LD77/07 | [FJ571066*] | |
| Bat astrovirus PaAstV/HK/AFCD11/05 | [EU847145*] | |
| Bat astrovirus HpAstV/GX/LC03/07 | [FJ571074*] | |
| Bat astrovirus HaAstV/GX/LS11/07 | [FJ571068*] | |
| Bat astrovirus MpAstV/HK/AFCD337/06 | [EU847155*] | |
| Bat astrovirus TmAstV/GX/LD38/07 | [FJ571065*] | |
| Bat astrovirus TmAstV/GX/LD04/07 | [FJ571069*] | |
| Bat astrovirus TmAstV/GX/LD27/07 | [FJ571070*] | |
| Bat astrovirus TmAstV/GX/DX19/07 | [FJ571071*] | |
| Bat astrovirus TmAstV/GX/LD45/07 | [FJ571072*] | |
| Bat astrovirus TmAstV/GX/LD54/07 | [FJ571073*] | |
| Bottlenose dolphin astrovirus 1 | [FJ890355*] | (BdAstV-1) |
| California sea lion astrovirus 1 | [FJ890351*] | (CslAstV-1) |
| California sea lion astrovirus 2 | [FJ890352*] | (CslAstV-2) |
| California sea lion astrovirus 3 | [FJ890353*] | (CslAstV-3) |
| Canine astrovirus 1 | [FM213330-2*] | (CaAstV-1) |
| Cheetah astrovirus | [EU650331-2*] | (ChAstV) |
| Capreolus capreolus astrovirus 1 | [HM447045*] | (CcAst-1) |
| Capreolus capreolus astrovirus 2 | [HM447046*] | (CcAst-2) |
| Human astrovirus MLB1 | [FJ402983] | (HAstV-MLB1) |

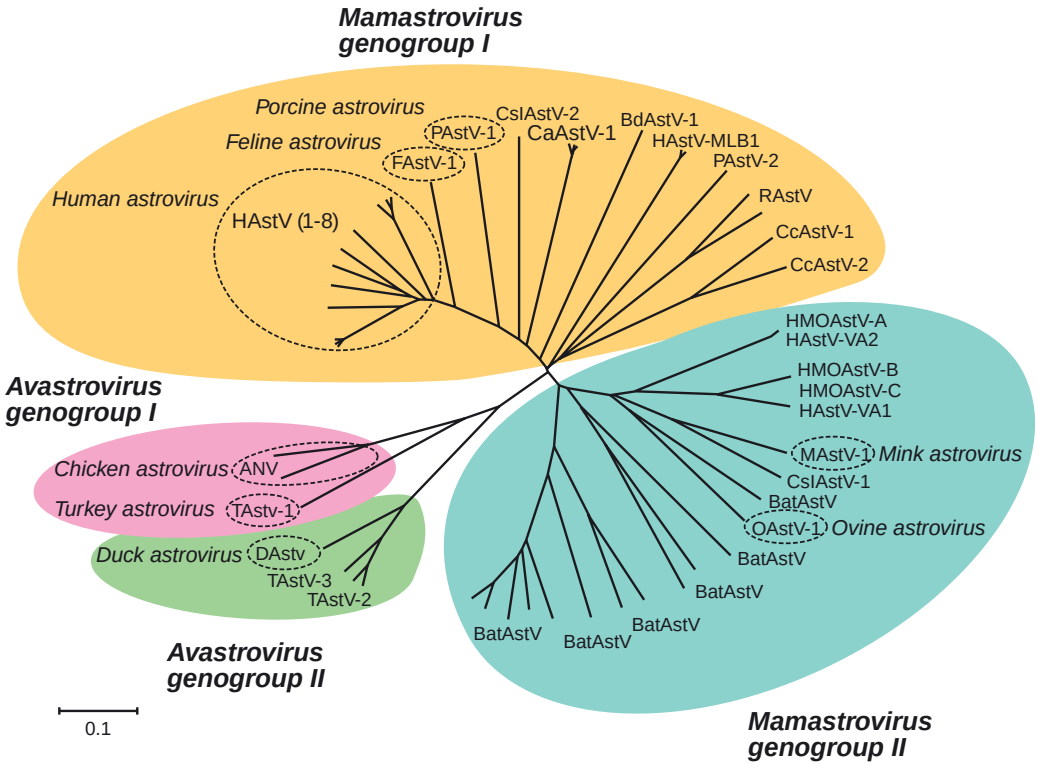


**Figure 4:** Phylogenetic relationships within the family *Astroviridae*. The predicted amino acid sequences of the entire capsid polyprotein were aligned using CLUSTALW2. The phylogenetic tree was generated using the neighbor-joining algorithm implemented in the MEGA4 program.

| | | |
|---|---|---|
| Human astrovirus MLB2 | [GQ502192*] | (HAstV-MLB2) |
| Human HMO astrovirus A | [GQ415660] | (HMOAstV-A) |
| (Human astrovirus VA2) | [GQ502193*] | (HAstV-VA2) |
| Human HMO astrovirus B | [GQ415661*] | (HMOAstV-B) |
| Human HMO astrovirus C | [GQ415662*] | (HMOAstV-C) |
| (Human astrovirus VA1) | [FJ973620] | (HAstV-VA1) |
| Porcine astrovirus 2 | [GU562296*] | (PAstV-2) |
| Rat astrovirus | [HM450381, HM450382] | (RAstV) |
| Steller sea lion astrovirus 1 | [FJ890354*] | (SslAstV-1) |

*Sequences do not comprise the complete genome.

## List of unassigned species in the family *Astroviridae*

None.

## Phylogenetic relationships within the family

Phylogenetic relationships within the family are depicted in Figure 4.

## Similarity with other taxa

None reported.

## Derivation of names

*Astro*: from Greek *astron*, "star", representing the star-like surface structure on virions.
*Av*: from Latin *avis*, "bird", representing avian host species.
*Mam*: from Latin *mamma*, "breast", representing mammalian host species.

## Further reading

### Journals and books

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The Astrovirus Pages: http://www.iah-virus.org/astroviridae/

## Contributed by

Bosch, A., Guix, S., Krishna, N.K., Méndez, E., Monroe, S.S., Pantin-Jackwood, M. and Schultz-Cherry, S.

# Family *Barnaviridae*

## Taxonomic structure of the family

Family *Barnaviridae*
Genus *Barnavirus*

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Barnavirus*

Type species *Mushroom bacilliform virus*

## Virion properties

### Morphology

Virions are bacilliform, non-enveloped and lack prominent surface projections. Typically, virions are 19 × 50 nm, but range between 18 and 20 nm in width and 48 and 53 nm in length (Figure 1). Optical diffraction patterns of the virions resemble those of virions of Alfalfa mosaic virus, suggesting a morphological subunit diameter of about 10 nm and a T = 1 icosahedral symmetry.

### Physicochemical and physical properties

Virion Mr is $7.1 \times 10^6$, buoyant density in $Cs_2SO_4$ is 1.32 g cm$^{-3}$. Virions are stable between pH 6 and 8 and ionic strength of 0.01 to 0.1M phosphate, and are insensitive to chloroform.

### Nucleic acid

Virions contain a single linear molecule of a positive sense ssRNA, 4.0 kb in size. The complete 4009 nt sequence of mushroom bacilliform virus (MBV) is available. The RNA has a linked VPg and appears to lack a poly(A) tail. RNA constitutes about 20% of virion weight.

### Proteins

Virions contain a single major CP of 21.9 kDa. There are probably 240 molecules in each capsid.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

The RNA genome (4009 nt) contains four major and three minor ORFs and has 5′- and 3′-UTRs of 60 nt and 250 nt, respectively. ORFs 1 to 4 encode polypeptides of 20, 73, 47 and 22 kDa, respectively. The deduced aa sequence of ORF2 contains putative serine protease motifs related to chymotrypsin. ORF3 encodes a putative RdRp and ORF4 encodes the CP. ORFs 5 to 7 encode 8, 6.5 and 6 kDa polypeptides, respectively. The polypeptides potentially encoded by ORFs 1, 5, 6 and 7 show no homology to known polypeptides (Figure 2).

In a cell-free system, genomic length RNA directs the synthesis of a 21 kDA and a 77 kDa polypeptide and several minor polypeptides of 18–60 kDa. The full-length genomic RNA and a sgRNA (0.9 kb) encoding ORF4 (CP) are found in infected cells. Virions accumulate singly or as aggregates in the cytoplasm.

## Antigenic properties

Virions are highly immunogenic.

Pos. ssRNA

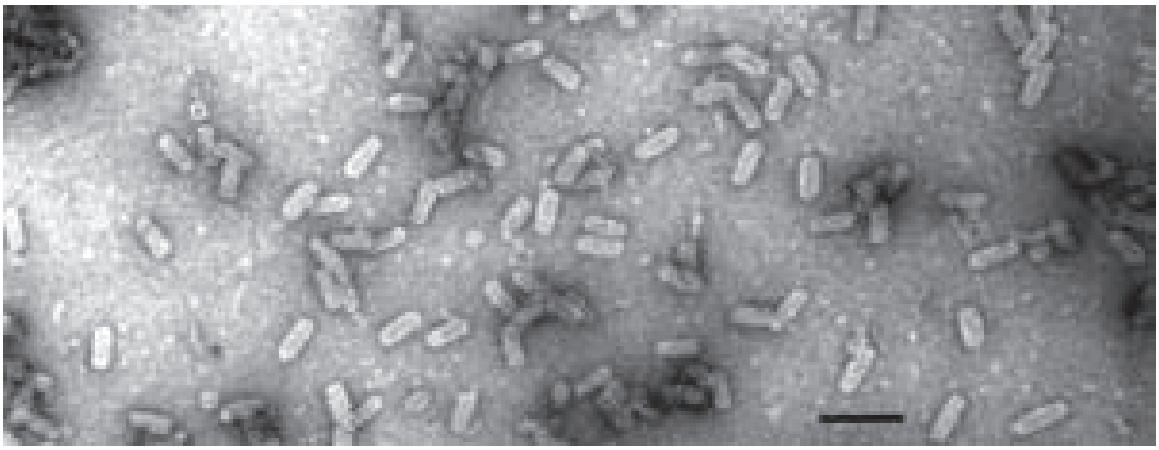

**Figure 1:** Negative contrast electron micrograph of particles of an isolate of Mushroom bacilliform virus. The bar represents 100 nm.

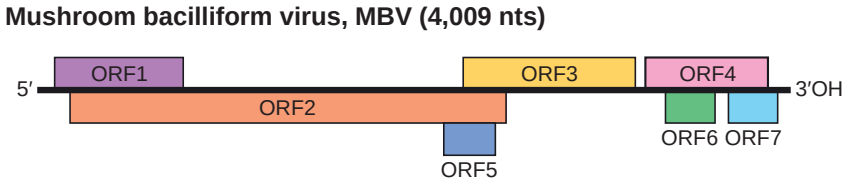


**Figure 2:** Genome organization of Mushroom bacilliform virus.

## Biological properties

The virus infects the common cultivated button mushroom (*Agaricus bisporus*). Bacilliform particles, which are morphologically similar to MBV, have been observed in the field mushroom *A. campestris*. Transmission is horizontal via mycelium and possibly basidiospores. Distribution of MBV coincides with that of the commercial cultivation of *A. bisporus*; the virus has been reported to occur in most major mushroom-growing countries. MBV is capable of autonomous replication, but commonly occurs as a double infection with a dsRNA virus (LaFrance isometric virus, LFIV) in mushrooms afflicted with La France disease. MBV is not required in pathogenesis involving LFIV, but it remains to be determined if it is a second, minor causal agent of LaFrance disease, the etiologic agent of an unrecognized pathology or benign. MBV RNA and LFIV dsRNA do not share sequence homology.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Barnavirus*

*Mushroom bacilliform virus*
Mushroom bacilliform virus-AUS LF-1 [U07551] (MBV- LF1)

Species names are in italic script; isolate names are in roman script. Sequence accession numbers and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Barnavirus* but have not been approved as species

None reported.

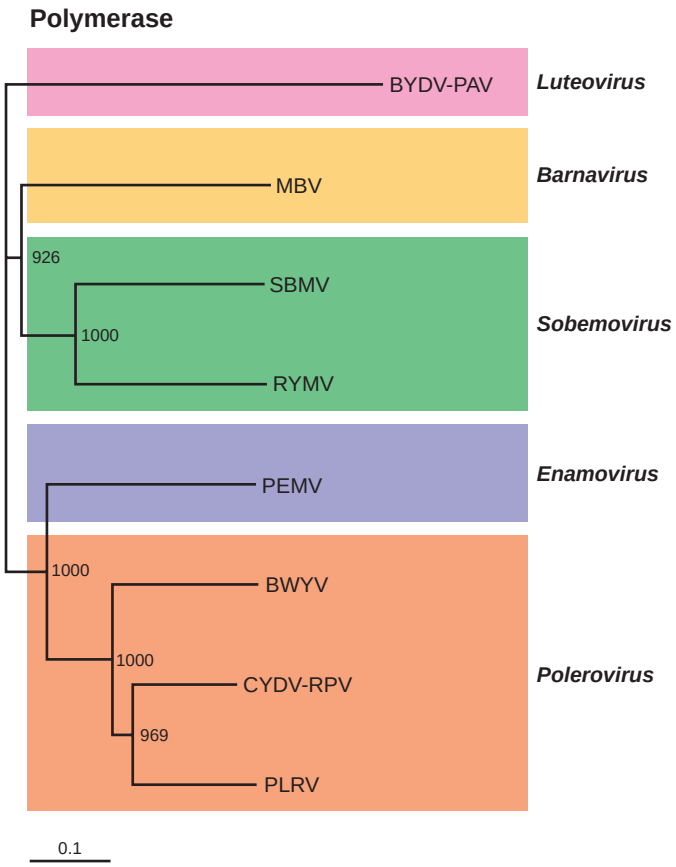


**Figure 3:** An unrooted neighbour-joining dendrogram (generated in Phylip) showing comparison of the aa sequence of the putative Mushroom bacilliform virus RdRp with those of selected sobemoviruses and poleroviruses, and an enamovirus. The luteovirus BYDV-PAV was included as an outgroup. Amino acid sequences were aligned with Clustal X and the tree was generated with Treeview. Bootstrap values (1000 replicates) are indicated. CYDV-RPV = cereal yellow dwarf virus-RPV; SBMV = southern bean mosaic virus; RYMV = rice yellow mottle virus; PEMV = pea enation mosaic virus-1; BWYV = beet western yellows virus; PLRV = potato leafroll virus. Accession numbers are AF218798 (BYDV-PAV), U07551 (MBV), AF055887 (SBMV), L20893 (RYMV), L04573 (PEMV) X13063 (BWYV), AF020090 (CYDV-RPV), AY138970 (PLRV).

## Phylogenetic relationships within the family

Not applicable.

## Similarity with other taxa

The amino acid sequences of the putative chymotrypsin-related serine protease and RdRp suggest an evolutionary relationship with some ssRNA positive sense plant viruses, particularly poleroviruses, sobemoviruses and enamoviruses (Figure 3).

## Derivation of name

*Barna*: from *ba*cilliform-shaped *RNA* viruses.

## Further reading

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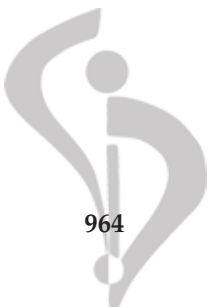
## Contributed by

Revill, P.A.



# Family *Bromoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Bromoviridae*** |
| Genus | ***Alfamovirus*** |
| Genus | ***Anulavirus*** |
| Genus | ***Bromovirus*** |
| Genus | ***Cucumovirus*** |
| Genus | ***Ilarvirus*** |
| Genus | ***Oleavirus*** |

## Virion properties

### Morphology

Virions are either spherical or quasi-spherical (Figure 1), having T = 3 icosahedral symmetry, and a diameter of 26–35 nm (genera *Anulavirus*, *Bromovirus*, *Cucumovirus* and *Ilarvirus*) or bacilliform (genera *Alfamovirus*, *Ilarvirus* and *Oleavirus*) having (within a species) constant diameters of 18–26 nm and lengths from 30 to 85 nm. Genomic RNAs are packaged in separate virions that may also contain sgRNAs, defective RNAs or satellite RNAs.

### Physicochemical and physical properties

The Mr of the virions varies from 3.5–6.9 $\times 10^6$, depending on the nucleic acid content. Virion Mr is constant among members of the genera *Bromovirus*, *Cucumovirus* and some *Ilarvirus* members, but varies among the remaining members of the family. The buoyant density of formaldehyde-fixed virions ranges from 1.35 to 1.37 g cm$^{-3}$ in CsCl. The $S_{20,w}$ varies from 63S to 99S. Virion integrity is dependent on RNA–protein interactions and virion RNA is susceptible to RNAse degradation *in situ* at neutral pH. Heat inactivation occurs at 60 °C in some genera, others have not been tested. In some cases, virions are unstable in the presence of divalent cations. Virions are generally stable in the presence of chloroform, but not in the presence of phenol. Virions are unstable in the presence of strong anionic detergents such as SDS, but can tolerate low concentrations of mild detergents such as Triton X-100.

### Nucleic acid

Total genome length is approximately 8 kb. Genomes consist of three linear, positive sense ssRNAs with 5′-terminal cap structures. The 3′ termini are not polyadenylated, but generally are highly conserved within a species or isolate, and form strong secondary structures. They are either tRNA-like and can be aminoacylated (genera *Bromovirus* and *Cucumovirus*) or form other structures that are not aminoacylated (genera *Alfamovirus*, *Anulavirus*, *Ilarvirus* and *Oleavirus*) (Table 1).

### Proteins

A single 20–24 kDa CP is expressed from a sgRNA. The CP is generally required for systemic movement and may be required for cell-to-cell spread in some cases.

### Lipids

There are no lipids associated with the virions.

### Carbohydrates

There are no carbohydrates associated with the virions.

## Genome organization and replication

RNA-1, -2, and -3 can act as mRNAs. The genomic RNA-1 and -2 each encodes a single large ORF. These proteins (1a and 2a) act with host factors as the viral replicase. In the genus *Cucumovirus* and in some members of the genus *Ilarvirus* (subgroups 1 and 2 only) a smaller 2b protein is expressed from an additional sgRNA that may or may not be encapsidated. These 2b proteins are involved in cell-to-cell movement and post-transcriptional gene silencing. The 3a protein is a movement protein and ORF3b encodes the coat protein, expressed from a sgRNA (Figure 2; Table 2).

There is no clear evidence of proteolytic or other post-translational processing. Virus replication occurs on cytoplasmic membranes via full length minus (−) strand synthesis and subsequent plus

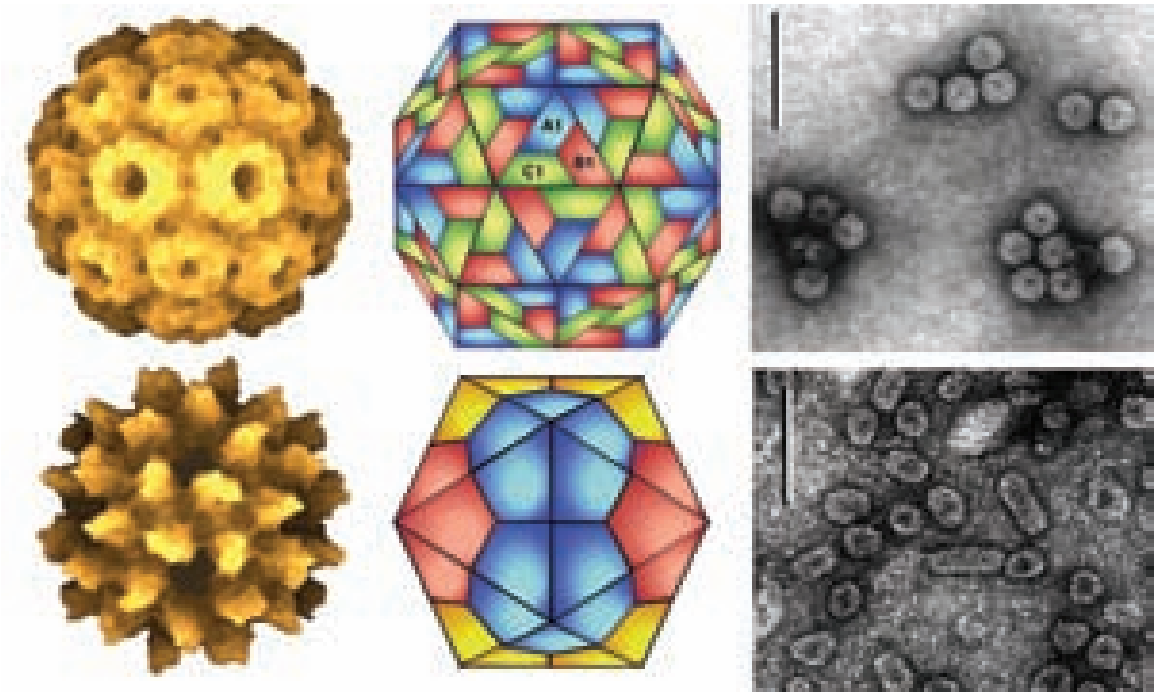


**Figure 1:** (Top left) Image reconstruction of a particle of cowpea chlorotic mottle virus (CCMV) (genus *Bromovirus*), showing pentamer and hexamer clustering in a T = 3 quasi-icosahedron (from Lucas *et al*. (2001). *J. Mol. Biol*., **317**, 95-108). (Top central) schematic representation of a T = 3 particle; (top right) negative contrast electron micrograph of particles of cucumber mosaic virus (CMV) (genus *Cucumovirus*) (courtesy of G. Kasdorf). (Bottom left) Electron density representation of a Ta particle of alfalfa mosaic virus (AMV) (genus *Alfamovirus*), showing T = 1 structure (from Kumar *et al*. (1997). *J. Virol*., **71**, 7911-7916). (Bottom center) Schematic representation of a T = 1 particle. (Bottom right) Negative contrast electron micrograph of particles of prune dwarf virus (PDV) (genus *Ilarvirus*) (courtesy of G. Kasdorf). The bars represent 100 nm.

**Table 1:** Genomic RNA sizes in nucleotide number for the type members of each genus

| Genus | Species | Strain | RNA-1 | RNA-2 | RNA-3 | 3′ term. | DIs/sat RNAs |
|---|---|---|---|---|---|---|---|
| *Alfamovirus* | AMV | 425 | 3,644 | 2,593 | 2,037 | Complex* | −/− |
| *Anulavirus* | PZSV | Tomato | 3,383 | 2,435 | 2,659 | Complex | −/− |
| *Bromovirus* | BMV | Russian | 3,234 | 2,865 | 2,117 | tRNA† | +/− |
| *Cucumovirus* | CMV | Fny | 3,357 | 3,050 | 2,216 | tRNA | +/+ |
| *Ilarvirus* | TSV | N.A. | 3,491 | 2,926 | 2,205 | Complex | −/− |
| *Oleavirus* | OLV-2 | N.A. | 3,126 | 2,734 | 2,438 | Complex | ?/? |

N.A.: not applicable, only one strain reported.
*Complex secondary structure.
†Aminoacylatable, with pseudoknot folding.

(+) strand synthesis. The sgRNAs are synthesized from the (−) template, and may or may not be found in the virions. The CP accumulates to high levels in infected cells, whereas the nonstructural proteins accumulate to much lower levels. Virions accumulate in the cytoplasm. The life cycle of the virus takes place predominantly in the cytoplasm.

## Antigenic properties

Native virions are generally poor immunogens and require stabilization with formaldehyde prior to use as antigens. There are few or no serological relationships between the genera, and weak relationships between species of the same genus.

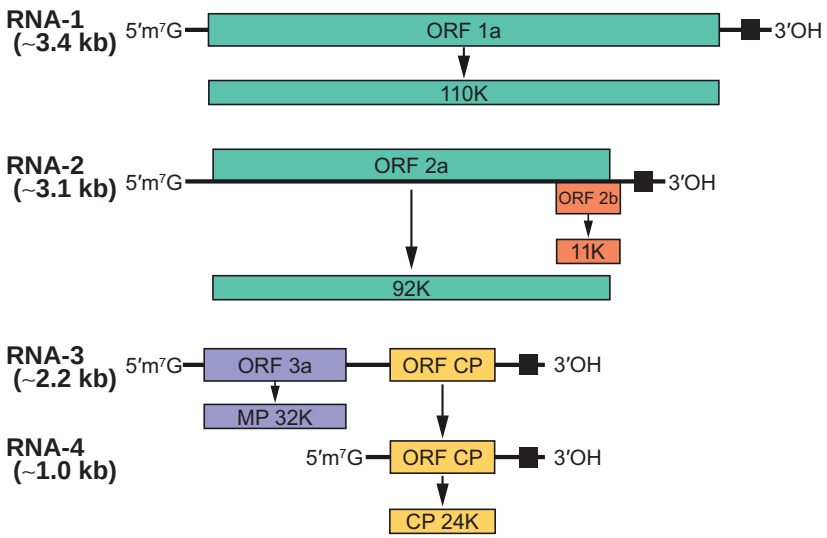



**Figure 2:** Schematic genome organization for members of the family *Bromoviridae*.

**Table 2:** Virus proteins, sizes and functional activities

| Protein | Size (kDa) | mRNA | Function* |
|---|---|---|---|
| 1a | 102.7–125.8 | RNA-1 | Mtr, helicase |
| 2a | 78.9–96.7 | RNA-2 | RdRp |
| 3a | 30.5–36.5 | RNA-3 | Cell to cell movement |
| CP | 19.8–26.2 | sgRNA-4† | Encapsidation, movement |

A 2b protein that is involved in cell-to-cell movement and post-transcriptional gene silencing is coded for by members of the genus *Cucumovirus* and some members of the genus *Ilarvirus* but is not found in other members of the genus *Ilarvirus* or in members of other genera within the family.
*Functions of 1a and 2a are putative in most cases, by analogy to related viruses.
†The sgRNA for the CP derived from RNA-3 is encapsidated in all but the genus *Oleavirus*.

## Biological properties

The natural host range of the viruses ranges from very narrow (genus *Bromovirus*) to extremely broad (genus *Cucumovirus*). They are predominantly transmitted by insects, in either a non-persistent manner or mechanically. Vectors have not been identified for some of the members of the family. They are distributed worldwide, and several are responsible for major disease epidemics in crop plants.

# Genus *Alfamovirus*

Type species *Alfalfa mosaic virus*

## Distinguishing features

Alfamoviruses are transmitted in a non-persistent manner by at least 14 aphid species. AMV shares many properties at the molecular level with members of the genus *Ilarvirus*. The CP is required to activate the genome, a feature shared with the ilarviruses. The CPs of AMV and ilarviruses can activate their respective genomes interchangeably.

## Virion properties

### Morphology

Virions are generally bacilliform, having a constant diameter of 18 nm, and varying from 30 to 57 nm in length, depending on the nucleic acid species encapsidated. The Mr values are from 3.5–6.9 $\times 10^6$. Virions can be separated into components on sucrose density gradients.

## Genome organization and replication

Replication is activated by CP binding to the complex 3′-end structure. CP from members of the genus *Ilarvirus* can also activate replication.

## Biological properties

The host range is very broad. The virus is transmitted by aphids in a non-persistent manner.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Alfamovirus*

| | | |
|---|---|---|
| *Alfalfa mosaic virus* | | |
| Alfalfa mosaic virus - 425 | RNA1:[L00163 = NC_001495]<br>RNA2:[X01572 = NC_002024]<br>RNA3:[K02703 = NC_002025] | (AMV-425) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Alfamovirus* but have not been approved as species

Cassava Ivorian bacilliform virus (CIBV)

# Genus *Anulavirus*

Type species ***Pelargonium zonate spot virus***

## Distinguishing features

The RNA 3 is slightly larger than the RNA 2, unlike other members of this family. The 2a protein is the smallest reported for viruses in the family (78.9 kDa).

## Virion properties

### Morphology

Virus particles are non-enveloped and quasi-spherical, with a diameter ranging from 25 to 35 nm, and have a poorly resolved surface structure.

### Physicochemical and physical properties

In sucrose density gradients, virions sediment as three components. In analytical centrifugation virions have single buoyant densities at equilibrium of 1.35 g cm$^{-3}$ in CsCl or 1.29 g cm$^{-3}$ in $Cs_2SO_4$.

## Genome organization and replication

Coat protein is not required for activation of the genome.

### Antigenic properties

Virions are weak immunogens and must be fixed before injection into rabbits to raise antibodies. Polyclonal antisera yield a single precipitin line in immuno-diffusion tests.

### Biological properties

The single member of the genus infects tomato and artichoke and is common in weeds (e.g. *Diplotaxis erucoides* where it is also seed-borne). The virus is present in/on the pollen that is carried on the bodies of thrips feeding on susceptible hosts. Infected cells have severe cytopathological alterations.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Anulavirus*

| | | |
|---|---|---|
| *Pelargonium zonate spot virus* | | |
| Pelargonium zonate spot virus - tomato | RNA1:[AJ272327 = NC_003649]<br>RNA2:[AJ272328 = NC_003650]<br>RNA3:[AJ272329 = NC_003651] | (PZSV-tomato) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Anulavirus* but have not been approved as species

None reported.

## GENUS *BROMOVIRUS*

Type species ***Brome mosaic virus***

### Distinguishing features

Beetle vectors are recorded for most bromoviruses but the efficiency of such transmission is low. A long-standing report of transmission by nematodes is regarded with suspicion.

### Virion properties

#### MORPHOLOGY

Virions are polyhedral, and all the same size, with a diameter of 27 nm.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virions prepared below pH 6.0 have $S_{20,w}$ of 88S, are stable to high salt and low detergent concentrations, and are nuclease- and protease-resistant. At pH 7.0 and above, virions swell to a diameter of 31 nm, $S_{20,w}$ decreases to 78S, salt and detergent stability decreases dramatically, and protein and RNA are susceptible to hydrolytic enzymes. This swelling is accompanied by conformational changes of the capsid that are detectable by physical and serological means.

#### NUCLEIC ACID

RNA 3′ termini are tRNA-like, are very similar in all viruses sequenced so far, and can be aminoacylated with tyrosine.

### Antigenic properties

All members are serologically related, with large antigenic differences between species.

## Biological properties

The natural host range is narrow, and is limited to a few plant hosts for each species. All species are thought to be beetle-transmitted, although BMV is inefficiently transmitted by aphids in a non-persistent manner.

## Species demarcation criteria in the genus

Criteria used for demarcation of species within the genus are:

- Host range
- Serological relationships
- Compatible replicase proteins (i.e. 1a and 2a proteins)
- Nucleotide sequence identity between species ranges from 50 to 80% depending on the gene used for comparison.

## List of species in the genus *Bromovirus*

| | | |
|---|---|---|
| *Broad bean mottle virus* | | |
| Broad bean mottle virus - Bawden | RNA1:[M65138 = NC_004008]<br>RNA2:[M64713 = NC_004007]<br>RNA3:[M60291 = NC_004006] | (BBMV-Bawden) |
| *Brome mosaic virus* | | |
| Brome mosaic virus - Russian | RNA1:[X02380 = NC_002026]<br>RNA2:[X01678 = NC_002027]<br>RNA3:[J02042 = NC_002028] | (BMV-RU) |
| *Cassia yellow blotch virus* | | |
| Cassia yellow blotch virus - KU1 | RNA1:[AB194806 = NC_006999]<br>RNA2:[AB194807 = NC_007000]<br>RNA3:[AB194808 = NC_007001] | (CYBV-KU1) |
| *Cowpea chlorotic mottle virus* | | |
| Cowpea chlorotic mottle virus – type | RNA1:[M65139 = NC_003543]<br>RNA2:[M28817 = NC_003541]<br>RNA3:[M28818 = NC_003542] | (CCMV-Type) |
| *Melandrium yellow fleck virus* | | |
| Melandrium yellow fleck virus - KU1 | RNA1:[AB444583 = NC_013266]<br>RNA2:[AB444584 = NC_013267]<br>RNA3:[AB444585 = NC_013268] | (MYFV –KU1) |
| *Spring beauty latent virus* | | |
| Spring beauty latent virus - KU1 | RNA1:[AB080598 = NC_004120]<br>RNA2:[AB080599 = NC_004121]<br>RNA3:[AB080600 = NC_004122] | (SBLV-KU1) |

Species names are in italic script; isolate and strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Bromovirus* but have not been approved as species

None reported.

# GENUS *CUCUMOVIRUS*

Type species ***Cucumber mosaic virus***

## Distinguishing features

Cucumoviruses are transmitted in a non-persistent manner by over 80 species of aphids in more than 30 genera. The RNA 2 is bistronic producing a 2b protein associated with the suppression of RNA interference.

## Virion properties

### Morphology

Virions are icosahedral, of uniform size and sedimentation properties. In electron micrographs they appear to have electron dense centers.

### Physicochemical and physical properties

Purified virions are labile, and are especially susceptible to anionic detergents and high ionic strength buffers that disrupt the RNA-protein interactions required for particle integrity. Most strains are unstable in the presence of $Mg^{2+}$, but at least one strain of cucumber mosaic virus (CMV) requires $Mg^{2+}$ for stability.

### Nucleic acid

The 3′ termini of all RNAs within a species are highly similar and can form tRNA-like structures that are aminoacylatable with tyrosine. Within a species, the 5′-NTR of RNA-1 and RNA-2 are also very similar. At least one strain of CMV can form defective RNAs that arise by deletions in the 3a ORF of RNA-3. Subgroup II strains of CMV encapsidate the sgRNA for the 2b ORF, called RNA-4a, and an additional small RNA of about 300 nt, called RNA-5, that is co-terminal with the 3′ ends of RNAs-3 and 4. In addition, CMV and peanut stunt virus (PSV) may harbor satellite RNAs of about 330 to 400 nt. The satellite RNAs are more common under experimental conditions than in field conditions, and may dramatically alter the symptoms of infection by the helper virus in certain hosts like tomato.

## Genome organization and replication

An additional ORF, the 2b ORF, is found in all cucumoviruses and has been shown to be active in CMV and tomato aspermy virus (TAV).

## Antigenic properties

CMV has been divided into two subgroups, based on serology. PSV also has more than one serological group. Sequence analysis has upheld the divisions, although the CMV subgroup I can be further divided into two groups by phylogenetic analyses.

## Biological properties

CMV has an extremely broad host range, infecting 85 distinct plant families, and up to 1000 species experimentally. The other cucumovirus species have narrower host ranges; PSV is largely limited to legumes and solanaceous hosts, and TAV predominantly infects composites and solanaceous plants. All species are transmitted by aphids in a non-persistent manner.

## Species demarcation criteria in the genus

Criteria used for demarcation of species within the genus are:

- Serological relatedness
- Compatibility of replicase proteins (1a and 2a proteins), but these distinctions may break down in the case of naturally occurring reassortants
- Sequence similarity.

Serology and nucleotide sequence similarity is used to distinguish subgroups within a species. Subgroups generally have at least 65% sequence identity.

## List of species in the genus *Cucumovirus*

*Cucumber mosaic virus*

| | | |
|---|---|---|
| Cucumber mosaic virus subgroup I strain Fny | RNA1:[D00356 = NC_002034]<br>RNA2:[D00355 = NC_002035]<br>RNA3:[D10538 = NC_001440] | (CMV-Fny) |

| | | |
|---|---|---|
| Cucumber mosaic virus subgroup II strain Q | RNA1:[X02733]<br>RNA2:[D00985]<br>RNA3:[M21464] | (CMV-Q) |
| *Peanut stunt virus*<br>(Robinia mosaic virus) | | |
| Peanut stunt virus - ER | RNA1:[U15728 = NC_002038]<br>RNA2:[U15729 = NC_002039]<br>RNA3:[U15730 = NC_002040] | (PSV-ER) |
| *Tomato aspermy virus*<br>(Chrysanthemum aspermy virus) | | |
| Tomato aspermy virus - V | RNA1:[D10044 = NC_003837]<br>RNA2:[D10663 = NC_003838]<br>RNA3:[AJ277268 = NC_003836] | (TAV-V) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Cucumovirus* but have not been approved as species

| | | |
|---|---|---|
| Gay feather mild mottle virus | RNA1:[FM881899 = NC_012134]<br>RNA2:[FM881900 = NC_012135]<br>RNA3:[FM881901 = NC_012136] | (GMMV) |

## Genus *Ilarvirus*

Type species ***Tobacco streak virus***

### Distinguishing features

Ilarviruses have been demonstrated to be transmitted mechanically by thrips feeding on pollen grains containing the virus. The RNA-2 of members of subgroups 1 and 2 is bicistronic producing a 2b protein which is inferred, because of functional homology, to serve the same function as the 2b protein of cucumoviruses.

### Virion properties

#### Morphology

Virions are quasi-isometric or occasionally bacilliform, and are about 30 nm in diameter.

#### Nucleic acid

There is a short region of sequence similarity at the 3′ ends of the RNAs.

### Genome organization and replication

The CP is required for activation of replication, but may be substituted with CP from alfalfa mosaic virus (genus *Alfamovirus*).

### Antigenic properties

Virions are "unpromising subjects for the raising of good antisera". Subgroups within the genus were based on the presence of serological relationships among some, but not all, members of each subgroup. Sequence data have supported many of the serological relationships but also show potential relationships among viruses not previously demonstrated as related. Some serological relationships have been reported between viruses in different subgroups and might be associated with conserved aa and secondary structures.

### Biological properties

The viruses mainly infect woody plants. Viruses are present in/on the pollen and may be transmitted when wind-blown pollen and populations of vector thrips are coincident on a susceptible host.

## Species demarcation criteria in the genus

Criteria used for demarcation of species within the genus are:

- Serology
- Host range
- Sequence similarity (Specific levels of sequence similarity have not been defined).

Ilarviruses were initially grouped on the basis of serological relationships. Sequence data have confirmed some of these groupings but have also shown that some species were grouped inappropriately. Currently accepted groupings of the viruses are indicated in the species list.

## List of species in the genus *Ilarvirus*

**Subgroup 1**

*Parietaria mottle virus*

| | | |
|---|---|---|
| Parietaria mottle virus – Caciagli | RNA1:[AY496068 = NC_005848]<br>RNA2:[AY496069 = NC_005849]<br>RNA3:[U35145 = NC_005854] | (PMoV - Caciagli) |

*Tobacco streak virus*

| | | |
|---|---|---|
| Tobacco streak virus – WC | RNA1:[U80934 = NC_003844]<br>RNA2:[U75538 = NC_003845]<br>RNA3:[X00435 = NC_002028] | (TSV-WC) |

**Subgroup 2**

*Asparagus virus 2*

| | | |
|---|---|---|
| Asparagus virus 2 - Mink | RNA1:[EU919666 = NC_011808]<br>RNA2:[EU919667 = NC_011809]<br>RNA3:[X86352 = NC_011807] | (AV-2 - Mink) |

*Citrus leaf rugose virus*

| | | |
|---|---|---|
| Citrus leaf rugose virus – Garnsey | RNA1:[U23715 = NC_003548]<br>RNA2:[U17726 = NC_003547]<br>RNA3:[U17390 = NC_003546] | (CiLRV - Garnsey) |

*Citrus variegation virus*

| | | |
|---|---|---|
| Citrus variegation virus - Garnsey | RNA1:[EF584664 = NC_009537]<br>RNA2:[EF584865 = NC_009538]<br>RNA3:[U17389 = NC_09536] | (CVV - Garnsey) |

*Elm mottle virus*
(Hydrangea mosaic virus)

| | | |
|---|---|---|
| Elm mottle virus - Jones | RNA1:[U57047 = NC_003569]<br>RNA2:[U34050 = NC_003568]<br>RNA3:[U85399 = NC_003570] | (EMoV - Jones) |

*Lilac ring mottle virus*

| | | |
|---|---|---|
| Lilac ring mottle virus - Netherlands | RNA1:[EU919668]<br>RNA2:[EU919669]<br>RNA3:[U17391] | (LiRMoV<br>-Netherlands) |

*Spinach latent virus*

| | | |
|---|---|---|
| Spinach latent virus - Bos | RNA1:[U93192 = NC_003808]<br>RNA2:[U93193 = NC_003809]<br>RNA3:[U93194 = NC_003810] | (SpLV - Bos) |

| | | |
|---|---|---|
| *Tulare apple mosaic virus* | | |
| Tulare apple mosaic virus - Garnsey | RNA1:[AF226160 = NC_003833]<br>RNA2:[AF226161 = NC_003834]<br>RNA3:[AF226162 = NC_003835] | (TaMV - Garnsey) |
| **Subgroup 3** | | |
| *Apple mosaic virus* | | |
| Apple mosaic virus | RNA1:[AF174584 = NC_003464]<br>RNA2:[AF174585 = NC_003465]<br>RNA3:[U15608 = NC_003480] | (ApMV) |
| *Blueberry shock virus* | | |
| Blueberry shock virus | | (BlShV) |
| *Prunus necrotic ringspot virus* | | |
| Prunus necrotic ringspot virus | RNA1:[AF278534 = NC_004362]<br>RNA2:[AF278535 = NC_004363]<br>RNA3:[U57046 = NC_004364] | (PNRSV) |
| **Subgroup 4** | | |
| *Fragaria chiloensis latent virus* | | |
| Fragaria chiloensis latent virus – Tzanetakis | RNA1:[AY682102 = NC_006566]<br>RNA2:[AY707771 = NC_006567]<br>RNA3:[AY707772 = NC_006568] | (FCiLV-Tzanetakis) |
| *Prune dwarf virus* | | |
| Prune dwarf virus | RNA1:[U57648 = NC_008039]<br>RNA2:[AF277662 = NC_008037]<br>RNA3:[L28145 = NC_008038] | (PDV) |
| **No relationships to other existing groups** | | |
| *American plum line pattern virus* | | |
| American plum line pattern virus - Parrish | RNA1:[AF235033 = NC_003451]<br>RNA2:[AF235165 = NC_003452]<br>RNA3:[AF235166 = NC_003453] | (APLPV -Parrish) |
| *Humulus japonicus latent virus* | | |
| Humulus japonicus latent virus – Adams | RNA1:[AY500236 = NC_006064]<br>RNA2:[AY500237 = NC_006065]<br>RNA3:[AY500238 = NC_006066] | (HJLV - Adams) |

Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Ilarvirus* but have not been approved as species

The potential subgroups to which these viruses will be assigned, once complete information and necessary approval as new species has been completed, are indicated.

| | | |
|---|---|---|
| **Subgroup 1** | | |
| Bacopa chlorosis virus | RNA1:[FJ607140]<br>RNA2:[FJ607141]<br>RNA3:[FJ607142] | (BaCV) |
| Blackberry chlorotic ringspot virus | RNA1:[DQ091193 = NC_011553]<br>RNA2:[DQ091194 = NC_011554]<br>RNA3:[DQ091195 = NC_011555] | (BCRV) |
| Strawberry necrotic shock virus | RNA1:[DQ318818 = NC_008708]<br>RNA2:[AY743591 = NC_008707]<br>RNA3: [AY363228 = NC_008706] | (SNSV) |
| Tomato necrotic spot virus | RNA3:[FJ236810] | (ToNSV) |

| | | |
|---|---|---|
| **Subgroup 3** | | |
| Lilac leaf chlorosis virus | RNA2:[FN669168]<br>RNA3:[FN669169] | (LLCV) |
| **Subgroup 4** | | |
| Viola white distortion virus | RNA3:[GU168941] | (VWDV) |

# GENUS *OLEAVIRUS*

Type species ***Olive latent virus 2***

## Distinguishing features

A fourth RNA with no apparent messenger activity, and which is slightly smaller than the RNA-3, is encapsidated by the virus.

## Virion properties

### MORPHOLOGY

Virions have different shape and size, ranging from quasi-spherical with a diameter of 26 nm, to bacilliform with lengths of 37, 43, 38, and 55 nm, and diameters of 18 nm. Particles up to 85 nm in length occasionally are present.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

In sucrose gradients, virions sediment as five or six components.

### NUCLEIC ACID

Virion RNA differs in size from that of other members of the family, encapsidating the three genomic RNAs and a sgRNA of about 2 kb, that is apparently not an mRNA. The sgRNA for the CP ORF is not encapsidated. Three additional RNAs of 200 to 550 nt are also present in virions. The 5′ termini of the genomic RNAs are capped, but not the 5′ terminus of the encapsidated sgRNA. The 3′ termini of the RNAs are similar to those of the genera *Alfamovirus* and *Ilarvirus*, but do not interact with CP to activate replication.

## Antigenic properties

Virions are efficient immunogens.

## Biological properties

The only known host is olive (*Olea europaea*), which is infected asymptomatically. The virus is transmitted by inoculations, but no insect vector is known.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Oleavirus*

| | | |
|---|---|---|
| *Olive latent virus 2* | | |
| Olive latent virus 2 – Italy | RNA1:[X94346 = NC_003673]<br>RNA2:[X94347 = NC_003674]<br>RNA3:[X76993 = NC_003671] | (OLV-2-Italy) |

Species names are in italic script; isolate names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Oleavirus* but have not been approved as species

None reported.

## Phylogenetic relationships within the family

Relationships within this family have been examined using the sequence of full-length genomic molecules, genes and gene products. The three genera with multiple members (*Bromovirus*, *Cucumovirus* and *Ilarvirus*) form clades that are distinct. The genera *Anulavirus* and *Oleavirus* are unique and distinct from other genera within the family. A longstanding debate as to whether AMV (genus *Alfamovirus*) should be regarded as a member of the genus *Ilarvirus* has yet to be resolved. Ilarviruses share many features at the molecular level with AMV. The CP of AMV will activate replication of ilarviruses and *vice versa*. The major difference between AMV and ilarviruses is in the mode of transmission. AMV is transmitted non-persistently by aphids and ilarviruses are transmitted through pollen and feeding of thrips. Studies defining recombination events in the genomes of members of the *Bromoviridae* suggest that AMV should be grouped with the ilarviruses.

## Similarity with other taxa

The viruses are members of the "alpha-like" supergroup, sharing sequence similarity in the 1a protein domains for Mtr and Hel activities, and in the 2a protein polymerase domain with members of the plant virus family *Virgaviridae* and order *Tymovirales*, and animal viruses in the family *Togaviridae*. The 3a proteins of bromoviruses and the 35 kDa protein of the members of the genus *Dianthovirus* (RCNMV) form a distinct "family" of movement-associated proteins. Raspberry bushy dwarf virus (RBDV) (genus *Idaeovirus*) is similar to bromoviruses in genome organization and in the sequence of certain genes. Like the members of the genus *Ilarvirus*, RBDV is transmitted in association with pollen.

## Derivation of names

*Alfamo*: from *alfa*lfa *mo*saic virus.
*Anula*: from Latin "*anular*" for the concentric symptom associated with infection by this virus.
*Bromo*: from *Bro*me *mo*saic, also, from *Bromus* (host of *Brome mosaic virus*).
*Cucumo*: from c*ucu*mber *mo*saic virus.
*Ilar*: from *i*sometric *la*bile *r*ingspot.
*Olea*: from the genus name of the host, olive (*Olea*).

## Further reading

See articles on this family and genera within the following sources:

*The Springer Index of Viruses* (Tidona, C.A. and Darai, G., Eds.), Springer Verlag, Berlin, 2001 and 2011.
*The Encyclopedia of Virology* (Mahey, B.W.J. and van Regenmortel, M.H.V., Eds.), Elsevier, Amsterdam, 2008.
*The Encyclopedia of Life Sciences*, Wiley Interscience, New York, 2010.
Codoñer, F.M. and Elena, S.F. (2008). The promiscuous evolutionary history of the *Bromoviridae*. *J Gen. Virol.*, **89**, 1739–1747.
Gallitelli, D., Finetti Sialer, M.M. and Martelli, G.P. (2005). Anulavirus a proposed new genus of plant viruses in the family Bromoviridae. *Arch. Virol.*, **150**, 407–411.
Martelli, G.P. and Grieco, F. (1997). *Oleavirus*, a new genus in the family *Bromoviridae*. *Arch. Virol.*, **142**, 1933–1936.
Palukaitis, P. and García-Arenal, F. (2003). Cucumoviruses. *Adv. Virus. Res*, **62**, 241–323.
Scott, S.W., Zimmerman, M.T. and Ge, X. (2003). Viruses in subgroup 2 of the genus Ilarvirus share both serological relationships and characteristics at the molecular level. *Arch. Virol*, **148**, 2063–2075.

## Contributed by

Bujarski, J., Figlerowicz, M., Gallitelli, D., Roossinck, M.J. and Scott, S.W.

# Family *Caliciviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Caliciviridae*** |
| Genus | ***Vesivirus*** |
| Genus | ***Lagovirus*** |
| Genus | ***Norovirus*** |
| Genus | ***Sapovirus*** |
| Genus | ***Nebovirus*** |

## Virion properties

### Morphology

Virions are non-enveloped with icosahedral symmetry. They are 27–40 nm in diameter by negative-stain electron microscopy and 35–40 nm by cryo-electron microscopy (Figure 1). The capsid is composed of 90 dimers of the major structural protein VP1 arranged on a T = 3 icosahedral lattice. In noroviruses, the VP1 forms a subunit comprised of a shell and two protruding domains (Figure 1). A characteristic feature of calicivirus capsid architecture is the 32 cup-shaped depressions at each of the icosahedral five-fold and three-fold axes. In negative-stain virus preparations, some cup-shaped depressions appear distinct and well defined, while in others these depressions are less prominent.

### Physicochemical and physical properties

Virion Mr is about $15 \times 10^6$. Virion buoyant density is 1.33–1.41 g cm$^{-3}$ in CsCl and 1.29 g cm$^{-3}$ in glycerol-potassium tartrate gradients. Virion $S_{20,w}$ is 170–187S. Physicochemical properties have been established for some members of the family. Generally, caliciviruses are stable in the environment and many strains are resistant to inactivation by heat and certain chemicals (ether, chloroform and mild detergents). Enteric caliciviruses are acid-stable.

### Nucleic acid

The genome consists of a linear ssRNA molecule of 7.4–8.3 kb. A protein (VPg, 10–15 kDa) is covalently attached to the 5′-terminus of the genomic RNA and the 3′-terminus is polyadenylated (Figure 2). Caliciviruses have conserved nucleotide motifs at the genomic 5′-terminus and at the junction of the coding sequences for the non-structural/structural proteins. A subgenomic RNA (sgRNA) of 2.2–2.4 kb is synthesized intracellularly and is apparently VPg-linked. The gene order for several caliciviruses was determined by transient expression and *in vitro* translation studies and cleavage mapping yielding at least six mature non-structural proteins, followed by two structural proteins (VP1 and VP2). All the non-structural proteins are encoded by a large polyprotein and expressed in the same order for each genus (Figure 2). In the case of lagoviruses, sapoviruses and noboviruses the open reading encoding the non-structural proteins is in-frame and contiguous with VP1.

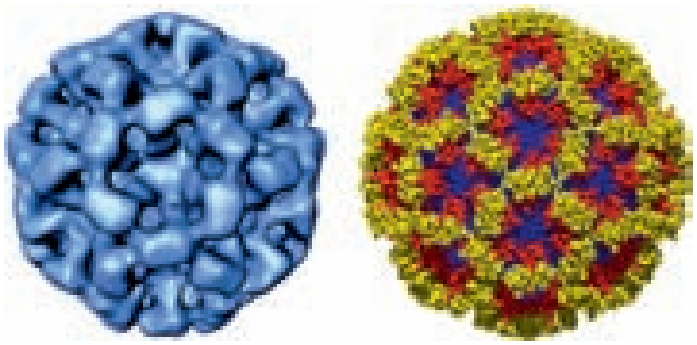


**Figure 1:** The structure of the calicivirus capsid exemplified by cryo-image reconstruction of recombinant Norwalk virus (NV)-like particles (rNV VLPs) (Left). X-ray structure of the Norwalk virus capsid (Right) with the Shell, Protruding 1, and Protruding 2 domains colored in blue, red and yellow, respectively. (Courtesy of B.V. Prasad.)

Pos. ssRNA

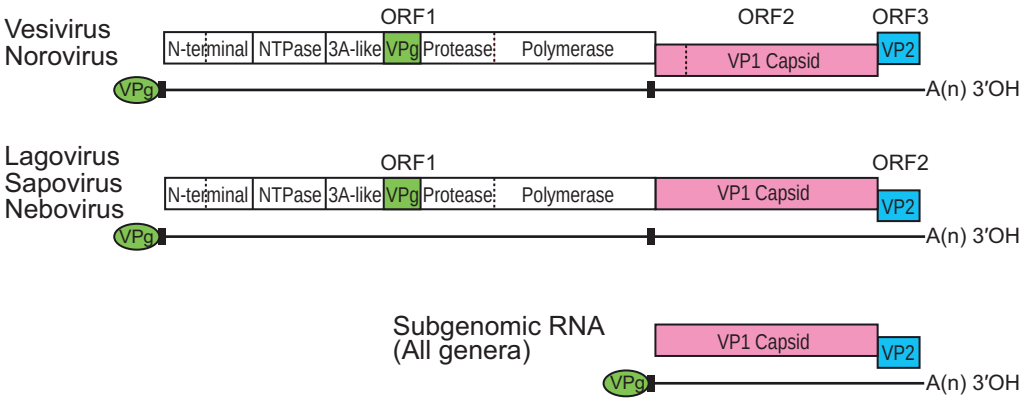



**Figure 2:** Reading frame usage and gene order in the *Caliciviridae*. Caliciviruses have a positive-strand RNA genome (7.4–8.3 kb), depicted by the horizontal black lines, which carry a protein (VPg) covalently linked to the 5′-terminus. The genome also contains a characteristic, short repeated sequence (▮) at the 5′-terminus and at the start of the VP1 gene. The genome (of each genus as indicated) is organized into at least two or three major ORFs as indicated. Structural genes are shown as pink (VP1) and blue (VP2) and the VPg gene is shown as green. In all genera, the structural proteins VP1 and VP2 are produced during replication from an abundant subgenomic RNA transcript that is co-terminal with the 3′-terminus of the genome. The ORF1 cleavage events mediated by the viral cysteine protease vary among the genera, but the gene order of the nonstructural proteins is conserved. Conserved cleavage sites are indicated with a solid vertical line; cleavage sites that vary in usage among genera are indicated with a broken vertical line. (Re-designed by Rachel Skilton.)

### Proteins

Virions are predominantly comprised from one major capsid protein, VP1 (58–60 kDa). A second minor structural protein named VP2 (8.5–23 kDa) has been found in association with FCV and RHDV virions, and Norwalk virus-like particles. Some non-structural proteins have sequence and motif homology with those of the family *Picornaviridae* replicative enzymes and include 2C (NTPase), 3C (cysteine protease) and 3D (RNA-dependent RNA polymerase) domains. The calicivirus VPg (10–15 kDa) is covalently linked to the viral RNA and maps to the region of the calicivirus genome analogous to the 3B region of picornaviruses, but without having an apparent amino acid homology with those of picornaviruses (Figure 2).

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

Caliciviruses have single stranded, positive sense genomic RNA (positive-strand RNA) organized into either two or three major ORFs (see genus descriptions). The non-structural proteins are encoded in the 5′-terminus of the genome and the structural proteins in the 3′-terminus (Figure 2). Replication occurs in the cytoplasm and two major positive-strand RNA species are found in infected cells: (1) the genome-sized, positive-strand RNA serves as the template for translation of a large polyprotein that undergoes cleavage by a virus-encoded protease to form the mature non-structural proteins and (2) a subgenomic-sized, positive-strand RNA, co-terminal with the 3′- terminus of the genome is the template for translation of the VP1 as well as the 3′-terminal ORF product VP2. A dsRNA corresponding in size to full-length genomic RNA has been identified in FCV and San Miguel sea lion virus (SMSV)-infected cells, indicating that replication occurs via a negative-strand intermediate.

## Antigenic properties

Taking each genus in turn, the prototype virus for the vesiviruses is vesicular exanthema of swine virus (VESV) isolated from pigs and 13 serotypes are recognized by serum neutralization assays. Following the isolation of SMSV, which is the progenitor of VESV, additional vesivirus serotypes

were recovered from at least 28 marine and other species. These viruses (now more than 40 serotypes) have been grouped together phylogenetically as the "marine vesiviruses". FCV represents the second species within the vesiviruses. Although initial studies of FCV indicated there were multiple serotypes, examinations of large collections of viruses and feline antisera obtained from experimental infections in specific pathogen-free cats led to recognition of extensive cross-reactions and a consensus that FCV represents a single serotype, albeit with antigenic variants.

Within the lagoviruses, cross-challenge studies in the natural host and experiments with monoclonal antibodies indicate that RHDV and European brown hare syndrome virus (EBHSV) are antigenically distinct.

Antigenic types have been defined by cross-challenge studies, immune electron microscopy or solid phase immune electron microscopy for non-cultivatable viruses in the genera *Norovirus* and *Sapovirus*. By contrast, viruses in the *Nebovirus* genus appear to fall into a single, closely related antigenic grouping.

For some non-cultivable viruses that are members of the genera *Lagovirus*, *Norovirus* and *Sapovirus*, recombinant virus-like particles (rVLPs) can be produced by expression of VP1 in mammalian, insect and plant systems. These VLPs are highly immunogenic and similar in antigenicity to native virions and they are useful to study the structure and antigenicity of virus particles.

## Biological properties

Caliciviruses have been isolated from a broad range of vertebrates. Except for some vesiviruses, individual caliciviruses generally exhibit a natural host restriction and this has presented a considerable problem with studying specific viruses for which there is no productive cell culture system, e.g. human noroviruses. However, at least one example virus from each genus can now be cultivated except for the neboviruses.

The marine vesiviruses have both a broad host range and a wide cell tropism. The diversity in host species of the marine vesiviruses was not initially recognized. For example, the VESVs isolated from swine from 1932 through 1956 were considered swine viruses and those subsequently isolated from ocean or other sources were either designated SMSVs or named after the original host species from which they were first isolated (e.g. walrus, cetacean, reptile). All these marine vesiviruses including those isolated from bystander host species are closely related genetically and generally lack host specificity.

Transmission of caliciviruses is via direct contact with an infected host or indirectly via contact with faecal material, vomitus or respiratory secretions, contaminated food, water and fomites. In general, no biologic vectors appear to be involved in transmission, however, mechanical, arthropod vector transmission of RHDV and VESV has been described.

Caliciviruses are associated with a number of disease syndromes. Marine vesiviruses (e.g. VESV) produced clinical signs in swine that were indistinguishable from foot-and-mouth disease, and include vesicles in the mouth, tongue, lips, snout and feet at the coronary band and between the digits. In addition, the virus may cause encephalitis, myocarditis, fever, diarrhoea, abortion, hepatitis, pneumonia and hemorrhage and failure of recovered animals to thrive. FCV in cats is predominantly associated with oral ulceration and rhinitis, and causes persistent infections. Recently, a more severe disease termed virulent-systemic feline calicivirus disease has been recognized, associated with epithelial cytolysis and systemic vascular compromise in susceptible cats, leading to severe oedema and high mortality.

In the genus *Lagovirus*, RHDV is associated with a generalized viraemic infection in which there is massive liver necrosis that triggers a disseminated intravascular coagulation and rapid death in rabbits greater than three months of age. Non-virulent viruses related to RHDV have been described. EBHSV is similar to RHDV but appears to be less virulent.

Human caliciviruses in the genera *Norovirus* and *Sapovirus* induce a generally self-limiting gastroenteritis. Vomiting is a consistent and prominent symptom; other symptoms may include nausea, diarrhoea, abdominal cramping, fever and malaise. Prolonged disease is seen in immunocompromised individuals. Neboviruses cause diarrhoea and mild upper intestinal lesions in calves.

# GENUS *VESIVIRUS*

Type species ***Vesicular exanthema of swine virus***

## Distinguishing features

The species in this genus form a distinct clade within the family (Figure 3). The genome is organized into three major ORFs. ORF1 encodes the non-structural polyproteins. ORF2 encodes the major structural capsid protein that is translated as a larger precursor protein before cleavage into the mature VP1, a feature that appears unique to this genus. ORF1 and ORF2 of the viruses in this genus are separated by either 2 nt (GC for FCV strains) or 5 nt (CCACT/C for marine vesiviruses). A third ORF (ORF3) encodes VP2 a small, basic protein and overlaps by one nt with ORF2 in a −1 frameshift. The ORF3 product has been detected in FCV-infected cells and is essential for infectious virus production.

## Biological properties

Most members of this genus can be readily propagated in cell culture. FCV grows most efficiently in cells of feline origin; *in vivo*, the primary site of replication is the upper respiratory tract. Primary isolation of most marine vesiviruses is possible in African green monkey kidney or porcine kidney cells. When tested approximately half of marine vesiviruses have produced skin vesicles in swine, horses and sometimes cattle and vesicles have occurred naturally in dogs, pinnipeds, cetaceans and primates (including man).

## Species demarcation criteria in the genus

Members of a *Vesivirus* species share a major phylogenetic branch within the genus and a common genome organization, however, demarcation into two species is on the basis of phylogenetic differences, subtle variation in genome structure and host range.

The virus VESV $A_{48}$ is the prototype virus of the genus *Vesivirus*. This virus and those isolated from marine species are phylogenetically grouped together and colloquially referred to as "marine vesiviruses". Feline calicivirus (FCV) is also recognized as a separate species within the genus. A collection of representative vesivirus isolates from which some sequences have been used to generate Figure 3 are shown in the table below.

## List of species in the genus *Vesivirus*

| | | |
|---|---|---|
| *Feline calicivirus* | | |
| Feline calicivirus [CFI/68] | [U13992] | (FCV) |
| *Vesicular exanthema of swine virus* | | |
| Vesicular exanthema of swine virus A48 [Fontana/48] | [U76874] | (VESV-A48) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table with more complete list of virus isolates can be found online on Science Direct®, www.sciencedirect.com.

## List of other related viruses which may be members of the genus *Vesivirus* but have not been approved as species

| | | |
|---|---|---|
| Canine calicivirus [No. 48] | [AB070225] | (CaCV) |
| 2117 CHOcalicivirus | [AY343325] | (2117) |
| Mink calicivirus [MV-20] | [AF338407] | (MCV) |

# Genus *Lagovirus*

Type species *Rabbit hemorrhagic disease virus*

## Distinguishing features

The strains in this genus form a distinct clade within the family. The genome is organized into two major ORFs. ORF1 encodes the non-structural polyprotein, with the major structural protein gene (VP1) in frame with the non-structural polyprotein coding sequence. The translated region of ORF2 overlaps ORF1 by 5 nt in the RHDV genome and 5 nt in the EBHSV genomes. The ORF2 encodes a small protein (VP2) of unknown function that has been identified as a minor structural component in the RHDV virion.

## Biological properties

These viruses have characteristically been associated with infection in rabbits and hares (lagomorphs), and can cause epidemics with high mortality in these animals. There is no productive continuous cell culture system for lagoviruses but recently infection of a rabbit kidney cell line with RHDV has been reported.

## Species demarcation criteria in the genus

Members of a *Lagovirus* species, either RHDV or EBHSV, share a major phylogenetic branch within the genus, common genome organization, natural host range and cross-protection antigens. Examples of lagoviruses are shown in Table 3.

## List of species in the genus *Lagovirus*

| | | |
|---|---|---|
| *European brown hare syndrome virus* | | |
| EBHSV [FRG] | [U09199] | (EBHSV-FRG) |
| *Rabbit hemorrhagic disease virus* | | |
| RHDV [FRG/89] | [M67473] | (RHDV-FRG) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
Full table with more complete list of virus isolates can be found online on Science Direct®, www.sciencedirect.com.

## List of other related viruses which may be members of the genus *Lagovirus* but have not been approved as species

None reported.

# Genus *Norovirus*

Type species *Norwalk virus*

## Distinguishing features

The strains in this genus form a distinct clade within the family. The full-length genome sequence is available for many strains (100+). The first complete genomes to be sequenced were for Norwalk virus, Southampton virus and Lordsdale virus and showed that the genome is organized into three major ORFs. ORF1 encodes the non-structural polyprotein. ORF2 encodes the major structural capsid protein (VP1) and overlaps by 14 nt with ORF1 in the Norwalk and Southampton virus strains and by 17 nt in the Lordsdale virus strain, resulting in a −2 frameshift of ORF2 in all three viruses. ORF3 overlaps by one nt with ORF2 in a −1 frameshift and encodes a small virion-associated protein (VP2). Detailed phylogenetic analysis indicated there are at least five "genogroups" (labelled GI to GV). Examples of the key viruses that form the genogroups are shown in Figure 3 and are listed below. Genogroup I (GI) is made up of eight genotypes, GII contains 19 genotypes, GIII and GIV each have two genotypes and GV has only one.

### Biological properties

Noroviruses have been detected in several mammalian species. Human noroviruses belong to GI, GII and GIV. Porcine (GII), bovine and ovine noroviruses (GIII) have been described and murine noroviruses currently constitute GV. However, serological evidence suggests other rodent noroviruses exist and thus viruses in GV are likely not to be exclusively murine in origin. At present only murine noroviruses can be grown in cell culture. Other mammalian species can be experimentally infected with human noroviruses. Furthermore, noroviruses closely related to human noroviruses have been identified from other mammalian species, but it is not clear whether these are the natural hosts or bystanders that have become naturally infected. Recombination between viruses in the same genotype and between genogroups occurs.

### Species demarcation criteria in the genus

Not applicable as only one species is currently recognized. However, the range of host species and the nature and extent of diversity of the noroviruses will need further characterization to delineate species criteria.

### List of species in the genus *Norovirus*

There is only one species, *Norwalk virus*, however, representatives of each genogroup are shown below:

| | | |
|---|---|---|
| *Norwalk virus* | | |
| GI [Hu/Norwalk virus 8FiiA/68/US] | [M87661] | (GI-NV) |
| GII [Hu/Hawaii virus/1971/US] | [U07611] | (GII-HV) |
| GIII [Bo/Jena/1980/GE] | [AJ011099] | (GIII-Jena) |
| GIV [Hu/Alphatron/98-2/1998/NL] | [AF195847] | (GIV-Alphatron) |
| GV [Mu/Murine norovirus 1/2002/US] | [AY228235] | (GV-MNV1) |

Species names are in italic script. Names of genogroups (G) followed by representative virus designations are in roman script. Host abbreviations are: Hu, human: Bo, bovine. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed. Full table with more complete list of virus isolates can be found online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Norovirus* but have not been approved as species

None reported.

## GENUS *SAPOVIRUS*

Type species ***Sapporo virus***

### Distinguishing features

The viruses in this genus form a distinct clade within the family. The first full-length genomic sequence available was for the human virus Manchester virus and subsequently for a range of viruses including the "porcine enteric calicivirus" (PEC strain Cowden). The Manchester virus genome is organized into three ORFs, two with known function. ORF1 encodes the non-structural polyprotein together with the major structural capsid protein gene (VP1) in frame with the non-structural polyprotein coding sequence. In PEC, ORF2 overlaps ORF1 in a −1 frameshift and encodes a predicted small protein likely the equivalent of VP2 in noroviruses. ORF3 begins 11 nt downstream from the predicted start codon of the VP1 in a +1 frameshift and encodes a predicted protein of ~160 aa. In some strains of this genus, and in the PEC Cowden strain, ORF3 is absent. Viruses in the genus are highly heterogeneous and can be divided on the basis of phylogenetic analysis of VP1 into five genogroups; GI, GII, GIV and GV contain human sapoviruses whereas porcine sapoviruses are found only in GIII (Figure 3). Each sapovirus genogroup can be subdivided into numerous genotypes. Examples of sapoviruses representing the genogroups are shown below.

### Biological properties

None of the human sapoviruses can be propagated in cell culture. However, PEC can be grown in porcine kidney cells as long as the media is supplemented with bile salts to induce intracellular signals to permit virus replication. Sapovirus infection most commonly occurs in children under the age of 5 years and is especially characterized by outbreaks in closed settings. However, recent molecular epidemiological studies have suggested that the number of outbreaks in which individuals of all age groups are infected may be increasing. Expression of VP1 in recombinant systems usually results in the production of VLPs. Antiserum raised against these VLPs and cross-reactivity studies using these VLPs and antiserum suggests that the antigenic differences amongst sapoviruses mirror the phylogenetic differences observed by genogrouping. Inter-genogroup and intra-genogroup recombination has been documented.

### List of species in the genus *Sapovirus*

The prototype strain for the species is the Sapporo virus, however, there is only a partial genome sequence available for this virus. Other representative viruses (one for each genogroup) are shown below.

| | | |
|---|---|---|
| *Sapporo virus* | | |
| GI [Hu/Sapporo/1982/JP] | [U65427] | (GI-SV) |
| GI [Hu/Manchester/1993/UK] | [X86560] | (GI-Man) |
| GII [Hu/Bristol/1998/UK] | [AJ249939] | (GII-Bristol) |
| GIII [Po/PEC Cowden] | [AF182760] | (GIII-Cowden) |
| GIV [Hu/Hou7-1181/1990/US] | [AF435814] | (GIV-Houston) |
| GV [Hu/Arg39/1995/ARG] | [AY289803] | (GV-Arg39) |

Species names are in italic script. Names of genogroups (G) followed by representative virus designations are in roman script. Host abbreviations are: Hu, human; Po, swine. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed. The genome sequence for Sapporo virus is only partial, therefore the first complete sapovirus genome sequence is for Manchester virus.
Full table with more complete list of virus isolates can be found online on Science Direct®, www.sciencedirect.com.

### List of other related viruses which may be members of the genus *Sapovirus* but have not been approved as species

None reported.

## GENUS *NEBOVIRUS*

Type species ***Newbury-1 virus***

### Distinguishing features

The viruses in this genus have been detected only in calves thus far and they form a distinct clade within the family. The complete genome sequence is available for several strains including Newbury "agent"-1, the prototype virus from the UK, and BEC-NB (Nebraska) virus described in the USA. The nebovirus genome is organized into two major ORFs. ORF1 encodes the non-structural polyprotein, with the major structural capsid protein (VP1) gene in frame with the non-structural polyprotein. VP2 is frameshifted +2 and begins 2 nts after the termination of ORF1.

### Biological properties

Neboviruses have been known since the 1980s but constitute the most recently defined calicivirus genus. They cause endemic diarrhoeal disease in calves and the severity of disease in experimentally infected calves is considered equal or worse than that caused by genogroup 3 bovine noroviruses. Neboviruses cannot be grown in cell culture and therefore information on their biological properties is limited. There appear to be two phylogenetically distinct polymerase types but the VP1 sequence is conserved between the prototype viruses, Newbury agent −1 and BEC NB virus. This suggests that, like other enteric caliciviruses, neboviruses may also undergo recombination.

## Species demarcation criteria in the genus

Not applicable as only one species is currently recognized. Additional characterization of the viruses in this genus will be required in order to delineate species criteria.

## List of species in the genus *Nebovirus*

| | | |
|---|---|---|
| *Newbury-1 virus* | | |
| Newbury-1 virus UK-1976 | [DQ013304] | (Newbury1) |
| Bovine calicivirus CV23-OH | [AY082890] | (CV23-OH) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Nebovirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Caliciviridae*

None reported.

## List of other related viruses which may be members of the family *Caliciviridae* but have not been approved as species

In 2008, a positive-strand RNA virus (Tulane virus) was isolated from stool samples taken from rhesus macaques held in captivity. The genome sequence and organisation (three ORFs) of this virus show the characteristic structure of caliciviruses (as defined in Figure 2) although with 6714 nt it is some 600 nt shorter than previously described calicivirus genomes. Whilst Tulane virions share similar morphology and density (1.7 g $cm^{-3}$) to other caliciviruses, phylogenetic analysis of the Tulane virus genome placed it on a deeply rooted branch separate from the other calicivirus genera. Although no disease entity has been associated with the Tulane virus, it appears to be an enteric virus and is cultivatable in a continuous macaque kidney cell line. The genus name Recovirus (for *r*hesus *e*nteric *c*alicivirus) has been proposed within the scientific literature with Tulane virus as the prototype.

In 2009 a report described a novel calicivirus isolated from pigs in Quebec, Canada. Genomic analysis revealed a positive sense genome of 6409 nt encoding two major ORFs. Phylogenetic analysis showed that these viruses form a unique cluster with a common root with the noroviruses and the Tulane virus. The genus name Valovirus was proposed with the St-Valérien virus as the prototype.

In October 2010 the complete genome sequence of a chicken calicivirus was submitted to the public databases. Analysis of this sequence suggests that it may form a novel calicivirus species and genus.

| | | |
|---|---|---|
| Tulane virus | [EU391643] | (TulaV) |
| St-Valérien virus | [FJ355928] | (ValoV) |
| Chicken calicivirus | [HQ010042] | (ChCV) |

## Phylogenetic relationships within the family

There are five recognized genera within the family *Caliciviridae*. The phylogenetic relationships amongst them are summarized in Figure 3.

## Similarity with other taxa

Caliciviruses have some properties similar to the viruses in the order *Picornavirales* and family *Potyviridae* in that they possess a genome-linked protein, VPg, at the 5′-terminus and a poly(A) tract at the 3′-terminus of the genome. The viral RNA-dependent RNA polymerase and the protease of caliciviruses share sequence homology with the picornaviruses.

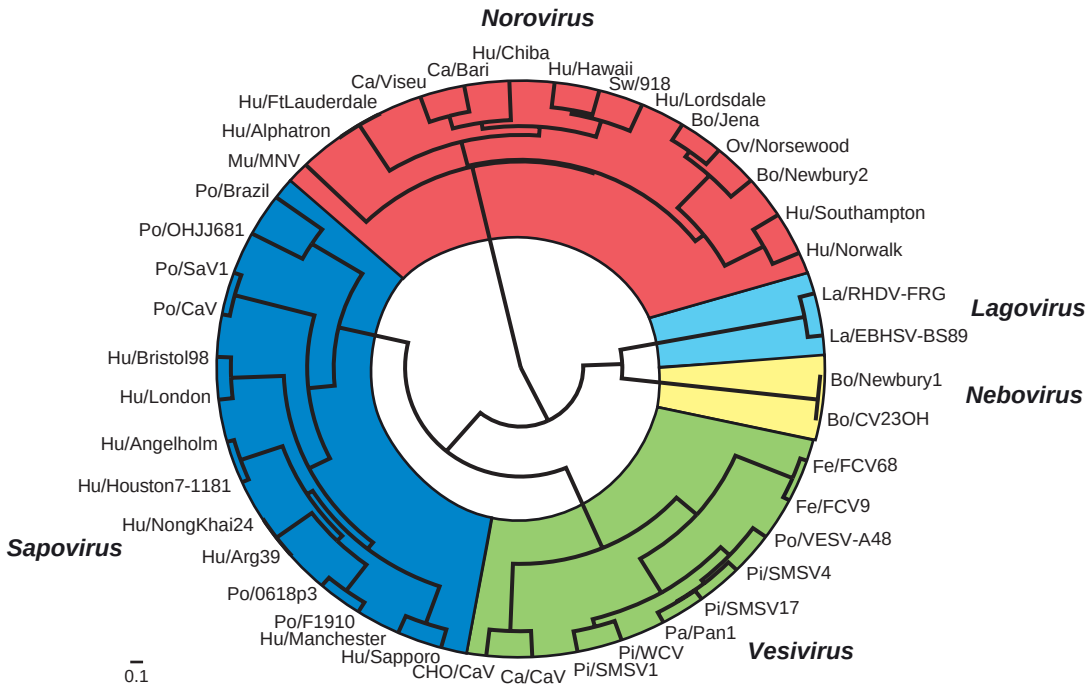



**Figure 3:** Phylogenetic relationships in the family *Caliciviridae*. The noroviruses are highlighted in red, lagoviruses in light blue, nebo viruses in yellow, vesiviruses in green and sapoviruses in blue. Two prototype viruses were included to represent each genogroup, For some genera, additional strains were included to demonstrate circulation in different species (e.g., Po/Sw917 in norovirus GII). Genera are defined by amino acid p-distance of up to 0.7, genera cut-offs were expanded to include all representative genogroups within a genus. The amino acids of full length VP1 capsid sequences were aligned in SeaView 4.2 using MUSCLE with a gap cost of −5 and neighbor-joining clustering method for the 1st and 2nd iterations. Bayesian phylogenetic analysis was run on BEAST without a molecular clock for $10^6$ iterations using the WAG amino acid substitution and Yule speciation tree model. Parameters and length of the run was verified by using Tracer, part of the BEAST suite of programs. All genera are supported by posterior distribution value of 1.0 (100% of sampled trees). The scale bar represents amino acid substitutions per site. (Courtesy of Everardo Vega.)

## Derivation of names

*Calici*: from Latin *calix*, "cup" or "goblet", from cup-shaped depressions on the virion surface observed by electron microscopy.
*Vesi*: from the type species name *vesi*cular exanthema of swine virus.
*Lago*: from *Lago*morpha, the mammalian host order for the prototype strain rabbit haemorrhagic disease virus.
*Noro*: modified from the type species name Norwalk virus.
*Sapo*: modified from the type species name Sapporo virus.
*Nebo*: derived by using the first two letters from *Ne*wbury and *Ne*braska, the first two isolates to be studied together with *bo* for bovine the only host species from which these viruses have been described.

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## Contributed by

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# Family *Closteroviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Closteroviridae* |
| Genus | *Closterovirus* |
| Genus | *Ampelovirus* |
| Genus | *Crinivirus* |

## Virion properties

### Morphology

Virions are helically constructed filaments with a pitch of the primary helix in the range of 3.4–3.8 nm, containing about 10 protein subunits per turn of the helix and showing a central hole of 3–4 nm (Figure 1 top). The very flexuous and open structure of the particles is the most conspicuous trait of members of the family. Virions have a diameter of about 12 nm and lengths ranging from 650 nm (species with fragmented genome) to over 2000 nm (species with monopartite genome). The fragility of virions and a tendency to end-to-end aggregation contribute to the fact that a range of lengths is often given for single viruses. Two types of coat proteins, the major CP and a CP analog, or minor CP (CPm), are the most abundant protein components involved in the formation of (most) closterovirid particles. CPm encapsidates the 600–700 5′-terminal nucleotides of the viral RNA, coating one extremity (75–100 nm) of the closterovirid particle, as shown for isolates of beet yellows virus (BYV), carrot yellow leaf virus (CYLV), citrus tristeza virus (CTV), grapevine leafroll-associated virus 2 (GLRaV-2) and lettuce infectious yellows virus (LIYV), thus forming a distinct structure for which the terms "rattlesnake", "heterodimeric" or "bipolar" have been coined (Figure 1 bottom).

### Physicochemical and physical properties

Virions usually sediment as a single band in sucrose or $Cs_2SO_4$ gradients. $S_{20,w}$ is around 130–140, buoyant density is 1.33 g cm$^{-3}$ in CsCl and 1.257 g cm$^{-3}$ in $Cs_2SO_4$. Virions of several species are degraded by CsCl and are unstable in high salt concentration, resist moderately high temperatures (thermal inactivation is around 45–55 °C) and organic solvents, but are sensitive to RNase and chelation.

### Nucleic acid

Regardless of the genome type, monopartite or fragmented, virions contain a single molecule of linear, positive sense, single stranded RNA, constituting 5–6% of the particle weight. Genome size is related to particle length, ranging from 13,000 to slightly over 19,000 nucleotides. The 5′ end of the genome is likely to be capped. The 3′ end is not polyadenylated and does not possess a tRNA-like structure, but has several hairpin structures and a putative pseudoknot essential for replication.

### Proteins

Structural proteins of most members of the family consist of a major CP and of a diverged copy of it denoted minor CP (CPm), with a size ranging from 22 to 46 kDa (CP) and 23 to 80 kDa (CPm), according to the individual species. A group of ampeloviruses with a small-sized genome (ca. 13,000 nt) apparently lacks a true CPm. With BYV, and presumably for most other members of the family, CPm is required for the assembly of the 5′-extremity of the virion, the protein of about 60 kDa is required for incorporation of both HSP70h and CPm to virions, which also incorporate a 20 kDa protein that may form the tip segment of the virion head.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

Members of the family have one of the largest genomes among plant viruses because of sequence duplication and acquisition of nonviral coding sequences (e.g., protease, HSP70 protein) via RNA

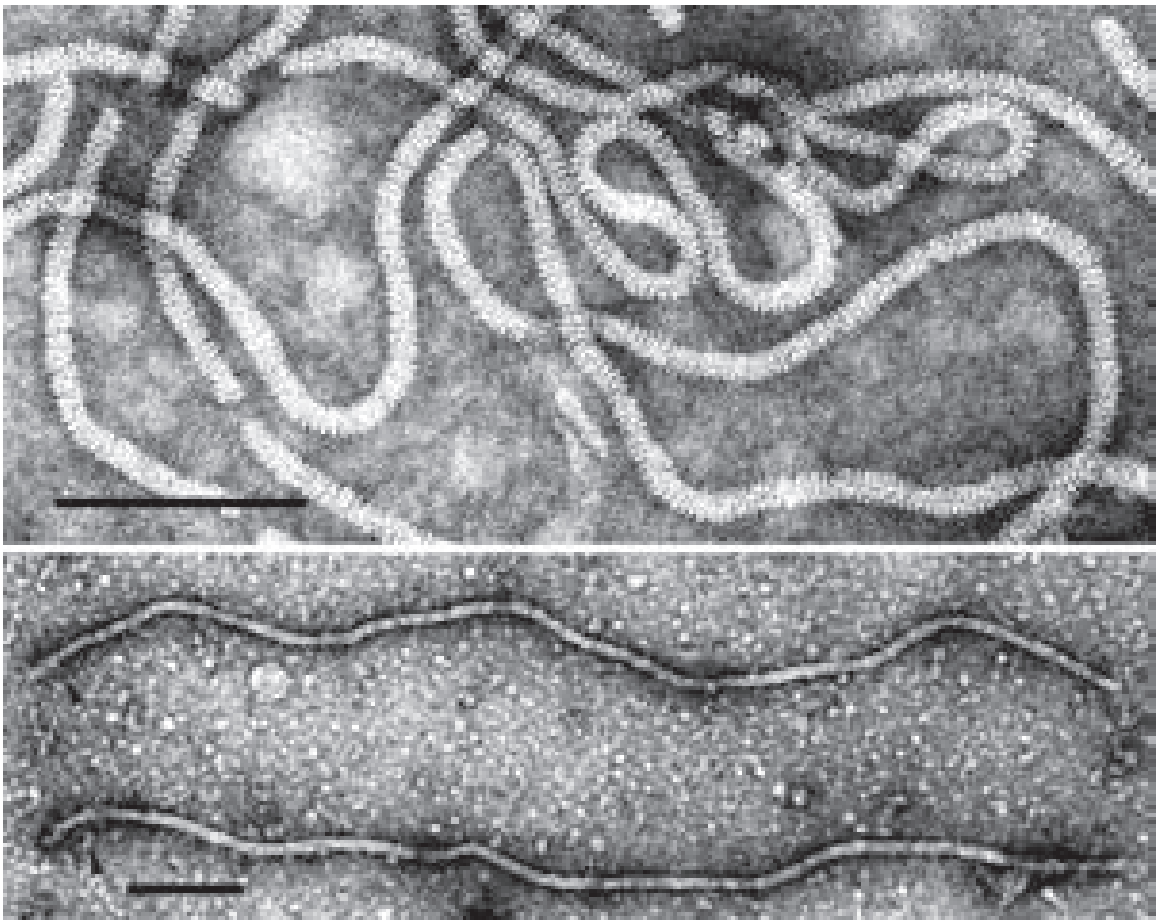

**Figure 1:** (Top) Negative contrast electron micrograph of virions of citrus tristeza virus (CTV) (genus *Closterovirus*). Particles of all members of the genera *Ampelovirus* and *Crinivirus* have a similar morphology. The bar represents 100 nm. (Courtesy of R.G. Milne.) (Bottom) Beet yellows virus (BYV) particles showing a decorated extremity (arrow heads) following exposure to an antiserum to the N-terminal peptide expressed from the minor CP gene. The bar represents 100 nm. (Courtesy of D.E. Lesemann.)

recombination. Recombination may also explain differences in genome organization between genera and members of the same genus. Genome organization, i.e. the number and relative position of the ORFs differs between the genera and/or individual viral species. However, the complex ORF1a–ORF1b invariably encodes the replication-related proteins, with methyl-transferase (Mtr), helicase (Hel), and RNA-dependent RNA polymerase (RdRp) conserved domains. Downstream ORFs, which encode in 5′→3′ direction a 6K small hydrophobic protein, the HSP70h, the ~60 kDa protein, the CP and CPm, form a five-gene module which is conserved, with few modifications, among most members of the family analysed so far. The HSP70h and the ~60 kDa proteins are integral virion components present in all the sequenced members of the family. The functions postulated for HSP70h are: mediation of cell-to-cell movement through plasmodesmata, involvement in the assembly of multisubunit complexes for genome replication and/or subgenomic RNAs synthesis, and assembly of virus particles. The ~60 kDa protein is required for incorporation of both HSP70h and CPm to virion heads. The duplication of the capsid protein gene seems to be the only example of such condition among viruses with elongated particles. In general, capsid proteins and their homologs (CPm) show a significant degree of sequence conservation and the duplicate copies probably retain the general spatial folding and some crucial properties of the CPs. Notable exception are a group of ampeloviruses with the smallest genomes in the family [e.g. grapevine leafroll-associated virus 4 (GLRaV-4), GLRaV-5, GLRaV-6, GLRaV-9, pineapple mealybug wilt-associated virus 1(PMWaV-1) and PMWaV-3] which do not appear to possess CPm. The genome expression strategy is based on: (i) proteolytic processing of the polyprotein encoded by ORF1a; (ii) +1

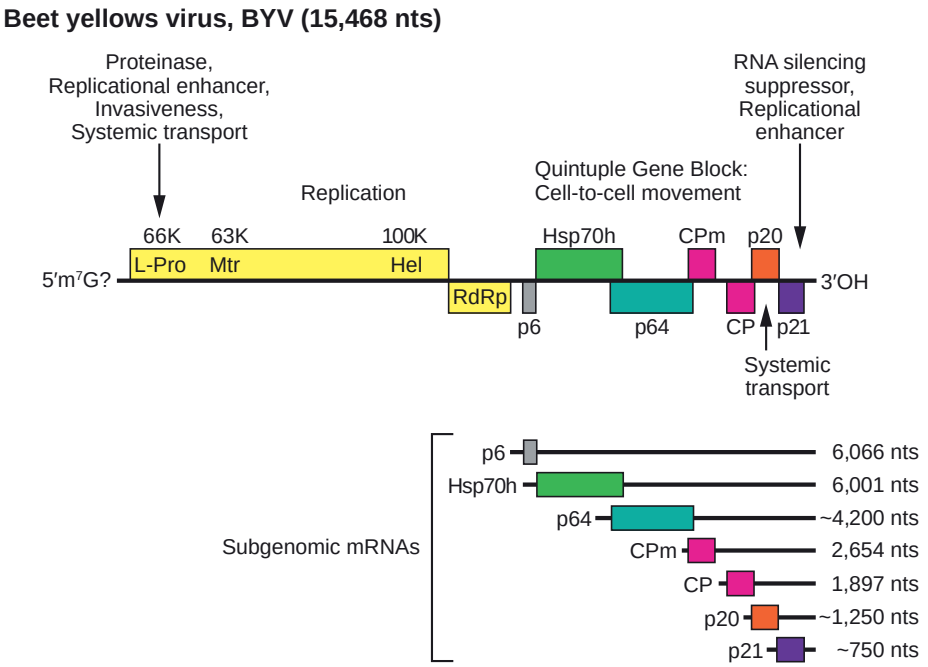


**Figure 2:** Genome organization and strategy of replication of beet yellows virus (BYV) showing the relative position of the ORFs, their expression products, and the 3′ nested set of sgRNAs. L-Pro, leader proteinase (66K); Mtr, methyltransferase (63K); Hel, helicase (100K); RdRp, RNA polymerase; HSP70h; ~60 kDa protein; CP, coat protein; CPm, minor capsid protein. The five boxes under "cell-to-cell movement" represent the five gene block conserved among most closteroviruses. (From Dolja, 2003; with permission.)

ribosomal frameshift for the expression of the RdRp domain encoded by ORF1b, a mechanism not found in other (+)RNA plant viruses; (iii) expression of the downstream ORFs via the formation of a nested set of 3′ co-terminal sub-genomic RNAs (sgRNAs). The dsRNA patterns are very complex and variable among species, reflecting the different numbers and sizes of the ORFs present in individual genomes and, in some cases, the existence of defective RNAs. Replication occurs in the cytoplasm, possibly in association with endoplasmic reticulum-derived membranous vesicles and vesiculated mitochondria. From an evolutionary point of view, closteroviruses represent a monophyletic virus lineage that might have evolved from a smaller filamentous virus when higher plants have differentiated. This progenitor virus, thought to be composed of three genes encoding replication-associated proteins, a protein (p6) with affinity for cell membranes, and a single coat protein, acquired the HSP70h and a ~60 kDa protein derived from a fusion of two domains, N-terminal domain of unknown evolutionary provenance, and a duplicated capsid protein domain. Under the pressure of further modular evolutionary events, i.e. duplication of the coat protein gene, acquisition of diverse suppressors of RNA silencing and of additional genes acquired via horizontal gene transfer (e.g. papain-like cysteine proteinase and AlkB domains), this family ancestor gave rise to the progenitors of the three extant genera of the family. One of these genera (Crinivirus), differentiated further by splitting its genome in two or three genome components.

## Antigenic properties

Virion proteins are moderately antigenic. Most virus species within genera are serologically unrelated or distantly related to one another. No intergeneric serological relationship has been detected.

## Biological properties

The natural and experimental host ranges of individual virus species are usually restricted, except for a few members of the genus *Crinivirus*. Disease symptoms are of the yellowing type (i.e. stunting, rolling, yellowing or reddening of the leaves, small and late ripening fruits), or pitting and/or grooving of the woody cylinder of woody hosts. Infection is systemic, but usually limited to

**Table 1:** Distinguishing properties of the genera in the family *Closteroviridae*

| Genus | Virion length (nm) | RNA species (No.) | Genome size (kb) | ORF (No.) | Replicase (kDa) | HSP70h (kDa) | CP (kDa) | CPm (kDa) | Vector |
|---|---|---|---|---|---|---|---|---|---|
| *Closterovirus* | 1350–2000 | 1 | 14.5–19.3 | 8–12 | 349–367 | 65–67 | 22–25 | 24–27 | Aphids |
| *Ampelovirus* | 1400–2000 | 1 | 13.0–18.5 | 7–12 | 245–293 | 57–59 | 28–36 | 50–56 | Mealybugs |
| *Crinivirus* | 750–900 | 2 or 3 | 15.6–17.9 | 9–13 | 267–280 | 62–65 | 28–29 | 53–80 | Whiteflies |

the phloem, which may necrotize to a varying extent. Few species of the genus *Closterovirus* are transmissible by mechanical inoculation, though with difficulty, but none of those in the genera *Ampelovirus* and *Crinivirus*. In vegetatively propagated crops long distance virus dissemination is primarily through infected propagating material. Some species can be transmitted through dodder (*Cuscuta* spp.). Transmission through seeds has not been ultimately proven. According to the genus, natural vectors are aphids, whiteflies, pseudococcid mealybugs and soft scale insects. Transmission is semi-persistent regardless of the type of vector. Geographical distribution varies from restricted to widespread, depending on the virus species, most of which occur in temperate or subtropical regions. Virions are usually found in the phloem (sieve tubes, companion cells, phloem parenchyma), occasionally in the mesophyll and epidermis. Ultrastructural modifications arise by membrane proliferation, degeneration and vesiculation of mitochondria, and formation of inclusion bodies. These are made up of aggregates of virions or membranous vesicles, or a combination of the two. Virions accumulate in conspicuous cross-banded fibrous masses or, more typically, in more or less loose bundles intermingled with single or clustered membranous vesicles. Inclusions of this type are one of the hallmarks of the family. The vesicles contain a fibrillar network and derive either from the endoplasmic reticulum, or from peripheral vesiculation of mitochondria.

## Species and genus demarcation criteria within the family

Traits that largely characterize the family and that are the basis of the current classification are:

- Morphology, substructure and size of virus particles.
- Very large, positive sense, single stranded RNA genome with an organization (number and order of genes) quite distinct from those of other plant viruses.
- Possession of unique genes coding for a homolog of the cellular HSP70 heat shock protein (HSP70h) and for a duplicated, diverged copy of the capsid protein (minor protein, CPm).
- Close phylogenetic relationships in replication-related proteins (Mtr, Hel and RdRp).
- Genome expression strategy based on ribosomal frameshift, proteolytic processing and production of subgenomic messenger RNAs.
- Induction of specific cytopathic structures in infected cells, consisting of cytoplasmic aggregates of virus particles intermingled with single or clustered membranous vesicles.
- Specific tissue tropism (members are mostly phloem-limited).
- Natural transmission by aphids, mealybugs or whiteflies in a semi-persistent manner; experimental transmission by mechanical inoculation very difficult or not possible.

See Table 1.

# Genus *Closterovirus*

Type species ***Beet yellows virus***

## Distinguishing features

The genus comprises species with particle length above 1200 nm, and monopartite RNA genome, 14.5–19.3 kb in size, in which CPm is located upstream of the CP gene. Natural transmission by aphids.

## Virion properties

### Morphology

Particle morphology largely conforms to that of other members of the family. Virions are of one size, ranging from 1350 to 2000 nm in length. CTV has also smaller than full-length particles that may encapsidate subgenomic or multiple species of defective RNAs (D-RNA) containing all of the *cis*-acting sequences required for replication. sgRNAs may be involved in the construction of recombinant D-RNAs.

### Physicochemical and physical properties

According to the species, infectivity is inactivated at temperatures between 40 and 55 °C, is retained for 1 to 4 days at room temperature, up to 1 year in frozen sap, 2 years in dried leaf material, 5 years

in lyophilized preparations stored at −20 °C, and is destroyed at pH lower than 6. $A_{260}/A_{280}$ ratio is around 1.20 but some members [BYV, carnation necrotic fleck virus (CNFV), burdock yellows virus (BuYV)] lack tryptophan, which results in a higher ratio (1.4–1.8) for the virions. $S_{20,w}$ ranges from 130 (BYV) to 140 (CTV), buoyant density is 1.33 g cm$^{-3}$ in CsCl (BYV and CTV) and 1.257 g cm$^{-3}$ in $Cs_2SO_4$ (CTV).

### Nucleic acid

Virions contain a single molecule of linear, positive sense, single stranded RNA from 14.5 to 19.3 kb in size. Multiple double stranded RNA (dsRNA) species occur in infected tissues, the largest of which is usually the replicative form of the entire genome. sgRNAs generate a range of smaller dsRNAs. With CTV, the presence of D-RNA makes the dsRNA pattern of virus isolates more complex than that of other members of the genus.

## Genome organization and replication

Sequenced members of the genus *Closterovirus* show three types of genome organization exemplified by BYV (Figure 2), CTV (Figure 3) and BYSV: (i) BYV contains eight ORFs flanked by 5′ and 3′ UTRs of 107 and 181 nt, respectively; (ii) CTV has 12 ORFs and UTRs of 107 nt at the 5′ end and 275 nt at the 3′ end. It differs from the BYV genome in having two papain-like protease domains in ORF1a, an extra 5′ proximal ORF (ORF2) encoding a 33 kDa product with no similarity to any other protein in databases, and two extra 3′ proximal ORFs (ORF9 and ORF11); (iii) BYSV has 10 ORFs and a 3′ UTR 241 nt in size, a length intermediate between that of the BYSV and CTV UTRs. A further difference with the BYV genome rests in the presence of an extra ORF (ORF2) encoding a 30 kDa polypeptide with no similarity to any other protein in databases. This ORF is located downstream of ORF1b, i.e. in the same position as the unrelated CTV ORF2. Thus, the organization of BYSV genomes is intermediate between that of BYV and CTV, suggesting that these three viruses might represent three distinct stages in closterovirus evolution. Non-structural proteins common to all members of the genus are: (i) a large polypeptide (over 300 kDa) containing the conserved domains of papain-like protease (P-Pro), methyltransferase (Mtr), and helicase (Hel); (ii) a ~50 kDa protein with all sequence motifs of viral RdRp of the "alpha-like" supergroup of positive-strand RNA viruses; (iii) a 6 kDa hydrophobic protein with membrane-binding properties; (iv) the homolog of the cellular HSP70 heat-shock proteins (HSP70h); (v) a 55–64 kDa product, referred to as the ~60 kDa protein. Some of the structural and non structural proteins function as suppressors of the RNA silencing plant defence machinery. For instance, CP, p20 and p23 proteins of CTV have suppressor activity, much the same as the homologs of p21 of BYSV, BYV, and GLRaV-2. CTV p23 is a unique protein in the family and has a nucleolar localization. Silencing suppressors contribute to the accumulation of virus particles and are important determinants of pathogenesis.

## Antigenic properties

No serological relationships reported among different virus species of the genus. Monoclonal antibodies have been produced to BYV, CTV and GLRaV-2 and polyclonal antisera have been raised to BYV, CTV and CYLV from fusion proteins obtained in bacterial expression systems. Polyclonal antisera have been raised to normal capsid proteins of BYV, BYSV, GLRaV-2 and BuYV.

## Biological properties

Most of the members of the genus infect herbaceous hosts (weeds, vegetable and flower crops) or shrubs (raspberry). Notable exceptions are CTV, GLRaV-2 and a couple of fig viruses [fig leaf mottle-associated virus 1 (FLMaV1) and fig mild mottle virus (FMMV)] which infect woody hosts. Symptoms are primarily of the yellowing type (rolling, yellowing or reddening of the leaves). Some CTV strains induce stem pitting. Transmission is by aphids with a semipersistent modality. The range of vectors varies for individual viruses from rather wide to restricted. For instance, BYV is transmitted by 23 aphid species (*Myzus persicae* and *Aphis fabae* being the main natural vectors), CTV by seven species (*Toxoptera citricida* and *Aphis gossypii* being the most efficient vectors) and a number of other viruses [e.g. CNFV, WYLV, BuYV, mint virus 1 (MV-1)] by a single aphid species, or do not have known vectors (e.g. GLRaV-2). Some species can be transmitted by inoculation of sap, though with difficulty (e.g. CTV, GLRaV-2, BYV), but none is transmitted through seeds. Species

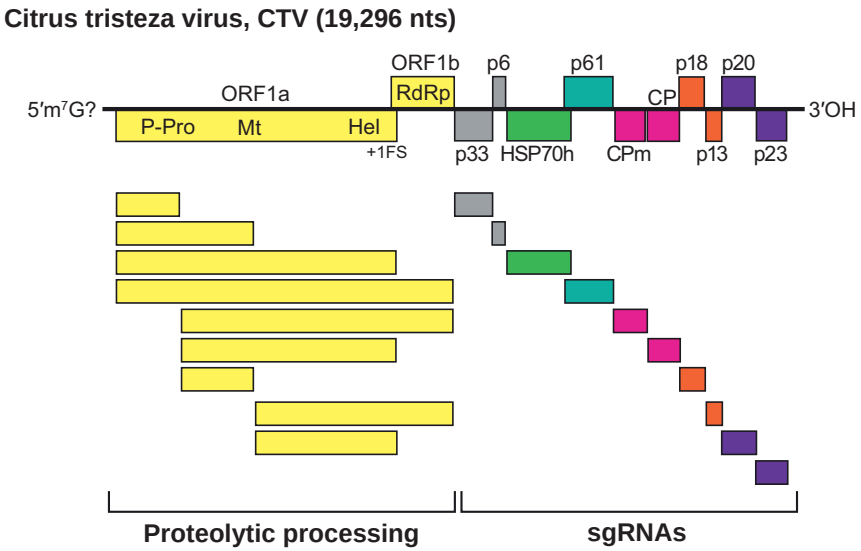


**Figure 3:** Organization and expression strategy of genomes characteristic of citrus tristeza virus showing the relative position of the ORFs and their expression products. +1 FS the putative ribosomal frameshift. The solid lines at the bottom of the figure define the two genomic regions expressed through proteolytic processing of the polyprotein precursor(s) and through the formation of a nested set of 3′ co-terminal sgRNAs. P-Pro, papain-like protease; Mt, methytransferase; Hel, helicase; RdRp, RNA polymerase; HSP70h, ~60 kDa protein; CPm, minor capsid protein; CP, capsid protein. (From Karasev and Hilf, 1997; with permission.)

infecting vegetatively propagated hosts (citrus, grapevine, raspberry) are transmitted by grafting and disseminated over long distances with propagating material. Geographic distribution ranges from very wide (e.g. CTV, GLRaV-2, BYV) to restricted (e.g. BuYV, MV-1, WYLV). The membranous vesicles with a fibrillar content derive from the endoplasmic reticulum.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Particle size.
- Size of CP, as determined by deduced amino acid sequence data.
- Serological specificity using discriminatory monoclonal or polyclonal antibodies.
- Genome structure and organization (number and relative location of the ORFs).
- Amino acid sequence of relevant gene products (polymerase, CP, HSP70h) differing by more than 25%.
- Vector species and specificity.
- Magnitude and specificity of natural and experimental host range.
- Cytopathological features (i.e., aspect of inclusion bodies and origin of cytoplasmic vesicles).

## List of species in the genus *Closterovirus*

| | | |
|---|---|---|
| *Beet yellow stunt virus* | | |
| Beet yellow stunt virus-California | [U51931*] | (BYSV-CA) |
| Beet yellows virus | | |
| Beet yellows virus-4 | [AF190581] | (BYV-4) |
| *Burdock yellows virus* | | |
| Burdock yellows virus-Japan | | (BuYV-JA) |
| *Carnation necrotic fleck virus* | | |
| Carnation necrotic fleck virus-Yunnan | [EU884443*] | (CNFV-YN) |
| *Carrot yellow leaf virus* | | |
| Carrot yellow leaf virus-Germany | [FJ869862 = NC_013007] | (CYLV-DE) |
| (Heracleum virus 6) | | |
| (Hogweed virus 6) | | |

| | | |
|---|---|---|
| *Citrus tristeza virus* | | |
| Citrus tristeza virus strain T30 | [AF260651] | (CTV-T30, mild isolate) |
| Citrus tristeza virus strain, T36 | [U16304 = NC_001661] | (CTV-T36, intermediate severity) |
| Citrus tristeza virus strain VT | [U56902] | (CTV-VT, severe isolate) |
| *Grapevine leafroll-associated virus 2* | | |
| (Grapevine rootstock stem lesion-associated virus) | [AF314061 = NC_004724] | (GRSLaV) |
| Grapevine leafroll-associated virus 2 - PN | [AY881628 = NC_007448] | (GLRaV-2-PN) |
| *Mint virus 1* | | |
| Mint virus 1-454.004 | [AY792620 = NC_006944] | (MV-1-454.004) |
| *Wheat yellow leaf virus* | | |
| Wheat yellow leaf virus-Japan | | (WYLV-JA) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

### List of other related viruses which may be members of the genus *Closterovirus* but have not been approved as species

| | | |
|---|---|---|
| Alligatorweed stunting virus | | (AWSV) |
| Clover yellows virus | | (CYV) |
| Dendrobium vein necrosis virus | | (DVNV) |
| Festuca necrosis virus | | (FNV) |
| Fig leaf mottle-associated virus 1 | [AM279676*] | (FLMaV-1) |
| Fig mild mottle virus | [FJ611959*] | (FMMV) |
| Raspberry leaf mottle virus | [DQ357218 = NC_008585] | (RLMV) |
| Strawberry chlorotic fleck-associated virus | [DQ860839 = NC_008366] | (SCFaV) |

*Sequences do not comprise the complete genome.

## Genus *Ampelovirus*

Type species ***Grapevine leafroll-associated virus 3***

### Distinguishing features

The genus comprises species with particles 1400–2000 nm long, monopartite genome 13.0–18.5 kb in size, transmitted by pseudococcid mealybugs and soft scale insects.

### Virion properties

#### Morphology

Particle morphology largely conforms to that of other members of the family.

#### Physicochemical and physical properties

Information is very limited, except for the size of CP and CPm, as deduced from sequence data.

#### Nucleic acid

Virions contain a single molecule of linear, positive sense, single stranded RNA from 13.0 to 18.5 kb in size. Multiple double stranded RNA (dsRNA) species occur in infected tissues, the largest of which is usually the replicative form of the entire genome. Smaller dsRNA are thought to be replicative forms of subgenomic RNAs.

### Genome organization and replication

The genus *Ampelovirus* shows a wide variation in genome size and organization. At one extreme there are grapevine leafroll-associated virus 1 (GLRaV-1) and GLRaV-3, which has the largest genome of all (18,498 nt). GLRaV-3 has 12 ORFs, coding for the replication related proteins (ORFs 1a and 1b), two small hydrophobic proteins (6 kDa), the HSP70h, the ~60–kDa protein, CP, CPm

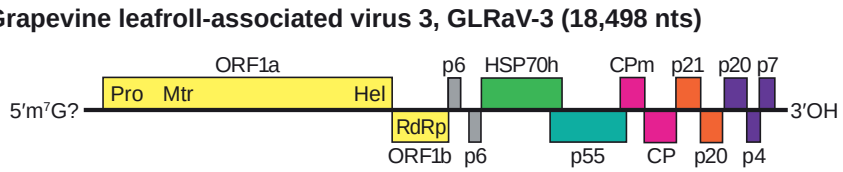


**Figure 4:** Genome organization of grapevine leafroll-associated virus 3, showing the relative position of the ORFs and their expression products. Pro, papain-like protease; Mtr, methytransferase; Hel, helicase; RdRp, RNA polymerase; HSP70h; ~60 kDa protein; CPm, minor capsid protein; CP, capsid protein.

and five additional proteins 21, 20, 20, 4 and 7 kDa in size, respectively (Figure 4). The 5′ UTR and 3′ UTR are 737 and 277 nt in size, respectively. GLRaV-1 differs from all other members of the genus in encoding two copies of the CPm. At the other extreme there is a group of viruses infecting grapevine [e.g, grapevine leafroll-associated viruses 5 and 9 (GLRaV-5 and -9)] and pineapple [e.g. pineapple mealybug wilt-associated viruses 1 and 3 (PMWaV-1 and -3)]. All these viruses have a genome made up of seven ORFs and lack the CPm. PMWaV-1, a representative of this group, has a genome 13,071 nt in size, beginning with a 535 nt UTR at the 5′ end, followed by the ORFs expressing, respectively, the replication related proteins, a 6 kDa hydrophobic protein, the HSP70h, the ~60 kDa protein, the CP and a 24 kDa protein. A UTR 132 nt in size terminates the genome. Replication occurs in the cytoplasm, likely in association with membranous vesicles, derived either from the endoplasmic reticulum or from peripheral vesiculation and disruption of mitochondria (GLRaV-1, GLRaV-3). Structural and non-structural proteins are similar in type and function to those reported for the genus *Closterovirus*.

## Antigenic properties

Polyclonal antisera and monoclonal antibodies have been raised to most of the members of the genus. A recombinant single-chain variable fragment antibody was synthesized to GLRaV-3. GLRaV-1 and GLRaV-3 are distantly serologically related based on cross-reactivity to a monoclonal antibody to GLRaV-1. GLRaV-4, -5, -6 and -9 show serological interrelations when tested with polyclonal antisera or monoclonal antibodies. Grapevine leafroll-associated virus 7 (GLRaV-7), an unassigned member of the family, is also distantly related to the four above species. The three pineapple mealybug wilt-associated viruses are serologically unrelated to one another.

## Biological properties

The majority of extant ampelovirus species are recorded from woody hosts (grapevine, plum, fig) and pineapple. According to the host, they induce rolling yellowing and reddening of the leaves (grapevine), stem pitting (plum), wilting or symptomless infections (pineapple). Natural vectors are mealybugs which transmit with a semipersistent modality. The range of vectors varies for individual viruses from rather wide to restricted. For instance, GLRaV-1 is transmitted by species of several genera of pseudococcid mealybugs (*Heliococcus*, *Phenacoccus*, *Pseudococcus*) and soft scale insects (*Pulvinaria*, *Neopulvinaria* and *Parthenolecanium*); GLRaV-3 by pseudococcid mealybugs (*Planococcus*, *Pseudococcus*, *Heliococcus*, *Phenacoccus*) and soft scale insects (*Pulvinaria*, *Neopulvinaria*, *Parthenolecnium*, *Coccus*, *Saissetia*, *Parasaissetia* and *Ceroplastes*), whereas GLRaV-5 is transmitted by *Pseudoccocus*, *Planococcus* and *Ceroplastes* spp. Vectors of pineapple ampeloviruses are two species of the genus *Dysmicoccus*, and LChV-2 is transmitted by *Phenacoccus aceris.* None of the viruses is transmitted through seed or mechanically. All persist in plant parts used for propagation and are disseminated with them over long distances. Geographical distribution is very wide.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Particle size.
- Size of CP, as determined by deduced amino acid sequence data.

- Serological specificity using discriminatory monoclonal or polyclonal antibodies.
- Genome structure and organization (number and relative location and size of the ORFs).
- Amino acid sequence of relevant gene products (polymerase, CP, HSP70h) differing by more than 25%.
- Vector species and specificity.
- Magnitude and specificity of natural and experimental host range.
- Cytopathological features (i.e., aspect of inclusion bodies and origin of cytoplasmic vesicles).

### List of species in the genus *Ampelovirus*

| | | |
|---|---|---|
| *Grapevine leafroll-associated virus 1* | | |
| Grapevine leafroll-associated virus 1-Australia | [AF195822*] | (GLRaV-1-AUS) |
| *Grapevine leafroll-associated virus 3* | | |
| Grapevine leafroll-associated virus 3-NY1 | [AF037268 = NC_004667] | (GLRaV-3-NY1) |
| *Grapevine leafroll-associated virus 5* | | |
| Grapevine leafroll-associated virus 5-US | [AF233934*] | (GLRaV-5-US) |
| *Little cherry virus 2* | | |
| Little cherry virus 2-USA6b | [AF531505 = NC_005065] | (LChV-2-USA6b) |
| *Pineapple mealybug wilt-associated virus 1* | | |
| Pineapple mealybug wilt-associated virus 1-Hawaii | [AF414119 = NC_010178] | (PMWaV-1-HI) |
| *Pineapple mealybug wilt-associated virus 2* | | |
| Pineapple mealybug wilt-associated virus 2-Hawaii | [AF283103*] | (PMWaV-2-HI) |
| *Pineapple mealybug wilt-associated virus 3* | | |
| Pineapple mealybug wilt-associated virus 3-Hawaii | [DQ399259*] | (PMWaV-3-HI) |
| *Plum bark necrosis stem pitting-associated virus* | | |
| Plum bark necrosis stem pitting-associated virus-US | [EF546442 = NC_009992] | (PBNSPaV-US) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

### List of other related viruses which may be members of the genus *Ampelovirus* but have not been approved as species

| | | |
|---|---|---|
| Fig leaf mottle-associated virus 2 | [FJ473383*] | (FLMaV-2) |
| Grapevine leafroll-associated virus 4 | [DQ325516*] | (GLRaV-4) |
| Grapevine leafroll-associated virus 6 | [AJ496796*] | (GLRaV-6) |
| Grapevine leafroll-associated virus 9 | [AY297819*] | (GLRaV-9) |
| Grapevine leafroll-associated Carnelian virus | [FJ907331] | (GLRaV-Car) |
| Grapevine leafroll-associated virus Pr | [AM182328 = NC_011702] | (GLRaV-Pr) |
| Sugarcane mild mosaic virus | | (SMMV) |

*Sequences do not comprise the complete genome.

## Genus *Crinivirus*

Type species ***Lettuce infectious yellows virus***

### Distinguishing features

The genus comprises species transmitted by whiteflies. Virions usually have two modal lengths (650–850 and 700–900 nm) and a bipartite genome, but potato yellow vein virus (PYVV) has a tripartite genome.

### Virion properties

#### Morphology

Particle morphology largely conforms to that of other members of the family.

#### Physicochemical and physical properties

Information is very limited, except for the size of CP and CPm, as deduced from sequence data.

### Nucleic acid

Virions contain a single molecule of linear, positive sense, single stranded RNA with size ranging from 7801 to 9127 nt (RNA-1) and from 7903 to 8530 nt (RNA-2) in species with bipartite genome. The RNA size of PYVV, the only species with a tripartite genome, is 8035 nt (RNA-1), 5339 nt (RNA-2) and 3892 nt (RNA-3).

## Genome organization and replication

The genome of most criniviruses (e.g. LIYV) is divided between two linear, positive sense, single stranded RNAs totalling 15.6–17.9 kb in size (Figure 5), but PYVV possesses a tripartite genome. All molecules are needed for infectivity and are separately encapsidated. RNA-1 of LIYV contains three ORFs, i.e. the ORF1a–ORF1b complex plus a 3′-most ORF coding for a 32 kDa protein with no similarity to any protein in databases. This ORF is similar in size and location to ORF2 of CTV and BYSV but the respective expression products are not related. RNA-1 has 5′ and 3′ UTRs of 97 and 219 nucleotides, respectively. As with other members of the family, the ORF1a–ORF1b complex codes for the replication-related proteins including the RNA-dependent RNA polymerase (RdRp). RNA-2 has seven ORFs flanked by a 5′ UTR of 326 nt and a 3′ UTR of 187 nt. RNA2 contains the five-gene module which, however, differs from that of members of the genus *Closterovirus* by the insertion of an extra gene (ORF4) upstream of the CP gene. As to PYVV: (i) RNA-1 (8035 nt in size) is composed of three ORFs, i.e. the ORF1a-ORF1b complex and a 7 kDa hydrophobic protein containing a potential transmembrane helix; (ii) RNA-2 (5,339 nt in size) comprising five predicted ORFs that encode, in the order, the HSP70h; a 7 kDa protein similar to a comparable protein of cucurbit yellow stunting disorder virus (CYSDV); the ~60 kDa protein; a 9.8 kDa product with no significant similarity to any other sequence in database; the 28.2 kDa putative CP; (iii) RNA-3 (3892 nt) has three potential ORFs coding for a protein 4 kDa in size with no counterpart with other proteins in the family and no significant sequence homology in database; the 77.5 kDa CPm, and a 26.4 kDa protein present in other members of the genus. In all criniviruses, the order of the CP and CPm ORFs is reversed compared to that of species in the genus *Closterovirus*. Sweet potato chlorotic stunt virus (SPCSV) and tomato chlorosis virus (ToCV) have a particularly large CPm (75–80 kDa), compared to LIYV (53 kDa). Replication occurs in the cytoplasm, likely in association with membranous vesicles, derived from the endoplasmic reticulum or from vesiculated mitochondria (CYSDV). Structural and non-structural proteins are similar in type and function to those reported for the genus *Closterovirus*. Both genomic RNAs of ToCV encode RNA silencing suppressors, e.g. the p22 protein in RNA-1 and CP and CPm in RNA-2. The p25 protein of CYSDV, the viral RNAse III and the p22 gene present in a few isolates of SPCSV also have suppressor activity.

## Antigenic properties

Monoclonal antibodies have been produced to proteins of SPCSV. Antisera have been raised from structural and non structural proteins produced as fusion proteins in bacterial expression systems (SPCSV and LIYV) or from CPs [tomato infectious chlorosis virus (TICV), LIYV, lettuce chlorosis

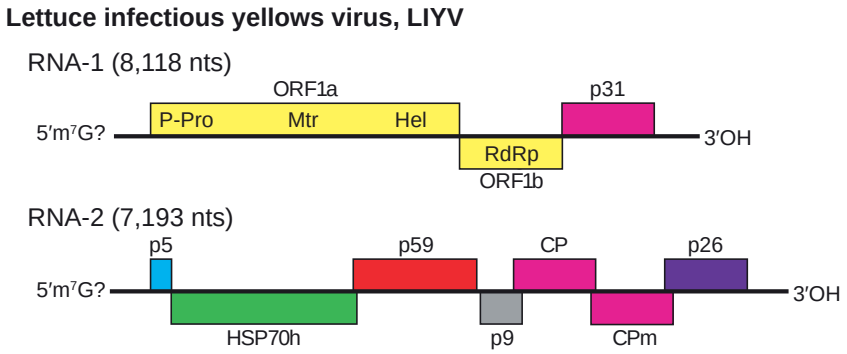


**Figure 5:** Genome organization of lettuce infectious yellows virus showing the relative position of the ORFs and their expression products. P-Pro, papain-like protease; Mtr, methyltranferase; Hel, helicase; RdRp, RNA polymerase; HSP70h; ~60 kDa protein; CP capsid protein; CPm, minor capsid protein.

Pos. ssRNA

virus (LCV) and ToCV]. Generally, there are no detectable interspecific serological relationships. TICV and ToCV, however, are distantly serologically related.

## Biological properties

Criniviruses infect primarily herbaceous hosts, in which they induce extensive chlorosis to yellow discoloration of the leaves, often accompanied by stunting. They are transmitted semi-persistently by whiteflies of the genera *Trialeurodes* and *Bemisia*. Persistence and specificity of transmission by their respective vectors have been used as characters for species differentiation. Thus, the viruses of group 1 [PYVV, blackberry yellow vein-associated virus (BYVaV), beet pseudoyellows virus (BPYV) and strawberry pallidosis-associated virus (SpaV)] are transmitted by *T. vaporariorum*, viruses of group 2 [ToCV, SPCSV, CYSDV and bean yellow disorder virus (BYDV)] by *B. tabaci*, whereas one of the viruses of group 3 is transmitted by *B. tabaci* (LIYV) and the other by *T. vaporariorum* (TICV). These groups were identified by comparative phylogenetic analyses of RdRp amino acid sequences. None of the viruses is transmitted through seed or mechanically. Geographical distribution varies from restricted (e.g. BYVaV) to very wide. Some emerging viruses (e.g. CYSDV, TICV and ToCV) are being increasingly recorded from a number of European, American and Asiatic countries. The membranous vesicles with a fibrillar content derive from the endoplasmic reticulum and/or from vesiculated mitochondria (CYSDV).

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Particle size.
- Size of CP, as determined by deduced amino acid sequence data.
- Serological specificity using discriminatory monoclonal or polyclonal antibodies.
- Genome structure and organization (number and relative location of the ORFs).
- Amino acid sequence of relevant gene products (polymerase, CP, HSP70h) differing by more than 25%.
- Vector species and specificity.
- Magnitude and specificity of natural and experimental host range.
- Cytopathological features (i.e., aspect of inclusion bodies and origin of cytoplasmic vesicles).

## List of species in the genus *Crinivirus*

| | | |
|---|---|---|
| *Abutilon yellows virus* | | |
| Abutilon yellows virus-California | [AY42270*] | (AbYV-CA) |
| *Bean yellow disorder virus* | | |
| Bean yellow disorder virus-Bn03 | [EU191904 = NC_010560, EU191905 = NC_010561] | (BYDV-Bn03) |
| *Beet pseudoyellows virus* | | |
| Beet pseudoyellows virus-Maryland | [AY330918 = NC_005209, AY330919 = NC_005210] | (BPYV-MD) |
| (Cucumber chlorotic spot virus) | | (CCSV) |
| (Cucumber yellows virus) | [AB085612, AB085613] | (CYV) |
| *Blackberry yellow vein-associated virus* | | |
| Blackberry yellow vein-associated virus-South Carolina | [AY776334 = NC_006962, AY776335 = NC_006963] | (BYVaV-SC) |
| *Cucurbit yellow stunting disorder virus* | | |
| Cucurbit yellow stunting disorder virus-Spain | [AJ537493, AJ439690] | (CYSDV-ES) |
| *Lettuce chlorosis virus* | | |
| Lettuce chlorosis virus-California | [FJ380118 = NC_012909, FJ380119 = NC_012910] | (LCV-CA) |
| *Lettuce infectious yellows virus* | | |
| Lettuce infectious yellows virus-California | [U15440 = NC_003617, U15441 = NC_003618 ] | (LIYV-CA) |
| *Potato yellow vein virus* | | |

| | | |
|---|---|---|
| Potato yellow vein virus-Peru | [AJ557128 = NC-006061, AJ557129 = NC-006062, AJ508757 = NC-006063] | (PYVV-Peru) |
| *Strawberry pallidosis-associated virus* | | |
| Strawberry pallidosis-associated virus-M1 | [AY488137 = NC_005895, AY488138 = NC_005896] | (SpaV-M1) |
| *Sweet potato chlorotic stunt virus* | | |
| Sweet potato chlorotic stunt virus-EA | [AJ428554 = NC_004123, AJ428555 = NC_004124] | (SPCSV-EA) |
| *Tomato chlorosis virus* | | |
| Tomato chlorosis virus-Florida | [AY903447 = NC_007340, AY903448 = NC_007341] | (ToCV-FL) |
| *Tomato infectious chlorosis virus* | | |
| Tomato infectious chlorosis virus-California | [FJ815440 = NC_013258, FJ814441 = NC_013259] | (TICV-CA) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

## List of other related viruses which may be members of the genus *Crinivirus* but have not been approved as species

| | | |
|---|---|---|
| Diodia vein chlorosis virus | [GQ376201*, GQ225585*] | (DVCV) |
| Cucurbit chlorotic yellows virus | [AB523788, AB523789] | (CCYV) |

*Sequences do not comprise the complete genome.

## List of unassigned species in the family *Closteroviridae*

| | | |
|---|---|---|
| *Mint vein banding-associated virus* | | |
| Mint vein banding-associated virus-US | [AY548173*] | (MVBaV-US) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequence does not comprise the complete genome.

## List of other related viruses which may be members of the family *Closteroviridae* but have not been approved as species

| | | |
|---|---|---|
| Grapevine leafroll-associated virus 7 | [EF093187*] | (GLRaV-7) |
| Little cherry virus 1 | [Y10237 = NC_001836] | (LChV-1) |
| Olive leaf yellowing-associated virus | [AJ440010*] | (OLYaV) |

*Sequences do not comprise the complete genome.

## Phylogenetic relationships within the family

Phylogenetic relationships within the family are depicted in Figure 6.

## Similarity with other taxa

Virions of some of the genera of the families *Alphaflexiviridae* (*Allexivirus*) and *Betaflexiviridae* (*Capillovirus*, *Trichovirus*, *Vitivirus*, *Citrivirus* and *Foveavirus*) have the same particle morphology as those of the family *Closteroviridae*. However, the sequence of the CP of members of this family has little similarity with that of CPs of viruses in the above genera, and major differences exist in genome size and organization, and in strategy of expression. Replication-associated proteins (RdRp, Mtr and Hel) contain signature sequences homologous to those of other taxa of the "alpha-like" supergroup of ssRNA viruses, the closest affinity being with the families *Bromoviridae* and *Virgaviridae*. The replication strategy, based on polyprotein processing, translational frameshifting and multiple sgRNA generation, closely resembles that of viruses in the families *Coronaviridae* and

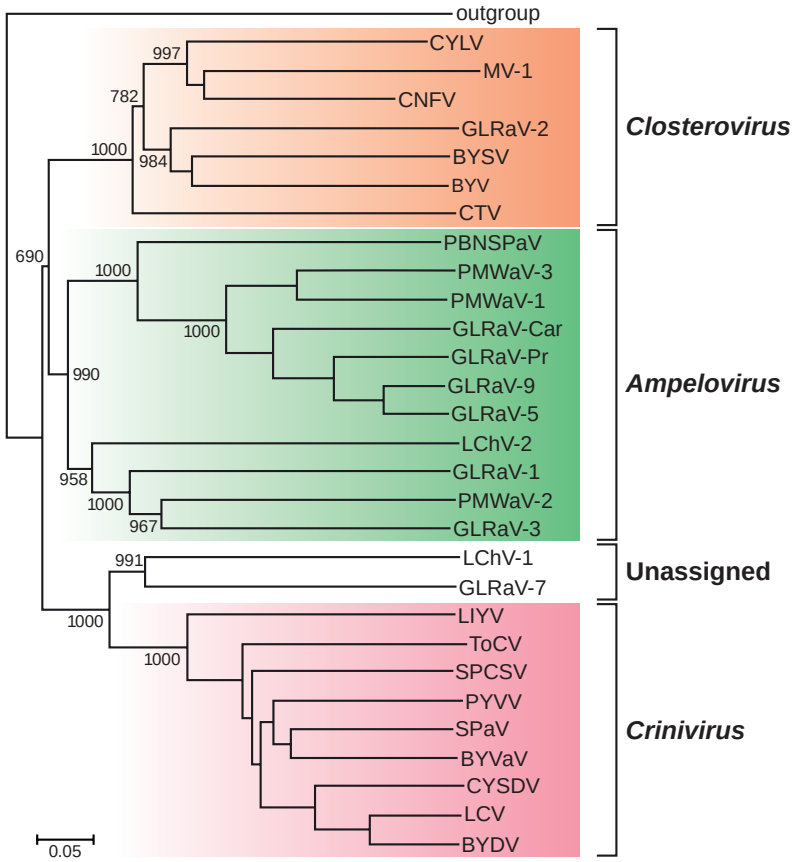


**Figure 6:** Phylogenetic tree showing the relationships between the species and genera of the family *Closteroviridae* based on the sequence of the HSP70h gene. The neighbor-joining tree was produced and bootstrapped using CLUSTAL W. Branch lengths are proportional to sequence distances. All abbreviations and accession numbers can be found in the "List of Species" in the description. GLRaV-7 and LChV-1 are unassigned viruses in the family. (Courtesy of P. Saldarelli.)

*Arteriviridae.* However, unlike closteroviruses, the RdRp of coronaviruses and arteriviruses belong to the "picorna-like" supergroup of polymerases. Hence, the transcriptional strategy of members of the family *Closteroviridae* follows the mechanism of other "alpha-like" viruses, and is dissimilar from the discontinuous, leader-primed transcription of coronaviruses and arteriviruses.

## Derivation of names

*Ampelo*: from Greek *ampelos*, "grapevine", the host of the type species of the genus.
*Clostero*: from Greek *kloster*, "spindle, thread".
*Crini*: from Latin *crinis*, "hair", from the appearance of the very long thread-like particles.

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### Supplementary reading

A supplementary list of references is available online on Science Direct®, www.sciencedirect.com.

## Contributed by

Martelli, G.P., Agranovsky, A.A., Bar-Joseph, M., Boscia, D., Candresse, T., Coutts, R.H.A., Dolja, V.V., Hu, J.S., Jelkmann, W., Karasev, A.V., Martin, R.R., Minafra, A., Namba, S. and Vetten, H.J.

# FAMILY *FLAVIVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Flaviviridae*** |
| Genus | ***Flavivirus*** |
| Genus | ***Pestivirus*** |
| Genus | ***Hepacivirus*** |

## Virion properties

### MORPHOLOGY

Virions are 40–60 nm in diameter, spherical in shape and contain a lipid envelope. The capsid is composed of a single protein and the envelope contains two or three virus-encoded membrane proteins. Specific descriptions of the three individual genera and a tentative fourth genus are given in the corresponding sections.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

The virion Mr, buoyant density, sedimentation coefficient and other physicochemical properties differ among the members of the genera and are described separately in the corresponding sections.

### NUCLEIC ACID

Genomes are positive sense ssRNA of approximately 11, 12.3 and 9.6 kb for members of the genera *Flavivirus*, *Pestivirus* and *Hepacivirus*, respectively. All members of the family lack a 3′-terminal poly(A) tract. Only the genomes of flaviviruses contain a 5′-terminal type I cap structure.

### PROTEINS

The virions of members of the family have a single, small basic capsid (C) and two (flavivirus and hepacivirus) or three (pestivirus) membrane-associated envelope proteins. Viruses that are candidates for inclusion in a possible fourth genus appear to lack a complete nucleocapsid protein gene. The nonstructural proteins contain sequence motifs characteristic of a serine protease, RNA helicase and RdRp that are encoded at similar locations along the genome in all genera. Further details of specific functional properties are given in the corresponding sections of the individual genera.

### LIPIDS

Lipids present in virions are derived from host cell membranes and make up 17% of the total virion weight in the case of flaviviruses. The lipid content of pesti- and hepaciviruses has not been determined.

### CARBOHYDRATES

Virions contain carbohydrates in the form of glycolipids and glycoproteins.

## Genome organization and replication

The genomic RNA of all members of the family has a similar organization and is the viral mRNA found in infected cells. It contains a single long ORF flanked by 5′- and 3′-terminal non-coding regions (NCRs) that form specific secondary structures required for genome replication and translation. Flaviviruses, but not pestiviruses or hepaciviruses, produce a unique, subgenomic, small (0.3–0.5 kb) non-coding RNA derived from 3′-NCR of genomic RNA, which is essential for virus replication in cells and modulates pathogenicity in animals. Translation-initiation of genomic RNA is cap-dependent in the case of flaviviruses, whereas IRES elements are present in the other genera. Viral proteins are synthesized as part of a polyprotein that is co- and post-translationally cleaved by viral and cellular proteases. The structural proteins are contained in the N-terminal portion of this polyprotein and the non-structural proteins in the remainder. The latter include a serine protease, an RNA helicase and the RdRp. RNA synthesis occurs in the cytoplasm in association with modified cellular membranes via synthesis of full-length negative-strand intermediates. Virion assembly, including acquisition of a glycoprotein-containing lipid envelope, occurs by budding through intracellular membranes. Viral particles are transported in cytoplasmic vesicles through the secretory pathway before they are released by exocytosis, as shown for members of the genus *Flavivirus* and assumed for members of the other genera.

### Antigenic properties

The genera are antigenically unrelated, but serological cross-reactivity exists among members within each genus.

### Biological properties

The biological properties of the three genera exhibit different characteristics and are described in the corresponding sections.

## GENUS *FLAVIVIRUS*

Type species ***Yellow fever virus***

### Distinguishing features

The 5′ end of the genome possesses a type I cap ($m^7$GpppAmp) not seen in the other genera. Most flaviviruses are transmitted to vertebrate hosts by arthropod vectors, mosquitoes or ticks, in which they actively replicate. Some flaviviruses are zoonotic agents transmitted between rodents or bats without known arthropod vectors.

### Virion properties

#### MORPHOLOGY

Virions are 50 nm in diameter and spherical in shape (Figure 1). Two virus forms can be distinguished. Mature virions contain two virus encoded membrane-associated proteins, E and M. Intracellular immature virions contain the precursor prM instead of M, which is proteolytically cleaved during maturation. In certain instances, partially mature/immature forms are also released from infected cells. The atomic structure of the major envelope protein E from tick-borne encephalitis virus (TBEV), dengue virus (DENV) and West Nile virus (WNV) has been determined by X-ray crystallography. It is a dimeric, rod-shaped molecule that is oriented parallel to the membrane and does not form spike-like projections in its neutral pH conformation. Image reconstructions from cryo-electron micrographs (Figure 1) have shown that the virion envelope has icosahedral symmetry, in which E protein dimers are organized in a herringbone-like arrangement.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virion Mr has not been precisely determined. Mature virions sediment at about 200S and have a buoyant density of about 1.19 g cm$^{-3}$ in sucrose. Viruses are stable at slightly alkaline pH 8.0 but are

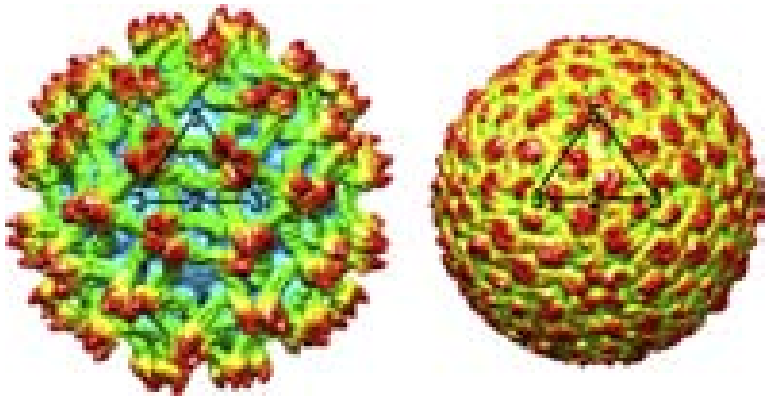

**Figure 1:** Three-dimensional cryo-electron microscopic reconstructions of immature (left) and mature (right) particles of an isolate of dengue virus (courtesy of M. Rossmann). Shown is a surface rendering of immature dengue virus at 12.5A resolution (left) and mature DENV at 10A resolution (right). The viruses are depicted to scale, but not colored to scale. Triangles outline one icosahedral unit.

readily inactivated at acidic pH, temperatures above 40 °C, organic solvents, detergents, ultraviolet light and gamma-irradiation.

### Nucleic acid

The virion RNA of flaviviruses is a positive sense infectious ssRNA of about 11 kb. The 5′ end of the genome possesses a type I cap (m-7GpppAmp) followed by the conserved dinucleotide AG. The 3′ ends lack a terminal poly(A) tract and terminate with the conserved dinucleotide CU.

### Proteins

Virions contain three structural proteins: C (11 kDa), E (50 kDa), the major envelope protein, and either prM (26 kDa), in immature virions, or M (8 kDa), in mature virions. The E protein is the viral hemagglutinin, which mediates both receptor binding and acid pH-dependent fusion activity after uptake by receptor-mediated endocytosis. Seven nonstructural proteins are synthesized in infected cells: NS1 (46 kDa), NS2A (22 kDa), NS2B (14 kDa), NS3 (70 kDa), NS4A (16 kDa), NS4B (27 kDa) and NS5 (103 kDa). NS1 has multiple forms and roles, with a cell-associated form functioning in viral RNA replication and a secreted form that regulates complement activation. One such form, a NS1′ protein, is the product of a −1 ribosomal frameshift and plays a role in viral neuroinvasiveness. NS3 is a multifunctional protein. The N-terminal one-third of the protein forms the viral serine protease complex together with NS2B that is involved in processing the polyprotein. The C-terminal portion of NS3 contains an RNA helicase domain involved in RNA replication, as well as an RNA triphosphatase activity that is probably involved in formation of the 5′-terminal cap structure of the viral RNA. NS5 is the largest and most highly conserved flavivirus protein. It is a multifunctional protein that acts as the viral RdRp and also possesses methyltransferase activity involved in the modification of the viral cap structure.

### Lipids

Virions contain about 17% lipid by weight; lipids are derived from host cell membranes.

### Carbohydrates

Virions contain about 9% carbohydrate by weight (glycolipids, glycoproteins); their composition and structure are dependent on the host cell (vertebrate or arthropod). N-glycosylation sites are present in the proteins prM (1 to 3 sites), E (0 to 2 sites) and NS1 (1 to 3 sites).

## Genome organization and replication

The genomic RNA represents the only viral messenger RNA in flavivirus-infected cells. It consists of a single long ORF of more than 10,000 nt that codes for all structural and non-structural proteins and is flanked by NCRs at the 5′- and 3′-terminal ends (Figure 2).

Both the 5′-NCR and the 3′-NCR contain RNA sequence motifs that are involved in viral RNA translation, replication and possibly packaging. Although RNA secondary structure and function of some elements are conserved, sequence composition, length and exact localization can vary considerably between different members of the genus, in particular between tick-borne and mosquito-borne flaviviruses. In some cases, the 3′-NCR of tick-borne encephalitis virus contains an internal poly(A) tract. Flavivirus infection induces dramatic rearrangements of cellular membrane structures within the perinuclear endoplasmic reticulum (ER) and causes the formation of ER-derived vesicular packets that most likely represent the sites of viral replication. After translation of the incoming genomic RNA, RNA replication begins with synthesis of complementary negative strands, which are then used as templates to produce additional genome-length positive-stranded molecules. These are synthesized by a semi-conservative mechanism involving replicative intermediates (containing double stranded regions as well as nascent single stranded molecules) and replicative forms (duplex RNA molecules). Translation usually starts at the first AUG of the ORF, but may also occur at a second in-frame AUG located 12 to 14 codons downstream in mosquito-borne flaviviruses. The polyprotein is processed by cellular proteases and the viral NS2B-NS3 serine protease to give rise to the mature structural and nonstructural proteins. Protein topology with respect to the ER and cytoplasm is determined by internal signal and stop-transfer sequences. Virus particles can first be observed in the rough endoplasmic reticulum, which is believed to be the site of virus assembly. These immature virions are then transported through the membrane systems of the host secretory pathway to the cell surface where exocytosis occurs. Shortly before virion release, the prM protein is cleaved by

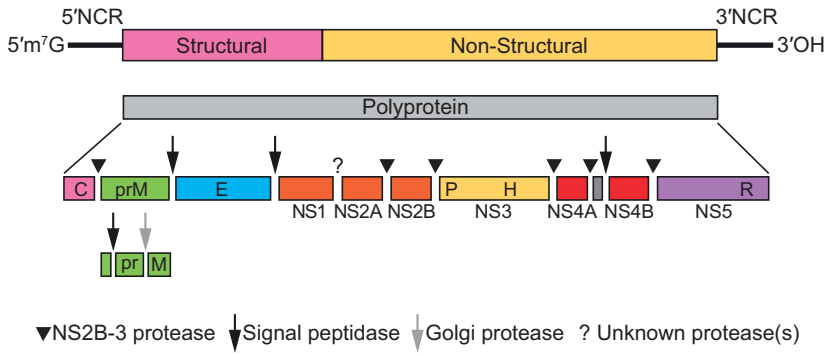


**Figure 2:** Flavivirus genome organization (not to scale) and polyprotein processing. The virion RNA is about 11 kb in size. At the top is the viral genome with the structural and nonstructural protein coding regions and the 5′- and 3′-NCRs. Boxes below the genome indicate viral proteins generated by the proteolytic processing cascade. P, H, and R symbols indicate the localization of the NS3 protease, the NS3 RNA helicase, and the NS5 RdRp, respectively.

furin or a furin-like cellular protease to generate mature virions. Flavivirus-infected cells also release a noninfectious subviral particle that has a lower sedimentation coefficient than whole virus (70S vs. 200S) and exhibits hemagglutination activity (slowly sedimenting hemagglutinin; SHA).

## Antigenic properties

All flaviviruses are serologically related, which can be demonstrated by binding assays such as ELISA and by hemagglutination-inhibition using polyclonal and monoclonal antibodies. Neutralization assays are more discriminating and have been used to define several serocomplexes of more closely related flaviviruses (see "List of species in the genus"). The envelope protein E is the major target for neutralizing antibodies and induces protective immunity. The E protein also induces flavivirus cross-reactive non-neutralizing antibodies. Antigenic sites involved in neutralization have been mapped to each of the three structural domains of the E protein. Antibodies to prM can also mediate immunity, probably by neutralizing viruses with partially uncleaved prM. The NS1 protein can also induce antibodies that protect infected animals from lethal infection.

## Biological properties

### Host range

Flaviviruses can infect a variety of vertebrate species and in many cases arthropods. Some viruses have a limited vertebrate host range (e.g., only primates), others can infect and replicate in a wide variety of species (mammals, birds, etc.). Arthropods are usually infected when they feed on a vertebrate host during viremia, but non-viremic transmission has also been described for tick-borne flaviviruses. A new group of flaviviruses that appear only to infect mosquitoes is now recognized. The prototype of these flaviviruses was tentatively assigned to the genus *Flavivirus* following the discovery of cell fusing agent virus. However, several genetically related but distinct insect-only flaviviruses have now been identified and will need to be considered as a possible separate group of viruses within the genus.

### Transmission

Most flaviviruses are arthropod-borne viruses that are maintained in nature by transmission from hematophagous arthropod vectors to vertebrate hosts. About 50% of known flaviviruses are mosquito-borne, 28% are tick-borne and the rest are zoonotic agents transmitted between rodents or bats without known arthropod vectors. For some flaviviruses, the transmission cycle has not yet been identified. In certain instances, flaviviruses can be transmitted to humans by blood products, organ transplantation, non-pasteurized milk or aerosols. In the arthropod vectors, the viruses may also be passed on trans-ovarially or vertically (mosquitoes, ticks) and trans-stadially (ticks). The mechanisms of virus transmission involving the insect-only flaviviruses may include vertical transmission but other mechanisms need to be considered to explain the success with which these viruses appear to have dispersed globally.

### Geographical distribution

Flaviviruses have a world-wide distribution but individual species are restricted to specific endemic or epidemic areas (e.g., yellow fever virus in tropical and subtropical regions of Africa and South America; dengue virus in tropical areas of Asia, Oceania, Africa, Australia and the Americas; Japanese encephalitis virus in South-East Asia; tick-borne encephalitis virus in Europe and Northern Asia).

### Pathogenicity

More than 50% of known flaviviruses have been associated with human disease, including the most important human pathogens: yellow fever virus, dengue virus, Japanese encephalitis virus, West Nile virus and tick-borne encephalitis virus. Flavivirus-induced diseases may be associated with symptoms of the central nervous system (e.g., meningitis, encephalitis), fever, arthralgia, rash and hemorrhagic fever. Several flaviviruses are pathogenic for domestic or wild animals (turkey, pig, horse, sheep, dog, grouse, muskrat) and cause economically important diseases.

## Species demarcation criteria in the genus

Species demarcation criteria in the genus include:

- Nucleotide and deduced amino acid sequence data.
- Antigenic characteristics.
- Geographic association.
- Vector association.
- Host association.
- Disease association.
- Ecological characteristics.

Species demarcation considers a combination of each of the criteria listed above. While nucleotide sequence relatedness and the resulting phylogenies are important criteria for species demarcation, the other listed criteria may be particularly useful in demarcation of genetically closely related viruses. For example far-eastern (FE) strains of tick-borne encephalitis virus exhibit distinct ecological differences when compared with Omsk hemorrhagic fever virus (OHFV) despite the fact that they are genetically relatively closely related. TBEV-FEs are associated predominantly with *Ixodes persulcatus* ticks in forest environments in far-east Russia, whereas OHFV is found in the Steppe regions of western Siberia associated particularly with *Dermacentor* spp. and to a lesser extent with *Ixodes* spp. These viruses are also antigenically distinguishable in neutralization tests that employ convalescent sera.

Louping ill virus (LIV) and TBEV provide another example where, despite their close genetic relationships and similar host ranges, they display different ecologies (moorlands versus forests), pathogenicities (red grouse, sheep/goats versus humans) and geographical distributions (UK versus Europe/Eurasia), thus justifying their classification as distinct species.

On the other hand (like poliovirus with its three serotypes), the four dengue virus serotypes comprise a single species, despite being phylogenetically and antigenically quite distinct. This is justified by the fact that they co-circulate in the same geographical areas and ecological habitats, and that they exploit identical vectors, exhibit similar life cycles and disease manifestations.

## List of species in the genus *Flavivirus*

| | | |
|---|---|---|
| **Tick-borne** | | |
| **Mammalian tick-borne virus group:** | | |
| *Gadgets Gully virus* | | |
| Gadgets Gully virus | [DQ235145] | (GGYV) |
| *Kyasanur Forest disease virus* | | |
| Kyasanur Forest disease virus | [AY323490] | (KFDV) |
| Alkhumra hemorrhagic fever virus | [AF331718] | (AHFV) |
| *Langat virus* | | |
| Langat virus | [AF253419] | (LGTV) |

| | | |
|---|---|---|
| *Louping ill virus* | | |
| British subtype | [D12937] | (LIV-Brit) |
| Irish subtype | [X86784] | (LIV-Ir) |
| Spanish subtype | [DQ235152 | (LIV-Spain) |
| Turkish sheep encephalitis virus subtype | [DQ235151] | (TSEV) |
| Greek goat encephalitis virus subtype | [DQ235153] | (GGEV) |
| *Omsk hemorrhagic fever virus* | | |
| Omsk hemorrhagic fever virus | [AY323489] | (OHFV) |
| *Powassan virus* | | |
| Powassan virus | [L06436] | (POWV) |
| *Royal Farm virus* | | |
| Royal Farm virus | [DQ235149] | (RFV) |
| *Tick-borne encephalitis virus* | | |
| European subtype | [M27157, M33668] | (TBEV-Eur) |
| Far Eastern subtype | [X07755] | (TBEV-FE) |
| Siberian subtype | [L40361] | (TBEV-Sib) |
| **Seabird tick-borne virus group:** | | |
| *Meaban virus* | | |
| Meaban virus | [DQ235144] | (MEAV) |
| *Saumarez Reef virus* | | |
| Saumarez Reef virus | [DQ235150] | (SREV) |
| *Tyuleniy virus* | | |
| Tyuleniy virus | [DQ235148] | (TYUV) |
| **Kadam virus group (probably tick-borne):** | | |
| *Kadam virus* | | |
| Kadam virus | [DQ235146] | (KADV) |
| **Mosquito-borne** | | |
| **Aroa virus group:** | | |
| *Aroa virus* | | |
| Aroa virus | [AF013362] | (AROAV) |
| Bussuquara virus | [AF013366] | (BSQV) |
| Iguape virus | [AF013375] | (IGUV) |
| Naranjal virus | [AF013390] | (NJLV) |
| **Dengue virus group:** | | |
| *Dengue virus* | | |
| Dengue virus 1 | [U88536] | (DENV-1) |
| Dengue virus 2 | [M19197] | (DENV-2) |
| Dengue virus 3 | [M93130] | (DENV-3) |
| Dengue virus 4 | [AF326573] | (DENV-4) |
| **Japanese encephalitis virus group:** | | |
| *Cacipacore virus* | | |
| Cacipacore virus | [AF013367] | (CPCV) |
| *Japanese encephalitis virus* | | |
| Japanese encephalitis virus | [M18370] | (JEV) |
| *Koutango virus* | | |
| Koutango virus | [AF013384] | (KOUV) |
| *Murray Valley encephalitis virus* | | |
| Alfuy virus | [AF013360] | (ALFV) |
| Murray Valley encephalitis virus | [AF151266] | (MVEV) |
| *St Louis encephalitis virus* | | |
| St. Louis encephalitis virus | [DQ525916] | (SLEV) |
| *Usutu virus* | | |
| Usutu virus | [AF013412] | (USUV) |
| *West Nile virus* | | |
| Kunjin virus | [D00246] | (KUNV) |
| West Nile virus | [M12294] | (WNV) |
| *Yaounde virus* | | |
| Yaounde virus | [AF013413] | (YAOV) |
| **Kokobera virus group:** | | |
| *Kokobera virus* | | |
| Kokobera virus | [AF013383] | (KOKV) |
| Stratford virus | [AF013407] | (STRV) |

| | | |
|---|---|---|
| **Ntaya virus group:** | | |
| *Bagaza virus* | | |
| Bagaza virus | [AF013363] | (BAGV) |
| *Ilheus virus* | | |
| Ilheus virus | [AF013376] | (ILHV) |
| Rocio virus | [AF013397] | (ROCV) |
| *Israel turkey meningoencephalitis virus* | | |
| Israel turkey meningoencephalitis virus | [AF013377] | (ITV) |
| *Ntaya virus* | | |
| Ntaya virus | [AF013392] | (NTAV) |
| *Tembusu virus* | | |
| Tembusu virus | [AF013408] | (TMUV) |
| *Zika virus* | | |
| Zika virus | [DQ859059] | (ZIKV) |
| **Yellow fever virus group:** | | |
| *Sepik virus* | | |
| Sepik virus | [DQ859063] | (SEPV) |
| *Wesselsbron virus* | | |
| Wesselsbron virus | [DQ859058] | (WSLV) |
| *Yellow fever virus* | | |
| Yellow fever virus | [X03700] | (YFV) |
| **Probably mosquito-borne** | | |
| **Kedougou virus group:** | | |
| *Kedougou virus* | | |
| Kedougou virus | [DQ859061] | (KEDV) |
| **Edge Hill virus group:** | | |
| *Banzi virus* | | |
| Banzi virus | [DQ859056] | (BANV) |
| *Bouboui virus* | | |
| Bouboui virus | [DQ859057] | (BOUV) |
| *Edge Hill virus* | | |
| Edge Hill virus | [DQ859060] | (EHV) |
| *Jugra virus* | | |
| Jugra virus | [DQ859066] | (JUGV) |
| *Saboya virus* | | |
| Potiskum virus | [DQ859067] | (POTV) |
| Saboya virus | [DQ859062] | (SABV) |
| *Uganda S virus* | | |
| Uganda S virus | [DQ859065] | (UGSV) |
| **Species with no known arthropod vector** | | |
| **Entebbe bat virus group:** | | |
| *Entebbe bat virus* | | |
| Entebbe bat virus | [AF013373] | (ENTV) |
| Sokoluk virus | [AF013405] | (SOKV) |
| *Yokose virus* | | |
| Yokose virus | [AF013414] | (YOKV) |
| **Modoc virus group:** | | |
| *Apoi virus* | | |
| Apoi virus | [AF160193] | (APOIV) |
| *Cowbone Ridge virus* | | |
| Cowbone Ridge virus | [AF013370] | (CRV) |
| *Jutiapa virus* | | |
| Jutiapa virus | [AF013379] | (JUTV) |
| *Modoc virus* | | |
| Modoc virus | [AJ242984] | (MODV) |
| *Sal Vieja virus* | | |
| Sal Vieja virus | [AF013401] | (SVV) |
| *San Perlita virus* | | |
| San Perlita virus | [AF013402] | (SPV) |
| **Rio Bravo virus group:** | | |
| *Bukalasa bat virus* | | |
| Bukalasa bat virus | [AF013365] | (BBV) |

| | | |
|---|---|---|
| *Carey Island virus* | | |
| Carey Island virus | [AF013368] | (CIV) |
| *Dakar bat virus* | | |
| Dakar bat virus | [AF013371] | (DBV) |
| *Montana myotis leukoencephalitis virus* | | |
| Montana myotis leukoencephalitis virus | [AJ299445] | (MMLV) |
| *Phnom Penh bat virus* | | |
| Batu Cave virus | [AF013369] | (BCV) |
| Phnom Penh bat virus | [AF013394] | (PPBV) |
| *Rio Bravo virus* | | |
| Rio Bravo virus | [AF144692] | (RBV) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses that may be members of the genus *Flavivirus* but have not been approved as species

| | | |
|---|---|---|
| **Mammalian tick-borne** | | |
| Karshi virus | [DQ235147] | (KSIV) |
| **Mosquito-borne** | | |
| Spondweni virus | [DQ859064] | (SPOV) |
| **Probably arthropod-borne** | | |
| Aedes flavivirus | [AB488408] | (AEFV) |
| Cell fusing agent virus | [M91671] | (CFAV) |
| Culex flavivirus | [GQ165808] | (CXFV) |
| Kamiti River virus | [AY149905] | (KRV) |
| Nakiwogo virus | [GQ165809] | (NAKV) |
| Quang Binh virus | [FJ644291] | (QBV) |
| **Viruses with no known arthropod vector** | | |
| Chaoyang virus | [FJ883471] | (CHAOV) |
| Lammi virus | [FJ606789] | (LAMV) |
| Ngoye virus | [DQ400858] | (NGOV) |
| Nounané virus | [EU159426] | (NOUV) |
| Tamana bat virus | [AF286080] | (TABV) |

## Genus *Pestivirus*

Type species ***Bovine viral diarrhea virus 1***

### Distinguishing features

Relative to the other genera, pestiviruses encode two unique gene products, namely $N^{pro}$ and $E^{rns}$. The first protein of the ORF, nonstructural protein $N^{pro}$, which possesses an autoproteolytic activity and is responsible for its release from the nascent polyprotein, is not essential for virus replication in cell culture. One of the three viral envelope glycoproteins, $E^{rns}$, possesses an intrinsic RNase activity. Both of these unique pestivirus proteins are involved in repression of the host type I IFN response. Two biotypes of pestiviruses, cytopathogenic (cp) and non-cytopathogenic (noncp) viruses, are distinguished by their ability to cause cytopathic effects in cell culture.

### Virion properties

#### Morphology

Virions are 40–60 nm in diameter and spherical in shape (Figure 3). The virion envelope has 10–12 nm ring-like subunits on its surface. Structure and symmetry of the core have not been characterized.

#### Physicochemical and physical properties

Virion Mr has not been determined precisely. Buoyant density in sucrose is 1.10–1.15 g $cm^{-3}$; $S_{20,W}$ is 140–150S. Virion infectivity is stable over a relatively broad pH range, but unstable at temperatures above 40 °C. Organic solvents and detergents rapidly inactivate these viruses.

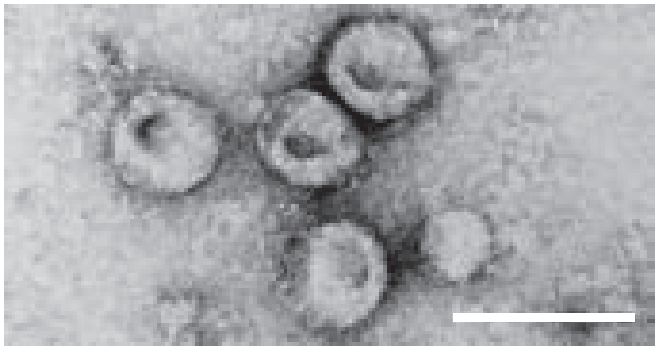

**Figure 3:** Negative contrast electron micrograph of particles of an isolate of bovine viral diarrhea virus 1. The bar represents 100 nm. (From M. König, with permission.)

### Nucleic acid

The virion RNA is a positive sense, infectious molecule of ssRNA about 12.3 kb in size. The 5′-NCR contains an IRES and is about 370–385 nt in length. The 3′-NCR, with about 185–273 nt, is complex and contains a region with variable sequences and a highly conserved terminal region. Genomic RNA contains a single ORF spanning the viral genome. For some cp pestivirus strains, a small and variable segment of host cell or viral nucleic acid is integrated into particular regions (often within NS2 or directly upstream of NS3) of the viral genome, sometimes accompanied by viral gene duplications or deletions. Other cp pestiviruses contain only viral gene duplications involving all or part of the $N^{pro}$ and NS3 to NS4B protein-coding regions, resulting in genomic RNA of up to about 16.5 kb. In all cases, the single large ORF is maintained. Finally, cp viruses may also arise by deletion of large portions of their genomes. Such defective genomes can be rescued by co-infecting intact helper viruses.

### Proteins

Virions are composed of four structural proteins: a basic nucleocapsid core protein, C (14 kDa) and three envelope glycoproteins, $E^{rns}$ (gp44/48), E1 (gp33) and E2 (gp55). All three glycoproteins exist as intermolecular disulfide-linked complexes: $E^{rns}$ homodimers, E1-E2 heterodimers and E2 homodimers. The $E^{rns}$ protein possesses an intrinsic RNase activity. Pestiviruses encode eight nonstructural (NS) proteins among which $N^{pro}$ (23 kDa), p7 (7 kDa) and NS2 (40 kDa) are not necessary for RNA replication. $N^{pro}$ is a proteinase that auto-catalytically releases itself from the nascent polyprotein. Nonstructural protein p7 is presumed to have a role in virus maturation. NS2-3 (120 kDa) is a multifunctional protein. The N-terminal 40% (NS2) is hydrophobic and contains a zinc finger motif for binding of a divalent metal ion. NS2 is a cysteine protease that is responsible for processing of NS2-3 to give rise to NS2 and NS3. NS3 (80 kDa) acts as both a serine protease involved in polyprotein processing and an RNA helicase/NTPase involved in RNA replication. NS2-3 is found after infection with all pestiviruses. In cells infected with cp pestiviruses, large amounts of NS3 can be detected. For noncp BVDV, noncp BDV and CSFV strains, only a minor fraction of NS2-3 is cleaved into NS2 and NS3, so that sometimes the cleavage products are difficult to detect. The NS4A (7 kDa) protein acts as a cofactor to the NS3 protease activity. The role of NS4B (33 kDa) is unknown. NS5A (58 kDa) represents a phosphorylated protein and presumably also plays a yet to be identified role in RNA replication. NS5B (75 kDa) possesses RdRp activity.

### Lipids

The viruses are enveloped, but no reports have described the lipid composition.

### Carbohydrates

All virus envelope glycoproteins contain N-linked glycans.

## Genome organization and replication

The genomic RNA contains a single large ORF encoding a polyprotein of about 3900 aa that is preceded by a 5′-NCR of about 370–385 nt and followed by a 3′-NCR of about 185–273 nt. The gene order is 5′-$N^{pro}$-C-$E^{rns}$-E1-E2-p7-NS2-3(NS2-NS3)-NS4A-NS4B-NS5A-NS5B-3′ (Figure 4).

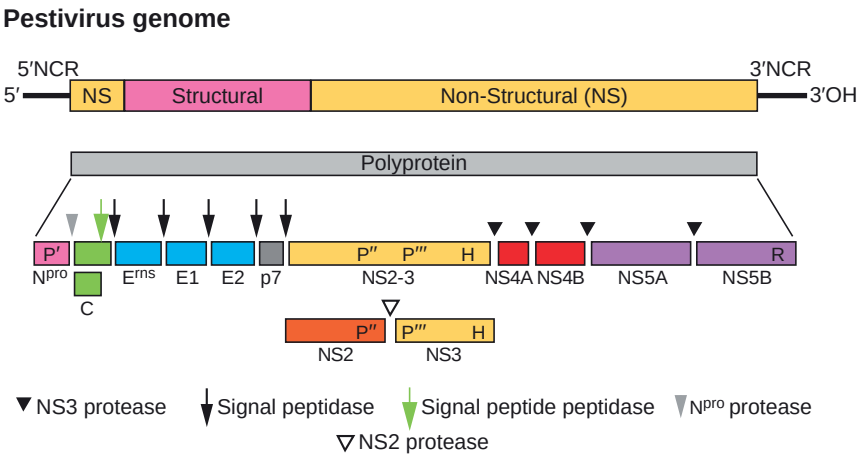


**Figure 4:** Pestivirus genome organization (not to scale) and polyprotein processing. The RNA is usually about 12.3 kb in size (depending on the virus). The 5′-NCR is about 370–385 nt, the ORF about 11.7 kb and the 3′-NCR is 185-273 nt. The viral nonstructural proteins are indicated as NS. P′, P″, P‴, H and R symbols indicate the localization of the $N^{pro}$ protease, the NS2 protease, the NS3 protease, the NS3 RNA helicase and the NS5B RdRp, respectively. The proteases and proteolytic steps involved in the generation of individual proteins are indicated. In noncp BVD viruses, NS2-3 cleavage is detectable only for a short time early after infection. In cp BVD viruses, NS3 is produced continuously in addition to NS2-3.

Pestivirus replication is initiated by receptor-mediated endocytosis involving most likely more than one cell surface molecule and viral glycoproteins $E^{rns}$ and E2. CD46 has been shown to function as a cellular receptor for BVDV but is not by itself sufficient to mediate infection. After endocytosis and uncoating, the genome RNA serves as mRNA; there are no subgenomic mRNA molecules. Translation initiation occurs by a cap-independent internal initiation mechanism involving an IRES within the 5′-NCR of the RNA. Polyprotein processing occurs co- and post-translationally by both cellular and viral proteases. Nonstructural protein $N^{pro}$, the first protein of the ORF, auto-proteolytically removes itself from the nascent polyprotein by cleavage at the $N^{pro}$/C site. Downstream cleavages that produce structural proteins C, $E^{rns}$, E1 and E2 as well as p7 are mediated by cellular signal peptide peptidase and signal peptidase(s). Glycoprotein translocation to the endoplasmic reticulum occurs by an internal signal sequence, within the C-terminal region of the C protein. Cleavage between E2 and p7 is not complete, leading to two intracellular forms of E2 with different C-termini. Depending on the pestivirus biotype, NS2-3 either remains mostly intact or is found at reduced levels together with high amounts of its N- and C-terminal products NS2 and NS3. The increased generation of NS3 in cp pestiviruses is in most cases due to RNA recombination. Most cp pestiviruses have gene insertions, deletions, duplications or rearrangements that result in enhanced NS3 production. The NS3/NS2-3 serine protease activity is responsible for all processing events downstream of NS3. NS4A facilitates cleavages by the NS3 protease of sites 4B/5A and 5A/5B.

RNA replication occurs most likely in association with intracytoplasmic membranes, presumably in a replication complex composed of viral RNA and viral nonstructural proteins. Nonstructural proteins NS3, 4A, 4B, 5A and 5B are necessary for RNA replication; only NS5A can be provided in *trans*. Replicative forms of pestiviral RNA have been detected. The ratio of positive-to-negative sense RNA in cells 12 hours post-infection is about 10. RNA synthesis is resistant to actinomycin D. Virus maturation is poorly understood. However, viral proteins are not found on the cell surface, suggesting that viruses mature in intracellular vesicles and are released by exocytosis. Considerable amounts of infectious virus remain cell-associated. Host cell RNA and protein synthesis continues throughout infection.

## Antigenic properties

Pestiviruses are antigenically related, and cross-reactive epitopes are found for all members. Separate antigenic determinants defined by monoclonal antibodies (Mabs) have also been identified. Antigenic variation is particularly pronounced among isolates of BVDV and BDV. The

N-terminal portion of E2 contains an antigenically hypervariable region. Mab binding patterns are generally consistent with the genetic relatedness of viruses.

Infected animals mount potent antibody responses to two structural glycoproteins ($E^{rns}$, E2) and to the NS2-3/NS3 protein, while antibody responses to other virus-encoded polypeptides are weak or not detectable. $E^{rns}$ and E2 are able to induce protection independently. Mabs reactive with these proteins can neutralize virus infectivity.

## Biological properties

Pestiviruses infect pigs and ruminants, including cattle, sheep, goats and wild ruminants. There are no invertebrate hosts. Transmission occurs by direct and indirect contact (e.g., nasal or urine secretion, feces, contaminated food, etc.). Transplacental transmission occurs in all host species. Pestivirus infections may be subclinical or produce a range of clinical conditions including acute diarrhea, acute hemorrhagic syndrome, acute fatal disease, and a wasting disease. Transplacental infection can result in fetal death, congenital abnormalities, or lifelong persistent infection. Fatal mucosal disease can occur in cattle persistently infected with noncp viruses when a cp virus is generated by mutation or introduced by superinfection. Pestivirus infections of livestock are economically important worldwide.

Experimental infection models have not been established for bovine or ovine pestiviruses outside their natural mammalian hosts. However, CSFV can be adapted to propagate in rabbits. Cells derived from natural host species (bovine, porcine, ovine) support virus replication. Most virus isolates are noncp and can establish persistent infections in cell culture. Infectious noncp BVDV is often present in bovine serum products used for cell culture. Cp pestiviruses induce extensive cytopathology and form virus plaques under appropriate conditions. Death of cp pestivirus infected cells is due to apoptosis. No hemagglutinating activity has been found associated with pestiviruses.

## Species demarcation criteria in the genus

Species demarcation criteria in the genus include:

- Nucleotide and deduced amino acid sequence relatedness.
- Antigenic relatedness.
- Host of origin.

Pestivirus species demarcation considers several parameters and their relationship to the type viruses of the four currently recognized species (BVDV-1 NADL; BVDV-2 890; BDV X818; and CSFV A187). Nucleotide sequence relatedness is an important criterion for pestivirus species demarcation. For example, the 5′-NCR sequences among the four currently recognized species are over 15% divergent. In most cases, the degree of genetic variation within the 5′-NCR will allow species demarcation. However, in some cases the nt sequence relatedness may be ambiguous and must be complemented with additional comparative analyses. Convalescent animal sera generated against members of a given species (e.g., *Bovine viral diarrhea virus 1*) generally show a several-fold higher neutralization titer against viruses of the same species than against viruses from the other species. Finally, differences in host of origin and disease can assist in species identification.

For example, *Bovine viral diarrhea virus 1* and *Classical swine fever virus* are considered different species because their members differ from each other by: (i) at least 25% at the sequence level (complete genomes), (ii) at least 10-fold difference in neutralization titer in cross-neutralization tests with polyclonal immune sera, and (iii) host range, in that under natural conditions CSFV infects only pigs while BVDV-1 infects ruminants as well as pigs.

The two species BVDV-1 and BVDV-2 are often referred to as genotypes 1 and 2 of BVDV. The genetic and antigenic differences between BVDV-1 and BVDV-2 are comparable to the ones among isolates of species BDV, which has been classified into several genotypes (e.g. BDV-1, -2, etc). For CSFV three genotypes (CSFV-1, CSFV-2, and CSFV-3) are recognized. In addition, the genotypes of these *Pestivirus* species can be further divided into subgroups.

In order to officially establish a novel pestivirus species or genotype, the complete genomic sequence of at least one virus isolate together with data on antigenic relatedness should be provided.

### List of species in the genus *Pestivirus*

| | | |
|---|---|---|
| *Border disease virus* | | |
| Border disease virus 1a BD31 | [U70263] | (BDV-1 BD31) |
| Border disease virus 1a X818 | [AF037405] | (BDV-1 X818) |
| Border disease virus 2 Reindeer-1 | [AF144618] | (BDV-2 V60) |
| Border disease virus 3 Gifhorn | [GQ902940] | (BDV-3 Gifhorn) |
| Border disease virus 4 Chamois-1 | [GU270877] | (BDV-4 H2121) |
| *Bovine viral diarrhea virus 1* | | |
| Bovine viral diarrhea virus 1a NADL | [M31182] | (BVDV-1a NADL) |
| Bovine viral diarrhea virus 1a SD1 | [M96751] | (BVDV-1a SD1) |
| Bovine viral diarrhea virus 1b CP7 | [U63479] | (BVDV-1b CP7) |
| Bovine viral diarrhea virus 1b Osloss | [M96687] | (BVDV-1b Osloss) |
| *Bovine viral diarrhea virus 2* | | |
| Bovine viral diarrhea virus 2 C413 | [AF002227] | (BVDV-2 C413) |
| Bovine viral diarrhea virus 2 NewYork'93 | [AF502399] | (BVDV-2 NY93) |
| Bovine viral diarrhea virus 2 890 | [U18059] | (BVDV-2 890) |
| *Classical swine fever virus* (Hog cholera virus) | | |
| Classical swine fever virus 1.1 Alfort/187 | [X87939] | (CSFV-1.1 A187) |
| Classical swine fever virus 1.1 C-strain | [Z46258] | (CSFV-1.1 C) |
| Classical swine fever virus 1.2 Brescia | [M31768] | (CSFV-1.2 Brescia) |
| Classical swine fever virus 2.3 Alfort-Tübingen | [J04358] | (CSFV-2.3 Alfort-T) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses that may be members of the genus *Pestivirus* but have not been approved as species

| | | |
|---|---|---|
| Atypical pestivirus | [FJ040215] | Th/04_KhonKaen |
| Bungowannah virus | [EF100713] | Bungo |
| Giraffe-1 pestivirus | [AF144617] | Giraffe-1 (H138) |
| Pronghorn antelope pestivirus | [AY781152] | Pronghorn |

## GENUS *HEPACIVIRUS*

Type species ***Hepatitis C virus***

### Distinguishing features

Hepatitis C virus (HCV) is transmitted between humans, principally via exposure to contaminated blood or blood products. There is no known invertebrate vector. Hepaciviruses differ from members of the genera *Flavivirus* and *Pestivirus* by their limited ability to be propagated in cell culture, since only a single HCV strain, JFH1, has been found to efficiently infect cultured cells, and only one specific human hepatoma cell line (Huh7) is susceptible to infection with this strain. In the HCV precursor protein, the NS2-3 junction is auto-catalytically cleaved by Zn-dependent NS2-3 protease activity.

### Virion properties

#### MORPHOLOGY

Virions are about 50 nm in diameter, as determined by filtration and electron microscopy. They are spherical in shape and contain a lipid envelope, as determined by electron microscopy and inactivation by chloroform. The viral core is spherical and about 30 nm in diameter. Detailed structural properties have not been determined.

### Physicochemical and physical properties

Virion Mr has not been determined. Buoyant density in sucrose is predominantly about 1.06 g cm$^{-3}$ for virus recovered from serum during acute infections while more dense forms (ca. 1.15–1.18 g cm$^{-3}$) predominate when recovered from the serum of chronically infected individuals. The lower density results from physical association of the virion with serum very-low-density lipoproteins (VLDLs). The higher density results from the binding of serum antibodies to the virion. A buoyant density range in isosmotic iodixanol gradients of 1.01–1.10 g cm$^{-3}$ has been measured for HCV recovered from hepatoma cells infected with HCV; the different densities are believed to be due to differences in the association with low-density lipoproteins/VLDL components. The $S_{20,w}$ is equal to or greater than 150S. The virus is stable in buffer at pH 8.0–8.7. Virions are sensitive to heat, organic solvents and detergents.

### Nucleic acid

Virions of HCV contain a single positive sense, infectious ssRNA (Figure 5). The genome length is about 9.6 kb. The 5′-NCR contains an IRES and is about 340 nt long. The 3′-NCR contains a sequence-variable region of about 50 nt, a polypyrimidine-rich region averaging about 100 nt (but highly variable in length) and a highly conserved 98 nt long 3′-terminal region with three defined stem-loop RNA secondary structures. There are at least two seed sites in the HCV 5′-NCR for the liver abundant microRNA miR-122 that are required for efficient HCV replication.

A divergent strain of the genus named "GB virus B" has a closely similar genome organization to HCV. However, it has a longer 5′-NCR of 445 nt with an HCV-like IRES structure. Furthermore, the RNA encodes a shorter polyprotein of 2864 aa, and with a substantial sequence divergence to HCV, with only 28% aa identity between the encoded polyproteins, compared to within the *Hepatitis C virus* species itself (>60% aa identity).

### Proteins

The HCV virion consists of at least three proteins: the nucleocapsid core protein C (p19-21), and two envelope glycoproteins, E1 (gp31) and E2 (gp70). An additional protein, p7 (believed to have properties of an ion channel protein important in viral assembly) is incompletely cleaved from a precursor of E2 to yield E2-p7 and p7, but it is not known whether these are virion structural components. In GB virus B, there is a corresponding protein, p13, that apparently can be cleaved into p7 and p6 proteins. The two envelope glycoproteins can associate as non-covalent heterodimers; recent data, however, indicate that they are covalently linked complexes in virions. The recognized nonstructural proteins include NS2 (21 kDa protein that, before cleavage, is part of a Zn-dependent cysteine protease that bridges NS2 and NS3 and mediates autocatalytic cleavage of the NS2/NS3 junction, and is involved with virus assembly and release), NS3 (70 kDa protein with additional serine protease, helicase and NTPase acitivities; the NS3 protease cleaves the remaining junctions between nonstructural proteins), NS4A (6 kDa cofactor essential for trans NS3 serine protease activity), NS4B (27 kDa protein that induces a membranous replication complex at the endoplasmic reticulum), NS5A (a serine phosphoprotein of unknown specific function, but critical for viral replication and assembly, that exists in 56 and 58 kDa forms, depending on the degree of phosphorylation) and NS5B (68 kDa protein with RdRp activity).

### Lipids

Virions have a lipid bi-layer envelope. Historically, based on observed removal of the viral envelope and loss of infectivity following exposure to solvents or detergents, the presence of lipids was inferred. Recently, it has become apparent that the host lipid metabolism plays a critical role in the viral life cycle.

### Carbohydrates

The E1 and E2 glycoproteins contain numerous N-linked glycosylation sites, and the demonstration of carbohydrate associated with the products of these two HCV genes expressed as recombinant proteins or in HCV retroviral pseudo-particles is consistent with the presence of carbohydrates in virions. E1 and E2 are TM type I glycoproteins, with C terminal ER retention signals, anchored within the lumen of the endoplasmic reticulum. They are apparently masked when budding occurs allowing the virion to move through the secretory pathway. Recent data in culture systems indicate that N-linked glycans of E1 remain in the high-mannose chains lacking complex carbohydrate, whereas those of E2 are modified. Glucosylation influences E1–E2 heterodimer formation, folding and assembly and release of virions.

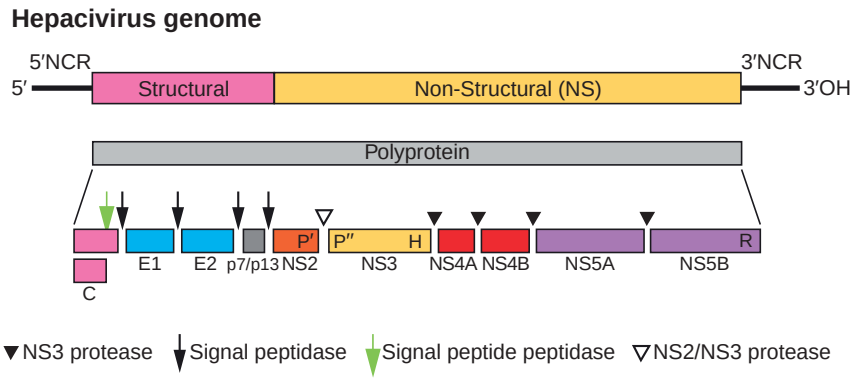


**Figure 5:** Hepacivirus genome organization (not to scale) and polyprotein processing. For species *Hepatitis C virus*, the RNA is about 9.6 kb in size. The 5′-NCR is about 340 nt, the 3′-NCR is about 250 nt, and the ORF is about 9 kb. The species HCV has a p7 protein between E2 and NS2; the other possible member of the genus, GB virus B, has a p13 protein, which can be cleaved into p7 and p6. The host and viral proteases involved in cleavage of the polyprotein are indicated. The cleavage by host signal peptide peptidase (at the C-terminus of core) is indicated by an open arrow; the cleavages by host signal peptidase (remaining sites) are indicated by filled arrows. The locations of the NS2-3 protease, NS3 protease, NS3 RNA helicase and NS5B RdRp are indicated by P′, P″, H and R, respectively.

## Genome organization and replication

The genome contains a single large ORF encoding a polyprotein of about 3000 aa (Figure 5). The gene order is 5′-C-E1-E2-p7-NS2-NS3-NS4A-NS4B-NS5A-NS5B-3′. All three structural proteins (C, E1, E2) are encoded within the amino-terminal portion of the large ORF. Immediately downstream is a small protein, p7 (HCV) or p13 (GB virus B), followed by the nonstructural proteins in the 3′ portion of the ORF. Replication is poorly understood but occurs in association with intracytoplasmic membranes. Replicative forms of viral RNA have been detected in liver tissue. The genomic RNA is translated into a polyprotein that is rapidly processed both co- and post-translationally by host and viral proteases. Translation initiation occurs via an IRES within the 5′-NCR, which also contains several closely spaced AUGs. Translocation of the structural glycoproteins to the endoplasmic reticulum probably occurs via internal signal sequences. Cleavage of the structural proteins is mediated by host cell signal peptidases, and signal peptide peptidase. With the exception of the p7/NS2 signalase cleavage, viral proteases cleave all non-structural protein junctions. Virus assembly is believed to occur by budding into vesicles from the endoplasmic reticulum.

## Antigenic properties

Virus-specific antibodies to recombinant-expressed structural proteins (C, E1 and E2) and non-structural proteins (principally NS3, NS4 and NS5) have been detected in individuals infected with HCV. Both linear and conformational epitopes are believed to be involved in the humoral immune response of the host to infection. Significant antigenic diversity throughout the genome is reflected in heterogeneity in the humoral immune response, especially to the product of the NS4 gene. In HCV, high variability is found in the N-terminal 27 aa of E2 (hypervariable region 1; HVR1). The HVR1 contains an HCV neutralization epitope and escape variants of HVR1 are positively selected by the host humoral immune response. Other neutralization epitopes have been identified in E2 outside of HVR1, and at least one neutralization epitope has been identified in E1. Cell-mediated immune responses to all HCV proteins have been detected; it is believed that such responses are associated with amelioration or resolution of infection. With the recent development of the JFH1 culture system, and of intra- and intergenotypic genotype 1-7 JFH1-based recombinant viruses with strain-specific structural proteins, it is now possible to carry out *in vitro* virus neutralization assays.

## Biological properties

### Host range

Humans are the natural host and apparent reservoir of hepatitis C virus. The virus can be transmitted experimentally to chimpanzees. No other natural host has been identified. The natural host for GB virus B is not known.

### Transmission

Hepatitis C virus is transmitted almost exclusively by parenteral exposure to blood, blood products and objects contaminated with blood. Effective screening of blood donors and implementation of inactivation procedures have virtually eliminated the transmission of HCV via blood and blood products, but other routes of exposure, principally via blood-contaminated syringes, are now the most important recognized risk factors in developed countries. Sexual and mother-to-child transmission has been documented, but are relatively uncommon.

### Geographical distribution

HCV has a worldwide distribution. Antibody-based epidemiological studies suggest that about 0.1–2% of the populations of developed countries may be infected with HCV, but antibody prevalence as high as 20% has been detected in some developing countries. The high prevalence of antibody to HCV is thought to be the result of using contaminated needles and syringes in such countries. Most estimates suggest that about 3% of the world population has been infected with HCV, and that more than 150 million people are chronically infected. There are about 4 million new infections per year.

### Pathogenicity

Infections range from subclinical to acute and chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. Persistence of the virus occurs in 60–80% of HCV infections, depending on the population studied. In about 20% of the cases, persistent HCV will progress to chronic active hepatitis and cirrhosis, usually over the course of many years. Patients with liver cirrhosis have an approximately 5% risk per year of developing hepatocellular carcinoma.

Persistent HCV infection has been epidemiologically linked to primary liver cancer, cryptogenic cirrhosis and some forms of autoimmune hepatitis. Extrahepatic manifestations of HCV infection include mixed cryoglobulinemia with associated membrano-proliferative glomerulonephritis and, possibly, porphyria cutanea tarda, Sjögren's-like syndromes and other autoimmune conditions.

Like HCV, GB virus B causes hepatitis and replicates in the liver. However, it only infects tamarins and owl monkeys, not humans or chimpanzees. GB virus B causes self-limiting hepatitis in experimentally infected tamarins and owl monkeys, whereas HCV typically causes chronic hepatitis in man and chimpanzees. Only one variant of GB virus B has been identified to date, in contrast to hundreds of HCV variants.

### Cell tropism

HCV has been reported to replicate in several cell lines derived from hepatocytes and lymphocytes, but virus growth has only been sufficient for practical application of these systems in a human hepatoma cell line, Huh7 cells and derivatives. *In vivo*, HCV replicates in hepatocytes and possibly lymphocytes.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Hepacivirus*

The genus *Hepacivirus* comprises a single species, *Hepatitis C virus*. However, the species can be classified into seven genetic groups (termed genotypes; see table below), based upon the genome-wide heterogeneity of isolates recovered throughout the world. These differ from each other by about 30–35% at the nt level. Within each genotype, there are a number of subtypes, differing from each other by about 15–25% at the nt level. Although genotypes are distinct genetically, discrimination of subtypes is less clear, particularly in areas of high diversity such as sub-Saharan Africa and South-East Asia. Because systematic serological typing by virus neutralization has not been performed to date, and because major genotypes do not have any other taxonomic characteristics except, in some cases, geographic distribution and differences in treatment response, the seven genetic groups of HCV currently comprise one species. Complete or near complete genomic sequences have been obtained from each of the seven genotypes of HCV, and functionality (replication competence) has been demonstrated by intrahepatic chimpanzee inoculation of RNA transcripts from cDNA clones of genotypes 1a [strains H77 (AF011751 and AF009606), HCV-1 (AF271632), HC-TN (EF62148)], 1b [Con1 (AJ238799), HCV-N (AF139594)], 2a [HC-J6 (AF177036) and JFH1 (AB047639)], 3a [S52 (GU814264)] and 4a [ED43 (GU814264)].

| | | |
|---|---|---|
| *Hepatitis C virus* | | |
| HCV genotype 1a /HPCPLYPRE | [M62321] | (HCV-1) |
| HCV genotype 1b /HPCJCG | [D90208] | (J) |
| HCV genotype 2a /HPCPOLP | [D00944] | (J6) |
| HCV genotype 2b /HPCJ8G | [D01221] | (J8) |
| HCV genotype 3a /HPCEGS | [D17763] | (NZL-1) |
| HCV genotype 3k /HPCJK049E1 | [D63821] | (JK049) |
| HCV genotype 4a /HCV4APOLY | [Y11604] | (ED43) |
| HCV genotype 4d | [DQ516083] | (24) |
| HCV genotype 5a /HCV1480 | [Y13184] | (HCV1480) |
| HCV genotype 6a /HCV12083 | [Y12083] | (EUHK2) |
| HCV genotype 6g /HPCJK046E2 | [D63822] | (JK046) |
| HCV genotype 7a | [EF108306] | (QC-69) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses that may be members of the genus *Hepacivirus* but have not been approved as species

GB virus B comprises one isolate, and its functionality (replication competence) has been demonstrated by intrahepatic inoculation of RNA transcripts from a cDNA clone into tamarins (AF179612).

| | | |
|---|---|---|
| GB virus B | [U22304] | (GBV-B) |

## Phylogenetic relationships within the family

### Divergent members of the family *Flaviviridae*: evidence for a possible fourth genus

Viruses identified in humans (GBV-C, also known as hepatitis G virus; HGV), chimpanzees (GBV-Ctro), a range of New World monkey species (GBV-A), and a fruit bat (GBV-D), show distant sequence relatedness to other members of the family *Flaviviridae*, forming a distinct cluster based on phylogenetic analysis of RdRp (Figure 6) and helicase sequences. In addition to their separate phylogenetic position, they show several differences in genome organization from *Hepacivirus* and other *Flaviviridae* genera (including an IRES structurally unrelated to those of hepaciviruses and pestiviruses, and the apparent absence of a gene encoding a nucleocapsid protein). These differences merit consideration of this cluster as a separate genus.

GBV-A and GBV-A-like agents are a group of related viruses that have been identified in at least six species of New World monkeys. They do not cause hepatitis in the unique host species of each virus nor in other susceptible species. Their organ site of replication has not been identified and, although the viruses are transmissible via blood, their natural route of transmission is unknown. They cause persistent infection in their respective host species.

GBV-C (or HGV) infects humans worldwide, as well as chimpanzees and potentially other apes. In both humans and chimpanzees, frequencies of viremia range from 2 to 10%. Although GBV-C is transmitted via blood and blood products, its primary route of infection is through sexual contact and from mother-to-child. GBV-C establishes persistent infections in a proportion of those infected (<25%), recovery being associated with seroconversion for anti-E2 antibodies (viremic individuals remain seronegative). Although originally described as a hepatitis virus, it rarely, if ever, causes hepatitis, and its pathogenicity and organ site of replication remain controversial. Some studies suggested that lymphocytes might be its primary site of replication.

GBV-D is the most genetically divergent member of this group. It was detected and genetically characterized from sera from an Old World frugivorous bat (*Pteropus giganteus*). Infected bats similarly show no evidence of hepatitis, while its frequent detection in serum of this bat species indicates likely persistence of infection.

These viruses show greatest similarity in overall genomic organization and sequence relatedness to hepaciviruses (Figure 6), but differ in that they appear to lack a complete nucleocapsid protein gene, they lack the type 3 IRES found in hepaciviruses (including GBV-B) and pestiviruses, and their 3′-NCR is less complex than that of the hepaciviruses.

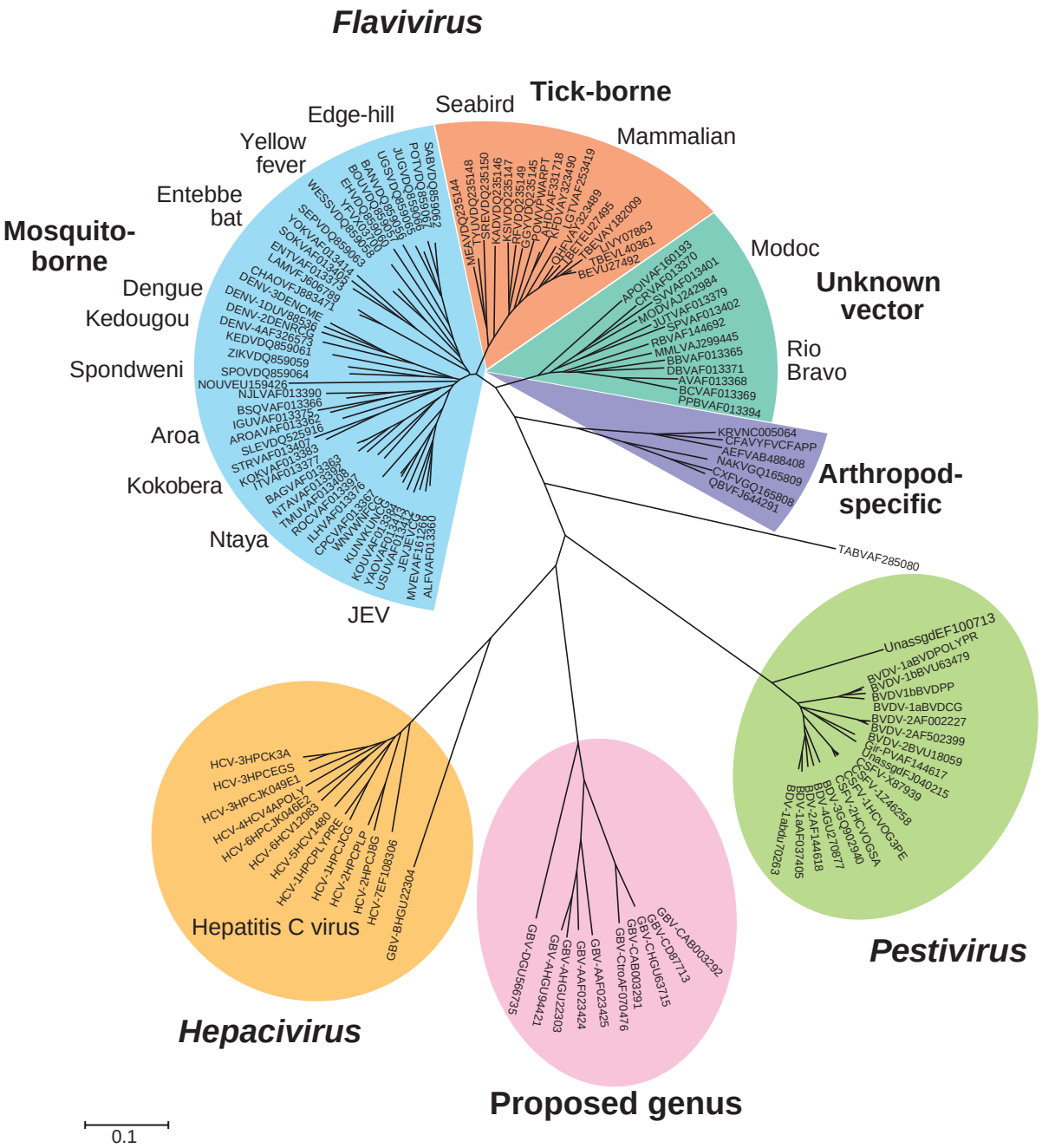


**Figure 6:** Phylogeny of the conserved sequences in the RdRp (NS5 or NS5B) of classified members of the family *Flaviviridae*. Partial gene sequences (292 aa, positions 7704–8550 numbered as in the HCV-1 genome, AF011751) from the representative strains listed in each table. CLUSTALW was used to create a multiple alignment for the aa sequences which was verified by alignment of the known motifs in the region. An unrooted phylogenetic tree was constructed from the sequence alignment by neighbor-joining of (uncorrected) amino acid p-distances using the MEGA version 4.1 package. The virus names corresponding to the abbreviations can be found in the "List of species" in each genus. Note that the Entebbe virus group comprising Entebbe bat virus, Sokoluk virus and Yokose virus have no known vector but group phylogenetically within the mosquito-borne flaviviruses. Nounané, Chaoyang, Ngoye and Lammi viruses are not currently assigned to a specific virus group but also group within the mosquito-borne viruses.

Pos. ssRNA

The names currently assigned to viruses in this putative genus are regarded as unsuitable for several reasons, being based on a patient's name (initials GB) or an unproven disease association (HGV). The letter suffixes (-A, -C and -D) do not indicate host origin or genetic relationships. Proposals for a revision of their nomenclature and assignment of a fourth genus in the family *Flaviviridae* are under consideration.

| | | |
|---|---|---|
| GBV-A | [U94421] | (Alab) |
| GBV-C | [U63715] | (GBV-EA) |
| GBV-C | [U45966] | (HGV-R10291) |
| GBV-C(chimpanzee) | [AF070476] | (GBV-Ctro) |
| GBV-D | [GU566735] | (GBV-D strain 93) |

Viruses in the divergent group of GB flaviviruses, members of a possible fourth genus

## Similarity with other taxa

The RdRp of flaviviruses shows distant similarity with those of some plant virus families (e.g. *Tombusviridae*) and has been assigned into RNA virus supergroup 3. However, virion structure and other viral structural and nonstructural genes are distinct and likely non-homologous.

## Derivation of names

*Flavi*: from Latin *flavus*, "yellow".
*Pesti*: from Latin *pestis*, "plague".
*Hepaci*: from Greek *hepar*, *hepatos*, "liver".

## Further reading

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## Contributed by

Simmonds, P., Becher, P., Collett, M.S., Gould, E.A., Heinz, F.X., Meyers, G., Monath, T., Pletnev, A., Rice, C.M., Stiasny, K., Thiel, H.-J., Weiner, A. and Bukh, J.

The chapter in the Eighth ICTV Report, which served as the template for this chapter, was contributed by Thiel, H.-J., Collett, M.S., Gould, E.A., Heinz, F.X., Houghton, M., Meyers, G., Purcell, R.H. and Rice, C.M.

# Family *Hepeviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Hepeviridae*** |
| Genus | ***Hepevirus*** |

## Virion properties

### Morphology

Virions of hepatitis E virus (HEV) are icosahedral, non-enveloped, spherical particles with a diameter of approximately 27–34 nm (Figure 1). The capsid is formed by capsomeres consisting of homodimers of a single capsid protein forming the virus shell. Each capsid protein contains three linear domains forming distinct structural elements: S (the continuous capsid), P1 (three-fold protrusions), and P2 (two-fold spikes). Each domain contains a putative polysaccharide-binding site that may interact with cellular receptors. Native T = 3 capsid contains flat dimers, with less curvature than those of T = 1 virus-like particles (Figure 2).

Virion particles of avian hepatitis E virus (Figure 3) revealed by negative staining EM of bile samples from chickens with hepatitis–splenomegaly syndrome are similar in size and morphology to members of the genus *Hepevirus*.

### Physicochemical and physical properties

Virion buoyant density is 1.35 to 1.40 g cm$^{-3}$ in CsCl and 1.29 g cm$^{-3}$ in glycerol and potassium tartrate gradients. Virion $S_{20,w}$ is 183S. Virion is sensitive to low-temperature storage (between −70 °C and +8 °C) and iodinated disinfectants. The virion of hepatitis E virus is more heat-labile than is hepatitis A virus: hepatitis E virus was about 50% inactivated at 56 °C and almost totally inactivated (96%) at 60 °C for 1 h. Liver suspensions containing avian hepatitis E virus remained infectious after treatment with chloroform and ether but lost infectivity after incubating at 56 °C for 1 h or 37 °C for 6 h. Viral infectivity in liver suspensions was reduced 1000-fold after treatment with 0.05% Tween-20, 0.1% NP40 and 0.05% formalin.

### Nucleic acid

The HEV genome is a linear, positive sense, ssRNA molecule of approximately 7.2 kb, with a 5′-m$^7$G cap structure and a 3′-poly(A) tail. The genome of avian hepatitis E virus is similar but only about 6.6 kb in size.

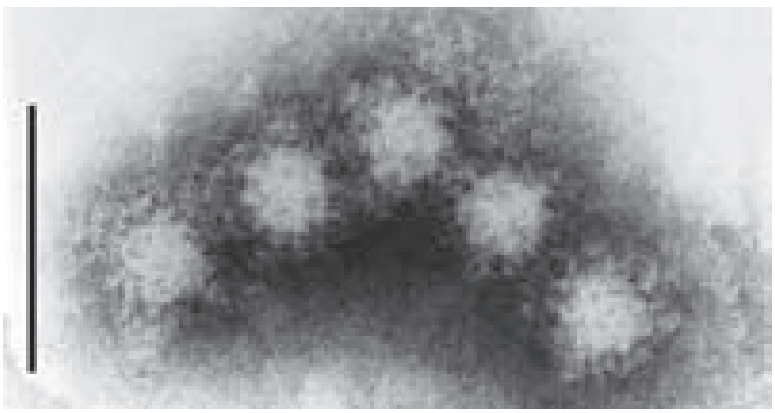

**Figure 1:** Negative contrast electron micrograph of virions of an isolate of hepatitis E virus, in the bile fluid from a monkey challenged with the genotype 2 Mexican strain of hepatitis E virus. The bar represents 100 nm. (From Ticehurst *et al*. (1992). *J. Infect. Dis.*, **165**, 835–845; with permission.)

Pos. ssRNA

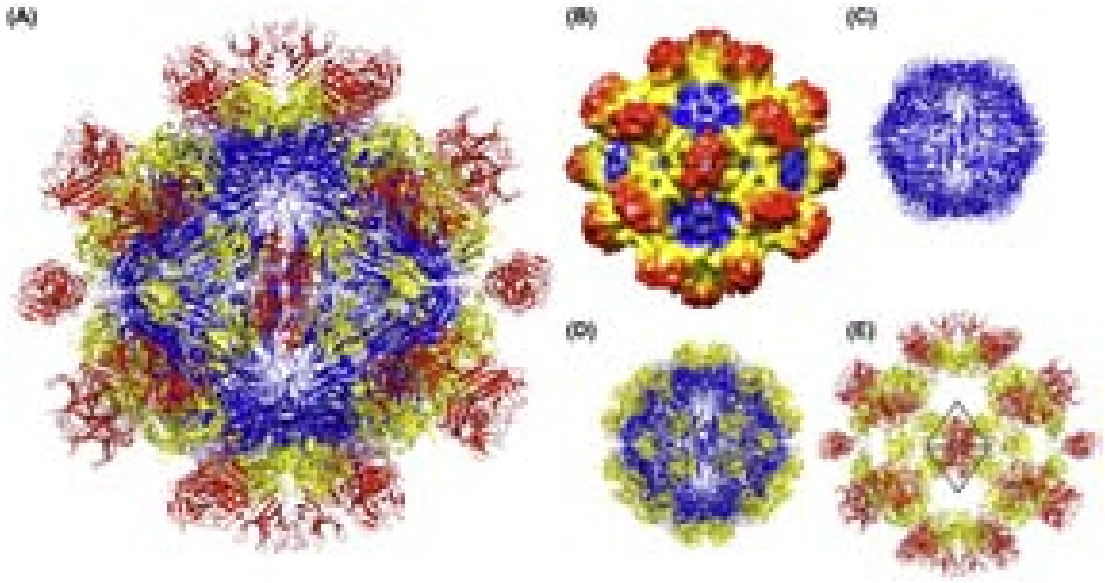


**Figure 2:** Structure of the hepatitis E virus-like particle (VLP) ($T = 1$). (A) Crystal structure of hepatitis E virus VLP. The three domains, S, P1 and P2 are colored blue, yellow and red, respectively. The VLP is positioned in a standard orientation with the 3 2-fold icosahedral symmetry axes aligned along the vertical, horizontal, and viewing directions, respectively. (B) Cryo-EM reconstruction at 14 Å resolution. The surface is colored by radial depth cue from blue, yellow, to red. (C) Hepatitis E virus VLP with only the S domain. (D) VLP with S and P1 domains. (E) VLP with P1 and P2 domains. (From Guu *et al.* (2009). *Proc. Natl Acad. Sci., U S A*, **106**, 12992–12997; with permission.)

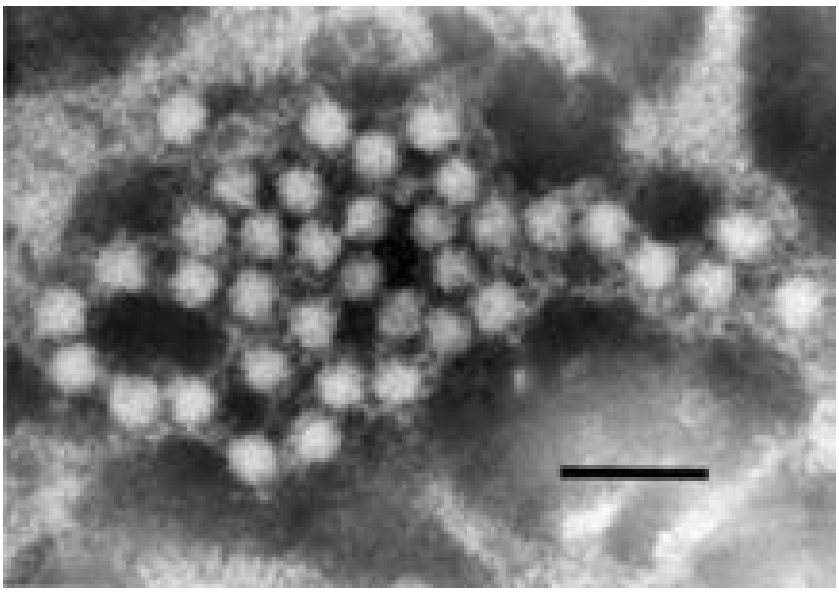

**Figure 3:** Electron micrograph of 30–35 nm diameter particles of Avian hepatitis E virus. The virus particles were detected from a bile sample of a chicken with hepatitis–splenomegaly syndrome. Bar = 100 nm. (From Haqshenas *et al.* (2001). *J. Gen. Virol.*, **82**, 2449–2462; with permission.)

## Proteins

Virions are constructed from a major capsid protein encoded by the second open reading frame (ORF2), and the CP may be proteolytically processed. A small immunoreactive protein (12.5 kDa) encoded by the third ORF (ORF3) has been identified and shown to exhibit multiple functions associated with virion morphogenesis and viral pathogenesis. Non-structural proteins encoded by the

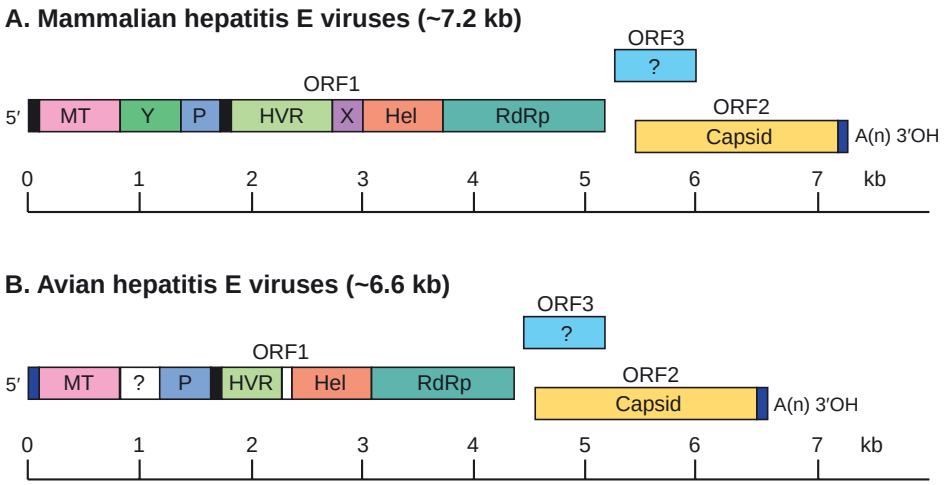


**Figure 4:** Schematic diagram of the genomic organization of hepatitis E virus: a short 5′ non-coding region (NCR), a 3′ NCR, and three ORFs. ORF2 and ORF3 overlap each other but neither overlaps ORF1. ORF1 encodes non-structural proteins including putative functional domains; ORF2 encodes capsid protein and ORF3 encodes a small phosphoprotein with a multi-functional C-terminal region. MT, methytransferase; Y, "Y" domain; P, a papain-like cystein protease; HVR, a hypervariable region that is dispensable for virus infectivity; Hel, helicase; RdRp, RNA-dependent RNA polymerase (From Meng, X.J. (2008). Hepatitis E virus (*Hepevirus*). In: *Encyclopedia of Virology* (5 vols), 3rd edn (B.W.J. Mahy and M.H.V. van Regenmortel, Eds.), Oxford, Elsevier, pp. 377–383; with permission.)

first major ORF (ORF1) have limited similarity with the "alpha-like supergroup" of viruses and contain domains consistent with a Mtr, RNA helicase, papain-like cysteine protease, and RdRp. The translational and posttranslational processes of the non-structural polyprotein remain unresolved. It remains unclear whether the non-structural polyprotein functions as a single protein with multiple functional domains or as individually-cleaved smaller proteins. RdRp, Mtr/guanylyltransferase and NTPase/RNA helicase activities have been experimentally demonstrated for ORF1 recombinant proteins.

### Lipids

None reported.

### Carbohydrates

Evidence for glycosylation of the major CP has been reported following its expression in mammalian cells. The CP sequence contains three potential sites for N-linked glycosylation and a signal peptide sequence at its N terminus. Mutations within the CP glycosylation sites prevent the formation of infectious virus particles, although the lethal effect is due to altered protein structure rather than elimination of glycosylation.

## Genome organization and replication

The RNA genome of HEV is organized into three ORFs, with the non-structural proteins encoded toward the 5′ end of the genome and the structural protein(s) toward the 3′ end. Capped genomic RNA is infectious for chickens, pigs, rhesus monkeys and chimpanzees. The 5′-NCR is only about 26 nt long, and a cap structure has been identified in the 5′ end of the viral genome and may play a role in the initiation of hepatitis E virus replication. The 3′-NTR contains a *cis*-reactive element. ORF1 encodes the non-structural polyprotein. ORF2 encodes the major CP, and binds to cell surface heparan sulfate proteoglycans (HSPGs) in liver cells. The ORF3 encodes a small phosphoprotein (113–114 aa) with a multifunctional C-terminal region. ORF2 overlaps ORF3, but neither overlaps with ORF1 (Figure 4). A bicistronic subgenomic mRNA encoding both ORF2 and ORF3 proteins has been identified.

Although avian hepatitis E virus shares only about 50% nucleotide sequence identity with HEV isolates, the genomic organization and functional motifs are relatively conserved between them (Figure 4).

## Antigenic properties

A single serotype of HEV has been described, with extensive cross-reactivity among circulating human and swine strains. Antibodies cross-reactive with capsid protein epitopes of human strains have been reported in various animal species but the viruses responsible for the cross-seropositivity were genetically identified only in pig, chicken, deer, mongoose, rat and rabbit. Avian hepatitis E virus also cross-reacts serologically with strains of HEV, and common antigenic epitopes have been identified in the capsid protein.

## Biological properties

HEV is associated in humans with outbreaks and sporadic cases of enterically transmitted acute hepatitis. The virus is considered endemic in tropical and subtropical countries of Asia and Africa, as well as in Mexico, but antibody prevalence studies suggest a global distribution of this virus. HEV is a recognized zoonotic virus, and pigs and more likely other animal species are reservoirs.

Transmission is via the fecal–oral route. Sporadic cases of human hepatitis E have been reported in both industrialized and developing countries, although epidemics occur only in developing countries. The sources of infection appear to be different for epidemics and sporadic cases: contaminated drinking water is the main source for epidemics, whereas the risk factors for sporadic cases include shellfish, contaminated animal meats and direct contacts with infected animals. Human-to-human transmission seems rare in hepatitis E epidemics. Genotypes 1 and 2 strains are restricted to humans, whereas genotypes 3 and 4 strains have a broader host range and are zoonotic. Interspecies transmission of genotypes 3 and 4 hepatitis E virus between swine and non-human primates has been experimentally demonstrated. Pig handlers in both developing and industrialized countries are shown to be at increased risk of hepatitis E virus infection. Sporadic cases of acute hepatitis E have been epidemiologically and genetically linked to the consumption of contaminated raw and undercooked animal meats.

Avian hepatitis E virus infection in chickens is widespread and approximately 71% of chicken flocks and 30% of chickens in the United States were positive for IgG antibodies to the virus. Avian hepatitis E virus infected turkeys but failed to infect two rhesus monkeys, suggesting that the virus is likely not zoonotic. Attempts to experimentally infect mice and pigs with avian hepatitis E virus were unsuccessful. In chickens experimentally infected with avian hepatitis E virus, replicating viruses were detected in livers as well as in several extrahepatic tissues, indicating that the virus replicates not only in the liver but in the gastrointestinal tissues as well.

# Genus *Hepevirus*

Type species ***Hepatitis E virus***

## Distinguishing features

Viruses within the genus *Hepevirus* infect mammals, and thus far strains within this genus have been genetically identified from human, pig, mongoose, deer, rat and rabbit.

## List of species in the genus *Hepevirus*

| | | |
|---|---|---|
| *Hepatitis E virus* | | |
| Hepatitis E virus 1 (Burma) | [M73218] | (HEV-1) |
| Hepatitis E virus 2 (Mexico) | [M74506] | (HEV-2) |
| Hepatitis E virus 3 (Meng) | [AF082843] | (HEV-3) |
| Hepatitis E virus 4 (T1) | [AJ272108] | (HEV-4) |

Species names are in italic script; names of strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

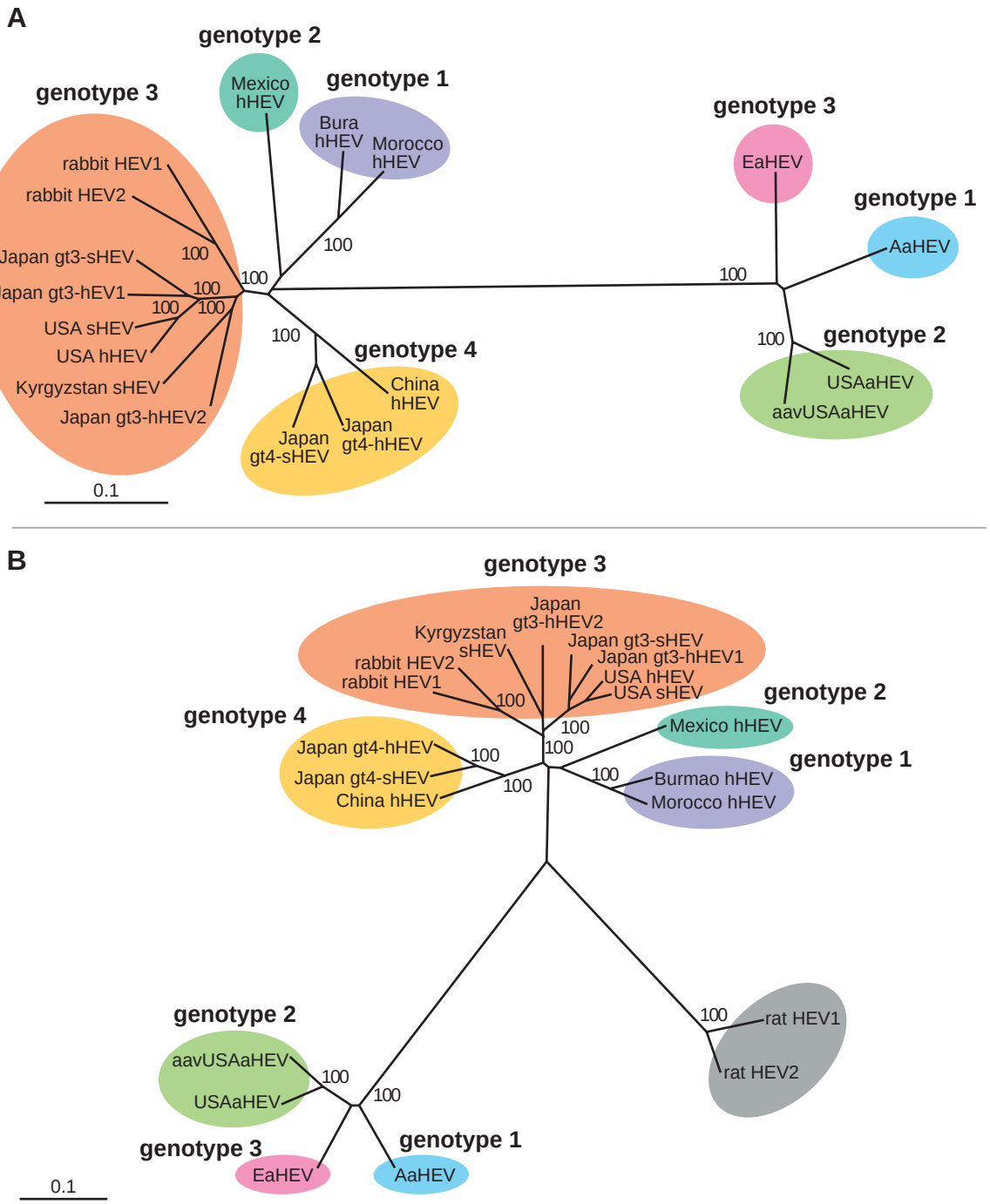


**Figure 5:** Phylogenetic trees depicting the relationship between strains of mammalian hepatitis E virus in the genus *Hepevirus* and the unassigned species *Avian hepatitis E virus* (courtesy of Hiroaki Okamoto). (A) A phylogenetic tree based on the full-length genomic sequences of representative hepatitis E virus strains including the four major genotypes of mammalian hepatitis E virus, the newly-identified rabbit hepatitis E virus and the three genotypes of Avian hepatitis E virus; (B) A phylogenetic tree based upon partial sequence (1545 nt) of the rat hepatitis E virus along with other hepatitis E virus strains in Figure 6A. GenBank accession numbers for the strains used in these analyses are Burma hHEV (M73218); Morocco hHEV (AY230202); Mexico hHEV (M74506); USA hHEV (AF060669); USA sHEV (AF082843); Japan gt3-hHEV1 (AP003430); Japan gt3-sHEV (AB073912); Japan gt3-hHEV2 (AB248520); Kyrgyzstan sHEV (AF455784); China hHEV (AJ272108); Japan gt4-sHEV (AB097811); Japan gt4-hHEV (AB220973); USAaHEV (AY535004); aavUSAaHEV (EF206691); AaHEV (AM943647); EaHEV (AM943646); rabbit HEV1 (FJ906895); rabbit HEV2 (FJ906896); rat HEV1 (GQ504009); and rat HEV2 (GQ504010).

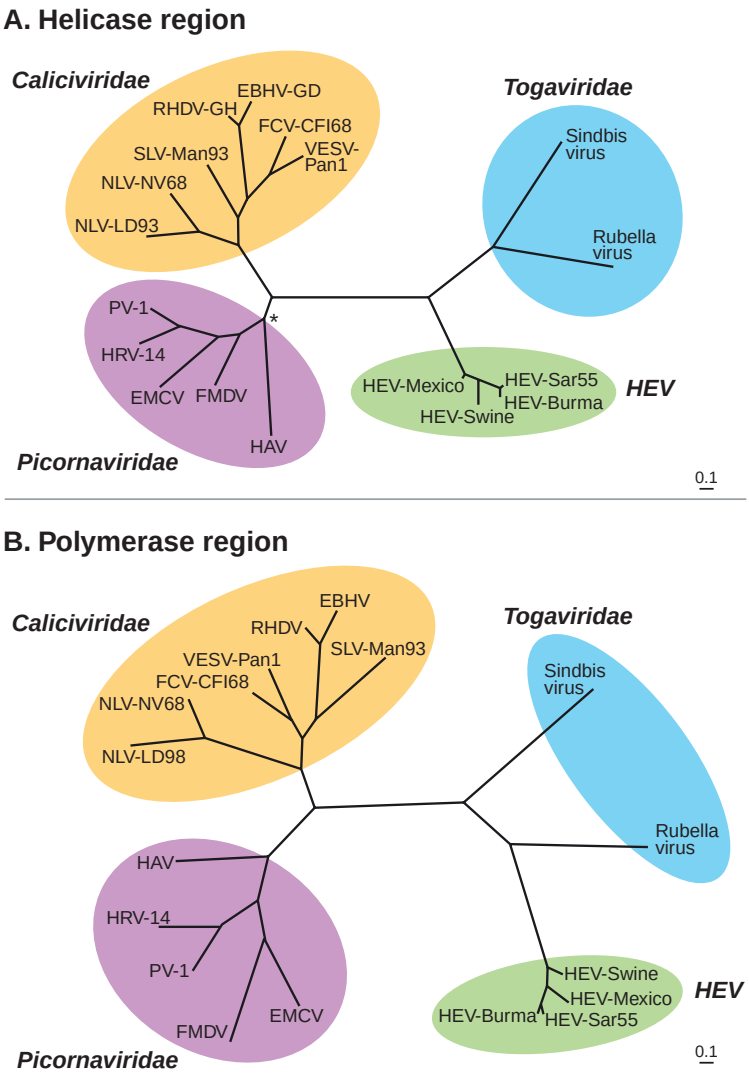


**Figure 6:** Phylogenetic relationships of hepatitis E virus with members of the families *Picornaviridae*, *Caliciviridae* and *Togaviridae*. The helicase (Hel) and polymerase (Pol) regions of the genome were analyzed (courtesy of T. Berke and D.O. Matson). (A) Partial gene sequences (200 aa) from the proposed helicase region were used for the phylogenetic analysis and included representative strains from each family. Clustal W v1.7 was used to create a multiple alignment for the aa sequences, which was verified by alignment of known motifs in the region (e.g. GxGKS/T). The nt sequences were added and aligned by hand using the corresponding aa sequences as a template resulting in a consensus length of 608 nt. A phylogenetic tree was constructed from the nt sequence alignment using the maximum likelihood algorithm in the program DNAML from the PHYLIP 3.52c package within UNIX environment. For the algorithm, the global rearrangement option was invoked and the order of sequence input was randomized ten times. Other menu options were left as default. The resultant tree is unrooted and the phylogenetic distances are in the unit of expected number of substitutions per site. Branch points of the resulting tree had a confidence level of $P<0.01$ ($P<0.05$*). GenBank accession numbers for the strains in this analysis were M87661 (Norwalk virus, NLV-NV68), X86557 (Lordsdale virus, NLV-LD93), U52086 (primate calicivirus, VESV-Pan1), U13992 (feline calicivirus, FCV-CFI/68), Z69620 (European brown hare syndrome virus, EBHV-GD), M67473 (rabbit hemorrhagic disease virus, RHDV-GH), X86560 (Sapporo virus, SLV-Man93), J02281 (human poliovirus 1, PV-1), K02121 (Human rhinovirus type 14, HRV-14), M22458 (encephalomyocarditis virus, EMCV), X00429 (foot-and-mouth disease virus, FMDV), K02990 (hepatitis A virus, HAV), M15240 (rubella virus), J02363 (Sindbis virus), M73218 (hepatitis E virus, HEV-Burma), M80581 (hepatitis E virus, HEV-Sar55), M74506 (hepatitis E virus, HEV-Mexico), AF011921 (hepatitis E virus, HEV-Swine). (B) Partial gene sequences (200 aa) from the proposed polymerase region were used for the phylogenetic analysis and included representative strains from each family. Clustal W v1.7 was used to create a multiple alignment for the aa sequences, which was verified by alignment of known motifs in the region (e.g. SGxxxTxxxMT/S, GDD). The nt sequences were added and aligned by hand using the corresponding aa sequences as template resulting in a consensus length of 590 nt. A phylogenetic tree was constructed as described above and GenBank accession numbers for the strains in this analysis were identical to those above.

## List of other related viruses which may be members of the genus *Hepevirus* but have not been approved as species

| | | |
|---|---|---|
| Rat hepatitis E virus | [GQ504009*] | (Rat HEV) |

*Sequence does not comprise the complete genome.

A novel strain of hepevirus was detected from fecal samples of wild Norway rats (*Rattus norvegicus*) in Germany. Based on the available partial sequence, the rat hepevirus shares approximately 50–60% nucleotide sequence identity to human and avian strains, respectively. The rat hepevirus thus appears to be a new genotype in the genus *Hepevirus*.

## List of unassigned species in the family *Hepeviridae*

*Avian hepatitis E virus*

| | | |
|---|---|---|
| Avian hepatitis E virus 1 (Australia) | [AM943647] | (avian HEV-1) |
| Avian hepatitis E virus 2 (USA) | [AY535004] | (avian HEV-2) |
| Avian hepatitis E virus 3 (Europe) | [AM943646] | (avian HEV-3) |

Species names are in italic script; names of strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## Phylogenetic relationships within the family

Currently, hepatitis E virus, the only species in the genus *Hepevirus*, includes the four recognized major genotypes of mammalian hepeviruses: genotype 1 (Burmese-like Asian strains), genotype 2 (a single Mexican strain and some African strains), genotype 3 (strains from sporadic human cases in industrialized countries, and animal strains from pig, deer and mongoose), and genotype 4 (strains from sporadic human cases in Asia, and swine strains from pigs). A rabbit hepatitis E virus was recently identified from farmed rabbits in China. It shares 74%, 73%, 78–79% and 74–75% nucleotide sequence identity to genotypes 1, 2, 3, 4 of hepatitis E virus and 46–47% identity to avian hepatitis E virus and appears to be a distant variant of HEV genotype 3 (Figure 5A). The rat hepatitis E virus appears to be a new genotype within the genus Hepevirus (Figure 5B).

Avian hepatitis E virus is phylogenetically distinct from mammalian hepeviruses, and forms a distinct clade within the family *Hepeviridae* that may justify creation of a new genus. At least three genotypes of *Avian hepatitis E virus* have been identified from chickens worldwide (Figure 5A and 5B): genotype 1 from chickens in Australia, genotype 2 from chickens in the USA, and genotype 3 from chickens in Europe. The inter-genotype nucleotide sequence identity among the three genotypes is approximately 82%.

## Similarity with other taxa

HEV is similar to members of the family *Caliciviridae* based on the superficial structural morphology as revealed by electron microscopy, and its genome organization. However, HEV does not share significant sequence homology with members of the family *Caliciviridae*. The Cap structure at the 5′ end of the HEV genome is absent from caliciviruses. HEV shows highest, but limited, amino acid sequence similarity in its replicative enzymes with *Rubella virus* and alphaviruses of the family *Togaviridae* and with plant furoviruses (Figure 6). The capping enzyme of HEV has properties very similar to those of viruses within the "alphavirus-like supergroup".

## Derivation of name

*Hepe*: from *hepa*titis *E* virus.

## Further reading

### Journals and books

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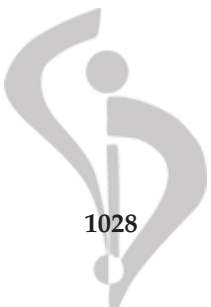
### Websites

VIPR Virus Pathogen Resource: http://www.viprbrc.org

## Contributed by

Meng, X.J., Anderson, D.A., Arankalle, V.A., Emerson, S.U., Harrison, T.J., Jameel, S. and Okamoto, H.



# Family *Hypoviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Hypoviridae*** |
| Genus | ***Hypovirus*** |

Since only one genus is currently recognized, the family description corresponds to the genus description.

# Genus *Hypovirus*

Type species ***Cryphonectria hypovirus 1***

## Virion properties

### Morphology

No true virions are associated with members of this family. Pleomorphic vesicles 50–80 nm in diameter, devoid of any detectable viral structural proteins but containing replicative form dsRNA and polymerase activity, are the only virus-associated particles that can be isolated from infected fungal tissue. (See Figure 1.)

### Physicochemical and physical properties

Mr of vesicles is unknown. They have a buoyant density in CsCl of approximately 1.27–1.3 g $cm^{-3}$ and sediment through sucrose as a broad component of approximately 200S. Their pH stability is unknown. The vesicles can be purified in pH 5.0 buffer and resuspended in pH 7.0 buffer. pH optimum for polymerase activity *in vitro* is 8.0; the optimum $Mg^{++}$ concentration for polymerase

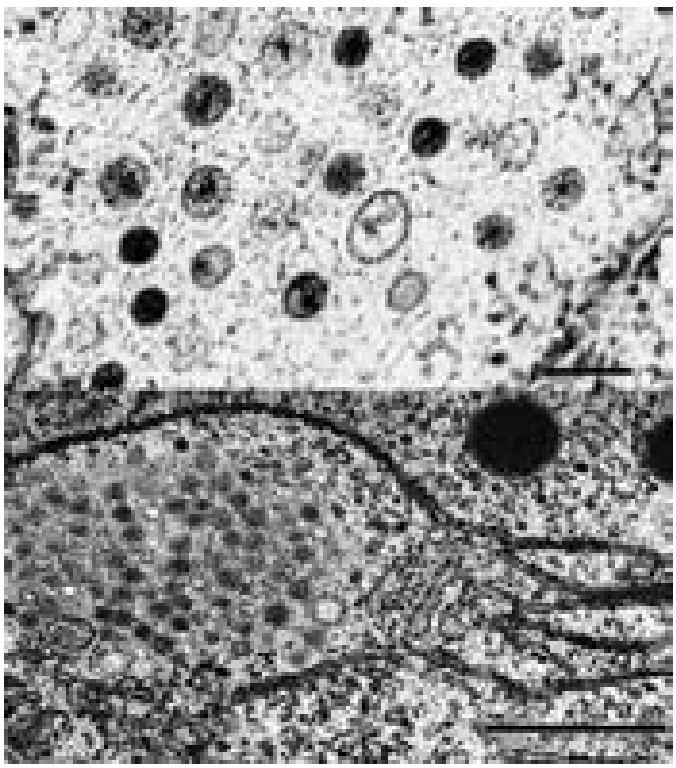


**Figure 1:** (Top) Thin section showing vesicles in fungal tissue; (bottom) thin section showing vesicle aggregate in fungal tissue surrounded by rough ER (from Newhouse *et al*. (1983). *Can. J. Bot.*, **61**, 389–399). The bar represents 100 nm.

activity is 5 mM. Activity decreases dramatically at pH less than 7.0 or more than 9.0. The vesicles are unstable when heated, or dispersed in lipid solvents. Optimal temperature for polymerase activity is 30 °C; temperatures over 40 °C inactivate polymerase activity. Deoxycholate at concentrations of more than 0.5% inactivates polymerase activity.

### Nucleic acid

Vesicles contain linear dsRNA, 9–13 kbp in size, that of one isolate of *Cryphonectria hypovirus 1*, the type species, being 12,712 nt. Apparently only one strand is employed in mRNA transcription. The coding (positive) strand contains a short 3′-poly(A) tail, which is 20–30 nt in length when analyzed as a component of the dsRNA.

In the absence of an identifiable virion RNA, hypoviruses were originally classified, along with several other fungal viruses, as dsRNA viruses. However, the ability of positive sense ssRNA, not dsRNA, to initiate infection is consistent with their current classification among the positive strand RNA viruses. The predominant dsRNA form found in infected mycelia appears to be replicative intermediate or replicative form RNA. The accumulation of plus strand RNA, by contrast, is low due to the RNA silencing antiviral defense response of the fungal host, but greatly increases in fungal strains in which the corresponding dicer or argonaute genes have been disrupted. The presence of shorter-than-full-length, internally deleted, defective interfering (DI) replicative form dsRNA molecules is common among some members, and satellite-like dsRNAs are present in other members. The host RNA silencing pathway has been reported to promote DI RNA production. No function has been ascribed to any ancillary dsRNA. The 5′-terminus of the positive strand of dsRNA from Cryphonectria hypovirus 1 (CHV-1) is blocked, but the nature of the blocking group is unknown. The 5′-terminus of the negative strand is unblocked. Both 5′-termini of dsRNA from Cryphonectria hypovirus 3-GH2 (CHV-3/GH2) are unblocked.

### Proteins

No structural proteins have been described for members of this family. Functions have been assigned to several nonstructural polypeptides encoded by members of the family. The 5′-proximal coding domain, ORFA, of CHV-1/EP713 RNA encodes a papain-like protease, p29, and a highly basic protein, p40, derived, respectively, from the N-terminus and C-terminus of polyprotein p69, by a p29-mediated cleavage event (Figure 2). A presumptive NS protein identified *in vitro* and *in vivo*, p29, has been shown by DNA-mediated transformation to contribute to suppression of host pigmentation, reduced sporulation, reduced laccase accumulation, to serve as a suppressor of host RNA silencing, and to promote RNA recombination of a co-infecting mycoreovirus. Protein p40

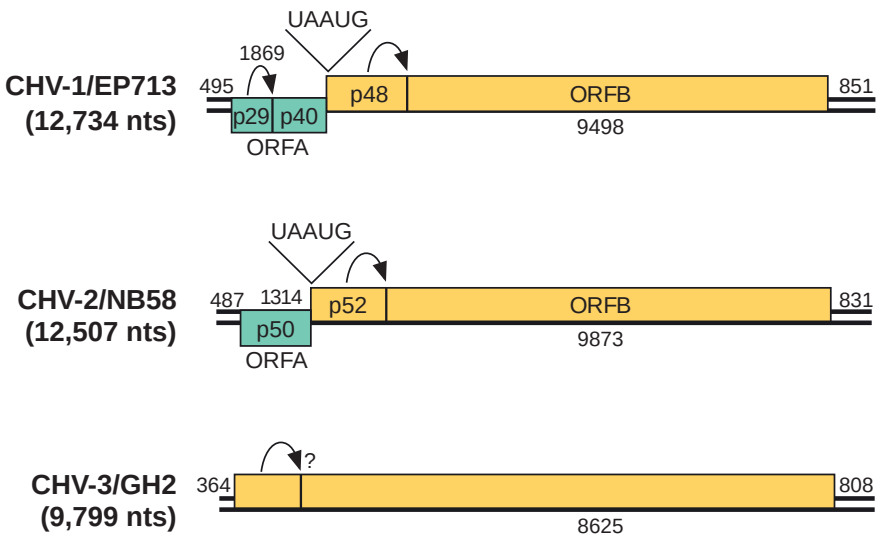



**Figure 2:** Genome organization of three members of the family *Hypoviridae*. Arrows represent known or suspected sites of autoproteolysis. Genome organization of Cryphonectria hypovirus 4 (CHV-4), which is 9.1 kb, is similar to Cryphonectria hypovirus 3 (CHV-3), but it is unknown whether CHV-4 undergoes autoproteolysis.

enhances viral RNA accumulation. RdRp activity is associated with isolated vesicles of CHV-1/EP713. The calculated size of the CHV-1 ORFB product, which contains putative RNA polymerase and helicase domains and a second papain-like protease, p48, is approximately $250 \times 10^3$ based on deduced aa sequence from cDNA clones, but no protein of that size has yet been isolated from vesicles. Smaller virus-encoded proteins have been identified in the vesicle-associated polymerase complex, suggesting extensive processing of replication proteins and that ORFB processing occurs *in vivo.* There are no known external viral proteins. The polymerase transcribes ssRNA molecules *in vitro* that correspond in size to full-length dsRNA. Approximately 90% of the polymerase products *in vitro* are of positive polarity. The p48 protein is required for initiation but not maintenance of viral RNA replication. CHV-3 and CHV-4 each contains a putative UDP glycosyltransferase-encoding domain that is not present in CHV-1 or CHV-2, although the specific function of this domain has not been examined.

### Lipids

Host-derived lipids make up the vesicles that encapsulate the viral dsRNA.

### Carbohydrates

Carbohydrates similar to those involved in fungal cell wall synthesis are associated with vesicles.

## Genome organization and replication

A 5′-leader of approximately 300–500 nt, including several AUG triplets, precedes the AUG codon that initiates the first long ORF. The viral coding region may be expressed from a single long ORF, or may be divided into two ORFs. If two ORFs are present, the shorter, 5′-proximal ORF is designated ORFA. Its product may or may not be autocatalytically cleaved, depending on the virus. The UAA termination sequence at the end of ORFA is part of the pentanucleotide UAAUG in all members with two ORFs investigated to date. The AUG of the UAAUG pentanucleotide initiates the other long ORF, ORFB. The N-terminal product of ORFB is a papain-like cysteine protease that autocatalytically releases from the growing polypeptide chain (e.g., P48 for CHV-1/EP713 or P52 for CHV-2/NB58). No further processing *in vitro* has been demonstrated for the remaining $300 \times 10^3$ polypeptide from this ORF. Phylogenetic relatedness to members of the positive sense, ssRNA genus *Potyvirus* has been demonstrated by comparisons of protease, polymerase and helicase domains, although these domains are positioned differently in the two families.

## Antigenic properties

No antibody has ever been raised from virus particle preparations. Anti-dsRNA antibodies were used to confirm the replicative form dsRNA constituent by immuno-electron microscopy. Antibodies directed against CHV1-EP713-encoded p29 were used to demonstrate association with the *trans*-Golgi network membranes. Antibodies directed against the conserved RNA polymerase domain of ORFB, expressed in bacteria, were used to identify an 87 kDa protein in a CHV-1/EP713 infected isolate.

## Biological properties

Confirmed members infect the chestnut blight fungus, *Cryphonectria parasitica*. Confirmed members result in reduced virulence (hypovirulence) on chestnut trees and altered fungal morphology in culture, but many possible family members have little or no discernible effect on the fungal host. Some possible members infect other filamentous fungi, e.g., *Sclerotinia sclerotiorum*. Infection of fungal mycelium is known only through fusion, or anastomosis, of infected hyphae with uninfected hyphae. The transmission rate through asexual spores (conidia) varies from a few to close to 100%. Transmission through sexual spores (ascospores) is not known to occur. Transmission via cell-free extracts has not been demonstrated but transfection of protoplasts with full-length synthetic transcripts has been successful for hypoviruses CHV-1/EP713, CHV-1/Euro7 and CHV-1/EP721. Confirmed members have been identified throughout chestnut-growing areas of Europe, North America and Asia. DsRNA-containing vesicles have been associated with abnormal Golgi apparati in freeze-substituted thin sections. No nuclear or mitochondrial associations, nor virus-associated inclusions, have been noted.

## Species demarcation criteria in the genus

Species are differentiated based on major differences in genetic organization or genome expression, as well as by major differences in nucleic acid sequence identity. Thus, CHV-1 differs from CHV-2 in the presence or absence, respectively, of a papain-like proteinase in ORFA. CHV-1 and CHV-2 isolates share less than 60% overall aa sequence identity with each other. CHV-3 and CHV-4 each contain a single ORF, but isolates of the two species share less than 50% overall sequence identity. Infection by CHV-1 isolates results in a white or near-white phenotype in the fungus; CHV-2 infection results in an orange-brown phenotype; CHV-3 and CHV-4 isolates have little effect on fungal pigment. Infection by members of any of the four species may reduce fungal virulence.

## List of species in the genus *Hypovirus*

| | | |
|---|---|---|
| *Cryphonectria hypovirus 1* | | |
| Cryphonectria hypovirus 1 - EP713 | [M57938] | (CHV-1/EP713) |
| Cryphonectria hypovirus 1 - Euro7 | [AF082191] | (CHV-1/Euro7) |
| Cryphonectria hypovirus 1 - EP721 | [DQ861913] | (CHV-1/EP721) |
| *Cryphonectria hypovirus 2* | | |
| Cryphonectria hypovirus 2 - NB58 | [L29010] | (CHV-2/NB58) |
| *Cryphonectria hypovirus 3* | | |
| Cryphonectria hypovirus 3 - GH2 | [AF188515] | (CHV-3/GH2) |
| *Cryphonectria hypovirus 4* | | |
| Cryphonectria hypovirus 4 - SR2 | [AY307099] | (CHV-4/SR2) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Hypovirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Hypoviridae*

None reported.

## Phylogenetic relationships within the family

The hierarchy of relationships among members of this family is unknown. Many strains and isolates have been identified by RNA hybridization, RFLP, or nt sequence analysis. Among the four species, CHV-1 and CHV-2 are more closely related to each other and CHV-3 and CHV-4 are more closely related to each other.

## Similarity with other taxa

The possession of an undivided positive sense RNA genome, expressed via long polyprotein precursors, demonstrates an affinity with viruses of the picornavirus supergroup. Deduced aa sequences of polymerase, helicase and protease motifs of members of the family *Hypoviridae* suggest that their closest relatives are bymoviruses in the family *Potyviridae*.

## Derivation of name

*Hypo*: from *hypo*virulence.

## Further reading

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Dawe, A.L. and Nuss, D.L. (2001). Hypoviruses and chestnut blight: Exploiting viruses to understand and modulate fungal pathogenesis. *Annu. Rev. Genet.*, **35**, 1–29.
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Smart, C.D., Yuan, W., Foglia, R., Nuss, D.L., Fulbright, D.W. and Hillman, B.I. (1999). *Cryphonectria hypovirus 3*, a virus species in the Family *Hypoviridae* with a single open reading frame. *Virology*, **265**, 66–73.

## Contributed by

Nuss, D.L. and Hillman, B.I.

# Family *Leviviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Leviviridae* |
| Genus | *Levivirus* |
| Genus | *Allolevivirus* |

## Virion properties

### Morphology

Virions are spherical and exhibit icosahedral symmetry (T = 3) with a diameter of about 26 nm. There is no envelope (Figure 1).

### Physicochemical and physical properties

Virion Mr varies from 3.6 to $4.2 \times 10^6$ depending on the genus. The $S_{20,w}$ value is 80–84S and buoyant density in CsCl is 1.46 g cm$^{-3}$. Infectivity is ether-, chloroform- and low-pH-resistant, but is sensitive to RNase and detergents. Inactivation by UV light and chemicals is comparable to that of other icosahedral viruses containing ssRNA.

### Nucleic acid

Virions contain one molecule of positive sense ssRNA of 3466–4276 nt: size and gene arrangement vary with genus. RNA makes up 39% of the virion weight. The 5′ nucleotide carries a triphosphate, while at the 3′ terminus a non-templated A residue is added by the replicase (see Figures 2,4).

### Proteins

The capsid contains 180 copies of CP (14 kDa) arranged in an icosahedron. The structure of the protein shell of several ssRNA phages has been solved by X-ray crystallography, and shows 60 quasi-symmetric AB- and 30 symmetric CC′-dimers. The A and C subunits are situated around the three-fold axes, and the B subunits around the five-fold axes of the icosahedron. CP has no structural similarity to those of eukaryote icosahedral RNA viruses. The X-ray structure of the capsid in a complex with the 19 nt operator shows interaction of the dimers with this hairpin. Operators found in the various phages are shown in Figure 3.

Each virion contains one copy of the A-protein (35–61 kDa), which is required for maturation of the virion and for pilus attachment (Figure 1). Alloleviviruses also contain several copies of the readthrough protein in their capsid.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

Members of the family *Leviviridae* that propagate in *E. coli* infect by adsorption to the sides of F(ertility) pili. (Non-coliphages such as Pseudomonas phage PP7 (PP7) and Acinetobacter phage AP205 (AP205) bind to other pili.) This event leads to cleavage of the A-protein and release of the RNA from the virion into the bacterium. The infecting RNA encodes a replicase, which assembles with three host proteins (ribosomal protein S1 and translation elongation factors EF-Tu and EF-Ts) to form the active RNA polymerase. A fourth protein, called Host Factor, not associated with the polymerase complex but acting directly on the RNA, is needed for synthesis of the minus strand. Members of the two genera use different Host Factors. Plus-strand synthesis requires, besides the virus-coded replicase, only EF-Tu and EF-Ts as cofactors. Late in infection coat-protein dimers act as translational repressors of the replicase gene by binding to an RNA hairpin, the operator that contains the start site of this gene. This protein–RNA complex is considered to also be the nucleation site for encapsidation. Virions assemble in the cytoplasm around phage RNA. It is unknown at which point the A-protein (and readthrough protein) is assembled in the virion but it is assumed to be an early step since the A-protein cannot be incorporated into preformed virions lacking the

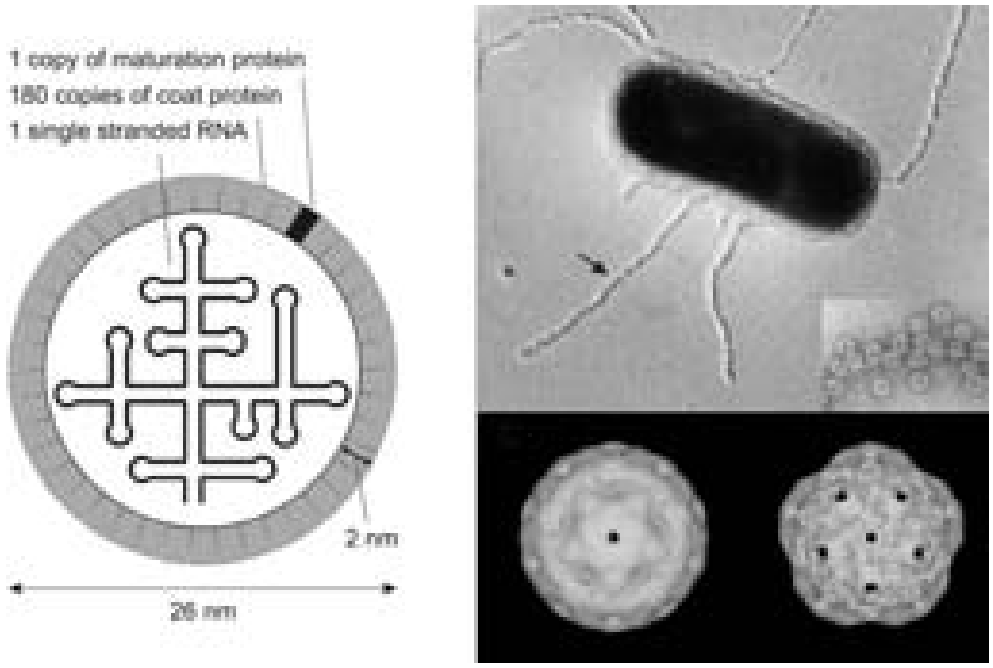


**Figure 1:** (Left) Schematic representation of a levivirus: the RNA inside the virion is highly structured. (Upper right) *Escherichia coli* bacterium with Enterobacteria phage MS2 (MS2) particles attached to its F-pili (courtesy A.B. Jacobson). The inset is a -pilus with phage- enlargement. (Courtesy R.I. Koning and H.K. Koerten.) (Lower right) Image reconstruction obtained from cryo-electron microscopy of MS2 phages. View from outside (left) and inside (right). (Courtesy R.I. Koning and H.K. Koerten.)

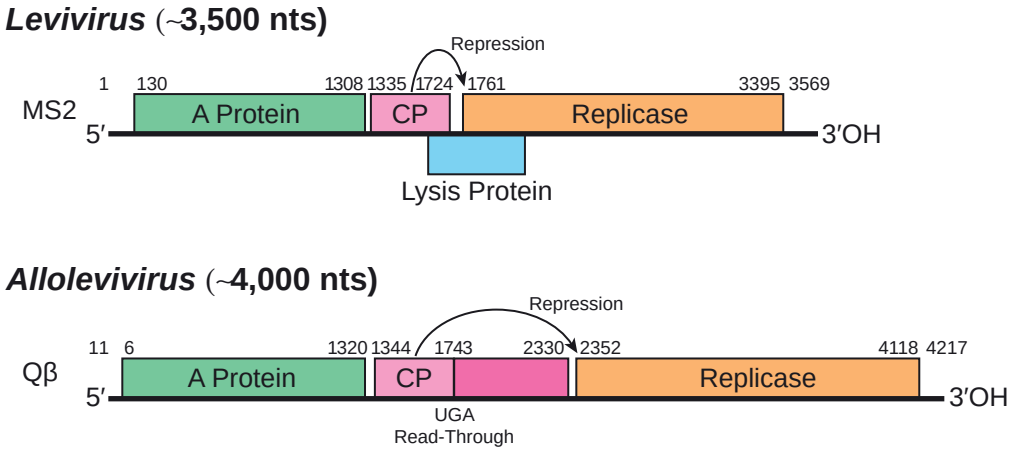


**Figure 2:** General genetic map of a representative levivirus – Enterobacteria phage MS2 (MS2) – and an allolevivirus – Enterobacteria phage Qβ (Qβ). The maturation protein is also called A-protein. The lysis gene overlaps the replicase gene in a +1 frameshift. Arrows indicate repression of replicase translation by capsid protein binding to an RNA hairpin structure (the operator) present at the start of the gene. The UGA nonsense codon (nt 1743) is occasionally (ca. 6%) misread as tryptophan to produce the readthrough protein.

protein. Infection usually results in cell lysis releasing thousands of phages per cell. The lysis protein short-circuits the membrane potential and somehow activates the bacterial autolysins leading to degradation of the peptidoglycan network.

## Antigenic properties

Members of the family *Leviviridae* are highly antigenic.

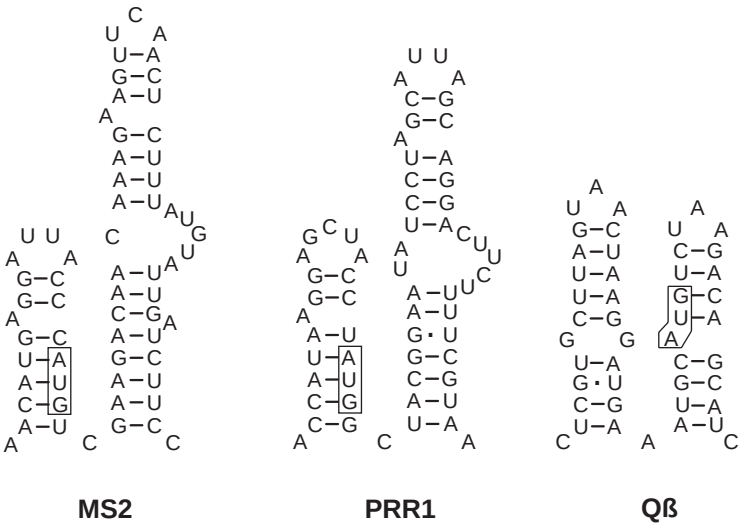


**Figure 3:** 2D structures of operator hairpins in several RNA phages. Note the presence of a second, putative operator hairpin in all three phages. The structure shown for PRR1 has not been confirmed yet.

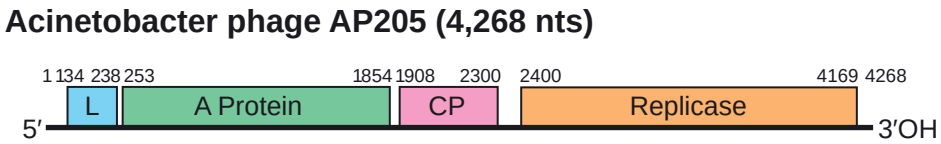


**Figure 4:** Genetic map of Acinetobacter phage AP205 (AP205). Note the location of the tentative lysis gene at the 5-terminus. AP205 is unusually long for a levivirus. This map corrects the one previously published (Klovins, J. *et al.* (2002). *J. Gen. Virol.* (2002), **83**, 1523–1533). A: A-protein; CP: capsid protein; R: replicase; L: lysis.

## Biological properties

Members of the family *Leviviridae* occur worldwide and are abundantly present in sewage, waste water, animal and human faeces. In Asia a particular geographic distribution has been noticed with respect to the four levivirus species. It has also been proposed that the various species have a preference for particular hosts, e.g. members of *Enterobacteria phage Qbeta* are found predominantly in human waste. The evidence is not conclusive. RNA bacteriophages are harmless for humans. Members of the family *Leviviridae* not only infect enterobacteria but also species of the genera *Caulobacter*, *Pseudomonas* and *Acinetobacter* and probably many other Gram-negative bacteria, provided they express the appropriate pili on their surface. RNA coliphages are often used as indicators for the presence of enteroviruses in waste and surface water. There is renewed interest in phage therapy to combat bacterial infections.

# Genus *Levivirus*

Type species ***Enterobacteria phage MS2***

## Distinguishing features

Leviviruses contain the short version of the genome and have a separate gene for cell lysis, which partly overlaps the replicase coding region in the +1 reading frame (see Figure 2). Overlap with the CP gene is variable. Genome size ranges from 3466 for GA (*Enterobacteria phage BZ13*) to 3577 for fr (*Enterobacteria phage MS2*) (Figure 2). Leviviruses and allolevivirus use different Host Factors for their polymerase holoenzyme. The levivirus Host Factor has been isolated but has not been genetically identified. Generally, the replicases from leviviruses poorly replicate allolevivirus RNA and *vice versa.*

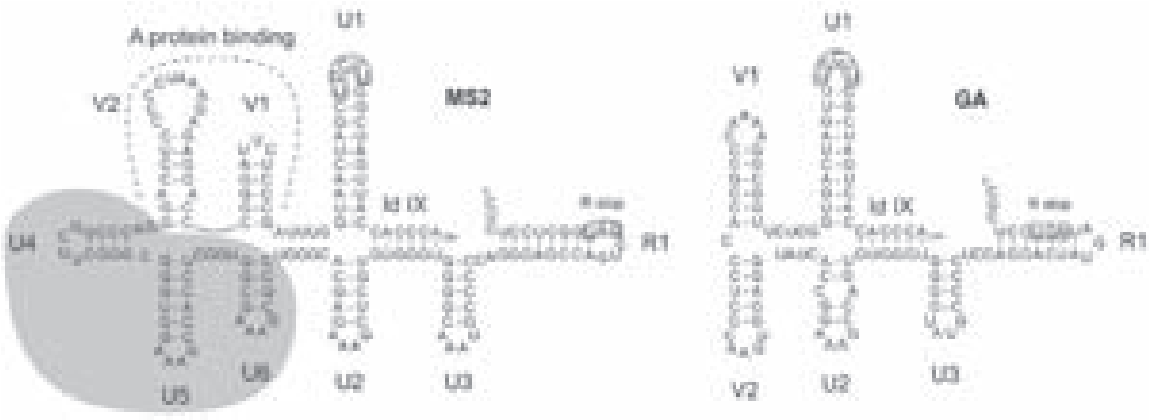


**Figure 5:** Comparison of the RNA folding in the 3′UTR of Enterobacteria phage MS2 (MS2) and Enterobacteria phage GA (GA). GA lacks the three stem-loops U4, U5 and U6. In MS2 stem-loops V1 and V2 are part of the A-protein binding site. The other part of the protein's binding site is located around nt 400.

The sequence of RNA of AP205, an RNA phage growing on *Acinetobacter* tentatively identified its lysis gene in the unusual location of the 5′ end. The absence of a readthrough protein was taken as criterion to classify AP205 as a levivirus (Figure 4).

Recently, the genome sequence of PRR1 (3,573 nt), a phage with a broad host range, was determined; its genetic map is identical to that of the leviviruses.

## Genome organization and replication

Figure 2 shows the map of the levivirus genome. Lysis and replicase synthesis are dependent on translation of the CP gene: early CP nonsense mutants are deficient in replicase and lysis protein synthesis. Translational starts at the lysis gene were shown to be reinitiations by ribosomes that had completed CP-gene reading but had not yet detached themselves from the message. A small fraction of these ribosomes manages to back up to the lysis start. Part of the replicase ribosome binding site is base-paired to an upstream sequence located in the coat coding region. A ribosome translating the CP cistron disrupts this interaction, thereby exposing the replicase start site (when not blocked by a CP dimer, which is the case late in infection). The CP gene is freely accessible to ribosomes.

Maturation or A-protein is translated from a specific RNA folding intermediate which has an accessible ribosome-binding site. This intermediate exists for a short time on nascent strands. Full-length RNA reaches an equilibrium folding in which the start site of the A-protein gene is inaccessible. It is believed that the purpose of these control mechanisms is to facilitate the switch from translation of the viral RNA to its replication. One of the binding sites of the replicase holoenzyme is the start of the CP gene. Binding of the enzyme to this site squeezes out ribosomes from CP, lysis and replicase genes. At this stage the A-protein gene is folded in its ribosome-inaccessible state and replication can proceed without interference from translation.

The polymerase of GA has been purified, that of MS2 may be unstable. Except for the Host Factor the polymerases of leviviruses and alloleviviruses contain the same subunits.

## Antigenic properties

Antigenic specificity is distinct from that of members of the genus *Allolevivirus*.

## Species demarcation criteria in the genus

A major difference between members of the species *Enterobacteria phage MS2* and *Enterobacteria phage BZ13* (formerly called subgroups I and II) is a deletion of about 60 nt in the 3′-UTR of

members of *Enterobacteria phage BZ13*, comprising three small RNA hairpins (Figure 5). There is also a 35 nt deletion in the replicase gene of members of *Enterobacteria phage BZ13* producing a shorter hairpin stem. Furthermore, the percentage of aa or nt sequence identity is dramatically lower between the two species than between strains within a species. Species can also be distinguished by serological means and by species-specific antisense DNA probes.

### List of species in the genus *Levivirus*

| | | |
|---|---|---|
| *Enterobacteria phage BZ13* | | |
| Enterobacteria phage GA | [X03869] | (GA) |
| Enterobacteria phage JP34 | [J04343] | (JP34) |
| Enterobacteria phage KU1 | [AF227250] | (KU1) |
| Enterobacteria phage TH1 | [AB218930] | (TH1) |
| Enterobacteria phage BZ13 | [FJ483839] | (BZ13) |
| Enterobacteria phage MS2 | | |
| Enterobacteria phage MS2 | [V00642] | (MS2) |
| Enterobacteria phage f2 | | (f2) |
| *Enterobacteria phage fr* | [X15031] | (fr) |
| Enterobacteria phage JP501 | [AF227251] | (JP501) |
| Enterobacteria phage M12 | [AF195778] | (M12) |
| Enterobacteria phage R17 | [EF108465] | (R17) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Levivirus* but have not been approved as species

| | | |
|---|---|---|
| Acinetobacter phage AP205 | [AF334111] | (AP205) |
| Pseudomonas phage PP7 | [X80191] | (PP7) |
| Pseudomonas phage PRR1 | [DQ836063] | (PRR1) |

## GENUS *ALLOLEVIVIRUS*

Type species ***Enterobacteria phage Qbeta***

### Distinguishing features

Alloleviviruses contain the longer version of the genome (Figure 2). The extra RNA encodes a C-terminal extension of CP arising by occasional suppression of the CP gene termination codon. The readthrough protein is present at about 12 copies per virion. Together with the A-protein, it is necessary for infection. Its precise role is not known. There is no separate lysis gene. Cell lysis is a secondary function of the A-protein. Genome length varies between 4217 nt for Qβ (*Enterobacteria phage Qbeta*) and 4276 nt for SP (*Enterobacteria phage F1*) (Figure 2).

### Genome organization and replication

Genome organization is shown Figure 2. The RNA polymerase of Qβ has been purified and the enzyme can amplify Qβ RNA *in vitro*. The crystal structure of the enzyme has been solved. The Host Factor has been purified and genetically characterized. It is the product of the *hfq* gene. In the uninfected cell the protein functions in the transition to stationary phase. In particular, it stimulates translation of the mRNA encoding the $\sigma^{38}$ protein involved in transcription of stationary phase genes. Hfq is a sequence non-specific ssRNA binding protein with some preference for A-residues. It is heat-resistant and acts as a pentamer. The protein helps the polymerase to get access to the 3′ end of the plus strand, which exists in a base-paired and therefore inactive state. In Figure 6 the secondary structure of the 3′UTR of Qβ RNA is shown; the 3′-terminal 6 nt are taken up in long-distance interaction with ld IX.

Although the polymerases are specific for their own RNA, the interaction with RNA involves host-encoded subunits (EF-Tu, S1 and Hfq) that have no sequence specificity. An important contribution

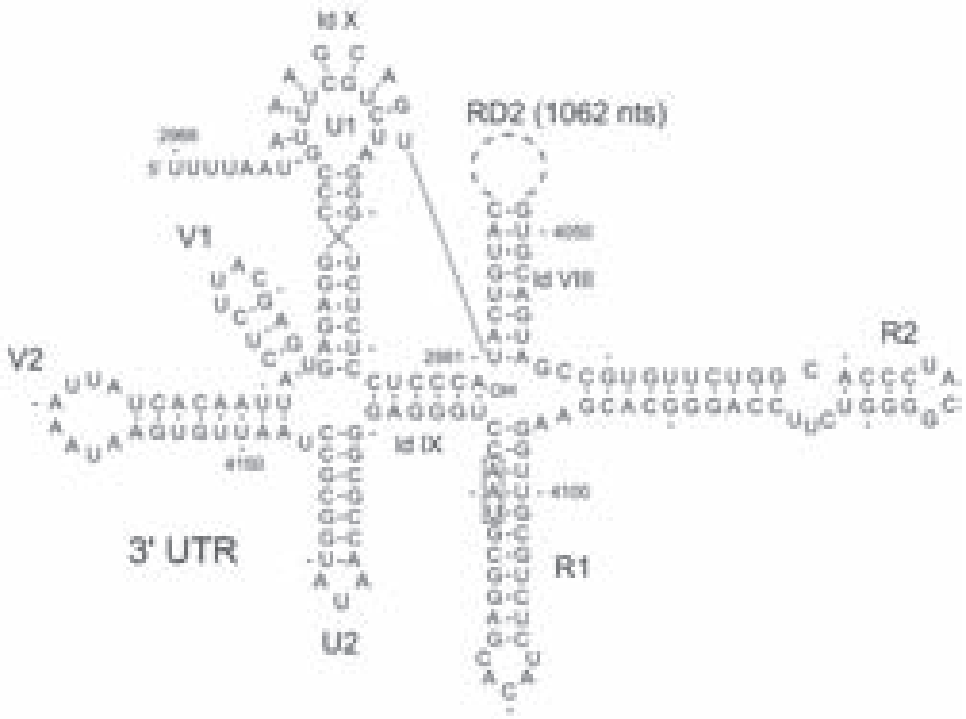


**Figure 6:** RNA secondary structure for Enterobacteria phage Qβ (Qβ) RNA from nt 2966 to the 3′ end (nt 4217) marked as $A_{OH}$. The UAA stop codon (nt 4119) of the replicase gene is boxed. Replicase Domain 2 (RD2) containing 1062 nt has been replaced by a dotted circle. Breaking two or three basepairs in the central pseudoknot (ldX) or ldVIII abolishes replication. However, breaking the pairs in ld IX, which buries the 3′-terminal nucleotides, stimulates replication. Production of minus strand is also inhibited by deletion of stem-loops U1, V1, V2 or U2. (R1 and R2 were not tested.)

to template activity is provided by the higher order structure of Qβ RNA (Figure 6). For instance, destroying two out of the eight base pairs that make up the central pseudoknot in Qβ RNA, here indicated as ld X, lowers replication 100-fold. The higher order structures of the RNAs of phages PP7 (not allocated to a current species) and SP (*Enterobacteria phage F1*) are shown in Figure 7.

The switch from translation to replication is as in leviviruses and was first formulated for Qβ. Control of the maturation protein is slightly different. The time window for producing the A-protein is not set by the lifetime of a folding intermediate, as for MS2, but by the time it takes the polymerase to move from about position 60, the start of the A-protein gene, to about position 470 where the complement to the Shine–Dalgarno sequence of the A-protein gene is located. Once this complement is synthesized, pairing between the two regions blocks furthers translation.

## Antigenic properties

Antigenic specificity is distinct from that of members of the genus *Levivirus*.

## Species demarcation criteria in the genus

The major difference between *Enterobacteria phage Qbeta* and *Enterobacteria phage F1* (formerly called subgroups III and IV respectively) is a deletion of about 90 nt in the maturation-protein gene of Qβ, corresponding to a bifurcated hairpin. There is also the extra stem-loop (V1) in the 3′UTR of members of *Enterobacteria phage Qbeta* that is lacking in members of *Enterobacteria phage F1*. Species can also be differentiated by serological criteria and by species-specific antisense DNA probes. Finally, the percentage of aa or nt sequence identity is dramatically lower between the two species than between strains within a species.

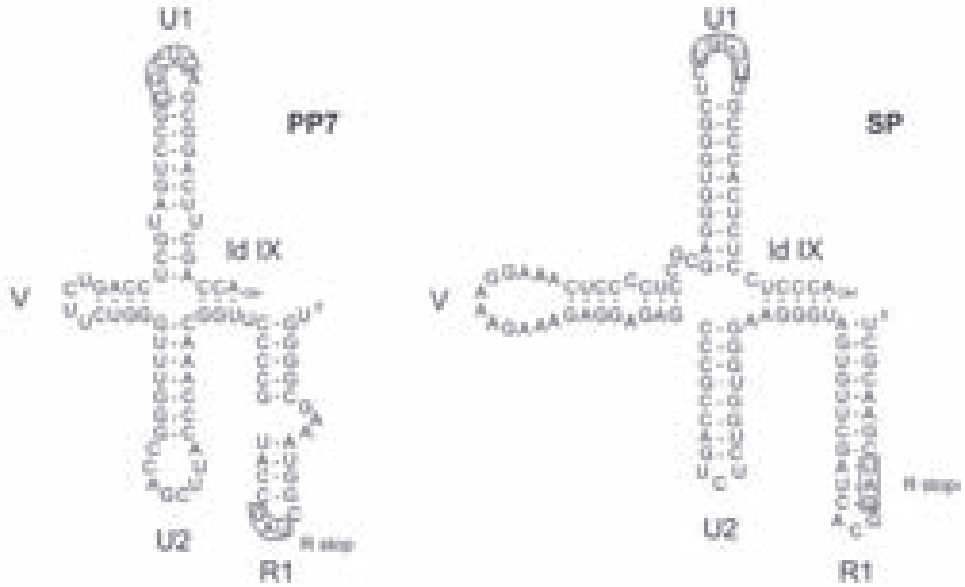


**Figure 7:** RNA secondary structure in the 3′UTR of Pseudomonas phage PP7 (PP7) and Enterobacteria phage SP (SP). The folding of PP7 RNA is much more like that of SP RNA than that of either MS2 or GA (Figure 5). Compared to MS2 the stem-loops U3, U4, U5, U6 and one of the two V-loops are missing. The boxed sequence in the loop of hairpin U1 is conserved in all viruses of the family *Leviviridae*. The sequence is part of the central pseudoknot in Qβ. The pseudoknot is believed to exist also in the other phages.

## List of species in the genus *Allolevivirus*

| | | |
|---|---|---|
| *Enterobacteria phage F1* | | |
| Enterobacteria phage F1 | [EF068134] | (F1) |
| Enterobacteria phage ID2 | | (ID2) |
| Enterobacteria phage NL95 | [AF059243] | (NL95) |
| Enterobacteria phage SP | [X07489] | (SP) |
| Enterobacteria phage TW28 | | (TW28) |
| *Enterobacteria phage Qbeta* | | |
| Enterobacteria phage Qβ | [AY099114] | (Qβ) |
| Enterobacteria phage M11 | [AF052431] | (M11) |
| Enterobacteria phage MX1 | [AF059242] | (MX1) |
| Enterobacteria phage ST | | (ST) |
| Enterobacteria phage TW18 | [FJ483840] | (TW18) |
| Enterobacteria phage VK | [EU372698] | (VK) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Allolevivirus* but have not been approved as species

None reported.

## List of other related viruses which may be members of the family *Leviviridae* but have not been approved as species

| | | |
|---|---|---|
| Caulobacter phage φCb2 | | (φCb2) |
| Caulobacter phage φCb4 | | (φCb4) |
| Caulobacter phage φCb5 | [HM066936] | (φCb5) |
| Caulobacter phage φCb8r | | (φCb8r) |
| Caulobacter phage φCb9 | | (φCb9) |

| | |
|---|---|
| Caulobacter phage φCb12r | (φCb12r) |
| Caulobacter phage φCb23r | (φCb23r) |
| Caulobacter phage φCP2 | (φCP2) |
| Caulobacter phage φCP18 | (φCP18) |
| Caulobacter phage φCr14 | (φCr14) |
| Caulobacter phage φCr28 | (φCr28) |
| Enterobacteria phage B6 | (B6) |
| Enterobacteria phage B7 | (B7) |
| Enterobacteria phage C-1 | (C-1) |
| Enterobacteria phage C2 | (C2) |
| Enterobacteria phage fcan | (fcan) |
| Enterobacteria phage Folac | (Folac) |
| Enterobacteria phage Iα | (Iα) |
| Enterobacteria phage M | (M) |
| Enterobacteria phage pilHα | (pilHα) |
| Enterobacteria phage R23 | (R23) |
| Enterobacteria phage R34 | (R34) |
| Enterobacteria phage ZG/1 | (ZG/1) |
| Enterobacteria phage ZIK/1 | (ZIK/1) |
| Enterobacteria phage ZJ/1 | (ZJ/1) |
| Enterobacteria phage ZL/3 | (ZL/3) |
| Enterobacteria phage ZS/3 | (ZS/3) |
| Enterobacteria phage α15 | (α15) |
| Enterobacteria phage β | (β) |
| Enterobacteria phage 2 | ( 2) |
| Enterobacteria phage τ | (τ) |
| (other enterobacteriophages, with many plasmid specificities, have been reported). | |
| Pseudomonas phage 7s | (7s) |

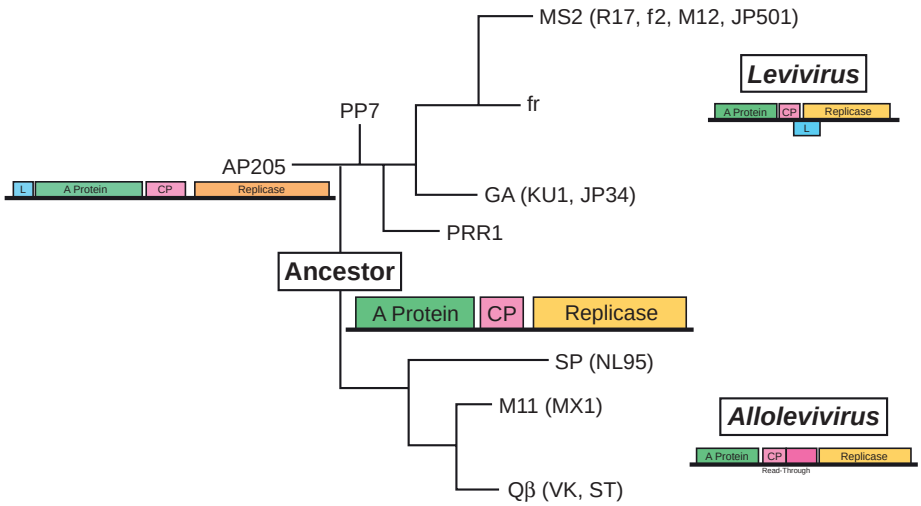


**Figure 8:** Proposed phylogenetic tree for the family *Leviviridae*. Distances are arbitrary. The ancestor only has the three basic genes. Lysis is effected by the A-protein as it still is today in Qβ. Presumably, fitness of the ancestor was restricted by the double function of the A-protein (Bollback, J.P. and Huelsenbeck, J.P. (2001). *J. Mol. Evol.*, **52**, 117–128). The leviviruses solved the problem by evolving a separate lysis protein either encoded on a vacant region of the genome (AP205) or resulting from a ribosomal restart following translation termination at the end of the capsid gene (other leviviruses). Once restrictions on the A-protein were relaxed the gene could evolve in various directions to better fulfill its remaining function: virion maturation and infection. Two features of leviviruses can be explained by this scenario: first, lysis genes have variable startpoints (even between MS2 and fr or between GA and KU1) and secondly, of the three "old" genes, the A-protein gene shows the lowest sequence conservation. The alloleviviruses solved the dual-function problem by transferring part of the maturation and infection function to a new protein, readthrough, which arose by an insertion between coat and replicase genes. Presumably, this allowed A-protein to improve its lysis function. Such a scenario would provide a different reason why also in the alloleviviruses A-protein is least conserved of the "old" genes. Abbreviations of virus names are provided in the tables.

## Phylogenetic relationships within the family

A tentative phylogenetic tree of the family *Leviviridae* is given in Figure 8. Relationships have been based first on deeply rooted features such as the genetic map and second on similarity in RNA folding, in particular the one present at the 3′UTR which is conserved in its outline. As a result there is a fundamental split between leviviruses and alloleviviruses because they have different maps. The two non-coli leviviruses AP205 and PP7 have been placed closer to the ancestor than the coli leviviruses because they have the same folding of their 3′UTR as the alloleviviruses (see Figure 7). As a result MS2 and GA are closer to the non-coliphages than to coliphage Qβ. PP7 is placed closer to MS2 than AP205 because AP205 has its lysis gene in a different position. PP7 has it in the same position as MS2, fr and GA. PRR1 is positioned between PP7 and the group II phages (GA) owing to the folding of its 3′UTR.

In this scheme, the ancestor contains only the three basic genes and the A-protein has the double function of lysis and maturation (infection). We assume that its 3′UTR is folded in the simple way of PP7 (AP205) and Qβ (SP) (see Figure 7).

The subdivision of each genus into two species is based on criteria explained above. Based on the sequence it is possible to make subtle distinctions between strains within a species. For example, MS2, R17, f2, M12 and JP501 are extremely close (ca. 95% identity) whereas fr is much further away (ca. 80% identity), has some features of members of *Enterobacteria phage BZ13*, but is still clearly a member of *Enterobacteria phage MS2*.

## Similarity with other taxa

Not reported.

## Derivation of names

*Levi*: from Latin *levis*, "light".
*Allo*: from Greek *allon*, "other".

## Further reading

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## Contributed by

van Duin, J. and Olsthoorn, R.C.L.

# FAMILY *LUTEOVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Luteoviridae*** |
| Genus | ***Luteovirus*** |
| Genus | ***Polerovirus*** |
| Genus | ***Enamovirus*** |

## Virion properties

### MORPHOLOGY

Virions are 25 to 30 nm in diameter, hexagonal in outline and have no envelope (Figure 1). Amino acid sequence homology modelling using the X-ray crystal structure of rice yellow mottle virus (genus *Sobemovirus*) as a guide suggests that particles have 180 subunits arranged in T = 3 icosahedra. Particles are composed of two CPs that encapsidate a genomic single stranded RNA.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virion Mr is 5.6–6.0 $\times 10^6$; buoyant density in CsCl is 1.39–1.42 g $cm^{-3}$; $S_{20,w}$ is 106–127S. Virions are moderately stable and are insensitive to treatment with chloroform or non-ionic detergents, but are disrupted by prolonged treatment with high concentrations of salts. Luteovirus and polerovirus particles are insensitive to freezing.

### NUCLEIC ACID

Virions contain a single molecule of infectious, linear, positive sense ssRNA. The genome size is fairly uniform ranging from 5.6 kb to 6.0 kb. The RNAs do not have a 3′-terminal poly(A) tract. A small protein (VPg) is covalently linked to the 5′ end of the genomic RNAs of poleroviruses and the one enamovirus. The 5′ termini of barley yellow dwarf virus-PAV (BYDV-PAV) genomic RNA can be phosphorylated after treatment with alkaline phosphatase suggesting that the 5′ termini of luteovirus genomic RNAs are phosphorylated.

### PROTEINS

There is a single major CP of 21–23 kDa encoded by ORF3 and smaller amounts of a "readthrough" protein, which is a fusion of the products of ORF3 and that of the contiguous ORF5. The readthrough protein may be associated with aphid transmission and/or virus particle stability.

### LIPIDS

None reported.

### CARBOHYDRATES

None reported.

## Genome organization and replication

Genomic RNAs of members of the *Luteoviridae* contain five or six ORFs that are predicted to encode proteins of between 4 and 132 kDa (Table 1; Figure 2). In poleroviruses and by extension the enamovirus, ORF0 (not present in members of the genus *Luteovirus*) encodes a symptom and host range determinant that functions as a suppressor of RNA silencing. Where present, it overlaps with ORF1. ORFs 1 and 2 overlap and encode the replication-related proteins. The major CP is encoded by ORF3, which is followed in frame by ORF5. The product of ORF4 (overlapping completely with ORF3, but absent from enaomovirus genomic RNA) has been shown to be required for long-distance movement of some luteoviruses and poleroviruses. Some luteovirus and polerovirus genomes contain an ORF 6, predicted to encode a small (≤6 kDa) protein, but no functions have been assigned to the proteins. Genera can be distinguished on the basis of the arrangements and sizes of the ORFs. Replication-related proteins encoded by ORFs 1 and 2 of the luteoviruses are not homologous to products of the corresponding ORFs of poleroviruses and the enamovirus. They are most similar to those of viruses in the family *Tombusviridae*, while the products of ORFs 1 and 2 of the poleroviruses and the enamovirus are related to those of sobemoviruses.

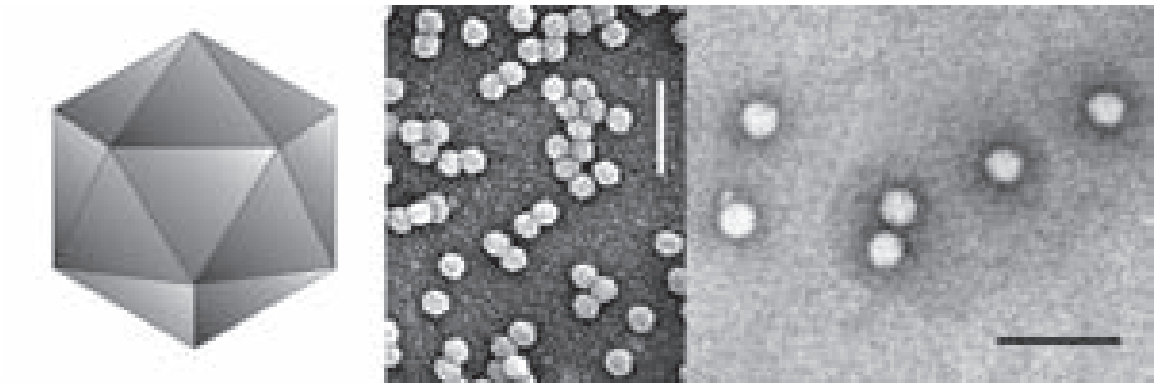

**Figure 1:** (Left) Diagram of the proposed structure of luteovirus particles. (Center) Negative contrast electron micrograph of particles of barley yellow dwarf virus-PAV (BYDV-PAV) and (right) pea enation mosaic virus-1 (PEMV-1), isolated by means of sucrose density gradient centrifugation and stained with uranyl acetate. Bars represent 100 nm.

**Table 1:** Proteins of the different ORFs with sizes (kDa) and possible function(s)

| ORF | *Luteovirus* | *Polerovirus* | *Enamovirus* | Function of product |
|---|---|---|---|---|
| 0 | NA | 28–30 | 34 | Suppressor of RNA silencing |
| 1 | 39–42 | 66–72 | 84 | Helicase motifs in luteoviruses; protease and VPg in polero- and enamoviruses |
| 1+2 | 99–103 | 116–121 | 132 | RNA-dependent RNA polymerase |
| 3 | 22 | 22–23 | 21 | Major coat protein |
| 4 | 16–21 | 17–21 | NA | Probable MP |
| 3+5 | 72–80 | 67–80 | 55 | Minor coat protein expressed as C-terminal fusion to core coat protein; possible aphid transmission and virus particle stability factor |
| 6 | 4–7 | 7–9 | NA | Unknown |

The differences among luteoviruses, poleroviruses and the enamovirus are principally in the 5′ end of the genome. ORFs 0, 1 and 2 are translated from the genomic RNA. ORF2 is translated by frameshift from ORF1 and thus shares an amino terminus with the product of ORF1. Polerovirus and enamovirus VPgs are cleaved from the products of ORF1 by upstream serine protease domains. ORFs 3, 4 (luteoviruses and poleroviruses) and 5 are expressed from a subgenomic RNA (sgRNA). ORF1 (poleroviruses and the enamovirus) and ORF4 (luteoviruses and poleroviruses) are expressed by leaky scanning. ORF5 is translated via a readthrough of the termination codon at the end of ORF3. Luteoviruses produce one or two additional sgRNAs, the larger of which from BYDV-PAV contains ORF6. Some poleroviruses produce additional sgRNAs.

There are no data on post-translational modification. Particles of some strains of cereal yellow dwarf virus-RPV (CYDV-RPV) contain 322 nt satellite RNAs. Virions of some isolates that consist of pea enation mosaic virus-1 (PEMV-1) together with the umbravirus, pea enation mosaic virus-2 (PEMV-2), contain 717-nt satellite RNAs in addition to genomic RNAs.

## Antigenic properties

Luteovirus and polerovirus particles are strongly immunogenic. Species within a genus are more closely related serologically than are species in different genera. Serological relationships may be detected when comparing disrupted virus particles that are not detectable when intact virions are tested. In gel diffusion assays, aphid-transmissible isolates sometimes display antigenic determinants that are absent from aphid-non-transmissible isolates. No serological relationships have been reported between enamoviruses and either luteoviruses or poleroviruses.

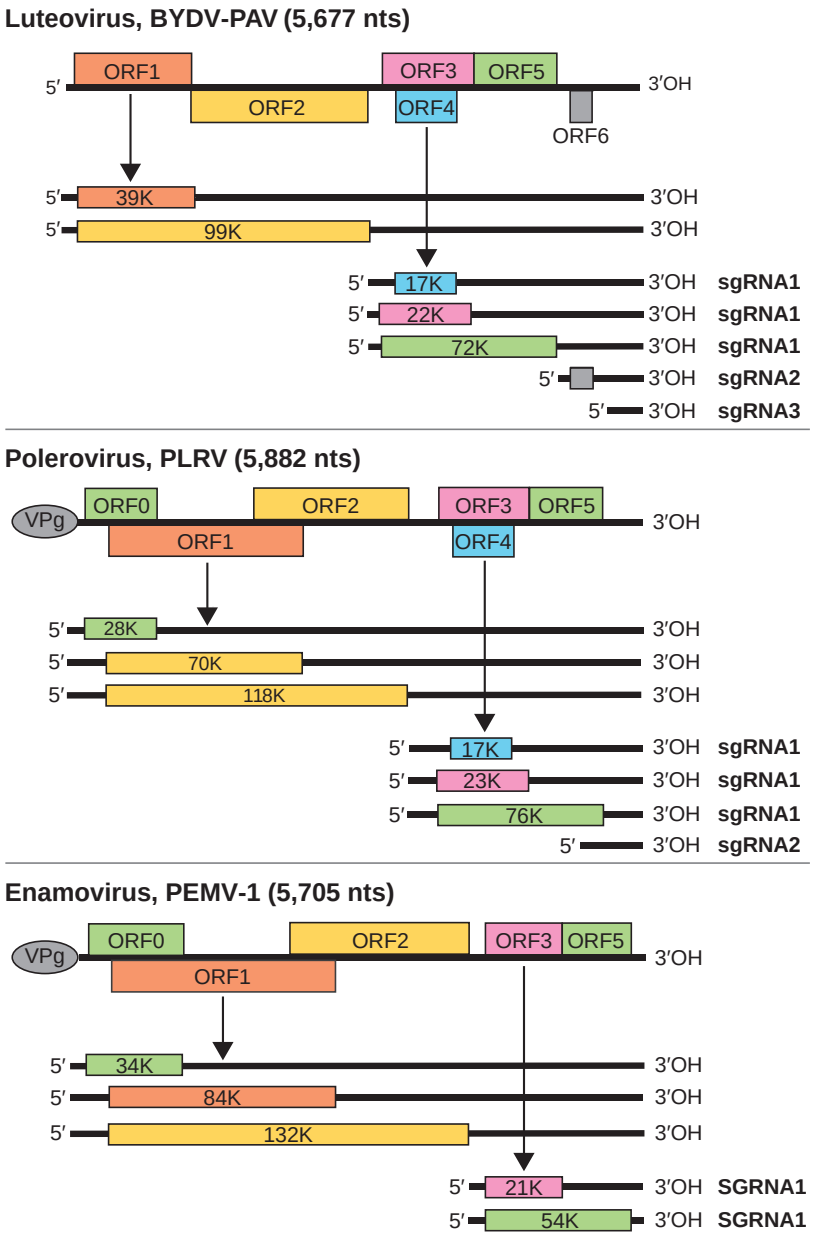


**Figure 2:** Diagram of the genome organization and map of the translation products typical of viruses in each genus of the family *Luteoviridae*. Solid lines represent RNA; boxes represent ORFs; thinner boxes represent translation products; grey ovals represent VPgs.

## Biological properties

### Host range

Several members of the family *Luteoviridae* have host ranges largely restricted to one plant family. For example, BYDVs and CYDVs infect several species in the family *Poaceae*, bean leafroll virus (BLRV) and soybean dwarf virus (SbDV) infect mainly legumes, and carrot red leaf virus infects mainly plants in the family *Umbelliferae*. Other members of the family *Luteoviridae* infect plants in several or many different families. For example, beet western yellows virus (BWYV) infects more than 150 species of plants in over 20 families.

### Geographic distribution

Members of the family *Luteoviridae* have been reported from Arctic, temperate, sub-tropical, and tropical regions. Some of the viruses are found worldwide, such as BYDV, BWYV and potato leaf-roll virus (PLRV). Others have more restricted distributions, such as tobacco necrotic dwarf virus, which has been reported only from Japan, and groundnut rosette assistor virus, which has been reported from south Saharan countries in Africa.

### Transmission

Transmission is in a circulative, non-propagative manner by specific aphid vectors. Viruses are acquired by phloem feeding, enter the hemocoel of the aphid via the hindgut (e.g., BYDV-PAV) or posterior midgut (e.g., PLRV) by a receptor mediated transport process, circulate in the hemolymph and enter the accessory salivary gland by a second receptor mediated transport event. Inoculation results from introduction of viruliferous saliva into the phloem tissues via the salivary duct during aphid feeding. PEMV-1 is readily transmitted mechanically, a property dependent on its multiplication in cells co-infected with PEMV-2 (*Umbravirus*).

### Cytopathology

Luteovirus and polerovirus particles are largely confined to phloem cells; PEMV-1, with PEMV-2, is found in phloem and mesophyll tissues. Virus particles are found in both the nuclei and cytoplasm of infected cells. Luteoviruses and poleroviruses often cause phloem necrosis that spreads from inoculated sieve elements and causes symptoms by inhibiting translocation, slowing plant growth and inducing the loss of chlorophyll, which results in characteristic yellowing and dwarfing of infected plants.

## Genus *Luteovirus*

Type species ***Barley yellow dwarf virus-PAV***

### Distinguishing features

Genome properties are the key features. There is no ORF0 and frameshift from ORF1 into ORF2 occurs at the termination codon of ORF1, and ORF1 and ORF2 overlap by less than 20 nt. ORF1 and ORF2 encode replication-related proteins that are most similar to those of viruses in the family *Tombusviridae*. The length of the non-coding sequence between ORF2 and ORF3 is about 100 nt. There is no evidence for the presence of a 5′ genome-linked protein. ORF4 is present and contained within ORF3. ORF5 is greater than 1350 nt in length.

### Virion properties

#### Physicochemical and physical properties

Virion buoyant density in CsCl is 1.39–1.40 g cm$^{-3}$; $S_{20,w}$ is 106–118S.

#### Nucleic acid

Sizes of positive sense ssRNA genomes are between 5,677 nt for BYDV-PAV and 5,964 nt for BLRV.

### Genome organization and replication

In addition to the characters listed under distinguishing features, viruses within the genus produce two or three subgenomic RNAs from minus-strand templates, the largest of which expresses ORFs 3-5. The 3′-noncoding region contains a transcription enhancer that interacts with the 5′-nontranslated regions of genomic and large subgenomic RNAs to effect cap independent translation initiation.

### Species demarcation criteria in the genus

Criteria used to demarcate species of the genus include:

- Differences in breadth and specificity of host range
- Failure of cross-protection in either one-way or two-way relationships

- Differences in serological specificity with discriminatory polyclonal or monoclonal antibodies
- Differences in amino acid sequence identity of any gene product of greater than 10%.

## List of species in the genus *Luteovirus*

| | | |
|---|---|---|
| *Barley yellow dwarf virus-MAV* | | |
| Barley yellow dwarf virus-MAV - PS1 | [D01213 = NC_003680] | (BYDV-MAV-PS1) |
| *Barley yellow dwarf virus-PAS* | | |
| Barley yellow dwarf virus-PAS - 129 | [AF218798 = NC_002160] | (BYDV-PAS-129) |
| *Barley yellow dwarf virus-PAV* | | |
| (Barley yellow dwarf virus-rgv = rice giallume) | | |
| Barley yellow dwarf virus-PAV - Australia | [X07653 = NC_004750] | (BYDV-PAV-AUS) |
| *Bean leafroll virus* | | |
| (Legume yellows virus) | | |
| (Michigan alfalfa virus) | | |
| (Pea leafroll virus) | | |
| Bean leafroll virus - Michigan | [AF441393 = NC_003369] | (BLRV-MI) |
| *Rose spring dwarf-associated virus* | | |
| Rose spring dwarf-associated virus - California | [EU024678 = NC_010806] | (RSDaV-CA) |
| *Soybean dwarf virus* | | |
| (Subterranean clover red leaf virus) | | |
| Soybean dwarf virus - Tas-1 | [L24049 = NC_003056] | (SbDV-Tas-1) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Luteovirus* but have not been approved as species

| | | |
|---|---|---|
| Barley yellow dwarf virus-GAV | [AY220739 = NC_004666] | (BYDV-GAV) |

# GENUS *POLEROVIRUS*

Type species ***Potato leafroll virus***

## Distinguishing features

Polerovirus genomic RNAs have VPgs linked to their 5′ termini and possess an ORF0 and a non-coding region between ORF2 and ORF3 of about 200 nt. ORF1 and ORF2 encode replication-related proteins, which are most similar to those of sobemoviruses. The frameshift from ORF1 into ORF2 occurs upstream of the termination of ORF1, and ORFs 1 and 2 overlap by more than 400 nt. Polerovirus genomes differ from those of the enamovirus in that ORF4 is present within ORF3 and ORF5 is greater than 1200 nt.

## Virion properties

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virion buoyant density in CsCl is 1.39–1.42 g $cm^{-3}$; $S_{20,w}$ is 115–127S.

### NUCLEIC ACID

Sizes of ssRNA genomes are between 5,641 nt for turnip yellows virus and 5,987 nt for PLRV.

## Genome organization and replication

See "Distinguishing features" above.

## Species demarcation criteria in the genus

See criteria for the genus *Luteovirus.*

## List of species in the genus *Polerovirus*

| | | |
|---|---|---|
| *Beet chlorosis virus* | | |
| Beet chlorosis virus - 2a | [AF352024 = NC_002766] | (BChV-2a) |
| *Beet mild yellowing virus* | | |
| Beet mild yellowing virus - 2ITB | [X83110 = NC_003491] | (BMYV-2ITB) |
| *Beet western yellows virus* | | |
| (Malva yellows virus) | | |
| (Turnip mild yellows virus) | | |
| Beet western yellows virus - USA | [AF473561 = NC_004756] | (BWYV-US) |
| *Carrot red leaf virus* | | |
| Carrot red leaf virus - UK1 | [AY695933 = NC_006265] | (CtLRV-UK1) |
| *Cereal yellow dwarf virus-RPS* | | |
| Cereal yellow dwarf virus-RPS - Mex1 | [AF235168 = NC_002198] | (CYDV-RPS-Mex1) |
| *Cereal yellow dwarf virus-RPV* | | |
| Cereal yellow dwarf virus-RPV - NY | [L25299 = NC_004751] | (CYDV-RPV-NY) |
| *Chickpea chlorotic stunt virus* | | |
| Chickpea chlorotic stunt virus - Et-fb-am1 | [AY956384 = NC_008249] | (CpCSV-Et-fb-am1) |
| *Cucurbit aphid-borne yellows virus* | | |
| Cucurbit aphid-borne yellows virus - N | [X76931 = NC_003688] | (CABYV-N) |
| *Melon aphid-borne yellows virus* | | |
| Melon aphid-borne yellows virus - Beijing | [EU000534 = NC_010809] | (MABYV-BJ) |
| *Potato leafroll virus* | | |
| (Solanum yellows virus) | | |
| (Tomato yellow top virus) | | |
| Potato leafroll virus - UK:Scotland | [D00530 = NC_001747] | (PLRV-UK) |
| *Sugarcane yellow leaf virus* | | |
| Sugarcane yellow leaf virus - Florida | [AF157029 = NC_000874] | (ScYLV-FL) |
| *Tobacco vein distorting virus* | | |
| Tobacco vein distorting virus - China:Longlin | [EF529624 = NC_010732] | (TVDV-CN) |
| *Turnip yellows virus* | | |
| Turnip yellows virus - FL-1 | [X13063 = NC_003743] | (TuYV-FL1) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Polerovirus* but have not been approved as species

| | | |
|---|---|---|
| Cotton leafroll dwarf virus | [GQ379224*] | (CLRDV) |
| Suakwa aphid-borne yellows virus | [FJ425878*] | (SABYV) |

*Sequences do not comprise the complete genome.

# Genus *Enamovirus*

Type species ***Pea enation mosaic virus-1***

## Distinguishing features

Enamovirus (PEMV-1) genomic RNA contains an ORF0, but does not contain an ORF4 (present in luteoviruses and poleroviruses). The non-coding intergenic region between ORF2 and ORF3 is about 200 nt in length. ORF1 and ORF2 encode replication-related proteins that are most similar to those of sobemoviruses. Frameshift from ORF1 into ORF2 occurs upstream of the termination of ORF1, and ORF1 and ORF2 overlap by more than 400 nt. The PEMV-1 genome contains an ORF5 of about 900 nt.

## Virion properties

### Physicochemical and physical properties

Enamovirus virions have a Mr of about $5.6 \times 10^6$, buoyant densities in CsCl of 1.42 g cm$^{-3}$, and $S_{20,w}$ of 107–122S.

## Genome organization and replication

Genomic RNA of PEMV-1 is 5,706 nt and has a 5′ VPg.

## Antigenic properties

Virions produced in plants infected with PEMV-1 together with PEMV-2 (*Umbravirus*) are moderately antigenic.

## Biological properties

PEMV-1 occurs as part of a complex with PEMV-2 (*Umbravirus*) and induces mosaic symptoms and enations. Unlike other members of the family *Luteoviridae*, PEMV-1 is readily transmitted mechanically, a property dependent on its multiplication in cells co-infected with PEMV-2. Aphid transmissibility is conferred by PEMV-1, but can be lost after several mechanical passages. Virions are found in mesophyll tissue as well as in vascular tissue. The genome of PEMV-1 is capable of autonomous replication in protoplasts, but is dependent on PEMV-2 to support systemic invasion.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Enamovirus*

| | | |
|---|---|---|
| *Pea enation mosaic virus-1* | | |
| Pea enation mosaic virus-1 - WSG | [L04573 = NC_003629] | (PEMV-1-WSG) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Enamovirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Luteoviridae*

| | | |
|---|---|---|
| *Barley yellow dwarf virus-GPV* | | |
| Barley yellow dwarf virus-GPV - 04FX6 | [EF174408*] | (BYDV-GPV-04FX6) |
| Wheat yellow dwarf virus-RPV | [FM865413 = NC_012931] | (WYDV-RPV) |
| *Barley yellow dwarf virus-RMV* | | |
| Barley yellow dwarf virus-RMV - Illinois | [Z14123*] | (BYDV-RMV-IL) |
| *Barley yellow dwarf virus-bv* | | |
| Barley yellow dwarf virus-SGV - NY | [AY541038*] | (BYDV-SGV-NY) |
| *Chickpea stunt disease associated virus* | | |
| Chickpea stunt disease associated virus - IC | [Y11530*] | (CpSDaV-IC) |
| *Groundnut rosette assistor virus* | | |
| Groundnut rosette assistor virus - M16GCP | [AF195824*] | (GRAV-M16GCP) |
| *Indonesian soybean dwarf virus* | | |
| Indonesian soybean dwarf virus - IND | | (ISDV-IND) |
| *Sweet potato leaf speckling virus* | | |
| Sweet potato leaf speckling virus - Peru | [DQ655700*] | (SPLSV-Peru) |
| *Tobacco necrotic dwarf virus* | | |
| Tobacco necrotic dwarf virus - Japan | | (TNDV-JA) |

Species names are in italic script; names of isolates, strains and synonyms are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

## List of other related viruses which may be members of the family *Luteoviridae* but have not been approved as species

| | | |
|---|---|---|
| Chickpea yellows virus | [GQ118150*] | (CpYV) |
| Lentil stunt virus | [GQ118152*] | (LSV) |

*Sequences do not comprise the complete genome.

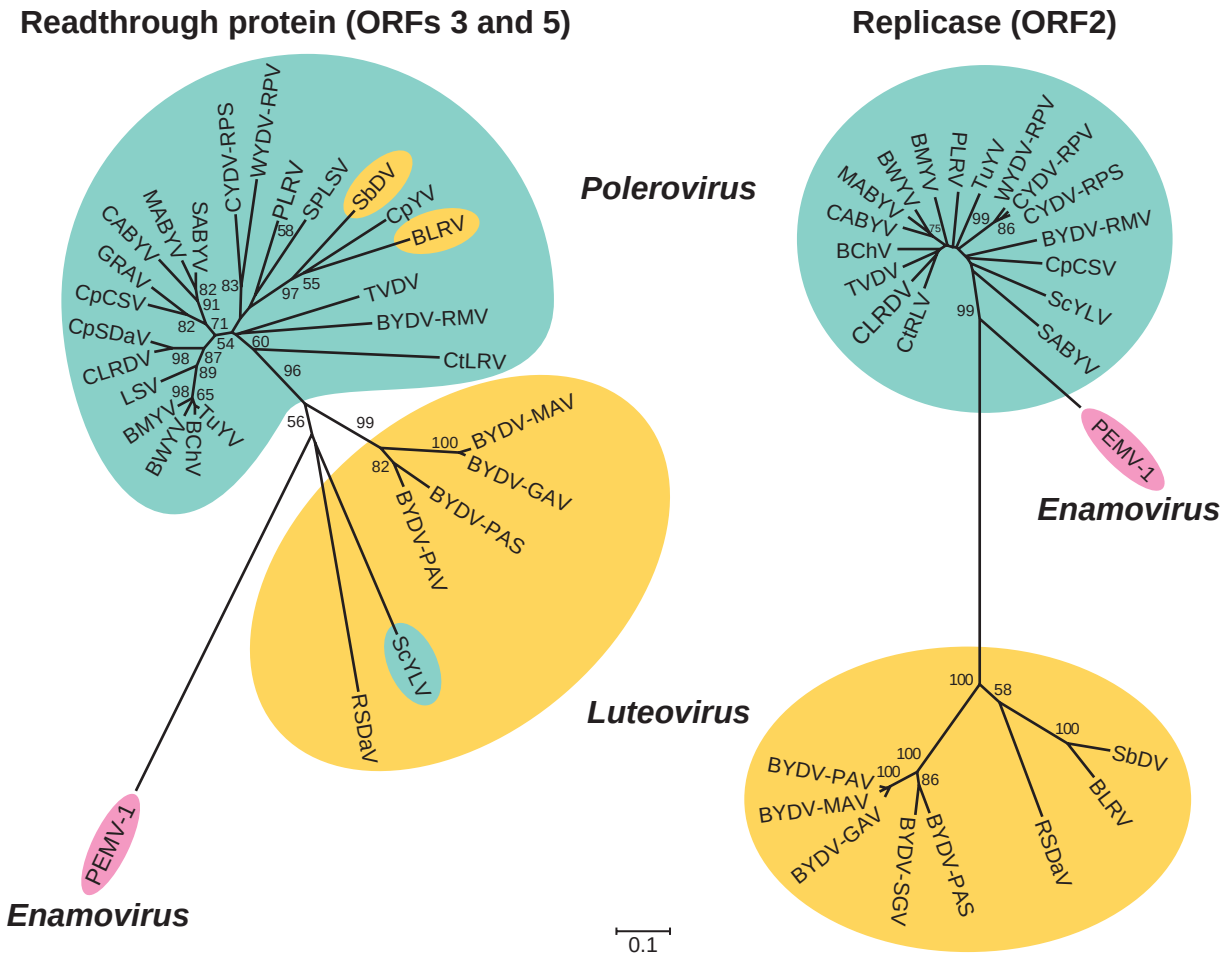


**Figure 3:** Phylogenetic analyses of the (left) readthrough protein (ORFs 3 and 5) and (right) polymerase (ORF2) sequences of representatives of species in the family *Luteoviridae*. Amino acid sequences were aligned with CLUSTALX and neighbour-joining trees constructed with MEGA 4. Bootstrap values above 50% are indicated.

## Phylogenetic relationships within the family

The three genera within the family *Luteoviridae* share very similar structural protein genes (ORFs 3 and 5) whose products show varying levels of serological relatedness. Phylogenetic analysis of the predicted amino acid sequences of the polymerases (ORF2) clearly separate the members of the family *Luteoviridae* into the three genera (Figure 3).

The nucleotide sequences of BLRV and SbDV (genus *Luteovirus*) lack ORF0, like those of luteoviruses, and the predicted amino acid sequences of their replication proteins are similar to those of the luteoviruses. However, their structural proteins are more closely related to those of poleroviruses (Figure 3). Conversely, sugarcane yellow leaf virus (genus *Polerovirus*) contains an ORF0 and its ORFs 1 and 2 are most closely related to those of other poleroviruses, whereas ORFs 3 and 4 are most closely related to those of the luteoviruses and ORF5 is most closely related to the readthrough protein gene of the enamovirus. These viruses may be recombinants between the genera.

## Similarity with other taxa

Viruses in the family *Luteoviridae* have replication-related and structural proteins that are sufficiently similar to those in other genera to suggest evolutionary relationships. The putative luteovirus polymerases resemble those of members of the family *Tombusviridae*. In contrast, polymerases

of poleroviruses and enamoviruses resemble those of viruses in the genus *Sobemovirus*. These polymerase types are thought to be very distant in evolutionary terms. The CP amino acid sequences of PLRV and rice yellow mottle virus, a sobemovirus, share 33% similarity, which has been used to predict the structure of PLRV and other members of the family *Luteoviridae*. It has been suggested that the genomes of the *Luteoviridae* originated by recombination between ancestral genomes containing the structural protein genes characteristic of the family *Luteoviridae* and genomes containing either of the two polymerase types.

## Derivation of names

*Enamo*: from pea *ena*tion *mo*saic virus.
*Luteo*: from Latin *luteus*, "yellow".
*Polero*: from *po*tato *le*af *ro*ll virus.

## Further reading

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## Contributed by

Domier, L.L.

# Family *Narnaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Narnaviridae*** |
| Genus | ***Narnavirus*** |
| Genus | ***Mitovirus*** |

Viruses in the family *Narnaviridae* consist of a single molecule of non-encapsidated positive-strand RNA of 2.3–2.9 kb, which encodes a single protein of 80–104 kDa with amino acid sequence motifs characteristic of an RNA dependent RNA polymerase (RdRp).

# Genus *Narnavirus*

Type species ***Saccharomyces 20S RNA narnavirus***

## Virion properties

### Morphology

No true virions are found associated with members of this genus. The genomes, however, are associated with their RdRps forming ribonucleoprotein complexes in 1:1 stoichiometry. Genetic and biochemical evidence show that they are located in the cytoplasm.

### Physicochemical and physical properties

The ribonucleoprotein complex sediments through a sucrose gradient with a sedimentation coefficient of about 20S. These complexes are quite stable at pH 9.0 and have *in vitro* RNA polymerase activity that synthesizes mainly 20S RNA, and a minor amount of complementary strands.

### Nucleic acid

The Saccharomyces 20S RNA narnavirus (ScNV-20S) genome is a linear ssRNA of 2.5 kb in size with a high G+C content (ca. 60%). There is no poly(A) tail at the 3′ end and it is not known whether the 5′ end is capped. It is present in a high copy number under stress conditions, such as growth under nitrogen starvation, reaching up to 100,000 copies/cell.

### Proteins

No structural proteins have been described for members of this family. ScNV-20S has a coding capacity for a protein of 91 kDa (p91), with sequences conserved among RdRps. The conserved sequences are more similar to those of replicases of ssRNA enterobacteria phages than polymerases of members of the family *Totiviridae* in the same host. This protein is quite basic (estimated pI of 11) and has ssRNA binding activity. Protein p91 is essential for replication and responsible for the *in vitro* RdRp activity that synthesizes 20S RNA. P91 does not undergo proteolytic processing after translation. Studies using antibodies against this protein show that it is expressed in yeast cells grown exponentially or under induction conditions.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

ScNV-20S has one ORF that encodes p91, and there are no ORFs with coding capacity larger than 100 aa in the complementary strand. The ORF for p91 spans almost the entire sequence of 20S RNA, with a short untranslated leader sequence at the 5′ end (12 nt) and an UTR at the 3′ end of 12 nt (Figure 1). Two replication models for 20S RNA have been proposed based on the similarity of p91 to the replicases of RNA enterobacteria phages and the replication intermediates obtained in the *in vitro* RNA polymerase reaction. One model is similar to the replication cycle of ssRNA enterobacteria phages such as Qβ; that is, ScNV-20S is copied into the complementary strands and these

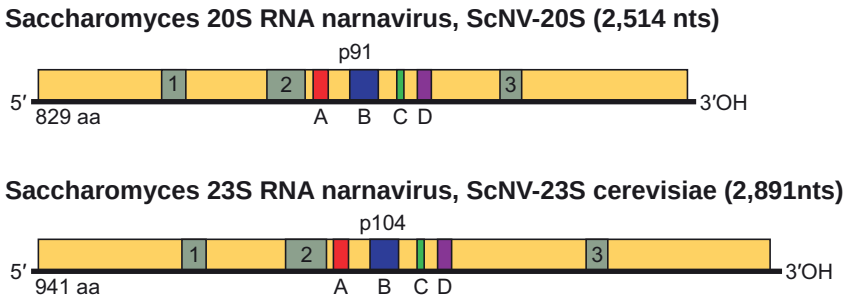


**Figure 1:** Genomic organization of Saccharomyces 20S RNA narnavirus (ScNV-20S) and Saccharomyces 23S RNA narnavirus (ScNV-23S) and the proteins encoded on them (p91 and p104, respectively). Sequence motifs (A to D) conserved in RdRp are boxed and shaded. Motifs 1, 2 and 3 are present only in p91 and p104.

copies serve as templates for 20S RNA synthesis. Annealing of 20S RNA and its complementary strand gives a double-stranded form of ScNV-20S. This dsRNA called W can be easily isolated from all ScNV-20S-containing yeast strains. The other model hypothesizes that W dsRNA is the replicative form of ScNV-20S. At present, available data support the first model. Recently, a reverse genetics system for ScNV-20S has been established. Like native viruses, viruses generated from cDNA vectors can be transmitted to daughter cells indefinitely without the vector or any selection.

## Antigenic properties

No antibody has been raised from virus particle preparations.

## Biological properties

ScNV-20S infects more than 90% of laboratory strains of the baker's yeast *Saccharomyces cerevisiae*. Some strains isolated from the brewery industry also have been found to carry ScNV-20S. There is no phenotype associated with the presence of this RNA. Like other viruses of fungi, there is no extracellular stage in the ScNV-20S life cycle. Transmission takes place through mating or cytoplasmic mixing. These viruses are very stable. Known curing procedures that eliminate members of the family *Totiviridae* in the same host, such us growth at high temperature, or addition of either cycloheximide, acridine orange, or guanidine HCl, do not eliminate ScNV-20S.

## Species demarcation criteria in the genus

Narnaviruses generally replicate stably within the cell as the cells grow. Virus strains of the same species are expected to segregate relative to each other as the cells grow, whereas those of different species should be stably co-maintained. Viruses of the same species should be similarly affected by host chromosomal mutations. Viruses that can recombine or exchange segments with each other to give viable progeny should be considered the same species. Although these biological criteria are the prime determinants of species, sequence criteria also are used. Less than 50% sequence identity at the protein level generally reflects a species difference. None of the above criteria is absolute, but narnaviruses described so far leave little doubt about species demarcation. For example, ScNV-20S and ScNV-23S are only 30% identical in the 439 aa region of highest similarity. More important, they are stably compatible with each other in the same yeast strain.

## List of species in the genus *Narnavirus*

| | | |
|---|---|---|
| *Saccharomyces 20S RNA narnavirus* | | |
| Saccharomyces 20S RNA narnavirus 37-4C | [AF039063] | (ScNV-20S-37-4C) |
| *Saccharomyces 23S RNA narnavirus* | | |
| Saccharomyces 23S RNA narnavirus 37-4C | [U90136] | (ScNV-23S-37-4C) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Narnavirus* but have not been approved as species

None reported.

## GENUS *MITOVIRUS*

Type species *Cryphonectria mitovirus 1*

### Virion properties

#### MORPHOLOGY

No true virions are found associated with members of this genus. Genetic and biochemical evidence shows that they are located in the mitochondria.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Mitochondrial fractions isolated in sucrose gradients contain mitovirus ds- and ssRNA. No subfractionation of mitochondria has been achieved.

#### NUCLEIC ACID

The virus genome consists of a single molecule of RNA of 2.3–2.7 kb. Double-stranded RNAs in this size range can be isolated from mitochondria of infected isolates. Single-stranded RNA of the same size, and corresponding to the coding strand of the dsRNA, is present in infected tissue in greater molar amount than the dsRNA. The 5′ and 3′ sequences can be folded into stable stem-loop structures. For some mitoviruses, the 5′ and 3′ sequences are complementary. The coding strand has 62–73% A+U residues, but no poly(A) tail is associated with the 3′ end.

#### PROTEINS

No structural proteins are known to be associated with the virus ssRNA or dsRNA.

#### LIPIDS

None reported.

#### CARBOHYDRATES

None reported.

### Genome organization and replication

The putative coding strand is predicted to be translatable only in mitochondria, not in the cytoplasm. When mitochondrial codon usage is invoked (UGA coding for tryptophan), the deduced translation product is a protein of 80–97 kDa, containing RdRp motifs. RdRp activity and an 80 kDa RdRp protein have been detected in mitochondria from an infected *Ophiostoma novo-ulmi* isolate. No large polypeptide is predicted from the complementary strand of any mitovirus.

### Antigenic properties

No antibody has ever been raised from virus particle preparations.

### Biological properties

Mitoviruses have been found in isolates of the chestnut blight fungus, *Cryphonectria parasitica*, Dutch elm disease fungi, *Ophiostoma novo-ulmi* and *O. ulmi*, and *Sclerotinia homoeocarpa*, the cause of dollar spot of turf grass. Fungal isolates may contain one or several mitoviruses. Some, but not all, member viruses reduce virulence of the fungus (i.e., cause "hypovirulence"). Mitoviruses are localized in mitochondria. They can be transmitted to uninfected strains by hyphal fusion (anastomosis). The transmission rate through asexual spores (conidia) is virus-specific and varies from 10 to 100%. In *C. parasitica*, transmission through sexual spores (ascospores) occurs at 20–50% when the infected parent is the female in matings, but does not occur when the infected parent is male in matings. In *O. novo-ulmi*, viruses are usually excluded from ascospores, even when both parents are infected.

Identical mitoviruses have been found in *O. novo-ulmi* and *O. ulmi*, and a strain of *Ophiostoma mitovirus 3a* has been reported in *Sclerotinia homoeocarpa*, suggesting that both interspecies and intergenus virus transmission occurs in nature.

## Species demarcation criteria in the genus

Species demarcation criteria have not been precisely defined. However, amino acid sequence identities of putative RdRp proteins between the different mitovirus species so far defined are less than 40%. Amino acid sequence identities of putative RdRp proteins between strains of the same mitovirus species are greater than 90%.

## List of species in the genus *Mitovirus*

| | | |
|---|---|---|
| *Cryphonectria mitovirus 1* | | |
| Cryphonectria mitovirus 1 cpNB631 | [L31849] | (CMV-1-cpNB631) |
| *Ophiostoma mitovirus 3a* | | |
| Ophiostoma mitovirus 3a OnuLd | [AJ004930] | (OMV-3a-OnuLd) |
| Sclerotinia homoeocarpa mitovirus | [AY172454] | |
| *Ophiostoma mitovirus 4* | | |
| Ophiostoma mitovirus 4 OnuLd | [AJ132754] | (OMV-4-OnuLd) |
| *Ophiostoma mitovirus 5* | | |
| Ophiostoma mitovirus 5 OnuLd | [AJ132755] | (OMV-5-OnuLd) |
| *Ophiostoma mitovirus 6* | | |
| Ophiostoma mitovirus 6 OnuLd | [AJ132756] | (OMV-6-OnuLd) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Mitovirus* but have not been approved as species

| | | |
|---|---|---|
| Botrytis mitovirus 1 | [EF580100] | (BMV-1) |
| Gremmeniella mitovirus S1 | [AF534641] | (GMV-S1) |
| Helicobasidium mitovirus 1 | [AB110977] | (HMV-1) |
| Ophiostoma mitovirus 1a | [AM087548] | (OMV-1a) |
| Ophiostoma mitovirus 1b | [AM087549] | (OMV-1b) |
| Ophiostoma mitovirus 2 | | (OMV-2) |
| Ophiostoma mitovirus 3b | [AM087550] | (OMV-3b) |
| Thielaviopsis mitovirus 1 | [AY563138] | (TMV-1) |

## List of other related viruses which may be members of the family *Narnaviridae* but have not been approved as species

| | | |
|---|---|---|
| Rhizoctonia virus M2 | [U51331] | (RVM2) |

## Phylogenetic relationships within the family

In a neighbor-joining phylogenetic tree based on aa sequences of the putative RdRp proteins, the mitovirus and narnavirus genera are clearly distinguished, but nevertheless form a significant cluster (Figure 2). The putative RdRp protein of the unassigned virus, Rhizoctonia virus M2 (RVM2), clusters with those of the mitoviruses (Figure 2). However, since only a small proportion of RVM2 co-purifies with mitochondria with most of it being found in the cytoplasm, RVM2 does not use the mitochondrial code. Furthermore, there is evidence for a RVM2 DNA copy in the host genome. These findings suggest significant differences between RVM2 and the mitoviruses.

## Similarity with other taxa

The putative RdRp proteins of narnaviruses and mitoviruses are distantly related to those of bacteriophages in the family *Leviviridae* (Figure 2). Furthermore, the 3′-end secondary structures of members of the genus *Narnavirus* resemble those of coliphages in the family *Leviviridae*. In a neighbor-joining phylogenetic tree of families of fungus viruses and related viruses in other taxa, based

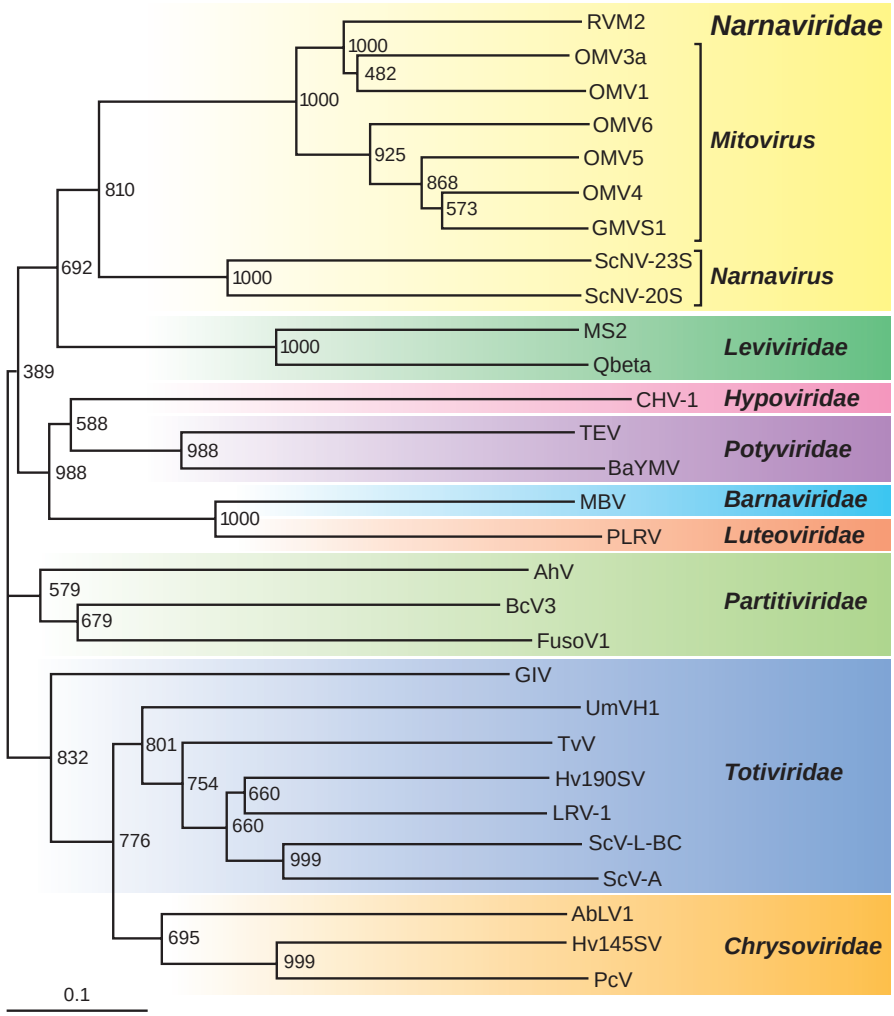


**Figure 2:** Phylogenetic tree based on aa sequences of motifs A to E (Hong *et al.* 1998) of the putative RdRp proteins of members of the family *Narnaviridae*, other families of RNA viruses of fungi and related viruses in other host taxa, and the family *Leviviridae* of RNA bacteriophages. Sequence alignments and the neighbor-joining tree were made using the Clustal X program. Bootstrap numbers (1000 replicates) are shown on the nodes. Abbreviations and sequence acquisition numbers are: AbVL1, Agaricus bisporus virus L1 X94361; AhV, Atkinsonella hypoxylon virus L39126; BaYMV, Barley yellow mosaic virus D01091; BcV3, Beet cryptic virus 3 S63913; CHV1, Cryphonectria hypovirus 1 M57938; CMV1, Cryphonectria mitovirus 1 L31849; FusoV1, Fusarium solani virus 1 D55668; GlV, Giardia lamblia virus L13218; GMVS1, Gremmeniella mitovirus S1 AF534641; Hv145SV, Helminthosporium victoriae 145S virus AF297176; Hv190SV, Helminthosporium victoriae 190S virus U41345; LRV1, Leishmania RNA virus 1-1 M92355; MBV, Mushroom bacilliform virus U07551; MS2, Enterobacteria phage MS2 GB-PH:MS2CG; OMV3a, Ophiostoma mitovirus 3a AJ004930; OMV4, Ophiostoma mitovirus 4 AJ132754; OMV5, Ophiostoma mitovirus 5 AJ132755; OMV6, Ophiostoma mitovirus 6 AJ132756; PcV, Penicillium chrysogenum virus AF296439; PLRV, Potato leafroll virus X14600; Qbeta, Enterobacteria phage Qβ AY099114; RVM2, Rhizoctonia virus M2 U51331; ScV-L-A, Saccharomyces cerevisiae virus L-A J04692; ScV-L-BC, Saccharomyces cerevisiae virus L-BC U01060; ScNV-20S, Saccharomyces 20S RNA narnavirus M63893; ScNV-23S, Saccharomyces 23S RNA narnavirus M86595; TEV, Tobacco etch virus M15239; TvV, Trichomonas vaginalis virus U08999; UmVH1, Ustilago maydis virus H1 U01059.

on aa sequences of the putative RdRp proteins, the families *Narnaviridae* and *Leviviridae* form a cluster with 69.2% bootstrap support (Figure 2).

## Derivation of names

*Narna*: from *na*ked *RNA* virus.
*Mito*: from *mito*chondrial.

## Further reading

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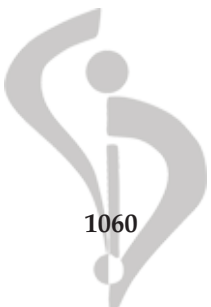
## Contributed by

Hillman, B.I. and Esteban, R.



# Family *Nodaviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Nodaviridae* |
| Genus | *Alphanodavirus* |
| Genus | *Betanodavirus* |

# Genus *Alphanodavirus*

Type species *Nodamura virus*

## Virion properties

### Morphology

Virions are non-enveloped, roughly spherical in shape, 32–33 nm in diameter and have icosahedral symmetry (T = 3). No distinct surface structure is seen by electron microscopy of negatively stained preparations. Empty shells are seldom seen in virus preparations. (See Figure 1.)

### Physicochemical and physical properties

Virion Mr is about $9 \times 10^6$; $S_{20,w}$ is 135–145S. Virion buoyant density in CsCl is 1.30–1.34 g cm$^{-3}$ (varies with species). Infectivity of aqueous suspensions is stable to extraction with chloroform. Infectivity of Nodamura virus (NoV), black beetle virus (BBV), or Flock House virus (FHV) is stable at room temperature in 1% sodium dodecyl sulfate but Boolarra virus (BoV) is inactivated. Virions are stable at acid pH. The RNA content of the virion is about 16%.

### Nucleic acid

The genome consists of two molecules of positive sense ssRNA: RNA1 (Mr $1.1 \times 10^6$, 3.1 kb) and RNA2 (Mr $0.48 \times 10^6$, 1.4 kb). Both molecules are required for infectivity, and both are encapsidated in the same virus particle. Both RNA molecules are capped at their 5′ ends with cap zero structures and lack poly(A) tails at their 3′ ends. RNA 3′ ends cannot be chemically derivatized even after treatment with denaturing solvents, indicating that the expected 3′-terminal-OH groups are unreactive.

### Proteins

The capsid consists of 180 protein subunits (protomers) arranged on a T = 3 surface lattice. Each protomer is composed of a single CP (protein α) or the two products of its cleavage (proteins β and γ). Mass spectrometry of the FHV CP indicates that the initiating methionine is removed. Thus, for FHV, the capsid proteins are: protein α: (44 kDa), aa 2-407; protein β: (39 kDa), aa 2–363; protein γ: (4 kDa), aa 364–407. Morphogenesis involves the formation of a non-infectious provirion, which acquires infectivity by autocatalytic cleavage of protein α to form proteins β and γ. Maturation cleavage is often incomplete and virions typically contain residual uncleaved protein α.

### Lipids

None.

### Carbohydrates

None.

## Genome organization and replication

Alphanodaviruses replicate in the cytoplasm of infected cells (Figure 2). RNA synthesis is resistant to actinomycin D. Infected cells contain three ssRNAs: RNA1 (Mr $1.1 \times 10^6$; 3.1 kb); RNA2 (Mr $0.48 \times 10^6$; 1.4 kb) and a sgRNA3 (Mr $1.10.13 \times 10^6$; 0.39 kb), whose nt sequence corresponds to the 3′ end of RNA1 (387 nt in the case of FHV). The 3′ end of all three RNAs is chemically unreactive. Unlike RNAs 1 and 2, RNA3 is not packaged into virions. RNA1 encodes protein A (112 kDa), which is the catalytic subunit of the viral RdRp. RNA2 encodes protein α, the CP precursor (44 kDa). Depending on the virus species, RNA3 encodes one or two small proteins (proteins B1 and B2, 11 kDa). B1 is encoded in the same ORF as protein A. Protein B2 is encoded in an overlapping ORF. BoV RNA3 does not encode protein B1 but all known alphanodavirus RNA3 molecules encode protein B2. Protein B2 of FHV functions as a suppressor of RNA interference (RNAi)

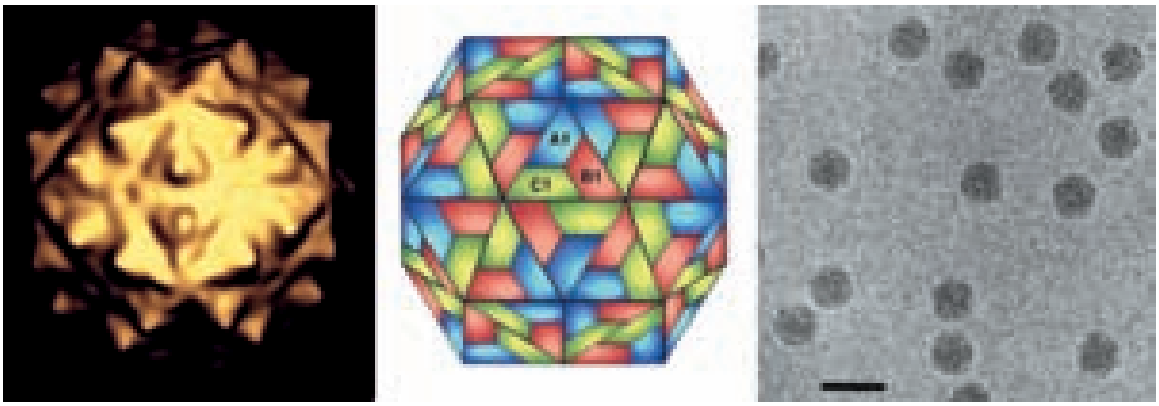


**Figure 1:** (Left) Image reconstruction of a particle of Flock House virus (FHV). (Center) Schematic representation of a T = 3 icosahedral lattice. (Right) Cryo-electron micrograph of particles of FHV; the bar represents 50 nm. (Courtesy of N. Olson and T. Baker.)

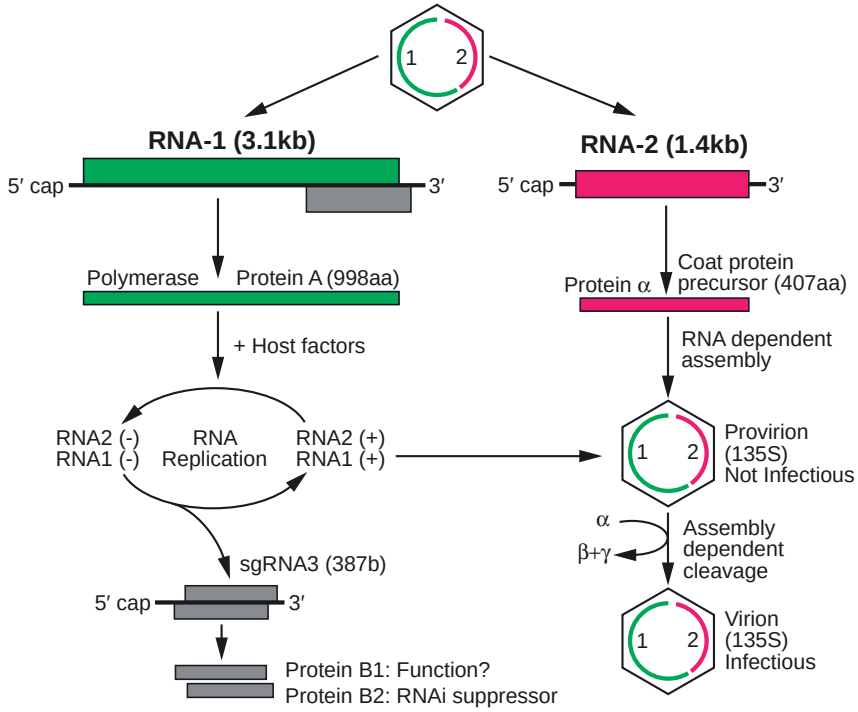



**Figure 2:** *Alphanodavirus* (Flock House virus; FHV) genome organization and strategy of replication. (Adapted from L.A. Ball and K.L. Johnson.)

in *Drosphila melanogaster*, cultured *Drosophila melanogaster* cells (Schneider's line 2), tobacco plants (*Nicotiana benthamiana*), and *Caenorhabditis elegans*. Similarly, NoV B2 suppresses RNAi in cultured mammalian and mosquito cells. The function of protein B1 is unknown. Cells transfected with isolated RNA1 synthesize RNA1 and overproduce RNA3, but do not make RNA2. RNA2 replication strongly inhibits synthesis of RNA3 and the translation of RNA2 suppresses the translation of RNA1.

## Antigenic properties

NoV, BBV, FHV and BoV are cross-reactive by double-diffusion immunoprecipitation tests, but all four members represent different serotypes (neutralization titer of each antiserum less than 0.5% in heterotypic crosses). In constrast, PaV and NoV are not cross-reactive by gel immunodiffusion tests.

## Biological properties

### Host range

**Nature:** All species of alphanodaviruses were isolated in nature from insects, although serological data suggest that NoV also naturally infects pigs and perhaps herons. NoV seems to be unique among the nodaviruses in its ability to infect both vertebrates and invertebrates. It is also very unusual in being able to kill both insect and mammalian hosts. The other alphanodaviruses do not show strict specificity for particular insect hosts.

**Laboratory:** In the laboratory, most alphanodaviruses can be propagated in larvae of the common wax moth, *Galleria mellonella*, where they cause paralysis and death. FHV, BBV, and BoV grow well in cultured *Drosophila melanogaster* cells and form plaques on monolayers of these cells. Defective-interfering particles are readily formed unless the viruses are passaged at low multiplicity of infection. FHV, isolated from grass grubs (*Costelytra zealandica*), also infects several other insect species, including adult common fruit flies (*Drosophila melanogaster)*, tsetse flies (*Glossina morsitans morsitans*), reduviid bugs (*Rhosnius prolixus*) and several mosquito species (*Aedes aegypti*, *Culex pipiens pipiens*, *Armigeres subalbatus* and *Anopheles gambiae*). FHV can be propagated in mammalian cells, plants (*Nicotiana benthamiana)*, yeast (*Saccharomyces cerevisiae)* and nematodes (*Caenorhabditis elegans)*. Persistent FHV infections, with subsequent resistance to superinfection, occur readily in cultured *Drosophila melanogaster* cells. NoV, isolated from mosquitoes, also causes paralysis and death in suckling mice and suckling hamsters. NoV infects cultured cells from both mosquito and mammalian species, but not those of *Drosophila melanogaster*. Interestingly, NoV infection is delayed in mammalian cells compared to that seen in mosquito cells. NoV can also be propagated by transfecting mosquito, vertebrate, or yeast (*Saccharomyces cerevisiae*) cell cultures with virion RNA or cloned cDNA copies of genomic RNAs at temperatures up to 37 °C. PaV infects cultured cells from *Spodoptera exigua* (beet armyworm), *Helicoverpa zea* (corn earworm), and *Aedes albopictus* mosquitoes, but not those of *Drosophila melanogaster* or *Spodoptera frugiperda* (fall armyworm). Infectious cDNA clones of the genomic RNAs of FHV, NoV and PaV allow the initiation of their respective replicative cycle in many cell types on transfection of plasmid DNA or *in vitro* transcripts.

### Transmission

NoV is transmissible to suckling mice by *Aedes aegypti* mosquitoes. It causes paralysis and death when injected into suckling mice and suckling hamsters, but no disease in adult animals. In their insect hosts, alphanodaviruses typically cause stunting, paralysis, and death.

## Species demarcation criteria in the genus

The following criteria can be applied to the demarcation of species within the *Alphanodavirus* genus:

- **Biological properties** (host range, vectors, mode of transmission). Since the natural host ranges of the nodaviruses have generally not been examined in detail but may in some cases be broad, virus isolation from a new host is not, in itself, evidence of a new nodavirus species.
- **Antigenic properties.** Antisera raised against different isolates or strains of a single nodavirus species should exhibit high levels of cross-reactivity in Western blot and/or neutralization analyses. Lower levels of cross-reactivity in these assays using antisera against all previously recognized nodavirus species can provide evidence of a novel nodavirus.
- **Virion physical/physicochemical characteristics.**
  - **Virion electrophoretic mobility.** Intact virus particles migrate with characteristic electrophoretic mobilities in non-denaturing agarose gel, so virion mobility should be compared with those of other nodavirus species.
  - **Sedimentation coefficient, buoyant density.** Virion sedimentation coefficient and buoyant density should be compared with those of other nodavirus species.
- **Structural protein characteristics.** The electrophoretic mobilities in SDS-PAGE of the CP precursor or its cleavage products should be compared with those of other nodavirus species.
- **Genome molecular characteristics.**
  - **RNA electrophoretic mobilities.** In the absence of sequence information, the electrophoretic mobilities of the viral genomic RNAs should be compared with those of other nodaviruses.

- **RNA hybridization properties.** In the absence of differences in RNA electrophoretic mobilities, the molecular hybridization properties of the viral genomic RNAs should be compared with those of other nodaviruses.
- **Genome sequence characteristics.** The nt sequence of the two genomic RNAs should be compared with those of other nodaviruses. Because the nodavirus genome is segmented, reassortment can occur and the two genome segments may have distinct evolutionary lineages.

**Application of these criteria:** In practice, while the five criteria above may be suggestive of a new species, definitive demarcation is based on the nt sequence of the viral CP gene. The two closest recognized species are BBV and FHV, whose RNA2 sequences show 80% identity at the nt level and 87% identity at the aa sequence level. Their RNA1 sequences, however, are 99% identical.

### List of species in the genus *Alphanodavirus*

| | | |
|---|---|---|
| *Black beetle virus* | | |
| Black beetle virus | RNA1[X02396 = NC_001411]<br>RNA2[X00956 = NC_002037] | (BBV) |
| *Boolarra virus* | | |
| Boolarra virus | RNA1[AF329080 = NC_004142]<br>RNA2[X15960 = NC_004145] | (BoV) |
| *Flock House virus* | | |
| Flock House virus | RNA1[X77156 = NC_004146]<br>RNA2[X15959 = NC_004144] | (FHV) |
| *Nodamura virus* | | |
| Nodamura virus | RNA1[AF174533 = NC_002690]<br>RNA2[AF174534 = NC_002691] | (NoV) |
| *Pariacoto virus* | | |
| Pariacoto virus | RNA1[AF171942 = NC_003691]<br>RNA2[AF171943 = NC_003692] | (PaV) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Alphanodavirus* but have not been approved as species

| | |
|---|---|
| Drosophila line 1 virus | (DLV) |
| Gypsy moth virus | (GMV) |
| Lymantria ninayi virus Greenwood | (LNV) |
| Manawatu virus | (MwV) |
| New Zealand virus | (NZV) |

## Genus *Betanodavirus*

Type species ***Striped jack nervous necrosis virus***

### Virion properties

#### Morphology

Virions are non-enveloped, spherical in shape, 25–30 nm in diameter, and have icosahedral symmetry (T=3). Distinct surface protrusions are observed by electron microscopy of negatively stained preparations (Figure 3). Image reconstruction of virus-like particles of Malabaricus grouper nervous necrosis virus (MGNNV) indicates that the CP of betanodaviruses has a two domain structure compared to the single domain structure of the CP of alphanodaviruses. The average diameter of the particle is 37 nm. In contrast with most alphanodaviruses, empty particles have been seen by electron microscopy of some preparations of betanodaviruses.

#### Physicochemical and physical properties

Virion buoyant density in CsCl of striped jack nervous necrosis virus (SJNNV) has not been reported but that of Dicentrarchus labrax encephalitis virus (DlEV) is about 1.31–1.36 g cm$^{-3}$.

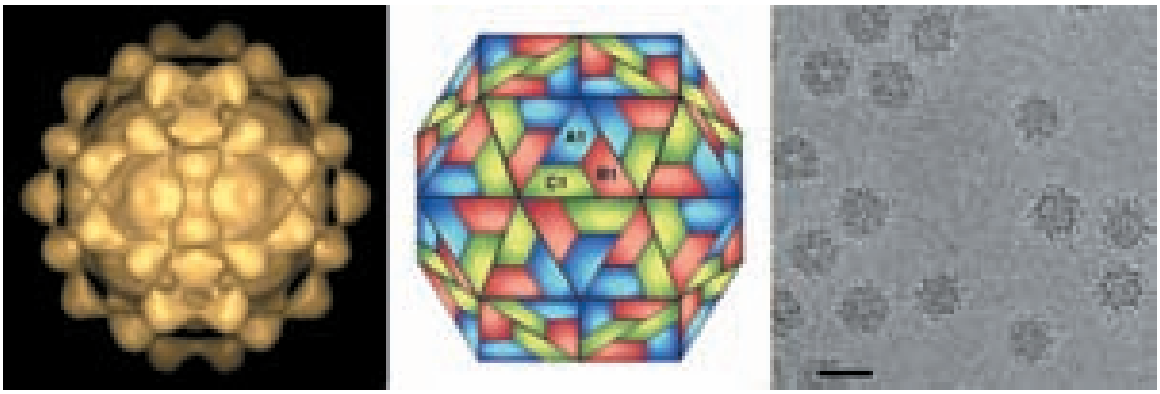


**Figure 3:** (Left) Image reconstruction of virus-like particles of Malabaricus grouper nervous necrosis virus (MGNNV) generated in *Spodoptera frugiperda* cells from a recombinant baculovirus expressing the MGNNV coat protein gene. (Center) Schematic representation of a T = 3 icosahedral lattice. (Right) Cryo-electron micrograph of virus-like particles of MGNNV; the bar represents 40 nm. (Courtesy of L. Tang and J.E. Johnson.)

Virions of DlEV are stable between pH 2 and 9 and resistant to heating at 56 °C for 30 min. Infectivity is resistant to extraction of virions with chloroform.

### Nucleic acid

The genome consists of two molecules of positive sense ssRNA: RNA1 (Mr $1.01 \times 10^6$, 3.1 kb) and RNA2 (Mr $0.49 \times 10^6$, 1.4 kb). Both RNA molecules are encapsidated in the same virus particle, and both are required for infectivity. Both molecules are capped at their 5′ ends and lack poly(A) tails at their 3′ ends.

### Proteins

Betanodavirus capsids contain 180 copies of a single structural protein of 42 kDa. In contrast to alphanodaviruses, maturation cleavage of this protein is not observed.

### Lipids

None.

### Carbohydrates

None.

## Genome organization and replication

The betanodaviruses replicate in the cytoplasm. Infected cells contain three ssRNAs: RNA1 (Mr $1.01 \times 10^6$; 3.1 kb); RNA2 (Mr $0.49 \times 10^6$; 1.4 kb) and a sgRNA3 (Mr about $0.13 \times 10^6$; 0.4 kb) derived from RNA1. RNA3 is not packaged into virions. RNA1 encodes protein 1a (110 kDa), the RdRp. RNA2 encodes protein 2a (42 kDa), the CP. SJNNV RNA3 encodes protein B2 and has a potent RNA silencing-suppression activity, as in alphanodaviruses.

## Antigenic properties

Betanodaviruses are cross-reactive by immunoblot analysis using polyclonal antisera but differential reactivity is observed with monoclonal antibodies. Virus neutralization with polyclonal antisera divides four betanodaviruses into three serotypes; serotype A for SJNNV, serotype B for TPNNV, and serotype C for RGNNV and BFNNV.

## Biological properties

### Host range

**Nature:** All species of the betanodaviruses were isolated from larvae, juvenile or adult marine fish, in which they cause "viral nervous necrosis" or "viral encephalopathy and retinopathy" associated with behavioral abnormalities and high mortalities. SJNNV and TPNNV have a limited host range: striped jack for SJNNV and tiger puffer for TPNNV. In contrast, RGNNV and BFNNV have a wide

range of host fish; RGNNV is isolated from warm-water fishes and BFNNV is isolated from cold-water fishes. These diseases cause significant problems for the marine aquaculture industry.

**Laboratory:** Betanodaviruses replicate in cultured cells from striped snakehead fish (SNN-1 and E-11) and other cells derived from fish; GF-1 and others from groupers, SBL from sea bass, TV-1 from turbot, and SAF-1 from gilthead sea bream. A low level of virus replication is observed in mammalian (COS-1 and HeLa) cells.

**Transmission:** Betanodavirus antigens and/or CP genes are detected in eggs, larvae and ovaries of some inapparently infected hatchery-reared fish species, and the CP gene is frequently detected in a variety of wild fishes without any disease signs, indicating both horizontal and vertical modes of transmission of the virus.

## Species demarcation criteria in the genus

The species demarcation criteria applied above for the alphanodaviruses also apply to betanodaviruses.

## List of species in the genus *Betanodavirus*

| | | |
|---|---|---|
| *Barfin flounder nervous necrosis virus* | | |
| Barfin flounder nervous necrosis virus - BF93Hok | RNA1[EU826137 = NC_011063] RNA2[EU826138 = NC_011064] | (BFNNV- BF93Hok) |
| *Redspotted grouper nervous necrosis virus* | | |
| Redspotted grouper nervous necrosis virus - SGWak97 | RNA1[AY324869 = NC_008040] RNA2[AY324870 = NC_008041] | (RGNNV- SGWak97) |
| *Striped jack nervous necrosis virus* | | |
| Striped jack nervous necrosis virus | RNA1[AB056571 = NC_003448] RNA2[AB056572 = NC_003449] | (SJNNV) |
| *Tiger puffer nervous necrosis virus* | | |
| Tiger puffer nervous necrosis virus - TPKag93 | RNA1[EU236148 = NC_013640] RNA2[EU236149 = NC_013641] | (TPNNV - TPKag93) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Betanodavirus* but have not been approved as species

| | | |
|---|---|---|
| Atlantic cod nervous necrosis virus | RNA1[EF433472] RNA2[EF433468] | (ACNNV) |
| Atlantic halibut nodavirus | RNA1[AJ401165] RNA2[AJ245641] | (AHNV) |
| Dicentrarchus labrax encephalitis virus | RNA2[U39876] | (DlEV) |
| Dragon grouper nervous necrosis virus | RNA1[AY721616] RNA2[AF245004, AY721615] | (DGNNV) |
| Greasy grouper nervous necrosis virus | RNA1[AF319555] RNA2[AF318942] | (GGNNV) |
| Japanese flounder nervous necrosis virus | RNA1[FJ748760] RNA2[D38527] | (JFNNV) |
| Lates calcarifer encephalitis virus | RNA2[EF591371] | (LcEV) |
| Malabaricus grouper nervous necrosis virus | RNA2[AF245003] | (MGNNV) |
| Seabass nervous necrosis virus | RNA2[Y08700] | (SBNNV) |
| Solea senegalensis nervous necrosis virus | RNA1[FJ803911] RNA2[AJ698113] | (SSNNV) |
| Turbot nodavirus | RNA2[AJ608266] | (TNV) |

## List of unassigned species in the family *Nodaviridae*

None reported.

## List of other related viruses which may be members of the family *Nodaviridae* but have not been approved as species

| | | |
|---|---|---|
| Macrobrachium rosenbergii nodavirus | RNA1[AY231436] RNA2[AY222840] | (MrNV) |
| Penaeus vannamei nodavirus | RNA2[EF137180] | (PvNV) |
| Wuhan nodavirus | RNA1[AY962576] RNA2[DQ233638] | (WhNV) |

## Phylogenetic relationships within the family

Within the alphanodaviruses, CP sequences are 44–87% identical to one another at the aa level, whereas within the betanodaviruses, CP aa sequence identities are 80% or greater. The phylogenetic relationship between betanodavirus species and the viruses not assigned to any species has not yet been rigorously defined. However, the sequences of RNA2 of RGNNV, GGNNV, MGNNV and DGNNV are >95% identical at both the nt and aa level suggesting that future reclassification of some of these as strains of current betanodavirus species may be possible. The CP aa sequences of the alphanodaviruses are only about 10% identical to those of the betanodaviruses, insufficient to indicate common ancestry.

## Similarity with other taxa

The omegatetraviruses such as Nudaurelia capensis omega virus (NωV) and Helicoverpa armigera stunt virus (HaSV) contain bipartite ssRNA genomes, but their RNAs are about twice the size of nodavirus RNAs and they have no 3′-terminal blockage. Tetraviruses also have larger capsids with T = 4 icosahedral symmetry.

## Derivation of names

The name of the family *Nodaviridae* is derived from that of the type species of the alphanodaviruses, *Nodamura virus*, named in turn after its place of isolation in Japan. By convention, the members of the alphanodavirus genus are named after their places of isolation (BoV, FHV, NoV and PaV) or the host from which they were isolated (BBV from the black beetle, *Heteronychus arator*). Betanodaviruses are pathogens of fish causing "viral nervous necrosis" and the members of the betanodavirus genus are therefore named after the host fish from which they were isolated, followed by NNV (nervous necrosis virus).

## Further reading

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Thiéry, R., Cozien, J., de Boisseson, C., Kerbart-Boscher, S. and Nevarez, L. (2004). Genomic classification of new betanodavirus isolates by phylogenetic analysis of the coat protein gene suggests a low host-fish species specificity. *J. Gen. Virol.*, **85**, 3079–3087.

## Contributed by

Thiéry, R., Johnson, K.L., Nakai, T., Schneemann, A., Bonami, J.R. and Lightner, D.V.

# Family *Potyviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Potyviridae*** |
| Genus | ***Potyvirus*** |
| Genus | ***Ipomovirus*** |
| Genus | ***Macluravirus*** |
| Genus | ***Rymovirus*** |
| Genus | ***Tritimovirus*** |
| Genus | ***Brambyvirus*** |
| Genus | ***Bymovirus*** |

## Distinguishing features

The family *Potyviridae* consists of plant viruses with a single stranded, positive sense RNA genome and flexuous, filamentous particles. Genomes have a VPg covalently linked to the 5′ end and the 3′ terminus is polyadenylated. Genomes encode a large polyprotein that is self-cleaved into a set of functional proteins. Gene order and protein sequences are conserved throughout the family.

## Virion properties

### Morphology

Virions are flexuous filaments with no envelope and are 11–15 nm in diameter, with a helical pitch of about 3.4 nm (Figure 1). Particle lengths of members of some of the six genera differ. Members of the genera *Potyvirus*, *Ipomovirus*, *Macluravirus*, *Rymovirus*, *Tritimovirus*, *Brambyvirus* and the unassigned viruses are monopartite with particle modal lengths of 650–900 nm; members of the genus *Bymovirus* are bipartite with particles of two modal lengths of 250–300 and 500–600 nm.

### Physicochemical and physical properties

Virions of viruses in the genera *Potyvirus* and *Rymovirus* have a density in CsCl of about 1.31 g cm$^{-3}$ and $S_{20,w}$ of 137–160S. Those of the genus *Bymovirus* have a density in CsCl of about 1.29 g cm$^{-3}$.

### Nucleic acid

Viruses in all genera except *Bymovirus* have a single molecule of positive sense, ssRNA, 9.3–10.8 kb in size. Virions are infectious. A VPg of about 24 kDa is covalently linked to the 5′-terminal nt. A polyadenylate tract (20 to 160 adenosines) is present at the 3′ terminus. Bymoviruses have two positive sense, ssRNA molecules; RNA-1 is 7.3–7.6 kb in size and RNA-2 is 3.5–3.7 kb in size. Both RNAs have 3′-terminal polyadenylate tracts and probably a VPg at the 5′ termini.

### Proteins

Virions contain one type of CP of 28.5–47 kDa. N- and C-terminal residues are positioned on the exterior of the virion. Mild trypsin treatment removes N- and C-terminal segments, leaving a trypsin-resistant core of about 24 kDa. Plant proteases may degrade the CP *in vivo*, as occurs *in vitro* during purification using some procedures or from certain hosts. All potyvirus CPs display significant aa sequence identity in the trypsin-resistant core, but little identity in their N and C-terminal segments.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

The genomic RNA (each genomic RNA for the genus *Bymovirus*) encodes a single major polyprotein. This then undergoes co- and post-translational proteolytic processing by three viral-encoded

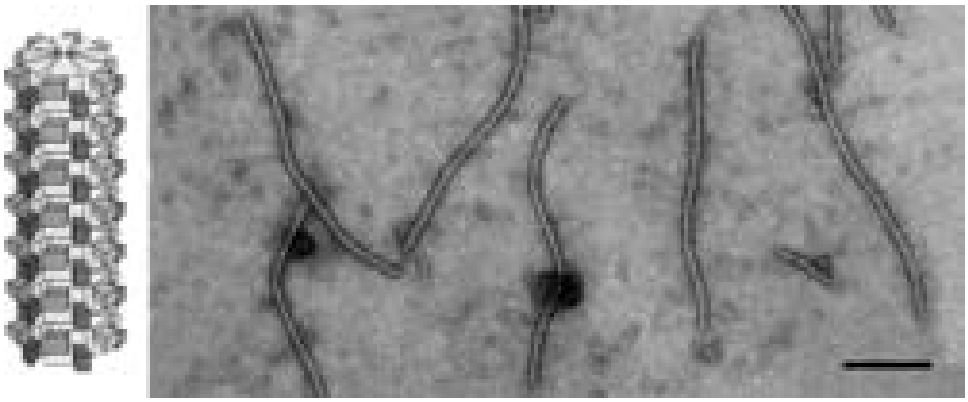

**Figure 1:** (Left) Schematic diagram of a potyvirus particle. The N-terminal (ca. 30 aa; large rectangle) and C-terminal (ca.19 aa; small rectangle) of the CP molecules are exposed on the surface of the intact virus particle (from Shukla and Ward (1989). *Adv. Virus Res.*, **36**, 273-314). (Right) Negative contrast electron micrograph of particles of an isolate of *Plum pox virus*, stained with 1% PTA, pH 6.0. The bar represents 200 nm. (Courtesy of I.M. Roberts.)

proteinases to form individual gene products. Genomic RNA replicates via the production of a full-length negative sense RNA. While there are exceptions noted in the relevant genus descriptions, the polyprotein of the majority of monopartite viruses in the family is cleaved into ten products which show conservation of sequence and organisation. As shown in Figure 2, these products are:

P1: Of all the potyvirid proteins, P1 is the least conserved in sequence and the most variable in size. It plays a significant role in virus replication probably due to the stimulation of the gene silencing suppressor HC-Pro. A serine protease domain towards the C-terminus cleaves the P1 from the polyprotein, typically at Tyr/Phe-Ser.
HC-Pro (Helper Component-Protease): the HC-Pro protein has roles in suppression of gene silencing and in vector transmission. A cysteine protease domain towards the C-terminus cleaves it from the remainder of the downstream polyprotein, typically at Gly-Gly.
P3: Involved in virus replication and appears to be significant in host range and symptom development.
6K1: The function of this small protein is not known.
CI (Cylindrical Inclusion protein): This protein has helicase activity and accumulates in inclusion bodies in the cytoplasm of infected plant cells.
6K2: A small transmembrane protein probably anchoring the replication complex to the ER.
VPg (Viral Protein genome-linked): Attached to the 5′ terminus of the genome and belongs to a class of intrinsically disordered proteins. It plays multiple roles in the viral infection cycle. It is essential for virus replication and translation, interacting with one or several isoforms of the eIF4E translation initiation factor. It is involved in suppression of RNA silencing.
NIa-Pro: Serine-like cysteine protease responsible for cleavage of most sites in the polyprotein, typically at Gln/Glu-(Ser/Gly/Ala).
NIb: The RNA-dependent RNA polymerase.
CP: Viral coat protein that also has roles in virus movement, genome amplification and vector transmission.

Recent studies have shown the presence of an additional short ORF (PIPO = "pretty interesting potyvirus ORF") embedded within the P3 cistron and expressed as a P3_PIPO fusion product via ribosomal frameshifting. This has now been identified throughout the family and has been shown to be essential for virus intercellular movement.

## Antigenic properties

The viral proteins are moderately immunogenic; there are serological relationships among members. An epitope of the CP in the conserved internal trypsin-resistant core has been identified that is similar in most members of the family.

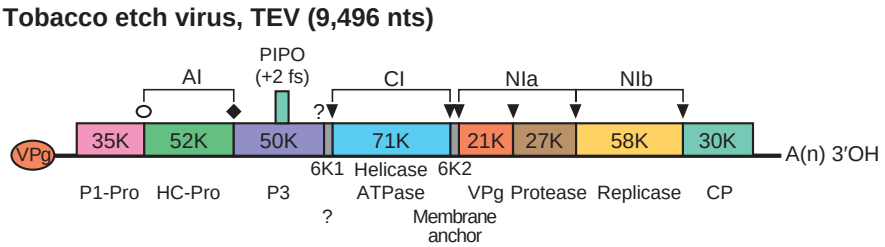


**Figure 2:** Genomic map of a member of the genus *Potyvirus*, using a strain of *Tobacco etch virus* as an example. The ssRNA genome is represented by a line and an open box representing the ORF translated into a polyprotein. Functions associated with the mature proteins proteolytically processed from the polyprotein are shown. VPg, genome-linked viral protein covalently attached to the 5′-terminal nt (represented by the oval at the 5′ end); P1-Pro, a protein with a proteolytic activity responsible for cleavage at typically Tyr/Phe-Ser (○); HC-Pro, a protein with aphid transmission helper-component activity and proteolytic activity responsible for cleavage at typically Gly-Gly (◆); Pro, serine-like proteolytic activity responsible for cleavage at Gln/Glu-(Ser/Gly/Ala) (▼). Some of these proteins of particular viruses of the family *Potyviridae* aggregate to form inclusion bodies during infection. The protein involved and the particular type of inclusion body is shown above the genetic map; AI, amorphous inclusion; CI, cylindrical-shaped inclusion body found in the cytoplasm; NIa and NIb, small and large nuclear inclusion proteins, respectively, which aggregate in the nucleus to form a nuclear inclusion body. The small ORF PIPO is putatively translated by +2 frameshift of the polyprotein ORF, and its product is expressed as a fusion with the N-terminal part of P3.

## Biological properties

### Inclusion body formation

All members of the family *Potyviridae* form cytoplasmic cylindrical inclusion (CI) bodies during infection. The CI is an array of a 70 kDa viral protein that possesses ATPase and helicase activities. Some potyviruses induce nuclear inclusion bodies that are co-crystals of two viral-encoded proteins – NIa and NIb – present in equimolar amounts. The small nuclear inclusion (NIa) protein (49 kDa) is a polyprotein consisting of the VPg and proteinase. Amorphous inclusion bodies are also evident in the cytoplasm during certain potyvirus infections and represent aggregations of the protein HC-Pro and perhaps other non-structural proteins.

### Host range

Some members have a narrow host range, most members infect an intermediate number of plants, and a few members infect species in up to 30 families. Transmission to most hosts is readily accomplished by mechanical inoculation. Many viruses are widely distributed. Distribution is aided by seed transmission in some cases.

### Transmission

Potyvirids are vectored by a variety of organisms. Members of the genera *Potyvirus* and *Macluravirus* have aphid vectors that transmit in a non-persistent, non-circulative manner. A helper component and a particular CP aa triplet (i.e., DAG for some potyviruses) are required for aphid transmission. Rymoviruses and tritimoviruses are transmitted by eriophyid mites, in a semi-persistent manner. Bymoviruses are transmitted by root-infecting vectors in the order *Plasmodiophorales*, once described as fungi but now classified as Cercozoa. Ipomoviruses appear to be transmitted by whiteflies.

## Species demarcation criteria

Throughout the family, species are distinguished by the following criteria:

- Genome sequence relatedness: different species have CP aa sequence identity less than about 80%; and nt sequence identity less than 76% either in the CP or over the whole genome. There are also differences in polyprotein cleavage sites.
- Host range and key host reactions; lack of cross protection.
- Different inclusion body morphology.
- Antigenic properties: serological relatedness may help in distinguishing species.

# Genus *Potyvirus*

Type species ***Potato virus Y***

## Distinguishing features

The largest genus in the family contains viruses transmitted by aphids in a non-persistent manner.

## Virion properties

### Morphology

Virions are flexuous filaments, 680–900 nm long and 11–13 nm wide, with helical symmetry and a pitch of about 3.4 nm. Particles of some viruses are longer in the presence of divalent cations than in the presence of EDTA.

### Physicochemical and physical properties

Virion $S_{20,w}$ is 137–160S; density in CsCl is 1.31 g cm$^{-3}$; $E^{0.1\%}_{1\,cm,\,260\,nm}$ = 2.4–2.7.

### Nucleic acid

Virions contain a single molecule of linear, positive sense ssRNA, about 9.7 kb in size; virions contain 5% RNA by weight.

### Proteins

Virions contain a single CP, 30–47 kDa in size. The CP of most isolates of the type species, PVY, contains 267 aa.

## Genome organization and replication

The genome is organized as described earlier (Figure 2).

## Antigenic properties

Virions are moderately immunogenic; there are serological relationships among many members. Some monoclonal antibodies react with most aphid-transmitted potyviruses. The CP aa sequence identity among aphid-transmitted viruses is 40–70%. Some viruses are serologically related to viruses in the genera *Rymovirus* and *Bymovirus*.

## Biological properties

Many individual viruses have a narrow host range, but a few infect plant species in up to 30 host families. The viruses are transmitted by aphids in a non-persistent manner and are transmissible experimentally by mechanical inoculation. Some isolates are inefficiently transmitted by aphids and others are not transmissible by aphids at all. This is apparently due to mutations within the helper component and/or CP cistrons. Some viruses are seed-transmitted.

## List of species in the genus *Potyvirus*

| | | |
|---|---|---|
| *Algerian watermelon mosaic virus* | | |
| Algerian watermelon mosaic virus-Algeria: H4 | [EU410442 = NC_010736] | (AWMV-H4) |
| *Alstroemeria mosaic virus* | | |
| Alstroemeria mosaic virus-O1 | [AB158522*] | (AlMV-O1) |
| *Alternanthera mild mosaic virus* | | |
| Alternanthera mild mosaic virus-Brazil | [EF442668*] | (AltMMV-BR) |
| *Amaranthus leaf mottle virus* | | |
| Amaranthus leaf mottle virus-Italy | [AJ580095*] | (AmLMV-I) |
| *Amazon lily mosaic virus* | | |
| Amazon lily mosaic virus-Japan | [AB158523*] | (ALiMV-JP) |
| *Angelica virus Y* | | |
| Angelica virus Y-USA:g | [EF488741*] | (AVY-g) |

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|---|---|---|
| *Apium virus Y* | | |
| Apium virus Y-USA: Ce | [HM363516 = NC_014905] | (ApVY-Ce) |
| *Araujia mosaic virus* | | |
| Araujia mosaic virus-Argentina: ARG1973 | [EF710625*] | (ArjMV-ARG1973) |
| *Arracacha mottle virus* | | |
| Arracacha mottle virus-Brazil:C17 | [DQ925486*] | (AMoV-C17) |
| *Artichoke latent virus* | | |
| Artichoke latent virus-California | | (ArLV-Cal) |
| *Asparagus virus 1* | | |
| Asparagus virus 1-Germany | | (AV1-DE) |
| *Banana bract mosaic virus* | | |
| Banana bract mosaic virus-Philippines | [DQ851496 = NC_009745] | (BBrMV-PH) |
| *Basella rugose mosaic virus* | | |
| Basella rugose mosaic virus-Taiwan:AC | [DQ821938 = NC_009741] | (BaRMV-AC) |
| Peace lily mosaic virus | [DQ851494] | (PcLMV) |
| *Bean common mosaic necrosis virus* | | |
| Bean common mosaic necrosis virus-USA:NL-3 | [AY282577] | (BCMNV-NL3) |
| *Bean common mosaic virus* | | |
| Bean common mosaic virus-NL4 | [DQ666332] | (BCMV-NL4) |
| Azuki bean mosaic virus | [AB012663*] | (AzBMV) |
| Blackeye cowpea mosaic virus | [AJ312437 = NC_003397] | (BlCMV) |
| Dendrobium mosaic virus | [U23564*] | (DeMV) |
| Peanut stripe virus | [U34972] | (PStV) |
| Yam bean mosaic virus | [AB289438*] | (YBMV) |
| *Bean yellow mosaic virus* | | |
| Bean yellow mosaic virus-MB4 | [D83749 = NC_003492] | (BYMV-MB4) |
| *Beet mosaic virus* | | |
| Beet mosaic virus-Wa | [AY206394 = NC_005304] | (BtMV-Wa) |
| *Bidens mottle virus* | | |
| Bidens mottle virus-Taiwan:B12 | [EU250210] | (BiMoV-B12) |
| Sunflower chlorotic spot virus | [AF538686*] | (SuCSV) |
| *Brugmansia suaveolens mottle virus* | | |
| Brugmansia suaveolens mottle virus-Brazil | [AB551370 = NC_014536] | (BsMoV-BR) |
| *Butterfly flower mosaic virus* | | |
| Butterfly flower mosaic virus-China:HZ | [AM774001*] | (BFMV-HZ) |
| *Calanthe mild mosaic virus* | | |
| Calanthe mild mosaic virus-Japan | [AB011404*] | (CalMMV-JP) |
| *Canna yellow streak virus* | | |
| Canna yellow streak virus-UK | [GQ421689 = NC_013261] | (CaYSV-UK) |
| *Carnation vein mottle virus* | | |
| Carnation vein mottle virus-Japan | [AB017630*] | (CVMoV-JP) |
| *Carrot thin leaf virus* | | |
| Carrot thin leaf virus-Australia | [AF203530*] | (CTLV-AU) |
| *Carrot virus Y* | | |
| Carrot virus Y-Australia:Victoria | [AF203537*] | (CarVY-VI) |
| *Celery mosaic virus* | | |
| Celery mosaic virus-Netherlands | [AF203531*] | (CeMV-NL) |
| *Ceratobium mosaic virus* | | |
| Ceratobium mosaic virus-Australia:13 | [AF022442*] | (CerMV-13) |
| *Chilli ringspot virus* | | |
| Chilli ringspot virus-Vietnam:C8 | [DQ925438*] | (ChiRSV-C8) |
| *Chilli veinal mottle virus* | | |
| Chilli veinal mottle virus-Pepper vein banding virus | [AJ237843 = NC_005778] | (ChiVMV-PVB) |
| *Chinese artichoke mosaic virus* | | |
| Chinese artichoke mosaic virus-Japan | [AB099711*] | (ChAMV-JP) |
| *Clitoria virus Y* | | |
| Clitoria virus Y-Australia:Queensland | [AF228515*] | (ClVY-QD) |
| *Clover yellow vein virus* | | |
| Clover yellow vein virus-30 | [AB011819 = NC_003536] | (ClYVV-30) |
| *Cocksfoot streak virus* | | |
| Cocksfoot streak virus-Germany | [AF499738 = NC_003742] | (CSV-DE) |

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|---|---|---|
| *Colombian datura virus* | | |
| Colombian datura virus-Germany:Br1 | [AJ237921*] | (CDV-Br1) |
| Petunia flower mottle virus | [AF030689*] | (PFMoV) |
| *Commelina mosaic virus* | | |
| Commelina mosaic virus-Florida | | (ComMV-FL) |
| *Cowpea aphid-borne mosaic virus* | | |
| Cowpea aphid-borne mosaic virus-Zimbabwe | [AF348210 = NC_004013] | (CABMV-ZM) |
| *Cowpea green vein banding virus* | | |
| Cowpea green vein banding virus-Brazil | | (CGVBV-BR) |
| *Cypripedium virus Y* | | |
| Cypripedium virus Y-UK:CP | [AF185954*] | (CypVY-CP) |
| *Daphne mosaic virus* | | |
| Daphne mosaic virus-Czech Republic | [DQ299908 = NC_008028] | (DapMV-CZ) |
| *Dasheen mosaic virus* | | |
| Dasheen mosaic virus-China: M13 | [AJ298033 = NC_003537] | (DsMV-M13) |
| Vanilla mosaic virus | [AJ616719*] | (VanMV) |
| *Datura shoestring virus* | | |
| Datura shoestring virus-India:Simla | | (DSSV-IN) |
| *Diuris virus Y* | | |
| Diuris virus Y-Australia | [AF203527*] | (DiVY-AU) |
| *East Asian passiflora virus* | | |
| East Asian passiflora virus-Japan:AO | [AB246773 = NC_007728] | (EAPV-AO) |
| *Endive necrotic mosaic virus* | | |
| Endive necrotic mosaic virus-Germany 1/85 | [AJ223827*] | (ENMV-1/85) |
| *Euphorbia ringspot virus* | | |
| Euphorbia ringspot virus-USA | [AY697300*] | (EuRSV-US) |
| *Freesia mosaic virus* | | |
| Freesia mosaic virus-South Korea | [GU214748] | (FreMV-KO) |
| *Fritillary virus Y* | | |
| Fritillary virus Y-China:Pan'an | [AM039800 = NC_010954] | (FVY-PA) |
| *Gloriosa stripe mosaic virus* | | |
| Gloriosa stripe mosaic virus-Netherlands | [EU042761*] | (GSMV-NL) |
| *Groundnut eyespot virus* | | |
| Groundnut eyespot virus-Ivory Coast | | (GEV-IC) |
| *Guinea grass mosaic virus* | | |
| Guinea grass mosaic virus-Ivory Coast | | (GGMV-IC) |
| *Hardenbergia mosaic virus* | | |
| Hardenbergia mosaic virus-Australia: BB-6 | [DQ898188*] | (HaMV-BB-6) |
| *Helenium virus Y* | | |
| Helenium virus Y-Germany | | (HVY-DE) |
| *Henbane mosaic virus* | | |
| Henbane mosaic virus-Hungary:PHYS/H | [AM184113*] | (HMV-PHYS/H) |
| *Hibbertia virus Y* | | |
| Hibbertia virus Y-Australia: New South Wales | [AF228516*] | (HiVY-NSW) |
| *Hippeastrum mosaic virus* | | |
| Hippeastrum mosaic virus-Amaryllis | [AY566239*] | (HiMV-AM) |
| *Hyacinth mosaic virus* | | |
| Hyacinth mosaic virus-Netherlands | [EF203681*] | (HyaMV-NL) |
| *Iris fulva mosaic virus* | | |
| Iris fulva mosaic virus-USA:Massachusetts | | (IFMV-US) |
| *Iris mild mosaic virus* | | |
| Iris mild mosaic virus-New Zealand:DC4a | [DQ436918*] | (IMMV-DC4a) |
| *Iris severe mosaic virus* | | |
| Iris severe mosaic virus-Netherlands | [X75939*] | (ISMV-NL) |
| *Japanese yam mosaic virus* | | |
| Japanese yam mosaic virus-mild | [AB027007 = NC_000947] | (JYMV-mild) |
| *Johnsongrass mosaic virus* | | |
| Johnsongrass mosaic virus-Australia | [Z26920 = NC_003606] | (JGMV-AU) |
| *Kalanchoë mosaic virus* | | |
| Kalanchoë mosaic virus-Netherlands:F39 | [GQ497731*] | (KMV-F39) |
| *Konjac mosaic virus* | | |
| Konjac mosaic virus-Japan: F | [AB219545 = NC_007913] | (KoMV-F) |
| Zantedeschia mosaic virus | [AF470620*] | (ZaMV) |
| Japanese hornwort mosaic virus | [AB081518*] | (JHMV) |

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|---|---|---|
| *Leek yellow stripe virus* | | |
| Leek yellow stripe virus-China:Yuhang | [AJ307057 = NC_004011] | (LYSV-YH) |
| *Lettuce mosaic virus* | | |
| Lettuce mosaic virus-E | [X97705 = NC_003605] | (LMV-E) |
| *Lily mottle virus* | | |
| Lily mottle virus-China:Sb | [AJ564636 = NC_005288] | (LMoV-Sb) |
| *Lycoris mild mottle virus* | | |
| Lycoris mild mottle virus-Taiwan | [AF399672*] | (LyMMoV-TW) |
| *Maize dwarf mosaic virus* | | |
| Maize dwarf mosaic virus-Bulgaria | [AJ001691 = NC_003377] | (MDMV-BU) |
| *Malva vein clearing virus* | | |
| Malva vein clearing virus-Italy:DS-Ba-01 | [FM212972*] | (MVCV-DS-Ba-01) |
| *Meadow saffron breaking virus* | | |
| Meadow saffron breaking virus-France | [AY388995*] | (MSBV-FR) |
| *Moroccan watermelon mosaic virus* | | |
| Moroccan watermelon mosaic virus-Tunisia:TN05-76 | [EF579955 = NC_009995*] | (MWMV-TN05-76) |
| *Narcissus degeneration virus* | | |
| Narcissus degeneration virus-China:Zhangzhou | [AM182028 = NC_008824] | (NDV-ZZ) |
| *Narcissus late season yellows virus* | | |
| Narcissus late season yellows virus-China:Hangzhou 2 | [AJ493579*] | (NLSYV-HZ2) |
| *Narcissus yellow stripe virus* | | |
| Narcissus yellow stripe virus-China:Zhangzhou | [AM158908 = NC_011541] | (NYSV-ZZ) |
| *Nerine yellow stripe virus* | | |
| Nerine yellow stripe virus-Netherlands:Ne800 | [EF362621*] | (NeYSV-Ne800) |
| *Nothoscordum mosaic virus* | | |
| Nothoscordum mosaic virus-Canary Islands | | (NoMV-CAY) |
| *Onion yellow dwarf virus* | | |
| Onion yellow dwarf virus-China:Yuhang | [AJ510223 = NC_005029] | (OYDV-YH) |
| *Ornithogalum mosaic virus* | | |
| Ornithogalum mosaic virus-O | [D00615*] | (OrMV-O) |
| *Ornithogalum virus 2* | | |
| Ornithogalum virus 2-Japan:Akita, Oga | [AB271783*] | (OrV2-AO) |
| *Ornithogalum virus 3* | | |
| Ornithogalum virus 3-Japan | [AB282754*] | (OrV3-JP) |
| *Papaya leaf distortion mosaic virus* | | |
| Papaya leaf distortion mosaic virus-Japan:P | [AB088221] | (PLDMV-P) |
| *Papaya ringspot virus* | | |
| Papaya ringspot virus-Hawaii | [S46722 = X67673] | (PRSV-HAT ) |
| *Parsnip mosaic virus* | | |
| Parsnip mosaic virus-UK:Scotland | | (ParMV-UK) |
| *Passiflora chlorosis virus* | | |
| Passiflora chlorosis virus-LAJ-2006 | [DQ860147*] | (PaChV-Pangda15) |
| *Passion fruit woodiness virus* | | |
| Passion fruit woodiness virus-Australia:MU2 | [HQ122652 = NC_014790] | (PWV-MU2) |
| *Pea seed-borne mosaic virus* | | |
| Pea seed-borne mosaic virus-DPD1 | [D10930 = NC_001671] | (PSbMV-DPD1) |
| *Peanut mottle virus* | | |
| Peanut mottle virus-M | [AF023848 = NC_002600] | (PeMoV-M) |
| *Pennisetum mosaic virus* | | |
| Pennisetum mosaic virus-China:B | [AY642590 = NC_007147] | (PenMV-B) |
| *Pepper mottle virus* | | |
| Pepper mottle virus-California | [M96425 = NC_001517] | (PepMoV-Cal) |
| *Pepper severe mosaic virus* | | |
| Pepper severe mosaic virus-South Korea | [AM181350 = NC_008393] | (PepSMV-KO) |
| *Pepper veinal mottle virus* | | |
| Pepper veinal mottle virus-P | [DQ645484 = NC_011918] | (PVMV-P) |
| *Pepper yellow mosaic virus* | | |
| Pepper yellow mosaic virus-Brazil:Pi-15 | [AB541985 = NC_014327] | (PepYMV-Pi15) |
| *Peru tomato mosaic virus* | | |
| Peru tomato mosaic virus-Peru:PPK13 | [AJ437280 = NC_004573] | (PTV-PPK13) |
| *Pfaffia mosaic virus* | | |
| Pfaffia mosaic virus-Brazil | [AY485276*] | (PfMV-BR) |

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| *Pleione virus Y* | | |
| Pleione virus Y-Australia | [AF185958*] | (PlVY-AU) |
| *Plum pox virus* | | |
| Plum pox virus-NAT | [D13751 = NC_001445] | (PPV-NAT) |
| *Pokeweed mosaic virus* | | |
| Pokeweed mosaic virus-USA | | (PkMV-USA) |
| *Potato virus A* | | |
| Potato virus A-Hungary: B11 | [AJ296311 = NC_004039] | (PVA-B11) |
| Tamarillo mosaic virus | [AJ131403] | (TamMV) |
| *Potato virus V* | | |
| Potato virus V-UK:DV 42 | [AJ243766 = NC_004010] | (PVV-DV42) |
| *Potato virus Y* | | |
| Potato virus Y-France:O | [X12456 = NC_001616] | (PVY-O) |
| Potato virus Y-Hungary:N | [M95491] | (PVY-N) |
| Potato virus Y-France:C | [AJ890348] | (PVY-C) |
| Bidens mosaic virus | [AY960150*] | (BiMV) |
| *Ranunculus leaf distortion virus* | | |
| Ranunculus leaf distortion virus-Italy:RN122 | [DQ152190*] | (RanLDV-RN122) |
| *Ranunculus mild mosaic virus* | | |
| Ranunculus mild mosaic virus-Italy:RN129 | [DQ152191*] | (RanMMV-RN129) |
| *Ranunculus mosaic virus* | | |
| Ranunculus mosaic virus-Italy:RN136 | [DQ152192*] | (RanMV-RN136) |
| *Rhopalanthe virus Y* | | |
| Rhopalanthe virus Y-Australia | [AF185956*] | (RhVY-AU) |
| *Sarcochilus virus Y* | | |
| Sarcochilus virus Y-Australia | [AF185957*] | (SaVY-AU) |
| *Scallion mosaic virus* | | |
| Scallion mosaic virus-China:Hangzhou | [AJ316084 = NC_003399] | (ScaMV-HZ) |
| *Shallot yellow stripe virus* | | |
| Shallot yellow stripe virus-China:ZQ2 | [AJ865076 = NC_007433] | (SYSV-ZQ2) |
| *Sorghum mosaic virus* | | |
| Sorghum mosaic virus-China:Xiaoshan | [AJ310197 = NC_004035] | (SrMV-Xiaoshan) |
| *Soybean mosaic virus* | | |
| Soybean mosaic virus-N | [D00507 = NC_002634] | (SMV-N) |
| *Spiranthes mosaic virus 3* | | |
| Spiranthes mosaic virus 3-USA | [AY685218*] | (SpMV3-USA) |
| *Sugarcane mosaic virus* | | |
| Sugarcane mosaic virus-China:Hangzhou | [AJ297628 = NC_003398] | (SCMV-HZ) |
| *Sunflower mosaic virus* | | |
| Sunflower mosaic virus-USA:Texas | [AF465545*] | (SuMV-TX) |
| *Sweet potato feathery mottle virus* | | |
| Sweet potato feathery mottle virus-S | [D86371 = NC_001841] | (SPFMV-S) |
| *Sweet potato latent virus* | | |
| Sweet potato latent virus-Taiwan | [X84012*] | (SPLV-TW) |
| *Sweet potato mild speckling virus* | | |
| Sweet potato mild speckling virus-Argentina | [U61228*] | (SPMSV-AR) |
| *Sweet potato virus 2* | | |
| Sweet potato virus 2-Nigeria | [AY232437*] | (SPV2-NG) |
| *Sweet potato virus G* | | |
| Sweet potato virus G-Peru:Hua2 | [EU218528*] | (SPVG-Hua2) |
| *Telfairia mosaic virus* | | |
| Telfairia mosaic virus-Nigeria | | (TeMV-NI) |
| *Telosma mosaic virus* | | |
| Telosma mosaic virus-Vietnam:Hanoi | [DQ851493 = NC_009742] | (TelMV-VN) |
| *Thunberg fritillary mosaic virus* | | |
| Thunberg fritillary mosaic virus-China: Ningbo | [AJ851866 = NC_007180] | (TFMV-NB) |
| *Tobacco etch virus* | | |
| Tobacco etch virus-HAT | [M11458 = NC_001555] | (TEV-HAT ) |
| *Tobacco vein banding mosaic virus* | | |
| Tobacco vein banding mosaic virus-China:YND | [EF219408 = NC_009994] | (TVBMV-YND) |
| *Tobacco vein mottling virus* | | |
| Tobacco vein mottling virus-S | [U38621] | (TVMV-S) |
| *Tradescantia mild mosaic virus* | | |
| Tradescantia mild mosaic virus-Italy:IFA195 | [AY861351*] | (TraMMV-IFA195) |

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| *Tropaeolum mosaic virus* | | |
| Tropaeolum mosaic virus-Ecuador:Mashua | | (TrMV-EC) |
| *Tuberose mild mosaic virus* | | |
| Tuberose mild mosaic virus-Taiwan | [AF062926*] | (TuMMV-TW) |
| *Tuberose mild mottle virus* | | |
| Tuberose mild mottle virus-China:Hangzhou | [AJ581528*] | (TuMMoV-HZ) |
| *Tulip breaking virus* | | |
| Tulip breaking virus-India | | (TBV-IN) |
| *Tulip mosaic virus* | | |
| Tulip mosaic virus-Japan | [X63630*] | (TulMV-JP) |
| *Turnip mosaic virus* | | |
| Turnip mosaic virus-UK1 | [AF169561 = NC_002509] | (TuMV-UK1) |
| *Twisted-stalk chlorotic streak virus* | | |
| Twisted-stalk chlorotic streak virus-Alaska:Denali 2001 | [AY954248*] | (TSCSV-Denali 2001) |
| *Vallota mosaic virus* | | |
| Vallota mosaic virus-USA:Beltsville | [EF441726*] | (ValMV-BV) |
| *Watermelon leaf mottle virus* | | |
| Watermelon leaf mottle virus-Florida | [AF028004*] | (WLMV-FL) |
| *Watermelon mosaic virus* | | |
| Watermelon mosaic virus-Fr | [AY437609 = NC_006262] | (WMV-Fr) |
| *Wild potato mosaic virus* | | |
| Wild potato mosaic virus-Peru | [AJ437279 = NC_004426] | (WPMV-Peru) |
| *Wild tomato mosaic virus* | | |
| Wild tomato mosaic virus-Vietnam: Laichau | [DQ851495 = NC_009744] | (WTMV-VN) |
| *Wisteria vein mosaic virus* | | |
| Wisteria vein mosaic virus-China:Beijing | [AY656816 = NC_007216] | (WVMV-BJ) |
| *Yam mild mosaic virus* | | |
| Yam mild mosaic virus-Papua New Guinea | [AB022424*] | (YMMV-PNG) |
| *Yam mosaic virus* | | |
| Yam mosaic virus-Ivory Coast | [U42596 = NC_004752] | (YMV-IC) |
| *Zantedeschia mild mosaic virus* | | |
| Zantedeschia mild mosaic virus-Taiwan | [AY626825 = NC_011560] | (ZaMMV-TW) |
| *Zea mosaic virus* | | |
| Zea mosaic virus-Israel | [AF228693*] | (ZeMV-IS) |
| *Zucchini yellow fleck virus* | | |
| Zucchini yellow fleck virus-Italy | [DQ641510*] | (ZYFV-IT) |
| *Zucchini yellow mosaic virus* | | |
| Zucchini yellow mosaic virus-Taiwan:TN3 | [AF127929 = NC_003224] | (ZYMV-TN3 ) |

Species names are in italic script; names of strains, isolates and synonyms are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Partial sequence including the coat protein; complete genome sequence not available.

## List of other related viruses which may be members of the genus *Potyvirus* but have not been approved as species

| | | |
|---|---|---|
| Ammi majus latent virus | [AB361564*] | (AmLV) |
| Anemone mosaic virus | [EU042755*] | (AnMV) |
| Arisaema potyvirus 1 | [FJ546415*] | (APV1) |
| Arisaema potyvirus 2 | [FJ546416*] | (APV2) |
| Begonia flower breaking virus | [FJ539085*] | (BFBV) |
| Calla lily latent virus | [EF105297] | (CLLV) |
| Catharanthus mosaic virus | [DQ365928*] | (CatMV) |
| Chickpea yellow mosaic virus | [AF527879*] | (CpYMV) |
| Christmas bell potyvirus | [EF427894] | (CBPV) |
| Delphinium vein-clearing virus | [FJ349327*] | (DVCV) |
| Ecuadorian rocoto virus | [EU495234*] | (EcRV) |
| Impatiens flower break virus | [AY864851*] | (IFBV) |
| Lupin mosaic virus | [EU847625 = NC_014898] | (LuMV) |
| Muscari mosaic virus | [EU042752*] | (MuMV) |
| Omphalodes virus Y | [AY974328*] | (OmVY) |
| Ornamental onion stripe mosaic virus | [EU042750*] | (OOSMV) |
| Ornithogalum necrotic mosaic virus | [FJ159380*] | (OrNMV) |

| | | |
|---|---|---|
| Ornithogalum stripe mosaic virus | [FJ159376*] | (OrSMV) |
| Ornithogalum virus 4 | [EU042753*] | (OrV4) |
| Panax virus Y | [GQ916624 = NC_014252] | (PnVY) |
| Passiflora foetida virus Y | [DQ112219*] | (PfVY) |
| Siratro 1 virus Y | [DQ098900*] | (SVY1) |
| Siratro 2 virus Y | [DQ098901*] | (SVY2) |
| Snowdrop virus Y | [EU927399*] | (SnVY) |
| Sunflower chlorotic mottle virus | [GU181199 = NC_014038] | (SCMoV) |
| Stenomesson mosaic virus | [EU042757*] | (StMV) |
| Sweet potato virus C | [AB509453] | (SPVC) |
| Trillium crinkled leaf virus | [FJ648825*] | (TCLV) |
| Vanilla distortion mosaic virus | [AY943944*] | (VDMV) |
| Veltheimia mosaic virus | [EF203686*] | (VelMV) |
| Veltheimia virus Y | [EU684971*] | (VelVY) |
| Verbena virus Y | [EU564817 = NC_010735] | (VerVY) |

*Partial sequence including the coat protein; complete genome sequence not available.

# Genus *Brambyvirus*

Type species ***Blackberry virus Y***

## Distinguishing features

This is a monotypic genus. The single species is distinguished from all other members of the family by having a very large P1 protein (83.6 k Da) containing an AlkB domain. It is also phylogenetically distinct.

## Virion properties

### Morphology

Virions are flexuous filaments 800 × 11–15 nm in size.

### Physicochemical and physical properties

No information.

### Nucleic acid

Virions contain a single molecule of linear positive sense ssRNA with a 3′-poly(A) terminus. Virion RNA is about 11 kb in size.

### Proteins

There is a single CP of 40.9 kDa.

## Genome organization and replication

Apart from the size of the P1, the genome organization is identical to that of most monopartite viruses in the family *Potyviridae* (Figure 2).

## Antigenic properties

The virus could not be detected by a universal potyvirus monoclonal antibody but there are no additional data.

## Biological properties

### Host range

The virus has been reported only from wild and cultivated blackberry (*Rubus* sp.) where it is often symptomless but is also a component of a complex of viruses. It is not known to cause symptoms in any herbaceous test host.

### Transmission
The virus is presumed to be transmitted by an aerial vector that has not yet been identified.

## Species demarcation criteria in the genus
Not applicable.

## List of species in the genus *Brambyvirus*

*Blackberry virus Y*
Blackberry virus Y-Arkansas 3 [AY994084 = NC_008558] (BlVY-Ark3)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Brambyvirus* but have not been approved as species
None reported.

# Genus *Ipomovirus*

Type species ***Sweet potato mild mottle virus***

## Distinguishing features
Ipomoviruses are distinguished from other genera by their mode of transmission (whiteflies) and by phylogenetic analyses.

## Virion properties

### Morphology
Virions are flexuous filaments 800–950 nm long.

### Physicochemical and physical properties
Virion $S_{20,w}$ is 155S for sweet potato mild mottle virus (SPMMV).

### Nucleic acid
Virions contain a positive sense ssRNA, with a 3′-poly(A) terminus.

### Proteins
The viral CP is a single polypeptide of 302–378 aa (35–41 kDa).

## Genome organization and replication
Ipomovirus genomes consist of 9069–10818 nt excluding the 3′-terminal poly(A) tail and encode a polyprotein of 2902–3456 aa (Figure 3). These viruses exhibit unusual structural variability. The structure and organization of the SPMMV genome is similar to *Potyvirus*, but some motifs of HC-Pro and CP characteristic of *Potyvirus* are incomplete or missing, which may account for its vector relations. The unusually large P1 (83 kDa) of SPMMV contains no obvious AlkB domain and hence differs from *Brambyvirus*. Cucumber vein yellowing and squash vein yellowing viruses (CVYV and SqVYV) differ from SPMMV by containing two P1-like serine proteases (P1a and P1b) but no HC-Pro. P1b functions as a suppressor of RNA silencing. Cassava brown streak virus (CBSV) differs from SPMMV by having no HC-Pro and, also, from CVYV and SqVYV by having only P1b which suppresses silencing. Additionally, CBSV contains a Maf/HAM1-like sequence recombined into the NIb/CP junction which can accommodate heterologous genes in engineered infectious potyvirus clones. Homology of HAM1h with cellular Maf/HAM1 NTP pyrophosphatases suggests that HAMh1 might intercept non-canonical NTPs to reduce mutation rates of viral RNA.

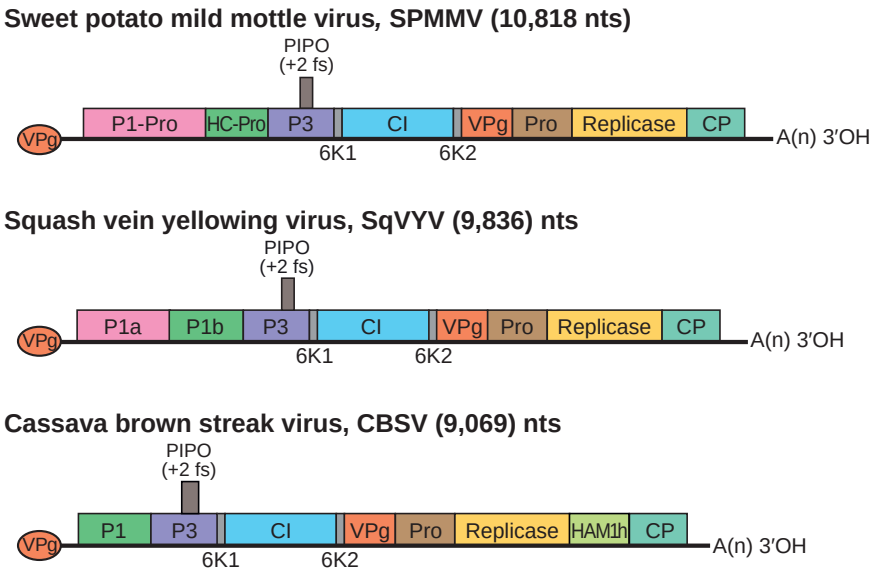


**Figure 3:** Genomic maps of the ipomoviruses sweet potato mild mottle virus (SPMMV), squash vein yellowing virus (SqVYV) and cassava brown streak virus (CBSV). The ssRNA genome is represented by a line and an open box representing the ORF translated into a polyprotein. Conventions are as for the potyvirus genome organization map (Figure 2). Activities of most gene products are postulated by analogy with genus *Potyvirus*. CVYV and SqVYV contain two P1-like serine proteases (P1a and P1b), of which P1b functions as a suppressor of RNA silencing. CBSV also contain P1b which suppresses silencing and, additionally, carries a Maf/HAM1-like sequence recombined into the NIb/CP junction. HAMh1 might intercept non-canonical NTPs to reduce mutation rates of viral RNA.

## Antigenic properties

Moderately immunogenic. No serological relationships with other members of the family *Potyviridae* have been found.

## Biological properties

### Host range

The natural host range of SPMMV is wide, with more than nine families susceptible, whereas the host range of CBSV, CVYV and SqVYV is less known apart from the hosts which they have been found to infect in the field.

### Transmission

CBSV, CVYV and SqVYV are transmitted by the whitefly *Bemisia tabaci* in a non-persistent manner. *B. tabaci* may also be the vector of SPMMV, but this is not fully confirmed. All ipomoviruses are transmissible experimentally by mechanical inoculation and by grafting.

## List of species in the genus *Ipomovirus*

| | | |
|---|---|---|
| *Cassava brown streak virus* | | |
| Cassava brown streak virus-Tanzania:KOR6 | [GU563327] | (CBSV-KOR6) |
| *Cucumber vein yellowing virus* | | |
| Cucumber vein yellowing virus-Spain:ALM32 | [AY578085 = NC_006941] | (CVYV-ALM32) |
| *Squash vein yellowing virus* | | |
| Squash vein yellowing virus-Florida | [EU259611 = NC_010521] | (SqVYV-FL) |
| *Sweet potato mild mottle virus* | | |
| Sweet potato mild mottle virus-East Africa | [Z73124 = NC_003797] | (SPMMV-EA) |

Species names are in italic script; names of strains, isolates and synonyms are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Ipomovirus* but have not been approved as species

| | | |
|---|---|---|
| Ugandan cassava brown streak virus | [FJ039520], [FN434109]* | (UCBSV) |

*Two strains sharing 87% nucleotide sequence identity.

## Genus *Macluravirus*

Type species ***Maclura mosaic virus***

### Distinguishing features

Macluraviruses resemble members of the genus *Potyvirus* in their transmission by aphids but virions are slightly shorter. They form a distinct group in phylogenetic analyses and have different polyprotein consensus cleavage sites.

### Virion properties

#### Morphology

Virions are flexuous filaments mostly 650–675 nm × 13–16 nm.

#### Physicochemical and physical properties

Virion $S_{20,w}$ is 155–158S; density in CsCl is 1.31–1.33 g cm$^{-3}$.

#### Nucleic acid

Virions contain one molecule of linear positive sense, ssRNA. RNA is about 8.0 kb.

#### Proteins

Macluraviruses have a single CP species of 33–34 kDa.

### Genome organization and replication

Complete genomes of macluraviruses are not yet available. The aa sequences of macluravirus CPs show limited (14–23%) identity with CP sequences of some aphid-transmitted potyviruses. Macluraviruses show significant aa sequence identity in portions of the replicase protein with viruses in other genera of the family *Potyviridae*. The macluraviruses seem to have a genome organization and replication strategy typical of viruses in the family *Potyviridae*.

### Antigenic properties

Moderately immunogenic. No serological relationships to members of the genus *Potyvirus* have been found except for a weak reaction between Maclura mosaic virus (MacMV) and bean yellow mosaic virus.

### Biological properties

#### Host range

Current information suggests that most viruses have a narrow host range, infecting species in up to nine host families.

#### Transmission

The viruses are transmitted by aphids in a non-persistent manner and experimentally by mechanical inoculation.

### List of species in the genus *Macluravirus*

| | | |
|---|---|---|
| *Alpinia mosaic virus* | | |
| Alpinia mosaic virus-Taiwan | [AF499025*] | (AlpMV-TW) |

| | | |
|---|---|---|
| *Cardamom mosaic virus* | | |
| Cardamom mosaic virus-India:Yelsur | [AF189125*] | (CdMV-YE) |
| *Maclura mosaic virus* | | |
| Maclura mosaic virus-UK | [U58771*] | (MacMV-UK) |
| *Chinese yam necrotic mosaic virus* | | |
| Chinese yam necrotic mosaic virus-Japan | [AB044386*] | (ChYNMV-JP) |
| *Narcissus latent virus* | | |
| Narcissus latent virus-New Zealand | [DQ450199*] | (NLV-NZ) |
| *Ranunculus latent virus* | | |
| Ranunculus latent virus-RN128 | [DQ152193*] | (RanLV-RN128) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Partial sequence including the coat protein; complete genome sequence not available.

### List of other related viruses which may be members of the genus *Macluravirus* but have not been approved as species

| | | |
|---|---|---|
| Yam chlorotic necrotic mosaic virus | [FM997910*] | (YCNMV) |

*Partial sequence including the coat protein; complete genome sequence not available.

## Genus *Rymovirus*

Type species ***Ryegrass mosaic virus***

### Distinguishing features

This genus contains only three viruses presumably transmitted by host-adapted populations of eriophyid mite species in a semi-persistent manner. The rymoviruses share a reciprocal monophyletic relationship with species of the genus *Potyvirus* (see Figure 6).

### Virion properties

#### Morphology

Virions are flexuous filaments 690–720 nm × 11–15 nm in size.

#### Physicochemical and physical properties

Virion density in CsCl is 1.325 g $cm^{-3}$ (for ryegrass mosaic virus, RGMV). Virion $S_{20,w}$ is 165–166S for most members.

#### Nucleic acid

Virions contain a single molecule of linear positive sense ssRNA with a 3′-poly(A) terminus. Virion RNA is about 9.5 kb in size.

#### Proteins

Rymoviruses have one type of CP, with a theoretical Mr of 35,482 Da and an apparent Mr estimated by Western blots of 45 kDa for RGMV.

### Genome organization and replication

The complete genome sequences available for two isolates of RGMV and one each of Agropyron mosaic virus (AgMV) and Hordeum mosaic virus (HoMV) indicate that rymoviruses have a genome organization (see Figure 2) and replication strategy similar to other species of the *Potyviridae* with monopartite genomes.

### Antigenic properties

Particles of most rymoviruses are moderately immunogenic. HoMV and AgMV are serologically related.

## Biological properties

### Host range
Most rymoviruses have limited but widespread host ranges within the family *Gramineae* but some have relatively narrow host ranges.

### Transmission
Transmission by eriophyid mites and mechanical transmission have been reported for most members. The eriophyid mites transmitting rymoviruses are different from those transmitting tritimoviruses. The cereal rust mite *Abacarus hystrix* transmits both RGMV and AgMV, but only the former is efficiently transmitted. No vector is known for HoMV. Recent studies have revealed that host-associated populations of *A. hystrix* represent a species complex.

## List of species in the genus *Rymovirus*

| | | |
|---|---|---|
| *Agropyron mosaic virus* | | |
| Agropyron mosaic virus-USA:ND402 | [AY623626 = NC_005903] | (AgMV-ND402) |
| *Hordeum mosaic virus* | | |
| Hordeum mosaic virus-ATCC PV81 | [AY623627 = NC_005904] | (HoMV-PV81) |
| *Ryegrass mosaic virus* | | |
| Ryegrass mosaic virus-Denmark | [Y09854 = NC_001814] | (RGMV-DK) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Rymovirus* but have not been approved as species

None reported.

# Genus *Tritimovirus*

Type species ***Wheat streak mosaic virus***

## Distinguishing features

Tritimoviruses are transmitted by mites (but different from those that transmit rymoviruses). They form a distinctive phylogenetic cluster.

## Virion properties

### Morphology
Virions are flexuous filaments 690–700 nm long.

### Physicochemical and physical properties
Virion $S_{20,w}$ is 166S for wheat streak mosaic virus (WSMV).

### Nucleic acid
Virions contain a positive sense ssRNA, about 9.4–9.6 kb in size, with a 3′-poly(A) terminus.

### Proteins
The viral CP is a single peptide of about 349 aa for WSMV and 320 aa for brome streak mosaic virus (BrSMV). The Mr estimated by electrophoresis is about 42 kDa.

## Genome organization and replication

The WSMV genome consists of 9384 nt excluding the 3′-terminal poly(A) tail. Sequence analysis reveals an ORF of 3035 aa. The structure and organization of the WSMV genome is similar to those of other members of the family *Potyviridae* (see Figure 2) except the bymoviruses. Most known

potyvirus motifs are present in the polyprotein of WSMV. However, motifs in the putative helper-component and CP of BrSMV are incomplete or missing, which may account for different vector relations of the tritimoviruses. The WSMV CP sequence shows limited (22-25%) identity with CP sequences of some aphid-transmitted potyviruses. WSMV shows significant aa sequence identity with aphid-transmitted potyviruses in the cylindrical inclusion protein and portions of the nuclear inclusion proteins. WSMV RNA has been translated *in vitro* into several large proteins immunoprecipitable with WSMV CP antiserum, suggesting that WSMV uses a proteolytic processing strategy to express functional proteins such as the CP. Antiserum to TEV 58 kDa nuclear inclusion protein also reacts with *in vitro* translation products of WSMV. An *in vitro* translation product is precipitated with antiserum to HC-Pro helper component of an isolate of the species *Tobacco vein mottling virus*. Comparative sequence analyses show similarities with other members of the family *Potyviridae*, but these are limited to the nine mature proteins. WSMV is especially susceptible to proteinases *in planta* and has CP molecules of 42, 36 and 32 kDa; the two smaller proteins are parts of the 42 kDa protein.

## Antigenic properties

Moderately immunogenic. WSMV and oat necrotic mottle virus (ONMV) are serologically related to each other, but not to the other members of the family *Potyviridae*.

## Biological properties

### Host range

The viruses only affect hosts in the *Gramineae* but while WSMV has a wide host range BrSMV and ONMV have narrow ones.

### Transmission

WSMV and BrSMV are transmitted by eriophyid mites in a semi-persistent manner. HC-Pro of WSMV is required for mite transmission. All definitive tritimoviruses are transmissible experimentally by mechanical inoculation.

## List of species in the genus *Tritimovirus*

| | | |
|---|---|---|
| *Brome streak mosaic virus* | | |
| Brome streak mosaic virus-France-11-Cal | [Z48506 = NC_003501] | (BrSMV-FR) |
| *Oat necrotic mottle virus* | | |
| Oat necrotic mottle virus-Type-NE | [AY377938 = NC_005136] | (ONMV-NE) |
| *Wheat Eqlid mosaic virus* | | |
| Wheat Eqlid mosaic virus-Iran | [EF608612 = NC_009805] | (WEqMV-IR) |
| *Wheat streak mosaic virus* | | |
| Wheat streak mosaic virus-Sidney 81 | [AF057533 = NC_001886] | (WSMV-Sidney 81) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Tritimovirus* but have not been approved as species

| | | |
|---|---|---|
| Yellow oat-grass mosaic virus | [GQ259764*] | (YOgMV) |

*Partial sequence including the coat protein; complete genome sequence not available.

# Genus *Bymovirus*

Type species ***Barley yellow mosaic virus***

## Distinguishing features

Compared with other viruses in the family, members of the genus *Bymovirus* are distinct in having a divided (bipartite) genome and in being transmitted by the root-infecting parasite, *Polymyxa graminis* (*Plasmodiophorales*, a fungoid protist).

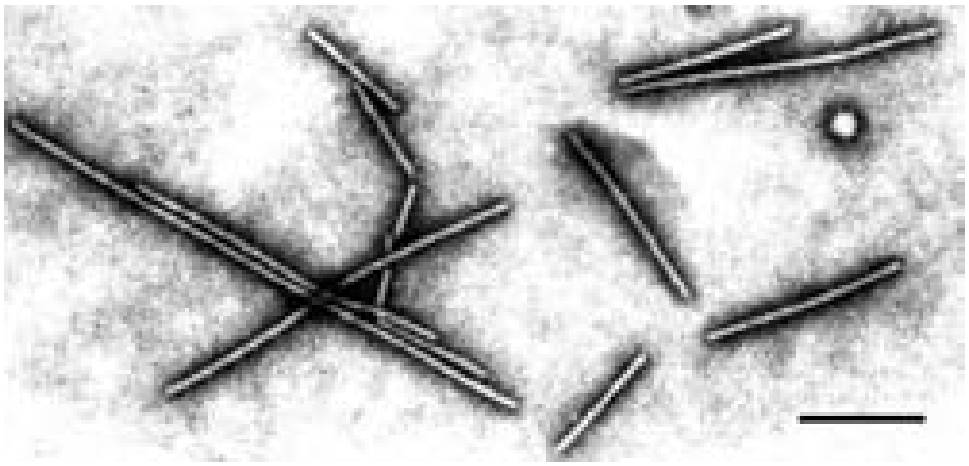

**Figure 4:** Virions of an isolate of barley yellow mosaic virus, stained with 1% PTA, pH 7.0. The bar represents 200 nm (from D. Lesemann).

## Virion properties

### Morphology

Virions are flexuous filaments of two modal lengths, 250–300 nm and 500-600 nm; both are 13 nm in width (Figure 4).

### Physicochemical and physical properties

Virion density in CsCl is 1.28–1.30 g $cm^{-3}$.

### Nucleic acid

Virions contain two molecules of linear positive sense, ssRNA. RNA-1 is 7.5–8.0 kb and RNA-2 is 3.5–4.0 kb; RNA makes up 5% by weight of particles. There is little base sequence identity between the two RNAs except in the 5′ UTR.

### Proteins

Virions have a single CP of 28.5–33 kDa. The CP of barley yellow mosaic virus isolates has 297 aa.

## Genome organization and replication

The two RNA molecules are translated initially into precursor polypeptides from which functional proteins are derived by proteolytic processing (Figure 5). The organization of RNA1 is similar to that of other potyviruses but without the P1 and HC-Pro proteins. The RNA2 polyprotein is unique to bymoviruses although the first (ca. 28 kDa) protein from RNA-2 has aa domains with sequence similarities to the potyvirus protein HC-Pro. The larger protein of RNA2 is believed to have a role in vector transmission.

## Antigenic properties

The viral proteins are moderately immunogenic; serological relationships exist among most members (except barley mild mosaic virus). The CP aa sequence identity among members is 35–74%.

## Biological properties

### Cytology

There are characteristic pinwheel-like inclusions and membranous network structures are formed in the cytoplasm of infected plant cells. No nuclear inclusions are found.

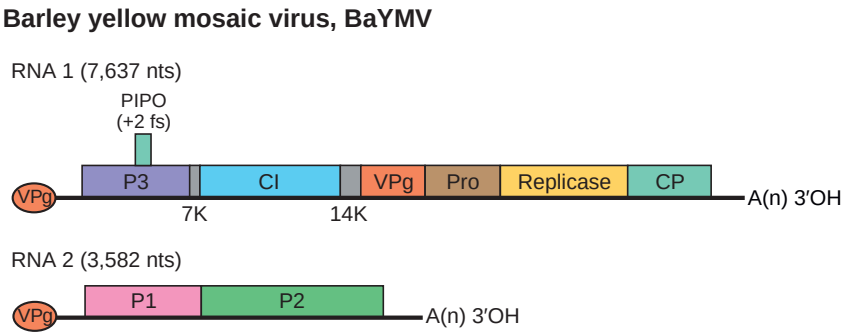


**Figure 5:** Genomic map of the bymovirus bipartite genome, using, as an example an isolate of barley yellow mosaic virus. Conventions are as for potyvirus genome organization map (Figure 2). Function of most gene products are postulated by analogy with genus *Potyvirus*. P1 corresponds to the C-terminal protease of HC-Pro.

### Host range

The host range of member viruses is narrow, restricted to the host family *Gramineae*. Each species has a very restricted host range; for example, the barley-infecting species do not infect wheat and vice versa.

### Transmission

These viruses are transmitted by *Polymyxa graminis* in a persistent manner, surviving in resting spores as long as these remain viable; it is transmissible experimentally by mechanical inoculation, sometimes with difficulty.

## List of species in the genus *Bymovirus*

| | | |
|---|---|---|
| *Barley mild mosaic virus* | | |
| Barley mild mosaic virus-fungally transmissible UK isolate | [Y10973 = NC_003483, X90904 = NC_003482,] | (BaMMV-F) |
| *Barley yellow mosaic virus* | | |
| Barley yellow mosaic virus-IIa | [D01091-2] | (BaYMV-IIa) |
| *Oat mosaic virus* | | |
| Oat mosaic virus-UK | [AJ306718-9 = NC_004016-7] | (OMV-UK) |
| *Rice necrosis mosaic virus* | | |
| Rice necrosis mosaic virus-Japan | [U95205*] | (RNMV-JA) |
| *Wheat spindle streak mosaic virus* | | |
| Wheat spindle streak mosaic virus-France | [X73883*] | (WSSMV-FR) |
| *Wheat yellow mosaic virus* | | |
| Wheat yellow mosaic virus-Japan | [D86634-5 = NC_002349-50] | (WYMV-JA) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Partial sequence of RNA1, including the coat protein; complete genome sequence not available.

## List of other related viruses which may be members of the genus *Bymovirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Potyviridae*

| | | |
|---|---|---|
| *Spartina mottle virus* | | |
| Spartina mottle virus-Germany | [AF491351*] | (SpMoV-DE) |
| *Sugarcane streak mosaic virus* | | |
| Sugarcane streak mosaic virus-Pakistan | [GQ388116] | (SCSMV-PAK) |
| *Tomato mild mottle virus* | | |
| Tomato mild mottle virus-Yemen | [AF359575*] | (TomMMoV-YM) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Partial sequence including the coat protein; complete genome sequence not available.

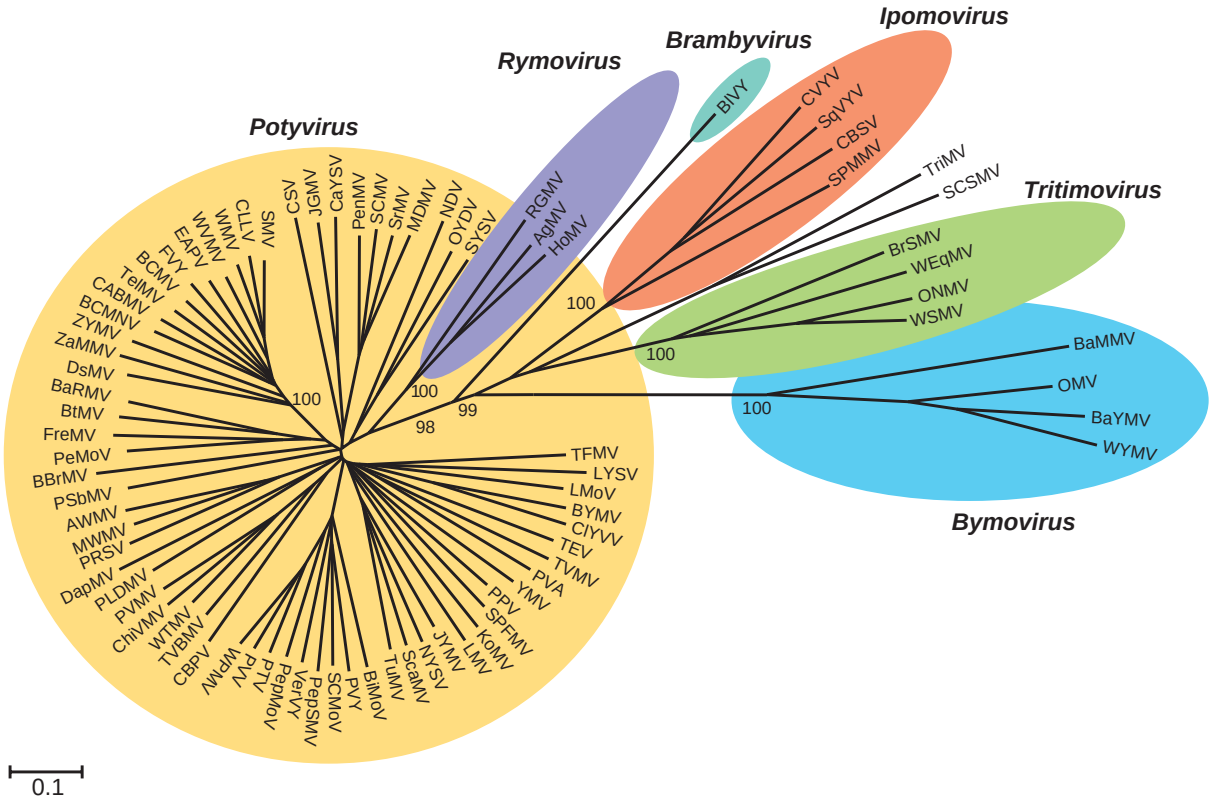


**Figure 6:** Unrooted phylogenetic tree based on the codon-aligned nucleotide sequences of the polyproteins of fully-sequenced members of the family *Potyviridae*. The tree uses the majority of the polyprotein (from the 6K1 cistron to the end of the coat protein). One representative sequence was chosen for each species. The analysis was done in MEGA4 (maximum composite likelihood distances) and the numbers on major branches indicate percentage of bootstrap support out of 10,000 bootstrap replications.

Tomato mild mottle virus is most closely related to members of the genus *Ipomovirus* but is transmitted by aphids in a non-circulative manner, whereas ipomoviruses are transmitted by the whitefly *Bemisia tabaci*. No vectors have been identified for the other unassigned members of the family.

## List of other related viruses which may be members of the family *Potyviridae* but have not been approved as species

Triticum mosaic virus-Kansas isolate U06-123 [FJ263671 = NC_012799] (TriMV)

There is a current proposal to make Triticum mosaic virus, recently described in the USA and transmitted by the same mite vector as tritimoviruses, the type species of a new genus, *Poacevirus*. If accepted, this genus would also contain the unassigned species *Sugarcane streak mosaic virus*, which is clearly related.

## Phylogenetic relationships within the family

Phylogenetic relationships within the family are depicted in Figures 6 and 7.

## Similarity with other taxa

Viruses in the family *Potyviridae* have similarity to members of the order *Picornavirales*. In particular, the genomes have a VPg at their 5′ termini and a poly(A) tract at their 3′ termini. Their genomes are expressed initially as high molecular weight polyprotein precursors which are processed by virus-encoded proteases. Gene products involved in replication are conserved in gene order and gene

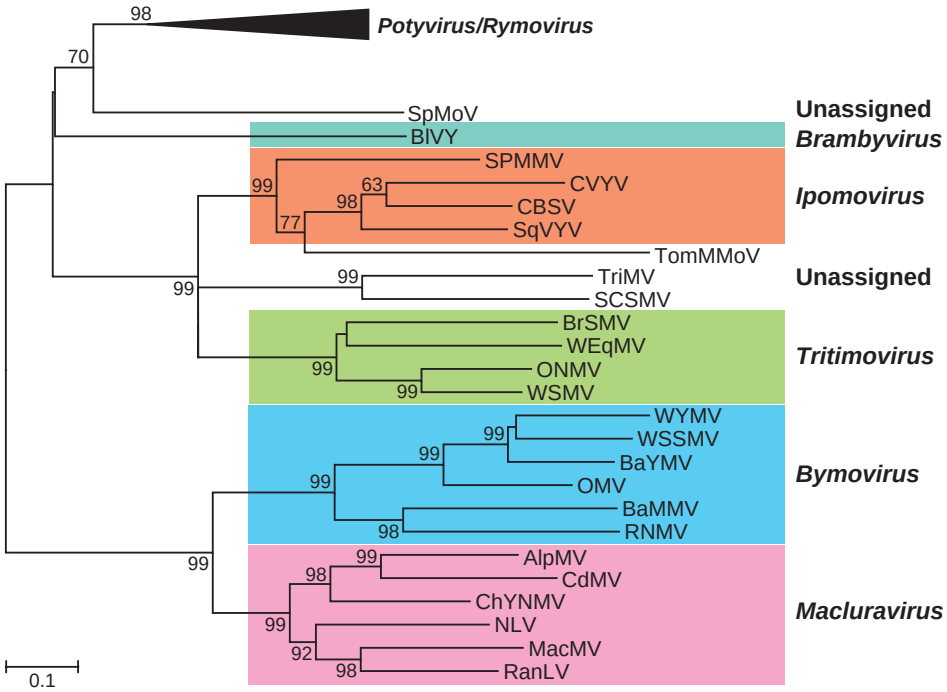


**Figure 7:** Phylogenetic (distance) tree based on the codon-aligned nucleotide sequences of the 3′ ends (partial NIb and complete coat protein) of the polyproteins of members of the family *Potyviridae*. The analysis was done in MEGA4 (maximum composite likelihood distances) and the numbers on branches indicate percentage of bootstrap support out of 10,000 bootstrap replications (where >60%). Because of the large number of sequences in the genus *Potyvirus*, the branch for this genus (and *Rymovirus*) has been collapsed. The tree is provided particularly to demonstrate the position of unassigned members of the family and those in the genus *Macluravirus*, where complete sequences are not available.

sequence. However, members of the *Picornavirales* have isometric particles, a much smaller VPg and a different type of helicase. There are also some sequence similarities to members of the family *Hypoviridae*.

## Derivation of names

*Bramby*: from *bramb*le, the host of the type species.
*Bymo*: from *b*arley *y*ellow *mo*saic.
*Ipomo*: from *Ipo*mea and *mo*saic.
*Maclura*: the genus name of the host of the type species.
*Poty*: from *pot*ato virus *Y.*
*Rymo*: from *ry*egrass *mo*saic.
*Tritimo*: from *Triti*cum and *mo*saic.

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## Contributed by

Adams, M.J., Zerbini, F.M., French, R., Rabenstein, F., Stenger, D.C. and Valkonen, J.P.T.

# Family *Tetraviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Tetraviridae* |
| Genus | *Betatetravirus* |
| Genus | *Omegatetravirus* |

# Genus *Betatetravirus*

Type species *Nudaurelia capensis beta virus*

## Virion properties

### Morphology

Virions are non-enveloped, roughly spherical, about 40 nm in diameter and exhibit T = 4 icosahedral shell *quasi*-symmetry. Distinct capsomers have been resolved by cryo-electron microscopy and image reconstruction (Figure 1). The genome consists of ssRNA. Viruses in the genus *Betatetravirus* have monopartite genomes.

### Physicochemical and physical properties

Virion Mr is about $18 \times 10^6$. Virion $S_{20,w}$ is 194–217S. Virion buoyant density in CsCl is usually 1.28–1.30 g cm$^{-3}$ but occasionally as high as 1.33 g cm$^{-3}$ (varies with species). Virions are stable over a broad range of pH and their infectivity can resist desiccation and protease treatment.

### Nucleic acid

Virions of Nudaurelia capensis beta viruses (NβV), which form the type species *Nudaurelia capensis beta virus*, contain a single, positive sense, ssRNA segment of approximately 6.6 kb (Mr $1.8 \times 10^6$) which represents about 10% of the particle mass. This genomic RNA (gRNA) is not polyadenylated at its 3′ end, nor blocked like nodaviral RNAs, but terminates instead with a distinctive tRNA-like structure. A pseudoknot is found instead at the 3′ ends of the gRNA of Euprosterna elaeasa virus (EeV) and Thosea asigna virus (TaV) (prototypes of species of the same name). These structures are absent at the 3′ end of the gRNAs of Providence virus (PrV; *Providence virus* prototype). A subgenomic message for the capsid proteins (CPs), which corresponds to the 3′ end of the genomic RNA, can also be encapsidated in some species.

### Proteins

Capsids of NβV consist of 240 protein subunits (protomers) arranged on a T = 4 surface lattice. Each protomer consists of the two cleavage products (large, L or β, 58.4 kDa and small, S or γ, 8 kDa), of a single CP precursor (α 66.4 kDa). Minor amounts of the un-cleaved precursor may be found in virions. Capsids of TaV, EeV, and PrV have similar protein compositions.

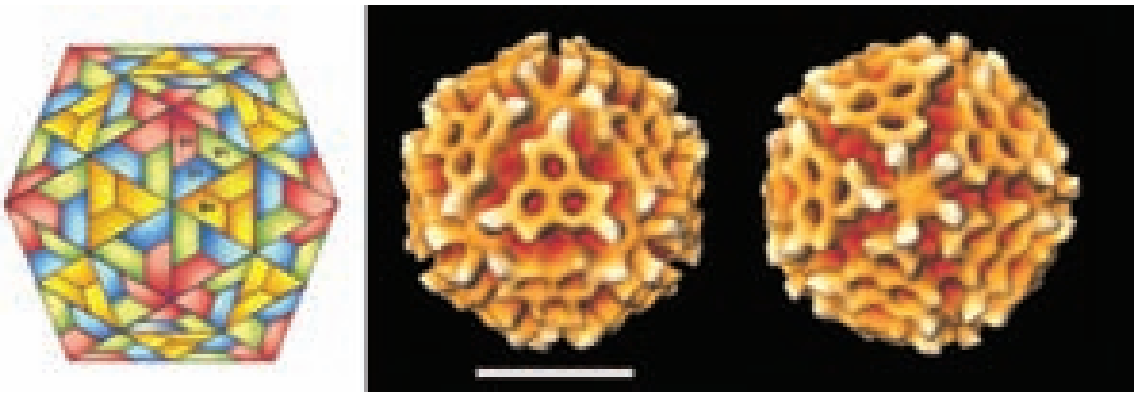

**Figure 1:** *Betatetravirus* capsid structure. (Left) Schematic representation of a T = 4 icosahedral lattice. (Center and Right) Cryo-electron microscopy image reconstruction of a particle of Nudaurelia capensis β virus (NβV) on the symmetry axis 3 and 5; the bar represents 20 nm. (Courtesy of H.R. Cheng, N. Olson and T. Baker.)

Pos. ssRNA

### Lipids
None reported.

### Carbohydrates
None reported.

## Genome organization and replication

Betatetraviruses replicate in the cytoplasm. Three distinct types of genomic organization have so far been found for betatetraviruses. The 6,625 nt gRNA of NβV, contains two ORFs that overlap for 1,517 nt: the first ORF at the 5′ end contains 1,821 codons and encodes the putative multidomain RNA replicase (204 kDa), which almost entirely overlaps the second ORF of 612 codons at the 3′ end that encodes the capsid protein precursor (66.4 kDa) (Figure 2). The replicase includes three highly conserved enzymatic domains, N7-methyltransferase (NMT), superfamily 1 RNA helicase (HEL1) and RNA-dependent RNA polymerase typical of the alpha-like supergroup (acRdRp) that are yet to be characterized experimentally.

The second type of gRNA is represented by the closely related TaV and EeV (5,714 and 5,698 nt, respectively). At the 5′ end is the longer ORF (1,257 codons) encoding the putative replicase of approximately 140 kDa. The viral replicase carries an RdRp domain with a non-canonical, permuted, CAB motif arrangement (pRdRp). At the N-terminus this domain is flanked by a short conserved signal implicated in priming replication and covalent binding to the 5′ end of RNAs (virus protein of genome, VPg). Two conserved domains flanking VPg-pRdRp have no resemblance to nmT or HEL1 domains. There is a much shorter overlap (536 and 529 nts for EeV and TaV, respectively) between the replicase and CP ORFs compared to that of NβV. The TaV and EeV CP ORFs are longer than that of NβV, yielding a putative precursor of 757 aa in length that undergoes NPGP-mediated (2A-like) processing to produce an amino terminal portion of 17 kDa (Figure 2) in addition to the capsid protein precursor of 65 kDa.

The third type of genomic organization is represented by PrV gRNA that has an intermediate size of 6,155 nt (Figure 2). At the 5′ end is an ORF of 3,659 nt comprising 1,233 amino acids encoding a protein of 130 kDa, of unknown function (ORF1) that includes an NPGP (2A-like) signal at the N-terminus. ORF1 (p130) almost completely overlaps with ORF2, encoding the viral replicase (2,934 nt, 978 amino acids and a protein of 104 kDa). ORF2 contains a readthrough stop codon that attenuates the synthesis of 104 kDa protein to produce a 40 kDa peptide from its N-terminus. The canonical RdRp domain, which is typical of the carmo-like supergroup, (ccRdRp) resides in the C-terminal half of the replicase, downstream of the readthrough stop and there is no evidence of nmT or HEL1 domains. The CP ORF is located immediately downstream of the replicase ORF, comprising 2,262 nt and 754 amino acids that undergoes NPGP-mediated (2A-like) processing at the second of two sites in the amino terminus of the protein, producing a peptide of 15 kDa and the 68 kDa capsid protein precursor.

During RNA replication of NβV, TaV and PrV, a subgenomic RNA (sgRNA), which represents the 3′ 2,500 nt of the genome is synthesized, and this serves as the mRNA for the CP precursor (Figure 2). There is a suggestion that the PrV sgRNA may replicate independently, and if confirmed, this, rather than the packaging of sgRNAs might blur the distinction between the monopartite genome organization of the betatetraviruses and the bipartite genome organization of the omegatetraviruses.

## Antigenic properties

Most of the members of the group are serologically interrelated but distinguishable. The majority of the isolates were identified on the basis of their serological reaction with antiserum raised against NβV.

## Biological properties

### Host range
**Nature:** All virus species were isolated from Lepidoptera species (moths and butterflies), principally from Saturniid, Limacodid and Noctuid moths and no replication in other animals has been detected. In larvae, virus replication is restricted predominantly to the cells of the midgut.

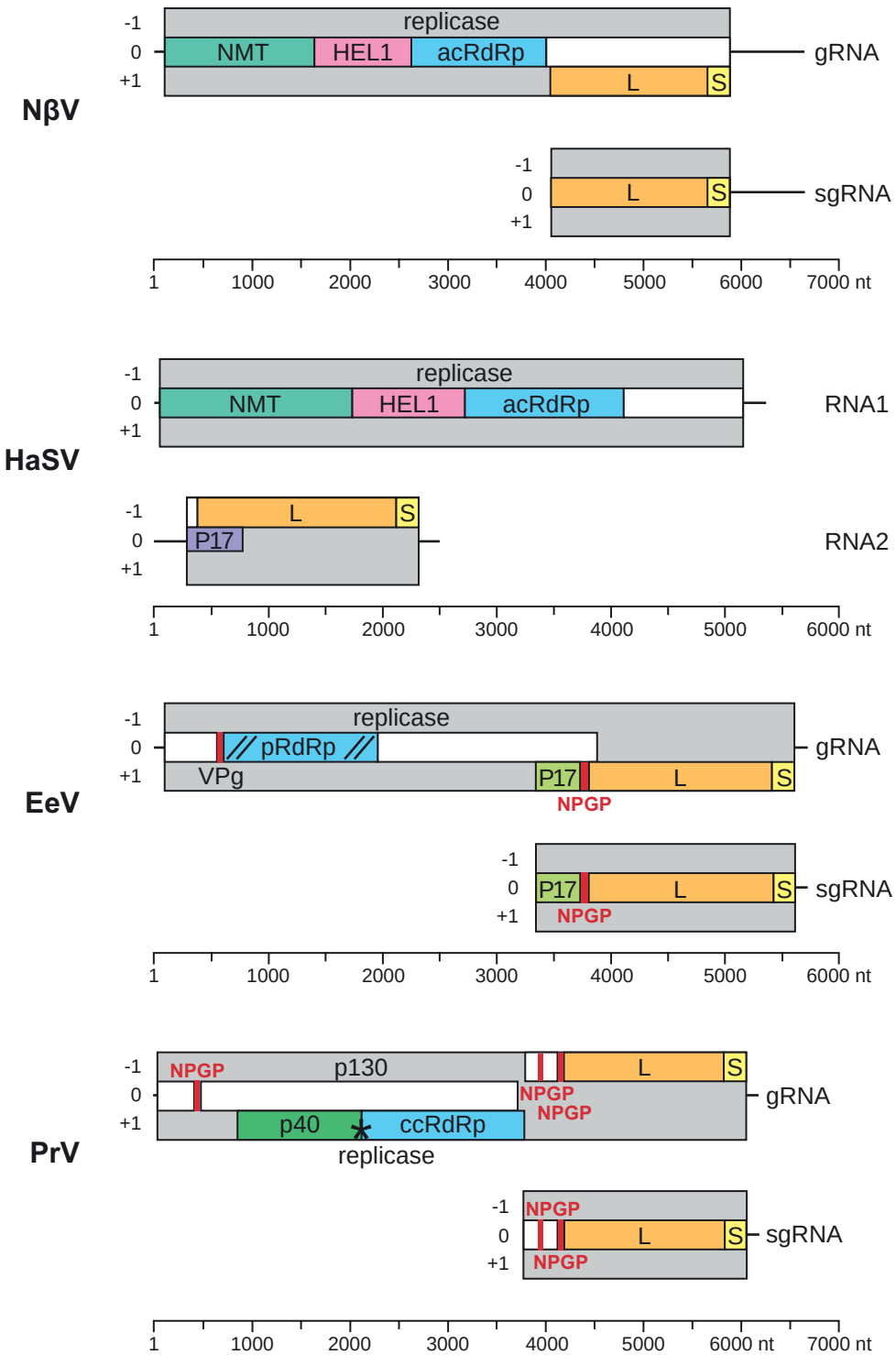


**Figure 2:** Tetravirus genome comparisons. The genome organization, including genome segments, ORFs and selected domains, is depicted for four tetraviruses, EeV, NβV, HaSV and PrV, using virus-specific scales. EeV, NβV and PrV have monopartite genomes that (are predicted to) yield sgRNAs, while HaSV has a bipartite genome (RNA1 and RNA2). The selected proteins and domains are labelled, pattern-coded and coloured to indicate homology. nmT: N7-methyltransferase; HEL1, superfamily 1 helicase; acRdRp: canonical RNA dependent RNA polymerase typical of alpha-like supergroup; ccRdRp: canonical RNA dependent RNA polymerase typical of carmo-like supergroup; pRdRp: non-canonical permuted RNA-dependent RNA polymerase most related to picorna-like supergroup; NPGP: 2A-like processing site. The sequence of the major (L or β) and minor (S or γ) capsid proteins are indicated as "L" and "S", respectively. (Modified from Figure 1 of Zeddam *et al*. (2010). *Virology*, **397**, 145-154.)

Pos. ssRNA

**Laboratory:** With the exception of PrV, no infections by members of the *Betatetravirus* genus have been achieved in cultured cells, even when gRNA was transfected directly into cells.

### Transmission

Oral transmission of NβV to *Antherea eucalypti* (the emperor gum moth) has been demonstrated experimentally. Oral transmission is implied by the midgut site of viral replication and by reports of some tetraviruses being used as sprayed insecticides in Malaysia (e.g. DtV; Darna trima virus). At high host densities, horizontal spread appears to be the major route of infection. Suggestive evidence exists for vertical transmission, which could be responsible for the observed persistence of tetraviruses within insect populations.

### Cytopathic effects

The viruses replicate primarily in the cytoplasm of gut cells of several Lepidoptera species. Crystalline arrays of virus particles are often seen within cytoplasmic vesicles. Different isolates vary considerably in pathogenicity; symptoms can vary from inapparent to acutely lethal infections.

## Species demarcation criteria in the genus

Viruses have been classified into the genus *Betatetravirus* based upon two properties: capsid of T = 4 symmetry and monopartite genome. The following criteria have been applied to the demarcation of species within the genus:

- **Biological properties (host range, vectors, mode of transmission).** Since the natural host-ranges of individual recognized tetravirus species appear to be narrow, virus isolation from a new host can provide suggestive evidence of a new tetravirus species.
- **Antigenic properties.** Antisera raised against different isolates or strains of a single tetravirus species should exhibit high levels of cross-reactivity in Western blot and/or neutralization analyses. Lower levels of cross-reactivity in these assays using antisera against previously recognized tetraviruses can provide evidence of a new tetravirus species.
- **Virion physical/physicochemical characteristics.** In the absence of more definitive criteria, significant (>5%) differences in virion sedimentation coefficient or buoyant density from those of all previously recognized tetravirus species can provide evidence of a new virus species.
- **Structural protein characteristics.** The electrophoretic mobilities in SDS-PAGE of the CP precursor or its cleavage products should be compared with those of other tetravirus species.
- **Genome molecular characteristics:**
  - **RNA electrophoretic mobilities.** In the absence of sequence information, the electrophoretic mobilities of the viral genomic RNAs should be compared with those of other tetraviruses.
  - **RNA hybridization properties.** In the absence of differences in RNA electrophoretic mobilities, the molecular hybridization properties of the viral genomic RNAs should be compared with those of other tetraviruses.
- **Genome sequence characteristics.** The nt sequences of the genomic RNA(s) should be compared with those of other tetraviruses.

**Application of these criteria:** In practice, while criteria 1–5 above may be suggestive of a new species, definitive demarcation has been based on the nucleotide sequence of the viral CP gene. Application of the sixth criterion to viruses of this genus led to suggestions, based on profound differences in the replicase genes accounting for a considerable portion of the genome, that TaV, EeV and PrV should be reclassified outside the *Tetraviridae*. A taxonomic proposal to this effect is currently under consideration.

## List of species in the genus *Betatetravirus*

*Antherea eucalypti virus*
Antherea eucalypti virus (AeV)
*Darna trima virus*
Darna trima virus (DtV)
*Dasychira pudibunda virus*
Dasychira pudibunda virus (DpV)
(Calliteara pudibunda virus) (CpV)

| | | |
|---|---|---|
| *Euprosterna elaeasa virus* | | |
| Euprosterna elaeasa virus | [AF461742] | (EeV) |
| *Nudaurelia capensis beta virus* | | |
| Nudaurelia capensis β virus | [AF102884] | (NβV) |
| *Philosamia cynthia x ricini virus* | | |
| Philosamia cynthia x ricini virus | | (PxV) |
| *Providence virus* | | |
| Providence virus | [GU991616] | (PrV) |
| *Pseudoplusia includens virus* | | |
| Pseudoplusia includens virus | | (PiV) |
| *Thosea asigna virus* | | |
| Thosea asigna virus | [AF062037*, AF282930*] | (TaV) |
| (Setothosea asigna virus) | | (SaV) |
| *Trichoplusia ni virus* | | |
| Trichoplusia ni virus | | (TnV) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

### List of other related viruses which may be members of the genus *Betatetravirus* but have not been approved as species

None reported.

## GENUS *OMEGATETRAVIRUS*

Type species ***Nudaurelia capensis omega virus***

### Virion properties

#### MORPHOLOGY

Virions are non-enveloped, roughly spherical, about 40 nm in diameter and exhibit T = 4 icosahedral shell *quasi*-symmetry. Distinct capsomers have been resolved by cryo-electron microscopy and image reconstruction (Figure 3). The genome consists of ssRNA. Unlike viruses in the genus *Betatetravirus*, viruses in the genus *Omegatetravirus* have bipartite genomes.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virion Mr is about $16\times10^6$. Virion $S_{20,w}$ is 194–217S. Virion buoyant density in CsCl is 1.28–1.30 g cm$^{-3}$ (varies with species). Virions of Helicoverpa armigera stunt virus (HaSV) are stable between pH 3.0 and 11.0 and at temperatures up to 55 °C and are resistant to protease treatment.

#### NUCLEIC ACID

Virions of viruses of the type species *Nudaurelia capensis omega virus* that have the same name and commonly abbreviated as NωV, contain two positive sense, ssRNA segments of approximately 5,300 nt (RNA1, Mr $1.75\times10^6$) and 2,450 nt (RNA2, Mr $0.8\times10^6$). These genomic RNAs are capped at their 5′ ends but their 3′ ends are not polyadenylated, nor blocked. Like NβV, omegatetraviral RNAs terminate with a distinctive tRNA-like structure. It is likely that omegatetraviruses encapsidate both genomic RNAs within a single particle, in which case the RNAs would represent about 10% of the particle mass.

#### PROTEINS

Capsids of the type strain, NωV, consist of 240 protein subunits (protomers) arranged on a T = 4 surface lattice. Each protomer consists of the two cleavage products (L or β, 62 kDa and S or γ, 7.8 kDa) of a single CP precursor (α, 69.8 kDa). Overall, the CPs of NωV and NβV share less than 20% aa sequence identity, indicating that viruses of the two genera have substantially diverged with respect to their capsid sequences.

#### LIPIDS

None reported.

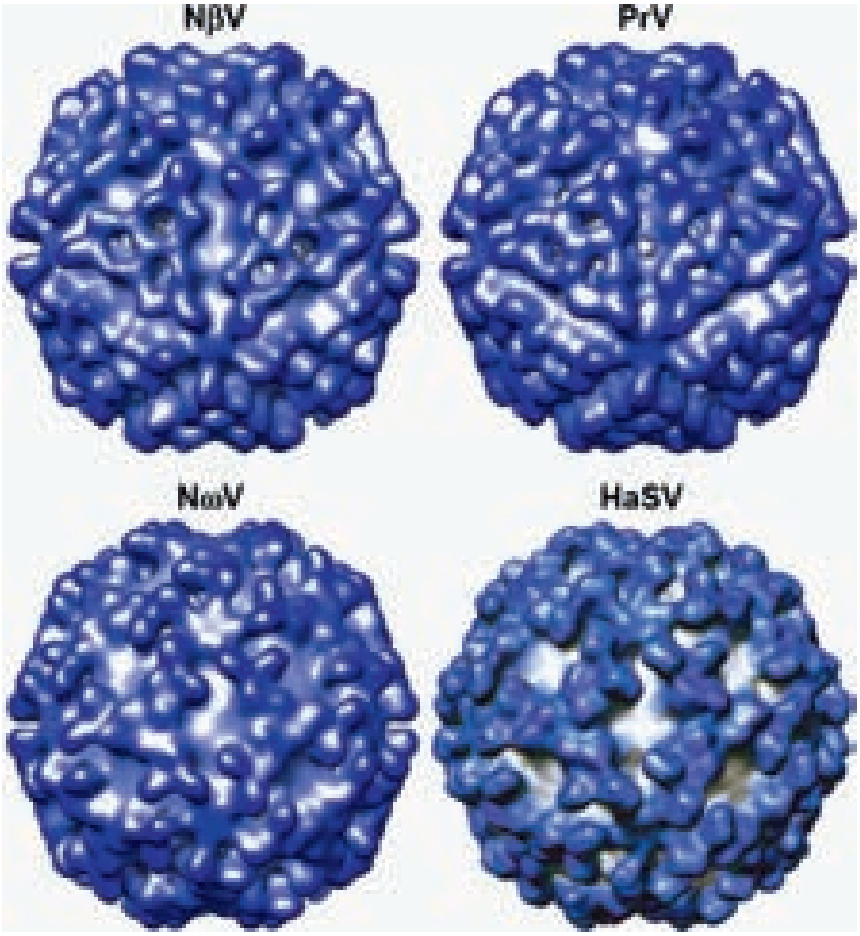


**Figure 3:** Comparison of tetravirus capsid morphologies. Cryo-electron microscopy reconstructions of capsids of the omegatetraviruses, Nudaurelia capensis ω virus (NωV) and Helicoverpa armigera stunt virus (HaSV) and the betatetraviruses, NβV and PrV. The structures, viewed down their two-fold axes, were determined at resolutions of between 25 and 30 Å. (Image taken from Figure 2 of Speir and Johnson (2008); with permission.)

### Carbohydrates

None reported.

## Genome organization and replication

As with the betatetraviruses, omegatetraviruses replicate in the cytoplasm. Studies in tissue culture cells show that the HaSV replicase is localized within the cytoplasm and associates with membranes derived from the endocytic pathway. The viral genome consists of two unique molecules of RNA, probably encapsidated in the same virus particle. The NωV genome has not yet been fully sequenced but the partial sequence available for RNA1 indicates that this virus has a similar genome organization to that of two other omegatetraviruses that have been fully characterized in this respect. These omegatetraviruses form separate species with identical or similar names (Table 2). RNA1 (HaSV: Mr $1.75 \times 10^6$; 5.3 kb), encodes the RNA replicase, 1,704 aa and 1,649 aa in HaSV and Dendrolimus punctatus tetravirus (DpTV), respectively, with a domain organization similar to that of the betatetravirus NβV (Figure 1). RNA1 also encodes three small ORFs of unknown function that overlap with the 3′ end of the replicase ORF. RNA2 (HaSV: Mr $0.8 \times 10^6$; 2.45 kb) encodes the CP precursor (70 kDa for NωV and DpTV and 71 kDa for HaSV). A 157-codon ORF precedes and partially overlaps the CP ORF; it encodes a non-structural protein (p17), which is encapsidated and involved in packaging of viral RNA. Separate dsRNAs that correspond to the two genome segments are observed in HaSV-infected cells.

## Antigenic properties

NωV and DpTV are serologically related, but display no serological relationship with more distantly related HaSV.

## Biological properties

### Host range

**Nature:** All species were isolated from Lepidoptera species, principally from Saturniid, Limacodid and Noctuid moths.

**Laboratory:** No infections by members of the genus *Omegatetravirus* have yet been achieved in cultured cells, but infectious HaSV particles were produced by plant protoplasts transfected with plasmids carrying full-length cDNAs that corresponded to the viral genome segments.

### Transmission

As with the betatetraviruses, oral transmission is implied by the midgut site of viral replication. At high host densities, horizontal spread appears to be the major route of infection, but evidence exists for vertical transmission which might be responsible for the observed persistence of tetraviruses within insect populations.

### Cytopathic effects

The viruses replicate primarily in the cytoplasm of midgut cells of the larvae of several Lepidoptera species. Crystalline arrays of virus particles are often seen within cytoplasmic vesicles. There is a considerable range of pathogenicity with different isolates, and symptoms can vary from unapparent to acutely lethal infections.

## Species demarcation criteria in the genus

Viruses have been classified into the genus *Omegatetravirus* based upon two properties: capsid of T = 4 symmetry and bipartite genome. The species demarcation criteria of betatetraviruses also apply to omegatetraviruses. Also, because the genome of omegatetraviruses is segmented, re-assortment is possible and the two genome segments may have different evolutionary histories. However, no chimeric omegatetraviruses have yet been detected. In contrast, it was proposed that the ancestral bi-segmented omegatetravirus has evolved by recombination from two mono-segmented betatetraviruses of the NβV and PrV types, respectively. This proposal suggested that the NβV-like parent supplied genomic RNA encoding the replicase, while the other PrV-like parent supplied a subgenomic RNA encoding CP, which subsequently evolved into the second segment by mutation and autonomization.

## List of species in the genus *Omegatetravirus*

| | | |
|---|---|---|
| *Dendronimus punctatus virus* | | |
| Dendronimus punctatus tetravirus | RNA1 [AY594352]<br>RNA2 [AY594353] | (DpTV) |
| *Helicoverpa armigera stunt virus* | | |
| Helicoverpa armigera stunt virus | RNA1 [U18246]<br>RNA2 [L37299] | (HaSV) |
| *Nudaurelia capensis omega virus* | | |
| Nudaurelia capensis ω virus | RNA2 [S43937] | (NωV) |

Species names are in italic script; strain names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Omegatetravirus* but have not been approved as species

None reported.

## List of other related viruses which may be members of the family *Tetraviridae* but have not been approved as species

| | |
|---|---|
| Acherontia atropas virus | (AaV) |
| Agraulis vanillae virus | (AvV) |
| Callimorpha quadripuntata virus | (CqV) |
| Eucocytis meeki virus | (EmV) |
| Euploea corea virus | (EcV) |
| Hyalophora cecropia virus | (HcV) |
| Hypocritae jacobeae virus | (HjV) |
| Lymantria ninayi virus | (LnV) |
| Saturnia pavonia virus | (SpV) |
| Setora nitens virus | (SnV) |
| Nudaurelia capensis ϵ virus* | (NϵV) |
| Nudaurelia capensis ζ virus | (NζV) |
| Nudaurelia capensis ψ virus | (NψV) |

*NϵV resembles the tetraviruses in appearance but is serologically unrelated to any known species.

## Phylogenetic relationships within the family

Two major clusters are evident in the tetravirus capsid phylogram, with one comprising NβV, TaV and EeV, and the other DpTV, HaSV, NωV and PrV (Figure 4). HaSV, NωV and DpTV appear to have evolved more recently, sharing an early common ancestor with PrV, while the closely related TaV and EeV share a most recent common ancestor with NβV. It is striking that in each of these clusters, both CP processing strategies, with and without involvement of the NPGP signal, are present.

In contrast to the CPs, the replicases of the tetraviruses for which genomic sequences have been determined fall into three distinct phylogenetic groups that do not reflect the taxonomic demarcation (Figure 5). The first of these groups includes the betatetravirus NβV and the omegatetraviruses HaSV, DpTV and probably also NωV (although no complete sequence has been published for the replicase of NωV, unpublished data show it to be very closely related to that of HaSV). Replicases of these viruses include three conserved domains, nmT, HEL1 and acRdRp, and cluster together with the similarly organized replicases of a dozen other ssRNA+ virus families with the mammalian *Hepeviridae* family being the closest (Figure 5; see also below). The second group includes the betatetraviruses TaV and EeV. The replicases of these viruses are also multi-domain proteins with

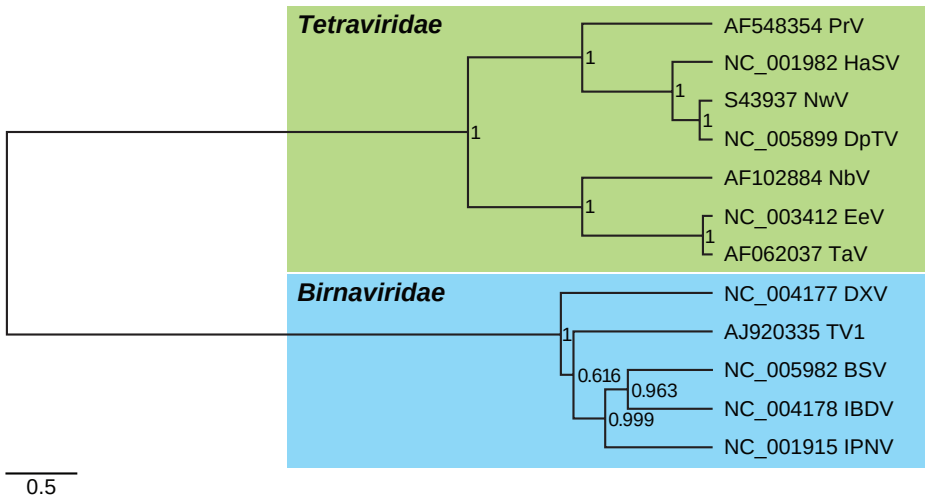


**Figure 4:** Phylogeny of tetravirus capsid protein. The tree for seven tetraviruses is based on an amino acid alignment of the jelly-roll domain of L protein (661 positions) and was rooted using the jelly-roll domain of S proteins of five birnaviruses as an outgroup. Numbers at branch points provide Bayesian posterior probability support values and the evolutionary scale is indicated by the bar of 0.5 amino acid substitutions per site on average. (Modified from Figure 5b (left part) of Zeddam *et al.* (2010). *Virology*, **397**, 145–154.)

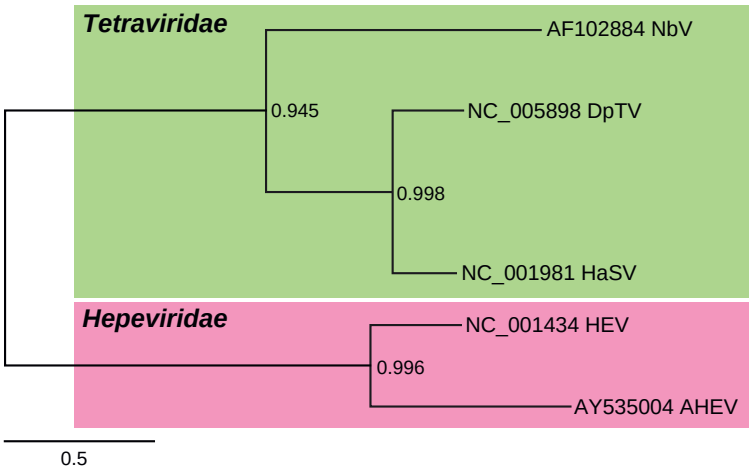


**Figure 5:** Phylogeny of prototypic tetravirus replicases. The tree for prototypic tetraviruses and DpTV is based on an amino acid alignment of the HEL1 and acRdRp domains (832 positions) and was rooted using avian and human Hepatitis E viruses as an outgroup. Numbers at branch points provide Bayesian posterior probability support values and the evolutionary scale is indicated by the bar of 0.5 amino acid substitutions per site on average. (From Figure 5b (right part) of Zeddam *et al.* (2010). *Virology*, **397**, 145–154.)

VPg and pRdRp being two domains with provisionally assigned functions. The phylogenetic neighborhood of the TaV, EeV pRdRps includes diverse viruses that are discussed below but not those of the NβV/HaSV group (Figure 6). There is a third distinct RdRp lineage within the tetraviruses represented by PrV, whose RdRp clusters with ssRNA+ plant viruses distinct from those that form either of the above two groups (Figure 7).

## Similarity with other taxa

The distinguishing feature of viruses currently classified within the family *Tetraviridae* is the unique T = 4 quasi-symmetry of their capsid architecture. Thus it is not surprising that their CPs form a monophyletic group. The jelly-roll fold β subunits of CPs, which are used to build capsid, are most closely but still distantly related to those of the CPs of nodaviruses and dsRNA birnaviruses having T = 3 and T = 13 capsids, respectively. It has been speculated that the tetravirus capsid might have evolved from a nodavirus-like ancestor through a process that included insertion of an immunoglobulin-like protein domain coding sequence (either acquired or evolved through sequence duplication) within the CP gene. This hypothesis is supported by structural studies that show that the PrV and NωV capsids are structurally conserved with respect to their jelly-roll folds and Ig-like domains, but not with respect to the structure and function of their N and C-termini. These domains, which form the molecular switch determining the T = 4 architecture of PrV capsid, are closely related to those of nodaviruses that possess a T = 3 capsid architecture.

Comparative analysis of currently available non-structural protein sequences split tetraviruses into three distinct lineages, prototyped by NβV/HaSV, TaV/EeV and PrV, respectively, within three different virus supergroups. The replicases of NβV, DpTV and HaSV resemble those of the "alphavirus-like" supergroup, having the distinct nmT-HEL1-acRdRp domain organization and through phylogenetic clustering with viruses having these three domains. The replicases of TaV and EeV lack both nmT and HEL1 domains. Furthermore, their pRdRp domain has a unique C-A-B motif arrangement in the palm subdomain of the active site that differs from the canonical A-B-C arrangement found in the other tetraviruses, all "alphavirus-like" viruses and indeed almost all known template-dependent polynucleotide polymerases (viral and cellular) carrying the palm sub-domain. Interestingly, the same C-A-B permutation of the three motifs is also found in replicases of all dsRNA birnaviruses. This rearrangement is a result of migration of about 22 aa residues encompassing motif C between two internal positions, separated by about 110 aa, in a conserved region of about 400 aa. The permuted TaV, EeV and birnavirus enzymes form a minor, deeply separated

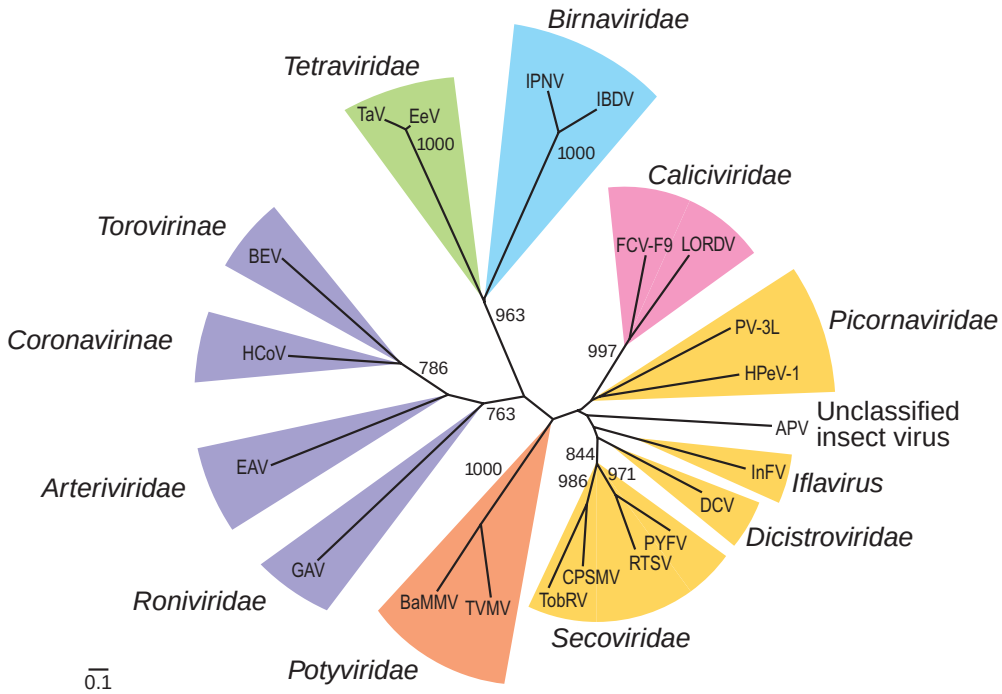


**Figure 6:** Unrooted phenogram showing the relationships of the RdRps of the tetraviruses TaV and EeV to other virus families and viruses in the "picornavirus-like supercluster". The pRdRps of TaV, EeV and the birnaviruses were converted into the canonical form by relocating the motif C sequence (18–20 aa) downstream of the motif B, as in canonical polymerase motifs. These sequences were aligned with those of polymerases from representative viruses in the *Picornaviridae*, *Dicistroviridae*, *Secoviridae*, *Iflaviridae*, *Caliciviridae*, *Potyviridae*, *Coronavirinae*, *Torovirinae*, *Roniviridae*, *Arteriviridae* and unclassified insect viruses. Using an extended, gap-free version of the alignment containing 332 informative characters, an unrooted neighbor-joining tree was inferred by the ClustalX1.81 program. All bifurcations with support in > 700 out of 1000 bootstraps are indicated. Different groups of viruses are highlighted. Virus families and groups, viruses included in the analysis, abbreviations ( ) and the NCBI protein (unless otherwise specified) IDs [ ] are as follows: *Picornaviridae*: human poliovirus type 3 Leon strain (PV-3L) [130503] and human parechovirus 1 (HpeV-1) [6174922]; *Iflaviridae*: infectious flacherie virus (InFV) [3025415]; unclassified insect virus Acyrthosiphon pisum virus (APV) [7520835]; *Dicistroviridae*: Drosophila C virus (DCV) [2388673]; *Secoviridae*: rice tungro spherical virus (RTSV) [9627951], parsnip yellow fleck virus (PYFV) [464431], cowpea severe mosaic virus (CPSMV) [549316] and tobacco ringspot virus (TobRV) [1255221]; *Caliciviridae*: feline calicivirus F9 (FCV-F9) [130538] and Lordsdale virus (LORDV) [1709710]; *Potyviridae*: tobacco vein mottling virus (TVMV) [8247947] and barley mild mosaic virus (BaMMV) [1905770]; *Coronavirinae*: human coronavirus 229E (HCoV) [12175747]; *Torovirinae*: Berne torovirus (BEV) [94017]; *Arteriviridae*: equine arteritis virus (EAV) [14583262]; *Roniviridae*: gill-associated virus (GAV) [9082018]; *Tetraviridae*: Thosea asigna virus (TaV) [AF82930; nt sequence] and Euprosterna elaeasa virus (EeV) [AF461742; nt sequence]; *Birnaviridae*: infectious pancreatic necrosis virus (IPNV) [133634] and infectious bursal disease virus (IBDV) [1296811]. *Coronaviridae*, *Arteriviridae* and *Roniviridae* belong to the order *Nidovirales*. (Modified from Gorbalenya *et al.*, 2002.)

cluster in the RdRp tree that also includes viruses of the "picornavirus-like supercluster" and the order *Nidovirales*. The pRdRp domains of these viruses are also flanked by the uniquely conserved VPg signal and another poorly characterized domain from the N-terminus. A similar arrangement is also found in DAV. Thus, replicases of TaV/EeV and birnaviruses (and possibly DAV) may represent their own virus supercluster.

The RdRp of PrV also lacks the nmT and HEL1 domains, but unlike the TaV/EeV cluster, has a canonical A-B-C palm subdomain. The PrV replicase clusters with ssRNA + plant viruses, being most closely related to the umbra- and tombusviruses, which belong to the third virus supergroup, the carmo-like viruses of other families and therefore appears to belong to a third lineage.

These complex and incongruent relationships of CP and replicase proteins imply that viruses currently classified as tetraviruses on the basis of their CP may form a polyphyletic group based upon

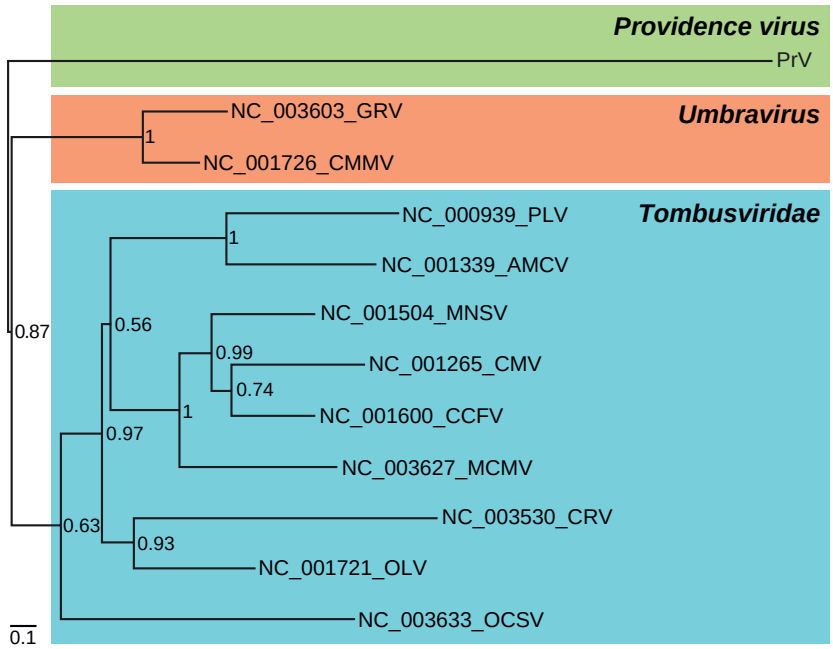


**Figure 7:** Phylogeny of Providence virus (PrV) and related tombus- and umbravirus RdRps. The tree is based on an amino acid alignment of the C-terminal half of the replicase starting after the read-through stop codon (578 positions) encoding putative RdRp and was midpoint pseudo-rooted. Poorly conserved alignment termini were discarded from the analysis. Tombus- and umbraviruses encode RdRps that are the closest to the PrV RdRp as determined in a protein Blast analysis. The RdRp tree was calculated and depicted following procedures and a style adopted by Zeddam *et al.* (2010) (see Figures 4 and 5). The relaxed lognormal molecular clock model allowing different branches of the tree to "evolve" at different rates was used. Numbers at branch points provide Bayesian posterior probability support values and the evolutionary scale is indicated by the bar of 0.1 amino acid substitutions per site on average. RefSeq accession numbers are indicated next to the virus names. This tree is by Lauber and Gorbalenya (unpublished) using the PrV sequence (GenBank accession no: AF548354).

the properties of their replicase. According to a recently developed evolutionary model, TaV and EeV resemble the most recent common tetravirus ancestor while other tetraviruses descend from more recent ancestors that originated through recombination(s) with viruses of other families and/or intrafamily recombination. To acknowledge these gross differences, TaV/EeV and, probably PrV, are proposed to be placed in two new families separate from the prototypic tetraviruses. Future revision of tetravirus taxonomy is expected to accommodate these suggestions. A further question is whether additional families need to be defined to include viruses like DAV whose replicases are related to TaV/EeV but whose capsid is of the T = 3 type.

## Derivation of names

*Nudaurelia capensis* is the emperor pine moth.
*Tetra*: from Greek *tettares*, meaning "four", as T = 4.

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Zeddam, J.-L., Gordon, K.H.J., Lauber, C., Felipe Alves, C.A., Luke, B.T., Hanzlik, T.N., Ward, V.K. and Gorbalenya, A.E. (2010). Euprosterna elaeasa virus genome sequence and evolution of the *Tetraviridae* family: Emergence of bipartite genomes and conservation of the VPg signal with the dsRNA *Birnaviridae* family. *Virology*, **397**, 145–154.

## Contributed by

Dorrington, R.A., Gorbalenya, A.E., Gordon, K.H.J, Lauber, C. and Ward, V.K.

# FAMILY *TOGAVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Togaviridae*** |
| Genus | ***Alphavirus*** |
| Genus | ***Rubivirus*** |

## Virion properties

### MORPHOLOGY

Alphavirus virions are assembled into icosahedral particles of approximately 70 nm in diameter and a molecular mass of $5.2 \times 10^7$ daltons. The particle core (40 nm) consists of 240 copies of the capsid protein (C or CP) arranged in a T4 symmetry surrounding the genomic RNA. However, in rubiviruses (Rubella virus, RUBV), the core particle consists of multiple CP disulfide-linked homodimers with the genomic RNA. The nucleocapsid core is covered by a lipid bilayer containing heterodimers of the two virally encoded glycoproteins, E2 and E1, which form a regular icosahedral surface lattice. The E1/E2 heterodimers of Sindbis virus (SINV) have been shown to be capable of producing three distinct morphological structures of varying dimensions. These heterodimers undergo conformational changes during the process of cellular entry. The lipid bilayer is derived from the cellular plasma membrane. RUBV virions are just over 60 nm in diameter and exhibit a T3 symmetry rather than a T4 as seen with the alphaviruses. Structural analysis of RUBV has been limited by an inability to purify virions free of cell-derived membranes. (See Figure 1.)

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Alphaviruses have a buoyant density in sucrose gradients of 1.20 g cm$^{-3}$ while that of RUBV is slightly less at 1.18 to 1.19 g cm$^{-3}$. The sedimentation coefficient of togavirus virions ranges from 240S to 350S with most of the variability associated with RUBV (Alphaviruses have an $S_{20,w}$ of 280S). Denaturing agents including formaldehyde and beta-propiolactone, detergents, acid, and lipid solvents readily eliminate infectivity. Togavirus infectivity is also abrogated by heat inactivation with virions being fairly heat labile; treatment at 58 °C results in a half-life of minutes. Exposure to ultravirolet light or radiation also results in loss of infectivity.

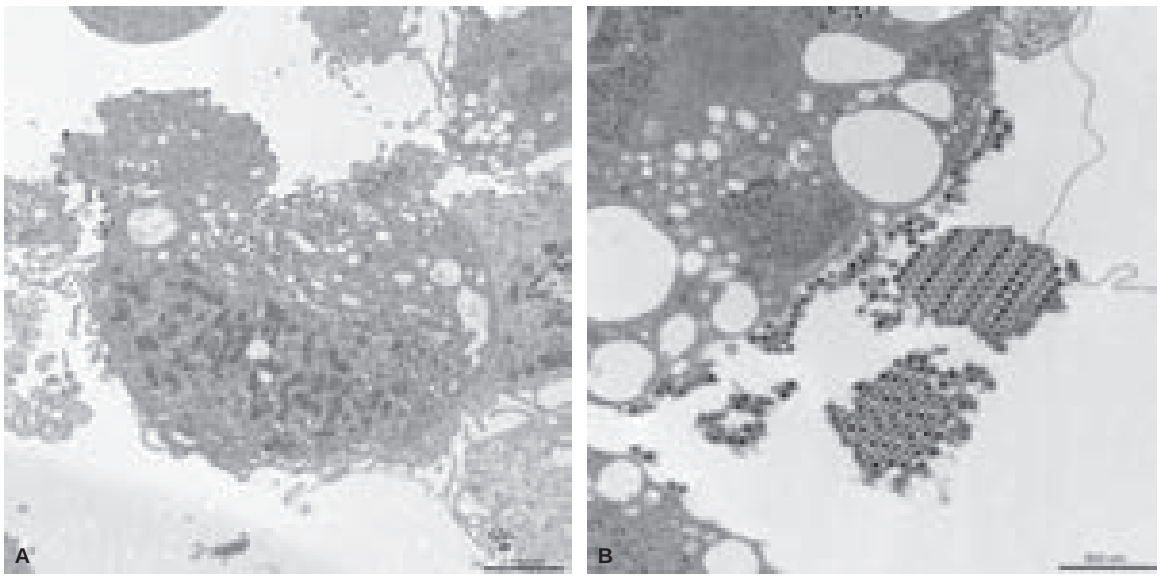


**Figure 1:** Thin section electron micrographs of Chikungunya virus in Vero E6 cells. Infected cells showed an abundance of viral particles that tended to associate with the plasma membrane. (Courtesy of Cynthia Goldsmith, MS and James A. Comer, PhD, CDC.)

### Nucleic acid

All togaviruses contain a single strand of positive sense RNA as their genetic material. The size ranges from 9.7 to 11.8 kb for the alphaviruses and is approximately 9.8–10.0 kb for RUBV. The RNA contains a 5′-terminal $m^7G$ cap and a 3′-terminal poly-A tail.

### Proteins

There are four non-structural proteins, designated nsP1-4, which encode the viral replication machinery of the togaviruses. These proteins are not found in intact virions but only in infected cells. The structural proteins consist of the capsid protein (C or CP), the two surface envelope glycoproteins (E1 and E2) and two small peptides (E3 and 6K) which serve as leader peptides for E2 and E1 respectively but are not present in all alphaviruses. These peptides are not present in the rubiviruses.

### Lipids

Virion associated lipids are derived from the host cell membranes during the process of virus maturation and budding. Budding occurs at the plasma membrane only for the alphaviruses while RUBV buds from both intracellular and surface membranes. These lipids make up approximately 30% of the total weight of the virion and their composition varies with the cell from which the virions were derived. When alphaviruses replicate in mammalian cells, particles have significantly more cholesterol than when the virus is propagated in invertebrate cells.

### Carbohydrates

Both E1 and E2 glycoproteins of alphaviruses contain N-linked glycans while O-linked glycans are present only on the RUBV E2. Alteration of the glycosylation can negatively affect viral replication. In RUBV, carbohydrates make up 10% of the mass of E1 and 30–40% of E2.

## Genome organization and replication

The RNA genome encodes the non-structural proteins in a single ORF immediately after a 5′ non-coding region. The proteins are oriented as 5′ nsP1 (methyltransferase and guanyltransferase) – nsP2 (helicase and protease) – nsP3 (phosphoprotein integral in minus strand synthesis) – nsP4 (RNA dependent RNA polymerase) – 3′. There is a stop codon present between the nsP3 and nsP4 genes in the majority of alphaviruses resulting in a limited amount of nsP1234 generated by inefficient read-through. The non-structural genes occupy approximately two-thirds of the genome. Immediately downstream of the non-structural ORF, there is a promoter for transcription of the subgenomic mRNA from which the structural polyprotein is translated. The structural proteins include the capsid (C/CP), E3, E2, 6K, and E1 proteins. The structural ORF is followed by a non-coding region of varying length (range 77–609 nucleotides) and finally, a polyadenylation signal. (See Figure 2.)

While RUBV and the alphaviruses share homology in the *cis*-acting elements at the 5′ end of the genome and in the subgenomic promoter region, there are major differences in their replication processes. In alphaviruses, the initial polyproteins generated, P123 and/or P1234, are cleaved in *trans* by the viral protease activity of the nsP2 protein. The P123 precursor protein combined with nsP4 form the replication complex associated with minus-strand replication. This negative-strand serves as the template in the synthesis of a full-length, positive sense RNA that will eventually be encapsidated as well as a subgenomic 26S mRNA that encodes the viral structural proteins. Cleavage of the P123 polyprotein generates nsP1, nsP2 and nsP3 individual proteins which are involved in positive-strand synthesis. In RUBV, the polyprotein precursor is cleaved either in *cis* or *trans* into two products, P150 and P90, by a protease located near the C-terminus of P150. The order of functional motifs in the RUBV genome is different from that of the alphaviruses with P90 polyprotein containing both helicase and replicase motifs.

The alphavirus subgenomic mRNA is translated into a single polyprotein from which individual structural proteins are produced by both viral and cellular proteases. RUBV CP lacks the viral autoprotease activity of the alphavirus C protein and thus relies completely upon cellular endopeptidases for structural protein cleavage. The glycoproteins that are produced are inserted into the endoplasmic reticulum during translation and are translocated to the plasma membrane for alphaviruses or to intracellular membranes for RUBV. Upon generation of sufficient C protein, this protein assembles with the viral RNA to form the viral nucleocapsids. This process occurs in the

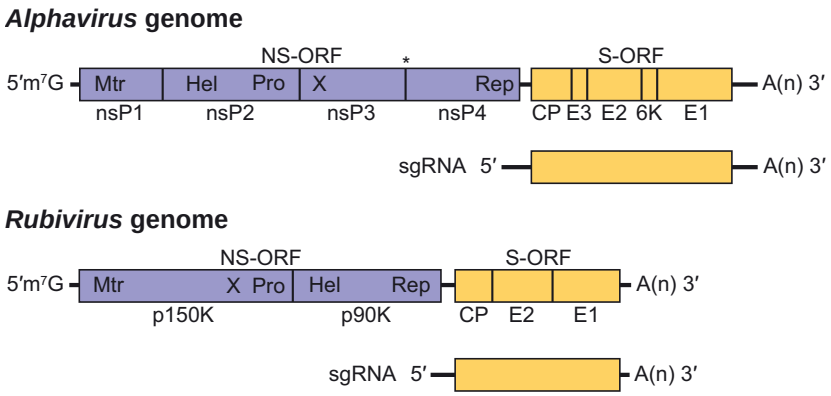


**Figure 2:** Togavirus genomic coding strategies. Shown are comparative schematic representations of the alphavirus and rubivirus genomic RNAs with UTRs represented as solid black lines and ORFs as open boxes (NS-ORF=non-structural protein ORF; S-ORF=structural protein ORF). Within each ORF, the coding sequences for the proteins processed from the translation product of the ORF are delineated. The asterisk between nsP3 and nsP4 in the alphavirus NS-ORF indicates the stop codon present in some alphaviruses that must be translationally read through to produce a precursor containing nsP4. Additionally, within the NS-ORFs, the locations of motifs associated with the following activities are indicated: (Mtr) methyl transferase, (Pro) protease, (Hel) helicase, (X) unknown function, and (Rep) replicase. The sequences encompassed by the sgRNA are also shown. (Courtesy of T. K. Frey.)

cytosol for alphaviruses and during the budding process associated with the Golgi apparatus for RUBV. Budding through the plasma membrane (alphaviruses) or the Golgi and plasma membrane (RUBV) leads to the acquisition of a lipid envelope containing the two membrane glycoproteins.

### Antigenic properties

The alphaviruses were originally described as Group A arboviruses based upon their antigenic cross-relationships. Using specific serological testing, antigenic complexes were developed where all members of a particular complex were closely related to each other. Eight (nine including the fish alphaviruses) such complexes are described whose members, for the most part, are also genetically clustered. The members of the two genera of the *Togaviridae* are antigenically quite distinct from each other with no detectable antigenic homology.

### Biological properties

Alphaviruses are, for the most part, arthropod-borne viruses which replicate in both their invertebrate vectors and their vertebrate hosts. (See Figure 3.) The salmonid alphaviruses and southern elephant seal virus, which cause disease in several species of fish and marine mammals, are exceptions because they seemingly lack an invertebrate vector. Similarly, RUBV appears to only infect mammals via the respiratory route but tends to develop persistent infections in contrast to the alphaviruses which are cytopathic to vertebrate cells. Both alphaviruses and rubiviruses have a global distribution and are capable of causing significant human illness. RUBV causes a mild febrile illness in children and adults but can cause significant teratology if a woman is infected during her first trimester. Alphaviruses cause disease ranging from mild febrile illness to prolonged arthritis to encephalitis.

## Genus *Alphavirus*

Type species ***Sindbis virus***

### Distinguishing features

Alphaviruses are transmitted between vertebrate hosts via the bite of a persistently infected arthropod vector, most frequently a mosquito. Each distinct virus has a preferred invertebrate vector and infection of the vector is typically lifelong and non-cytopathic. Vertebrate hosts can

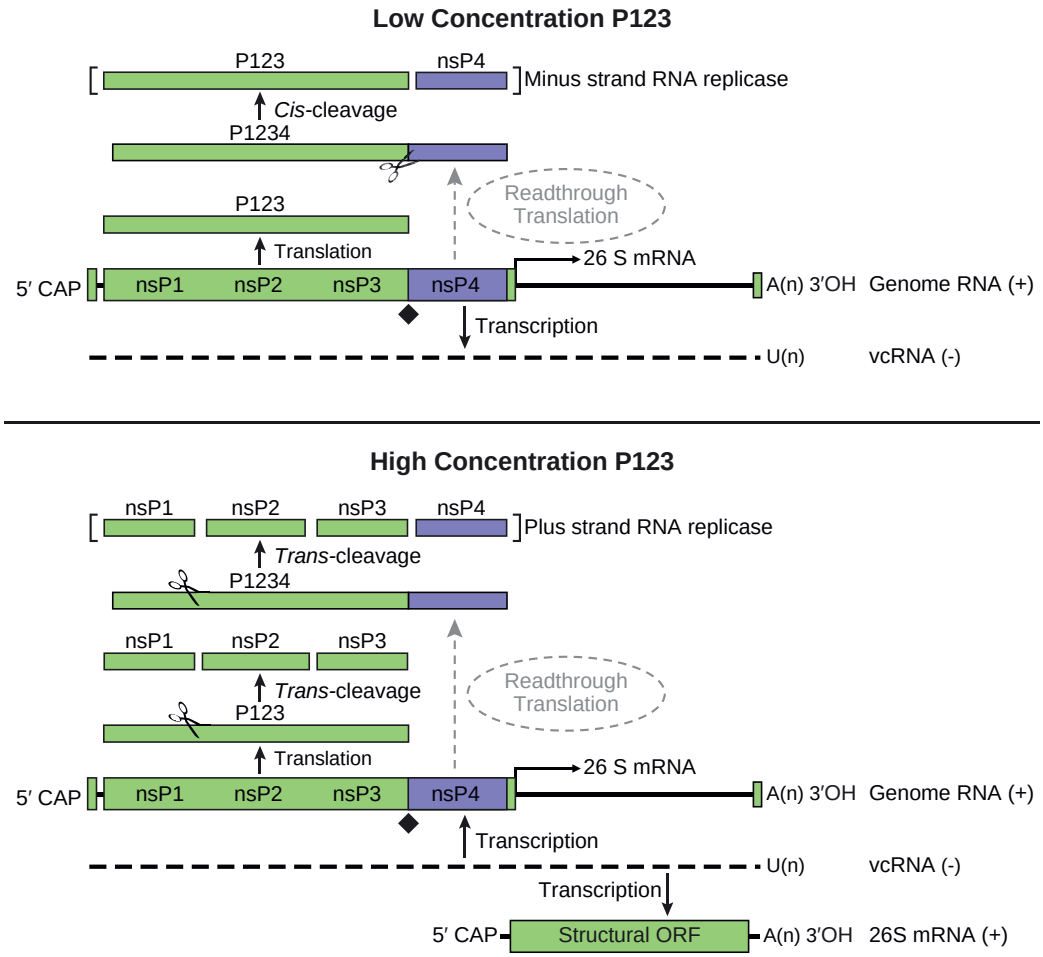


**Figure 3:** Model for the processing of the alphavirus nonstructural polyprotein during replication. When low levels of P123 are present, *cis*-cleavage of P1234 generates the minus-strand RNA replicase of the virus. This results in primarily negative sense RNA being generated from the incoming genomic RNA of the virus (upper panel). As the level of the *trans*-acting protease P123 rises in the infected cell, cleavage in *trans* generates other RNA replicase complexes. This results in a shift by the virus from the production of primarily negative sense RNA to primarily positive sense RNA. Eventually, replicase complexes capable of producing negative sense RNA will no longer be present in the infected cell resulting in the complete cessation of negative sense RNA synthesis (lower panel). The presence of a leaky opal termination codon (depicted as a black diamond) in the virus genome is believed to lead to a more rapid buildup of P123 in the infected cell, and thus a more rapid conversion to the production of positive sense RNA by the virus. (Courtesy of Dr Kevin Myles; modified from Powers, A.M. (2008). Togaviruses: Alphaviruses. In: *Encyclopedia of Virology*, 3rd edn (B.W.J. Mahy and M.H.V. Van Regenmortel, Eds.), Oxford, Elsevier, pp. 96-100.)

also vary significantly with distinct viruses infecting mammals, birds, fish, reptiles and amphibians. Geographically, alphaviruses span the globe yet many of the viruses are found in highly limited geographic areas with specific ecological conditions suited to their survival. Severe disease (encephalitis) is associated primarily with New World geography while febrile illness and arthralgia are more globally distributed. While the overriding common characteristics are genome organization and arthropod-borne status, the alphaviruses are a widely diverse group of viruses in virtually all other characteristics.

## Species demarcation criteria in the genus

Distinct species within the same antigenic complex generally show at least 21% nucleotide and 8% amino acid sequence divergence. Viruses in different serocomplexes typically diverge by over 38% at the nucleotide level and 40% at the amino acid level. Members of different species usually have distinct ecological and transmission patterns.

## List of species in the genus *Alphavirus*

| | | |
|---|---|---|
| *Aura virus* | | |
| Aura virus BeAR 10315 | [AF126284] | (AURAV-BeAR10315) |
| *Barmah Forest virus* | | |
| Barmah Forest virus BH2193 | [U73745] | (BFV-BH2193) |
| *Bebaru virus* | | |
| Bebaru virus MM2354 | [U94595*, AF339480*] | (BEBV-MM2354) |
| *Cabassou virus* | | |
| Cabassou virus CaAr 508 | [AF075259] | (CABV-CaAr508) |
| *Chikungunya virus* | | |
| Chikungunya virus 37997 | [AY726732] | (CHIKV-37997) |
| *Eastern equine encephalitis virus* | | |
| Eastern equine encephalitis virus BeAr436087 | [EF151503] | (EEEV-BeAr436087) |
| *Everglades virus* | | |
| Everglades virus Fe3-7c | [AF075251] | (EVEV-Fe37c) |
| *Fort Morgan virus* | | |
| Fort Morgan virus CM4-146 | [GQ281603] | (FMV-CM4-146) |
| Buggy Creek virus 81V8122 | [AF339474*, U94607*, U60403*] | |
| *Getah virus* | | |
| Getah virus M1 | [EU015061] | (GETV-M1) |
| *Highlands J virus* | | |
| Highlands J virus B-230 | [GQ227789] | (HJV-B230) |
| *Mayaro virus* | | |
| Mayaro virus Brazil | [AF237947] | (MAYV - Brazil) |
| *Middelburg virus* | | |
| Middelburg virus MIDV857 | [EF536323] | (MIDV-857) |
| *Mosso das Pedras virus* | | |
| Mosso das Pedras virus 78V3531 | [AF075257] | (MDPV-78V3531) |
| *Mucambo virus* | | |
| Mucambo virus BeAn 8 | [AF075253] | (MUCV-BeAn8) |
| *Ndumu virus* | | |
| Ndumu virus SaAr 2204 | [U94600*, AF339487*] | (NDUV-SaAr2204) |
| *O'nyong-nyong virus* | | |
| O'nyong-nyong virus SG650 | [AF079456] | (ONNV-SG650) |
| *Pixuna virus* | | |
| Pixuna virus BeAr35645 | [AF075256] | (PIXV-BeAr35645) |
| *Rio Negro virus* | | |
| Rio Negro virus AG80-663 | [AF075258] | (RNV-AG80-663) |
| *Ross River virus* | | |
| Ross River virus NB5092 | [M20162] | (RRV-NB5092) |
| Sagiyama virus | [AB032553] | |
| *Salmon pancreas disease virus* | | |
| Salmon pancreas disease virus F93125 | [AJ316244] | (SPDV-F93125) |
| Sleeping disease virus | [AJ316246] | |
| *Semliki Forest virus* | | |
| Semliki Forest virus 42S | [X04129] | (SFV-42S) |
| *Sindbis virus* | | |
| Babanki virus DakAry 251 | [U94604*, AF339477*] | |
| Kyzylagach virus LEIV 65A | [U94605*, AF339478*] | |
| Ockelbo virus Edsbyn | [M69205] | |
| Sindbis virus hssp | [J02363] | (SINV-hssp) |
| *Southern elephant seal virus* | | |
| Southern elephant seal virus Macquarie Island | [AF315122*] | (SESV-McQI) |
| *Tonate virus* | | |
| Tonate virus CaAn 410d | [AF075254] | (TONV-CaAn410d) |
| *Trocara virus* | | |
| Trocara virus BeAr422431 | [AF252265*] | (TROV-BeAr422431) |
| *Una virus* | | |
| Una virus BeAr 13136 | [U94603*, AF339481*] | (UNAV-BeAr13136) |
| *Venezuelan equine encephalitis virus* | | |
| Venezuelan equine encephalitis virus Trinidad donkey | [L01442] | (VEEV-TRD) |

| | | |
|---|---|---|
| *Western equine encephalitis virus* | | |
| Western equine encephalitis virus McMillan | [GQ287640] | (WEEV-McMillan) |
| *Whataroa virus* | | |
| Whataroa virus M78 | [U94606*, AF339479*] | (WHAV-M78) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

### List of other related viruses which may be members of the genus *Alphavirus* but have not been approved as species

None reported.

## GENUS *RUBIVIRUS*

Type species ***Rubella virus***

### Distinguishing features

In contrast to the alphaviruses which are arthropod-borne, RUBV is transmitted by aerosol. The illness, known as rubella or German measles, generally consists of fever and rash; complications are rare. However, if a pregnant woman is infected with RUBV during the first trimester there is a 20% chance of the fetus developing congenital rubella syndrome (CRS). Birth defects due to CRS include deafness, cataracts, heart defects, mental retardation, and liver and spleen damage.

CRS infants shed virus for up to six months. Such persistence contrasts with alphavirus infections which are cleared within days or weeks. RUBV is endemic worldwide and is vaccine preventable.

### Species demarcation criteria in the genus

Not applicable.

### List of species in the genus *Rubivirus*

| | | |
|---|---|---|
| *Rubella virus* | | |
| Rubella virus RA 27/3 | [L78917] | (RUBV-RA27/3) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Rubivirus* but have not been approved as species

None reported.

### List of unassigned species in the family *Togaviridae*

None reported.

### Phylogenetic relationships within the family

The alphaviruses have been extensively sequenced and phylogenetic comparisons made using whole genomes, E1, nsP1 and nsP4 genes. Distinct species within the same antigenic complex generally show at least 21% nucleotide and 8% amino acid sequence divergence. Viruses in different serocomplexes typically diverge by over 38% at the nucleotide level and 40% at the amino acid level. RUBV is quite distinct genetically from the alphaviruses demonstrating amino acid homology only within the non-structural proteins. (See Figure 4.)

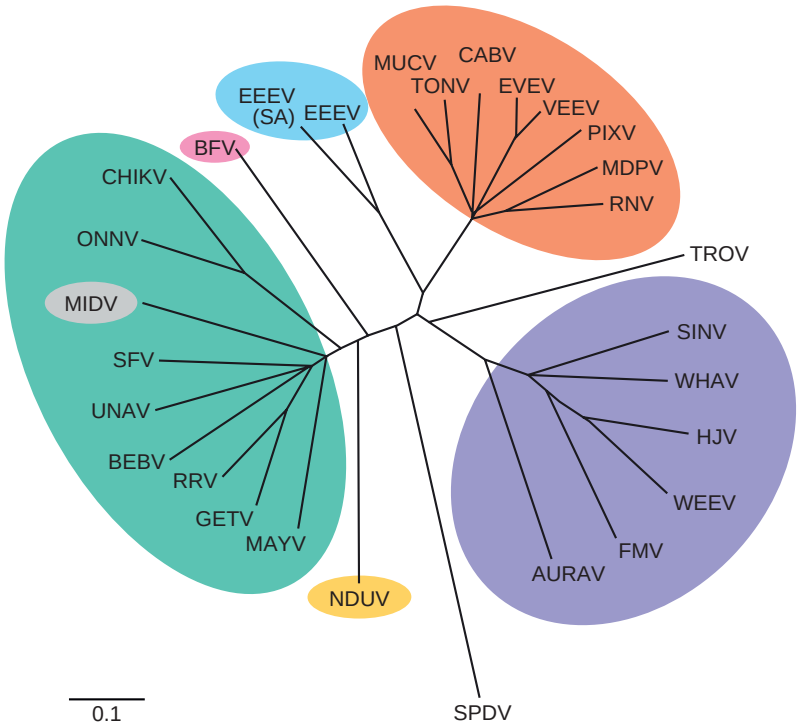


**Figure 4:** Unrooted phylogenetic tree of representative isolates of all alphavirus species generated from the E1 nucleotide sequences using the F84 algorithm of the neighbor-joining program (SESV and RUBV are not included because no homologous sequence for this region is available). Virus abbreviations are from the list of species in the genus *Alphavirus*. Antigenic complexes are indicated by colored circles. (From Powers, A.M. (2008). Togaviruses: Alphaviruses. In: *Encyclopedia of Virology*, 3rd edn (B.W.J. Mahy and M.H.V. Van Regenmortel, Eds.), Oxford, Elsevier, pp. 96-100.)

## Similarity with other taxa

Flaviviruses were previously included in the family *Togaviridae* suggesting some biological and virological similarities to the current members of this family. An atomic resolution crystal structure of an alphavirus E1 protein shows a folding pattern related to the E protein of flaviviruses, suggesting homology of at least some genes between these families. However, distinct genetic organization and replication led to the separation of these groups. There has also been the suggestion that similarities in replication proteins among members of RNA viruses from several plant families could imply an alphavirus-like superfamily exists.

## Derivation of names

*Alpha*: from Greek letter α.
*Rubi*: from Latin *rubeus*, "reddish".
*Toga*: from Latin *toga*, "cloak".

## Further reading

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Strauss, J.H. and Strauss, E.G. (1994). The alphaviruses: gene expression, replication, and evolution. *Microbiol. Rev.*, **58**, 491–562.

## Contributed by

Powers, A., Huang, H., Roehrig, J., Strauss, E. and Weaver, S.

# Family *Tombusviridae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Tombusviridae*** |
| Genus | ***Tombusvirus*** |
| Genus | ***Dianthovirus*** |
| Genus | ***Aureusvirus*** |
| Genus | ***Avenavirus*** |
| Genus | ***Carmovirus*** |
| Genus | ***Necrovirus*** |
| Genus | ***Panicovirus*** |
| Genus | ***Machlomovirus*** |

## Virion properties

### Morphology

Capsids exhibit T = 3 icosahedral symmetry and are composed of 180 identical protein subunits in three conformationally distinct states (A,B,C) (Figure 1). Capsids are formed with CP having one of two distinct phylogenetic origins. The virions from the genera *Aureusvirus*, *Avenavirus*, *Carmovirus*, *Dianthovirus* and *Tombusvirus* have a rounded outline, a granular surface and a diameter of about 32–35 nm. Each subunit folds into three distinct structural domains: R, the N-terminal internal domain interacting with RNA; S, the shell domain constituting the capsid backbone; and P, the protruding C-terminal domain, which gives the virus its granular appearance. P domains are clustered in pairs to form 90 projections. The S domain forms a beta barrel structure made up of eight β-strands. Two $Ca^{2+}$ binding sites stabilize contacts between adjacent S domains of the A, B and C subunits at the pseudo three-fold axis. The capsids of viruses in the genera *Machlomovirus*, *Necrovirus* and *Panicovirus* are composed of similarly structured CPs that lack the P domain and thus have a smooth appearance. Particles range in diameter between 30–32 nm, and the S domain is related to the CPs of sobemoviruses.

### Physicochemical and physical properties

Virions sediment as one component with an $S_{20,w}$ of 118–140S, have a buoyant density ranging from 1.34 to 1.36 g cm$^{-3}$ in CsCl, and a virion Mr of 8.2–8.9 × $10^6$. Virions are stable at acidic pH, but expand above pH 7 and in the presence of EDTA. Virions are resistant to elevated temperatures (thermal inactivation usually occurs above 80 °C) and are insensitive to organic solvents and non-ionic detergents.

### Nucleic acid

With the exception of those of the genus *Dianthovirus*, virions contain a single molecule of positive sense, linear ssRNA, that constitutes about 17% of the particle weight, and have a size ranging from 3.7 to 4.8 kb, depending on the genus. Dianthovirus virions contain two genomic RNAs of approximately 3.8 kb and 1.4 kb. The 5′-end of the genome is uncapped. The 3′-ends are not polyadenylated. dsRNAs corresponding in size to viral genomic and sgRNAs are present in infected tissues. DI RNAs occur in some genera. In addition, some members have satellite RNAs or satellite viruses associated with them.

### Proteins

Capsids are composed of one of two phylogenetically distinct groups of CP subunit. In those genera that have a CP lacking a protruding domain, the CP subunit ranges in size from 25–30 kDa. In those genera with a CP containing a P domain, the CP subunit ranges in size from 37 to 48 kDa.

### Lipids

None reported.

### Carbohydrates

None reported.

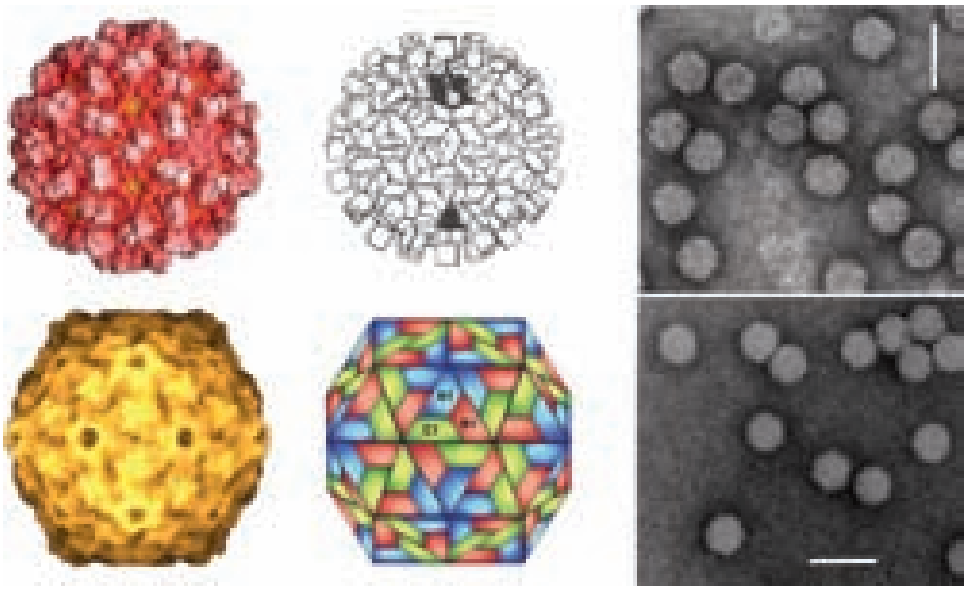

**Figure 1:** (Top row, left) Computer reconstruction of a tomato bushy stunt virus (TBSV) particle based on X-ray crystallography at 2.9 Å resolution (J.Y. Sgro, University of Wisconsin-Madison) (Olson *et al.* (1983). *J. Mol. Biol.*, **171**, 61-93). (Top row center) Diagrammatic representation of a T = 3 TBSV particle. A, B and C correspond to the three conformational states of the CP. (Top row, right) Negative contrast electron micrograph of TBSV particles. (Bottom row, left) Computer reconstruction of a tobacco necrosis virus A (TNV-A) particle, based on X-ray crystallography at 2.25 Å resolution (Oda *et al.* (2000). *J. Mol. Biol.*, **300**, 153-169). (Bottom row center) Schematic representation of the T = 3 structure of TNV-A particles. A, B, C correspond to the three conformational states of the CP. (Bottom row, right) Negative contrast electron micrograph of particles of TNV (species unidentified). The bars represent 50 nm.

## Genome organization and replication

Even though variability exists in the number and location of genes within members of the family, a number of organizational features are highly conserved (Figure 2). The unifying feature of the family is that each member species possesses a highly conserved polymerase that is interrupted by an in-frame termination codon that is periodically suppressed to express the core polymerase containing the canonical "GDD" motif. Dianthoviruses utilize a −1 ribosomal frameshifting mechanism to accomplish the same result. The polymerase is further characterized by possessing no obvious helicase or methlytransferase motifs. In this description, as well as those for each genus, the polymerase is labeled as a single ORF with the readthrough (RT) portion labeled ORF1-RT or ORF1-FS.

Genomes of members of the genera *Dianthovirus* and *Avenavirus* encode three ORFs, members of the genus *Necrovirus* and *Panicovirus* encode five ORFs, while all others encode four ORFs. In the genus *Machlomovirus* there is an additional putative terminator readthrough event to express an accessory ORF (Figure 2, ORF3-RT). Products of the 5′-proximal ORFs 1 and 1RT or 1FS are expressed by translation directly from the genomic RNA, whereas translation products of the internal and 3′-proximal ORFs are expressed from subgenomic RNAs (sgRNAs). Translation of the genome is cap-independent and is controlled by elements in the 3′-terminal untranslated region of the genome. These elements form base pairs with sequences in the 5′-terminal region of the template RNA, enhancing translation of RNA. For all genera, the CP ORF is either internal or 3′-proximal and requires the synthesis of a sgRNA for expression *in vivo*. In most cases, it has been demonstrated that the CP is not required for cell-to-cell movement but is required for or facilitates long distance movement.

Non-structural proteins include the phylogenetically conserved pre-readthrough proteins of 23–48 kDa and its 82–112 kDa readthrough products. Tombusviruses and aureusviruses encode a conserved 14–19 kDa protein (see Figure S2) which is associated with the suppression of silencing. Viruses in the family utilize at least four phylogenetically distinct MPs. Genomes of tombusviruses

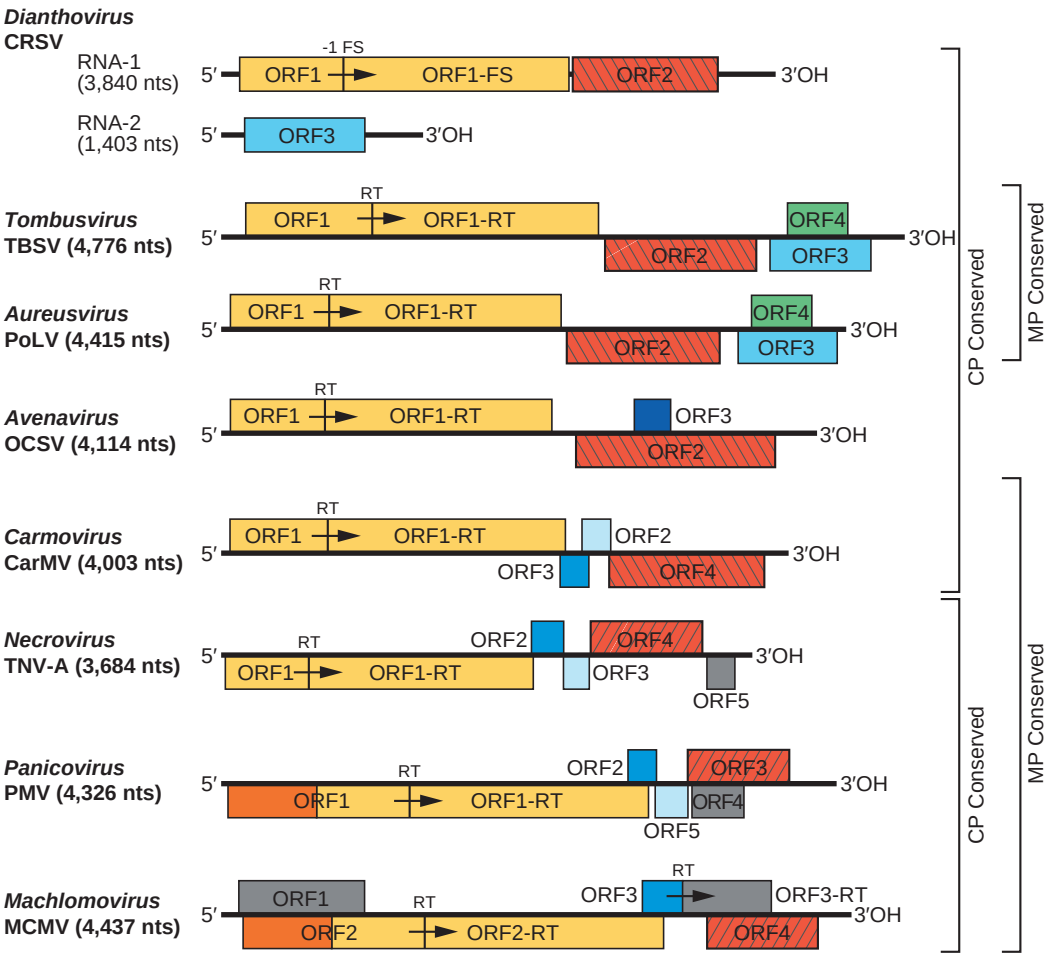


**Figure 2:** Genome organization of the type species for each genus in the family *Tombusviridae*. Boxes represent known and predicted ORFs. Similarly shaded boxes represent proteins with sequence conservation with the exception of the gray boxes which represent proteins unique to the indicated virus. The yellow boxes represent ORFs encoding the phylogenetically conserved polymerase. Red-hatched boxes represent CP encoding ORFs. Right-hatched boxes identify CPs lacking a protruding domain that are related to those of the genus *Sobemovirus* while those which are left-hatched represent tombusvirus-like CPs that contain protruding domains. Blue boxes represent viral MPs. In carmoviruses, necroviruses and panicoviruses the light-blue-shaded box corresponds to a second movement protein conserved among these viruses. In avenaviruses the dark-blue-shaded box corresponds to a potential movement protein that does not show sequence similarity to any of the other movement proteins encoded by the *Tombusviridae*. In tombusviruses and aureusviruses the green-shaded boxes correspond to the silencing suppressor protein. The orange-shaded region in PMV and MCMV are similar to each other but do not have sequence similarity with other members of the *Tombusviridae*. RT: translational readthrough of termination codon. −1FS: −1 ribosomal frameshifting event. CP: capsid protein.

and aureusviruses encode a conserved 22–27 kDa MP (see Figure S1) and the dianthovirus genome encodes a second type of MP of about 34 kDa. The carmoviruses, machlomoviruses, necroviruses and panicoviruses encode a 7–8 kDa MP (MP1) which contains a conserved carboxyl-terminus (see Figure S3). The carmoviruses, necroviruses and panicoviruses encode an additional conserved small 6–9 kDa polypeptide (MP2) (see Figure S4). Panicoviruses and machlomoviruses have additional accessory ORFs whose functions have not been determined.

Replication occurs in the cytoplasm, in membranous vesicles that may be associated with endoplasmic reticulum, or modified organelles such as peroxisomes, mitochondria and, more rarely, chloroplasts. Virions are present in the cytoplasm and occasionally in mitochondria and nuclei.

## Antigenic properties

Virions are efficient immunogens. Antisera yield single precipitin lines in immunodiffusion tests. Depending on the genus, serological cross-reactivity among species ranges from nil to near-homologous titers. Many serologically related strains have been identified in several species.

## Biological properties

### Host range

The natural host range of individual virus species is relatively narrow. Members can either infect monocotyledonous or dicotyledonous plants, but no species can infect both. The experimental host range is wide. Infection is often limited to the root system, but when hosts are invaded systemically, viruses enter all tissues. Many members induce a necrosis symptom in the foliar parts of the plant. Diseases are characterized by mottling, crinkling, necrosis and deformation of foliage. Some infections are symptomless in their natural hosts.

### Transmission

All members are readily transmitted by mechanical inoculation and through plant material used for propagation. Some may be transmitted by contact and through seeds. Viruses are often found in natural environments, i.e. surface waters and soils from which they can be acquired without assistance of vectors. Transmission by the chytrid fungus in the genus *Olpidium* and by beetles has also been reported for members of several genera. Most members can be transmitted through the soil either dependent on, or independent of, a biological vector.

### Geographical distribution

Geographical distribution of particular species varies from wide to restricted. The majority of the species occur in temperate regions. Legume-infecting carmoviruses and one possible member of the genus *Dianthovirus* have been recorded from tropical areas.

### Cytopathic effects

Distinctive cytopathological features occur in association with exceedingly high accumulations of virus particles in cells and "multivesicular bodies", i.e. cytoplasmic membranous inclusions originated from profoundly modified mitochondria and/or peroxisomes. Dense granules which may consist of coat protein aggregates are also found in infected cells.

## Genus demarcation criteria in the family

The list of criteria demarcating genera in the family are:

- Structural criteria: spherical virions with either a smooth or granular appearance.
- Genomic criteria: genome organization, number of genome segments, size of genome.
- Polymerase criteria: gene interrupted by a termination codon or a −1 ribosomal frameshifting element that is periodically read through.

# Genus *Tombusvirus*

Type species ***Tomato bushy stunt virus***

## Distinguishing features

The genome is approximately 4.7–4.8 kb and contains four ORFs. The CP ORF is located internally on the genomic RNA and is expressed *in vivo* from an approximate 2.2 kb sgRNA. ORFs 3 and 4 are 3′ proximally located and ORF4 is contained within ORF3 in a different reading frame. Both ORFs are expressed from a second sgRNA of about 0.9 kb. The genome organization and expression strategy are identical to that of aureusviruses. The tombusvirus ORF3 is significantly smaller and ORF4 significantly larger than that in the aureusviruses. The ORF3 MP shares sequence similarity with that of the aureusviruses (see Figure S1) whereas no sequence similarity is observed with other members of the family *Tombusviridae*. All members elicit formation of multivesicular inclusion

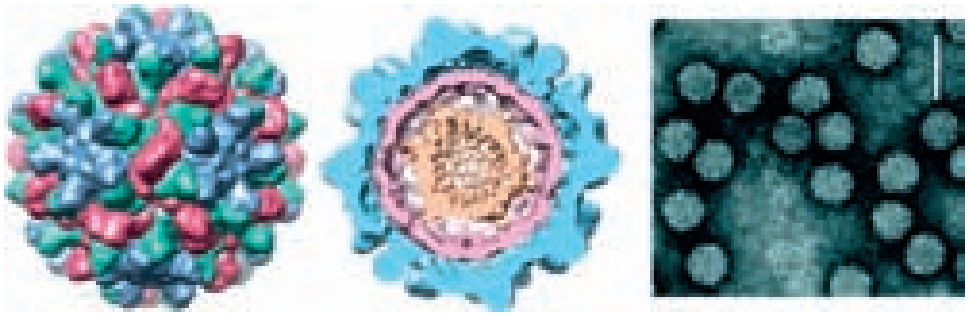

**Figure 3:** (Left) Cryo reconstruction of the CNV particle showing the P-domain pairs and the five-fold, three-fold and two-fold axes of symmetry. (Center) Cut away image of CNV particle showing features of the structured interior including the inner shell and the connections between it and the outer shell at the particle three-fold axes (cryo images kindly provided by Tom Smith, Donald Danforth Plant Science Centre, St Louis, MO). (Right) Negative contrast electron micrograph of TBSV particles. The bar represents 50 nm.

bodies. Diseases caused by tombusviruses prevail in temperate climates. All species are soil-borne, but only one, cucumber necrosis virus (CNV), has a recognized fungal vector (*Olpidium bornovanus*).

## Virion properties

### Morphology

Capsids are 32–35 nm T = 3 icosahedra and have the structure described above for family members containing a P domain. Recent cryo-electron microscopy studies show that the CNV particle consists of a structured inner shell that forms conspicuous connections with the outer shell at the particle three-fold axis (Figure 3). Viral RNA lies along the inner face of the outer shell and may also extend into the inner shell. The structured inner shell may serve as a scaffold for assembly of the particle.

### Physicochemical and physical properties

The virus sediments as one component with an $S_{20,w}$ of 132–140S, has a buoyant density of 1.34–1.36 g cm$^{-3}$ in CsCl, and a virion Mr of $8.9 \times 10^6$. The virion isoelectric point is pH 4.1. Particles exhibit an $A_{260}/A_{280}$ ratio of 1.64 and a thermal inactivation point of 80–90 °C. Longevity *in vitro* of 130–150 days has been reported. Virions have a dilution end point in excess of $10^{-6}$. Virions are insensitive to ether, chloroform and non-ionic detergents. Virions are stabilized by divalent cations.

### Nucleic acid

Nucleic acid represents 17% of the virion, and consists of one molecule of linear positive sense ssRNA. Total genome length is approximately 4.8 kb. The 3′ terminus has neither a poly(A) tract nor a terminal tRNA-like structure. A 3′-proximal segment is involved in facilitating cap-independent translation. In addition to genomic RNA, virions of some species harbor and package DI and/or satellite RNAs. sgRNAs may also be packaged into virions at various levels. sgRNAs are generated by premature termination during genome minus strand synthesis, followed by sgRNA production using the truncated minus strand RNA as template. Three virus-specific dsRNA species are found in infected cells. These correspond to the genomic RNA and the two sgRNAs.

## Genome organization and replication

The genomic RNA contains four ORFs (Figure 4). ORF1 encodes a 32–36 kDa protein. This protein has transmembrane binding regions for anchoring to peroxisomal or mitochondrial outer membranes and is involved in recruiting viral RNA to the site of replication. Readthrough (RT) of the ORF1 amber termination codon allows expression of a 92–95 kDa protein (ORF1-RT). Both the 32–36 and 92–95 kDa proteins are produced by translation of genomic RNA. The ORF1 and ORF1-RT-encoded proteins comprise part of the viral RNA-dependent RNA polymerase (RdRp) which is conserved among members of the *Tombusviridae* (see Figure S6). The polymerase is also distantly related to the flavivirus and pestivirus polymerases (see Figure S6). The internal ORF2 encodes the CP, which is expressed from the approximate 2.2 kb sgRNA1. The CP is less conserved than the

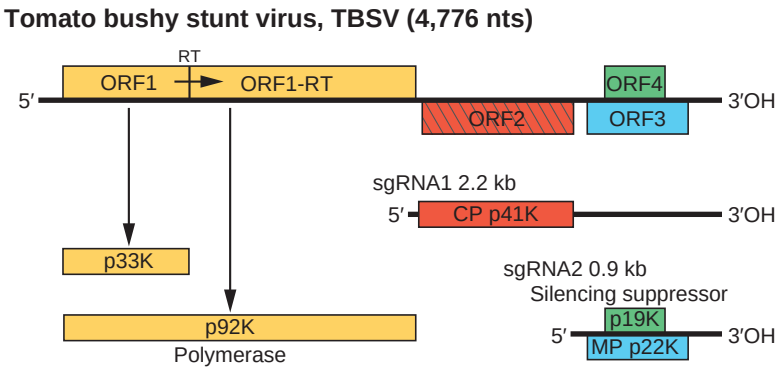


**Figure 4:** Genome organization and replication strategy of the tombusvirus tomato bushy stunt virus (TBSV). Boxes represent known and predicted ORFs with the sizes of the respective proteins (or readthrough products) shown on the horizontal bars below the ORFs. The yellow-shaded ORFs indicate polymerase proteins that have a high degree of sequence conservation within the family *Tombusviridae*. Left-hatched box identifies the CP that is conserved among other genera within the family *Tombusviridae* that have a protruding domain. The blue box shows the MP which shares sequence similarity to the MPs of aureusviruses (see Figure S1). The green box identifies the ORF encoding the silencing suppressor that shares sequence similarity with that of aureusviruses (see Figure S1). Lines underneath depict the two sgRNAs that are synthesized in infected cells. The bars underneath the sgRNAs correspond to the encoded proteins, i.e., the CP, the RNA silencing suppressor and the movement protein (MP). RT = amber termination codon that is read through.

polymerase. Two nested ORFs (ORF3 and ORF4) located at the 3′ terminus of the genome, encode proteins of about 22 kDa (p22) and about 19 kDa (p19), respectively. p22 is required for cell-to-cell movement, interacting with a host homeodomain leucine-zipper protein. p19 is a suppressor of RNA silencing. It also has a role in the systemic spread of the virus, and is involved in host specific development of necrosis. p22 and p19 are related to the ORF 4 and 5 proteins of aureusviruses (see Figures S1 and S2) and are not related to proteins encoded by other members of the *Tombusviridae*.

Genome replication is carried out by the virus encoded polymerase in conjunction with at least eight host factors. The replication process begins with the synthesis of a minus-strand RNA from a plus-strand template. The minus-strand is then used as template for the synthesis of the progeny genomes. Numerous long-range RNA/RNA interactions regulate viral RNA replication, transcription and translation. Cap-independent translation utilizes a Y-shaped structure in the 3′ region of the genome that base-pairs with sequences in the 5′ end of the genome. DI RNAs are generated during replication of some species following multiple and progressive deletions of genomic RNA. Cymbidium ringspot virus (CymRSV), carnation Italian ringspot virus (CIRV) and TBSV RNA can replicate in cells of the yeast *Saccharomyces cerevisiae*. Over 150 host proteins have been found to affect the efficiency of viral RNA replication in yeast. DI RNAs are also replicated in yeast.

## Antigenic properties

Most species are serologically interrelated, though to a variable extent. Species with serologically related virions are: artichoke mottled crinkle virus, pelargonium leaf curl virus and petunia asteroid mosaic virus (PAMV), which are closely related; Moroccan pepper virus (MPV) which is distantly related; eggplant mottled crinkle virus (EMCV), CIRV, Lato river virus and Neckar river virus (NRV) which are very distantly related. Species with serologically unrelated virions are CymRSV and CNV.

## Biological properties

### Host range

Most species have a narrow natural host range. However, most also have a wide experimental host range. Even though the host range of an individual species is restricted in nature, tombusviruses are present in a wide range of both monocotyledonous and dicotyledonous plants. Viruses tend to remain localized, forming a necrosis in artificially infected hosts. However, *Nicotiana clevelandii* and *N. benthamiana* are often systemically infected.

### Transmission

Members are readily transmitted mechanically in the field and experimentally. Most, if not all, species are soil-borne without the aid of a biological vector. They appear to be directly transmitted through the soil. CNV is transmitted by the chytrid fungus *Olpidium bornovanus*. Some species may be transmitted through the seed at a very low level.

### Geographical distribution

TBSV strains are present throughout North and South America, Europe, and the Mediterranean. Other tombusviruses are present wherever the primary hosts exist.

### Cytopathic effects

Virions are found in all parts of the host plant cells including cytoplasm, nuclei, nucleoli, mitochondria and cell vacuoles. Virus crystals are present in the cytoplasm of infected cells. Multivesicular bodies (MVBs), i.e. cytopathic structures made up of a main body surrounded by spherical to ovoid vesicles 80–150 nm in diameter, are consistently present in infected cells. MVBs originate from the proliferation of the limiting membrane of peroxisomes (e.g. CymRSV, TBSV and CNV) or mitochondria (CIRV), their formation being determined by an ORF1 encoded sequence as short as about 200 aa. MVBs do not contain virions. However, virions are present within seemingly intact mitochondria in cells infected by EMCV, CymRSV, grapevine Algerian latent virus, MPV, NRV, TBSV and occasionally PAMV.

## Species demarcation criteria in the genus

The list of species demarcation criteria in the genus is:

- Extent of serological relationship as determined by immunodiffusion (usually not below 3), and/or ELISA
- Less than 85% sequence identity in the CP
- Differential cytopathological features; organelles from which MVBs arise
- Natural host range
- Artificial host range reactions

## List of species in the genus *Tombusvirus*

| | | |
|---|---|---|
| *Artichoke mottled crinkle virus* | | |
| Artichoke mottled crinkle virus - Bari | [X62493 = NC_001339] | (AMCV-Bari) |
| *Carnation Italian ringspot virus* | | |
| Carnation Italian ringspot virus - Italy | [X85215 = NC_003500] | (CIRV-IT) |
| *Cucumber Bulgarian latent virus* | | |
| Cucumber Bulgarian latent virus - Bulg | [AY163842 = NC_004725] | (CBLV-Bulg) |
| *Cucumber necrosis virus* | | |
| Cucumber necrosis virus - Canada | [M25270 = NC_001469] | (CNV-Can) |
| *Cymbidium ringspot virus* | | |
| Cymbidium ringspot virus - UK | [X15511 = NC_003532] | (CymRSV-UK) |
| *Eggplant mottled crinkle virus* | | |
| Eggplant mottled crinkle virus - Israel | [FJ977166*] | (EMCV-Is) |
| Lisianthus necrosis virus - Taiwan | [DQ011234 = NC_007983] | (LNV-TW) |
| Pear latent virus - Italy | [AY100482 = NC_004723] | (PLV-IT) |
| *Grapevine Algerian latent virus* | | |
| Grapevine Algerian latent virus - Japan nipplefruit | [AY830918 = NC_011535] | (GALV-JA) |
| *Havel river virus* | | |
| Havel river virus - S | [AY370535*] | (HRV-S) |
| *Lato river virus* | | |
| Lato river virus - Italy | | (LRV-IT) |
| *Limonium flower distortion virus* | | |
| Limonium flower distortion virus - Lim2 | [AY500882*] | (LFDV-Lim2) |
| *Moroccan pepper virus* | | |
| Moroccan pepper virus - Italy | [AF540886*] | (MPV-IT) |
| *Neckar river virus* | | |
| Neckar river virus - Germany | [AY500887*] | (NRV-GR) |

| | | |
|---|---|---|
| *Pelargonium leaf curl virus* | | |
| Pelargonium leaf curl virus - Koenig | [AF290026*] | (PLCV-Koenig) |
| *Pelargonium necrotic spot virus* | | |
| Pelargonium necrotic spot virus - UPEV | [AJ607402 = NC_005285] | (PNSV-UPEV) |
| *Petunia asteroid mosaic virus* | | |
| Petunia asteroid mosaic virus - Lim1 | [AY500881*] | (PAMV-Lim1) |
| *Sikte waterborne virus* | | |
| Sikte waterborne virus - Lim6 | [AY500886*] | (SWBV-Lim6) |
| *Tomato bushy stunt virus* | | |
| Tomato bushy stunt virus - cherry | [M21958 = NC_001554] | (TBSV-cherry) |

*Sequences do not comprise the complete genome.

### List of other related viruses which may be members of the genus *Tombusvirus* but have not been approved as species

| | | |
|---|---|---|
| Lettuce necrotic stunt virus | [AJ288944] | (LNSV) |

## Genus *Dianthovirus*

Type species ***Carnation ringspot virus***

### Distinguishing features

Virions sediment in sucrose gradients as a single band of $S_{20,w}$ 126–135S. The genome is composed of two RNAs of approximately 3.8 and 1.4 kb (Figure 6). ORF1 encodes the polymerase and is interrupted by a ribosomal frameshifting event yielding a pre-frameshift approximate 27 kDa protein and an approximate 88 kDa frameshift polypeptide. The CP, possessing a protruding domain, is encoded by the 3′-proximal ORF on RNA1 and is expressed from an approximate 1.5 kb sgRNA *in vivo*. The ORF for the 34–35 kDa MP is in the monocistronic RNA2. The movement protein aa sequence is unique to the dianthoviruses. Members are transmitted through the soil without the aid of a biological vector.

### Virion properties

#### Morphology

Capsids are approximately 37 nm in diameter and have T = 3 icosahedral symmetry (Figure 5) as described above for family members containing a P domain. Cryo-electron microscopy has revealed that red clover necrotic mosaic virus (RCNMV) capsids contain a defined inner shell (Figure 5), consisting of a cage containing complexes of virion RNA and the N-terminal R-domain region of the CP. Virions undergo significant structural changes upon removal of the $Ca^{2+}$ and $Mg^{2+}$ ions that are found in the shell and interior of the capsid. Loss of these ions in the divalent cation depleted cytoplasm of cells may contribute to particle disassembly.

#### Physicochemical and physical properties

Virions sediment as one component in sucrose with $S_{20,w}$ of 126–135S. The buoyant density in CsCl is 1.363–1.366 g $cm^{-3}$, and the virion Mr is $8.6 \times 10^6$. Particles exhibit an $A_{260}/A_{280}$ ratio of 1.67 and a thermal inactivation point between 80–90 °C. A longevity *in vitro* of around 10 weeks has been reported for most members. Virions are insensitive to ether, chloroform and non-ionic detergents. Virions are stable at pH 6 and lower; alkaline conditions (pH 7–8) induce particle swelling. Virions are stabilized by divalent cations.

#### Nucleic acid

Virions contain two molecules of infectious linear positive sense ssRNA of 3.8 and 1.4 kb. The two genomic components are not capped. They do not contain a 3′-terminal poly(A) tract. Virions package either both genomic RNAs or multiple copies of RNA-2. Three virus-specific dsRNA species are found in infected cells. The largest and smallest dsRNAs correspond to the genomic RNA1 and RNA2, respectively. The intermediate sized dsRNA corresponds to the 1.5 kb sgRNA.

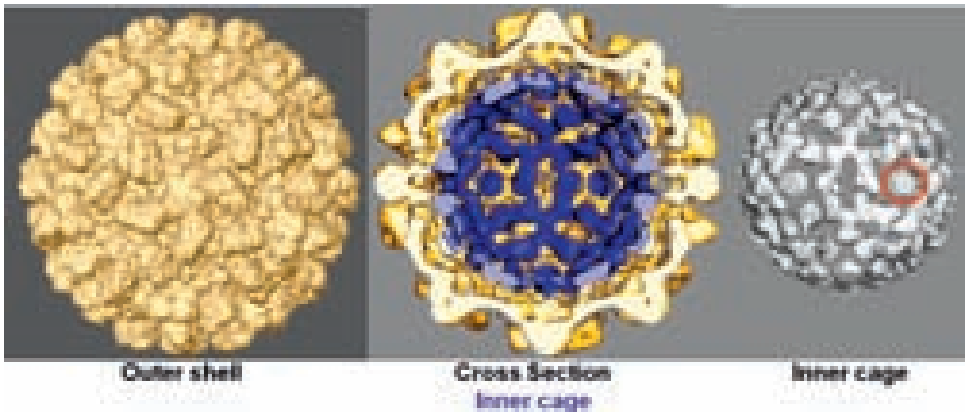


**Figure 5:** Cryo-EM of the RCNMV particle (from Baker, Sherman and Lommel, with permission; see also Sherman, M.B. *et al.* (2006). *J. Virol.*, **80**, 10395-10406). The RCNMV virion has an ordered internal shell as can be seen by the cross-section of the cryo structure (center panel). In addition to an outer shell (left panel, colored yellow) comprised entirely of coat protein, RCNMV has an additional internal ordered density (colored with shades of blue). Approximately 90% of RCNMV's density corresponds to the coat protein. Thus it is possible that the ordered density observed in the inner cage may correspond in part to ordered RNA.

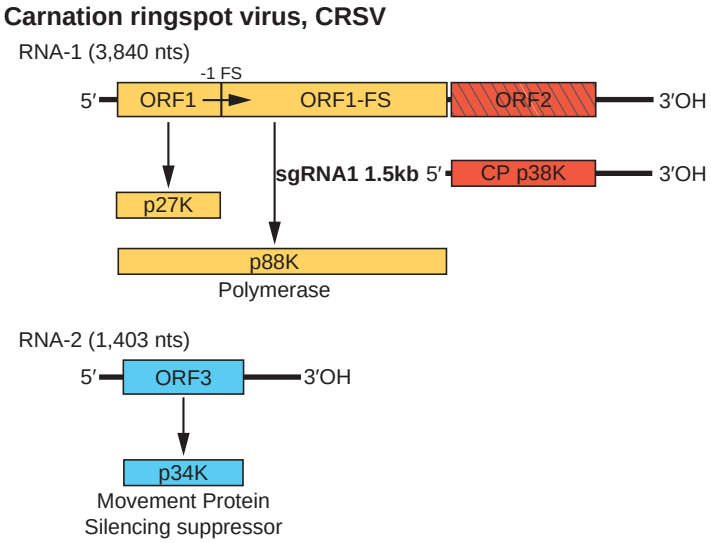


**Figure 6:** Genome organization and replication strategy of the dianthovirus carnation ringspot virus (CRSV). Boxes represent known ORFs with the sizes of the respective proteins (or readthrough products) indicated in the bars below. Yellow ORFs on RNA1 indicate polymerase proteins that have a high degree of sequence conservation within the family *Tombusviridae*. Left-hatched box on RNA1 identifies the CP that is conserved among other genera within the family *Tombusviridae* that have a protruding domain. The blue box on RNA2 identifies the ORF that encodes the MP which is also a silencing suppressor. The MP contains a region of sequence similarity with MPs in the other virus families (see text). The line under RNA1 depicts the 1.5 kb CP sgRNA. −1 FS = site of −1 ribosomal frameshifting.

## Genome organization and replication

RNA1 contains two ORFs. ORF1 is capable of encoding a 27 kDa protein. A −1 ribosomal frameshift event at the canonical shifty heptanucleotide allows translation to continue into ORF1-FS to produce the 88 kDa protein. The ORF1 and ORF1-FS encoded proteins form the viral polymerase. ORF2 encodes the 37–38 kDa CP. This ORF is expressed *in vivo* from the approximately 1.5 kb sgRNA. ORF3 on RNA2 encodes the 34–35 kDa MP. The RCNMV MP also serves as a silencing suppressor. For the sgRNA of RNA1 to be expressed, the loop of a stem-loop in RNA2 must base pair within the RNA1 sgRNA promoter. This same stem-loop interaction allows RNA-1 to become encapsidated

since RNA-2 contains the origin of assembly. Translation of RNA1 occurs in a cap-independent fashion requiring an element in the 3′ UTR that is similar to the barley yellow dwarf virus (BYDV) translational enhancer.

## Antigenic properties

Virus particles are moderately to highly immunogenic. Various serologically distinct strains have been identified. Antisera yield a single precipitin line in agar gel-diffusion assays. Monoclonal antibodies have been identified that cross-react between species.

## Biological properties

### Host range

In nature, dianthoviruses have moderately broad natural host ranges restricted to dicotyledonous plants. In the laboratory, the experimental host range is much broader, and includes a wide range of herbaceous species in the families *Solanaceae*, *Leguminosae*, *Cucurbitaceae* and *Compositae*. Most plants are infected locally (non-systemically).

### Transmission

The viruses are readily transmitted by mechanical inoculation; they are not known to be seed-transmitted. The viruses are not transmitted by insects, nematodes, or soil-inhabiting fungi, although there is a report that RCNMV is transmitted by *Olpidium*. However, viruses are readily transmitted through the soil without the aid of a biological vector.

### Geographic distribution

Dianthoviruses are widespread throughout the temperate regions of the world.

### Cytopathic effects

None reported.

## Species demarcation criteria in the genus

The list of species demarcation criteria in the genus is:

- Extent of serological relationship as determined by immunodiffusion and/or ELISA
- Extent of sequence identity between relevant gene products
- Less than 93% aa sequence identity in the polymerase
- Less than 75% aa sequence identity in the CP
- Ability to form pseudorecombinants with the two RNA components
- Transmission through the soil
- Natural host range
- Artificial host range reactions

## List of species in the genus *Dianthovirus*

| | RNA1 | RNA2 | |
|---|---|---|---|
| *Carnation ringspot virus* | | | |
| Carnation ringspot virus - Lommel | [L18870 = NC_003530] | [M88589 = NC_003531] | (CRSV-Lommel) |
| *Red clover necrotic mosaic virus* | | | |
| Red clover necrotic mosaic virus - Australia | [J04357 = NC_003756] | [X08021 = NC_003775] | (RCNMV-AUS) |
| *Sweet clover necrotic mosaic virus* | | | |
| Sweet clover necrotic mosaic virus - 59 | [L07884 = NC_003806] | [S46028 = NC_003807] | (SCNMV-59) |

Species names are in italic script; isolate names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

**List of other related viruses which may be members of the genus *Dianthovirus* but have not been approved as species**

| | RNA1 | RNA2 | |
|---|---|---|---|
| Rice virus X | [AB033715] | | (RVX) |

# Genus *Aureusvirus*

Type species ***Pothos latent virus***

## Distinguishing features

The virion is a 30 nm icosahedron that packages the genomic RNA of about 4.2–4.4 kb. The genome contains four ORFs. The CP ORF is located internally in the genomic RNA and is expressed *in vivo* from a sgRNA of about 2 kb. ORFs 3 and 4 are 3′-proximally located and ORF4 is nested within ORF3 in a different reading frame. Both ORFs are expressed from an approximate 0.8 kb sgRNA. The genome organization and expression strategy are identical to that of the tombusviruses. The polymerase and pre-readthrough regions of aureusviruses are most similar to those of tombusviruses (see Figure S6). The aureusvirus ORF3 is significantly larger and ORF4 is significantly smaller than those in the tombusviruses but their encoded proteins are similar to the corresponding ORFs of tombusviruses (Figures S1 and S2). Transmission occurs through the soil without (pothos latent virus; PoLV) or with (cucumber leaf spot virus; CLSV) the aid of a vector (*Olpidium bornovanus*).

## Virion properties

### Morphology

Virions are isometric with a rounded outline, a knobby surface and a diameter of about 30 nm (Figure 7). Based on comparative aa sequence alignments, the CP subunits likely contain the R, S and P domain structures described for TBSV and RCNMV. Similarly, particles are likely T = 3 icosahedra composed of 180 copies of the CP.

### Physicochemical and physical properties

Preparations of purified virus sediment as a single component in sucrose density gradients, and to equilibrium in solutions of CsCl and $Cs_2SO_4$. Buoyant density in CsCl and $Cs_2SO_4$ is 1.34–1.36 and 1.37 g cm$^{-3}$, respectively. The thermal inactivation point is above 80 °C. Virus particles resist organic solvents but are readily disrupted by SDS.

### Nucleic acid

Virions contain a single molecule of linear, uncapped, non-polyadenylated, positive sense ssRNA of about 4.2–4.4 kb constituting 17% of the particle weight. Virions can contain the two sgRNAs. Three

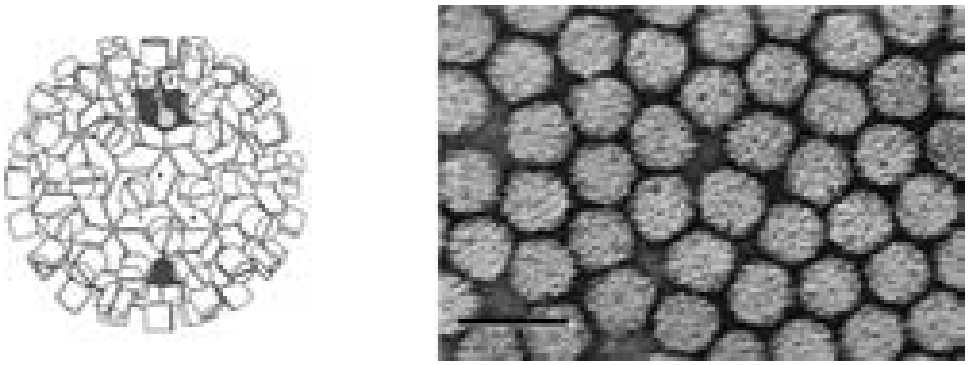

**Figure 7:** (Left) Diagrammatic representation of a T = 3 particle with A, B, C corresponding to the three conformational states of the CP subunit. (Right) Negative contrast electron micrograph of pothos latent virus particles. The bar represents 50 nm.

dsRNA species corresponding to the full-size genomic RNA and the two sgRNAs can be recovered from infected plants. Maize white line mosaic virus (MWLMV) supports the replication of a satellite virus but satellite RNAs or DI RNAs have not been reported for the other aureusvirus members.

## Genome organization and replication

The genome of PoLV contains four ORFs (Figure 8). ORF1 encodes a 25 kDa protein. The readthrough of its amber stop codon results in translation into ORF1-RT yielding an 84 kDa polymerase. ORF2 encodes the 40 kDa CP. ORF3 and 4 are nested in different reading frames and encode 27 and 14 kDa proteins, respectively. The 27 kDa protein is the MP and the 14 kDa protein is responsible for symptom severity and is a suppressor of RNA silencing. Both the MP and the silencing suppressor are related to those of tombusviruses (see Figures S1 and S2). The CP is important in regulating the synthesis of the 14 kDa protein, the excess production of which is lethal to infected plants. Two sgRNAs of 2.0 kb and 0.8 kb give rise to the 40 kDa CP and the 27 kDa and 14 kDa proteins, respectively. Translation of sgRNA2 involves a 3′-proximal enhancer element as found for TBSV but translation of sgRNA1 is controlled by a stem loop structure at its 5′ terminus. Replication may occur in the cytoplasm, possibly in association with nucleus-derived vesicles and vesiculated bodies. Virus particles accumulate in the cytoplasm.

## Antigenic properties

Virions are efficient immunogens. Polyclonal antisera yield a single precipitin line in immunodiffusion tests and uniformly decorate virus particles. Distant relationships were found with members of the genera *Tombusvirus* and *Carmovirus*.

## Biological properties

### Host range

Pothos (*Scindapsus aureus*) and pigeonpea (*Cajanus cajan*) are the only known natural hosts of PoLV, and cucumber (*Cucumis sativus*) is the natural host of CLSV. The experimental host range is moderately wide. Localized infections are induced in most hosts, except for *Nicotiana benthamiana* and *N. clevelandii*, which are systemically invaded. MWLMV is a soil-borne virus that causes disease on sweet corn and is found mainly in the Great Lakes region of the US and Canada.

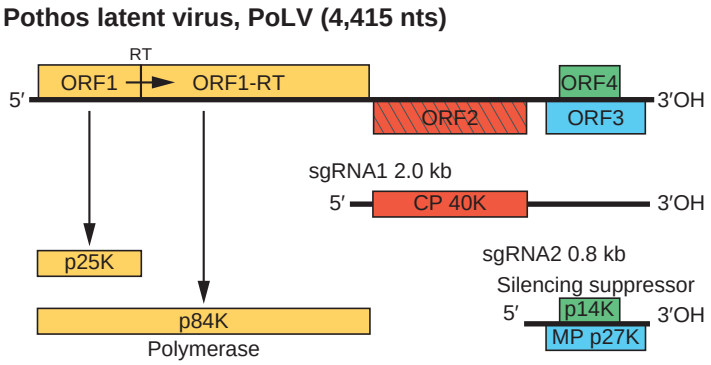


**Figure 8:** Genome organization and replication strategy of PoLV. Boxes represent known and predicted ORFs with the sizes of the respective proteins (or readthrough products) indicated in the bars below. Yellow ORFs indicate polymerase proteins that have a high degree of sequence conservation within the family *Tombusviridae*. Left-hatched box identifies the CP that is highly conserved among other genera within the family *Tombusviridae* that share a protruding domain. The blue box corresponds to the MP which shows sequence similarity to the MP of tombusviruses (see Figure S1). The green box identifies the silencing suppressor that shares sequence similarity with that of tombusviruses (see Figure S1). Lines underneath depict the two sgRNAs that are synthesized in infected cells and which allow for expression of the CP, p14K and the MP. RT = termination codon that is read through.

### Transmission

PoLV and CLSV are readily transmitted by mechanical inoculation. Natural transmission occurs through the soil or the circulating solution in hydroponics. CLSV is transmitted by the soil-inhabiting fungus *Olpidium bornovanus* and, to a low rate (ca. 1%), through seeds. MWLMV may be transmitted by the protist, *Polymyxa graminis*.

### Geographical distribution

Reported from several European countries, Canada, the US, Jordan and India.

### Cytopathic effects

PoLV is very invasive in systemically infected plants and is found in parenchyma and conducting tissues. Virus particles often form intracellular crystalline aggregates. Distinctive cytopathological features are the extensive vesiculation of the nuclear envelope and the single-membrane vesiculated bodies in the cytoplasm. The cytopathology of CLSV infections is characterized by occasional peripheral vesiculation of mitochondria and the nuclear envelope and by the presence of membranous cytoplasmic vesicles with fibrillar content. MWLMV infection is associated with lobate nuclei and particles are found in bleb-like evaginations of the tonoplast. Mitochondria contain electron-dense patches.

## Species demarcation criteria in the genus

The list of species demarcation criteria in the genus is:

- Serological specificity (known species are serologically unrelated)
- Extent of sequence identity between relevant gene products
- Less than 40% aa sequence identity of the CP
- Less than 80% aa sequence identity of the polymerase
- Differential cytopathological features
- Transmission by a vector
- Natural host range
- Artificial host range reactions

## List of species in the genus *Aureusvirus*

| | | |
|---|---|---|
| *Cucumber leaf spot virus* | | |
| Cucumber leaf spot virus - Israel | [DQ227315 = NC_007816] | (CLSV-IS) |
| *Johnsongrass chlorotic stripe mosaic virus* | | |
| Johnsongrass chlorotic stripe mosaic virus - Iran | [AJ557804 = NC_005287] | (JCSMV-IR) |
| *Maize white line mosaic virus* | | |
| Maize white line mosaic virus - US | [EF589670 = NC_009533] | (MWLMV-US) |
| *Pothos latent virus* | | |
| Pothos latent virus - India pigeonpea | [AJ243370 = NC_000939] | (PoLV-IN) |

Species names are in italic script; isolate names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Aureusvirus* but have not been approved as species

| | | |
|---|---|---|
| Sesame necrotic mosaic virus | [DQ367845*] | (SNMV) |

*Sequence does not comprise the complete genome.

# Genus *Avenavirus*

Type species ***Oat chlorotic stunt virus***

## Distinguishing features

This monotypic genus is distinguished from other genera in the family *Tombusviridae* because the CP is significantly larger (48K) than other members with a protruding domain and it shares limited

aa sequence identity in the coat protein (11%–28%) The genome organization is unique and is intermediate between the tombusviruses and carmoviruses. The 8K protein that overlaps the CP ORF does not share sequence similarity to the small proteins encoded by other *Tombusviridae* members.

## Virion properties

### Morphology

Particles are isometric and approximately 35 nm in diameter. Based on sequence similarity with the CPs of members of the family *Tombusviridae* that contain a protruding domain, it is assumed that the particle has T = 3 icosahedral symmetry and is composed of 180 protein subunits with R, S and P structural domains.

### Physicochemical and physical properties

No information.

### Nucleic acid

The genome is composed of a single positive sense ssRNA molecule of 4,114 nt. It is not known if the 5′ terminus is capped. The 3′ end does not possess a poly(A) tail. A single sgRNA of 1,772 nt is expressed in infected tissues and is encapsidated at low concentrations within the virions.

## Genome organization and replication

The genomic RNA contains three ORFs (Figure 9). ORF1 encodes a 23 kDa protein which can be read through to produce the 84 kDa polymerase. These proteins share sequence similarity with the pre-readthrough and readthrough proteins (polymerases) of other members of the *Tombusviridae* (Figure S6). ORF2 encodes the 48 kDa CP which also shares sequence similarity with other members of the *Tombusviridae* (Figure S5). ORF3 is within ORF2, in a different reading frame. An ORF encoding a protein with sequence similarity to the MPs of other *Tombusviridae* members is lacking. ORF3 encodes a unique 8 kDa polypeptide with unknown function but which may serve as a MP. A single 3′ co-terminal sgRNA is formed that presumably serves as template for the CP.

## Antigenic properties

The virus is a moderate immunogen**.** Antibodies do not cross-react with other icosahedral viruses of oats or with representative members of the genera *Carmovirus* and *Machlomovirus*.

## Biological properties

### Host range

The virus has only been identified and studied in oats (*Avena sativa*).

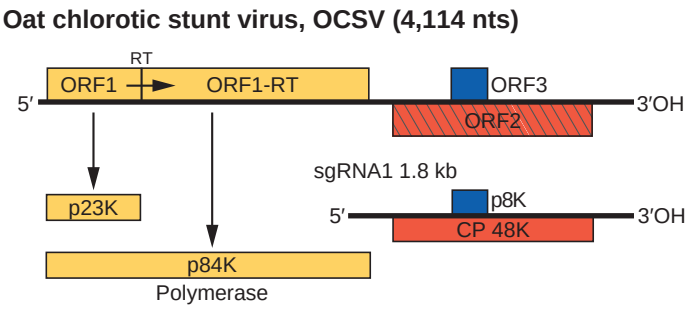


**Figure 9:** Genome organization of the avenavirus oat chlorotic stunt virus (OCSV). Boxes represent known and predicted ORFs with the sizes of the respective proteins (or readthrough products) indicated in the bars below. Yellow ORFs indicate polymerase proteins that have a high degree of sequence conservation within the family *Tombusviridae*. Left-hatched box identifies the CP that is highly conserved among other genera within the family *Tombusviridae* that share a protruding domain. The blue box identifies a putative cell-to-cell MP; this protein does not share sequence similarity with any of the other *Tombusviridae* MPs. RT = termination codon that is read through.

### Transmission
The virus is readily transmitted mechanically from oat plant to oat plant. Infection patterns in winter oat fields are consistent with the virus being soil-borne, and possibly transmitted by zoosporic fungi.

### Geographical distribution
This virus has only been reported in the United Kingdom.

### Cytopathic effects
None reported.

## Species demarcation criteria in the genus
Not applicable.

## List of species in the genus *Avenavirus*

| | | |
|---|---|---|
| *Oat chlorotic stunt virus* | | |
| Oat chlorotic stunt virus - UK | [X83964 = NC_003633] | (OCSV-UK) |

Species names are in italic script; isolate names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Avenavirus* but have not been approved as species
None reported.

# Genus *Carmovirus*

Type species ***Carnation mottle virus***

## Distinguishing features
The genomic RNA is about 4.0 kb in size and contains four ORFs. Translation of the genome yields a polypeptide of about 28 kDa encoded by ORF1 and a polypeptide of about 88 kDa (ORF1RT) originating from readthrough of the amber terminator of ORF1. ORFs 2 and 3 code for two small polypeptides of about 7 kDa and 9 kDa that are involved in cell-to-cell movement. The CP contains a P domain and is encoded by ORF4, which is 3′ co-terminal with genomic RNA. The genome organization is similar to that of necroviruses. The carmovirus CP contains a P-domain which is lacking in necroviruses. The ORFs 2, 3 and 4 polypeptides are translated from two sgRNAs with sizes of about 1.7 and 1.5 kb. Members of different species are not serologically related. Multivesicular bodies are formed only by some viruses. Most species are found in temperate regions. Those infecting legumes are reported from tropical areas. Several species are soil-borne. Melon necrotic spot virus (MNSV), cucumber soil-borne virus (CSBV) and squash necrosis virus (SqNV) are transmitted by *Olpidium bornovanus*.

## Virion properties

### Morphology
Virions are 32–35 nm in diameter and have T = 3 icosahedral symmetry (Figure 10). The isometric nucleocapsids have an obvious regular surface structure giving them a granular appearance in the electron microscope. As with TBSV, TNV and RCNMV, virions are composed of 180 identical protein subunits that fold into the distinctive R, S and P structural domains. The CP subunit ranges in size from about 35–42 kDa. The crystal structures of the carnation mottle virus (CarMV) and cowpea mottle virus (CPMoV) particle have been determined.

### Physicochemical and physical properties
Virions sediment as one component with an $S_{20,w}$ of 118–130S. The buoyant density of virions is 1.33–1.36 g cm$^{-3}$ in CsCl, and the Mr is $8.2 \times 10^6$. CarMV has an isoelectric point of pH 5.2. Particles exhibit an $A_{260}/A_{280}$ ratio of 1.48–1.66 and a thermal inactivation point of 95 °C. Longevity *in vitro*

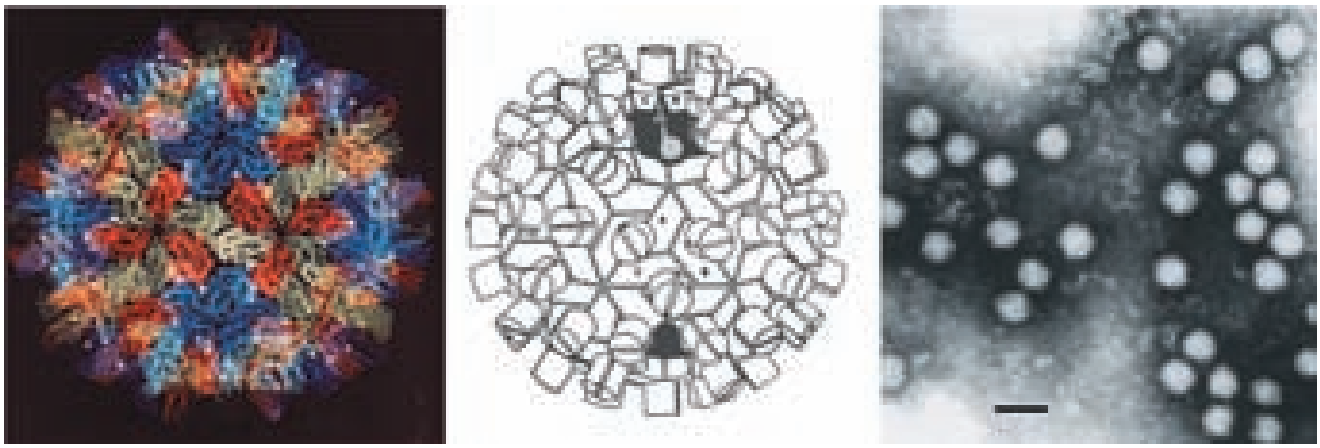

**Figure 10:** (Left) Computer reconstruction of a carnation mottle virus (CarMV) particle based on X-ray crystallography at 3.2 Å resolution (from Morgunova *et al.* (1994). *FEBS Letters*, **338**, 267-271; with permission). (Center) Diagrammatic representation of a carmovirus particle. (Right) Negative contrast electron micrograph of CarMV particles. The bar represents 50 nm.

of 395 days has been reported. Virions have dilution end points often in excess of $10^{-6}$. Virions are insensitive to ether, chloroform and non-ionic detergents, and are stabilized by divalent cations.

### Nucleic acid

Nucleic acid comprises 14% of the virion. Virions contain one molecule of linear positive-sense ssRNA. Total genome length varies between about 3.9 and 4.5 kb. The genome is not capped. The 3′-terminus lacks a poly(A) tract. Generally only the genomic RNA is encapsidated. However, some species also harbor and package DI and/or satellite RNAs and sgRNAs may also be packaged into virions at a very low level. Three virus-specific dsRNA species are found in infected cells. These correspond to the genomic and 2 sgRNAs.

## Genome organization and replication

The genomic RNA contains four ORFs (Figure 11). In CarMV, ORF1 encodes a 28 kDa protein which upon readthrough of the amber terminator codon yields an 88 kDa polymerase (see Figure S6). ORFs 2 and 3 encode 7 and 9 kDa proteins. The 7 kDa protein is an RNA binding protein and the 9 kDa protein is an integral membrane protein. These and the similar proteins encoded by other carmoviruses function together in cell-to-cell movement of viral RNA. The p7 protein of carmoviruses (MP1) is highly basic and shares sequence similarity with the small proteins encoded by necroviruses, the panicovirus panicum mosaic virus (PMV) and the machlomovirus maize chlorotic mottle virus (MCMV) (see Figure S3). The p9 protein (MP2) has hydrophobic regions and shares sequence similarity with a second small protein encoded by necroviruses and PMV (see Figure S4). Both ORFs 2 and 3 are thought to be expressed *in vivo* from the larger 1.7 kb sgRNA1 synthesized in infected cells. The ORF3 initiation codon is in a sub-optimal translational context and the ORF2 initiation codon is in an optimal translational context. Ribosome scanning allows translation of ORF3 from the 1.7 kb sgRNA1. ORF4 encodes the 38 kDa CP which is expressed *in vivo* from the approximate 1.5 kb sgRNA2. For turnip crinkle virus (TCV), hibiscus chlorotic ringspot virus and pelargonium flower break virus the CP has been shown to also be a silencing suppressor. The 5′ and 3′ terminal regions of TCV RNA contain well-characterized sequences regulating viral RNA synthesis. Sequences in the 3′ terminal UTR fold into a Y-shaped structure that control cap-independent translation likely via base pairing interaction with a loop within ORF1. The proximal positioning of the 3′ translation and replication elements likely controls relative levels of transcription and translation of the same viral RNA template.

## Antigenic properties

Virions are efficient immunogens. Polyclonal antisera yield a single precipitin line in immunodiffusion tests. Virus species are not serologically related.

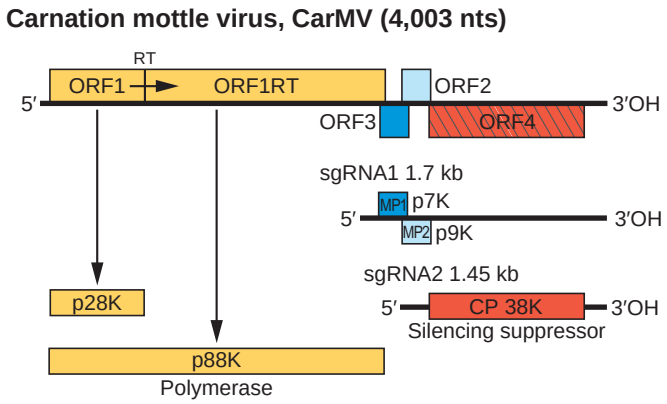


**Figure 11:** Genome organization and replication strategy of the carmovirus CarMV. Boxes represent known and predicted ORFs with the sizes of the respective proteins (or readthrough products) indicated within the horizontal bars below. Yellow ORFs indicate polymerase proteins that have a high degree of sequence conservation within the family *Tombusviridae*. Left-hatched box identifies the CP that is conserved among other genera in the family *Tombusviridae* that have a protruding domain. The dark blue box identifies one (MP1) of the two proteins involved in cell-to-cell movement that exhibits carboxyl-terminal sequence conservation with similar proteins in the machlomoviruses, necroviruses and panicoviruses (see Figure S2). The light-blue box identifies an ORF for a second movement protein (MP2), that shares sequence similarity with the small proteins encoded by viruses in the genera *Necrovirus* and *Panicovirus* (see Figure S3). Lines underneath the gene map depict the two sgRNAs that are synthesized in infected cells that encode MP1, MP2 and CP. RT = amber termination codon that is read through. The CP of CarMV is indicated as a suppressor of silencing by analogy to the suppressor activity of other carmovirus CPs (see text).

## Biological properties

### Host range

Most species have a narrow natural host range. However, most also have a wide experimental host range. Even though the host range of an individual species is restricted in nature, different carmoviruses infect a wide range of both monocotyledonous and dicotyledonous plants. Viruses tend to remain localized, forming necrosis in artificially infected hosts.

### Transmission

The virus is readily transmitted mechanically, under both experimental and natural conditions. CarMV has spread worldwide by the dispersal of infected carnation cuttings. Some species may be transmitted through seed at a low level. Several viruses are soil-borne, and MNSV, CSBV and SqNV are transmitted by *Olpidium bornovanus*. CPMoV, bean mild mosaic virus, and TCV are transmitted by beetles (Coleoptera).

### Geographical distribution

Probably distributed worldwide. Most species are found in temperate regions of the world. Those infecting legumes are reported from tropical areas.

### Cytopathic effects

In systemically infected plants virus particles are found in parenchyma and conducting tissues, sometimes forming intracellular crystalline aggregates. Membranous vesicles are produced from the endoplasmic reticulum. Multivesicular bodies are formed only in cells infected by some viruses.

## Species demarcation criteria in the genus

The list of species demarcation criteria in the genus is:

- Extent of serological relationship as determined by immunodiffusion and/or ELISA
- Extent of sequence identity between relevant gene products
- Less than 52% aa sequence identity of the CP

- Less than 57% aa sequence identity of the polymerase
- Cytopathological features. Presence or absence of multivesicular bodies
- Transmission by a fungal vector
- Natural host range
- Artificial host range reactions

## List of species in the genus *Carmovirus*

| | | |
|---|---|---|
| *Ahlum waterborne virus* | | |
| Ahlum waterborne virus - Germany | | (AWBV - GR) |
| *Angelonia flower break virus* | | |
| Angelonia flower break virus - Florida | [DQ219415 = NC_007733] | (AFBV-FL) |
| *Bean mild mosaic virus* | | |
| Bean mild mosaic virus - Colombia | | (BMMV-Col) |
| *Cardamine chlorotic fleck virus* | | |
| Cardamine chlorotic fleck virus - CL | [L16015 = NC_001600] | (CCFV-CL) |
| *Carnation mottle virus* | | |
| Carnation mottle virus - Shanghai | [AF192772 = NC_001265] | (CarMV-SHA) |
| *Cowpea mottle virus* | | |
| Cowpea mottle virus - Nigeria | [U20976 = NC_003535] | (CPMoV-NG) |
| *Cucumber soil-borne virus* | | |
| Cucumber soil-borne virus - Lebanon | | (CuSBV-LEB) |
| *Galinsoga mosaic virus* | | |
| Galinsoga mosaic virus - Queensland | [Y13463 = NC_001818] | (GaMV-QLD) |
| *Hibiscus chlorotic ringspot virus* | | |
| Hibiscus chlorotic ringspot virus - Singapore | [X86448 = NC_ 003608] | (HCRSV-SING) |
| *Japanese iris necrotic ring virus* | | |
| Japanese iris necrotic ring virus - Japan | [D86123 = NC_002187] | (JINRV-JA) |
| *Melon necrotic spot virus* | | |
| Melon necrotic spot virus - Netherlands | [M29671 = NC_001504] | (MNSV-Neth) |
| *Pea stem necrosis virus* | | |
| Pea stem necrosis virus - Japan | [AB086951 = NC_004995] | (PSNV-JA) |
| *Pelargonium flower break virus* | | |
| Pelargonium flower break virus - MZ10 | [AJ514833 = NC_005286] | (PFBV-MZ10) |
| *Saguaro cactus virus* | | |
| Saguaro cactus virus - Arizona | [U72332 = NC_001780] | (SgCV-Arizona) |
| *Turnip crinkle virus* | | |
| Turnip crinkle virus - UK | [M22445 = NC_003821] | (TCV-UK) |
| *Weddel waterborne virus* | | |
| Weddel waterborne virus - Germany | | (WWBV - GR) |

Species names are in italic script; isolate names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Carmovirus* but have not been approved as species

| | | |
|---|---|---|
| Blackgram mottle virus | | (BMoV) |
| Calibrachoa mottle virus | [GQ244431] | (CMoV) |
| Elderberry latent virus | [AY038066*] | (ElLDV) |
| Glycine mottle virus | | (GMoV) |
| Narcissus tip necrosis virus | | (NTNV) |
| Nootka lupine vein clearing virus | [EF207438 = NC_009017] | (NLVCV) |
| Pelargonium chlorotic ring pattern virus | [AY038069 = NC_005985] | (PCRPV) |
| Plantain virus 6 | | (PlV-6) |
| Soybean yellow mottle mosaic virus | [FJ457015 = NC_016643] | (SYMMV) |
| Squash necrosis virus | | (SqNV) |
| Tephrosia symptomless virus | | (TeSV) |

*Sequence does not comprise the complete genome.

# Genus *Necrovirus*

Type species ***Tobacco necrosis virus A***

## Distinguishing features

Virions sediment as a single component with an S20w of 118S. The genomic RNA is about 3.7 kb in size and contains four ORFs. A fifth potential smaller ORF is predicted within the 3′ leader of the type member tobacco necrosis virus-A (TNV-A). The genome organization and expression strategy are similar to members of the genus *Carmovirus*. Necroviruses have a small CP that forms smooth virions and is phylogenetically related to the sobemovirus CP. This feature distinguishes the necroviruses from carmoviruses, which have a larger CP with a protruding domain. ORFs 2 and 3 encode MPs.

## Virion properties

### Morphology

Virions are approximately 28 nm in diameter and exhibit T = 3 icosahedral symmetry. The particle has a smooth appearance (Figure 12). The crystal structure of TNV-A has been determined at 2.25 angstrom resolution. The tertiary and quaternary structures of TNV-A are most similar to those of sobemoviruses. As with other members of the *Tombusviridae*, the CP subunit has an internal R domain, a shell that has a beta-barrel structure and subunit contacts at the three-fold axis that are stabilized by $Ca^{2+}$. Necrovirus particles do not have a protruding domain.

### Physicochemical and physical properties

The virus sediments as one component with $S_{20,w}$ of 118S, and has a buoyant density of 1.40 g cm$^{-3}$ in CsCl. The particle has a Mr of $7.6 \times 10^6$. The thermal inactivation point is between 85 and 95 °C. The virion isoelectric point is pH 4.5. Virions are insensitive to ether, chloroform and non-ionic detergents.

### Nucleic acid

Virions contain one molecule of infectious linear positive sense ssRNA. The type species RNA is 3,684 nt. The 5′ end of the RNA is not capped, possessing a ppA... terminus. The RNA does not contain a 3′-terminal poly(A) tract. The virion packages exclusively the genomic RNA. Three virus-specific dsRNA species are found in infected cells. The size of the largest virus-specific dsRNA corresponds to that of the genomic RNA. The second largest 1.6 kb and the smallest 1.3 kb dsRNAs correspond to sgRNA1 and 2 respectively.

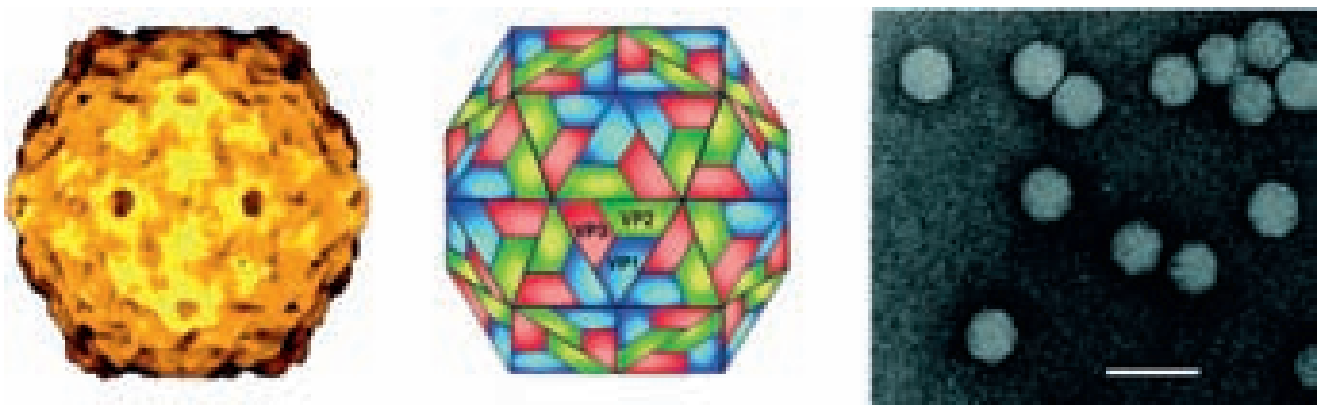


**Figure 12:** (Left) Computer reconstruction of a tobacco necrosis virus A (TNV-A) particle based on X-ray crystallography at 2.25 Å resolution (Oda *et al.* (2000). *J. Mol. Biol.*, **300**, 153-169). (Center) Diagrammatic representation of a necrovirus particle. VP1, VP2 and VP3 correspond to the three conformational states of the CP subunit. (Right) Negative contrast electron micrograph of TNV-A virions. The bar represents 50 nm.

## Genome organization and replication

The genomic RNA contains four ORFs (Figure 13). However, TNV-A also contains a small 3′ proximal ORF5. ORF1 is capable of encoding an approximate 23 kDa polypeptide. Readthrough of the ORF1 amber termination codon allows translation to continue into ORF1-RT for the expression of the 82 kDa polymerase. ORF2 can encode a 7–8 kDa polypeptide implicated in cell-to-cell movement. This protein shares sequence similarity with p7-like proteins (MP1) of carmoviruses, PMV and MCMV (see Figure S3). ORF3 encodes a 6–7 kDa polypeptide that shares sequence similarity to the second MP (MP2) encoded by carmoviruses and by PMV. ORF4 encodes the 29–30 kDa CP. ORF5, present only in TNV-A, encodes a 7 kDa protein. Two sgRNAs of 1.6 and 1.3 kb are synthesized in infected cells. The smaller sgRNA is the translational template for the CP and the larger is the translational template for the ORF2 and possibly ORF3 products. Translation of TNV-D RNAs is cap and polyA-independent and is regulated by sequences in the 3′-terminal region of the genome that are similar to translational enhancer elements utilized by RCNMV but distinct from those of tombusviruses and carmoviruses. The function, if any, of an ORF5 product is not known.

## Antigenic properties

Particles of necroviruses are moderately immunogenic. Species can be distinguished serologically. Antisera yield a single precipitin line in agar gel-diffusion assays.

## Biological properties

### Host range

Necroviruses have wide host ranges that include monocotyledonous and dicotyledonous plants. In nature, infections are typically restricted to roots. Experimental inoculations usually cause necrotic lesions on the inoculated leaves, but rarely result in systemic infection.

### Transmission

Virions are readily transmitted by mechanical inoculation. Member viruses are soil-borne. Some (TNV-A, TNV-D, beet black scorch virus; BBSV) are naturally transmitted by the chytrid fungus *Olpidium brassicae*, while others such as olive latent virus-1 (OLV-1) are transmitted through the soil without the apparent intervention of a vector.

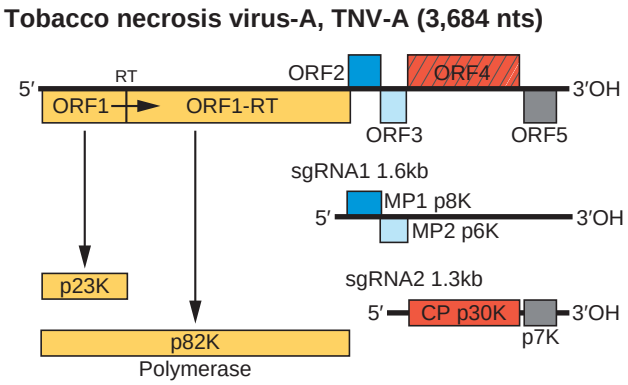


**Figure 13:** Genome organization and replication strategy of the necrovirus TNV-A. Boxes represent known and predicted ORFs with the sizes of the respective proteins (or readthrough products) indicated in the bars below. Shaded ORFs indicate polymerase proteins that have a high degree of sequence conservation within the family *Tombusviridae*. Right-hatched box identifies the CP that is highly conserved among other genera within the family *Tombusviridae* that lack a protruding domain. The dark-blue box identifies one (MP1) of the two proteins involved in cell-to-cell movement that exhibits carboxyl-terminal sequence conservation with similar proteins in the machlomoviruses, carmoviruses, and panicoviruses (see Figure S2). The light-blue box identifies an ORF for a second movement protein (MP2), that shares sequence similarity with the small proteins encoded by viruses in the genera *Carmovirus* and *Panicovirus* (see Figure S3). The ORF5 protein does not share sequence similarity with other viral proteins in the family *Tombusviridae*. Lines underneath the gene map depict the two sgRNAs that are synthesized in infected cells and which allow for the expression of MP1, MP2 and CP. RT = amber termination codon that can be read through.

### Geographical distribution

TNV-A and TNV-D are ubiquitous, OLV-1 was reported from several Mediterranean countries, leek white stripe virus (LWSV) from France, and BBSV from China and the US.

### Cytopathic effects

Virus particles occur, often in prominent crystalline arrays, in infected cells in all tissue types, including vessels. Clumps of electron-dense amorphous material resembling accumulations of excess coat protein are present in the cytoplasm of cells infected by some necroviruses. Membranous vesicles with fibrillar material, lining the tonoplast, or derived from the endoplasmic reticulum and accumulating in the cytoplasm are elicited by LWSV or OLV-1.

## Species demarcation criteria in the genus

- Extent of serological relationship as determined by immunodiffusion and/or ELISA
- Extent of sequence identity between relevant gene products
- Less than 93% aa sequence identity of the polymerase
- Less than 87% aa sequence identity of the CP
- Transmission by a fungal vector
- Natural host range
- Artificial host range reactions

## List of species in the genus *Necrovirus*

| | | |
|---|---|---|
| *Beet black scorch virus* | | |
| Beet black scorch virus - China | [AF452884 = NC_004452] | (BBSV-CN) |
| *Chenopodium necrosis virus* | | |
| Chenopodium necrosis virus - UK | | (ChNV-UK) |
| *Leek white stripe virus* | | |
| Leek white stripe virus - France | [X94560 = NC_001822] | (LWSV-FR) |
| *Olive latent virus 1* | | |
| Olive latent virus 1 - citrus | [X85989 = NC_001721] | (OLV-1) |
| *Olive mild mosaic virus* | | |
| Olive mild mosaic virus - GP | [AY616760 = NC_006939] | (OMMV-GP) |
| *Tobacco necrosis virus A* | | |
| Tobacco necrosis virus A - FM-1B | [M33002 = NC_001777] | (TNV-A-FM1B) |
| *Tobacco necrosis virus D* | | |
| Tobacco necrosis virus D - Hungary | [U62546 = NC_003487] | (TNV-D-HU) |

Species names are in italic script; isolate names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Necrovirus* but have not been approved as species

| | | |
|---|---|---|
| Carnation yellow stripe virus | | (CYSV) |
| Furcraea necrotic streak virus | [FJ768020] | (FNSV) |

# Genus *Panicovirus*

Type species ***Panicum mosaic virus***

## Distinguishing features

Virions sediment at $S_{20,w}$ 109S. The genomic RNA is 4.3 kb and contains four ORFs and a fifth ORF that initiates with a non-canonical GUG codon. The polymerase is larger than those encoded by other members of the family *Tombusviridae*. This is largely due to the presence of a region of about 20 kDa at the N-terminus of the polyprotein that is not conserved in other members of the family except the panicovirus PMV which shares a similar sequence. The virus produces a single 1.5 kb sgRNA that is a template for the expression of ORFs 2–5. The overall size and organization of the

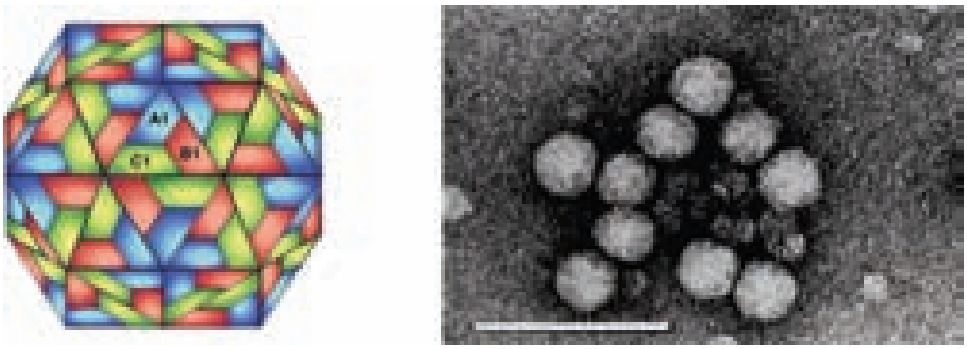

**Figure 14:** (Left) Diagrammatic representation of a particle of panicum mosaic virus (PMV). A, B and C correspond to the three conformational states of the CP subunit. (Right) Negative contrast electron micrograph of PMV particles. The bar represents 100 nm.

genome is similar to that of the genus *Machlomovirus*. However, viruses in the genus *Panicovirus* lack the additional 5′-proximally located ORF. The virus is restricted to monocotyledonous hosts.

## Virion properties

### Morphology

Virions are approximately 30 nm in diameter and exhibit icosahedral symmetry (Figure 14). Detailed capsid structure is not known. Based on CP sequence similarity, it is predicted that the capsid is structurally similar to the T = 3 capsid of southern bean mosaic virus (genus *Sobemovirus*).

### Physicochemical and physical properties

Virions sediment as one component with an $S_{20,w}$ of 109S, and have a buoyant density of 1.365 g cm$^{-3}$ in CsCl, and a virion Mr of $6.1 \times 10^6$. Virus stored in desiccated tissue retained infectivity after 12 years. The thermal inactivation point of the type strain is 85 °C, and 60 °C for the St Augustine grass decline virus strain. Virions are stable at pH 6 and lower. Virions are insensitive to ether, chloroform and non-ionic detergents. Virions are stabilized by divalent cations.

### Nucleic acid

Virions contain a single molecule of infectious linear positive sense ssRNA which is approximately 4.3 kb in length. The 5′ end of the RNA appears not to be capped. The RNA does not contain a 3′-terminal poly(A) tract. An approximately 1.5 kb sgRNA is also produced *in vivo* that appears not to be packaged into virions.

## Genome organization and replication

The genomic RNA contains five ORFs (Figure 15). ORF1 encodes a 48 kDa protein. Readthrough of the ORF1 amber termination codon allows the production of a 112 kDa protein. These proteins share sequence similarity with the polymerase proteins of other members of the family (Figure S6). ORF2 encodes an 8 kDa protein that is produced by the 1.5 kb sgRNA. p8 is required for cell-to-cell movement and shows sequence similarity to the MP1 of carmoviruses, necroviruses and machlomoviruses (Figure S3) The sgRNA also encodes p6.6 from a non-canonical start codon. This protein is similar to a similarly positioned ORF for a 6.8K protein in the possible panicovirus, cocksfoot mild mosaic virus (CMMV). These proteins are similar to the MP2 proteins encoded by other members of the *Tombusviridae* (see Figure S4). Experimental evidence exists that p15 from ORF 4, and the CP from ORF 3 are all important for movement in addition to p8 and p6.6. PMV contains a translational enhancer in the 3′-terminal region of the genome as do several other members of the family *Tombusviridae*.

## Antigenic properties

PMV particles are highly immunogenic**.** Antisera yield a single precipitin line in agar gel-diffusion assays. There are several serological strains of the type strain as well as the serologically distinct St Augustine grass decline virus strain.

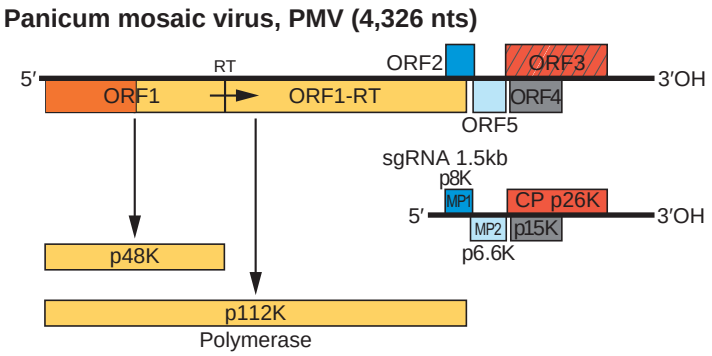


**Figure 15:** Genome organization and replication strategy of the panicovirus PMV. Boxes represent known and predicted ORFs with the sizes of the respective proteins (or readthrough products) indicated in the bars below. Shaded ORFs indicate polymerase proteins that have a high degree of sequence conservation within the family *Tombusviridae*. Right-hatched box identifies the CP that is highly conserved among other genera within the family *Tombusviridae* that lack a protruding domain. The dark-blue box identifies one (MP1) of the two proteins involved in cell-to-cell movement that exhibits carboxyl-terminal sequence conservation with like proteins in the machlomoviruses, necroviruses, and carmoviruses (see Figure S2). The light-blue box identifies an ORF for a second movement protein (MP2), that shares sequence similarity with the small proteins encoded by viruses in the genera *Necrovirus* and *Carmovirus* (see Figure S3). The 1.5 kb sgRNA is illustrated as a line below the genomic RNA and is believed to encode the four indicated proteins. RT = amber termination codon that can be read through. −1 FS = Site of a −1 ribosomal frameshift event.

## Biological properties

### Host range

In nature, PMV is restricted to grass species in the *Paniceae* tribe of the *Poaceae*. It is known to cause diseases of note in switch grass (*Panicum virgatum*), St Augustine grass (*Stenotaphrum secundatum*) and centipede grass (*Eremochloa ophiuroides*). In the laboratory, a number of additional species in the *Graminae* can be symptomless hosts. *Zea mays* is used as a propagation host for the type strain. St Augustine grass must be used as a propagation host for the St Augustine grass decline virus strain. CMMV infects grass species such as *Dactylis glomerata* (cocksfoot), *Phleum pratense* (Timothy), *Holcus* sp., *Bromus* sp. and *Festuca* sp.

### Transmission

The virus is readily transmitted by mechanical inoculation. The virus is typically transmitted by the transport and replanting of infected sod. There is one report that the St Augustine grass decline virus strain was seed-transmitted through *Setaria italica*.

### Geographic distribution

PMV has been reported in the US and Mexico. The type strain is widely distributed in a number of turf grasses throughout the central United States whereas the St Augustine grass decline virus strain is widely distributed throughout the southern US. CMMV is present in Europe and Canada.

### Pathogenicity, association with disease

The virus typically forms a systemic mosaic. More severe symptoms, including chlorotic mottling, stunting and seed yield reduction, occur in forage grasses when PMV is in a mixed infection with panicum mosaic satellite virus. The St Augustine grass decline virus strain can cause a severe disease on St Augustine grass with symptoms being more severe in the hot summer months.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Panicovirus*

*Panicum mosaic virus*
Panicum mosaic virus - Kansas [U55002 = NC_002598] (PMV-KAN)

Species names are in italic script; isolate names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

Pos. ssRNA

**List of other related viruses which may be members of the genus *Panicovirus* but have not been approved as species**

| | | |
|---|---|---|
| Cocksfoot mild mosaic virus | [EU081018 = NC_011108] | (CMMV) |
| Molinia streak virus | | (MoSV) |

# Genus *Machlomovirus*

Type species ***Maize chlorotic mottle virus***

## Distinguishing features

Virions sediment at an $S_{20,w}$ of 109S. The genomic RNA is about 4.4 kb and contains four ORFs. Like that of panicoviruses, the polymerase is larger, containing an N-terminal extension of about 20 kDa that is not present in the polymerases of other members of the family. Unlike other family members, the ORF for the polymerase is not the 5′-most proximal ORF. A 1.47 kb sgRNA is present in infected plants.. Apparently a 0.34 kb sgRNA is present in infected plants that does not contain an ORF for any viral protein. The overall size and organization of the genome is quite similar to that of the genus *Panicovirus*. However, genomes of machlomoviruses encode an additional 5′-proximally located ORF encoding a 32 kDa protein of unknown function. The virus is restricted to monocotyledonous hosts.

## Virion properties

### Morphology

Virions are approximately 30 nm in diameter and exhibit icosahedral symmetry (Figure 16). Detailed structure of virions is not known. Based on CP sequence similarity, it is predicted that the capsid is structurally similar to the T = 3 capsids of sobemoviruses.

### Physicochemical and physical properties

Mr of virions is $6.1 \times 10^6$; $S_{20,w}$ is 109S; buoyant density in CsCl is 1.365 g cm$^{-3}$. Virions are insensitive to ether, chloroform and non-ionic detergents. Virions are stable *in vitro* for up to 33 days and the thermal inactivation point of virions is between 80–85 °C. Virions are stable at pH 6 and lower. Virions are stabilized by divalent cations.

### Nucleic acid

Virions contain a single molecule of infectious linear positive sense ssRNA. The RNA is 4437 nt in length. The 5′ end of the RNA is probably uncapped The RNA does not contain a 3′-terminal poly(A) tract. Either a 1470 or an 1100 nt sgRNA is also packaged into virions at a very low level.

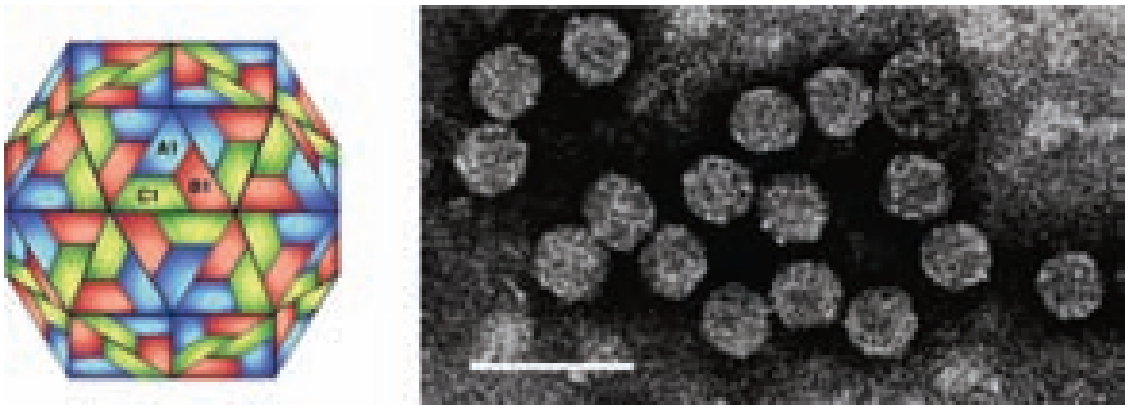

**Figure 16:** (Left) Diagrammatic representation of a particle of MCMV. VP1, VP2 and VP3 correspond to the three conformational states of the CP subunit. (Right) Negative contrast electron micrograph of MCMV virions. The bar represents 100 nm.

## Genome organization and replication

The genomic RNA contains four ORFs (Figure 17). ORF1 is capable of encoding a 32 kDa protein. ORF2 can encode a 48 kDa protein. Readthrough of the ORF2 amber termination codon allows for translation to continue into ORF2-RT, yielding a 112 kDa protein which shares sequence similarities with polymerase proteins of other members of the family (see Figure S6). ORF3 encodes a 7 kDa protein whose carboxyl- terminus is like those of the MP1 proteins encoded by the similarly located small ORFs in the genomes of carmoviruses, necroviruses and panicoviruses (see Figure S3). A protein corresponding to MP2 does not appear to be encoded by the MCMV genome. If the ORF3 opal termination codon is read through, a 33 kDa protein would be produced. ORF4 encodes the 25 kDa CP and appears to be translated from the 1.47 kb sgRNA. A second 0.34 kb sgRNA is made *in vivo*; however, it is not known if it acts as a mRNA. The functions of proteins encoded by ORF1 and the ORF3 readthrough product are not known.

## Antigenic properties

MCMV particles are moderately to highly immunogenic. Serological variants have been identified. Antisera yield a single precipitin line in agar gel-diffusion assays.

## Biological properties

### Host range

In nature, the virus systemically infects varieties of maize (*Zea mays*). In the laboratory, the virus is restricted to members of the family *Gramineae*.

### Transmission

The virus is readily transmitted by mechanical inoculation. The virus is also seed-transmitted. Kansas and Nebraska isolates can be transmitted by six species of chrysomelid beetles in the laboratory. A Hawaiian isolate is transmitted by thrips.

### Geographic distribution

The virus has been reported in Argentina, Mexico, Peru and the United States. Within the United States, the virus is restricted to the Republican River valley of Kansas and Nebraska, and to Kauai, Hawaii.

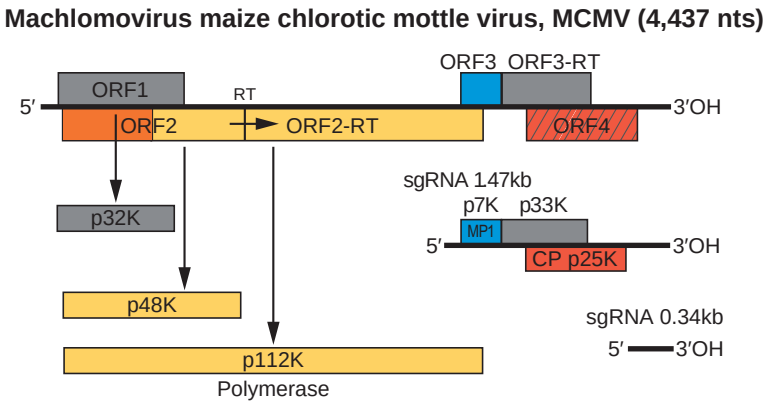


**Figure 17:** Genome organization and replication strategy of the machlomovirus MCMV. Boxes represent known and predicted ORFs with the sizes of the respective proteins (or readthrough products) indicated in the bars below. Shaded ORFs indicate polymerase proteins that have a high degree of sequence conservation within the family *Tombusviridae*. Right-hatched box identifies the CP that is highly conserved among those genera within the family *Tombusviridae* that lack a protruding domain. The blue box identifies the putative cell-to-cell MP that exhibits sequence conservation with similar proteins in the carmoviruses, necroviruses and panicoviruses (see Figure S2). The gray boxes identify ORFs having no significant sequence similarity with other viral proteins. The 1.47 kb sgRNA with its encoded proteins as well as the 0.34 kb sgRNA are illustrated as lines below the genomic RNA. RT = termination codon that is read through.

**Pathogenicity, association with disease**

MCMV causes a mild mosaic on maize in nature. When plants are also infected with one of several Graminae-specific potyviruses, a severe necrotic disease results, termed corn lethal necrosis.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Machlomovirus*

| | | |
|---|---|---|
| *Maize chlorotic mottle virus* | | |
| Maize chlorotic mottle virus - United States | [X14736 = NC_003627] | (MCMV-US) |

Species names are in italic script; isolate names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Machlomovirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Tombusviridae*

| | | |
|---|---|---|
| *Maize necrotic streak virus* | | |
| Maize necrotic streak virus – Arizona | [AF266518 = NC_007729] | (MNeSV-AZ) |
| *Pelargonium line pattern virus* | | |
| Pelargonium line pattern virus - PV-0193 | [AY613852 = NC_007017] | (PLPV-PV0193) |

Species names are in italic script; isolate names are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## Phylogenetic relationships within the family

The polymerase is highly conserved among members of the family *Tombusviridae* with the readthrough (or frameshift) portion showing the greatest level of conservation, i.e. 27–60% identity among type members compared to 2–20% identity in the pre-readthrough (pre-frameshift) region. Phylogenetic analyses (see Figure S6) show that overall the polymerases of members of a genus are more closely related to each other than they are to members of other genera consistent with the notion that such trees are a useful criterion for defining genera. An exception to this is the polymerase of the carmovirus galingsoga mosaic virus which appears to be more closely related to necroviruses. Also, necroviruses are divided into two major groupings: one that is associated with the tombusvirus/aureusvirus lineage and the other with the carmovirus/machlomovirus/panicovirus lineage.

Sequence comparisons of the MPs indicate that there are three main groupings that are phylogenetically unrelated. The dianthoviruses encode a 32 kDa MP which is not related to the MPs of other members of the family. The tombusviruses and aureusviruses share a similar movement protein (Figure 1). The remaining viruses with the exception of the machlomoviruses, encode two MPs that fall into two classes, MP1 and MP2 (see Figure S3,4). Machlomoviruses encode an MP1-like protein but not an MP2-like protein. Phylogenetic analysis of the MP2-like protein may be a useful taxonomic tool since members of a genus show a greater phylogenetic relationship to each other than they do to members of other genera (see Figure S4). MP1 comparisons would not appear to be useful since the phylogenetic groupings are weak and are not in agreement with current taxonomic groupings.

The CPs are conserved among all genera and overall the CP is a reliable indicator of taxonomic identity (see Figure S5). However, exceptions exist. The CPs of tombusviruses and aureusviruses do not separate into distinct groupings. Also, sometimes the CP of a virus does not group with other members of its genus. Examples include the CPs of the carmoviruses melon necrotic spot virus (MNSV) and pea stem necrosis virus (PSNV) that are present in a distinct lineage. This is also the case for the aureusvirus CLSV which does not group with the tombusvirus/aureusvirus lineage. On a structural level, the CPs fall into two main classes: those with a protruding domain

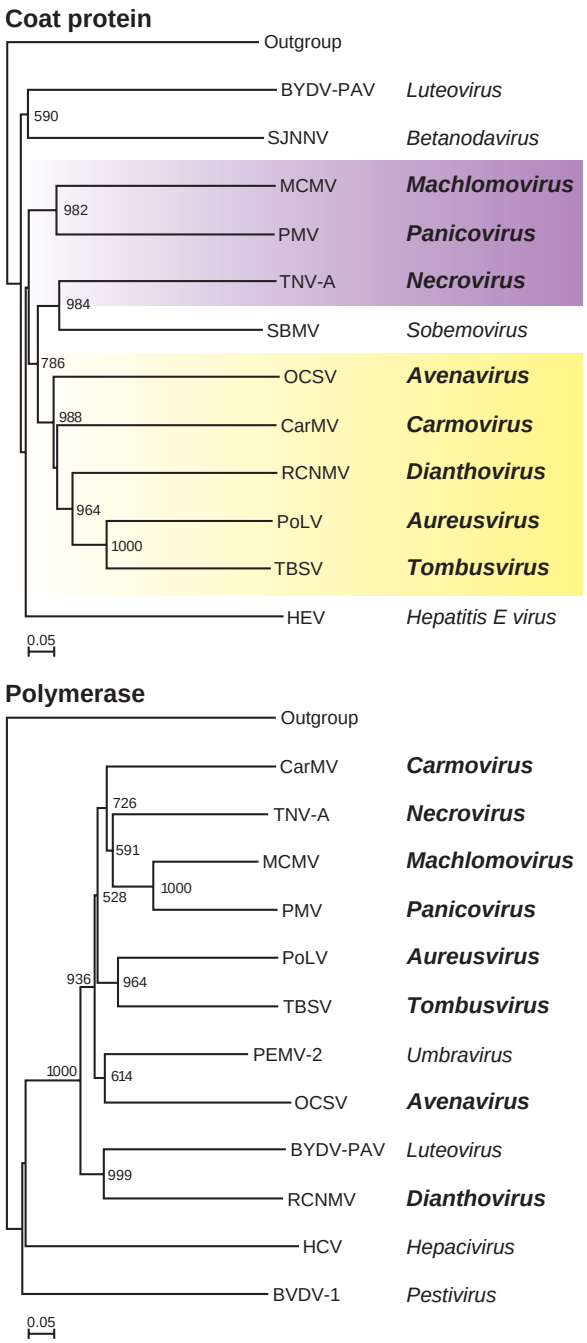


**Figure 18:** Phylogenetic analysis of CP and polymerase proteins of genera of the family *Tombusviridae* and relationships to other taxa. The coloured boxes encompass viruses with the two different morphological types of virions, smooth (purple) versus those with a protruding domain (yellow). Protein sequences were aligned and dendograms were constructed using ClustalX2. Genera of the family *Tombusviridae* are in bold whereas non-members are in regular type. The CP of plum pox virus (family *Potiviridae*) was used as an outgroup. Barley yellow dwarf virus-PAV is a luteovirus (family *Luteoviridae*) and southern bean mosaic virus (SBMV) is a sobemovirus. Striped jack nervous necrosis virus (SJNNV) is the type member of the genus *Betanodavirus* in the family *Nodaviridae*. A more extensive dendogram that includes the CPs of all members of the *Tombusviridae* is shown in Figure S5. Polymerase dendogram. Hepatitis virus C-1 (HCV-1) and bovine viral diarrhea virus (BVDV-1) are members of the genera *Hepacivirus* and *Pestivirus*, respectively in the family *Flaviviridae*. Pea enation mosaic virus-2 (PEMV-2) is an umbravirus in the family *Luteoviridae* and Hepatitis E virus is a hepevirus in the family *Hepeviridae*. The polymerase of tobacco mosaic virus (family *Virgaviridae*) was used as an outgroup for generation of the polymerase dendogram. A dendogram depicting relationships of the polymerases among all members of the *Tombusviridae* is shown in Figure S6.

(dianthoviruses, tombusviruses, aureusviruses, avenaviruses and carmoviruses) and those without a protruding domain (necroviruses, machlomoviruses and panicoviruses).

## Similarity with other taxa

The polymerases of members of the family *Tombusviridae* are related to the polymerases of the genus *Luteovirus*, as exemplified by barley yellow dwarf virus-PAV, as well as the genus *Umbravirus* as exemplified by pea enation mosaic virus-2 (see Figure 18; Figure S5). The luteovirus polymerase is most closely related to that of the dianthoviruses. The polymerases are also distantly related to those of the genera *Hepacivirus* and *Pestivirus* in the family *Flaviviridae*. The CPs of necroviruses are phylogenetically related to the CPs of sobemoviruses (Figure 18; Figure S5). The hepatitis E virus and the betanodavirus CPs are distantly related to those of members of the family *Tombusviridae* (Figure 18). The dianthovirus MP is phylogenetically related to the movement proteins of a diverse number of viruses including members of the families *Bromoviridae*, *Rhabdoviridae*, *Bunyaviridae*, *Virgaviridae* and the genus *Umbravirus.* It has recently been found that the MPs of tombusviruses and aureusviruses share significant sequence similarity with the MPs of members of the genus *Ourmiavirus* (see Figure S7).

## Derivation of names

*Aureus*: from the specific epithet of *Scindapsus aureus* (pothos), natural host of the virus.
*Avena*: from *Avena*, the generic name for oats.
*Carmo*: from *car*nation *mo*ttle.
*Diantho*: from *Dianthus*, the generic name of carnation.
*Machlomo*: from *ma*ize *chlo*rotic *mo*ttle.
*Necro*: from Greek *nekros*, "dead body".
*Panico*: from *panic*um mosaic.
*Tombus*: from *tom*ato *bus*hy stunt.

## Supplementary Figures

Supplementary Figures, Figure S1 to S7, are available online on Science Direct®, www.sciencedirect.com.

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## Contributed by

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# FAMILY *VIRGAVIRIDAE*

## Taxonomic structure of the family

| | |
|---|---|
| Family | ***Virgaviridae*** |
| Genus | ***Furovirus*** |
| Genus | ***Hordeivirus*** |
| Genus | ***Pecluvirus*** |
| Genus | ***Pomovirus*** |
| Genus | ***Tobamovirus*** |
| Genus | ***Tobravirus*** |

## Distinguishing features

The family *Virgaviridae* consists of plant viruses with rod-shaped virions, a single stranded RNA genome with a 3′-terminal tRNA-like structure and an alpha-like replication protein.

## Virion properties

### MORPHOLOGY

The non-enveloped, rod-shaped particles are helically constructed with a pitch of 2.3 to 2.5 nm and an axial canal. They are about 20 nm in diameter, with predominant lengths that depend upon the genus.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

The $S_{20,w}$ values range from 194 to 306 for large particles that encode the replication protein and 125 to 245 for smaller particles. Particles are stable at higher temperatures (60–90 °C) with the exception of pomoviruses, which lose infectivity at room temperature within a few hours.

### NUCLEIC ACID

The genome consists of positive sense ssRNA with 5′-cap ($m^7$GpppG) and a 3′-terminal tRNA-like structure. The number of genome components depends upon the genus.

### PROTEINS

The capsid comprises multiple copies of a single polypeptide of about 17–24 kDa, depending upon the genus. Some viruses encode an additional capsid protein (CP) produced by suppression of the CP gene stop codon to produce a larger readthrough (RT) protein of variable mass called the minor CP or CP-RT.

### LIPIDS

None reported.

### CARBOHYDRATES

None reported.

## Genome organization and replication

The largest (and 5′-most) ORF is an alpha-like replication protein with conserved methyltransferase (Mtr) and helicase (Hel) domains. This is believed to be translated directly from the genomic RNA. In all genera except *Hordeivirus*, the RNA-dependent RNA polymerase (RdRp) is expressed as the C-terminal part of this protein by readthrough of a leaky stop codon. Downstream genes are expressed from subgenomic RNAs, some of which may be bicistronic. In some genera, the viruses have a single cell-to-cell movement protein (MP) of the "30K" superfamily, while in other genera there is a triple gene block (TGB). There are differences in the number of genomic RNAs (1, 2 or 3 depending on the genus). Replication is cytoplasmic.

## Antigenic properties

Virions are moderately to strongly antigenic.

**Table 1:** Distinguishing properties of genera in the family *Virgaviridae*

| Genus | RNAs | RdRP[a] | MP[b] | CP[c] | 3′ structure[d] | Transmission |
|---|---|---|---|---|---|---|
| *Furovirus* | 2 | RT | "30K" | 19K + RT | t-RNA$^{Val}$ | "fungus" |
| *Hordeivirus* | 3 | Separate | TGB | 22K | t-RNA$^{Tyr}$ | seed |
| *Pecluvirus* | 2 | RT | TGB | 23K | t-RNA$^{Val}$ | "fungus" + seed |
| *Pomovirus* | 3 | RT | TGB | 20K + RT | t-RNA$^{Val}$ | "fungus" |
| *Tobamovirus* | 1 | RT | "30K" | 17–18K | t-RNA$^{His}$ | mechanical |
| *Tobravirus* | 2 | RT | "30K" | 22–24K | t-RNA$^{-}$ | nematode |

[a]Relation of RdRp to the replication protein (methyltransferase, helicase); RT, in a readthrough domain at the C-terminus.
[b]MP, Movement protein either of the "30K" superfamily or a triple gene block (TGB).
[c]CP, Coat protein size in kDa (with indication of RT, a readthrough domain at the C-terminus if present).
[d]t-RNA$^{Val/Tyr/His/-}$, t-RNA like structure accepting valine, tyrosine, histidine or not aminoacylated respectively.

## Biological properties

Biologically, the viruses are fairly diverse. They have been reported from a wide range of herbaceous and mono- and dicotyledonous plant species, but the host range of individual members is usually limited. All members can be transmitted experimentally by mechanical inoculation, and for those in the genus *Tobamovirus*, this is the only known means of transmission. In some genera, transmission is by soil-borne vectors, while members of the genus *Hordeivirus* are transmitted through pollen and seed.

## Genus demarcation criteria in the family

Genera are distinguished by the number of genomic RNAs, various features of genome organization, the type of cell-to-cell movement protein and the natural mode of transmission. These are summarized in Table 1.

# Genus *Furovirus*

Type species ***Soil-borne wheat mosaic virus***

## Distinguishing features

Furoviruses have a bipartite genome, a "30K"-like cell-to-cell movement protein and are transmitted by root-infecting vectors in the family *Plasmodiphorales*, once described as fungi but now classified as *Cercozoa*.

## Virion properties

### Morphology

Virions are non-enveloped hollow rods, which have helical symmetry. Virions are about 20 nm in diameter, with predominant lengths of 140–160 nm and 260–300 nm. The length distribution of the soil-borne wheat mosaic virus (SBWMV) short particles is broad, 80–160 nm, due to the presence of deletion mutants in some cultures (Figure 1).

### Physicochemical and physical properties

Virions sediment as two (or three) components; for SBWMV the $S_{20,w}$ values are 220–230S (long particles) and 170–225S (short particles), and 126–177S (deletion mutants). SBWMV loses infectivity in extracts of wheat kept at 60–65 °C for 10 min.

### Nucleic acid

Complete or almost complete nt sequences are available for all five species in the genus. The genome is bipartite, linear, positive sense ssRNA. RNA-1 is about 6–7 kb and RNA-2 about 3.5–3.6 kb. The RNA molecules of SBWMV have a 5′ cap ($m^7$GpppG) and in all of the species where the complete

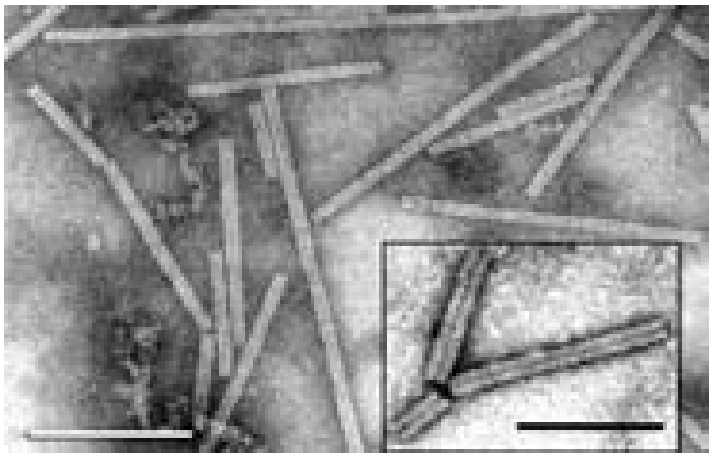

**Figure 1:** Negative contrast electron micrograph of stained (ammonium molybdate pH 7.0) particles of soil-borne wheat mosaic virus (SBWMV). The bar represents 200 nm. Inset: Negative contrast electron micrograph of particles SBWMV stained with 1% uranyl acetate. The bar represents 100 nm.

sequences have been determined there is a 3′-terminal tRNA-like structure with a putative anti-codon for valine. The 3′ terminus of SBWMV RNA was shown experimentally to accept valine.

### Proteins

The capsid comprises multiple copies of a single polypeptide of about 19–20.5 kDa. The CPs of SBWMV, Chinese wheat mosaic virus (CWMV), soil-borne cereal mosaic virus (SBCMV) and oat golden stripe virus (OGSV) comprise 176 aa with 76–82% aa homologies; they share only 46% homology with that of sorghum chlorotic spot virus (SrCSV). The CP gene terminates in a leaky (UGA) stop codon that can be suppressed to produce a read-through protein (ca. 85 kDa), which is thought to be involved in natural transmission by the plasmodiophorid vector. In addition to replicase proteins the furoviruses encode a single MP (ca. 37 kDa) and a cysteine rich protein (ca. 18 kDa) that is probably a suppressor of gene silencing.

## Genome organization and replication

Genome organization and structure is conserved between species but there are substantial differences in the nt sequences. SBWMV RNA-1 encodes a 150 kDa protein, a 209 kDa readthrough product and a 37 kDa protein (Figure 2). The 150 kDa protein contains Mtr and NTP-binding Hel motifs and the readthrough protein, in addition, contains RNA polymerase motifs, indicating that these proteins are involved in replication. The 37 kDa protein belongs to the "30K"-like cell-to-cell movement protein superfamily and is thought to be involved in virus movement as it shares partial sequence similarity to the MPs of dianthoviruses. RNA-2 encodes the CP (19 kDa), the sequence of which terminates in a UGA codon that can be suppressed to give a readthrough product of 84 kDa. A 25 kDa polypeptide is initiated from a CUG codon upstream of the CP AUG. An ORF towards the 3′ end of the RNA-2 encodes a 19 kDa protein that contains seven conserved cysteine residues. Products corresponding to the 37 kDa protein and the cysteine-rich 19 kDa protein were not found in *in vitro* transcription/translation experiments, and these proteins are thought to be expressed from sgRNAs. Spontaneous deletions in the CP readthrough domain occur on successive passage by manual inoculation, and in field isolates in older infected plants.

## Antigenic properties

Virions are immunogenic and the five virus species can be distinguished serologically.

## Biological properties

### Host range

The natural host ranges of furoviruses are narrow and confined to species within the *Graminae*. SBWMV induces green or yellow mosaic and stunting in winter wheat (*Triticum aestivum*) causing up to 80% yield loss in severely infected crops. It also may infect barley and rye. SBCMV infects

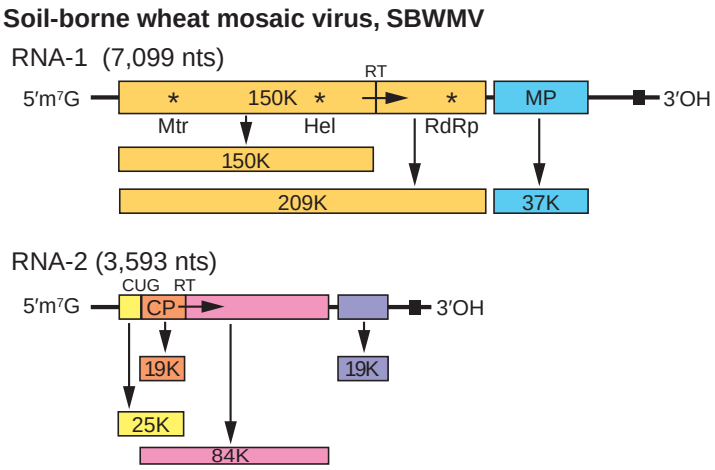


**Figure 2:** Genome organization of soil-borne wheat mosaic virus (SBWMV). The tRNA structure motifs at the 3′-ends of the RNAs are represented by a dark square, the methyl transferase (Met), Helicase (Hel) and RNA-dependent RNA polymerase (RdRp) motifs by asterisks and the readthrough of the polymerase and coat protein ORFs by RT and an arrow.

mainly wheat and triticale in Western and Southern Europe and mainly rye in Central and North-Eastern Europe. Both viruses are (not readily) mechanically transmissible to *Chenopodium quinoa*. OGSV infects oats (*Avena sativa*) but failed to infect wheat when plants were grown in viruliferous soil. Mechanically it can be transmitted to some *Nicotiana* and *Chenopodium* species. SrCSV infects *Sorghum bicolor*. Mechanically it can be transmitted to a range of species including *Chenopodium quinoa*, *C. amaranticolor*, *Nicotiana clevelandii*, *Arachis hypogaea*, *Zea mays* and *T. aestivum*.

### Transmission

The viruses are soil-borne, and *Polymyxa graminis* has been identified as a vector for SBWMV. Virions are thought to be carried within the motile zoospores. Soil containing the resting spores remains infectious for many years.

### Geographical distribution

Furoviruses are found in temperate regions worldwide including the United States of America, Europe, China, Japan.

### Cytopathic effects

Virions are found scattered, or in aggregates and inclusion bodies in the cytoplasm and vacuole. Inclusion bodies can be crystalline inclusions or comprise loose clusters of virus particles in association with masses of microtubules. Amorphous inclusion bodies can be seen in tissue sections by light microscopy.

## Species demarcation criteria in the genus

The species within the genus *Furovirus* are presently mainly differentiated on the basis of the nt sequences of their RNAs and the deduced aa sequences of their putative gene products. RNA-1 sequences of these viruses share 58–74% identity and RNA-2 46–80% (see Figure 3). SBWMV, SBCMV, CWMV and OGSV can be discriminated also by reactivity with selected monoclonal and polyclonal antibodies. OGSV and SrCSV differ in host range to SBWMV, SBCMV and CWMV. Especially the latter three viruses have similar biological properties and genetic reassortants can be formed with RNA-1 and RNA-2 of SBWMV (Nebraska), SBWMV (Japan) and SBCMV. With the other viruses this possibility has not yet been checked.

## List of species in the genus *Furovirus*

*Chinese wheat mosaic virus*

| | | |
|---|---|---|
| Chinese wheat mosaic virus-China:Yantai | [AJ012005 = NC_002359 + AJ012006 = NC_002356] | (CWMV-YT) |

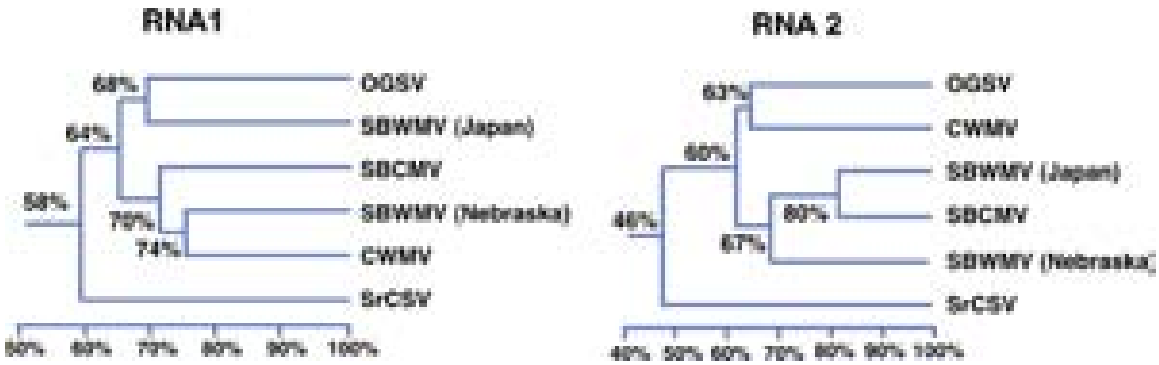


**Figure 3:** Percentage sequence identities of total RNAs of furoviruses.

| | | |
|---|---|---|
| *Oat golden stripe virus* | | |
| Oat golden stripe virus-UK | [AJ132578 = NC_002358 + AJ132579 = NC_002357] | (OGSV-UK) |
| *Soil-borne cereal mosaic virus* (European wheat mosaic virus) (Soil-borne rye mosaic virus) | | |
| Soil-borne cereal mosaic virus-France | [AJ132576 = NC_002351 + AJ132577 = NC_002330] | (SBCMV-FR) |
| *Soil-borne wheat mosaic virus* | | |
| Soil-borne wheat mosaic virus-USA:Nebraska | [L07937 = NC_002041 + L07938 = NC_002042] | (SBWMV-NE) |
| *Sorghum chlorotic spot virus* | | |
| Sorghum chlorotic spot virus-USA | [AB033691 = NC_004014 + AB033692 = NC_004015] | (SrCSV-USA) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Furovirus* but have not been approved as species

| | | |
|---|---|---|
| French barley mosaic virus | [AJ749657-8*] | |
| Japanese soil-borne wheat mosaic virus | [AB033689-70] | (JSBWMV) |

*Sequences do not comprise the complete genome.

## GENUS *HORDEIVIRUS*

Type species ***Barley stripe mosaic virus***

### Distinguishing features

Hordeiviruses have three genomic RNAs and a "triple gene block" set of cell-to-cell movement proteins. They differ from all other genera because the RNA-dependent RNA polymerase (RdRp) is encoded on a separate RNA (rather than by readthrough of a stop codon from an upstream replication protein).

### Virion properties

#### MORPHOLOGY

Virions are non-enveloped, elongated and rigid, about 20 × 110–150 nm in size; they are helically symmetrical with a pitch of 2.5 nm (Figure 4).

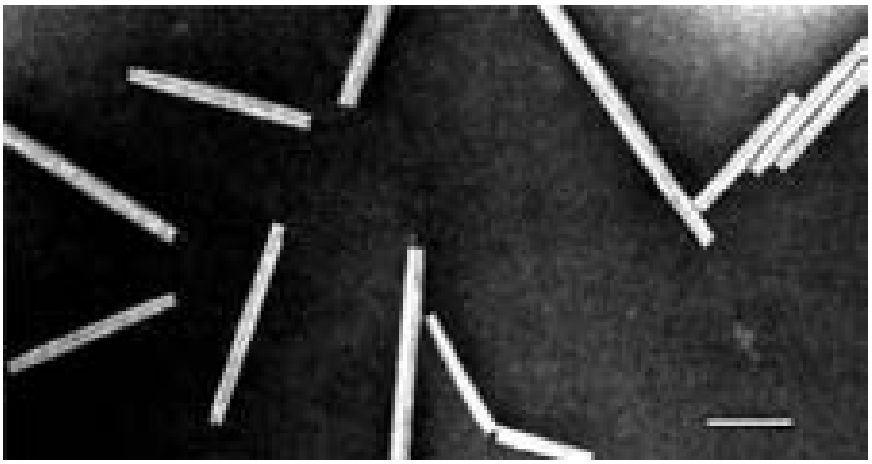

**Figure 4:** Electron micrograph of purified barley stripe mosaic virus (BSMV) particles stained with 2% uranyl acetate. The particles are approximately 20 nm wide and have a length that varies depending on the size of the encapsidated RNA. The field was selected to represent monomers, but often a range of heterodisperse end-to-end aggregates up to 1000 nm in length predominate in purified preparations. The particles in the top left, bottom center, and upper left side of the micrograph are end-to-end aggregates that occur during purification. The bar represents 150 nm.

### Physicochemical and physical properties

Barley stripe mosaic virus (BSMV) virions occur as heterodisperse sedimenting species with an $S_{20,w}$ of about 182–193S; other species have an $S_{20,w}$ of about 165–200S, depending on the virus. The BSMV isoelectric point is pH 4.5. Anionic detergents, added to purification buffers, increase virus yield by preventing particle aggregation. Thermal inactivation of infectivity occurs at 63–70 °C. Virions are stable and their survival in sap ranges from a few days to several weeks.

### Nucleic acid

Virions normally contain three positive sense ssRNAs. The RNAs are designated α, β and γ, and their respective sizes are 3.8, 3.2 and 2.8 kb (BSMV-ND18 strain), 3.7, 3.1 and 2.6 kb (Lychnis ringspot virus, LRSV), and 3.9, 3.6 and 3.2 kb (Poa semilatent virus, PSLV). The sizes of the α and β RNAs are similar between different strains of BSMV, whilst RNAγ varies in size. The ND18 RNAγ is 2.8 kb, that of the type strain is 3.2 kb. This difference is due to a 266 nt duplication near the 5′ end of the RNA that produces a γa protein of 87 kDa in the type strain compared to a 74 kDa γa ND18 protein. The Argentine mild strain contains mixtures of RNAγ species of 3.2, 2.8 and 2.6 kb. The 3.2 kb molecule contains a duplication similar to that of the type strain and the 2.6 kb RNA encodes a defective polymerase. No extensive hybridization can be detected between RNAs of BSMV, LRSV and PSLV. Each RNA has $m^7$GpppGUA at its 5′ end, and a highly conserved 238 nt (BSMV), 148 nt (LRSV), or 330 nt (PSLV) tRNA-like structure at the 3′ end. In the case of BSMV, this structure can be charged with tyrosine. In the BSMV and LRSV genomes, a poly(A) sequence that is variable in length separates the coding region from the tRNA-like structure; however, this sequence is not present in the PSLV genome. A close sequence similarity between the first 70 nt of RNAα and RNAγ of the CV17 strain of BSMV suggests that a natural recombination event has occurred between RNAα and RNAγ of this strain. A similar recombination appears to have occurred between the 5′-untranslated leaders of RNAα and RNAβ of LRSV. These results plus sequence duplications in RNAγ provide persuasive evidence that RNA recombination has had a substantial role in the evolution of hordeiviruses.

### Proteins

The virion capsid is constructed from subunits of a single protein. The CP of all species is 22 kDa in size, yet the proteins differ in electrophoretic mobility.

## Genome organization and replication

All three BSMV genomic RNAs are required for systemic infection of plants, but RNAs α and γ alone can infect protoplasts. The 5′- and 3′-NCR of each BSMV RNA are required for replication.

The hordeivirus genome encodes seven proteins as illustrated for BSMV in Figure 2. RNAα is monocistronic and encodes the αa protein (130 kDa in BSMV, 129 kDa in LRSV and 131 kDa in PSLV) that functions as the helicase subunit of the viral replicase. The αa protein has two conserved sequence domains, an amino-terminal Mtr and a carboxy-terminal NTPase/Hel. The 5′-terminal RNAβ ORFs (βa) of all three viruses encode a 22 kDa CP. The BSMV CP, which is dispensable for systemic movement of the virus, is more closely related to the PSLV CP (55.2% identity) than to the LRSV CP (41.5% identity). An intergenic region separates a "triple gene block" (TGB) that encodes three nonstructural proteins, βb (TGB1), βc (TGB3) and βd (TGB2), in which the βd protein overlaps the other two genes. In BSMV, The βb protein is expressed from a 2,450 nt sgRNA, and the βc and βd proteins are expressed from a second bicistronic 960 nt sgRNA with βc being expressed via a leaky scanning mechanism. In BSMV, a minor 23 kDa translational readthrough extension of the βd protein, designated βd′, is present in plants. However, genetic experiments have not identified a function for βd′, so it appears to be dispensable for infection in all local lesion and systemic hosts tested. The BSMV sgRNAβ1 and sgRNAβ2 promoters reside between positions −29 to −2 and −32 to −17 relative to the transcription initiation sites, respectively, and the nt sequences preceding the transcription initiation sites of these sgRNAs are conserved in LRSV and PSLV. The βb protein (58 kDa in BSMV, 50 kDa in LRSV, and 63 kDa in PSLV) contains a conserved NTPase/Hel domain. The BSMV βb protein binds RNA, NTPs and exhibits ATPase and helicase activity *in vitro*. The βc (17 kDa in BSMV, and 18 kDa in LRSV and PSLV) and βd (14 kDa in BSMV and LRSV, and 18 kDa in PSLV) proteins are hydrophobic and membrane-associated. Each of the BSMV TGB proteins (βb, βc and βd) is required for virus cell-to-cell movement in plants. RNAγ is bicistronic and encodes the γa polymerase subunit of the viral replicase (74 kDa in the BSMV-ND18 strain, 71 kDa in LRSV, and 84 kDa in PSLV), and the cysteine-rich γb protein (17 kDa in BSMV, 16 kDa in LRSV, and 20 kDa in PSLV) (see Figure 5).

The γa protein is variable in size because of the approximately 250 nt RNAγ repeated sequence present in different strains of BSMV. The BSMV γb protein is expressed from a 737 nt sgRNA and is a pathogenicity determinant that is involved in regulating expression of genes encoded by RNAβ. The sgRNAγ promoter is between nt −21 to +2 relative to its transcription start site, and this sequence has similarity to sequences upstream of the γb proteins in PSLV and LRSV. The BSMV γb protein has both RNA binding and zinc binding ability, participates in homologous interactions, and may act as a suppressor of post-transcriptional gene silencing. Translation of a functional αa protein is required for replication of RNAα *in cis*, whilst replication of RNAβ is dependent on the presence of the βa and βb intergenic region, and RNAγ requires approximately 600 nt of the 5′-terminal region. The TGB proteins on RNAβ (b, c, d) are required for cell-to-cell and systemic movement in plants, but the CP and βd′ are dispensable. The γb protein is also dispensable in some genetic backgrounds. A mutation in the 5′-leader sequence of the γa ORF interfered with systemic infection of *Nicotiana benthamiana*, suggesting that modulation of γa expression can affect movement. Full-length dsRNAs corresponding to all viral genomic ssRNAs can be isolated from infected plants. Virus particles accumulate predominantly in the cytoplasm and also in nuclei. Infected barley plants develop pronounced enlargements of the plasmodesmata that contain the βb protein, and prominent peripheral vesicles appear in proplastids and chloroplasts. These vesicles may be the sites of replication because antibodies raised against poly(I):poly(C) have detected dsRNA in proplastids from infected barley root tips.



## Antigenic properties

Hordeivirus particles are efficient immunogens. Member species are distantly related serologically with BSMV being more closely related to PSLV than to LRSV, which is in agreement with sequence analyses.

## Biological properties

### Host range

The native hosts of three viruses (ALBV, BSMV, PSLV) are grasses (family *Gramineae*); strains of LRSV occur naturally in dicotyledonous plants of the families *Caryophyllaceae* and *Labiatae*. Various

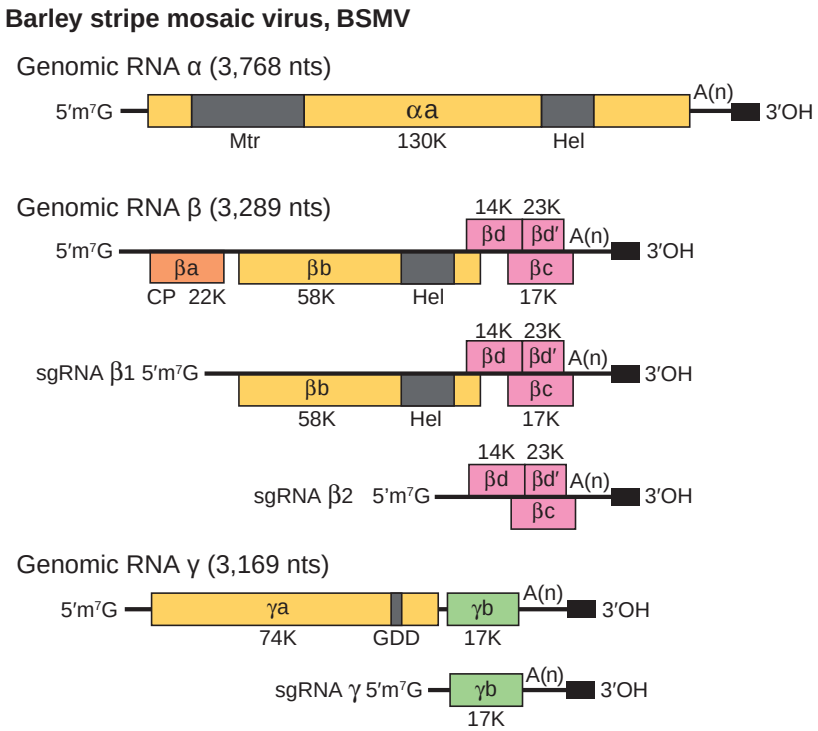


**Figure 5:** Genome organization of barley stripe mosaic virus (BSMV). The color rectangles and smaller solid black rectangles represent the ORFs, and the 3′-terminal tRNA-like structure, respectively. The 3′-proximal ORFs on each RNA terminate with an UAA that initiates the short poly (A) tract that directly precedes the 238 nt tRNA-like terminus. RNAα encodes a single protein, αa, with an amino-terminal methyl transferase domain (Mtr) and a carboxy-terminal helicase domain (Hel). This protein is referred to as the helicase subunit of the replicase (RdRp). RNAβ encodes five proteins: βa, the CP is translated from the genomic RNA; βb, a 58 kDa protein that contains a helicase domain. βb is translated from sgRNAβ1, whose promoter resides between positions −29 to −2 relative to the transcription start site; βc, a 17 kDa protein that is separated from the βb ORF by 173 nt; βd, a 14 kDa protein which overlaps the βb and the βc ORFs; and βd′, a 23 kDa translational extension product of unknown function. The βd, βd′, and βc proteins are translated from sgRNAβ2. The sgRNAβ2 promoter is located between nt –32 to –17 relative to its transcription start site. RNAγ encodes two proteins. The γa protein contains the GDD domain and is the polymerase subunit of the replicase. The cysteine-rich, 17 kDa γb protein has RNA binding ability, and is translated from a sgRNAγ, whose promoter lies between positions −21 to +2 relative to the transcription start site.

strains of these viruses elicit local lesions in *Chenopodium* species and are able to establish systemic infections in a common host, *Nicotiana benthamiana*.

### Transmission

BSMV and LRSV are efficiently seed-transmitted, and are transmitted less efficiently by pollen. Field spread from primary infection foci occurs efficiently by direct leaf contact. There are no known vectors for any members of the genus.

### Geographic distribution

ALBV has been reported only from Great Britain; BSMV occurs world-wide wherever barley is grown; LRSV (mentha strain) has been isolated in Hungary, and the type strain which is highly seed-transmissible in the family *Caryophyllaceae*, was initially discovered in California from seed of *Lychnis divaricata* introduced from Europe. PSLV has been recovered from *Poa palustris* isolated from two locations in Western Canada.

## Species demarcation criteria in the genus

Species differ in host range and are phylogenetically distinct in the genes studied. Precise molecular discrimination criteria have not been established because few sequences have been determined except for the type member.

## List of species in the genus *Hordeivirus*

| | | |
|---|---|---|
| *Anthoxanthum latent blanching virus* | | |
| Anthoxanthum latent blanching virus-UK:Aberystwyth | | (ALBV-ABER) |
| *Barley stripe mosaic virus* | | |
| Barley stripe mosaic virus-Type | [J04342 = NC_003469+ X03854 = NC_003481+ M16576 = NC_003478] | (BSMV-Type) |
| *Lychnis ringspot virus* | | |
| Lychnis ringspot virus-USA:California | [Z46351* + Z46353*] | (LRSV-CAL) |
| *Poa semilatent virus* | | |
| Poa semilatent virus-Canada | [M81486* + M81487*] | (PSLV-CAN) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

## List of other related viruses which may be members of the genus *Hordeivirus* but have not been approved as species

None reported.

# GENUS *PECLUVIRUS*

Type species ***Peanut clump virus***

## Distinguishing features

Pecluviruses have a bipartite genome, a "triple gene block" set of cell-to-cell movement proteins and are transmitted by root-infecting vectors in the family *Plasmodiphorales*, once described as fungi but now classified as *Cercozoa*.

## Virion properties

### MORPHOLOGY

Virions are rod-shaped of about 21 nm in diameter and of two predominant lengths of 190 and 245 nm (Figure 6). The length distribution of the short particles is broad and in some preparations an additional class of 160 nm is recognizable. Virions have helical symmetry with a pitch of 2.6 nm.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virions sediment as two major components with $S_{20,w}$ of 183S and 224S. Buoyant density in CsCl is 1.32 g cm$^{-3}$. Virion isoelectric point is pH 6.45. Thermal inactivation of infectivity occurs at 64 °C. Virions are stable in frozen leaves.

### NUCLEIC ACID

The genome consists of two molecules of linear positive sense ssRNA; RNA-1 of about 5,900 nt and RNA-2 of about 4,500 nt. RNAs are thought to have a 5′-cap structure but this has not been confirmed. The 3′ ends of the RNAs can fold into a tRNA-like structures and are not polyadenylated.

### PROTEINS

The virion CP subunits are 23 kDa.

## Genome organization and replication

RNA-1 contains two ORFs (Figure 7). The 5′ ORF encodes a 131 kDa protein, and suppression of a termination codon results in the synthesis of a readthrough protein of 191 kDa. The 3′ ORF encodes a 15 kDa protein. The proteins of 131 and 191 kDa contain NTP-binding, Hel and RNA polymerase

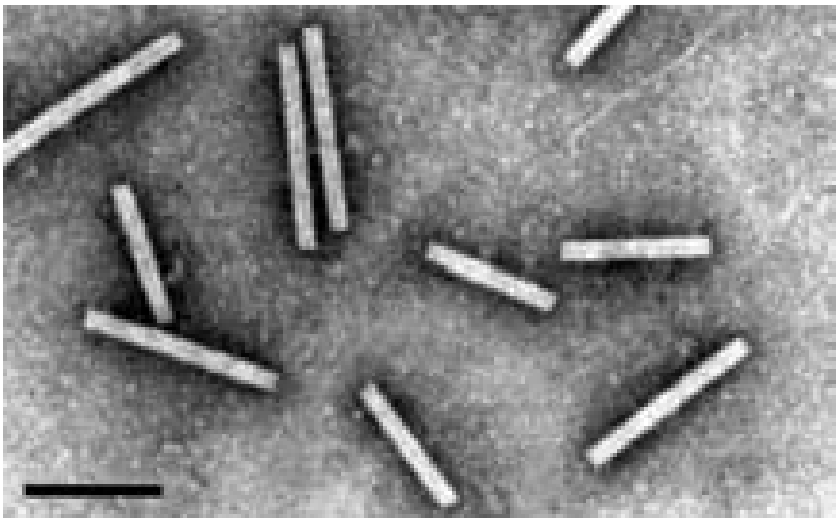

**Figure 6:** Negative contrast electron micrograph of virions of Indian peanut clump virus (L serotype) negatively stained with 2% phosphotungstic acid, pH 6. The bar represents 150 nm. (Courtesy, G.H. Duncan.)

motifs that make a putative replication complex. For peanut clump virus (PCV), these proteins are respectively 88%, 95% and 75% similar to the products of an isolate of one serotype of Indian peanut clump virus (IPCV). The 15 kDa protein is translated from a sgRNA. It is a suppressor of post-transcriptional gene silencing and is targeted to peroxisomes or related punctate bodies during infection. RNA-2 contains five ORFs: the ORF near the 5′ end encodes the CP, the second ORF which, in PCV RNA-1, overlaps the first ORF by 2 nts encodes a 39 kDa protein. This protein is expressed by leaky scanning and is thought to be involved in the transmission of PCV by its fungus vector. Further downstream, separated by a 135 nt intergenic region, is a triple gene block sequence that codes for proteins of 51, 14 and 17 kDa that are thought to be involved in the movement of virus from cell to cell. The proteins are probably expressed via sgRNAs, but these have not been clearly identified. The 3′-NCRs for PCV are 298 nt for RNA-1 and 275 nt for RNA-2; the last 96 nt are identical in both RNAs. The NCRs differ in size among isolates from the different serotypes of IPCV.

The two RNAs are required for systemic invasion of plants but RNA-1 is able to replicate in absence of RNA-2 in protoplasts. The virus is found in the cells of roots, stems and leaves of systemically infected plants.

## Antigenic properties

The virus is highly immunogenic. There is a great serological variability among isolates of PCV. IPCV isolates fall into one of three very distinct serotypes: IPCV-H, IPCV-L, IPCV-T. All are serologically distinct from PCV.

## Biological properties

### Host range

The natural host first reported was *Arachis hypogea* (groundnut, *Leguminosae*). Disease symptoms are stunting – mottle – mosaic – chlorotic ringspot. PCV infects *Sorghum arundinaceum*, usually symptomlessly. IPCV infects a number of cereal crops and graminaceous weeds, some symptomlessly and others to induce stunting. The experimental host range is wide and includes species of *Aizoaceae*, *Amaranthaceae*, *Chenopodiaceae*, *Cucurbitaceae*, *Graminae*, *Leguminosae*, *Scrophulariaceae* and *Solanaceae*. *Nicotiana benthamiana* and *Phaseolus vulgaris* are experimental propagation hosts, *Chenopodium amaranticolor* and *Chenopodium quinoa* are local lesions hosts.

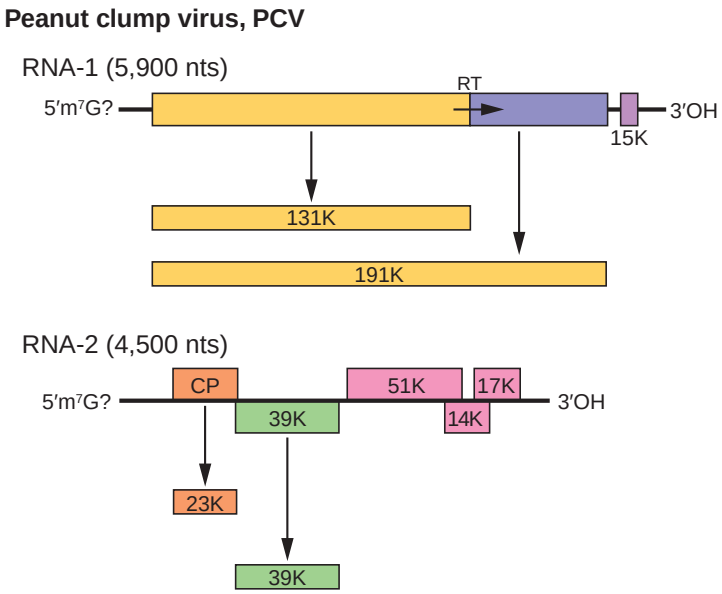


**Figure 7:** Genomic organization of peanut clump virus (PCV) RNAs. ORFs are indicated by rectangles and suppressible termination codon by an arrow (RT = readthrough).

### Transmission

The virus is transmitted naturally by *Polymyxa graminis* or by seed (in groundnuts). It is mechanically transmissible.

### Geographical distribution

PCV spreads in West Africa (Bénin, Burkina Faso, Congo, Côte d'Ivoire, Mali, Niger, Senegal and Pakistan). IPCV is widely distributed in India and Pakistan. A soil type favorable to the vector is a prerequisite for virus to cause disease.

## Species demarcation criteria in the genus

The species are distinguished by different reactions with particular antisera (heterologous reactions are weak or undetectable). Also, PCV occurs only in Africa whereas IPCV occurs in the Indian subcontinent. However, isolates of IPCV can be readily assigned to one of three serotypes as protein preparations made from particles of each serotype barely react with heterologous antisera in immunoblotting tests. Isolates of PCV are also heterogeneous in their reactions with a panel of monoclonal antibodies. Moreover, several of the proteins encoded by genes in RNA of the different serotypes of IPCV differ in sequence from corresponding proteins of other IPCV serotypes by about as much as each differs from the corresponding protein of one isolate of PCV.

## List of species in the genus *Pecluvirus*

| | | |
|---|---|---|
| *Indian peanut clump virus* | | |
| Indian peanut clump virus-Hyderabad serotype | [X99149 = NC_004729 + AF447397 = NC_004730] | (IPCV-H) |
| *Peanut clump virus* | | |
| Peanut clump virus-87/TGTA2 | [X78602 = NC_003672 + L07269 = NC_003668] | (PCV-87/TGTA2) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Pecluvirus* but have not been approved as species

None reported.

# Genus *Pomovirus*

Type species ***Potato mop-top virus***

## Distinguishing features

Pomoviruses have three genomic RNAs, a "triple gene block" set of cell-to-cell movement proteins and are transmitted by root-infecting vectors in the family *Plasmodiophorales*, once described as fungi but now classified as *Cercozoa*.

## Virion properties

### Morphology

The non-enveloped, rod-shaped particles are helically constructed with a pitch of 2.4 to 2.5 nm and an axial canal (Figure 8). They have predominant lengths of about 65–80, 150–160 and 290–310 nm and diameters of 18–20 nm. Crude extracts of plants infected with beet soil-borne virus (BSBV), beet virus Q (BVQ) and potato mop-top virus (PMTV) contain characteristic small bundles of a few side-by-side aggregated particles in addition to singly dispersed particles.

### Physicochemical and physical properties

Virions sediment as three components with $S_{20,w}$ of about 125S, 170S and 230S, respectively. In sap at room temperature, most of the infectivity is lost within a few hours.

### Nucleic acid

Virions contain three molecules of linear positive sense ssRNA of about 6, 3–3.5 and 2.5–3 kb, respectively. The sequence has been determined for all three RNA species of BSBV, BVQ, PMTV and broad bean necrosis virus (BBNV). The RNAs are probably capped at the 5′ end; their 3′ ends can be folded into tRNA-like structures that are preceded by a long hairpin-like structure and an upstream pseudoknot domain. The tRNA-like structures of pomoviruses like those of tymoviruses contain an anticodon for valine and are capable of high-efficiency valylation.

### Proteins

The major capsid protein (CP) species is 20 kDa in size. It is not needed for systemic infection. The CP readthrough protein may be detected in some PMTV particles near one extremity by means of immunogold labeling. Sequences in the CP readthrough protein are necessary for the transmission of PMTV by *Spongospora subterranea*. Yeast two-hybrid experiments revealed that the CP readthrough protein interacts with the triple gene block protein movement protein TGB1. In this

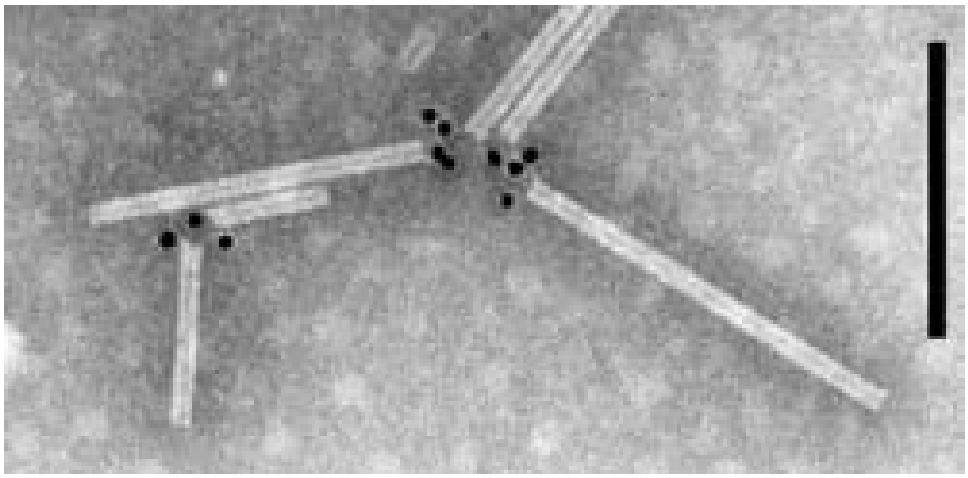

**Figure 8:** Negative contrast electron micrograph of particles of potato mop-top virus. The gold-labeling shows the binding of monoclonal antibody SCR 68 to one extremity of the particles. The bar represents 100 nm. (Courtesy I.M. Roberts.)

system, TGB proteins show self interactions and TGB2 and TGB3 interact with each other. TGB2 and TGB3 are membrane-associated and TGB2 binds ssRNA in a sequence nonspecific manner. It has been suggested that they may form a complex with PMTV RNA that is translocated and localized to the plasmodesmata by TGB3.

## Genome organization and replication

RNA-1 of PMTV has an ORF for a 148 kDa protein and a 206 kDa readthrough protein that are presumably involved in replication. The shorter ORF is terminated by an apparently suppressible UGA stop codon (Figure 9). Proteins of similar sizes are encoded on RNA-1 of BVQ, BBNV and BSBV. The smaller protein contains a Mtr motif in its N-terminal part and a Hel motif in its C-terminal part; the motifs for RdRp are found in the C-terminal part of the readthrough protein (Figure 9). The two proteins contain other highly conserved domains of unknown function in their N- and C-terminal parts, but their central regions (designated as "variable" in Figure 9) are highly specific for each virus. RNA-CP (in PMTV) and the second largest RNA (RNA 2 of the other pomoviruses) contains the CP gene, which terminates with a suppressible UAG stop codon and then continues in frame to form a CP readthrough protein gene that varies considerably in size between different pomoviruses, possibly because it readily undergoes internal deletions, and large deletions have been found in both natural and laboratory isolates of PMTV. PMTV RNA-CP was therefore originally designated as RNA-3. PMTV RNA-2 contains a gene for a cysteine-rich protein and a gene encoding a predicted 6 kDa glycine-rich protein in BBNV neither of which are found on the RNAs of BSBV and BVQ. A triple gene block (TGB) coding for proteins involved in viral movement is found on RNAs-3. TGB1 also contains Hel motifs. The sequences of the C-terminal part of TGB1, of the entire TGB2 and of the N-terminal part of TGB3 are highly conserved among pomoviruses. The replication mechanisms are unknown.

## Antigenic properties

Virions are moderately antigenic. Distant serological relationships have been found between the particles of BSBV and BVQ but not between those of the two beet viruses and PMTV. This is probably due to the fact that PMTV CP has ten extra amino acids on its immunodominant N-terminus that are missing in the two beet viruses. A conserved sequence EDSALNVAHQL is found in the CPs of PMTV, BSBV and BVQ. It contains an epitope for which the monoclonal antibody SCR 70 is specific and which is only detectable by Western blotting after disruption of the particles. Other epitopes are either exposed along the entire particle length, e.g. the immunodominant N-terminus, or are accessible only on one extremity (Figure 8). PMTV and BBNV show distant serological relationships to tobamoviruses.

## Biological properties

### Host range

The natural host range of pomoviruses is very narrow; only dicotyledonous hosts have been described.

### Transmission

Pomoviruses are transmitted by soil. *Spongospora subterranea* and *Polymyxa betae* have been identified as vectors for PMTV and BSBV, respectively. The viruses are also transmissible mechanically.

### Geographical distribution

Countries with temperate climate.

### Cytopathic effects

PMTV-infected cells contain in the cytoplasm virions aggregated in sheaves. Infections by BSBV and BVQ induce voluminous cytoplasmic inclusions which consist of hypertrophied endoplasmic reticulum, convoluted membrane accumulations, numerous small virion bundles and rarely compact virus aggregates.

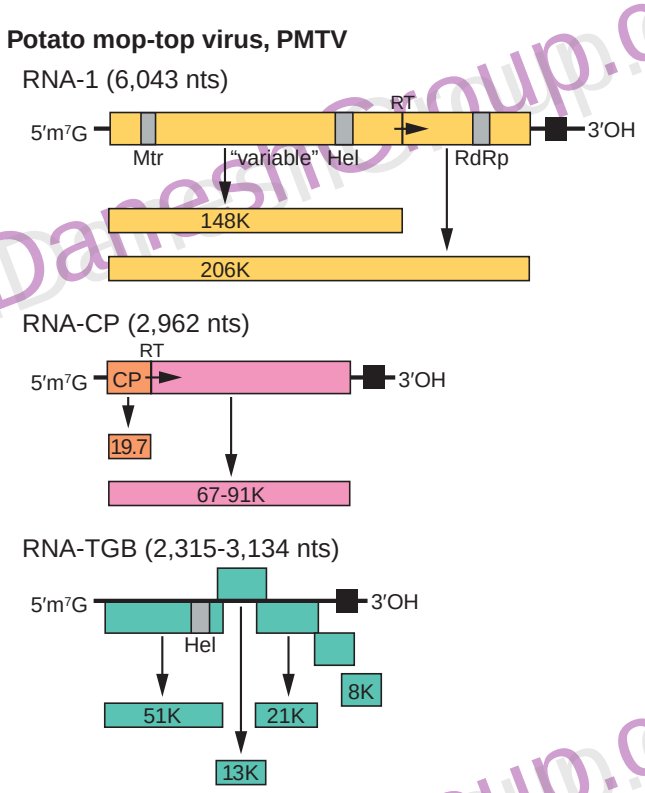


**Figure 9:** Genome organization typical of potato mop-top virus. Arrows indicate respectively the UGA and UAG stop codons that are thought to be suppressible, and solid squares indicate a 3′-terminal tRNA-like structure. Hel, helicase; Mt, methyltransferase; RdRp, RNA dependent RNA polymerase; RT, readthrough.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Differences in host range
- Effects in infected tissue: different inclusion body morphology
- Transmission: different vector species
- Serology: virions are distantly related serologically
- Genome: different numbers of genome components (presence or absence of a gene for a cysteine-rich protein)
- Sequence: less than about 80% identical over whole sequence
- Sequence: less than about 90% identical in CP amino acid sequence

## List of species in the genus *Pomovirus*

| | | |
|---|---|---|
| *Beet soil-borne virus* | | |
| Beet soil-borne virus-Ahlum | [Z97873 = NC_003520 + U64512 = NC_003518 + Z66493 = NC_003519] | (BSBV-Ahlum) |
| *Beet virus Q* | | |
| Beet virus Q-Germany | [AJ223596 = NC_003510 + AJ223597 = NC_003511 + AJ223598 = NC_003512] | (BVQ-DE) |
| *Broad bean necrosis virus* | | |
| Broad bean necrosis virus-Japan | [D86636 = NC_004423 + D86637 = NC_004424 + D86638 = NC_004425] | (BBNV-JP) |
| *Potato mop-top virus* | | |
| Potato mop-top virus-Sweden:Halland | [AJ238607 = NC_003723 + AJ243719 = NC_003724 + AJ277556 = NC_003725] | (PMTV-Sw) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

### List of other related viruses which may be members of the genus *Pomovirus* but have not been approved as species

None reported.

## GENUS *TOBAMOVIRUS*

Type species ***Tobacco mosaic virus***

### Distinguishing features

Tobamoviruses are the only members of the family to have an undivided genome. They have a "30K"-like cell-to-cell movement protein, are not vector-transmissible and when seed transmitted the embryo is not affected. It is easily the largest genus in the family for numbers of species.

### Virion properties

#### MORPHOLOGY

Virions are 18 nm in diameter and have a predominant length of 300–310 nm (Figure 10). Shorter virions produced by the encapsidation of sgRNA are a minor component of the virion population, although at least two species produce an abundant short virion 32–34 nm in length. Virions often form large crystalline arrays visible by light microscopy.

#### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virion Mr is $40 \times 10^6$. Buoyant density in CsCl is 1.325 g cm$^{-3}$. $S_{20,w}$ is 194S. Tobamoviruses have thermal inactivation points of 90 °C and survive in sap for many years.

#### NUCLEIC ACID

The genome is 6.3–6.6 kb in size. An approximately 70 nt long 5′-untranslated sequence contains many AAC repeats and few or no G residues. The 0.2–0.4 kb 3′-UTR contains sequences that can be folded into pseudoknots followed by 3′-terminal sequences that can be folded into a tRNA-like, amino acid-accepting structure. SgRNAs also contain a 5′-terminal cap and 3′-tRNA-like structure. The origin of assembly for encapsidation is usually located within the ORF for the MP, but within the ORF for the CP in the studied isolates of at least two species, *Cucumber green mottle mosaic virus* and *Sunn hemp mosaic virus*.

#### PROTEINS

Virions contain a single structural protein (17–18 kDa). Two nonstructural proteins are expressed directly from the genomic RNA: a 124–132 kDa protein terminated by an amber (UAG) stop codon and a 181–189 kDa protein produced by readthrough of this stop codon, both of which are required for efficient replication. A third nonstructural protein (28–31 kDa) is required for cell-to-cell and long-distance movement and belongs to the "30K"-like cell-to-cell movement proteins. The MP is associated with plasmodesmata and has single-stranded nucleic acid binding activity *in vitro*. The CP is not required for cell-to-cell movement, but has a role in vascular tissue dependent virus accumulation. The replication proteins have also been implicated in virus movement. The MP and CP are expressed from individual 3′-co-terminal sgRNAs. The MP is expressed early during infection, whereas the CP is expressed later, and at higher levels. The MP and CP are not required for replication in single cells. The N-terminal one-third of the 124–132 kDa protein has similarity with methyltransferase/guanylyl transferases whereas the C-terminal one-third of the 124–132 kDa protein has similarity with RNA helicases (including an NTP-binding motif). The readthrough domain of the 181–189 kDa protein has motifs common to RdRps.

### Genome organization and replication

The single genomic RNA encodes at least four proteins. The 124–132 kDa and 181–189 kDa replication proteins are translated directly from the genomic RNA. The 124–132 kDa replication protein contains the Mtr and Hel domains. The 181–189 kDa replication protein that also contains the

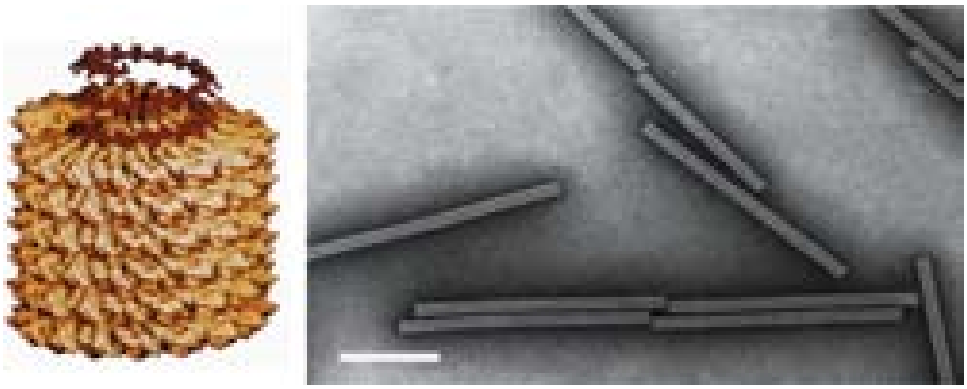

**Figure 10:** (Left) Model of particle of tobacco mosaic virus (TMV). Also shown is the RNA as it is thought to participate in the assembly process. (Right) Negative contrast electron micrograph of TMV particle stained with uranyl acetate. The bar represents 100 nm.

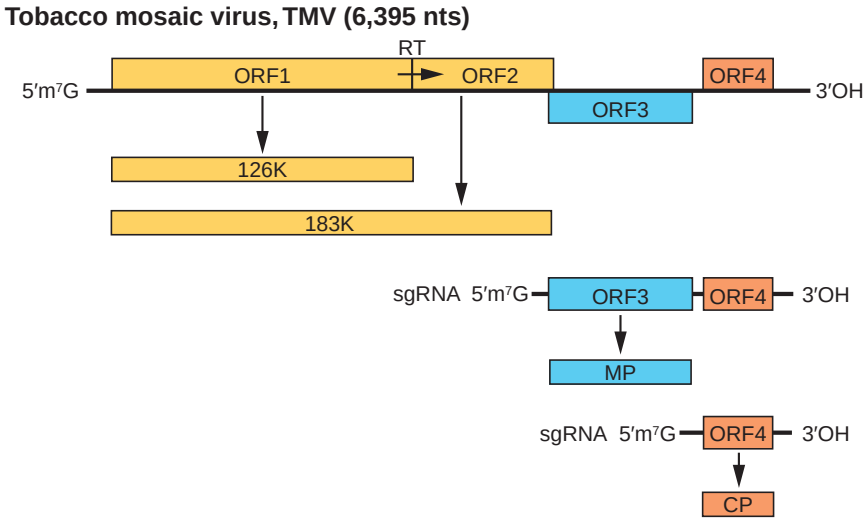


**Figure 11:** Genome organization of tobacco mosaic virus (TMV). Conserved replicase domains are indicated as shaded boxes. Genomic RNA is capped and is template for expression of the 126 and 183 kDa proteins. The 3′ distal movement and CP ORFs are expressed from individual 3′ co-terminal sgRNAs. CP = coat protein; MP = movement protein.

polymerase domain is synthesized by occasional readthrough of the leaky termination codon of the 124–132 kDa ORF. The 181–189 kDa replication protein is the only protein required for replication in single cells, although the 124–132 kDa replication protein is also required for efficient replication. The next ORFs encode the 28–31 kDa MP and 17–18 kDa CP, which are translated from their respective 3′ co-terminal sgRNAs, both of which contain a 5′ cap (Figure 11). In some species, the MP ORF overlaps both of the 181–189 kDa protein and CP ORFs, and in others does not overlap either ORF or overlaps one of the ORFs.

## Antigenic properties

The virions act as strong immunogens. Different species can be identified by intragel cross-absorption immunodiffusion tests using polyclonal antisera or by ELISA using monoclonal antibodies. Antigenic distances between individual species expressed as serological differentiation indices are correlated with the degree of sequence difference in their CPs.

## Biological properties

### Host range
Most species have moderate to wide host ranges under experimental conditions, although in nature host ranges are usually quite narrow. The viruses are found in all parts of host plants.

### Transmission
Transmission occurs without the help of vectors by contact between plants and sometimes by seed, although this occurs in the absence of infection of the embryo.

### Geographical distribution
Members of the genus are found throughout the world.

## Species demarcation criteria in the genus
Many tobamoviruses that were historically designated as strains of tobacco mosaic virus are now defined as separate species based on nucleotide sequence data.

The criteria demarcating species in the genus are:

- Sequence similarity: less than 10% overall nt sequence difference is considered to characterize strains of the same species, although most of the sequenced species have considerably less than 90% sequence identity
- Host range: however many of these viruses have wider and more overlapping host ranges in experimental rather than natural situations
- Antigenic relationships between the CPs

## List of species in the genus *Tobamovirus*

| | | |
|---|---|---|
| *Brugmansia mild mottle virus* | | |
| Brugmansia mild mottle virus-2373 | [AM398436 = NC_010944] | (BrMMV-2373) |
| *Cucumber fruit mottle mosaic virus* | | |
| Cucumber fruit mottle mosaic virus-Israel | [AF321057 = NC_002633] | (CFMMV-IS) |
| *Cucumber green mottle mosaic virus* | | |
| Cucumber green mottle mosaic virus-SH | [D12505 = NC_001801] | (CGMMV-SH) |
| *Frangipani mosaic virus* | | |
| Frangipani mosaic virus-China:Hainan | [AF165884*] | (FrMV-CN) |
| *Hibiscus latent Fort Pierce virus* | | |
| Hibiscus latent Fort Pierce virus-USA:Florida | [FJ196834*] | (HLFPV-FLA) |
| *Hibiscus latent Singapore virus* | | |
| Hibiscus latent Singapore virus-Singapore | [AF395898 = NC_008310] | (HLSV-SIN) |
| *Kyuri green mottle mosaic virus* | | |
| Kyuri green mottle mosaic virus-C1 | [AJ295948 = NC_003610] | (KGMMV-C1) |
| *Obuda pepper virus* | | |
| Obuda pepper virus-TMV-Ob | [D13438 = NC_003852] | (ObPV-TMVOb) |
| *Odontoglossum ringspot virus* | | |
| Odontoglossum ringspot virus-Korea | [X82130 = NC_001728] | (ORSV-KOR) |
| *Paprika mild mottle virus* | | |
| Paprika mild mottle virus-Japan | [AB089381 = NC_004106] | (PaMMV-JP) |
| *Pepper mild mottle virus* | | |
| Pepper mild mottle virus-S | [M81413 = NC_003630] | (PMMoV-S) |
| *Rehmannia mosaic virus* | | |
| Rehmannia mosaic virus-Henan | [EF375551 = NC_009041] | (RheMV-HN) |
| *Ribgrass mosaic virus* | | |
| Ribgrass mosaic virus-Stubbs | [U69271*] | (RMV-Stubbs) |
| *Sammons's Opuntia virus* | | |
| Sammons's Opuntia virus-USA:Arizona | | (SOV-AZ) |
| *Streptocarpus flower break virus* | | |
| Streptocarpus flower break virus-Germany | [AM040955 = NC_008365] | (SFBV-DE) |
| *Sunn-hemp mosaic virus* | | |
| Sunn-hemp mosaic virus-India | [U47034*] + [J02413*] | (SHMV-IN) |

| | | |
|---|---|---|
| *Tobacco latent virus* | | |
| Tobacco latent virus-Nigeria | [AY137775*] | (TLV-NIG) |
| *Tobacco mild green mosaic virus* | | |
| Tobacco mild green mosaic virus-Canary Islands | [M34077 = NC_001556] | (TMGMV-CY) |
| *Tobacco mosaic virus* | | |
| Tobacco mosaic virus-variant 1 | [V01408 = NC_001367] | (TMV-1) |
| *Tomato mosaic virus* | | |
| Tomato mosaic virus-Australia: Queensland | [AF332868 = NC_002692] | (ToMV-QLD) |
| *Turnip vein-clearing virus* | | |
| Turnip vein-clearing virus-OSU | [U03387 = NC_001873] | (TVCV-OSU) |
| *Ullucus mild mottle virus* | | |
| Ullucus mild mottle virus-Peru | | (UMMV-Peru) |
| *Wasabi mottle virus* | | |
| Wasabi mottle virus-Shizuoka | [AB017503 = NC_003355] | (WMoV-Shizuoka) |
| *Youcai mosaic virus* | | |
| Youcai mosaic virus-OSRMV | [U30944 = NC_004422] | (YMoV-OSRMV) |
| *Zucchini green mottle mosaic virus* | | |
| Zucchini green mottle mosaic virus-Type strain K | [AJ295949 = NC_003878] | (ZGMMV-K) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequences do not comprise the complete genome.

### List of other related viruses which may be members of the genus *Tobamovirus* but have not been approved as species

| | | |
|---|---|---|
| Abutilon yellow mosaic virus | [EU559678*] | (AbYMV) |
| Bell pepper mottle virus | [DQ355023 = NC_009642] | (BPMoV) |
| Cactus mild mottle virus | [EU043335 = NC_011803] | (CMMoV) |
| Cucumber mottle virus | [AB261167 = NC_008614] | (CuMoV) |
| Maracuja mosaic virus | [DQ356949 = NC_008716] | (MarMV) |
| Tropical soda apple mosaic virus | [AY956381-2*] | (TSAMV) |

*Sequences do not comprise the complete genome.

## Genus *Tobravirus*

Type species ***Tobacco rattle virus***

### Distinguishing features

Tobraviruses have a bipartite genome, a "30K"-like cell-to-cell movement protein and are transmitted by trichodorid nematodes.

### Virion properties

#### Morphology

Virions are tubular particles with no envelope (Figure 12). They are of two predominant lengths, (L) 180–215 nm and (S) ranging from 46 to 115 nm, depending on the isolate. Many strains produce in addition small amounts of shorter particles. The particle diameter is 21.3–23.1 nm by electron microscopy or 20.5–22.5 nm by X-ray diffraction, and there is a central canal 4–5 nm in diameter. Virions have helical symmetry with a pitch of 2.5 nm; the number of subunits per turn has been variously estimated as 25 or 32.

#### Physicochemical and physical properties

Virion Mr is 48–50 $\times 10^6$ (L particles) and 11–29 $\times 10^6$ (S particles). Buoyant density in CsCl is 1.306–1.324 g cm$^{-3}$. $S_{20,W}$ is 286–306S (L particles) and 155–245S (S particles). Virions are stable over a wide range of pH and ionic conditions and are resistant to many organic solvents, but are sensitive to treatment with EDTA. In *N. clevelandii* sap, the thermal inactivation point (10 min) of M-type isolates is 80–85 °C.

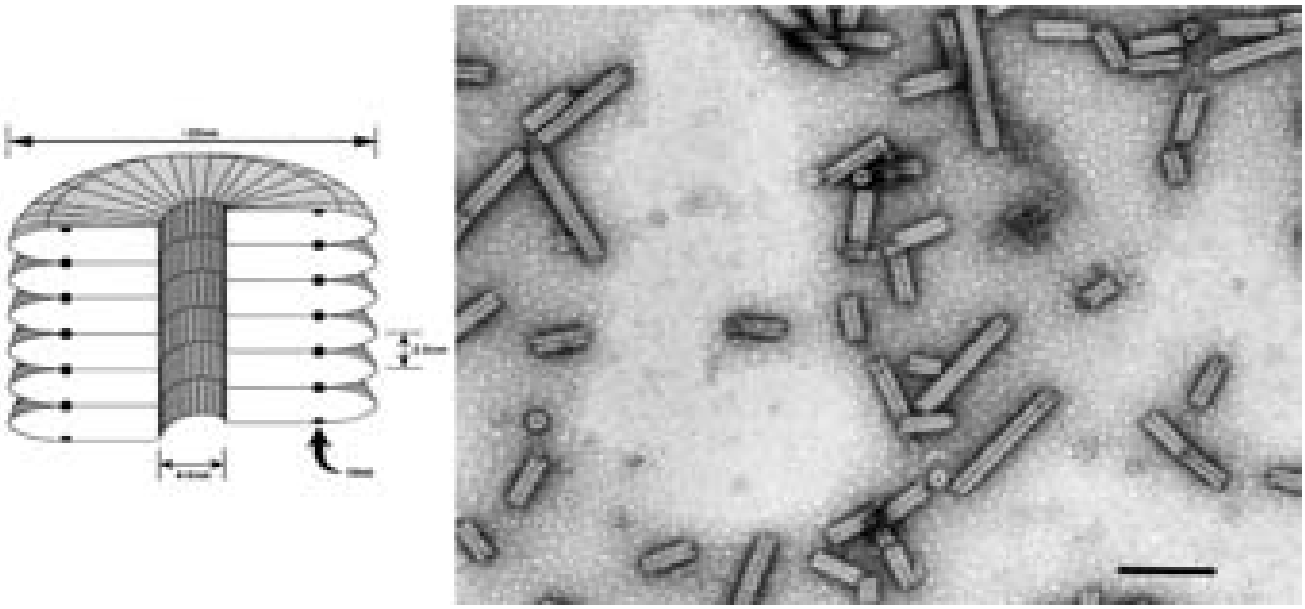

**Figure 12:** (Left) Diagram of a virion of tobacco rattle virus (TRV), in section. (Right) Negative contrast electron micrograph of particles of TRV. The bar represents 100 nm.

### Nucleic acid

The genome consists of two molecules of linear positive sense ssRNA; RNA-1 is about 6.8 kb and RNA-2 ranges from 1.8 kb to about 4.5 kb in size (varying in different isolates). The 5′ terminus is capped with the structure $m^7G^{5'}ppp^{5'}Ap$. There is no genome-linked protein or poly(A) tract. The 3′ terminus can adopt a tRNA-like structure that can be adenylated but not aminoacylated.

### Proteins

Virions contain a single structural protein of 22–24 kDa. RNA-1 codes for four nonstructural proteins: a 134–141 kDa protein terminated by an opal stop codon and a 194–201 kDa protein produced by readthrough of this stop codon, both of which are probably involved in RNA replication; a 29–30 kDa protein (P1a) "30K"-like cell-to-cell movement protein, which is involved in intercellular transport of the virus; and a 12–16 kDa protein (P1b), which is a suppressor of RNA silencing and determinant of seed transmission (of pea early-browning virus (PEBV) in pea). In addition to the virion structural protein, RNA-2 codes for two nonstructural proteins, P2b and P2c. The size of P2b ranges from 27 to 40 kDa in different isolates, and that of P2c from 18 to 33 kDa. P2b is absolutely required for transmission by nematodes, whereas mutation of the P2c gene affects nematode transmission in some strains but not in others. The genes for P2b and P2c are missing from some laboratory strains that have been maintained by mechanical transmission. RNA-2 of some tobravirus isolates contains an additional small ORF between the CP and P2b genes, which codes for a potential 9 kDa protein. RNA2 of tobacco rattle virus (TRV) isolate SYM has an unusual gene organization, with additional, novel genes being located 5′ of the CP gene.

## Genome organization and replication

RNA-1 is capable of independent replication and systemic spread in plants. The 134–141 kDa and 194–201 kDa replication proteins are translated directly from it, whereas P1a and P1b are translated from sgRNA species 1a and 1b, respectively. RNA-2 does not itself have messenger activity; the CP is translated from sgRNA-2a. The means by which the other RNA-2 encoded proteins are expressed is not known but probably also involves sgRNAs (Figure 13). There is sequence homology between RNA-1 and RNA-2 at both ends, but the extent of the homology varies between strains. In some strains, the homologous region at the 3′ end is large enough to include some or all of the P1a and P1b genes of RNA-1, but it is not known if these genes are expressed from RNA-2. Accumulation of virus particles is sensitive to cycloheximide but not to chloramphenicol,

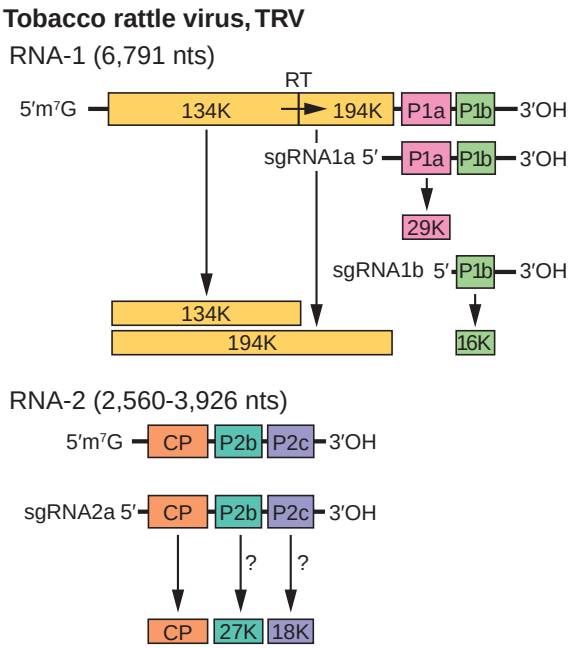


**Figure 13:** Genome organization and strategy of expression of tobacco rattle virus (TRV). The means by which P2b and P2c are expressed is unknown.

suggesting that cytoplasmic ribosomes are involved in viral protein synthesis. Virions accumulate in the cytoplasm. L particles of pepper ringspot virus (PepRSV) become radially arranged around mitochondria, which are often distorted, and in cells infected with some TRV isolates, "X-bodies" largely composed of abnormal mitochondria and containing small aggregates of virus particles may be produced.

## Antigenic properties

Tobravirus particles are moderately immunogenic. There is little or no serological relationship between members of the genus, and considerable antigenic heterogeneity among different isolates of the same virus.

## Biological properties

The host ranges are wide, including members of more than 50 monocotyledonous and dicotyledonous plant families. The natural vectors are nematodes in the genera *Trichodorus* and *Paratrichodorus* (*Trichodoridae*), different species being specific for particular virus strains. Adults and juveniles can transmit, but virus is probably not retained through the molt. Ingested virus particles become attached to the esophageal wall of the nematodes, and are thought to be released by salivary gland secretions and introduced into susceptible root cells during exploratory feeding probes. Virus can be retained for many months by non-feeding nematodes. There is no evidence for multiplication of virus in the vector and it is probably not transmitted through nematode eggs. The viruses are transmitted through seed of many host species. TRV occurs in Europe (including Russia), Japan, New Zealand and North America; PEBV occurs in Europe and North Africa, and PepRSV occurs in South America. TRV causes diseases in a wide variety of crop plants as well as weeds and other wild plants, including spraing (corky ringspot) and stem mottle in potato, rattle in tobacco, streaky mottle in narcissus and tulip, ringspot in aster, notched leaf in gladiolus, malaria in hyacinth and yellow blotch in sugar beet. PEBV is the cause of diseases in several legumes, including broad bean yellow band, distorting mosaic of bean and pea early-browning. PepRSV causes diseases in artichoke, pepper and tomato.

Most tissues of systemically invaded plants can become infected, but in many species virus remains localized at the initial infection site in the roots. In some virus–host combinations, notably TRV in

some potato cultivars, limited systemic invasion occurs, and virus may not be passed on to all the vegetative progeny of infected mother plants.

Normal particle-producing isolates (called M-type) are readily transmitted by inoculation with sap and by nematodes. Other isolates (called nm-type) have only RNA-1, do not produce particles, are transmitted with difficulty by inoculation with sap, and are probably not transmitted by nematodes. nm-type isolates are obtained from M-type isolates by using inocula containing only L particles, and are also found in naturally infected potato plants. They often cause more necrosis in plants than do their parent M-type cultures.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Nucleotide sequences of RNA-1 show <75% identity
- Interspecific pseudo-recombinant isolates cannot be made
- Host ranges differ in specific hosts (e.g. legumes)
- RNA-2 sequences and serological relationships are of limited value

## List of species in the genus *Tobravirus*

| | | |
|---|---|---|
| *Pea early-browning virus* | | |
| Pea early-browning virus-SP5 | [X14006 = NC_002036 + X51828 = NC_001368] | (PEBV-SP5) |
| *Pepper ringspot virus* | | |
| Pepper ringspot virus-CAM | [L23972 = NC_003669 + X03241 = NC_003670] | (PepRSV-CAM) |
| *Tobacco rattle virus* | | |
| Tobacco rattle virus-PpK20 | [AF166084 = NC_003805 + Z36974 = NC_003811] | (TRV-PpK20) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Tobravirus* but have not been approved as species

None reported.

## List of unassigned species in the family *Virgaviridae*

None.

## List of other related viruses which may be members of the family *Virgaviridae* but have not been approved as species

| | | |
|---|---|---|
| Nicotiana velutina mosaic virus | [D00906*] | (NVMV) |

*Sequence does not comprise the complete genome.

## Phylogenetic relationships within the family

Best phylogenetic trees are obtained using the replication protein (or conserved domains within it) as these occupy the majority of the genome. However, the different genera usually separate reliably, regardless of the gene used (Figure 14). In both the replication protein and the coat protein, the monopartite genus *Tobamovirus* separates substantially from the remaining genera. *Furovirus* and *Pomovirus* occur together on the same branch in all trees as do *Pecluvirus* and *Hordeivirus*.

Within genera, only the genus *Tobamovirus* is large enough for particular subgroupings to be distinguished. Here, there are clearly groupings of closely-related viruses infecting similar hosts. The most obvious are those infecting cucurbits (CFMMV, CGMMV, KGMMV, ZGMMV), cruciferous plants (RMV, TVCV, WMoV, YoMV) and solanaceous plants (BrMMV, ObPV, PaMMV, PMMoV, TMGMV, TMV, ToMV).

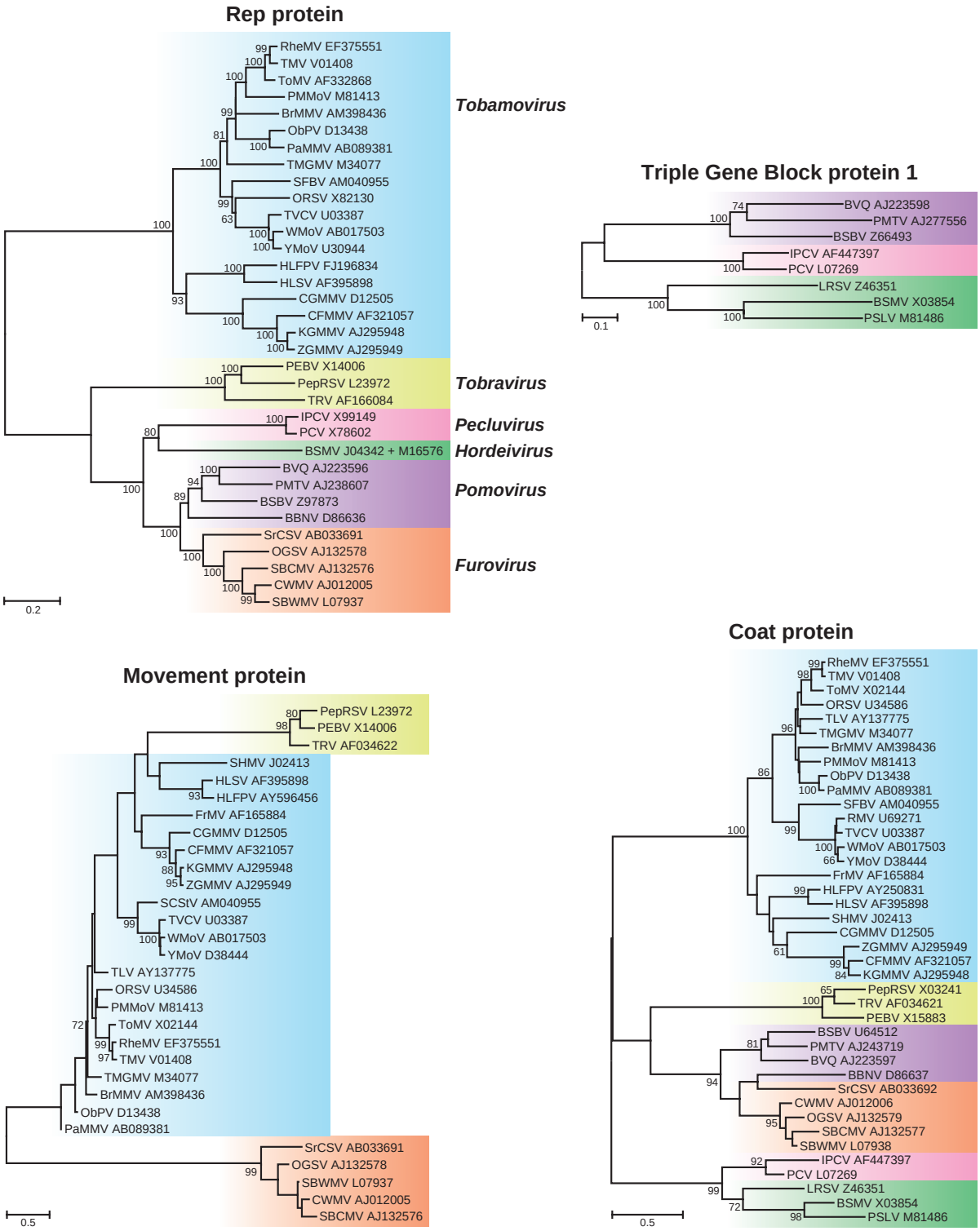


**Figure 14:** Phylogenetic (distance) trees based on the amino acid sequences of the entire replication protein, the Triple Gene Block protein 1 (TGB1), the movement protein and the coat protein. A single representative isolate of each sequenced species in the family was included. Numbers on branches indicate percentage of bootstrap support out of 1,000 bootstrap replications (when >60%). The scale indicates JTT amino acid distances. Trees produced in MEGA4. The BSMV replication protein was combined from two different genome components.

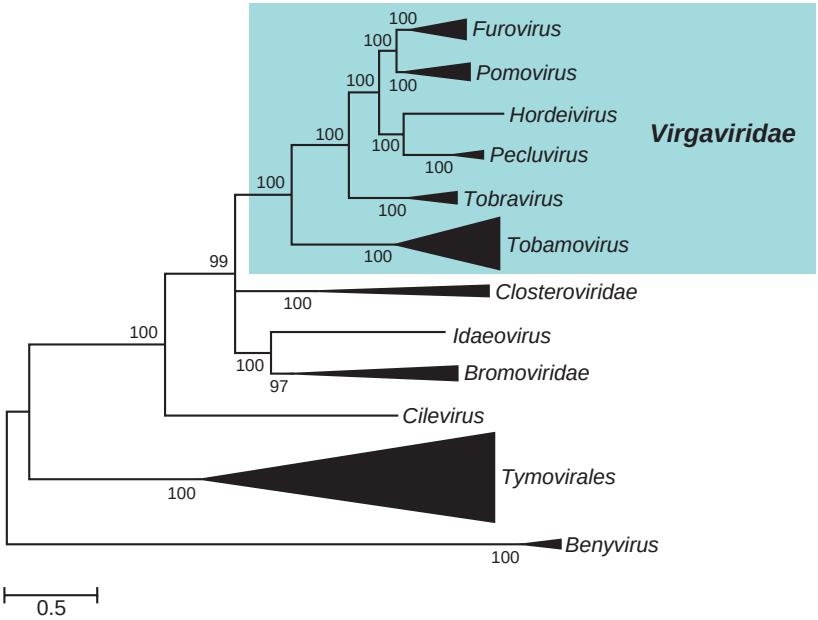


**Figure 15:** Bayesian phylogenetic tree of the nucleotide sequences of the fused Met–Hel–RdRp domains of the members of the six genera included in the family *Virgaviridae* together with some other related viruses. A total of 500 amino acid positions corresponding to 1,500 nt positions were used for the alignment. The tree was generated from a back-translated amino-acid alignment using MrBayes v3.1.2, employing the general time reversible model with gamma-shaped rate variation with a proportion of invariable sites; 1,000,000 generations of MCMC analysis were performed, to the point at which the average standard generation of split frequency between two parallel runs had reached 0.009565. Posterior probability values are indicated on the corresponding branches. Nearly identical trees were obtained by neighbour-joining and maximum composite likelihood methods. Genera and families (which are all monophyletic) have been collapsed into a triangle, the length of which corresponds to the variation found within the clade.

## Similarity with other taxa

The replication proteins are related to those of other viruses with alpha-like replicases, and more particularly to the families *Closteroviridae* and *Bromoviridae* and the genus *Idaeovirus*. The only genus with rod-shaped virions excluded from the family is *Benyvirus*, because this is much more distantly related in phylogenetic analyses and because (unlike the members of the *Virgaviridae*) its members have a polyadenylated genome and a polymerase that is processed by autocatalytic protease activity (Figure 15).

## Derivation of names

*Furo*: from *fu*ngus-borne, *ro*d-shaped virus.
*Hordei*: from *hordeus*, Latin name of the primary host of the type species virus of the genus *Hordeivirus*.
*Peclu*: from *pe*anut *clu*mp virus.
*Pomo*: from *po*tato *mo*p-top virus.
*Tobra* : *tob*acco *ra*ttle virus.
*Tobamo*: from *toba*cco *mo*saic virus.

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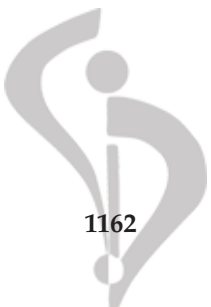
## Contributed by

Adams, M.J., Heinze, C., Jackson, A.O., Kreuze, J.F., Macfarlane, S.A. and Torrance, L.



# Genus *Benyvirus*

Type species ***Beet necrotic yellow vein virus***

## Distinguishing features

Multipartite rod-shaped viruses containing positive stranded RNA genomes with 5′ $m^7G$ cap and 3′ polyA and post-translational cleavage of the viral replicase.

## Virion properties

### Morphology

Viral particles consist of four to five non-enveloped, helically constructed rod-shaped structures, with an axial canal (Figure 1). They have predominant lengths of about 390, 265, 100, 85 and 80 to 65 nm and a constant diameter of 20 nm. The right-handed helix with a pitch of 2.6 nm has an axial repeat of four turns, involving 49 CP subunits, each occupying four nucleotides. Coat proteins constitute about 95% of the particle weight.

### Physicochemical and physical properties

The viral particles are sometimes unstable in sap with strong infectivity losses within one to five days at room temperature.

### Nucleic acid

In naturally infected plants, viruses contain four to five linear positive sense ssRNAs of about 6.7, 4.6, 1.8, 1.4 and 1.3 kb, respectively. The viral RNAs are capped at the 5′ end and, unlike the RNAs of all other rod-shaped plant viruses, are 3′-polyadenylated. Viral RNAs harbour a conserved 3′ structure essential for RNA replication initiation. After mechanical transmission to laboratory test plants, beet necrotic yellow vein virus (BNYVV) RNAs 3, 4 and 5 may carry deletions or may be lost entirely. The complete sequence has been determined for all five RNAs of different isolates of BNYVV and for the four RNAs of an isolate of beet soil-borne mosaic virus (BSBMV). The possible additional members rice stripe necrosis virus (RSNV) and burdock mottle virus (BdMV) apparently only contain two genomic RNAs.

### Proteins

The major CP species is 21–23 kDa in size. The minor CP amber readthrough protein is detected near one extremity of BNYVV particles and initiates virus assembly. The minor capsid protein contains a KTER motif in its C terminal part that is necessary for the transmission of the virions by *Polymyxa betae*.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

RNA-1 contains one large ORF coding for a replication-associated protein that is cleaved post-translationally. This proteolytic cleavage of the replicase distinguishes the benyviruses from all other viruses with rod-shaped particles, which have their replication-associated proteins encoded on two ORFs. *In vitro* translation of BNYVV RNA-1 may initiate at two sites: at the first AUG in the sequence at position 154 or at a downstream AUG at position 496. The resulting proteins of 237 and 220 kDa, respectively, both contain in their N-terminal part Mtr motifs, in their central part Hel and papain-like protease motifs (Prot) and in their C-terminal part RdRp motifs (Figure 2). RNA-2 of BNYVV contains six ORFs, i.e. the CP gene which is terminated by a suppressible amber stop codon (UAG), the CP readthrough protein gene, the TGB coding for TGB1, TGB2 and TGB3 (42, 13 and 15 kDa respectively) and a gene coding for P14 cysteine-rich protein, a viral posttranscriptional gene-silencing suppressor (14 kDa). The TGB ORFs and the P14 gene are expressed by means of

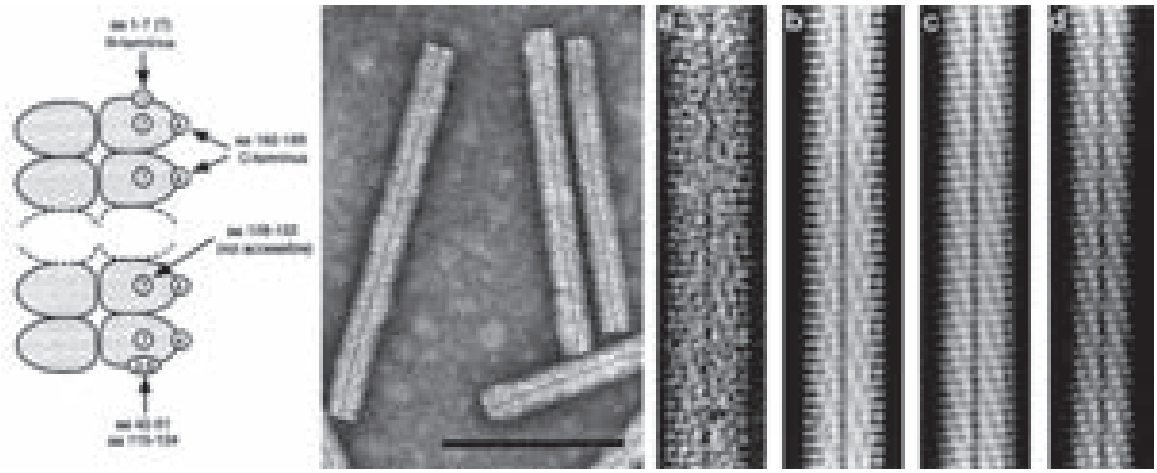


**Figure 1:** (Left) Scheme showing the accessibility to antibodies of various parts of the coat protein amino acid (aa) sequence in particles of beet necrotic yellow vein virus (BNYVV). Encircled numbers designate different epitopes. (Center) Negative contrast electron micrograph of stained purified particles of BNYVV. (Right) From left (a) negative contrast electron micrograph of a BNYVV particle and (b, c, d) computer-filtered micrographs of BNYVV particles (courtesy of A.C. Steven, from *Virology* **113**, 428 (1981); with permission). The bar represents 100 nm.

subgenomic RNAs (Figure 2). The three triple gene block (TGB)-encoded proteins (TGB1, TGB2 and TGB3) but not the CP are necessary for cell-to-cell movement but long distance movement requires CP. BNYVV TGB1 labeled with green fluorescent protein (GFP) on its N-terminus is targeted by TGB2 and TGB3 to punctate bodies associated with plasmodesmata. The N-terminal part of BNYVV TGB1 has nucleic acid binding activity and its C-terminal part contains consensus sequence motifs characteristic of an ATP/GTP-dependent helicase. The three proteins are found along membrane-rich peripheral bodies, thought to be derived from endoplasmic reticulum (ER). The MP of tobacco mosaic virus can functionally complement TGB proteins. RNAs 1 and 2 are sufficient for replication of BNYVV in the local lesion host *Chenopodium quinoa* or in the systemic host *Spinacia olacera*. The typical rhizomania symptoms in beet are produced only in the presence of RNA-3; RNA-4 greatly increases the transmission rate by *Polymyxa betae* and RNA-5 may modulate the type of symptoms formed. RNAs-3 and -4 are always present in natural BNYVV infections. BNYVV is able to replicate and encapsidate BSBMV RNA-3.

## Antigenic properties

BNYVV and BSBMV are distinct serologically. Epitope mapping has revealed portions of the amino acid sequence of BNYVV CP that are either exposed along the entire particle length, e.g. the immunodominant C-terminus, or are accessible only on one extremity or after disruption of the particles (Figure 1).

## Biological properties

BNYVV has been found at the cytoplasmic surface of mitochondria soon after infection. Later, virions of most BNYVV isolates are scattered throughout the cytoplasm of infected cells or occur in aggregates. More or less dense masses of particles arranged in parallel or angle-layer arrays may be formed. Depending on the isolate only one or both types of aggregates occur. Membranous accumulations of ER may also be found. The p25 protein and its tetrad sequence variability appear involved in the resistance breaking capabilities of the virus on sugar beet.

The natural host ranges of benyviruses are very narrow. *Beta vulgaris* is the natural host of BNYVV and BSBMV. Species of *Chenopodium* are infected experimentally, often only locally. *Nicotiana benthamiana*, *Spinacia olacera* and *Beta macrocarpa* are systemic hosts for BNYVV and BSBMV. In nature

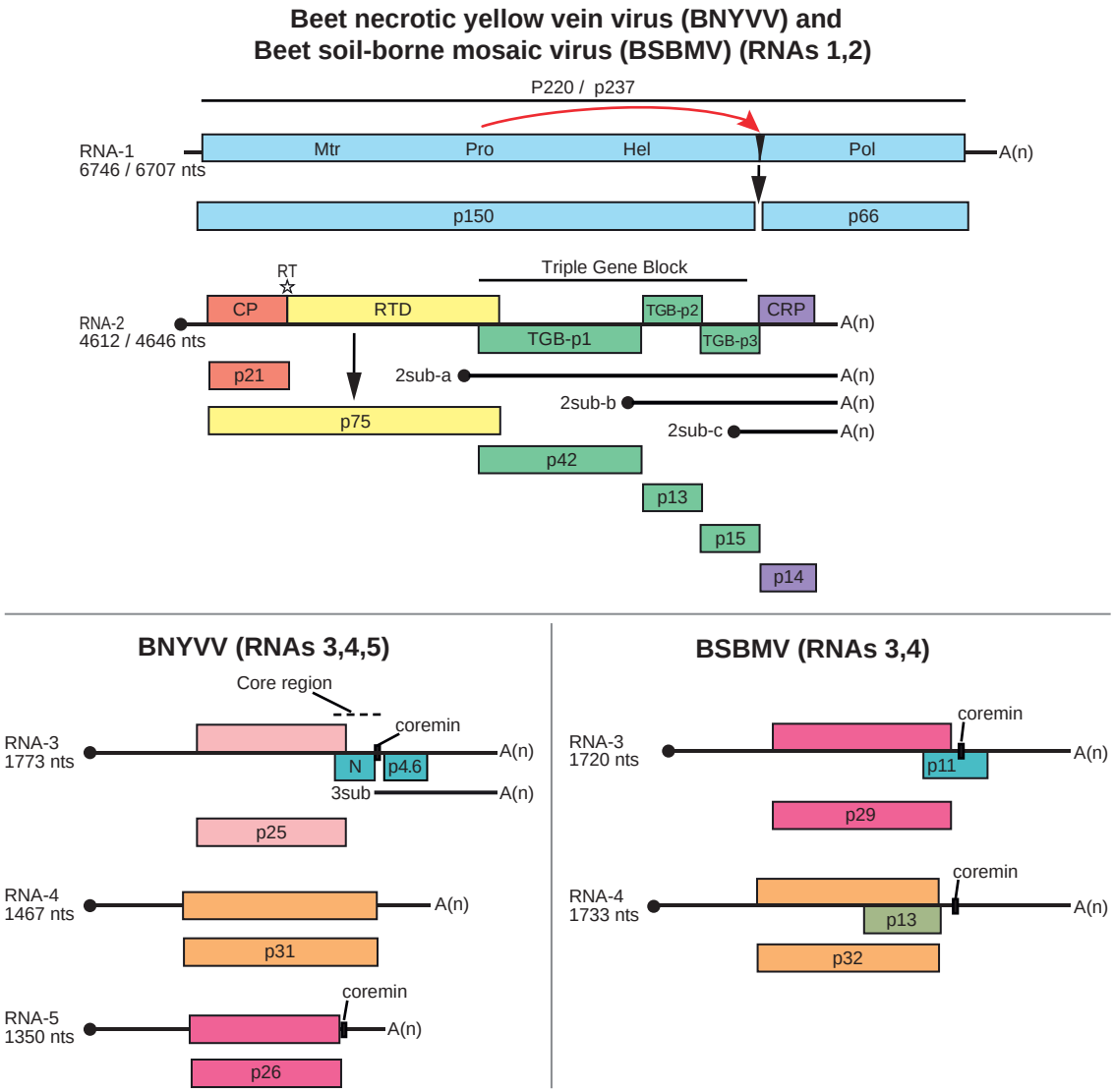



**Figure 2:** Genome organization and translation strategies of beet necrotic yellow vein virus (BNYVV) and beet soil-borne mosaic virus (BSBMV). The scheme indicates a self-cleavage of the replicase protein (red arrow and black triangle), a suppressible UAG stop codon (yellow star), $m^7$Gppp (black circle) and (An) the 3′ poly (A)-tails. Mtr, methyltransferase; Hel, helicase; Pro, protease; Pol, RNA polymerase; RT, readthrough; RTD, readthrough domain; sub, subgenomic; CRP, cysteine rich protein. N and p4.6 have never been detected. Nothing is known about BSBMV putative p11 and p13.



BNYVV and BSBMV are transmitted by *Polymyxa betae* and RNSV by *P. graminis*. The viruses are also mechanically transmissible.

BNYVV has spread to most sugar beet-growing areas worldwide. Variants (A-, B- or P-type) occur in different geographical areas and are distinguished by minor changes in the CP sequence, four amino acids in the p25 ORF (tetrad) and the presence of the RNA-5 species. BSBMV is widely distributed in the United States. RSNV occurs in Africa, and South and Central America. BdMV has been identified in a restricted area in Japan.

## Species demarcation criteria in the genus

Benyviruses are distinguished by coat protein sequence less than 90% identical, distant serological relations and distinct host ranges.

## List of species in the genus *Benyvirus*

| | | |
|---|---|---|
| *Beet necrotic yellow vein virus* | | |
| Beet necrotic yellow vein virus - F2, France (B type) | [X05147; X04197; M36894; M36896] | (BNYVV-B-Eur) |
| Beet necrotic yellow vein virus - S, Japan (A type) | [D84410; D84411; D84412; D84413; D63936] | (BNYVV-A-Jap) |
| Beet necrotic yellow vein virus - F72, France (P type) | [HM126464; HM117903; DQ682454; DQ682453; AY823407] | (BNYVV-P-Eur) |
| *Beet soil-borne mosaic virus* | | |
| Beet soil-borne mosaic virus - EA | [AF280539; AF061869; AF280540; AF280541] | (BSBMV-EA) |
| Beet soil-borne mosaic virus - MRM06,* USA | [EU410955; FJ424610] | (BSBMV-MRM06) |

*Complete RNA3 and 4 sequences only.
Species names are in italic script; names of strains and isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Benyvirus* but have not been approved as species

| | | |
|---|---|---|
| Burdock mottle virus | | (BdMV) |
| Rice stripe necrosis virus | [EU099844; EU099845] | (RSNV) |

## Phylogenetic relationships within the genus

The CP amino acid sequences of BNYVV and BSBMV share less than 60% homology (Figure 3). The percentages of identity between various non-structural proteins of the two viruses range between 38% (cysteine-rich protein) and 84% (replication-associated protein). RNA species of BNYVV and BSBMV share common structural motifs at their extremities but coding sequences differ. BNYVV p26 protein resembles BSBMV p29 (Figure 2) in sequence and functions. The "coremin" sequence of 20 nucleotides involved in the systemic movement of the virus is present on: BNYVV RNA-3 core sequence and RNA-5, BSBMV RNA-3 and RNA-4 (Figure 2) as well as on cucumber mosaic virus RNAs. The CP of BdMV shares 38% sequence identity with that of BNYVV. TGB1 of BdMV shares c. 50% identity with the corresponding proteins of BNYVV and BSBMV. A 2,239 nucleotide fragment of RSNV RNA-1 shares about 50% sequence identity with the corresponding regions of BNYVV and BSBMV RNA-1.

## Similarity with other taxa

Benyviruses are morphologically similar to other rod-shaped viruses that are classified in the family *Virgaviridae* (furo-, peclu-, pomo-, hordei-, tobra- and tobamoviruses). Conserved residues are found among the CP of these viruses, e.g. RF and FE in their central and C-terminal parts, respectively, which are presumably involved in the formation of salt bridges. Like pomo-, peclu- and hordeiviruses, but unlike furo-, tobamo- and tobraviruses, the benyviruses have their movement function encoded on a triple gene block. Sequence identities in the first and second triple gene block-encoded proteins reveal affinities not only to pomo- and hordei-, but also to potex- and carlaviruses. The Mtr, Hel and RdRp motifs in the replication-associated proteins show a higher degree of similarity to those of the viruses of the family *Togaviridae* (rubella virus) and of hepatitis virus E than to those of other rod-shaped plant viruses.

## Derivation of name

*Beny*: from *be*et *n*ecrotic *y*ellow vein virus.

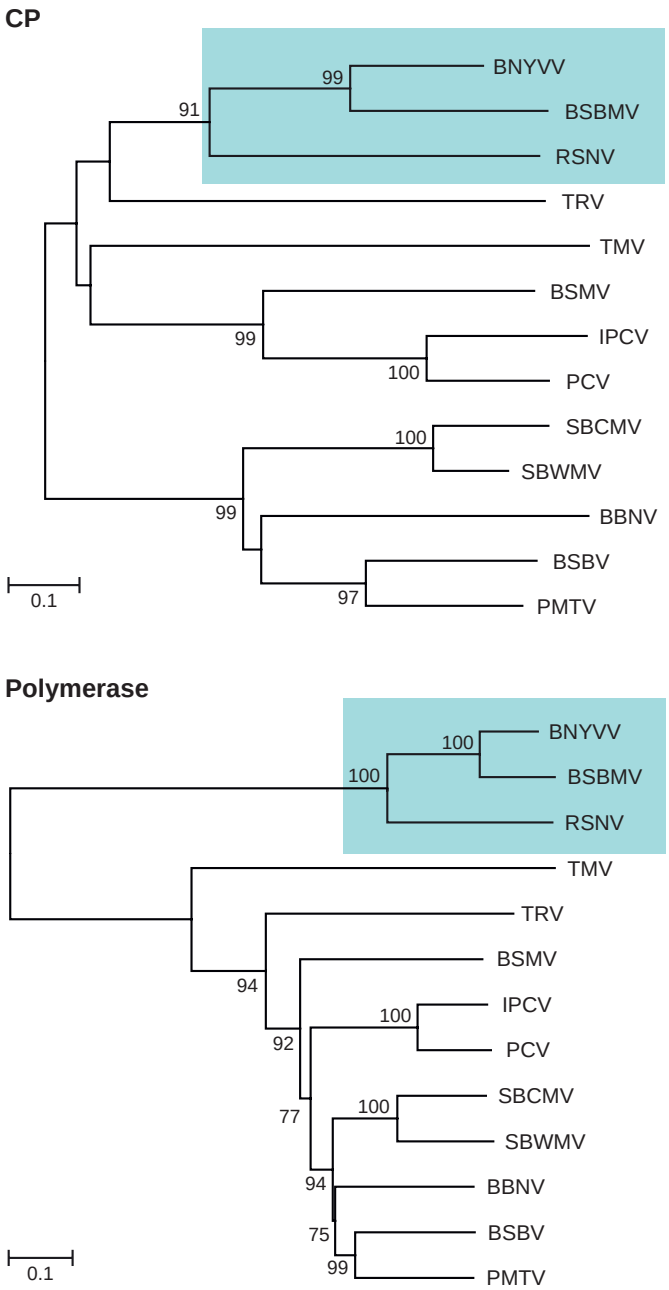



**Figure 3:** Phylogenetic (distance) tree based on the codon-aligned nucleotide sequences of the capsid (CP) and polymerase proteins of rod-shaped plant viruses. Numbers on branches indicate percentage of bootstrap support out of 10,000 bootstrap replications (when >60%). The scales indicate maximum composite likelihood distances. Trees produced in MEGA4. Viruses (or possible members) of the genus *Benyvirus* are highlighted. Other viruses are Genus *Pomovirus*: potato mop top virus (PMTV), beet soil-borne virus (BSBV), broad bean necrosis virus (BBNV); *Furovirus*: soil-borne cereal mosaic virus (SBCMV), soil-borne wheat mosaic virus (SBWMV); *Tobamovirus*: tobacco mosaic virus (TMV); *Tobravirus*: tobacco rattle virus (TRV); *Hordeivirus*: barley stripe mosaic virus (BSMV); *Pecluvirus*: Indian peanut clump virus (IPCV), peanut clump virus (PCV).

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## Contributed by

Gilmer, D. and Ratti, C.

# Genus *Cilevirus*

Type species ***Citrus leprosis virus C***

## Distinguishing features

This genus contains mite-transmitted viruses with bacilliform particles and a bipartite ssRNA genome that is polyadenylated.

## Virion properties

### Morphology

Virions are bacilliform, about 120–130 × 50–55 nm and are not enveloped (Figure 1). In ultra-thin sections of infected citrus plants, they can be seen in enclaves of the endoplasmic reticulum of mesophyll and vascular parenchyma (Figure 2).

### Physicochemical and physical properties

Virions have an *in vitro* thermal inactivation point between 55 and 60 °C. They remain viable for 6 days at 4 °C and 3 days at room temperature. Dilution end point is $10^{-3}$. Infectivity is retained for about 45 months in dried leaves. Virions of *Citrus leprosis virus C* (CiLV-C) can be isolated 24 h after inoculation from inoculated leaves of *Chenopodium quinoa* with very low titer of infection.

### Nucleic acid

Virions contain two molecules of linear, positive sense, ssRNA of about 8745 nt (RNA 1) and 4986 nt (RNA 2). The RNA molecules are polyadenylated in the 3′-termini, and contain a "cap" structure in the 5′-termini.

### Proteins

See section on genome organization and replication.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

The genome of CiLV-C is bipartite. ORF1 of RNA1 (frame +1) encodes a large polypeptide involved in viral replication. This has 2512aa (286 kDa) and contains conserved domains for methyltransferase (MTR), cysteine protease (C-Prot), helicase (Hel) and RNA dependent RNA polymerase (RdRp). ORF2 of RNA1 (frame +3) encodes a putative protein of 263aa (29 kDa) with no conserved domains but a very low similarity to the capsid proteins of members of the genus *Alphavirus*. This fact, associated with serological data obtained using antiserum against recombinant P29 suggest that the protein may be involved in virion encapsidation. This ORF is followed by a 3′ NTR of 228 nt and a poly (A) tail of undetermined length (Figure 3). RNA2 has four ORFs. ORF1 (frame +1) encodes a putative protein with 130aa (15 kDa). ORF2 (frame +3) encodes the largest putative protein of this RNA (537aa, 61 kDa). ORF3 (frame +3) codes for a putative protein of 32 kDa (297 aa) with conserved domains characteristic of a viral movement protein (MP). ORF4 (frame +1) codes for a putative protein with 214aa (24 kDa). None of the putative proteins encoded by ORFs 1, 2 and 4 of RNA2 have conserved domains or similarity with known sequences in databases. ORF4 of RNA2 is followed by a 3′ NTR of 232 nt and a poly (A) tail of undetermined length (Figure 3). The conserved sequence GAUAAAUCU was found at the 5′-termini of both RNAs 1 and 2 of CiLV-C. The virus has four subgenomic RNAs (sgRNA). The first one (sgRNA1, 796 nt) corresponds to nucleotide sequences of *p29* of RNA1, while the other three correspond to sequences of RNA2 (sgRNA2 of 3 kb corresponds to regions within *p61*, *p32* and *p24*, sgRNA3 of 1.5 kb transcribes regions of *p32* and *p24*, and sgRNA4 of 0.6 kb corresponds to *p24* sequence). Infected citrus leaves contain dsRNAs corresponding to double stranded forms of RNA1 and RNA2 as well as subgenomic RNAs.

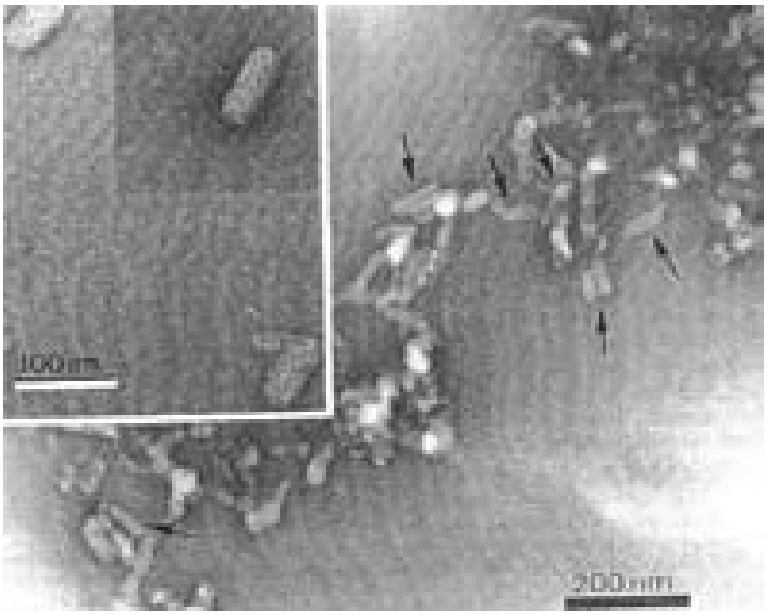


**Figure 1:** Electron micrograph of purified particles of an isolate of *Citrus leprosis virus C*. (Courtesy Addolorata Colariccio, Instituto Biológico, SP, Brazil.)

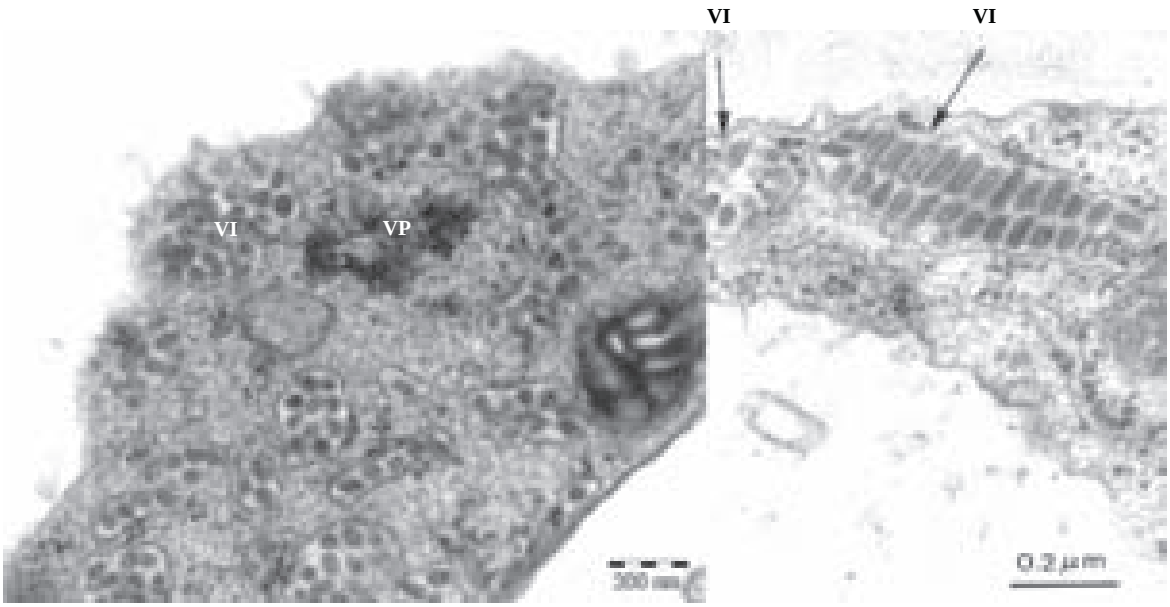


**Figure 2:** Electron micrographs exhibiting viroplasma (VP) and virions (VI) inside the endoplasmic reticulum in parenchyma cells of Pera sweet orange. (Courtesy Elliot W. Kitajima, ESALQ/USP, Brazil.)

## Antigenic properties

It has not been possible to purify the virion using standard procedures. In heterologous systems using *Eschericha coli*, P29 was highly immunogenic, yielding a high quality antiserum. All of the other proteins tested appear less immunogenic.

## Biological properties

Transmission occurs by all of the active stages of the mite vector *Brevipalpus* sp. (Acari: Tenuipalpidae): larvae, nymphs and adults. The virus circulates but, differently from what has been

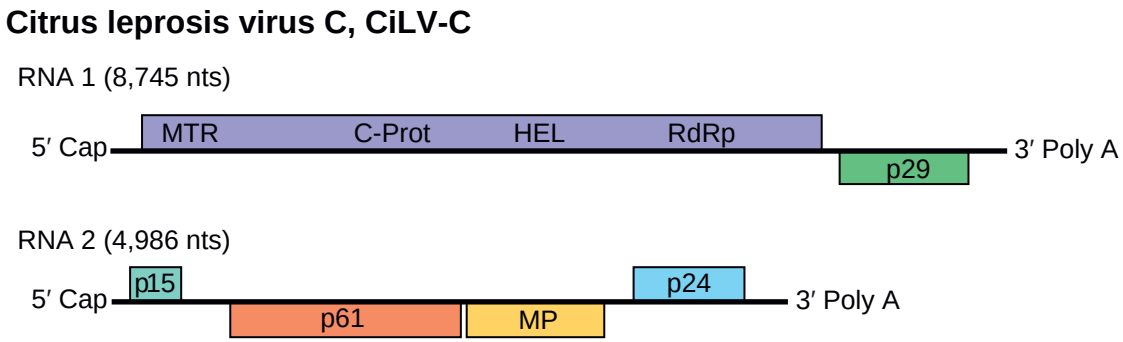


**Figure 3:** Schematic drawing of genome structure and organization of CiLV-C. Each RNA is presented as a line with the corresponding ORFs indicated by rectangles. ORFs located in different frames are represented above (+1) or below (+3) the lines. Functional domains attributed according to the translated sequences are indicated inside the rectangles. For those translated sequences with unidentified function, the approximate molecular weight is indicated inside the rectangles. MTR=methyltransferase domain; C-Prot=cysteine protease domain; HEL=helicase domain; RdRp=RNA-dependent RNA polymerase domain; MP=movement protein domain. (Reproduced from Locali-Fabris *et al.* (2006). *J. Gen Virol.*, **87**, 2721-2729; with permission.)

reported earlier, it does not seem to propagate in the vector. The virus was initially considered to have a very narrow host range, but in recent years the list of CiLV-C hosts has increased significantly. In nature, CiLV-C infects several species of *Citrus*. Sweet oranges (*Citrus sinensis*) are among the most susceptible species, along with *C. keraji*. Mandarins (*C. reticulata*, *C. reshni*, *C. deliciosa*, *C. lycopersiciformis*, *C. unshiu*, *C. depressa*) and few hybrids exhibit variable levels of resistance, but are symptomatic. The rutaceous plant *Swinglea glutinosa* has been recently reported as the first natural host of CiLV-C outside the genus *Citrus*. *Chenopodium quinoa*, *C. amaranticolor* and *Gomphrena globosa* are experimental hosts by mechanical inoculation only, while CiLV-C can also be experimentally transmitted to *Solanum violaefolium*, *Phaseolus vulgaris*, *Hibiscus rosa-sinensis*, *Malvaviscus arboreus*, *Grevilea robusta*, *Bixa orellana*, *Glycosmis pentaphylla* and *Commelina benghalensis* by the mite vector. Graft transmission of CiLV-C is possible experimentally but, contrary to what happens to other citrus viruses, it does not occur naturally. The virions remain confined to the localized lesions they induce, and are never spread systemically within the host plants. Typical symptoms are chlorotic or necrotic lesions, often exhibiting ringspot patterns in leaves, fruits and stems. Lesions can be raised in all affected organs but, in fruits, they can also be depressed. Affected leaves and fruits may drop prematurely. Heavy infection can eventually lead to the death of the tree. Typically from the Americas, CiLV-C is present in Paraguay, Argentina, Brazil, Bolivia, Venezuela, Colombia, Panama, Costa Rica, Guatemala, Nicaragua, Honduras, El Salvador and Mexico. Its first report was from the United States, but it has not been found in that country since the 1960s. There are unconfirmed reports of the occurrence of leprosis in Asia and Africa.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Cilevirus*

| | | |
|---|---|---|
| *Citrus leprosis virus C* | | |
| Citrus leprosis virus C - Cordeiropolis | RNA1 [DQ352194]RNA2 [DQ352195] | (CiLV-C-Cor) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Cilevirus* but have not been approved as species

| | | |
|---|---|---|
| Ligustrum ringspot virus | [HM164551*], [HM164552*] | (LigRSV) |
| Solanum violaefolium ringspot virus | [DQ 514336*] | (SvRSV) |
| Passion fruit green spot virus | [HM002746*], [HM002747*] | (PFGSV) |

*Sequences do not comprise the complete genome.

## Similarity with other taxa

CiLV-C and other possible species of the genus *Cilevirus* have unusual biological properties such as the inability to spread systemically within the host plants, the *Brevipalpus* mite transmission and the uncommon bacilliform morphology of the virions. Sequence analyses reveal conserved domains corresponding to the movement protein and replication-associated proteins of members of several positive sense, ssRNA genera such as *Furovirus*, *Bromovirus*, *Tobravirus* and *Tobamovirus*. However, none of the other four ORFs of CiLV-C or their translated proteins exhibit conserved domains or similarity with sequences available in the main sequence databases.

## Derivation of name

*Cilevirus*: from the genus of the natural host (*Citrus*) and the name of the disease caused by the type member virus (*le*prosis).

## Further reading

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## Contributed by

Locali-Fabris, E.C., Freitas-Astúa, J. and Machado, M.A.

# GENUS *IDAEOVIRUS*

Type species ***Raspberry bushy dwarf virus***

## Distinguishing features

A monotypic genus for a pollen transmitted virus with isometric particles containing two genomic and one subgenomic positive sense, single stranded RNAs.

## Virion properties

### MORPHOLOGY

Virions are isometric, about 33 nm in diameter and are not enveloped. They appear flattened in electron micrographs of preparations negatively stained with uranyl salts.

### PHYSICOCHEMICAL AND PHYSICAL PROPERTIES

Virion Mr is about $7.5 \times 10^6$ (calculated from the $S_{20,w}$ of 115S). The buoyant density of aldehyde-fixed particles in CsCl is $1.37\,g\,cm^{-3}$. Particles are readily disrupted in neutral chloride salts and by SDS.

### NUCLEIC ACID

Virion preparations contain three species of linear, positive sense, ssRNA of about 5.5 kb (RNA-1), 2.2 kb (RNA-2) and 1 kb (RNA-3). These RNA molecules are not polyadenylated.

## Genome organization and replication

The genome is bipartite. RNA-1 has a single, major ORF encoding a 188 kDa protein that contains sequence motifs characteristic of viral RNA helicases and polymerases. RNA-2 has two ORFs: that in the 5′-terminal half encodes a protein of about 39 kDa which has some slight sequence similarities with cell-to-cell movement proteins of other viruses; that in the 3′-terminal half encodes the 30 kDa CP. RNA-2 is probably a template for the production of RNA-3, that comprises the 3′-most 946 nt of RNA-2 and is a sgRNA for CP expression. The 3′-terminal non-coding 18 nt of RNA-1 and RNA-2 (and hence of RNA-3) are the same and the 3′-terminal 70 nt can be arranged in similar extensively base-paired structures. Infected leaves contain dsRNA corresponding in size to double stranded forms of RNA-1 and RNA-2. *In vitro* translation of RNA isolated from purified virus particles yields three major proteins, of about 190, 44 and 31 kDa (CP), which are the translation products, respectively, of RNA-1, RNA-2 and RNA-3. Using infectious clones it has been shown that RNA1 and RNA2 together have low infectivity but that the addition of RNA3 (and probably also virus coat protein itself, although these experiments have not been done) greatly stimulates virus replication. This resembles the mechanism of "genome activation" that operates also with Alfalfa mosaic virus and ilarviruses.

## Antigenic properties

Particles are moderate immunogens.

## Biological properties

In nature, the host range was thought to be confined to *Rubus* species, all but one in the subgenus *Idaeobatus*. However, RBDV has recently been found causing an infection in grapevines. The experimental host range of RBDV is fairly wide. The virus occurs in all tissues of the plant, including seed and pollen, and is transmitted in association with pollen, both vertically to the seed and horizontally to the pollinated plant. This is the only known method of natural spread. Experimentally, the virus can be transmitted by mechanical inoculation. The virus occurs throughout the world wherever raspberry is grown. Infection of raspberry and blackberry is often symptomless but in some cultivars may be associated with "yellows disease" and/or "crumbly fruit", a major economic problem in raspberry production. Confusingly, black raspberry necrosis virus is the main cause of bushy dwarf disease in Lloyd George raspberry. However, because the additional presence of RBDV in

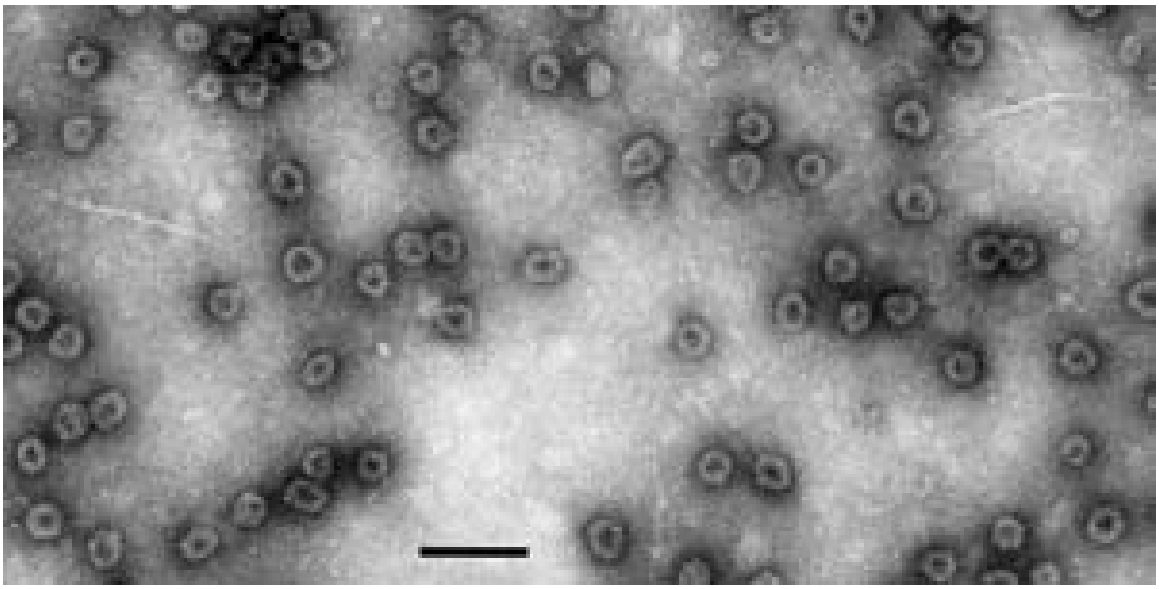

**Figure 1:** Negative contrast electron micrograph of particles of an isolate of *Raspberry bushy dwarf virus*, stained with uranyl formate/sodium hydroxide. The bar represents 100 nm.

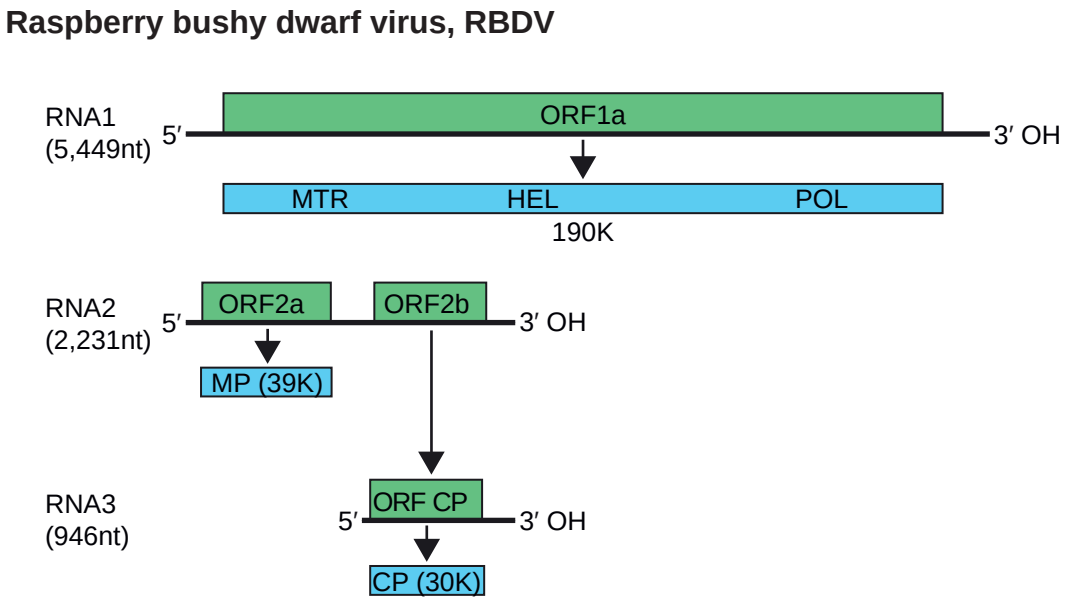


**Figure 2:** Scale diagram of RNA species found in particles of raspberry bushy dwarf virus (RBDV). The open boxes represent the ORFs. The positions of putative domains are indicated as "MTR", a methyltransferase motif, "HEL", an NTP-binding motif, "POL", an RNA-dependent RNA polymerase motif. MP signifies the putative movement protein role of the 39 kDa protein and CP is the coat protein.

plants contributes significantly to the intensity of the disease symptoms usually observed in the field, it can also be regarded as an integral component of the disease syndrome.

## Species demarcation criteria in the genus

Not applicable.

# Genus *Ourmiavirus*

Type species ***Ourmia melon virus***

## Distinguishing features

Plant viruses with unenveloped bacilliform virions composed of a single coat protein and three positive sense ssRNAs. Only three proteins are expressed. The virions possess a unique fine structure with a series of discrete lengths from 30 to 62 nm. The RdRp has closest similarity to that of the fungal virus genus *Narnavirus* (*Narnaviridae*); the movement protein is significantly similar to the MPs of tombusviruses (*Tombusviridae*); the coat protein shows limited similarity to the CPs of several plant and animal viruses. This combination of characters is not found in any other virus taxon.

## Virion properties

### Morphology

The bacilliform virions constitute a series of particles with conical ends (apparently hemi-icosahedra) and cylindrical bodies 18 nm in diameter. The bodies of the particles are composed of a series of double disks, the commonest particle having two disks (particle length 30 nm), a second common particle having three disks (particle length 37 nm) with rarer particles having four disks (particle length 45.5 nm) and six disks (particle length 62 nm). There is no envelope. (See Figures 1 and 2.)

### Physicochemical and physical properties

The Mr of virions and their sedimentation coefficients are not known. The buoyant density in CsCl of all particle sizes is 1.375 g cm$^{-3}$. The particles are stable near pH 7. Thermally, they are relatively stable; infectivity survives in crude sap after heating for 10 min at 70 °C but not 80 °C, and is retained after at least one freeze–thaw cycle. The particles survive in CsCl density gradients and survive treatment with chloroform but not n-butanol, and survive treatment with 1% Triton X-100 detergent.

### Nucleic acid

There are three positive sense ssRNAs. In Ourmia melon virus (OuMV) these have sizes of 2814, 1064 and 974 nt, with similar values for the other members of the genus.

### Proteins

For OuMV there is one structural protein of 23.8 kDa; the other two proteins are the RdRp (97.5 kDa) and the movement protein (31.6 kDa). Data are similar for the other two species.

### Lipids

None reported.

### Carbohydrates

None reported.

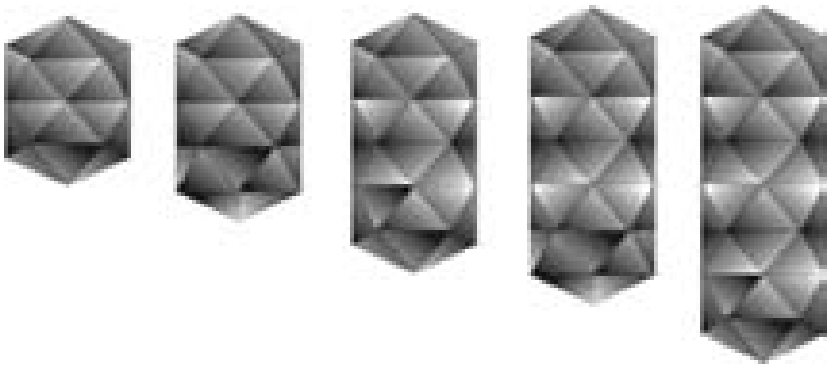

**Figure 1:** Diagram of virion surface of a member of the genus *Ourmiavirus*, showing arrangement of double disks and conical ends in particles of different length. Each row of five triangles represents a double disk.

Pos. ssRNA

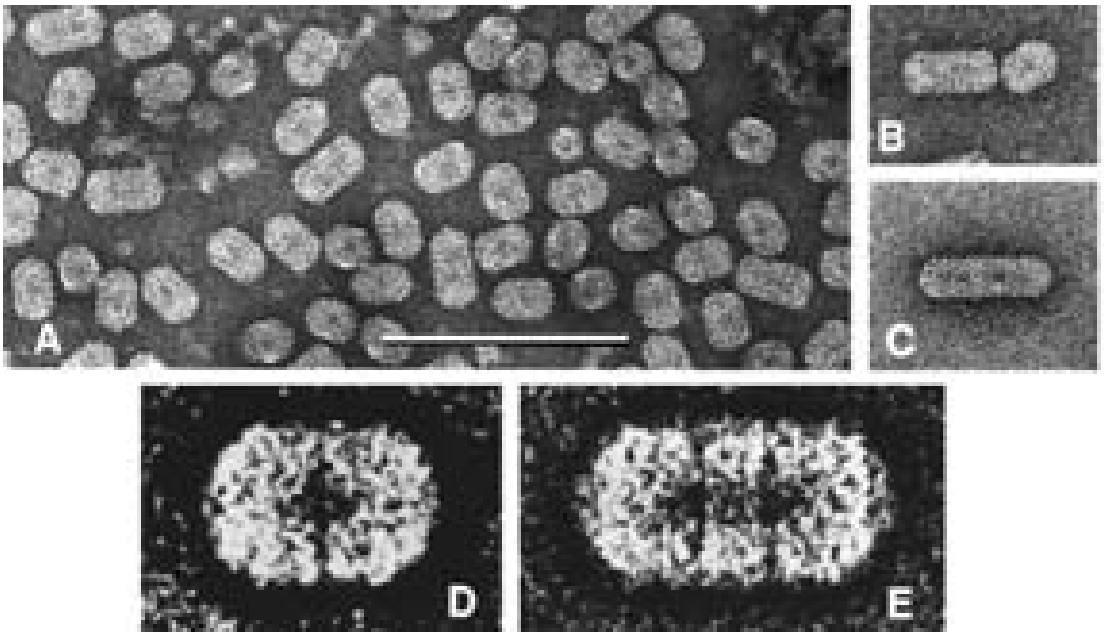


**Figure 2:** (A, B, C) Negative contrast electron micrographs (uranyl acetate) of purified particles of Ourmia melon virus. The bar represents 100 nm. D and E, features of the two commonest particle types, enhanced by photographic superimposition.

## Genome organization and replication

Each RNA has one ORF (Figure 3). RNA-1 encodes a protein carrying the GDD motif typical of RNA-dependent RNA polymerases (RdRps). There is evidence that RNA-2 encodes the cell-to-cell movement protein. The product of RNA-3 is the coat protein, which localizes to the nucleus. Synthesis of coat protein from actively replicating RNA-3 is necessary for both virion assembly and systemic infection of the host. There appear to be no subgenomic RNAs and no further proteins produced by read through. The movement protein may undergo post-translational modification. Details of replication are not known except that virions and the movement protein accumulate in the cytoplasm.

## Antigenic properties

The virions (i.e. the assembled coat protein) are good immunogens, as are the tubular structures associated with the movement protein. Antisera to these proteins did not react in Western blots to extracts of plants infected with either of the other two viruses in the genus.

## Biological properties

The type species can easily be mechanically transmitted to a rather wide range of dicot plants (about 40 species in 15 families reported), usually inducing systemic ringspots, mosaic and necrosis, with local lesions on some hosts. There is no particular tissue tropism. No vector has been identified but several species of weeds around infected fields are commonly also infected. No experimental transmission was obtained with several aphid species, the whiteflies *Trialeurodes* and *Bemisia* or a *Tetranychus* mite. Attempts at transmission through soil or irrigation water were negative. Experimental seed-transmission rates are 1–2% in *Nicotiana benthamiana* and *N. megalosiphon*. Different species in the genus occur in geographically diverse areas and on widely different hosts, though there are experimental hosts in common.

## Species demarcation criteria in the genus

- Not related serologically with respect to both the coat protein and the movement protein.
- Natural hosts are widely different.
- Less than 60% similarity between species in the coat protein aa sequence.

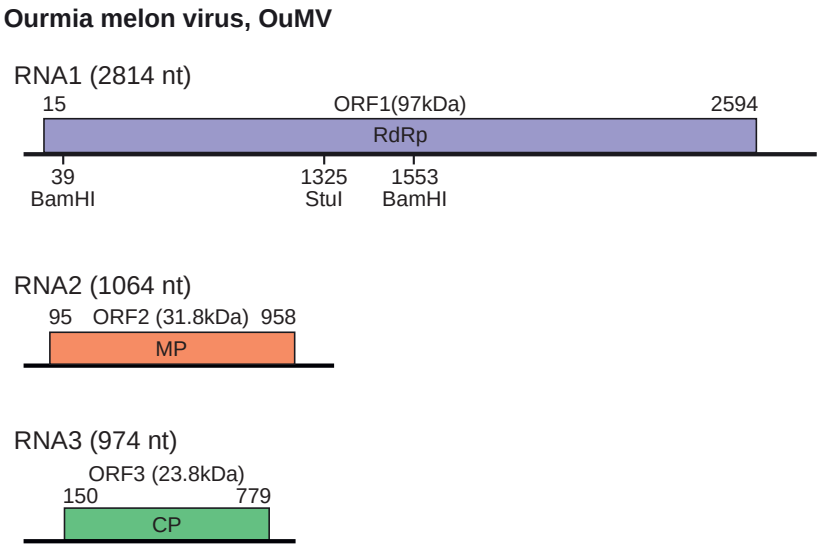


**Figure 3:** Diagram of the genome organization of Ourmia melon virus isolate VE9 showing the size of each RNA and the positions and sizes of the ORFs. CP, coat protein; MP, movement protein; RdRp, RNA-dependent RNA polymerase.

## List of species in the genus *Ourmiavirus*

| | | |
|---|---|---|
| *Ourmia melon virus* | | |
| Ourmia melon virus-VE9 | [RNA-1: EU770623; RNA-2: EU770624; RNA-3: EU770625] | (OuMV-VE9) |
| *Epirus cherry virus* | | |
| Epirus cherry virus-VE450 | [RNA-1: EU770620; RNA-2: EU770621; RNA-3: EU770622] | (EpCV-VE450) |
| *Cassava virus C* | | |
| Cassava virus C-IC | [RNA-1: FJ157981; RNA-2: FJ157982; RNA-3: FJ157983] | (CsVC-IC) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Ourmiavirus* but have not been approved as species

None reported.

## Similarity with other taxa

The genus *Ourmiavirus* stands isolated, having particles of unique morphology and a unique combination of phylogenetic affinities for the three RNAs. The RdRp encoded by RNA-1 has closest (although distant) affinity with the RdRp of the fungal virus *Narnavirus* (*Narnaviridae*). The movement protein encoded by RNA-2 has clear affinities with the MPs of viruses in the *Tombusviridae*. The coat protein shows distant affinities with the CPs of sobemo-, tombus- and luteoviruses (plant viruses) and nodaviruses (animal viruses).

## Derivation of names

*Ourmia*: from Ourmia (Urmia, Orumieh) in north-western Iran where the type virus was first found.
*Epirus*: from Epirus, Greece, where cherry virus was isolated.
*Cassava*: cassava virus C has been isolated from the cassava plant in various parts of Africa.

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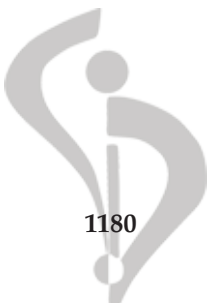
## Contributed by

Rastgou, M., Turina, M. and Milne, R.G.



# Genus *Polemovirus*

Type species ***Poinsettia latent virus***

## Distinguishing features

This is a monotypic genus containing a virus that has a close relationship to poleroviruses within the first three-quarters of its genome but to sobemoviruses in the last quarter.

## Virion properties

### Morphology

Virions are 34 nm in diameter, hexagonal in outline and have no envelope. They are similar to those of sobemoviruses and exhibit icosahedral symmetry (T = 3).

### Physicochemical and physical properties

These have not been studied but are probably similar to those of sobemoviruses.

### Nucleic acid

Particles contain a single molecule of positive sense ssRNA, about 4.6 kb in size. A 1.07 kb subgenomic RNA (sgRNA) molecule, co-terminal with the 3′ end of the genomic RNA, can be detected in infected cells and a larger sgRNA of 2.60 kb may also be present. The 3′ terminus is non-polyadenylated and is predicted to form a stable hairpin. It is likely that a VPg protein is covalently linked to the 5′ end of the genomic RNA.

### Proteins

There is one major structural protein of 33 kDa. A genome-linked (VPg) protein is predicted from the sequence data and by comparison with poleroviruses, but has not been detected *in vivo*.

### Lipids

None reported.

### Carbohydrates

None reported.

## Genome organization and replication

Four ORFs have been identified in the sequence determined (Figure 1). The first three ORFs resemble those of members of the genus *Polerovirus* (family *Luteoviridae*) and have been labelled similarly. The ORF0 product is therefore probably a suppressor of gene silencing. ORF1 contains helicase, putative VPg and protease domains, while ORF2 is an RNA-dependent RNA polymerase. As in poleroviruses, it is likely that ORFs 0, 1 and 2 are translated from the genomic RNA and that ORF2 is translated by frameshift from ORF1, thus sharing the same amino terminus. ORF3 encodes the viral coat protein and is believed to be translated from the sgRNA that can be detected in infected plants.

## Antigenic properties

Virions are strongly immunogenic.

## Biological properties

The virus occurs regularly in cultivated poinsettia plants throughout the world, apparently without causing symptoms. It is distributed by grafting and vegetative propagation of the host plant but its natural means of transmission is unknown. Its appearance in virus-free plants has lead to suggestions that transmission from soil may occur. It often occurs in association with poinsettia mosaic virus but the two viruses are serologically and genetically unrelated.

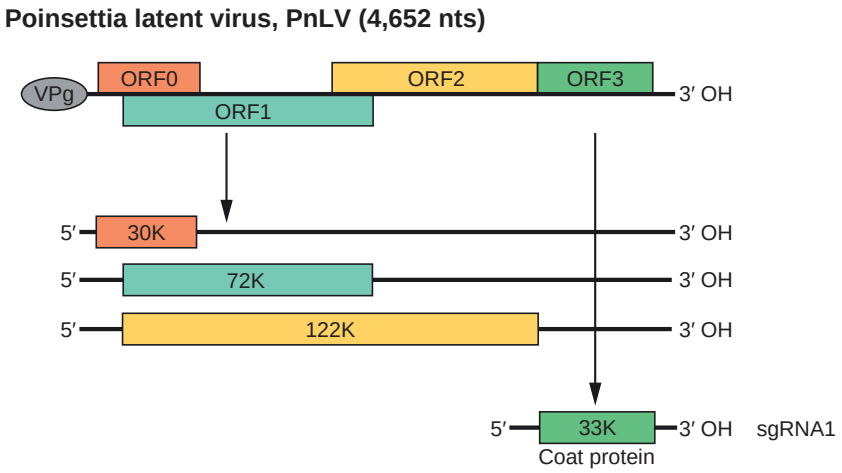



**Figure 1:** Diagram of the genome organization and map of the translation products of poinsettia latent virus.

## Species demarcation criteria in the genus

Not applicable.

## List of species in the genus *Polemovirus*

*Poinsettia latent virus*
(Poinsettia cryptic virus)
Poinsettia latent virus-Germany [AJ867490 = NC_011543] (PnLV-DE)

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Polemovirus* but have not been approved as species

None reported.

## Similarity with other taxa

The virus was initially named poinsettia cryptic virus but has now been shown to be unrelated to the cryptic viruses, which have dsRNA genomes. Sequence comparisons show that the 5′-section of the genome is similar in organization and sequence to members of the genus *Polerovirus* (family *Luteoviridae*), whereas the coat protein is most closely related to members of the genus *Sobemovirus* (Figure 2). The virus therefore probably arose by recombination although the exact history may be more involved. There is a complex network of putative recombination amongst members of the families *Luteoviridae* and *Tombusviridae* and the unassigned genera *Polemovirus* and *Sobemovirus* that is difficult to reflect by conventional hierarchical taxonomy.

## Derivation of name

*Polemo*: from genera *Polerovirus* and *Sobemovirus* to indicate the chimeric nature of the virus.

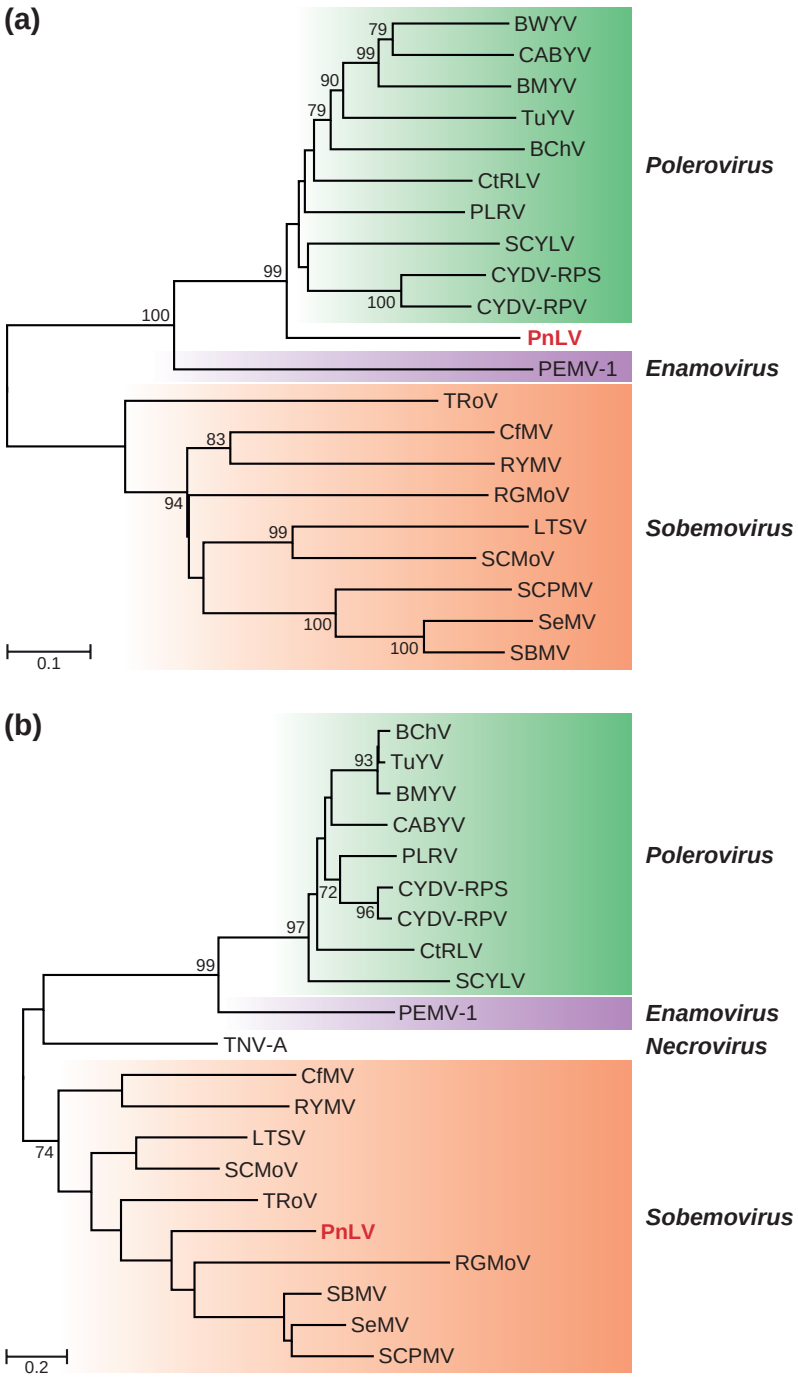



**Figure 2:** Phylogenetic (distance) trees of (a) the RdRp domain and (b) the coat protein nucleotide sequences of members of the genera *Polerovirus* and *Sobemovirus* showing the different placement of poinsettia latent virus (PnLV) in the two regions of the genome. Trees were prepared from codon-aligned sequences in MEGA4 (maximum composite likelihood distances and 10,000 bootstrap replicates). Bootstrap values >70% are shown at the nodes.

## Further reading

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## Contributed by

Adams, M.J. and Jeske, H.

# Genus *Sobemovirus*

Type species ***Southern bean mosaic virus***

## Distinguishing features

Sobemoviruses are similar in particle morphology, capsid stabilization, sedimentation coefficients, sizes of protein subunits and genomic RNA. All the sequenced sobemoviruses have a polycistronic positive sense single stranded RNA genome that consists of four ORFs. ORFs 1, 2a and 2b are all translated from the genomic RNA. Translation of ORF2a occurs via a leaky scanning mechanism. ORF2b is expressed as a fusion protein with ORF2a through a −1 ribosomal frameshift mechanism. The genome 3′-proximal ORF3 is translated from the subgenomic RNA.

## Virion properties

### Morphology

Virions are about 25–30 nm in diameter. They have a single tightly packed capsid layer with 180 subunits of about 26–31 kDa assembled on a T = 3 icosahedral lattice. Sobemoviruses are stabilized by divalent cations, pH-dependent protein–protein interactions and salt bridges between protein and RNA. Upon alkali treatment in the presence of divalent chelators, the capsid swells and becomes sensitive to enzymes and denaturants. (See Figure 1.)

### Physicochemical and physical properties

The virion Mr is about $6.6 \times 10^6$; $S_{20,w}$ is about 115S; density is about 1.36 g cm$^{-3}$ in CsCl (but virus forms two or more bands in $Cs_2SO_4$); particles swell reversibly in EDTA and higher pH with concomitant changes in capsid conformation and partial loss of stability.

### Nucleic acid

Particles contain a single molecule of positive sense ssRNA, about 4.0–4.5 kb in size. A subgenomic RNA molecule, co-terminal with the 3′ end of the genomic RNA, with a Mr of $0.3–0.4 \times 10^6$, is synthesized in the virus-infected cell. Both genomic and subgenomic RNAs are thought to have a viral genome-linked protein VPg covalently bound to their 5′ end. The 3′ terminus is non-polyadenylated and does not contain a tRNA-like structure. Several sobemoviruses encapsidate a circular viroid-like satellite RNA (220–390 nt).

### Proteins

The major structural protein of sobemoviruses is the coat protein. In addition, from the central part of the polyprotein a viral genome-linked protein VPg is processed. It is predicted that sobemovirus VPgs are natively unfolded proteins.



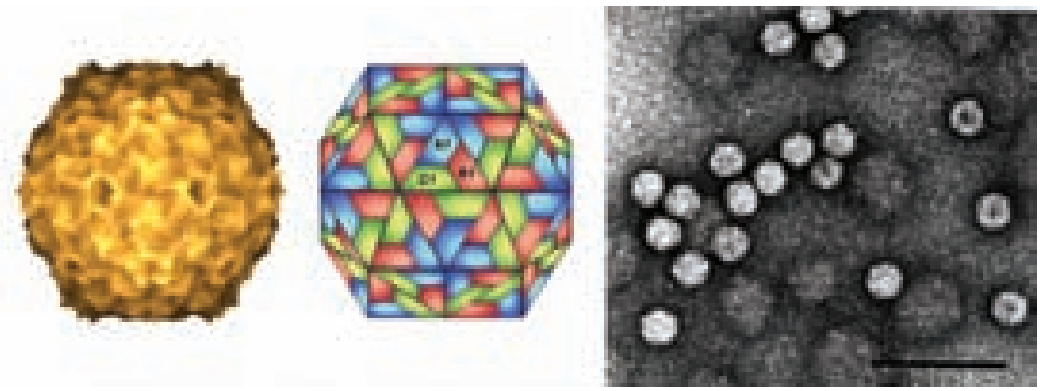

**Figure 1:** (Left) Electronic rendering of particles of southern bean mosaic virus (T = 3); (centre) diagrammatic representation of a T = 3 structure capsid; (right) negative contrast electron micrograph of rice yellow mottle virus particles stained in uranyl acetate. The bar represents 100 nm.

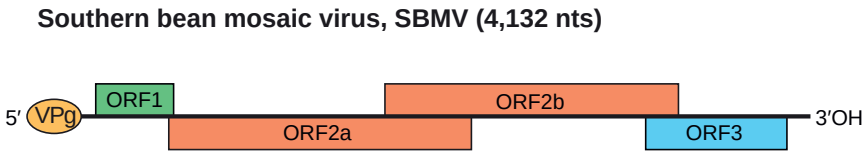


**Figure 2:** Diagram of the genome organization of southern bean mosaic virus (SBMV).

### Lipids
None reported.

### Carbohydrates
None reported.

## Genome organization and replication

It has been presumed earlier that the genus includes viruses with two different genome organizations. Recently it has been demonstrated that these differences were due to sequencing errors and in fact the genus has the common genome organization depicted in Figure 2.

The protein P1, encoded by the 5′ terminal ORF of the genomic RNA, is the suppressor of gene silencing. It is essential for the systemic spread of the virus. The translation initiation codon of P1 has a poor context, enabling ribosomes to bypass it often and, by the utilization of the leaky scanning mechanism, initiate from the genomic RNA the translation of the next ORF. This ORF encodes the viral polyprotein. The first part of this ORF, ORF2a, encodes the virus serine protease, VPg as well as C-terminal proteins P10 and P8 with ATPase and RNA binding properties, respectively. The processing of the P2a polyprotein takes place at consensus cleavage site E/T,S,N. Around 10% of ribosomes undergo −1 ribosomal frameshifting at the 3′ region of ORF2a and continue the translation on ORF2b. These ribosomes translate RNA-dependent RNA polymerase.

CP is translated from the subgenomic RNA (ORF3). The CP subunits are chemically identical but are not structurally equivalent. Three types of CP subunits termed A, B, C are related by quasi threefold axes of symmetry and are involved in different inter-subunit contacts. The CP has two distinct domains. The N-terminal R (random) domain is localized to the interior of the particle. The S (shell) domain which forms the surface of the particle displays a canonical β-barrel motif. The arrangement of the N-terminal part of the subunit plays a crucial role in determining the capsid size. The CP of sobemoviruses has been reported to be necessary for cell-to-cell as well as for long-distance movement.

The molecular details of sobemovirus replication are not well understood. Recently, primer independent initiation of RNA synthesis by sesbania mosaic virus recombinant RNA-dependent RNA polymerase was demonstrated. For the minus-strand synthesis a stem-loop at the 3′ end of the genome is necessary. For the plus-strand synthesis, a conserved aCAAa motif at the 5′ end may be involved. The cellular compartments where the replication takes place are unknown.

## Antigenic properties

Virions and coat proteins of sobemoviruses are efficient immunogens. A single precipitin line is formed in gel diffusion tests. There are distant serological relationships between some members of the genus. Several serotypes with different geographical origins have been identified in some species.

## Biological properties

### Host range
Sobemoviruses infect both dicotyledonous and monocotyledonous plants. However, the natural host range of each virus species is narrow except that sowbane mosaic virus (SoMV) can infect plants from several different families. Disease symptoms are mainly mosaic and mottle of infected leaves.

### Transmission
All sobemoviruses are readily transmitted mechanically and many are also transmitted by beetles. In contrast, blueberry shoestring virus is transmitted by aphids and velvet tobacco mottle virus by

mirids. SoMV has been reported to be transmitted by insects belonging to different orders (Diptera, Hemiptera, Thysanoptera), but this is probably non-specific mechanical transmission by the mouthparts of different insects. Several viruses in the genus are seed-transmissible.

### Geographical distribution

There are members with limited distribution (one continent or one country only) but some species are found worldwide.

### Cytopathic effects

Virions are found in both the cytoplasm and nuclei, and late in infection occur as large crystalline aggregates and inclusions in the cytoplasm and the vacuoles. Infected cells show cytoplasmic vacuolization. Occasionally the association of virus particles with chloroplast membranes and changes in chloroplast structure have been reported. Sobemoviruses have been found in mesophyll, epidermal as well as bundle sheath cells. In the course of systemic infection, viruses invade the phloem as well as the xylem and for some members virions are found in pit membranes of xylem cell walls.

## Species demarcation criteria in the genus

- Genome sequence relatedness: different species have overall genome sequence identity less than about 75%
- Host range
- Serological relatedness may help in distinguishing species

## List of species in the genus *Sobemovirus*

| | | |
|---|---|---|
| *Blueberry shoestring virus* | | |
| Blueberry shoestring virus - USA | | (BSSV-US) |
| *Cocksfoot mottle virus* | | |
| Cocksfoot mottle virus - Norway | [Z48630 = NC_002618] | (CfMV-NO) |
| *Lucerne transient streak virus* | | |
| Lucerne transient streak virus - New Zealand | [U31286 = NC_001696] | (LTSV-NZ) |
| *Rice yellow mottle virus* | | |
| Rice yellow mottle virus - Côte d'Ivoire: CI63 | [AJ608207] | (RYMV-CI63) |
| *Ryegrass mottle virus* | | |
| Ryegrass mottle virus - Japan | [EF091714] | (RGMoV-JP) |
| *Sesbania mosaic virus* | | |
| Sesbania mosaic virus - India | [AY004291 = NC_002568] | (SeMV-IN) |
| *Solanum nodiflorum mottle virus* | | |
| Solanum nodiflorum mottle virus - Australia | | (SNMoV-AUS) |
| *Southern bean mosaic virus* | | |
| Southern bean mosaic virus - Brazil | [DQ875594] | (SBMV-BR) |
| *Southern cowpea mosaic virus* | | |
| Southern cowpea mosaic virus - USA | [M23021 = NC_001625] | (SCPMV-US) |
| *Sowbane mosaic virus* (Rubus chlorotic mottle virus) | | |
| Sowbane mosaic virus - USA | [GQ845002] | (SoMV-US) |
| *Subterranean clover mottle virus* | | |
| Subterranean clover mottle virus - Australia | [AY376453] | (SCMoV-MJ) |
| *Turnip rosette virus* | | |
| Turnip rosette virus - UK | [AY177608 = NC_004553] | (TRoV-UK) |
| *Velvet tobacco mottle virus* | | |
| Velvet tobacco mottle virus - Australia-K1 | [HM754263] | (VTMoV-K1) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.

## List of other related viruses which may be members of the genus *Sobemovirus* but have not been approved as species

| | | |
|---|---|---|
| Cynosurus mottle virus | | (CnMoV) |
| Ginger chlorotic fleck virus | | (GCFV) |
| Imperata yellow mottle virus | [AM990928 = NC_011536] | (IYMV) |
| Papaya lethal yellowing virus | [GU066876*] | (PLYV) |
| Snake melon asteroid mosaic virus | [HM450304*] | (SMAMV) |

*Sequence does not comprise the complete genome.

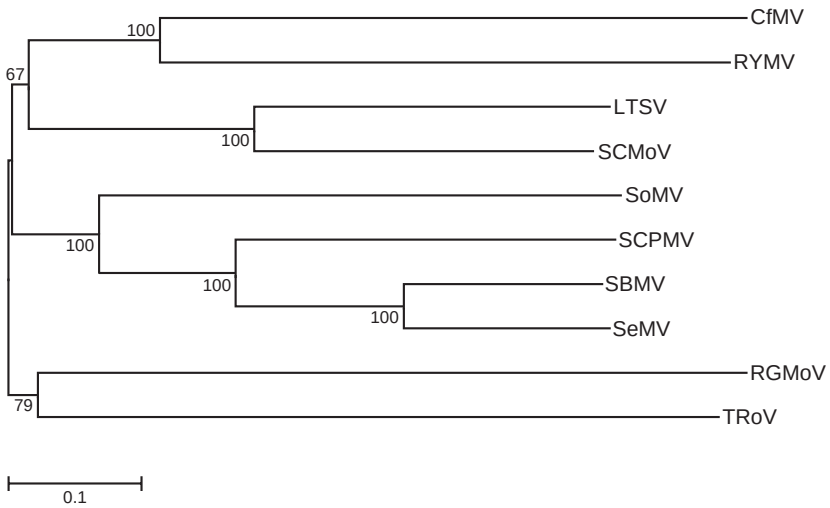


**Figure 3:** Phylogenetic tree inferred by the maximum composite likelihood model from the full sequences of members of the genus *Sobemovirus*. Mid-point rooting was applied and the percentage of bootstrap support of the branches shown at the nodes.

## Phylogenetic relationships within the genus

Phylogenetic relationships within the genus are depicted in Figure 3.

## Similarity with other taxa

The 5′ ends of the sobemoviral genomes together with ORF1 are unrelated to any other known viral genera. However, the middle parts of the genomes (encoding the successive domains Pro-VPg-RdRP) are similar to those of the genera *Polerovirus* and *Enamovirus* from the family *Luteoviridae*. In contrast, the 3′ part of the sobemoviral genome encoding the CP is more closely related to CP genes of the genus *Necrovirus* of the family *Tombusviridae*. These similarities suggest early recombination events during the evolution of these genera. This possibility is further supported by the existence of *Poinsettia latent virus*, the unique species of the genus *Polemovirus* whose sequence shows a close relationship to poleroviruses within the first three quarters of its genome (encoding the P1 and polyprotein), but rather to sobemoviruses in the last quarter (encoding the CP gene). The unique species of the family *Barnaviridae* has a genomic organization similar to that of sobemoviruses (except that it lacks ORF1 of sobemoviruses) and its Pro-VPg-RdRP and CP genes are related to homologous sequences of sobemoviruses. In addition, the putative protease and RdRp of positive sense ss RNA animal viruses of the family *Astroviridae* have similarities to those of sobemoviruses.

## Derivation of name

*Sobemo*: from the type species *Southern bean mosaic virus*.

## Further reading

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Fargette, D., Pinel, A., Abubakar, Z., Traoré, O., Brugidou, C., Fatogoma, S., Hébrard, E., Choisy, M., Séré, Y., Fauquet, C. and Konaté, G. (2004). Inferring the evolutionary history of *Rice yellow mottle virus* from genomic, phylogenetic and phylogeographic studies. *J. Virol.*, **78**, 3252–3261.

Gayathri, P., Satheshkumar, P., Prasad, K., Nair, S., Savithri, H. and Murthy, M. (2006). Crystal structure of the serine protease domain of *Sesbania mosaic virus* polyprotein and mutational analysis of residues forming the S1-binding pocket. *Virology*, **346**, 440–451.

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Mäkeläinen, K. and Mäkinen, K. (2005). Factors affecting translation at the programmed −1 ribosomal frameshifting site of *Cocksfoot mottle virus* RNA in vivo. *Nucleic Acids Res.*, **33**, 2239–2247.
Meier, M. and Truve, E. (2007). Sobemoviruses possess a common CfMV-like genomic organization. *Arch. Virol.*, **152**, 635–640.
Nair, S. and Savithri, H. (2010). Processing of SeMV polyproteins revisited. *Virology*, **396**, 106–117.
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Qu, C., Liljas, L., Opalka, N., Brugidou, C., Yeager, M., Beachy, R., Fauquet, C., Johnson, J. and Lin, T. (2000). 3D domain swapping of a molecular switch for quasi-equivalent symmetry modulates the stability of an icosahedral virus group. *Structure*, **8**, 1095–1103.

## Contributed by

Truve, E. and Fargette, D.

# Genus *Umbravirus*

Type species *Carrot mottle virus*

## Distinguishing features

Positive sense, single strand RNA viruses which do not form conventional virus particles and lack a coat protein ORF but are encapsidated with a helper virus.

## Virion properties

### Morphology

Umbraviruses do not form conventional virus particles, and the five genomes whose complete sequences are known lack plausible ORFs for capsid CPs. Umbraviruses rely on the CP of a helper virus, characteristically from a virus in the family *Luteoviridae*, for encapsidation and for transmission by the vector of the helper virus. However, in single infections by umbraviruses, the infectivity in buffer extracts of leaves is surprisingly stable, though very sensitive to treatment with organic solvents, suggesting that the infective RNA is protected in lipid-containing structures. In plants infected with carrot mottle virus (CMoV), enveloped structures about 52 nm in diameter (Figure 1A, B) occur in the vacuoles of infected cells and in partially purified preparations. These structures may be involved in virus replication and/or serve to protect the RNA. Similar structures occur in plants infected with the bean yellow vein-banding strain of *Pea enation mosaic virus-2* (BYVBV), groundnut rosette virus (GRV) and lettuce speckles mottle virus (LSMV), but no information is available for other umbraviruses. An infective fraction from GRV-infected tissues contained filamentous ribonucleoprotein (RNP) particles (Figure 1C) composed of viral RNA, the umbraviral ORF3 protein and the host nucleolar protein, fibrillarin.

### Physicochemical and physical properties

Infectivity in leaf extracts is stable for several hours at room temperature or several days at 5 °C, but is abolished by treatment with organic solvents. Partially purified preparations of CMoV consist predominantly of cell membranes but contain infective components which, because they have a sedimentation coefficient of about 270S and a buoyant density of about 1.15 g cm$^{-3}$ in CsCl, are probably the 52 nm-diameter enveloped structures observed in these preparations (Figure 1A, B). An infective fraction from GRV-infected tissue contained complexes with a buoyant density of 1.34–1.45 g cm$^{-3}$ consisting of filamentous RNP particles, composed of the umbraviral ORF3 protein, host fibrillarin and virus RNA (Figure 1C). The relationship between the enveloped and filamentous structures is unclear.

### Nucleic acid

Nucleic acid preparations made by extracting leaves with phenol are often much more infective than buffer extracts. The infective agent in these preparations is a ssRNA, but the preparations also contain abundant dsRNA. The genome consists of one linear segment of positive-sense ssRNA 4.0–4.2 kb in length. Umbravirus genomic RNAs are not polyadenylated at their 3′ ends and lack a 5′ cap structure. The 3′ UTR of pea enation mosaic virus (PEMV-2) genomic RNA contains the 100 nt cap-independent translational element which binds the eIF4E subunit of eukaryotic translation initiation factor eIF4F. In the case of GRV, a 900 nt ssRNA satellite RNA is essential for the encapsidation of the GRV genomic RNA with the coat protein of groundnut rosette assistor virus (unassigned member of the family *Luteoviridae*).

### Proteins

No conventional structural proteins are reported. The nucleotide sequences lack plausible ORFs for CPs but possess ORFs for four potential non-structural protein products (Figure 2).

### Lipids

Although no conventional virus particles are formed, the sensitivity to organic solvents, and low buoyant density, of the infective components in partially purified preparations of CMoV suggests that this infectivity is associated with lipid, probably of plant origin. The infective components probably correspond to the enveloped structures seen in sections of infected leaves.

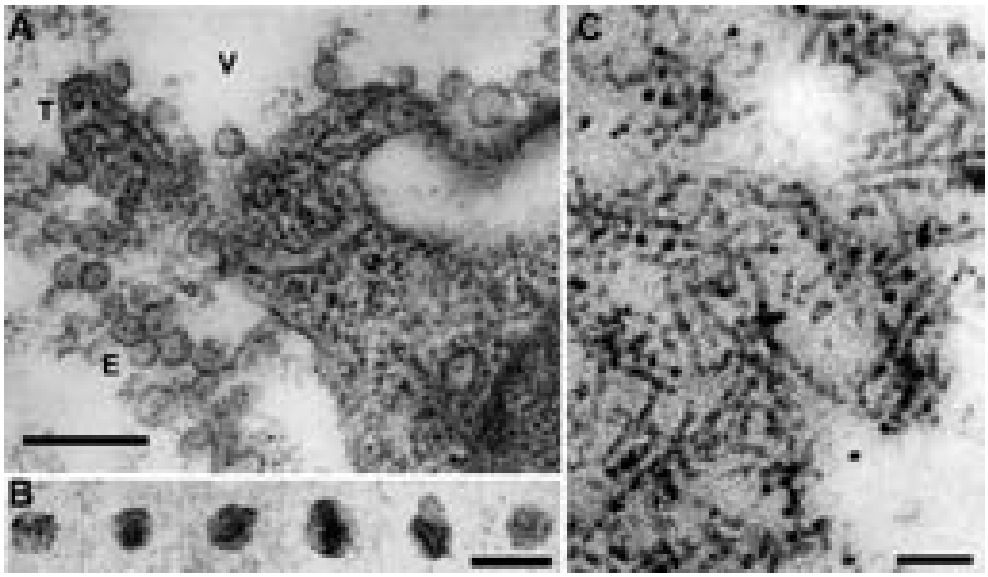


**Figure 1:** (A) Section of palisade mesophyll cell from a leaf of *Nicotiana clevelandii* systemically infected with carrot mottle virus (CMoV), showing enveloped structures (E) about 52 nm in diameter in the cell vacuole (V) in association with the tonoplast (T). The bar represents 250 nm. (B) Enveloped structures about 52 nm in diameter in a partially purified preparation from CMoV-infected *N. clevelandii*, stained with 2% uranyl acetate. The bar represents 100 nm. (C) Section of a *Nicotiana benthamiana* cell expressing the GRV ORF3 protein. The section shows filamentous ribonucleoprotein particles embedded in an electron-dense matrix. The section was labeled by *in situ* hybridization with a GRV-specific RNA probe. The bar represents 100 nm.

### Carbohydrates

None reported.

## Genome organization and replication

Figure 2 shows the genome organization of GRV; those of other umbraviruses are very similar. For each RNA, there is at the 5′ end a very short non-coding region preceding ORF1, which encodes a putative product of 31–37 kDa. ORF2, which slightly overlaps the end of ORF1, could encode a product of 63–65 kDa but lacks an AUG initiation codon near its 5′ end. However, immediately before the stop codon of ORF1 there is a 7 nt sequence that is associated with frameshifting in several plant and animal viruses, and it seems probable that ORF1 and ORF2 are translated as a single polypeptide of 94–98 kDa by a mechanism involving a −1 frameshift. The predicted product contains, in the ORF2 region, sequence motifs characteristic of viral RdRp. A short untranslated region separates ORF2 from ORF3 and ORF4, which overlap each other almost completely in different reading frames and each yield a putative product of 26–29 kDa. The ORF4 product contains sequences characteristic of plant virus MPs. The ORF3 product of different umbraviruses studied have up to 50% similarity to each other but no significant similarity to any other viral or non-viral proteins; their function is to protect viral RNA and enable its transport through the phloem.

Umbravirus-infected leaf tissue contains abundant dsRNA that is not itself infective but that becomes so when heat-denatured. Two dsRNA species are common to all umbraviruses: dsRNA-1 (ca. 4.2–4.8 kbp) and dsRNA-2 (ca. 1.1–1.5 kbp). cDNA copies of dsRNA-1 hybridize with dsRNA-2 and these molecules are thought to represent double stranded forms of, respectively, genomic and subgenomic ssRNA species. ORFs 3 and 4 are probably expressed from sgRNA. There is evidence for the presence in GRV-infected plants of two less-than-full-length RNA species of very similar size, close to that expected for such sgRNAs, and corresponding to that of dsRNA-2. The dsRNA-2 of CMoMV has been shown to include the sequences of ORFs 3 and 4, and the 3′ UTR.

Some umbraviruses possess one or more additional dsRNA species, associated in at least one instance (GRV) with the presence of a satellite RNA. PEMV-2 also has a satellite RNA, and each of these satellites can be supported by the helper virus of the other.

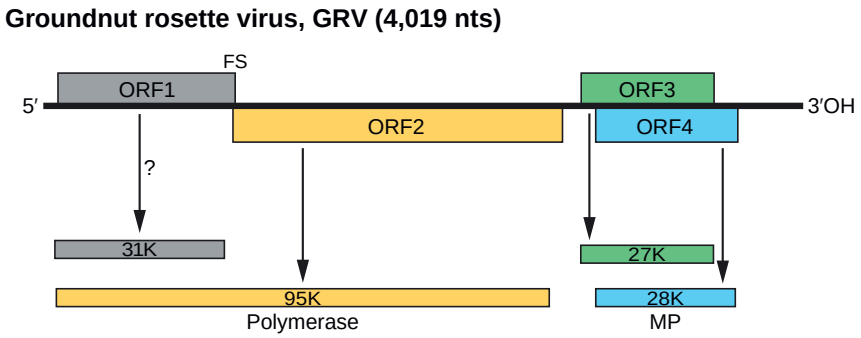


**Figure 2:** Diagram showing the genomic organization of groundnut rosette virus (GRV). The continuous horizontal line represents the genome RNA, and the numbered blocks the correspondingly numbered ORFs. The lower part of the diagram shows the predicted translation products, with their size. The potential product of ORF1 has not been shown to exist. The single product produced from ORFs 1 and 2, probably as a result of a −1 frameshift event (FS), is thought to be a polymerase because it contains, in the ORF2 region, sequences characteristic of viral RdRp. The ORF3 product functions to protect viral RNA and enable its transport through the phloem. The ORF4 product, marked MP, has a cell-to-cell movement function.

## Antigenic properties

None reported.

## Biological properties

### Host range

Individual umbraviruses are confined in nature to one or a few host plant species. Their experimental host ranges are broader but still restricted. The symptoms induced in infected plants are usually mottles or mosaics. Symptoms of GRV are greatly influenced by the associated satellite RNA.

### Transmission

Umbraviruses are transmissible, sometimes with difficulty, by mechanical inoculation. However, in nature each is dependent on a specific helper virus for transmission in a persistent (circulative, non-propagative) manner by aphids. All helper viruses that have been characterized are members of the family *Luteoviridae*. The mechanism of this dependence is encapsidation of the dependent virus RNA in the CP of the helper. In GRV, the satellite RNA plays an essential role in mediating this luteovirus-dependent aphid transmission. There is no evidence for multiplication of umbraviruses in the insect vector. Seed transmission has not been reported.

### Geographical distribution

CMoV and/or carrot mottle mimic virus and PEMV-2 apparently occur worldwide wherever their crop hosts are grown; other umbraviruses have a restricted distribution. Several umbraviruses, notably GRV, occur only in Africa.

### Pathogenicity

Although all umbraviruses depend on a helper virus, a member of the family *Luteoviridae*, for transmission by vector insects, several of them are as important as, or more important than, their helpers in the causation of disease symptoms. The umbravirus of greatest economic importance is GRV, which causes the most devastating virus disease of groundnut (peanut) in Africa. However, in this case it is a GRV-dependent satellite RNA that is the actual cause of the symptoms. In most instances umbraviruses have not been shown to contribute functions essential for the biological success of their associated helper viruses. However, a notable exception is PEMV-2, which is essential for the systemic spread of PEMV-1 in plants. PEMV-2 even allows PEMV-1 and another member of the family *Luteoviridae*, potato leafroll virus, to spread out of the phloem into mesophyll tissue and thereby to become transmissible by manual inoculation. Similarly, another member of the *Luteoviridae*, beet western yellows virus, has been reported to show limited manual transmissibility when in the presence of the umbravirus LSMV.

### Cytopathic effects

Umbraviruses, even in the absence of their helper viruses, exhibit rapid systemic spread in plants. They infect cells throughout the leaf, though presumably the aphid transmissible particles are restricted to the same tissues (in most instances the phloem) as the luteoviruses that provide their CP. In mesophyll cells infected with CMoV there is extensive development of cell wall outgrowths sheathing elongated plasmodesmatal tubules. The ORF3 protein of GRV targets and reorganizes Cajal bodies (CB) into multiple CB-like structures and then enters the nucleolus by causing fusion of these structures with the nucleolus. The nucleolar localization of the ORF3 protein is essential for subsequent formation of viral RNP particles capable of virus long-distance movement and systemic infection.

## Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

- Natural host range
- dsRNA band pattern (bearing in mind that some bands may represent satellite RNA species)
- Nucleotide sequence identity less than 70%
- Little or no hybridization with cDNA probes representing most parts of the genome

## List of species in the genus *Umbravirus*

| | | |
|---|---|---|
| *Carrot mottle mimic virus* | | |
| Carrot mottle mimic virus-Australia | [U57305 = NC_001726] | (CMoMV-AU) |
| *Carrot mottle virus* | | |
| Carrot mottle virus-Weddel | [FJ188473 = NC_011515] | (CMoV-We) |
| *Groundnut rosette virus* | | |
| Groundnut rosette virus-MC1 | [Z69910 = NC_003603] | (GRV-MC1) |
| *Lettuce speckles mottle virus* | | |
| Lettuce speckles mottle virus-California | | (LSMV-CA) |
| *Pea enation mosaic virus-2* | | |
| Bean yellow vein-banding virus | | (BYVBV) |
| Pea enation mosaic virus-2-WGS | [U03563] | (PEMV-2-WGS) |
| *Tobacco bushy top virus* | | |
| Tobacco bushy top virus-Baoshan | [AF431890 = NC_004366] | (TBTV-Bao) |
| *Tobacco mottle virus* | | |
| Tobacco mottle virus-Zimbabwe | [AY007231*] | (TMoV-ZW) |

Species names are in italic script; names of isolates and strains are in roman script. Sequence accession numbers [ ] and assigned abbreviations ( ) are also listed.
*Sequence does not comprise the complete genome.

## List of other related viruses which may be members of the genus *Umbravirus* but have not been approved as species

| | | |
|---|---|---|
| Opium poppies mosaic virus | [EU151723*] | (OPMV) |
| Sunflower crinkle virus | | (SuCV) |
| Sunflower yellow blotch virus | | (SuYBV) |
| Tobacco yellow vein virus | | (TYVV) |

*Sequence does not comprise the complete genome.

## Similarity with other taxa

Amino acid sequence comparisons show that the putative RdRps encoded by the genomic RNA of umbraviruses belong to the so-called supergroup 2 of RNA polymerases, as do those of viruses in the genera *Carmovirus*, *Dianthovirus*, *Luteovirus*, *Machlomovirus*, *Necrovirus* and *Tombusvirus* (Figure 3). Since these enzymes are the only universally conserved proteins of positive strand RNA viruses, the genus *Umbravirus* might be considered to be in or close to the family *Tombusviridae*.

## Derivation of names

*Umbra*: from Latin *umbra*, "a shadow". In English, a shadow is an uninvited guest that comes with an invited one.

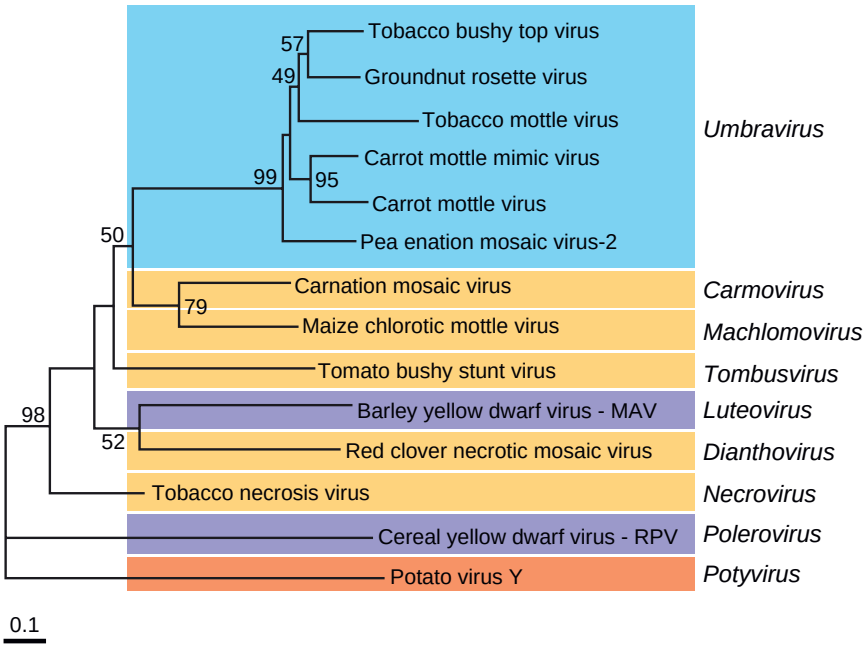


**Figure 3:** Phylogenetic relationships of the RdRps of umbraviruses and some other plant viruses. Amino acid sequences were aligned using the ClustalX2 program. The neighbour-joining trees were produced and bootstrapped using the PHYLIP package programs. Numbers at the nodes represent bootstrap values as percentages obtained from 2000 replications, shown only for branches supported by more than 40%. Length of branches is proportional to number of changes.

## Further reading


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Wang, Z., Treder, K. and Miller, W.A. (2009). Structure of a viral cap-independent translation element that functions via high affinity binding to the eIF4E subunit of eIF4F. *J. Biol. Chem.*, **284**, 14189–14202.


## Contributed by

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# Unassigned Viruses

Although many viruses have been classified into genera in this Report, a number of relatively well-characterized viruses are yet, for various reasons, to be assigned to existing genera/families or have been sufficiently distinguished from recognized viruses to form types and members for new genera. Some examples are listed here for which certain key characteristics are known, most notably a significant amount of sequence data along with some well-described biological and biophysical properties. Some of the unassigned viruses listed in the Eighth ICTV Report are now classified or are in the process of being classified into recognized taxa. There are, however, a number of viruses that are new to this list and the emphasis has been placed upon those viruses that are likely to represent as yet undescribed genera and families, and that underline the fact that developing a universally applicable virus taxonomy is a continuously evolving process.

# Archaeal Viruses

## Haloarcula phage SH1

This icosahedral phage was originally isolated from the archaeon *Haloarcula hispanica* and have virions that have no visible tail and are about 70 nm in diameter. The virions contain a genome of linear dsDNA of approximately 30 kb in length, and a lipid envelope. The virions have a T = 28 symmetry, which is shared with another archaeal virus, Thermus phage P23–77. The genome shares little homology with any other virus but the structure and lipid content indicates distant relationships with P23–77-like phages and the bacterial tectiviruses.

Sequence accession number: AY950802.

Bamford, D.H., Ravantti, J.J., Ronnholm, G., Laurinavicius, S., Kukkaro, P., Dyall-Smith, M., Somerharju, P., Kalkkinen, N. and Bamford, J.K. (2005). Constituents of SH1, a novel lipid-containing virus infecting the halophilic euryarchaeon Haloarcula hispanica. *J. Virol.*, **79**, 9097–9107.

## Haloarcula hispanica pleomorphic virus 1 (HHPV-1)

This pleiomorphic virus was isolated from the halophilic archaeon *Haloarcula hispanica*, and contains a circular dsDNA genome of around 8 kbp. The organization of the genome shows remarkable synteny and amino acid sequence similarity to the genome and predicted proteins of the archaeal virus HRPV-1, a ssDNA containing pleiomorphic virus from a *Halorubrum* sp. These viruses have no known relationships to any described genus.

Sequence accession number: GU321093.

Roine, E., Kukkaro, P., Paulin, L., Laurinavicius, S., Domanska, A., Somerharju, P. and Bamford, D.H. (2010). New, closely related haloarchaeal viral elements with different nucleic acid types. *J. Virol.*, **84**, 3682–3689.

## Halorubrum pleomorphic virus 1 (HRPV-1)

Originally isolated from a host belonging to the genus *Halorubrum*, this virus has an enveloped virion with pleiomorphic morphology and is approximately 44 × 55 nm in size. Like the archaeal virus HHPV-1, HRPV-1 has a ssDNA genome, in this instance of about 7 kb. The pleomorphic membrane vesicle carries the two major virion proteins: VP4 forms glycosylated spikes on the virion surface and VP3 resides on the inner surface of the membrane vesicle. Related to HHPV-1 but otherwise has no known relationships.

Sequence accession number: FJ685651.

Pietila, M.K., Roine, E., Paulin, L., Kalkkinen, N. and Bamford, D.H. (2009). A ssDNA virus infecting archaea: a new lineage of viruses with a membrane envelope. *Mol. Microbiol.*, **72**, 307–319.

### Thermus phage IN93

### Thermus phage P23-77

These two tailless icosahedral phages were originally isolated from the thermophilic archaea *Thermus aquaticus* and *T. thermophilus*. The virions are about 90 nm in diameter from vertex to vertex and have 15 nm projections on the vertices. The virion has T= 28 architecture and contains an internal lipid envelope and a circular dsDNA genome of 17–19 kbp. The two viruses have very similar ORF composition and translated sequences of the virion structural protein genes available show about 75% amino acid identity. These viruses share the unusual T=28 architecture with the archaeal Haloarcula phage SH1.

Sequence accession numbers: AB063393 (IN93), GQ403789 (P23-77).

Jalasvuori M., Jaatinen S.T., Laurinavicius S., Ahola-Iivarinen E., Kalkkinen N., Bamford D.H. and Bamford J.K. (2009). The closest relatives of icosahedral viruses of thermophilic bacteria are among viruses and plasmids of the halophilic archaea. *J. Virol.*, **83**, 9388–9397.

## Bacterial Viruses

### Mycoplasma phage MAV1

Mycoplasma phage MAV1 is a lysogenic phage of *Mycoplasma arthritidis* with ds linear DNA genome of about 16 kb. Lysogenic bacterial strains have been observed to have higher virulence in a murine arithritis model than non-lysogenic strains and a putative virulence factor in the phage genome has been identified; more recent studies have however questioned the effect of MAV1 on virulence in this model. The phage can be grown in a plaquing system and while isolated virions have not been observed, they are known to be proteinase K resistant.

Sequence accession number: AF074945.

Maniloff, J. and Dybvig, K. (2006). Mycoplasma phages. In R. Carpenter (Ed.), *The Bacteriophages.* New York, Oxford University Press, pp. 636–652.

### Staphylococcus phage P954

### Staphylococcus phage ROSA

### Staphylococcus phage PT1028

Staphylococcus phage P954 and Staphylococcus phage ROSA are phages of *Staphylococcus aureus* with dsDNA genomes of about 40 kbp. They show close relationships to a number of other phages with similar sized genomes isolated from the same host. Although the morphology of P954 and ROSA have not been reported, related viruses show a morphotype with isometric heads and long non-contractile tails. These phages and their relatives are probably members of the *Siphoviridae*. Staphylococcus phage PT1028 is a phage of *Staphylococcus aureus* with an unknown morphology and a dsDNA genome of around 20 kbp that shows little relationship to any other phage.

Sequence accession numbers: GQ398772 (P945), AY954961 (ROSA), AY954948 (PT1028).

Kwan, T., Liu, J, DuBow, M., Gros, P. and Pelletier, J. (2005). The complete genomes and proteomes of 27 *Staphylococcus areus* bacteriophages. *Proc. Natl Acad. Sci., U S A*, **102**, 5174–5179.

### Stenotrophomonas phage phiSMA9

A filamentous phage of the opportunistic nosocomial pathogen *Stenotrophomonas maltophilia*, this phage has a genome of 6.9 kb that encodes seven genes, the largest being an orthologue of the gene encoding the toxin of the filamentous bacteriophage of *Vibrio cholerae.*

Sequence accession number: AM040673.

Hagemann, M., Hasse, D. and Berg, G. (2006). Detection of a phage genome carrying a zonula occludens like toxin gene (zot) in clinical isolates of *Stenotrophomonas maltophilia*. *Arch. Microbiol.*, **185**, 449–458.

# Fungal Viruses

## Curvularia thermal tolerance virus (CThTV)

Curvularia thermal tolerance virus has a bipartite genome comprising two dsRNA segments, 2.2 and 1.8 kbp in size. A defective dsRNA of less than 1 kbp may also be present. Isometric particles, 27 nm in diameter, can be isolated from the infected fungal host and are thought to package the genomic dsRNAs. Each of the genomic dsRNAs has two ORFs; dsRNA1 ORF codes for replication proteins. The two ORFs of dsRNA 2 have no similarity to any known protein. Infection of the fungal endophyte *Curvularia protuberata* with CThTV confers thermal tolerance to its tropical panic grass host and allows both fungus and plant to grow at high soil temperatures.

Sequence accession numbers: EF120984/5.

Márquez, L.M., Redman, R.S., Rodriguez, R.J. and M.J. Roossinck. (2007). A virus in a fungus in a plant: Three-way symbiosis required for thermal tolerance. *Science*, **315**, 513–515.

## Diaporthe RNA virus (DRV)

Diaporthe RNA virus (DRV) was originally named Diaporthe ambigua RNA virus (DaRV). However, the host fungus was later correctly identified as *Diaporthe perjuncta*, not *D. ambigua*. No particles are associated with DRV and its ssRNA genome is 4,113 nt in length. DRV is associated with hypovirulence of its fungal host. It has two large ORFs present in the same reading frame, which are most likely translated by readthrough of a UAG stop codon in the central part of the genome. The longest possible translation product has a predicted molecular mass of about 125 kDa, which shows significant similarity to the nonstructural proteins of carmoviruses of the positive-strand RNA virus family *Tombusviridae*. Transcripts derived from full-length cDNA clones are infectious when inoculated to spheroplasts.

Sequence accession number: AF142094.

Moleleki, N., van Heerden, S.W., Wingfield, M.J., Wingfield, B.D. and Preisig, O. (2003). Transfection of *Diaporthe perjuncta* with Diaporthe RNA virus. *Appl. Environ. Microbiol.*, **69**, 3952–3956.

## Fusarium graminearum virus DK21 (FgV-DK21)

Fusarium graminearum virus DK21 confers a hypovirulent phenotype to its plant pathogenic fungal host *Fusarium graminearum*. The RNA genome (6624 nt in length) comprises five putative ORFs (ORF1-5) encoding proteins of 174, 17, 6.2, 4.8, and 41 kDa, respectively. The 5′ and 3′ UTRs are 53 and 46 nt in length. ORF1 encodes a putative RNA-dependent RNA polymerase, which is phylogenetically related to hypoviruses. The genome organization and expression strategy of FgV-DK21, however, are more similar to those of the plant ssRNA alphaflexiviruses. The putative proteins encoded in ORFs 2 through 5 appear to be expressed from at least two different subgenomic RNAs. No typical virions are associated with FgV-DK21.

Sequence accession number: AY533037.

Kwon, S.-J., Lim, W.-S., Park, S.-H., Park, M.-R. and Kim, K.-H. (2007). Molecular characterization of a dsRNA mycovirus, Fusarium graminearum virus-DK21, which is phylogenetically related to hypoviruses but has a genome organization and gene expression strategy resembling those of plant potex-like viruses. *Mol. Cells*, **23**, 304–315.

## Fusarium graminearum virus 3 (FgV3)

No particles are associated with FgV3. Its dsRNA genome comprises 9,098 bp and contains two ORFs; ORF1 codes for a protein of unknown function (145 kDa) and ORF2 codes for a putative RNA-dependent RNA polymerase (RdRp; 151 kDa). The two ORFs are separated by 143 nucleotides. The 5′ and 3′ UTRs are 865 and 44 bp long, respectively. Although FgV3 is closely related phylogenetically to members of the families *Totiviridae* and *Chrysoviridae*, it is placed outside of

their main clusters. FgV3 and FgV4 can co-infect *Fusarium graminearum*, the causal agent of important head and seedling blights of small grains.

Sequence accession number: GQ140626.

Yu, J., Kwon, S.-J., Lee, K.-M., Son, M. and Kim, K.-H. (2009). Complete nucleotide sequence of double-stranded RNA viruses from *Fusarium graminearum* strain DK3. *Arch. Virol*., **154**, 1855–1858.

## Fusarium graminearum virus 4 (FgV4)

The genome of FgV4 comprises two dsRNA segments (dsRNAs 1 and 2) of 2,383 bp and 1,739 bp, respectively. FgV4 dsRNA1 contains a single ORF, which has a conserved RdRp motif, whereas dsRNA2 contains two putative ORFs coding for products of unknown function. The 5′ and 3′ UTRs of dsRNAs 1 and 2 share conserved sequences, including stretches of 48 and 67 nucleotides with 100% identity. FgV4 does not code for a capsid protein and no typical virions can be isolated. FgV4 forms a distinct clade within the family *Partitiviridae.*

Sequence accession numbers: GQ140627/8.

Yu, J., Kwon, S.-J., Lee, K.-M., Son, M. and Kim, K.H. (2009). Complete nucleotide sequence of double-stranded RNA viruses from *Fusarium graminearum* strain DK3. *Arch. Virol*., **154**, 1855–1858.

## Oyster mushroom spherical virus (OMSV)

Virions are isometric 27 nm in diameter containing a monopartite positive sense ssRNA genome (5784 nt in length) and a coat protein of approximately 28.5 kDa. The OMSV genome comprises seven ORFs; ORF1 has the motifs of RNA-dependent RNA polymerases (RdRp), and helicase and ORF2 encodes a coat protein. None of the putative polypeptides potentially encoded by ORFs 3–7 have similarities to any known proteins. ORFs 3 to 6 are located within ORF1 sequence with a −1 frameshift, whereas ORF7 overlaps ORF2. Genomic organization and amino acid sequence analysis of RdRp and helicase domains are most similar to those of tymoviruses. OMSV is associated with a devastating oyster mushroom die-back disease.

Sequence accession number: AY182001.

Yu, H.J., Lim, D. and Lee, H.-S. (2003). Characterization of a novel single-stranded RNA mycovirus in *Pleurotus ostreatus*. *Virology*, **314**, 9–15.

## Sclerophthora macrospora virus A (SmV-A)

Virions are isometric 30 nm in diameter and contain three segments of positive-sense ssRNA (RNAs 1, 2, and 3). RNA 1 (2928 nt) contains the RdRp motif and RNA 2 (1981 nt) codes for a capsid protein. RNA 3 (977 nt) is a satellite RNA. Sequence analysis of RdRp (100 kDa) shows similarity to RdRps of nodaviruses. The amino acid sequence of the viral CP, on the other hand, shows similarity to those of members in the family *Tombusviridae*. The capsid of SmV-A comprises two capsid proteins, CP1 (43 kDa) and CP2 (39 kDa), both encoded in ORF2. CP2 is derived from CP1 via proteolytic cleavage. The genome organization of SmV-A is distinct from those of other known fungal RNA viruses.

Sequence accession numbers: AB083060-62.

Yokoi T., Yamashita S. and Hibi, T. (2003). The nucleotide sequence and genome organization of *Sclerophthora macrospora* virus A. *Virology*, **311**, 394–399.

## Sclerophthora macrospora virus B (SmV-B)

Particles are isometric, 32 nm in diameter, containing a monopartite positive sense ssRNA genome. The viral genome (5533 nt) has two large ORFs: ORF1 encodes a putative polyprotein (145 kDa) containing the motifs of chymotrypsin-related serine protease and RdRp, and ORF2 encodes a capsid protein (41 kDa). The genome arrangement of SmV-B is similar to those belonging to the genera *Sobemovirus*, *Barnavirus* and *Polerovirus*. The putative domains for the serine protease, VPg, RdRp, and the CP are located in this order from the 5′ terminus to the 3′ terminus. SmV B, however, is

distinctive since its genome has only two ORFs. *S. macrospora*, the plant pathogenic fungal host of SMV-B, is the causal agent of downy mildew of gramineous plants.

Sequence accession number: AB012756.

Yokoi, T., Takemoto, Y., Suzuki, M., Yamashita, S. and Hibi, T. (1999). The nucleotide sequence and genome organization of *Sclerophthora macrospora* virus B. *Virology*, **264**, 344–349.

### Sclerotinia sclerotiorum hypovirulence-associated DNA virus 1 (SsHADV-1)

SsHADV-1 is the first reported DNA mycovirus (viruses infecting fungi). It has a circular ssDNA genome of 2166 nt, coding for a replication initiation protein (Rep) and a coat protein (CP). SsHADV-1 is phylogenetically related to the geminiviruses based on Rep sequences. However, it is distinct from geminiviruses both in genome organization and particle morphology. Particles are non-twinned, isometric, 20–22 nm in diameter. SsHADV-1 confers hypovirulence to its plant pathogenic fungal host *Sclerotinia sclerotiorum*. Purified SsHADV-1 particles or viral DNA, isolated directly from mycelium, are infectious when transfected to virus-free fungal protoplasts. Transfected isolates exhibit the hypovirulence phenotype typical of the naturally infected isolate.

Sequence accession number: GQ365709.

Yua, X., Li, B., Fu, Y., Jiang, D., Ghabrial, S.A., Li, G., Peng, Y., Xie, J., Cheng, J., Huang, J. and Yi, X. (2010). A geminivirus-related DNA mycovirus that confers hypovirulence to a plant pathogenic fungus. *Proc. Natl Acad. Sci., U S A*, **107**, 8387–8392.

### Sclerotinia sclerotiorum RNA virus L (SsRV-L)

This virus is one of a number cloned and sequenced from dsRNAs isolated from a debilitated strain of the plant pathogenic fungus *Sclerotinia sclerotiorum*. The sequence of 6,043 nt has a single ORF encoding a protein with significant similarity to the replication proteins of the "alphavirus-like" supergroup of positive-strand RNA viruses. Phylogenetic analyses suggest a relationship to positive sense ssRNA viruses infecting plants (clostero-, beny- and tobamoviruses), insects (omegatetraviruses) and vertebrates (hepeviruses). There is evidence that the RNA can replicate independently within its fungus host but appears to have little effect on its growth. No virions have been observed and the sequence lacks any ORF that might encode a coat protein.

Sequence accession number: EU779934.

Liu, H., Fu, Y., Jiang, D., Li, G., Xie, J., Peng, Y., Yi, X. and Ghabrial, S.A. (2009). A novel mycovirus that is related to the human pathogen *Hepatitis E virus* and rubi-like viruses. *J. Virol.*, **83**, 1981–1991.

## Invertebrate Viruses

### Acyrthosiphon pisum virus (APV)

Acyrthosiphon pisum virus particles are icosahedral and 31 nm in diameter. The virus was isolated from *Acyrthosiphon pisum* (*Hemiptera: Aphididae*) but is able to infect many other aphid species. The virus capsid is composed of a major protein of 34 kDa and three minor proteins of 23, 24 and 66 kDa. The genome has been completely sequenced and consists of a single stranded polyadenylated ssRNA molecule of approximately 10 kb. The genome contains two large ORFs with ORF2 overlapping ORF1. The latter is thought to be expressed by a −1 translational frameshift. The CPs are encoded at the 3′ end of ORF1 and the 5′ end of ORF2. The ORF1 product contains motifs characteristic for RdRp, Hel and cysteine proteases.

Sequence accession number: AF024514.

Van der Wilk, F., Dullemans, A.M., Verbeek, M. and van den Heuvel, J.F.J.M. (1997). Nucleotide sequence and genomic organization of Acyrthosiphon pisum virus. *Virology*, **238**, 353–362.

## Chronic bee paralysis virus (CBPV)

The virus was first isolated from honey bees, *Apis mellifera* (*Hymenoptera*: *Apidae*), in the United Kingdom. The virions have an unusual anisometric shape and are heterogeneous in size, usually of about 60 nm in length but ranging in diameter from 20 to 30 nm. Purified virion preparations contain two ssRNA species (3674 nt and 2305 nt), the larger encoding the RdRp. Virions contain a single structural protein of 23.5 kDa. The virus is readily transmitted orally to adult honey bees and has been identified in colonies almost everywhere that honey bees are maintained.

Sequence accession numbers: EU122229/30.

Ribière, M., Olivier, V. and Blanchard P. (2010). Chronic bee paralysis: a disease and a virus like no other? *J. Invertebr. Pathol.*, **103** Suppl. 1, S120–S131.

## Drosophila A virus (DAV)

Originally isolated from a laboratory colony of *Drosophila melanogaster* in France, it has subsequently been found widely distributed in laboratory and natural populations of *Drosophila* spp. from around the world. The virions are 30 nm in diameter, have icosahedral T = 3 symmetry and comprise a single major coat protein of 42 kDa. The ssRNA genome is 4806 nucleotides long and contains two ORFs: one (5′) encoding an RdRp, and the other the major coat protein. The RdRp has a permuted organization similar to that found in members of the *Birnaviridae* and *Tetraviridae*.

Sequence accession number: FJ150422.

Ambrose, R.L., Lander, G.C., Maaty, W.S., Bothner, B., Johnson, J.E. and Johnson, K.N. (2009). Drosophila A virus is an unusual RNA virus with a T = 3 icosahedral core and permuted RNA-dependent RNA polymerase. *J. Gen. Virol.*, **90**, 2191–2200.

## Gryllus bimaculatus nudivirus (GbNV)

Gryllus bimaculatus nudivirus infects nymphs and adults of the cricket *Gryllus bimaculatus*. Like the other nudiviruses, Heliothis zea nudivirus 1 (HzNV-1) and Oryctes rhinoceros nudivirus (OrNV), the virions of GbNV have enveloped rod-shaped nucleocapsids similar in size to those of baculoviruses. The circular dsDNA genome of GbNV is about 97 kbp and encodes approximately 100 ORFs, 33 of which are shared with the other nudiviruses. Previously considered to be non-occluded baculoviruses, the nudiviruses are a distinct sister-group to the baculoviruses that probably warrant separate taxonomic treatment.

Sequence accession number: EF203088.

Wang, Y. and Jehle, J.A. (2009). Nudiviruses and other large, double-stranded circular DNA viruses of invertebrates: new insights on an old topic. *J. Invertebr. Pathol.*, **101**, 187–193.

## Heliothis zea virus 1 (HzV-1)

## Gonad specific virus (Hz2-V)

Heliothis zea virus 1 (also known as Heliothis zea nudivirus 1; HzNV-1) was isolated as a persistent virus of an insect cell line derived from *Helicoverpa zea* (*Lepidoptera: Noctuidae*), although the virus can also infect a number of other insect (lepidopteran) cell lines. Related to the nudiviruses Gryllus bimaculatus nudivirus and Oryctes rhinoceros nudivirus (OrNV), the HzV-1 genome consists of a single molecule of circular dsDNA, approximately 228 kbp in length. The closely related Gonad specific virus Hz2-V is found in larvae and adults of *Helicoverpa zea* and is associated with an agonadal disease.

Sequence accession number: AF451898.

Burand, J. (1998). Nudiviruses. In L. Miller and L.A. Ball (Eds.), *The Insect Viruses.* New York: Plenum Press. pp. 69–90.

## Kelp fly virus (KFV)

The virus was originally isolated from kelp fly, *Chaetocoelopa sydneyensis* (Diptera: Coelopidae) collected in New South Wales, Australia. The virus has isometric particles 29 nm in diameter with surface projections that gives the particles the appearance of a small reovirus. The genome comprises ssRNA of about 11kb. The genome encodes a single ORF with the CPs (75 and 28 kDa) towards the 5′ end of the genome and the RdRp towards the 3′ end. KFV is a structurally distinctive virus that is possibly a member of the *Picornavirales*.

Sequence accession number: DQ112227.

Hartley, C.J., Greenwood, D.R., Gilbert, R.J., Masoumi, A., Gordon, K.H., Hanzlik, T.N., Fry, E.E., Stuart, D.I. and Scotti, P.D. (2005). Kelp fly virus: a novel group of insect picorna-like viruses as defined by genome sequence analysis and a distinctive virion structure. *J. Virol.*, **79**, 13385–13398.

## Nora virus (NV)

Isolated from *Drosophila melanogaster,* the virions of Nora virus are approximately 30 nm in diameter. The virions contain a single species of a 11908 nt polyadenylated ssRNA, encoding four ORFs. The RdRp (ORF2) is towards the 5′ end (the other three ORFs are not closely related to any other viral sequences), and the putative coat protein encoding sequences are at the 3′ end of the genome. NV occurs commonly as a persistent infection in laboratory stocks of drosophila, the major site of replication is the intestine and the virus is readily transmitted horizontally.

Sequence accession number: DQ321720.

Habayeb, M.S., Ekengren, S.K. and Hultmark, D. (2006). Nora virus, a persistent virus in *Drosophila*, defines a new picorna-like virus family. *J. Gen. Virol.*, **87**, 3045–3051.

## Solenopsis invicta virus 2 (SINV-2)

SINV-2 was originally isolated from the red imported fire ant *Solenopsis invicta* and is an icosahedral ssRNA-containing virus with a genome of about 11 kb. The genome is organized with the structural proteins at the 5′ end and the RdRp at the 3′ end (like the picornaviruses and iflaviruses) but with an apparent polycistronic structure like the dicistroviruses. SINV-2 is a probable member of the *Picornavirales* but it is phylogenetically distinct from all other viruses in the order.

Sequence accession number: EF428566.

Valles, S.M., Strong, C.A. and Hashimoto, Y. (2007). A new positive-strand RNA virus with unique genome characteristics from the red imported fire ant, *Solenopsis invicta*. *Virology*, **365**, 457–463.

## Solenopsis invicta virus 3 (SINV-3)

An isometric virus approximately 27 nm in diameter with distinct surface projections, this ssRNA-containing virus of the red imported fire ant, *Solenopsis invicta*, has a genome of about 11.5 kb and a genomic organization similar to the dicistroviruses (5′RdRp, 3′CPs). The two coat proteins are approximately 41 and 36 kDa and appear to be driven by an IRES element (also like the dicistroviruses). However, phylogenetically SINV3, appear to be only distantly related to currently known dicistroviruses and its probable affinities are poorly understood.

Sequence accession number: FJ528584.

Valles, S.M. and Hashimoto, Y. (2009). Isolation and characterization of Solenopsis invicta virus 3, a new positive-strand RNA virus infecting the red imported fire ant, *Solenopsis invicta*. *Virology*, **388**, 354–361.

# Plant Viruses

## Japanese holly fern mottle virus (JHFMoV)

The virus has quasi-spherical particles 30–40 nm in diameter and two genomic ssRNAs of about 6.2 and 3.0 kb. It causes a disease in Japanese holly fern (*Cyrtomium falcatum*), and can be transmitted by grafting and through spores from infected plants. The larger RNA encodes a 214 kDa replication polyprotein and a putative 12 kDa protein of unknown function. RNA2 encodes three proteins: a 32 kDa movement protein, a 37 kDa protein and a 29 kDa coat protein. In phylogenetic analyses, the replication protein and movement protein show some relationships to those of ssRNA+viruses infecting angiosperms, more particularly in the genera *Idaeovirus* and *Umbravirus* respectively. The unique genome organization and distinctive host make this a candidate for a novel genus.

Sequence accession numbers: FJ907327/8.

Valverde, R.A. and Sabanadzovic, S. (2009). A novel plant virus with unique properties infecting Japanese holly fern. *J. Gen. Virol.*, **90**, 2542–2549.

## Orchid fleck virus (OFV)

Preparations of purified virus contain bacilliform and non-enveloped particles of 150 ×40 nm. Enveloped particles are sometimes seen in infected plant tissues. The genome comprises two molecules: RNA1 (6,413 nt) and RNA2 (6,001 nt). The RNAs have conserved and complementary terminal sequences. RNA1 contains five ORFs, and RNA2 has a single ORF encoding an RNA polymerase. Some of the encoded proteins, particularly the polymerase, have sequences similar to those of proteins of rhabdoviruses. OFV was previously classified as a tentative rhabdovirus and it has been suggested that it should be included in a new genus named *Dichorhabdovirus*. However, this poses a taxonomic challenge because the family *Rhabdoviridae* is included in the order *Mononegavirales* and includes only viruses with undivided genomes.

Sequence accession numbers: AB244417/8.

Kondo, H., Maeda, T., Shirako, Y. and Tamada, T. (2006). Orchid fleck is a rhabdovirus with an unusual bipartite genome. *J. Gen. Virol.*, **87**, 2413–2416.

## Southern tomato virus (STV)

The virus has a monopartite dsRNA genome of 3.5 kbp and is consistently associated with a disease of field and glasshouse-grown tomatoes in California, Mexico and Mississippi. No virions have been detected. The genome has two partially overlapping ORFs with a genomic organization resembling members of the family *Totiviridae*. However, there is very little sequence similarity to totiviruses (which infect fungi and protozoa) and a similarly remote relationship to members of the family *Partitiviridae*, some of which infect plants but which have a divided genome. The virus was efficiently transmitted by seed but not mechanically or by grafting.

Sequence accession number: EF442780.

Sabanadzovic, S., Vlaverde, R.A., Brown, J.K., Martin, R.R. and Tzanetakis, I.E. (2009). Southern tomato virus: the link between the families *Totiviridae* and *Partitiviridae*. *Virus Res.*, **140**, 130–137.

# Vertebrate Viruses

## Nyamanini virus (NYMV)

## Midway virus (MIDWV)

Nyamanini virus was first isolated from *Bubulcus ibis* (cattle egret) collected in 1957 in South Africa and has since been widely isolated from cattle egrets and ticks in Africa. Serologically related to

Midway Virus (originally isolated in 1966 from seabird ticks collected on several Pacific islands) both viruses have negative sense ssRNA genomes of about 11.5 kbp contained within roughly spherical, pleiomorphic enveloped virions of between 100 and 130 nm in diameter. Both viruses are pathogenic for certain laboratory vertebrates and cell cultures. Phylogenetic analysis of the deduced nucleocapsid and viral polymerase proteins indicate that these viruses form a distinct lineage in the order *Mononegavirales*.

Sequence accession numbers: FJ554526 (NYMV); FJ554525 (MIDWV).

Mihindukulasuriya, K.A., Nguyen, N.L., Wu, G., Huang, H.V., da Rosa, A.P., Popov, V.L., Tesh, R.B. and Wang, D. (2009). Nyamanini and midway viruses define a novel taxon of RNA viruses in the order *Mononegavirales*. *J. Virol.*, **83**, 5109–5116.

## Sea turtle tornovirus 1 (STTV1)

Sea turtle tornovirus 1 is a putative virus isolated from fibropapillomas collected from a green sea turtle. Identified in a fraction with buoyant density in CsCl of between 1.2 and 1.5 g cm$^{-3}$, it has a circular genome of approximately 1.8 kb. STTV1 has only weak amino acid level identities (25%) to chicken anaemia virus in short regions of its genome but most of the genome shows no homology with any other viral sequences.

Sequence accession numbers: EU867823.

Ng, T.F., Manire, C., Borrowman, K., Langer, T., Ehrhart, L. and Breitbart, M. (2009). Discovery of a novel single-stranded DNA virus from a sea turtle fibropapilloma by using viral metagenomics. *J. Virol.*, **83**, 2500–2509.

## Chimpanzee stool-associated circular virus (ChiSCV)

Originally isolated from stool samples collected from wild-living chimpanzees, chimpanzee stool-associated circular virus (ChiSCV) has a single-stranded circular DNA genome with organizational similarities to vertebrate circoviruses and plant geminiviruses, but with a different location for the stem-loop structure involved in rolling circle DNA replication.

Sequence accession numbers: GQ351272-78.

Blinkova, O., Victoria, J., Li, Y., Keele, B.F., Sanz, C., Ndjango, J.B., Peeters, M., Travis, D., Lonsdorf, E.V., Wilson, M.L., Pusey, A.E., Hahn, B.H. and Delwart, E.L. (2010). Novel circular DNA viruses in stool samples of wild-living chimpanzees. *J. Gen. Virol.*, **91**, 74–86.

## Contributed by

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# Satellites and Other Virus-dependent Nucleic Acids

## Definitions

Satellites are subviral agents which lack genes that could encode functions needed for replication. Thus for their multiplication they depend on the co-infection of a host cell with a helper virus. Satellite genomes have a substantial portion or all of their nucleotide sequences distinct from those of the genomes of their helper virus.

According to this definition, two major classes of satellites may be distinguished. **Satellite viruses** encode a structural protein that encapsidates their genome and so have nucleoprotein components distinct from those of their helper viruses. **Satellite nucleic acids** encode either non-structural proteins, or no proteins at all, and are encapsidated by the CP of helper viruses.

In addition to the true satellites, this chapter also describes subviral agents (nucleic acids) that depend upon viruses in a variety of ways. **Satellite-like nucleic acids** resemble satellites because they do not encode a replicase but differ because they encode a function necessary for the biological success of the associated virus. They can therefore be considered as components that remedy a deficiency in a defective virus. They have sometimes been classified as part of the genome of the virus they assist but they can also be dispensable because they are not always found in association with their helper virus. Examples include RNAs associated with groundnut rosette virus (genus *Umbravirus*) or with beet necrotic yellow vein virus (genus *Benyvirus*), that contribute to vector transmissibility and DNAs associated with begomoviruses (betasatellites) that encode a pathogenicity determinant.

A final group of agents described are nucleic acids capable of autonomous replication and which therefore are not strictly satellites although the term has sometimes been loosely applied to them. These agents are dependent on their helper viruses for various functions such as encapsidation, cell-to-cell and long-distance movement and vector transmission. Examples are the alphasatellites (DNAs that encode a replication initiator protein) or the RNAs associated with some poleroviruses that appear to encode a carmovirus-like RdRp.

The distinction between satellite nucleic acids, satellite-like nucleic acids and virus genomic components can be subtle and these agents are not always easy to categorize.

## Distinguishing features

Satellites are genetically distinct from their helper virus with a nucleotide sequence that is substantially different from that of their helper virus. However, the genomes of most satellites have short sequences, often at the termini, that are identical to the genome of the helper virus. This is presumably linked to the dependence of nucleic acids of both satellite and helper virus on the same viral polymerase and host-encoded proteins for replication. Satellites are distinct from defective interfering (DI) RNAs or defective RNAs because such RNAs are derived from their "helper" virus genomes. Nevertheless, satellite viruses may form their own DI RNAs that specifically interfere with the satellite virus genomic RNA, as has been shown for satellite panicum mosaic virus. Recombination can occur between satellites and their helper viruses. For example, chimeric molecules can be formed from a satellite RNA associated with turnip crinkle virus (genus *Carmovirus*) and parts of the helper virus genome.

Satellites do not constitute a homogeneous taxonomic group and are not formally classified into species and higher taxa by ICTV. The descriptions in this section are meant only to provide a classification framework and a nomenclature to assist in the description and identification of satellites and other virus-dependent nucleic acids. The arrangement adopted is based largely on features of the genetic material of the satellites. The physicochemical and biological features of the helper virus and of the helper virus host are important secondary characters.

There appears to be no taxonomic correlation between the viruses that are associated with satellites. Satellites would appear to have arisen independently a number of times during virus evolution. A

further complication is that some viruses are associated with more than one satellite and some satellites are supported by more than one species of helper virus. Satellites can even depend on both a second satellite and a helper virus for multiplication.

The first satellites characterized were mostly ssRNA satellites that use ssRNA plant viruses as helpers. It can be very difficult to distinguish between satellite RNA and viral genomic RNA (e. g., dsRNA satellites of fungus viruses) and it is very likely that other satellites, some with novel combinations of characters, remain to be discovered.

## Categories of satellites

**Satellite viruses:**

1. **Chronic bee-paralysis virus-associated satellite virus**
2. **Satellites that resemble tobacco necrosis satellite virus**
3. **Nodavirus-associated satellite virus**
4. **Adenovirus-associated satellite virus (*Dependovirus*)**
5. **Mimivirus-associated satellite virus (Sputnik, virophage)**

**Virus-dependent nucleic acids:**

6. **Single stranded DNAs**
   - **6a. Alphasatellites (encoding a replication initiator protein)**
   - **6b. Betasatellites (encoding a pathogenicity determinant)**
7. **Double stranded RNAs**
8. **Single stranded RNAs**
   - **8a. Large linear single stranded satellite RNAs**
   - **8b. Small linear single stranded satellite and satellite-like RNAs**
   - **8c. Small circular single stranded satellite RNAs**
   - **8d. Hepadnavirus-associated satellite-like RNAs (*Deltavirus*)**
   - **8e. Polerovirus-associated RNAs**

# Satellite Viruses

These satellites encode a structural protein to encapsidate their genomes. The satellite virus particles are antigenically, and usually morphologically, distinct from those of the helper virus. Five subgroups of satellite viruses are currently distinguished.

## 1. Chronic bee-paralysis associated satellite virus

Satellite virus particles are found in bees infected with the helper, chronic bee-paralysis virus (CBPV; a virus not yet classified). Particles are about 12 nm in diameter and serologically unrelated to those of CBPV. The satellite interferes with CBPV replication.

### List of group members

| | | |
|---|---|---|
| Chronic bee-paralysis satellite virus | | (CBPSV) |

## 2. Satellites that resemble tobacco necrosis satellite virus

These satellite virus particles are found in plant hosts in association with taxonomically diverse helper viruses. The T = 1 isometric particles are about 17 nm in diameter. The capsid consists of 60 copies of a single protein of 17–24 kDa, which is encoded by the satellite virus genome (positive sense ssRNA). The genomes of some satellite viruses contain a second ORF.

### List of group members

| | | |
|---|---|---|
| **Satellite viruses associated with viruses in the family *Tombusviridae*** | | |
| Maize white line mosaic satellite virus | [M55012] | (SMWLMV) |

| | | |
|---|---|---|
| Panicum mosaic satellite virus | [M17182] | (SPMV) |
| Tobacco necrosis satellite virus | [V01468] | (STNV) |
| **Satellite viruses associated with viruses in the family *Virgaviridae*** | | |
| Tobacco mosaic satellite virus | [M25782] | (STMV) |

## 3. Nodavirus-associated satellite virus

Satellite virus particles are found in *Macrobrachium rosenbergii* (giant river prawn) infected with Macrobrachium rosenbergii nodavirus (MrNV; a virus not yet classified but clearly related to viruses in the family *Nodaviridae*). The XSV (extra small virus) satellite virus particles are about 15 nm in diameter and serologically unrelated to those of MrNV. XSV is a positive-sense single-stranded RNA, about 800 bases in size, encoding a 17 kDa capsid protein. The mixed infection of MrNV and XSV is implicated in white spot disease of prawns.

### List of group members

| | | |
|---|---|---|
| Macrobrachium rosenbergii nodavirus XSV (extra small virus) | [AY247793] | (XSV) |

## 4. Adenovirus-associated satellite virus (*Dependovirus*)

Adenovirus-associated (AAV) satellite virus particles are found in humans, domesticated animals, fowl and in tissue or cell cultures as co-infections with a helper virus. The single stranded 5 kb DNA genome encodes three structural proteins (VP1, −2 and −3). The 26 nm T = 1 particles have a 10:1:1 ratio of VP3:VP2:VP1. Smaller particles about 12 nm in diameter only contain the 60 kDa VP3 protein. AAV satellite viruses are dependent on adenoviruses (or herpesviruses) for replication and cap functions. This group of satellites is anomalous, having been placed in a genus *Dependovirus* within the family *Parvoviridae*, although they meet all definitions for an authentic satellite virus. For more details see the section on genus *Dependovirus*.

### List of group members

See tables for the genus *Dependovirus* in the *Parvoviridae* chapter.

## 5. Mimivirus-associated satellite virus (Sputnik, virophage)

Acanthamoeba polyphaga mimivirus (genus *Mimivirus*) is an extremely large (ca. 1.2 Mbp) virus with a dsDNA genome that infects amoebae of the genus *Acanthamoeba*. A mimivirus strain (sometimes called mamavirus) isolated from *A. castellanii* supports a 50 nm T = 27 satellite virus, referred to as Sputnik (= satellite in Russian). The satellite virus has a circular dsDNA genome of 18 kbp that is predicted to code for about 22 proteins. Sputnik does not replicate in either host in the absence of the helper virus.

### List of group members

| | | |
|---|---|---|
| Acanthamoeba castellanii mamavirus-associated satellite virus (Sputnik) | [EU606015] | (Sputnik; virophage) |

# Virus-dependent Nucleic Acids

This category includes a diverse range of DNA and RNA molecules that do not encode a capsid protein but are packaged in capsids encoded by their helper virus. Those that encode a function necessary for the biological success of the associated virus are described as "satellite-like".

## 6. Single stranded DNAs

### 6a. Alphasatellites

These molecules are not strictly satellites because they encode a rolling-circle replication initiator protein (known as the replication-associated protein [Rep]) with similarity to the master Rep

encoding genomic components (DNA-R) of nanoviruses. They are capable of autonomous replication in host cells, have a stem-loop region containing the ubiquitous nonanucleotide TAA/GTATTAC, and depend on their helper viruses for encapsidation, movement in plants and insect transmission. Some are associated with viruses in the genus *Begomovirus* and are typically about 1.4 kb, half the size of their helper viruses. Others are associated with multipartite genome viruses of the family *Nanoviridae* and are approximately the same size (ca. 1 kb) as the genomic components of their helper viruses (but are not derived from them). The presence of alphasatellites in begomovirus and nanovirid infections may reduce symptom severity, suggesting interference akin to that seen with defective interfering DNAs. Recent results have shown that the Rep encoded by at least some alphasatellites associated with begomoviruses suppresses host defenses based on RNA interference.

## List of group members

| | | |
|---|---|---|
| **Begomovirus-associated alphasatellites** | | |
| Ageratum yellow vein alphasatellite | [AJ238493] | (AYVA) |
| Ageratum yellow vein India alphasatellite | [AJ512958] | (AYVIA) |
| Ageratum yellow vein Kenya alphasatellite | [AJ512960] | (AYVKA) |
| Ageratum yellow vein Pakistan alphasatellite | [AJ512949] | (AYVPKA) |
| Ageratum yellow vein Singapore alphasatellite | [AJ416153] | (AYVSGA) |
| Cotton leaf curl Dabwali alphasatellite | [AJ512957] | (CLCuDaA) |
| Cotton leaf curl Gezira alphasatellite | [FN554581] | (CLCuGeA) |
| Cotton leaf curl Multan alphasatellite | [GQ374450] | (CLCuMuA) |
| Cotton leaf curl Shadadpur alphasatellite | [AM711116] | (CLCuShA) |
| Duranta leaf curl alphasatellite | [FM179614] | (DuLCA) |
| Gossypium darwinii symptomless alphasatellite | [EU384606] | (GDarSLA) |
| Gossypium davidsonii symptomless alphasatellite | [EU384652] | (GDavSLA) |
| Gossypium mustilinum symptomless alphasatellite | [EU384662] | (GMusSLA) |
| Hibiscus leaf curl alphasatellite | [AJ579349] | (HLCuA) |
| Malvastrum yellow mosaic alphasatellite | [AM236763] | (MalYMA) |
| Malvastrum yellow mosaic Hainan alphasatellite | [AM236765] | (MalYMHnA) |
| Okra leaf curl alphasatellite | [AJ512954] | (OLCuA) |
| Okra leaf curl Barombi alphasatellite | [FM164739] | (OLCuBaA) |
| Okra leaf curl Burkina Faso alphasatellite | [FN554582] | (OLCuBFA) |
| Okra leaf curl Mali alphasatellite | [FN554580] | (OLCuMA) |
| Sida yellow vein Vietnam alphasatellite | [DQ641718] | (SiYVVA) |
| Tobacco curly shoot alphasatellite | [AJ579346] | (TbCSA) |
| Tomato leaf curl Pakistan alphasatellite | [FM164939] | (ToLCPKA) |
| Tomato yellow leaf curl China alphasatellite | [AJ579358] | (TYLCCNA) |
| **Babu- and nanovirus-associated alphasatellites** | | |
| Banana bunchy top S1 alphasatellite | [AF216221] | (BBTS1A) |
| Banana bunchy top S2 alphasatellite | [L32167] | (BBTS2A) |
| Banana bunchy top S3 alphasatellite | [AF416471] | (BBTS3A) |
| Banana bunchy top W1 alphasatellite | [L32166] | (BBTW1A) |
| Banana bunchy top Y alphasatellite | [FJ389724] | (BBTS2A) |
| Faba bean necrotic yellows C1 alphasatellite | [X80879] | (FBNYC1A) |
| Faba bean necrotic yellows C11 alphasatellite | [AJ005968] | (FBNYC11A) |
| Faba bean necrotic yellows C7 alphasatellite | [AJ005964] | (FBNYC7A) |
| Faba bean necrotic yellows C9 alphasatellite | [AJ005966] | (FBNYC9A) |
| Milk vetch dwarf C1 alphasatellite | [AB000920] | (MDC1A) |
| Milk vetch dwarf C10 alphasatellite | [AB009047] | (MDC10A) |
| Milk vetch dwarf C2 alphasatellite | [AB000921] | (MVDC2A) |
| Milk vetch dwarf C3 alphasatellite | [AB000922] | (MDC3A) |
| Subterranean clover stunt C2 alphasatellite | [U16731] | (SCSC2A) |
| Subterranean clover stunt C6 alphasatellite | [U16735] | (SCSC6A) |
| **Possible member** | | |
| Coconut foliar decay alphasatellite | [M29963] | (CFDA) |

## 6b. Betasatellites

These are satellite-like circular ssDNA components, usually about 1.3 kb in size that are associated with viruses in the genus *Begomovirus*. Although initially identified only in association with monopartite begomoviruses, recently they have been increasingly identified in association with bipartite

begomoviruses. All betasatellites have a stem-loop region containing the ubiquitous nonanucleotide TAATATTAC and an associated highly conserved sequence located immediately upstream (the function of which remains uncertain), a conserved ORF (termed βC1; encoding a protein that is a pathogenicity determinant and a suppressor of host defenses based on RNA interference), and an A-rich region that may reflect size adaptation for maintenance of the component by the helper begomovirus. The betasatellite DNAs readily recombine with the helper begomovirus genome and such recombinants may retain a biological activity similar to the parental betasatellite. Pairwise comparisons between sequences have shown that a sequence identity of about 78% is an appropriate demarcation threshold for distinguishing between betasatellites.

## List of group members

| | | |
|---|---|---|
| Ageratum leaf curl Cameroon betasatellite | [FM164738] | (ALCCMB) |
| Ageratum yellow leaf curl betasatellite | [AJ316041] | (AYLCB) |
| Ageratum yellow vein betasatellite | [AJ252072] | (AYVB) |
| Ageratum yellow vein Sri Lanka betasatellite | [AJ542493] | (AYVSLB) |
| Alternanthera yellow vein betasatellite | [DQ641716] | (AlYVB) |
| Bean leaf curl China betasatellite | [AM260730] | (BeLCCNB) |
| Bhendi yellow vein betasatellite | [AJ308425] | (BYVB) |
| Cardiospemum yellow leaf curl betasatellite | [AM933578] | (CarYLCB) |
| Chilli leaf curl betasatellite | [AJ316032] | (ChLCB) |
| Cotton leaf curl Gezira betasatellite | [AJ316039] | (CLCuGeB) |
| Cotton leaf curl Multan betasatellite | [AJ292769] | (CLCuMuB) |
| Croton yellow vein mosaic betasatellite | [AM410551] | (CroYVMB) |
| Emilia yellow vein betasatellite | [FJ869906] | (EmYVB) |
| Erectites yellow mosaic betasatellite | [DQ641713] | (ErYMB) |
| Eupatorium yellow vein betasatellite | [AJ438938] | (EpYVB) |
| Honeysuckle yellow vein betasatellite | [AJ316040] | (HYVB) |
| Honeysuckle yellow vein Japan betasatellite | [AB236324] | (HYVJB) |
| Honeysuckle yellow vein Kochi betasatellite | [AB236326] | (HYVKoB) |
| Honeysuckle yellow vein mosaic betasatellite | [AB287442] | (HYVMB) |
| Honeysuckle yellow vein mosaic Hyogo betasatellite | [AB182263] | (HYVMHyB) |
| Honeysuckle yellow vein mosaic Nara betasatellite | [AB287443] | (HYVMNaB) |
| Kenaf leaf curl betasatellite | [AY705381] | (KLCuB) |
| Leucas zeylanica yellow vein betasatellite | [GQ421324] | (LeZYVB) |
| Lindernia anagallis yellow vein betasatellite | [DQ641715] | (LaYVB) |
| Ludwigia yellow vein betasatellite | [AJ965541] | (LuYVB) |
| Luffa leaf distortion betasatellite | [EU557374] | (LuLDB) |
| Malvastrum leaf curl betasatellite | [AM072289] | (MaLCB) |
| Malvastrum yellow vein betasatellite | [AJ971459] | (MaYVB) |
| Malvastrum yellow vein Yunnan betasatellite | [AJ786712] | (MaYVYnB) |
| Mesta yellow vein mosaic betasatellite | [EF614160] | (MeYVMB) |
| Okra leaf curl betasatellite | [AJ316029] | (OLCuB) |
| Papaya leaf curl betasatellite | [AY230138] | (PaLCuB) |
| Radish leaf curl betasatellite | [EF175734] | (RaLCB) |
| Sida leaf curl betasatellite | [AM050732] | (SiLCuB) |
| Sida yellow mosaic China betasatellite | [AM048833] | (SiYMCNB) |
| Sida yellow vein betasatellite | [AJ967003] | (SiYVB) |
| Sida yellow vein mosaic betasatellite | [EU188921] | (SiYVMB) |
| Sida yellow vein Vietnam betasatellite | [DQ641712] | (SiYVVB) |
| Siegesbeckia yellow vein betasatellite | [AM230643] | (SgYVB) |
| Siegesbeckia yellow vein Guangxi betasatellite | [AM238695] | (SgYVGxB) |
| Tobacco curly shoot betasatellite | [AJ421485] | (TbCSB) |
| Tobacco leaf curl betasatellite | [AJ316033] | (TbLCB) |
| Tomato leaf curl Bangalore betasatellite | [AY428768] | (ToLCBaB) |
| Tomato leaf curl Bangladesh betasatellite | [AJ542489] | (ToLCBB) |
| Tomato leaf curl betasatellite | [AJ316036] | (ToLCB) |
| Tomato leaf curl China betasatellite | [AJ704609] | (ToLCCNB) |
| Tomato leaf curl Java betasatellite | [AB100306] | (ToLCJaB) |
| Tomato leaf curl Joydebpur betasatellite | [AJ966244] | (ToLCJoB) |
| Tomato leaf curl Karnataka betasatellite | [AY754813] | (ToLCKaB) |
| Tomato leaf curl Laos betasatellite | [AJ542491] | (ToLCLB) |

| | | |
|---|---|---|
| Tomato leaf curl Maharastra betasatellite | [AY838894] | (ToLCMaB) |
| Tomato leaf curl Patna betasatellite | [EU862324] | (ToLCPaB) |
| Tomato leaf curl Philippines betasatellite | [AB308071] | (ToLCPB) |
| Tomato leaf curl virus satellite | [U74627] | (ToLCV-sat) |
| Tomato yellow dwarf betasatellite | [AB294512] | (ToYDB) |
| Tomato yellow leaf curl China betasatellite | [AJ781297] | (TYLCCNB) |
| Tomato yellow leaf curl Thailand betasatellite | [AJ536621] | (TYLCTHB) |
| Tomato yellow leaf curl Vietnam betasatellite | [DQ641714] | (TYLCVB) |
| Tomato yellow leaf curl Yunnan betasatellite | [AJ421620] | (TYLCYnB) |
| Vernonia yellow vein betasatellite | [FN435836] | (VerYVB) |
| Zinnia leaf curl betasatellite | [AJ542499] | (ZLCuB) |

# 7. Double stranded RNAs

Most satellites in this category are found in association with viruses in the families *Totiviridae* and *Partitiviridae*. The dsRNAs range in size from 0.5 to 1.8 kbp and are encapsidated using the helper virus capsid protein. These particles often also contain a positive sense single stranded copy of the dsRNA. The satellite dsRNAs associated with helper viruses in the genus *Totivirus* encode a secreted preprotoxin that is lethal to sensitive cells (virus-free or containing helper virus only). The presence of satellites in helper totivirus cultures imparts self-protection against the secreted toxin and confers ecological advantage by killing competing virus- or satellite-free fungi. The satellite dsRNAs associated with partitiviruses do not code for functional proteins and their biological significance is not known.

## List of group members

| | | |
|---|---|---|
| **Satellites associated with viruses in the family *Totiviridae*** | | |
| M satellites of Saccharomyces cerevisiae L-A virus | | |
| M1 | [U78817] | (ScV-M1) |
| M2 | [X56987] | (ScV-M2) |
| M28 | | (ScV-M28) |
| M satellites of Ustilago maydis virus H | | |
| M-P1 | [M63149] | (UmV-P1) |
| M-P4 | [L12226] | (UmV-P4) |
| M-P6 | [P16948] | (UmV-P6) |
| Satellites of Trichomonas vaginalis T1 virus | [U15991] | (TVV-Sat*) |
| **Possible member** | | |
| M satellite of Zygosaccharomyces balii virus | [AF515592] | (ZbV-M) |
| **Satellites associated with viruses in the family *Partitiviridae*** | | |
| Satellite of Atkinsonella hypoxylon virus | [L39127] | (AhV-Seg3*) |
| Satellites of Discula destructiva virus 1 | | |
| dsRNA 3 | [AF316994] | (DdV-Seg3) |
| dsRNA4 | [AF316995] | (DdV-Seg4) |
| Satellite of Gremmeniella abietina virus MS1 | [AY089995] | (GaVMS1-Seg3) |
| Satellite of Penicillium stoloniferum virus F | [AY738338] | (PsVF-Seg3) |
| **Possible members** | | |
| Satellites of Amasya cherry disease-associated virus | | |
| Satellite A | [AM085138] | (ACDAV-SatA) |
| Satellite B | [AM085139] | (ACDAV-SatB) |
| Satellites of cherry chlorotic rusty spot -associated virus | | |
| Satellite A | [AM749118] | (CCRSAV-SatA) |
| Satellite B | [AM749119] | (CCRSAV-SatB) |
| Satellite C | [AM749120] | (CCRAV-SatC) |
| **Satellites associated with viruses in the family *Reoviridae*** | | |
| Bombyx mori cypovirus 1 satellite RNA | [AB183384] | (BmCpV1-Sat) |

*Abbreviations: Sat, satellite; Seg, segment.

# 8. Single stranded RNAs

## 8a. Large linear single stranded satellite RNAs

This category comprises satellites with genomes that are 0.8 to 1.5 kb in size and encode a non-structural protein that, at least in some cases, is essential for satellite RNA multiplication. Little sequence homology exists between the satellites and their helpers, some satellites can be exchanged

among different helper viruses. These satellites rarely modify the disease induced in host plants by the helper virus. Most are associated with helper viruses in the family *Secoviridae*.

## List of group members

| | |
|---|---|
| **Satellite RNAs associated with viruses in the family *Secoviridae*** | |
| Arabis mosaic virus large satellite RNA | [D00664] |
| Beet ringspot virus satellite RNA (TBRV-S serotype satellite RNA) | |
| Blackcurrant reversion virus satellite RNA | [AF112119] |
| Chicory yellow mottle virus large satellite RNA | [D00686] |
| Grapevine Bulgarian latent virus satellite RNA | |
| Grapevine fanleaf virus satellite RNA | [D00442] |
| Myrobalan latent ringspot virus satellite RNA | |
| Strawberry latent ringspot virus satellite RNA | [X69826] |
| Tomato black ring virus satellite RNA (TBRV-G serotype satellite RNA) | [X00978] |
| **Satellite RNAs associated with viruses in the family *Alphaflexiviridae*** | |
| Bamboo mosaic virus satellite RNA | [L22762] |
| **Possible member** | |
| Beet necrotic yellow vein virus RNA5* | [D63759] |

*Non-essential genome component that may be regarded as a satellite-like RNA. Beet necrotic yellow vein virus is a member of the genus *Benyvirus*.

## 8b. Small linear single stranded satellite and satellite-like RNAs

These satellites have a strictly linear genome of less than 0.7 kb that does not encode functional proteins. Some satellites can attenuate the symptoms induced by helper virus infection, whereas other satellites can exacerbate the symptoms.

## List of group members

| | |
|---|---|
| **Satellite RNAs associated with viruses in the family *Tombusviridae*** | |
| Artichoke mottled crinkle virus satellite RNA | |
| Black beet scorch virus satellite RNA | [AY394497] |
| Carnation Italian ringspot virus satellite RNA | |
| Cymbidium ringspot virus satellite RNA | [D00720] |
| Panicum mosaic virus satellite RNA | [M17182] |
| Pelargonium leaf curl virus satellite RNA | |
| Petunia asteroid mosaic virus satellite RNA | |
| Tobacco necrosis virus small satellite RNA | [E03054] |
| Tomato bushy stunt virus satellite RNA (several types) | [AF022787-8][FJ666076] |
| **Satellite RNAs associated with viruses in the family *Bromoviridae*** | |
| Cucumber mosaic virus satellite RNA (several types) | [X69136] |
| Peanut stunt virus satellite RNA | [Z98197] |
| **Satellite RNAs associated with viruses in the genus *Umbravirus*†** | |
| Carrot mottle mimic virus satellite RNA | [EU914919] |
| Groundnut rosette virus satellite RNA* | [Z29702] |
| Pea enation mosaic virus satellite RNA | [U03564] |
| Tobacco bushy top virus satellite RNA* | [AF510392] |

*These may be regarded as a satellite-like RNAs as they appear to be essential components of a disease complex.
†These in turn depend upon viruses in the family *Luteoviridae* for their encapsidation and transmission.

## 8c. Small circular single stranded satellite RNAs

These satellites have genomes that are about 350 nt long. Both circular and linear forms of the genome are found in infected cells. In some cases (e.g. the satellite RNA associated with tobacco ringspot virus, genus *Nepovirus*), replication involves self-cleavage of circular progeny molecules by an RNA-catalyzed reaction.

## List of group members

| | |
|---|---|
| **Satellite RNAs associated with viruses in the family *Secoviridae*** | |
| Arabis mosaic virus small satellite RNA | [M21212] |
| Chicory yellow mottle virus satellite RNA | [D00721] |
| Tobacco ringspot virus satellite RNA | [M14879] |

| | |
|---|---|
| **Satellite RNAs associated with viruses in the family *Luteoviridae*** | |
| Cereal yellow dwarf virus-RPV satellite RNA | [M63666] |
| **Satellite RNAs associated with viruses in the genus *Sobemovirus*** | |
| Lucerne transient streak virus satellite RNA | [X01984] |
| Rice yellow mottle virus satellite | [AF039909] |
| Solanum nodiflorum mottle virus satellite RNA | [J02386] |
| Subterranean clover mottle virus satellite RNA (2 types) | [M33000][M33001] |
| Velvet tobacco mottle virus satellite RNA | [J02439] |
| **Possible member** | |
| Cherry small circular viroid-like RNA | [Y12833] |

## 8d. Hepadnavirus-associated satellite-like RNAs (*Deltavirus*)

Hepatitis D virus (HDV, genus *Deltavirus*) has a single molecule of circular, negative sense 1.7 kb ssRNA that encodes a 24 kDa small protein (S-HDAg) and a 27 kDa large protein (L-HDAg). The ribonucleoprotein of HDV RNA and both HDAgs, are packaged within an envelope containing lipid and helper virus antigens. For complete replication and transmission, HDV also requires a host DNA dependent RNA polymerase II and HBsAg, a protein encoded by its helper virus, human hepatitis B virus (genus *Orthohepadnavirus*). HDV RNA is encapsidated in distinct virions by the HBsAg capsid protein of the helper virus. HDV is a serious human pathogen and has until now been classified as a virus, although it meets the definitions of a satellite-like RNA. For more details see the chapter on genus *Deltavirus*.

## List of group members

See chapter for genus *Deltavirus*.

## 8e. Polerovirus-associated RNAs

These ssRNA genomes are about 2.8–3 kb long and have two major ORFs. It is likely that the second ORF, which contains the classic RdRp motifs of the carmovirus supergroup, is translated by readthrough of the ORF1 amber stop codon. Additional small ORFs have been identified in some members. The RNA is capable of autonomous replication but appears to depend on a virus of the genus *Polerovirus* as helper virus for aphid transmission by encapsidating this RNA with the polerovirus coat protein to form aphid-transmissible hybrid virions. Some members increase the severity of disease symptoms.

## List of group members

| | | |
|---|---|---|
| Beet western yellows virus ST9-associated RNA | [L04281] | (BWYV ST9aRNA) |
| Carrot red leaf virus-associated RNA | [AF020617] | (CtRLVaRNA) |
| Tobacco vein distorting virus-associated RNA | [EF529625] | (TVDVaRNA) |

## Further reading

### Journals and books

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## Websites

Subviral RNA database: http://subviral.med.uottawa.ca/cgi-bin/home.cgi.

## Contributed by

Briddon, R.W., Ghabrial, S., Lin, N.-S., Palukaitis, P., Scholthof, K.-B.G. and Vetten, H.-J.

# VIROIDS

## Definition

Viroids are small, circular, single stranded, non-protein-coding RNAs that replicate autonomously when inoculated into higher plants. Some are pathogenic, but others replicate without eliciting symptoms in susceptible plant species. Unlike the genomes of conventional viruses, viroids are not protected by a protein capsid.

## Taxonomic structure of viroids

| | |
|---|---|
| Family | ***Avsunviroidae*** |
| Genus | ***Avsunviroid*** |
| Genus | ***Pelamoviroid*** |
| Genus | ***Elaviroid*** |
| Family | ***Pospiviroidae*** |
| Genus | ***Pospiviroid*** |
| Genus | ***Hostuviroid*** |
| Genus | ***Cocadviroid*** |
| Genus | ***Apscaviroid*** |
| Genus | ***Coleviroid*** |

## Physicochemical and physical properties

Viroid molecules, with a $M_r$ of 80–125 $\times 10^3$, display extensive internal base pairing and assume, in most cases, a rod-like or quasi-rod-like conformation *in vitro* that is about 50 nm in length (Figure 1). For some viroids, there is indirect evidence supporting this type of structure *in vivo*.

These structures denature by cooperative melting ($T_m$ in 10 mM $Na^+$ at about 50 °C) to single stranded circles of about 100 nm contour length. Viroids can also form metastable structures with hairpins that may be functionally important. However, at least two viroids, peach latent mosaic viroid (PLMVd) and chrysanthemum chlorotic mottle viroid (CChMVd), do not follow this rule, and their RNAs clearly appear to adopt branched conformations. There is experimental support for the contention that PLMVd and CChMVd have unique conformations because unlike other viroids, they are insoluble in 2 M LiCl. Some viroids also contain elements of tertiary structure like kissing-loops. Sequences vary from 246 to 401 nt in length and are rich in G+C (53–60%), except avocado sunblotch viroid (ASBVd) which has 38% G+C.

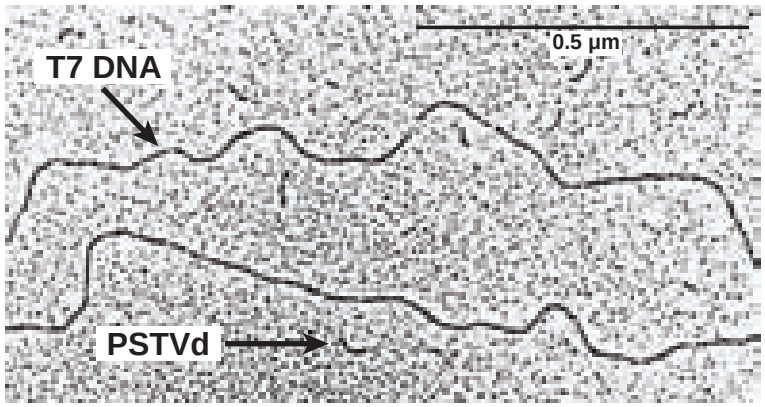


**Figure 1:** Electron micrograph of potato spindle tuber viroid (PSTVd) and viral DNA (coliphage T7 DNA) illustrating the comparative sizes and the rod-like structure of the viroid. The scale bar represents 0.5 µm. (From Diener, T.O. (1979). *Viroids and Viroid Diseases.* Wiley, New York; with permission.)

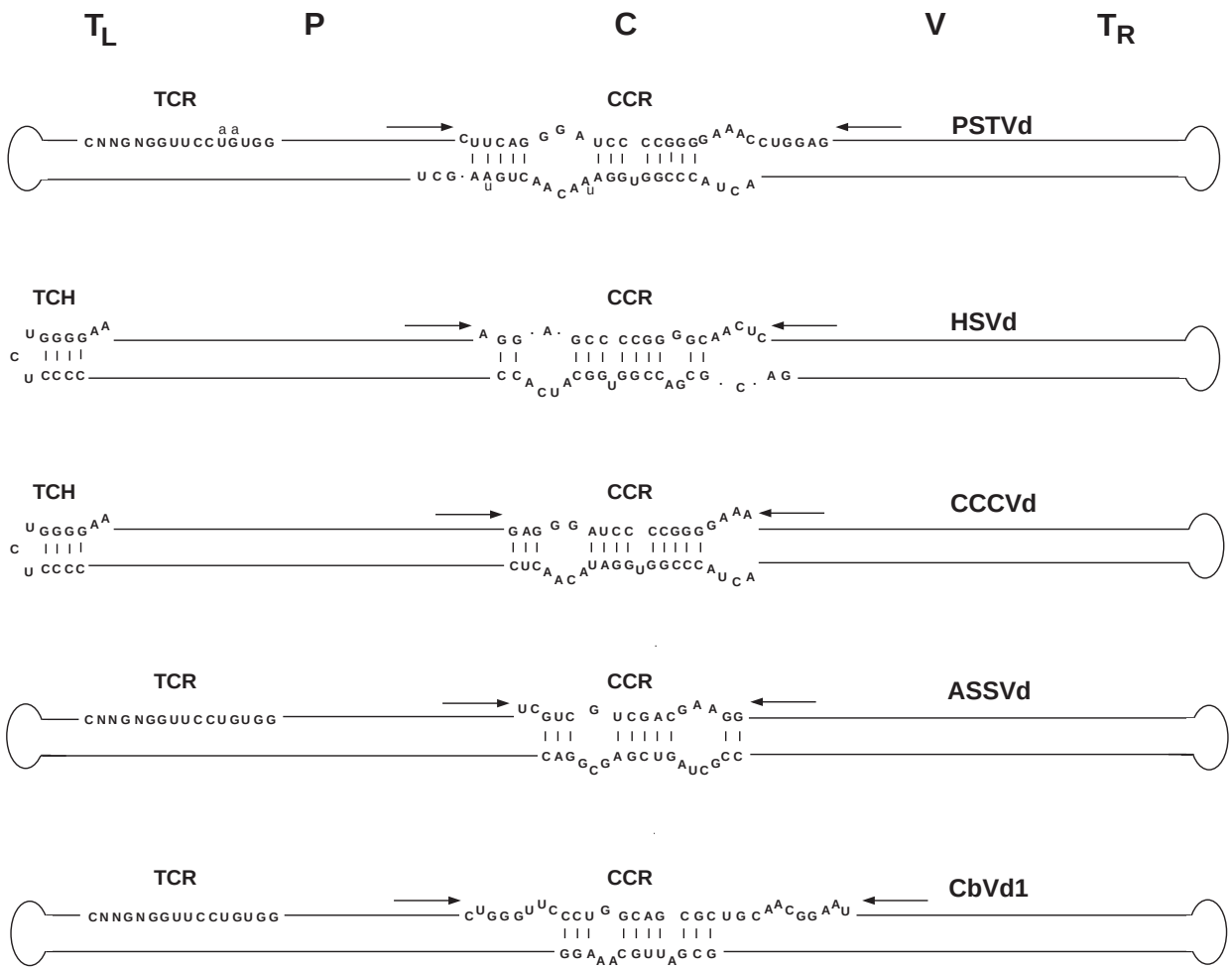


**Figure 2:** Rod-like structure models for viroids in the different genera of the family *Pospiviroidae*. The type species of each genus is indicated on the right and the approximate location of the five domains C (central), P (pathogenic), V (variable) and $T_L$ and $T_R$ (terminal left and right), at the top of the figure. The core nt of the central conserved region (CCR), terminal conserved region (TCR) and terminal conserved hairpin (TCH), are shown. Arrows indicate flanking sequences which form, together with the core nucleotides of the CCR upper strand, imperfect inverted repeats. The core nucleotides of the hop stunt viroid (HpSVd) CCR have been defined by comparison with columnea latent viroid (CLVd), which on the basis of other criteria (presence of the TCR, absence of the TCH and overall sequence similarity) is included in the genus *Pospiviroid*. Substitutions found in the CCR and TCR of iresine viroid 1 (IrVd-1) a member of the genus *Pospiviroid*, are indicated in lowercase. In the genus *Coleviroid* the TCR only exists in the two largest members coleus blumei viroid 2 (CbVd-2) and coleus blumei viroid 3 (CbVd-3). (Adapted from Flores, R. *et al*. (1997). Viroids: the non-coding genomes. *Semin. Virol.*, **8**, 65–73.)

## Genome organization and replication

All viroids except ASBVd, PLMVd, CChMVd and eggplant latent viroid (ELVd) share a model of five structural functional domains within the proposed rod-like secondary structure of minimal free energy: C (central), P (pathogenic), V (variable) and $T_R$ and $T_L$ (terminal right and left). The C domain contains a central conserved region (CCR) formed by two sets of conserved nucleotides located in the upper and lower strands, with those of the upper strand being flanked by an inverted repeat (Figure 2).

The upper strand of the CCR can form either a hairpin or, in oligomers, a double stranded structure possibly relevant in replication. Two other conserved sequence motifs are the terminal conserved region (TCR), found in all members of the genera *Pospiviroid* and *Apscaviroid* and in the two largest

members of the genus *Coleviroid*, and a terminal conserved hairpin (TCH) present in all members of the genera *Hostuviroid* and *Cocadviroid* (Figure 2). The conservation of these two motifs in sequence and location in the left terminal domain suggests their involvement in some critical functions. Coconut cadang-cadang viroid (CCCVd) is unusual in occurring as RNAs of different sizes, the larger ones having sequence repetitions of the smallest one; a similar situation has been found in citrus exocortis viroid (CEVd). Some viroids, the most striking examples being columnea latent viroid (CLVd) and australian grapevine viroid (AGVd), appear to have arisen by intermolecular RNA recombination events since they seem to consist of a more or less complex mosaic of sequences present in other viroids. Direct evidence in support of an ongoing recombination process has come from studies on the dynamic distribution over time of *Coleus* viroids coinfecting individual plants.

There is no evidence that viroids encode proteins and unlike viruses that primarily parasitize the host translation machinery, viroids mainly parasitize host transcription, possibly by subverting either nuclear RNA polymerase II (family *Pospiviroidae*), or one of the chloroplastic RNA polymerases (family *Avsunviroidae*) to accept RNA templates. Oligomers isolated from infected tissue may be replicative intermediates produced by a rolling circle mechanism with two variants (asymmetric and symmetric) and three steps (RNA polymerization, cleavage and ligation). Initiation of RNA synthesis in PSTVd and ASBVd appears to occur at specific sites, suggesting that it is promoter-driven. In the family *Avsunviroidae* oligomeric RNAs self-cleave *in vitro* and very probably *in vivo* through hammerhead structures to produce unit-length strands, but in the family *Pospiviroidae* cleavage is catalyzed by host factors (probably one or more of the RNase III-like proteins) (Figure 3). Ligation of PSTVd appears to be mediated by a nuclear RNA ligase, whereas this reaction has been proposed to be autocatalytic for PLMVd leading to unusual 2′, 5′-phosphodiester bonds at the ligation sites; alternatively, a chloroplastic RNA ligase might mediate this step.

## Antigenic properties

No antigenicity demonstrated.

## Biological properties

### Host range

Some viroids have wide host ranges in the angiosperms but others, particularly members of the family *Avsunviroidae*, have narrow host ranges. CCCVd and coconut tinangaja viroid (CTiVd) infect monocotyledons. Old cultivars of grapevine and citrus can harbor at least five different viroids. A single nucleotide substitution converts PSTVd from a non-infectious RNA to one that is infectious for *Nicotiana tabacum*.

### Symptoms

Some viroids have devastating effects, for example, CCCVd has killed millions of coconut palms in the Philippines, while others cause epinasty, rugosity, chlorosis and necrosis on leaves, internode shortening of stems leading to stunted plants, bark cracking, deformation and color alterations of fruits and storage organs, and delays in foliation, flowering and ripening. A few viroids induce only mild or no symptoms. Symptom expression is generally more pronounced at high temperature and light intensities.

Molecular determinants of specific symptoms have been mapped in the genome of members of both families. Small sequence changes can convert a severe strain into a symptomless strain (or *vice versa*). Direct interaction of the genomic viroid RNA with host factors, and involvement of or interference with the plant RNA silencing machinery, have been suggested as primary event(s) of symptom induction.

### Transmission

Viroids of horticultural species are transmitted mainly by vegetative propagation. In plants propagated via seeds, they may be transmitted mechanically or through seed or pollen. Only tomato planta macho viroid (TPMVd) is known to be efficiently transmitted by aphids.

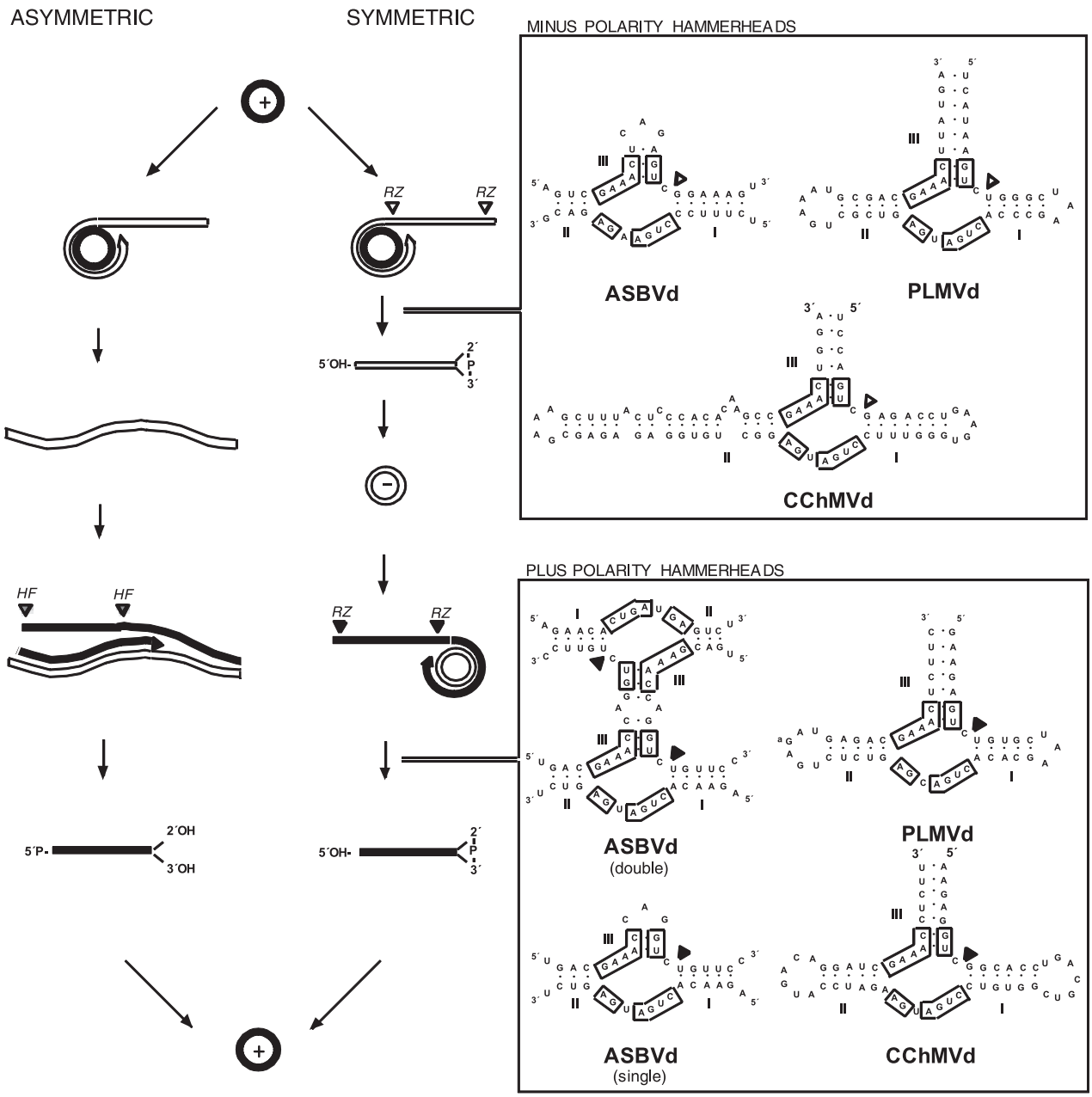


**Figure 3:** Models for viroid replication. The plus polarity (solid lines) is assigned by convention to the most abundant infectious RNA and the minus polarity (open lines) to its complementary strand. The alternative asymmetric and symmetric pathways involve one and two rolling circles, respectively. In the symmetric variant, cleavage of plus and minus multimeric strands is mediated by hammerhead ribozymes (RZ), which lead to linear monomeric RNAs with 5′-hydroxyl and 2′-3′-cyclic phosphodiester termini. Arrowheads denote the cleavage sites. The hammerhead structures that can be formed by avocado sunblotch viroid (ASBVd), peach latent mosaic viroid (PLMVd) and chrysanthemum chlorotic mottle viroid (CChMVd) RNAs are shown on the right; conserved nucleotides are boxed. In the asymmetric variant, cleavage of plus multimeric strands relies on a host factor (HF), which generates linear monomeric RNA containing probably 5′-phosphomonoester and 2′ and 3′-hydroxyl termini. (Adapted from Flores, R. *et al.* (1997). Viroids: the non-coding genomes. *Semin. Virol.*, **8**, 65–73.)

### Movement

To invade plants systematically, viroids must be able to move from the initially infected cells to the surrounding ones and then to the vascular system. Three different types of movement can be considered: intracellular, cell-to-cell and long-distance. PSTVd movement into the nucleus appears to be a cytoskeleton-independent process that is mediated by a specific and saturable receptor and involves recognition of a conserved sequence and/or structural motif in the upper central conserved region. How members of the family *Avsunviroidae* are transported into chloroplasts is unknown. Mutational analysis of PSTVd has shown that many of the loops in its rod-like secondary structure (see Figure 6) play a role in movement from cell-to-cell and across tissue boundaries, possibly by creating pockets that bind host proteins. *In vitro*, hop stunt viroid (HpSVd) can form a ribonucleoprotein complex with the phloem protein 2, a dimeric lectin able to move from cell-to-cell through plasmodesmata and toward sink tissues in the assimilate stream. These properties, together with its ability to bind RNA, suggest that this lectin facilitates the systemic movement of viroids. Access of PSTVd to floral and vegetative meristems is impaired, most likely by an RNA silencing mechanism. In contrast, a chloroplast replicating viroid, PLMVd, is able to enter the apical meristem of its natural host.

### Cross protection

Interactions at the level of symptom expression and viroid accumulation have been detected in plants co-infected by two strains of a viroid or even by two different viroids sharing extensive sequence similarities. Interactions of this class have been observed in the case of members belonging to both viroid families (see below), suggesting the possible existence of more than one mechanism of cross-protection between viroids. Alternatively, because RNA-mediated cross-protection in plant viruses is mechanistically similar to post-transcriptional gene silencing, cross-protection between viroids might occur through a similar mechanism in both families.

## Phylogenetic relationships within the viroids

Criteria such as the nuclear site of replication and accumulation, the presence and type of CCR, and the presence or absence of the two other conserved regions TCR and TCH, are used for classifying most viroids in family *Pospiviroidae*, with the two latter criteria serving for their allocation into genera. ASBVd, PLMVd, CChMVd and ELVd form a second family of viroids, the *Avsunviroidae*, without CCR but endowed with the ability to self-cleave via hammerhead ribozymes (Figure 3). The type of hammerhead structure, together with the G+C content and the solubility in 2M LiCl are used to demarcate species into genera. Essentially the same groupings are obtained by using phylogenetic trees derived from the whole sequences (Figure 4).

## Species demarcation criteria

An arbitrary level of less than 90% sequence similarity and distinct biological properties, particularly host range and symptoms, currently separate most species within each viroid genus. This second criterion is now mandatory for the creation of new viroid species. Where host range is restricted to a single botanical species not expressing symptoms, other distinct biological properties should be described (i.e. seed transmission, movement and distribution within the host, differential fitness in competition assays) to justify creation of a new viroid species. Due to the heterogeneous nature of viroid populations, infected plants often contain a spectrum of closely related variants, each showing more than 90% sequence similarity. One or a limited number of these variants may represent the bulk of the population.

# Family *Avsunviroidae*

## Taxonomic structure of the family

| | |
|---|---|
| Family | *Avsunviroidae* |
| Genus | *Avsunviroid* |
| Genus | *Pelamoviroid* |
| Genus | *Elaviroid* |

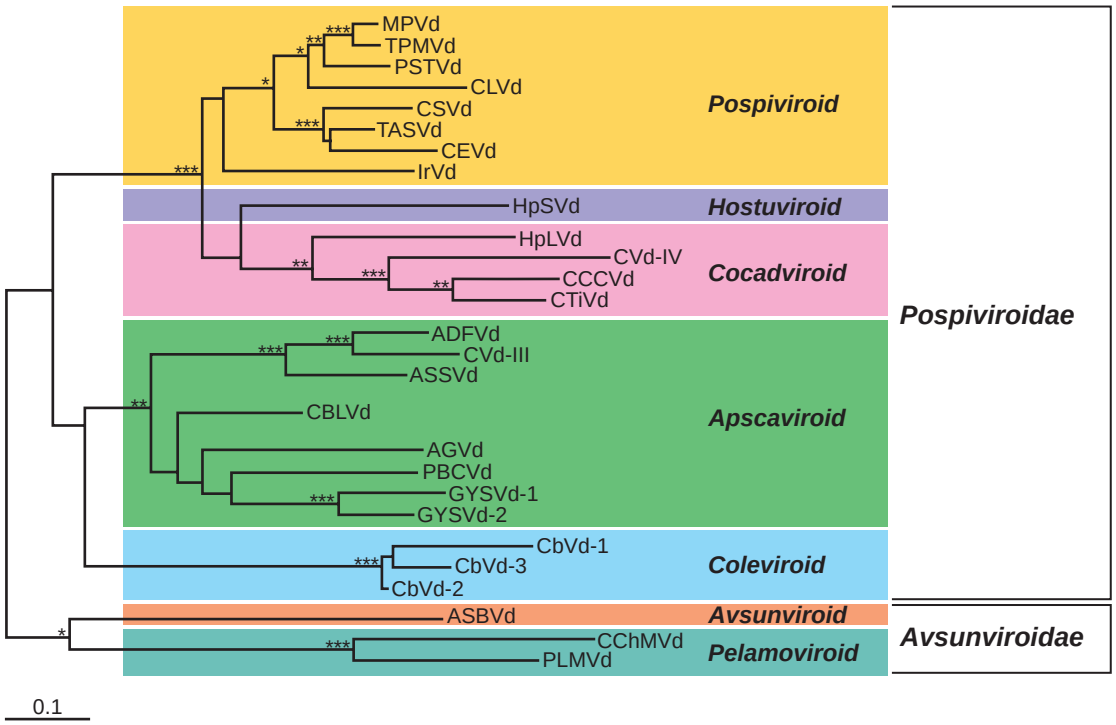


**Figure 4:** Consensus phylogenetic tree (based on 1000 replicates) obtained for viroids. Isolates indicated by ***, ** and * form monophyletic groups in more than 95%, 85% and 75% of the replicates, respectively. Numbering of sequences was based on the similarities found between the upper strands of the central conserved regions of members of the family *Pospiviroidae* and the upper-left-hand portions of the hammerhead structures of members of the family *Avsunviroidae* (Diener, T.O. (1991) Subviral pathogens of plants: viroids and viroidlike satellite RNAs. *FASEB J.*, **5**, 2808–2813). The alignment was performed with the Clustal X program, version 1.64 (gap opening and gap extension penalties 15 and 1, respectively) and minor manual adjustments between the conserved regions. Evolutionary distances were estimated according to the model of Jukes and Cantor and the phylogenetic tree was constructed by the neighbor-joining method using the MEGA program (version 1.01). (De la Peña and Flores, unpublished data.)

### Distinguishing features

Lack of a central conserved region (CCR). RNA self-cleavage is mediated by hammerhead ribozymes in strands of either polarity. Replication studies have been carried out with ASBVd and PLMVd, and the inferred mechanism (see below) is presumed to operate in the other members of the family.

## Genus *Avsunviroid*

Type species ***Avocado sunblotch viroid***

### Distinguishing features

A circular ssRNA between 246 and 250 nt depending on isolates and sequence variants. It is unique in having a base composition rich in A+U (62%) in contrast to the other viroids, which are rich in G+C (53–60%). The most stable secondary structure is a rod-like or quasi-rod-like conformation in which neither five domains nor a central conserved region (CCR) can be distinguished. It is soluble in 2 M LiCl as are typical viroids like PSTVd, ASSVd and CbVd-1 which also have most stable secondary structures that are rod-like or quasi-rod-like. Plus and minus strands of ASBVd can form

hammerhead structures. Because both single hammerhead structures of ASBVd are thermodynamically unstable, double hammerhead structures have been proposed to operate in the self-cleavage reactions, especially in that of the plus polarity RNA (see Figure 3). Replication occurs through a symmetric rolling-circle model since the minus circular monomer has been found in infected tissue (see Figure 3). ASBVd replicates and accumulates in chloroplasts.

### Biological properties

Found naturally only in avocado but it can be experimentally transmitted into other members of the family *Lauraceae.* Reported in USA, Mexico, Australia, Israel, Spain, South Africa and South America.

### List of species in the genus *Avsunviroid*

| | | | |
|---|---|---|---|
| *Avocado sunblotch viroid* | | | |
| Avocado sunblotch viroid - avocado | [J02020] | {247} | (ASBVd-avo) |

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ], length in nucleotides { } and assigned abbreviations ( ) are also listed.

### List of other related viroids which may be members of the genus *Avsunviroid* but have not been approved as species

None.

## GENUS *PELAMOVIROID*

Type species ***Peach latent mosaic viroid***

### Distinguishing features

Circular ssRNAs between 337 and 401 nt depending on isolates and sequence variants. The most stable secondary structure is a branched conformation, stabilized by a kissing-loop interaction, in which neither five domains nor a central conserved region (CCR) can be distinguished (Figure 5). RNAs are insoluble in 2 M LiCl. Thermodynamically stable single hammerhead structures can be formed in both strands, and plus and minus monomeric RNAs self-cleave *in vitro* as predicted by these structures (see Figure 3). Replication most probably occurs by a symmetric rolling-circle mechanism since the PLMVd minus circular monomers have been found in infected tissue (see Figure 3). PLMVd and, most likely, CChMVd, replicate and accumulate in the chloroplast.

### Biological properties

PLMVd and CChMVd appear to be restricted to their natural hosts and very closely related species, but some reports indicate that PLMVd naturally infects additional species within and outside the genus *Prunus.* Recorded in many peach and chrysanthemum-growing areas. Most isolates of PLMVd are non-symptomatic or incite a mosaic, but others, with a specific hairpin insertion of 12 nt, incite an extreme albinism (peach calico). Symptomatic and non-symptomatic isolates of CChMVd differ in the sequence of a hairpin tetraloop.

### List of species in the genus *Pelamoviroid*

| | | | |
|---|---|---|---|
| *Chrysanthemum chlorotic mottle viroid* | | | |
| Chrysanthemum chlorotic mottle viroid – chrysanthemum | [Y14700] | {399} | (CChMVd-chr) |
| *Peach latent mosaic viroid* | | | |
| Peach latent mosaic viroid – peach | [M83545] | {337} | (PLMVd-pch) |
| Peach latent mosaic viroid – calico (Peach calico) | [AJ550912] | {349} | (PLMVd-cal) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ], length in nucleotides { } and assigned abbreviations ( ) are also listed.

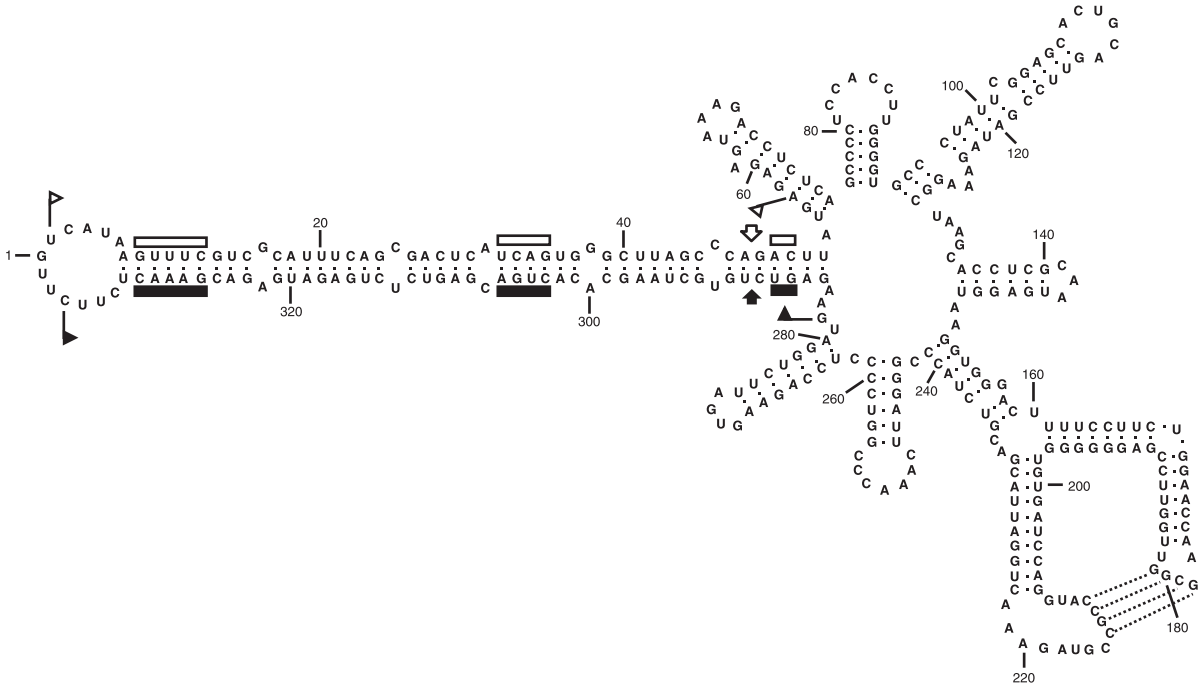

**Figure 5:** Primary and proposed branched secondary structure of lowest free energy for the reference sequence variant of an isolate of peach latent mosaic viroid. Plus and minus self-cleavage domains are delimited by flags, the 13 conserved residues present in most of the hammerhead structures are indicated by bars, and the self-cleavage sites are shown by arrows. Solid and open symbols refer to plus and minus polarities respectively. *In vitro* chemical and enzymatic probing has revealed the probable existence of a kissing-loop interaction between residues GCGG and CCGC (position 178–181 and 211–214, respectively) (Bussière *et al.*, 2000). (Adapted from Hernández, C. and Flores, R. (1992). Plus and minus RNAs of peach latent mosaic viroid self-cleave in vitro via hammerhead structures. *Proc. Natl Acad. Sci., U S A*, **89**, 3711–3715.)

### List of other related viroids which may be members of the genus *Pelamoviroid* but have not been approved as species

None.

## GENUS *ELAVIROID*

Type species ***Eggplant latent viroid***

### Distinguishing features

A circular ssRNA between 332 and 335 nt depending on isolates and sequence variants. Like most other viroids, ELVd is rich in G+C (57–59%). The most stable secondary structure is a quasi rod-like conformation with bifurcations at both termini. Neither five domains nor a central conserved region (CCR) can be distinguished. Like other viroids having a rod-like or quasi-rod-like conformation, ELVd is soluble in 2 M LiCl. Plus and minus strands of ELVd can form thermodynamically stable hammerhead structures. Because infected tissue contains circular forms of both plus and minus ELVd, replication appears to proceed through a symmetric rolling circle model – presumably in the chloroplast (see Figure 3).

### Biological properties

Found naturally in eggplant growing in eastern Spain. No symptoms observed under greenhouse conditions, and host range appears to be restricted. Seed-transmitted at relatively high rates

(16–26%), but attempts to transmit ELVd to tomato, chrysanthemum, cucumber and citron were unsuccessful.

### List of species in the genus *Elaviroid*

*Eggplant latent viroid*
Eggplant latent viroid – eggplant [AJ536612] {335} (ELVd-egg)

Species names are in italic script; names of isolates are in roman script. Sequence accession numbers [ ], length in nucleotides { } and assigned abbreviations ( ) are also listed.

### List of other related viroids which may be members of the genus *Elaviroid* but have not been approved as species

None.

## FAMILY *POSPIVIROIDAE*

### Taxonomic structure of the family

| | |
|---|---|
| Family | ***Pospiviroidae*** |
| Genus | ***Pospiviroid*** |
| Genus | ***Hostuviroid*** |
| Genus | ***Cocadviroid*** |
| Genus | ***Apscaviroid*** |
| Genus | ***Coleviroid*** |

### Distinguishing features

Presence of a central conserved region (CCR) and lack of RNA self-cleavage mediated by hammerhead ribozymes. According to the conserved residues there are basically three types of CCRs exemplified by those of PSTVd, apple scar skin viroid (ASSVd) and coleus blumei viroid 1 (CbVd-1). Genera *Pospiviroid*, *Hostuviroid* and *Cocadviroid* share an identical subset of nucleotides within their CCRs, which are, therefore, more closely related between themselves than with the CCRs of members of the genera *Apscaviroid* and *Coleviroid* (Figure 2). Replication studies have been done mostly with PSTVd, and the inferred mechanism (see below) is presumed to operate in the other members of the family as well.

## GENUS *POSPIVIROID*

Type species ***Potato spindle tuber viroid***

### Distinguishing features

Circular ssRNAs between 356 and 375 nt (excluding duplications) depending on species and sequence variants. The most stable secondary structure is a rod-like or quasi-rod-like conformation with five domains, a central conserved region (CCR) and a terminal conserved region (TCR) (Figure 2 and Figure 6). Replication, which takes place in the nucleus where the viroid also accumulates, occurs by an asymmetric rolling-circle mechanism since longer-than-unit minus strands have been found in infected tissue (see Figure 3).

### Biological properties

Pospiviroids are found world-wide and in a wide range of plants, mostly solanaceous species, but also in other hosts including citrus, avocado and some ornamentals. They can be experimentally transmitted to many other hosts in which symptom expression differs from symptomless to almost lethal. Some members, particularly TPMVd, can be transmitted by aphids under specific

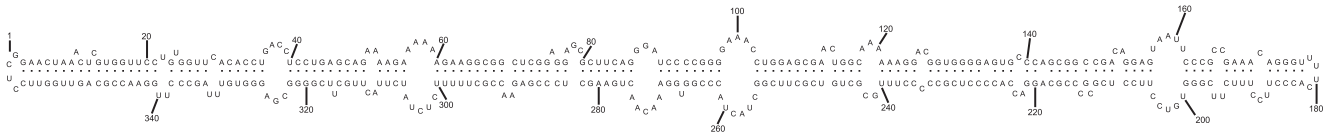

**Figure 6:** Primary and proposed rod-like secondary structure of lowest free energy for the reference sequence variant of potato spindle tuber viroid (PSTVd). (Adapted with modifications from Gross, H.J. *et al.* (1978). Nucleotide sequence and secondary structure of potato spindle tuber viroid. *Nature*, **273**, 203–208.)

ecological conditions. PSTVd is also aphid-transmissible when encapsidated in particles of potato leafroll virus.

### List of species in the genus *Pospiviroid*

| | | | |
|---|---|---|---|
| *Chrysanthemum stunt viroid* | | | |
| Chrysanthemum stunt viroid - chrysanthemum | [V01107] | {356} | (CSVd-chr) |
| *Citrus exocortis viroid* | | | |
| Citrus exocortis viroid - citrus | [M34917] | {371} | (CEVd-cit) |
| Citrus exocortis viroid - tomato (Indian tomato bunchy top viroid) | [X53716] | {372} | (CEVd-tom) |
| *Columnea latent viroid* | | | |
| Columnea latent viroid - columnea | [X15663] | {370} | (CLVd-col) |
| *Iresine viroid 1* | | | |
| Iresine viroid 1 - Iresine herbstii | [X95734] | {370} | (IrVd-1-ire) |
| *Mexican papita viroid* | | | |
| Mexican papita viroid - Solanum cardiophyllum | [L78454] | {360} | (MPVd-sol) |
| *Pepper chat fruit viroid* | | | |
| *Potato spindle tuber viroid* | [FJ409044] | {348} | (PCFVd-pep) |
| Potato spindle tuber viroid - intermediate | [V01465] | {359} | (PSTVd-int) |
| Potato spindle tuber viroid - tomato | [X17268] | {356} | (PSTVd-tom) |
| *Tomato apical stunt viroid* | | | |
| Tomato apical stunt viroid - tomato | [K00818] | {360} | (TASVd-tom) |
| *Tomato chlorotic dwarf viroid* | | | |
| Tomato chlorotic dwarf viroid - tomato | [AF162131] | {360} | (TCDVd-tom) |
| *Tomato planta macho viroid* | | | |
| Tomato planta macho viroid - tomato | [K00817] | {360} | (TPMVd-tom) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ] length in nucleotides { } and assigned abbreviations ( ) are also listed.

### List of other related viroids which may be members of the genus *Pospiviroid* but have not been approved as species

None.

## GENUS *HOSTUVIROID*

Type species ***Hop stunt viroid***

### Distinguishing features

A circular ssRNA of 295–303 nt depending on isolates and sequence variants. The most stable secondary structure is a rod-like or quasi-rod-like conformation with five domains, a central conserved region (CCR) similar to that of pospiviroids and cocadviroids, and a terminal conserved hairpin (TCH) (Figure 2). Replication occurs through an asymmetric rolling-circle model since longer-than-unit minus strands have been found in infected tissue (see Figure 3).

### Biological properties

HpSVd infects a very broad range of natural hosts and has been reported to be the causal agent of five different diseases. It is distributed worldwide.

### List of species in the genus *Hostuviroid*

| | | | |
|---|---|---|---|
| *Hop stunt viroid* | | | |
| Hop stunt viroid - hop | [X00009] | {297} | (HpSVd-hop) |
| Hop stunt viroid - citrus (Citrus cachexia viroid) | [AF131249] | {299} | (HpSVd-cit) |
| Hop stunt viroid - cucumber (Cucumber pale fruit viroid) | [X00524] | {303} | (HpSVd-cuc) |
| Hop stunt viroid - peach (Peach dapple viroid) | [D13765] | {297} | (HpSVd-pch) |
| Hop stunt viroid - plum (Plum dapple viroid) | [D13764] | {297} | (HpSVd-plu) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ], length in nucleotides { } and assigned abbreviations ( ) are also listed.

### List of other related viroids which may be members of the genus *Hostuviroid* but have not been approved as species

None.

## GENUS *COCADVIROID*

Type species ***Coconut cadang-cadang viroid***

### Distinguishing features

Circular ssRNAs of 246–301 nt depending on species and sequence variants. The most stable secondary structure is a rod-like or quasi-rod-like conformation with five domains, a central conserved region (CCR) similar to that of pospiviroids and hostuviroids, and a terminal conserved hairpin (TCH) (Figure 2). Single or double cytosine residues occur at a specific locus. The $T_R$ domain is variable as a result of 41, 50 or 55 nt duplications that generate large variants between 287 and 301 nt. As the disease progresses from the early to the medium stages the small forms are modified to the large forms. Dimeric forms of CCCVd occur with their corresponding monomeric forms. Replication most probably occurs through an asymmetric rolling-circle model by analogy with PSTVd (Figure 3). At the subnuclear level, CCCVd is concentrated in the nucleolus with the remainder distributed throughout the nucleoplasm.

### Biological properties

CCCVd and CTiVd are lethal to coconut palm, and have a host range restricted to members of the family *Arecaceae*. CCCVd has a geographic distribution that includes the Philippines, Malaysia and Sri Lanka. CTiVd is found in Guam. HpLVd and CBCVd have broader geographic distributions.

### List of species in the genus *Cocadviroid*

| | | | |
|---|---|---|---|
| *Citrus bark cracking viroid* | | | |
| Citrus bark cracking viroid - citrus (Citrus viroid IV) | [X14638] | {284} | (CBCVd-cit) |
| *Coconut cadang-cadang viroid* | | | |
| Coconut cadang-cadang viroid - coconut palm | [J02049] | {246} | ( (CCCVd-coc) |
| Coconut cadang-cadang viroid - oil palm | [DQ097183] | {297} | ( (CCCVd-op) |
| *Coconut tinangaja viroid* | | | |
| Coconut tinangaja viroid - coconut palm | [M20731] | {254} | (CTiVd-coc) |
| *Hop latent viroid* | | | |
| Hop latent viroid – hop | [X07397] | {256} | (HpLVd-hop) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ], length in nucleotides { } and assigned abbreviations ( ) are also listed.

### List of other related viroids which may be members of the genus *Cocadviroid* but have not been approved as species

None.

# GENUS *APSCAVIROID*

Type species ***Apple scar skin viroid***

## Distinguishing features

Circular ssRNAs between 306 and 369 nt depending on species and sequence variants. The most stable secondary structure is a rod-like or quasi-rod-like conformation with five domains, a central conserved region (CCR) and a terminal conserved region (TCR) (see Figure 2). Replication most probably occurs through an asymmetric rolling-circle model by analogy with PSTVd (see Figure 3).

## Biological properties

Known natural hosts are confined to genera *Malus*, *Pyrus*, *Citrus* and *Vitis*. Reported worldwide, particularly those affecting the genera *Citrus* and *Vitis*.

## List of species in the genus *Apscaviroid*

| | | | |
|---|---|---|---|
| *Apple dimple fruit viroid* | | | |
| Apple dimple fruit viroid - apple | [X99487] | {306} | (ADFVd-app) |
| *Apple scar skin viroid* | | | |
| Apple scar skin viroid - apple | [M36646] | {329} | (ASSVd-app) |
| Apple scar skin viroid - dapple apple (Dapple apple viroid) | [X71599] | {331} | (ASSVd-dap) |
| Apple scar skin viroid - Japanese pear (Japanese pear fruit dimple viroid) | | | (ASSVd-jpf) |
| Apple scar skin viroid - pear rusty skin (Pear rusty skin viroid) | | | (ASSVd-prs) |
| *Australian grapevine viroid* | | | |
| Australian grapevine viroid - grapevine | [X17101] | {369} | (AGVd-grp) |
| *Citrus bent leaf viroid* | | | |
| Citrus bent leaf viroid - citrus (Citrus viroid I) | [M74065] | {318} | (CBLVd-cit) |
| *Citrus dwarfing viroid* | | | |
| Citrus dwarfing viroid - IIIa (Citrus viroid IIIa) | [S76452] | {297} | (CDVd-IIIa) |
| Citrus dwarfing viroid - IIIb (Citrus viroid IIIb) | [AF184147] | {294} | (CDVd-IIIb) |
| *Citrus viroid V* | | | |
| Citrus viroid V - citrus | [EF617306] | {294} | (CVd V-cit) |
| *Citrus viroid VI* | | | |
| Citrus viroid VI - citrus (Citrus viroid-original source) | [AB019508] | {330} | (CVd VI-os) |
| *Grapevine yellow speckle viroid 1* | | | |
| Grapevine yellow speckle viroid 1 - grapevine | [X06904] | {367} | (GYSVd-1-grp) |
| *Grapevine yellow speckle viroid 2* | | | |
| Grapevine yellow speckle viroid 2 - grapevine | [J04348] | {363} | (GYSVd-2-grp) |
| *Pear blister canker viroid* | | | |
| Pear blister canker viroid - pear | [D12823] | {315} | (PBCVd-pr) |

Species names are in italic script; names of isolates are in roman script; names of synonyms are in roman script and parentheses. Sequence accession numbers [ ], length in nucleotides { } and assigned abbreviations ( ) are also listed.

## List of other related viroids which may be members of the genus *Apscaviroid* but have not been approved as species

| | | | |
|---|---|---|---|
| Apple fruit crinkle viroid | [E29032] | {371} | (AFCVd) |
| Grapevine yellow speckle viroid 3 | [DQ371462] | {366} | (GYSVd-3) |
| Persimmon latent viroid | [AB366022] | {396} | (PLVd) |

# GENUS *COLEVIROID*

Type species ***Coleus blumei viroid 1***

## Distinguishing features

Circular ssRNAs between 248 and 364 nt depending on species and sequence variants. The most stable secondary structure is a rod-like or quasi-rod-like conformation with five domains and a

central conserved region (CCR) different from that of other viroid genera, and a terminal conserved region (TCR) in the two largest members of the genus (see Figure 2). Replication most probably occurs through an asymmetric rolling-circle model by analogy with PSTVd (see Figure 3).

## Biological properties

Known hosts are confined to the genus *Coleus*. Identified in Brazil, Canada and Germany. Seed transmission of CbVd-1 (and presumably CbVd-2 and CbVd-3) is particularly efficient.

## Species demarcation criteria in the genus

Recombination events appear to be especially frequent in this genus. In the future, recognition of new species will be based on the standard criteria for all viroids.

## List of species in the genus *Coleviroid*

| | | | |
|---|---|---|---|
| *Coleus blumei viroid 1* | | | |
| Coleus blumei viroid 1 - coleus | [X52960] | {248} | (CbVd-1-cls) |
| *Coleus blumei viroid 2* | | | |
| Coleus blumei viroid 2 - coleus | [X95365] | {301} | (CbVd-2-cls) |
| *Coleus blumei viroid 3* | | | |
| Coleus blumei viroid 3 - coleus | [X95364] | {361} | (CbVd-3-cls) |

Species names are in italic script; names of isolates are in roman script. Sequence accessions [ ], length in nucleotides { } and assigned abbreviations ( ) are also listed.

## List of other related viroids which may be members of the genus *Coleviroid* but have not been approved as species

| | | | |
|---|---|---|---|
| Coleus blumei viroid 4 | [X97202] | {295} | (CbVd-4) |
| Coleus blumei viroid 5 | [FJ151370] | {274} | (CbVd-5) |
| Coleus blumei viroid 6 | [FJ615418] | {342} | (CbVd-6) |

## List of unassigned viroids which have not been approved as species

| | |
|---|---|
| Blueberry mosaic viroid-like RNA | (BluMVd-RNA) |
| Burdock stunt viroid | (BuSVd) |
| Nicotiana glutinosa stunt viroid | (NGSVd) |
| Pigeon pea mosaic mottle viroid | (PPMMoVd) |
| Tomato bunchy top viroid | (ToBTVd) |

## Derivation of names

*Viroid*: from the name given to the subviral agent of potato spindle tuber disease.
*Apsca*: from *ap*ple *sca*r skin viroid.
*Avsun*: *av*ocado *sun*blotch viroid.
*Cocad*: from *co*conut *cad*ang-cadang viroid.
*Cole*: from *Cole*us blumei viroid 1.
*Ela*: from *e*ggplant *la*tent viroid.
*Hostu*: from *ho*p *stu*nt viroid.
*Pelamo*: from *pe*ach *la*tent *mo*saic viroid.
*Pospi*: from *po*tato *spi*ndle tuber viroid.

## Further reading

### Journals and books

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Gas, M.E., Hernández, C., Flores, R. and Daròs, J.A. (2007). Processing of nuclear viroids in vivo: An interplay between RNA conformations. *PLoS Pathog.*, **3**, 1813–1826.
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Hadidi, A., Flores, R., Randles, J.W. and Semancik, J.S. (Eds.) (2003). *Viroids*. Collingwood, Australia: CSIRO Publishing.
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## Websites

Subviral RNA Database: http://subviral.med.uottawa.ca.
NCBI Entrez Viral Genomes (includes viroids): http://www.ncbi.nlm.nih.gov/genomes/GenomesHome.cgi?taxid = 10239.

## Contributed by

Owens, R.A., Flores, R., Di Serio, F., Li, S-F., Pallás, V., Randles, J.W., Sano, T. and Vidalakis, G.

# Fungal Prions

## Taxonomic structure of the fungal prions

**Prions**
- **[URE3] prion**
- **[PSI] prion**
- **[Het-s] prion**
- **[PIN] prion**
- **[β] prion**
- **[SWI] prion**
- **[OCT] prion**
- **[MOT3] prion**
- **[ISP] prion**

## Distinguishing features

The name "prion" means infectious protein. In yeast and filamentous fungi, infection by viruses occurs exclusively by cell–cell fusion with transmission of cytoplasm. Viruses are transmitted as non-chromosomal genetic elements (denoted in brackets as above). Likewise, one expects prions of these organisms to show similar behavior. There are now eight well-documented prions in yeast and filamentous fungi. Seven of these are due to self-propagating amyloids, while the eighth is an auto-activating protease.

## Prion properties

### Morphology

Prions are infectious proteins: altered forms of a normal cellular protein that may have lost their normal function, but have acquired the ability to change the normal form of the protein into the same abnormal form as themselves. In most cases they form filaments or filamentous aggregates, but this is not part of the definition of a prion, which in some cases could be based on a self-perpetuating covalent modification without substantial morphological effects. Amyloid filaments (Figure 1) are typically straight and unbranched. Some have a helical appearance. In several cases, amyloid formed *in vitro* of the recombinant prion protein has been shown to be infectious for yeast, transmitting the corresponding prion.

### Physicochemical and physical properties

Except for [β], each prion is a self-propagating amyloid form of the corresponding protein (see below). Infectious amyloids of the prion domains of Ure2p, Sup35p and Rnq1p have an in-register parallel β-sheet architecture (Figure 2). Infectious amyloid of the HET-s prion domain is a two-turn β-helix. The vacuolar protease B is processed into its mature active form in cells carrying the [β] prion; the [β] prion is simply the mature active form of protease B.

### Nucleic acid

None. The definition of a prion is that it is an infectious protein without the need for any accompanying nucleic acid, although the host genome must encode the protein.

### Proteins

| Prion | Protein | Function of the protein |
|---|---|---|
| [URE3] | Ure2p | Regulator of nitrogen catabolism |
| [PSI] | Sup35p | Translation termination |
| [Het-s] | HET-s | Heterokaryon incompatibility |
| [PIN] | Rnq1 | Unknown; detected by [PSI]-inducibility |
| [β] | Prb1p | Protease B: protein processing and degradation |
| [SWI] | Swi1p | Subunit of chromatin remodeling complex |
| [OCT] | Cyc8p | Transcription repressor with Tup1p |
| [MOT3] | Mot3p | Transcription factor |
| [ISP] | Sfp1 | Transcription factor |

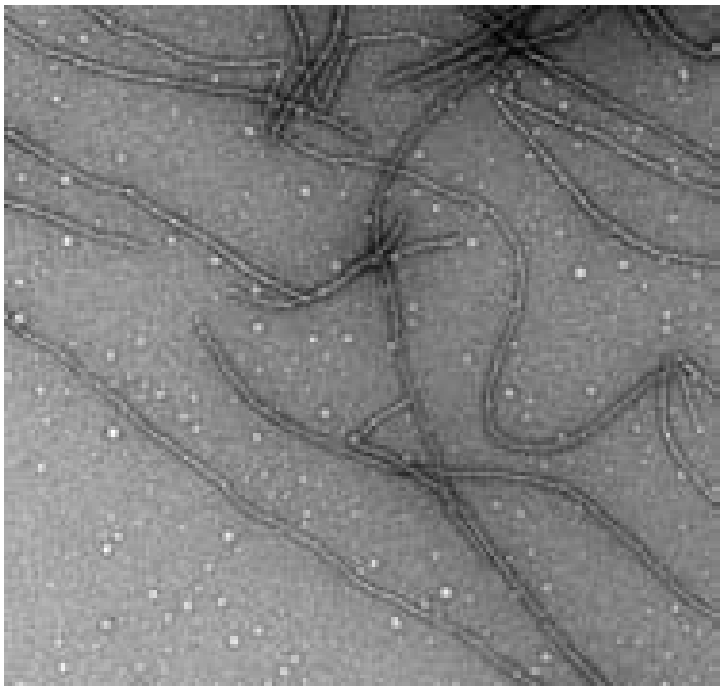

**Figure 1:** Amyloid filaments of the Sup35 protein prion domain (courtesy of Dr Frank Shewmaker, NIH). Amyloid is a filamentous protein polymer, with strands perpendicular to the long axis of the filament, relative protease-resistance of the protein and special dye staining properties.

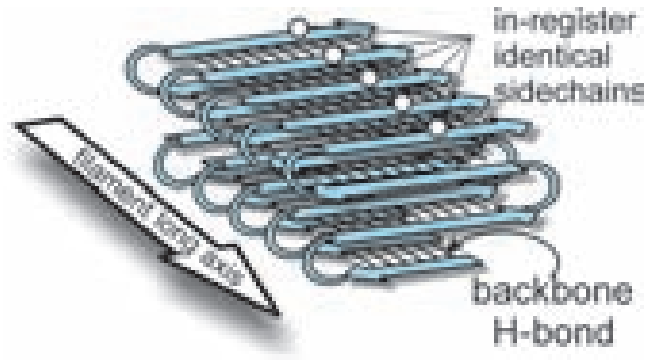


**Figure 2:** Architecture of yeast prion amyloid filaments is an in-register parallel β-sheet. The side chains of each residue form a row along the long axis of the filaments, and interactions among these identical side chains, such as the β-zipper of glutamine or asparagine side chains, enforce the in-register structure. The same interactions explain how conformational information is propagated from the filament to new molecules joining the ends.

### Lipids
None.

### Carbohydrates
None.

## Genome organization and replication

Each of the prions is inherited as a non-Mendelian genetic element (also called cytoplasmic genetic elements or non-chromosomal genetic elements). The "genetic material" in this case is the altered protein which is self-replicating by changing the normal form of the protein (which nonetheless

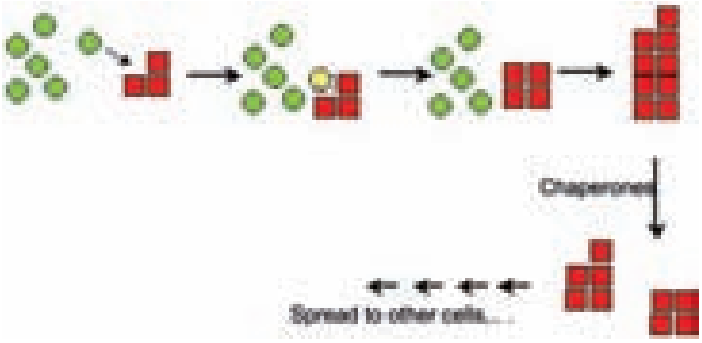


**Figure 3:** Amyloid prions replicate by elongation of amyloid filaments and scission of filaments by the action of chaperones to produce new filaments.

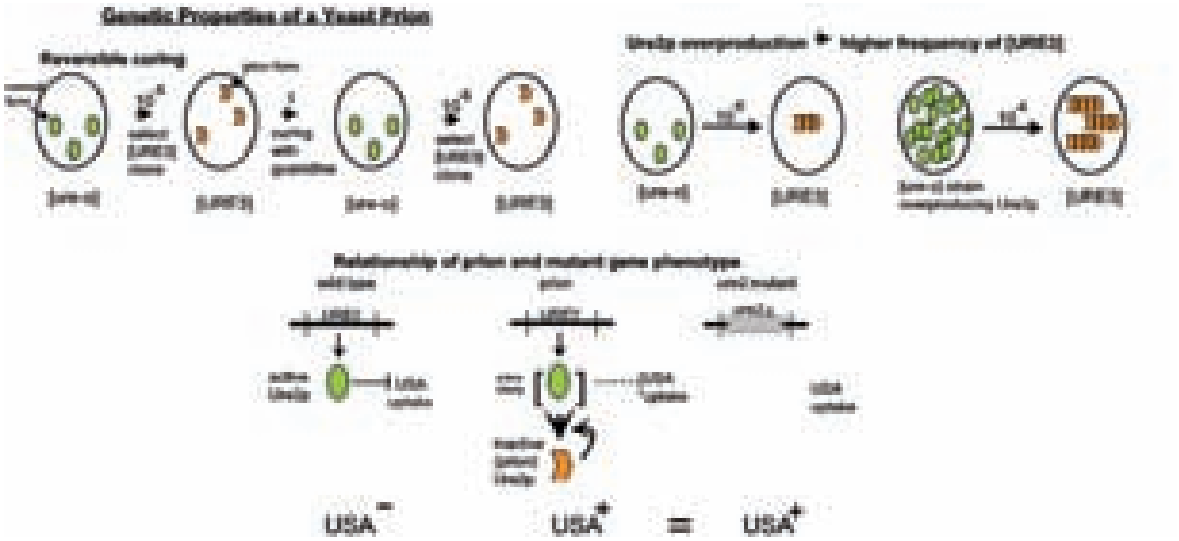


**Figure 4:** Genetic criteria for identification of prions.

has the same primary sequence) into the altered form. Thus altered Ure2p, Sup35p, Rnq1p, Prb1p, HETs, Swi1p, Cyc8p, Mot3p and Sfp1p are the "genomes"of [URE3], [PSI], [PIN], [β], [Het-s], [SWI], [OCT], [MOT3] and [ISP].

Each amyloid-forming prion protein has a prion domain. Deletions of the remainder of the molecule increase the frequency with which the prion form arises. Chaperones play an important role in all amyloid-related prions of *S. cerevisiae*, but different prions show different effects of altered chaperone activities. The propagation of all amyloid-based yeast prions requires Hsp104, and its cooperating chaperones, for the breakage of large fibers into smaller ones, to create new "seeds" (Figure 3).

## Biological properties

The yeast and fungal prions are passed from cell to cell during mating and hyphal anastomosis. There are no known natural vectors or extracellular transmission. Thus, fungal prions appear first as non-Mendelian genetic elements. Three biologic (genetic) criteria have been proposed to determine whether a given non-Mendelian genetic element is a prion (Figure 4).

- Prions are "reversibly curable", meaning that even if a prion can be cured from a strain, it can arise again spontaneously at some low frequency in the cured strain.
- Overexpression of the normal protein increases the frequency with which the prion form arises.

- The phenotype of the presence of the prion is the same as the phenotype of a recessive mutant in the gene encoding the protein. This gene first appears as a chromosomal gene necessary for the propagation of the prion. In some cases, the prion produces a phenotype by having an action beyond inactivation of the normal form. In this case, the phenotypic relation does not provide evidence that the non-Mendelian genetic element is a prion, but, of course, it is also not evidence against it being a prion. In all cases, the prion depends for its propagation on the gene for the protein.

Further, physical or chemical evidence for an alteration of the protein in cells carrying the prion should be demonstrated.

The [Het-s] and [β] prions are advantageous for their hosts and are present in essentially all of the respective wild-type strains. Most of the other prions produce a phenotype resulting from a deficiency of the normal protein. However, the [PIN] prion phenotype of priming generation of the [PSI] or [URE3] prion is not produced by deficiency of the corresponding protein, Rnq1p.

A feature of most prions is the existence of several prion "variants" (called "prion strains" in mammals) with different biological properties although the sequence of the prion protein is identical. The differences include phenotype intensity, stability of the prion, sensitivity to overproduction or deficiency of certain chaperones, and height of species barriers.

## Species demarcation criteria

Prions are divided into species based on the identity of the protein that makes up the infectious element. (Note: The formal taxonomy of viruses does not extend to unconventional agents like prions and prion "species" are not included in the ICTV Master Species List.)

# Species [URE3]

Host *Saccharomyces cerevisiae*

## Distinguishing features

[URE3] is a self-propagating amyloid form of the Ure2 protein, a regulator of nitrogen catabolism. In the prion form of the Ure2 protein, its nitrogen regulation activity is largely lost. This results in a slow growth phenotype as well as derepression of activities normally repressed by a good nitrogen source.

## Prion properties

### Morphology

The synthetic prion domain of Ure2p, $Ure2p^{1-65}$, forms amyloid *in vitro*, and induces a self-propagating amyloid formation by full-length native Ure2p purified from yeast. Ure2p amyloid filaments have a 4 nm core composed of the prion domain, surrounded by globular appendages composed of the C-terminal nitrogen regulation domain. Cells carrying the [URE3] prion contain a network of 25 nm diameter filaments containing the Ure2 protein (Figure 5).

### Physicochemical and physical properties

Ure2p is normally a soluble dimer, whose C-terminal domain is similar in sequence and structure to glutathione - S - transferases. Ure2p is more proteinase K-resistant in extracts of [URE3] strains than in wild-type strains, with the N-terminal prion domain the most protease resistant part. Ure2p amyloid formed *in vitro* likewise has a core composed of the prion domain which is the most protease-resistant part of the aggregate. The amyloid core of the filaments has beta-sheet structure. Solid-state NMR of prion domain amyloid filaments shows that they have an in-register parallel β-sheet structure. The prion domain changes from being unstructured in the normal soluble form to being in a very tight in-register parallel β-sheet structure in the amyloid (prion) form. The

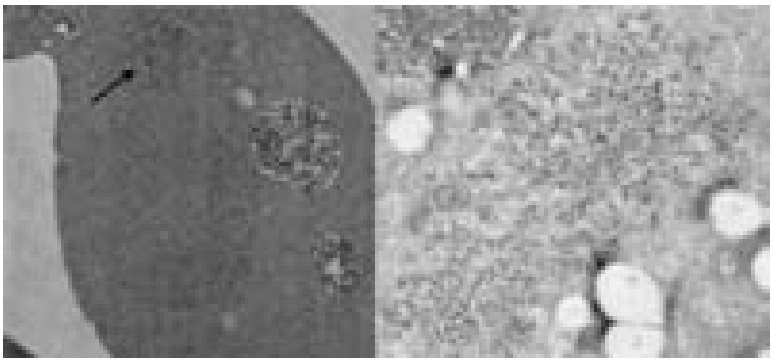

**Figure 5:** Amyloid filaments in cells of *S. cerevisiae* infected with the [URE3] prion. (From Speransky, V. *et al.* (2001). Prion filament networks in [URE3] cells if *Saccaromyces cerevisiae. J. Cell Biol.*, **153**, 1327-1335; with permission.)

C-terminal domain does not change significantly in structure on conversion from soluble to amyloid form.

### Proteins

Ure2p, in its altered form, is the primary component of [URE3]. The presence of the [PIN] prion also stimulates the rate of [URE3] prion generation.

## Genome organization and replication

The *URE2* gene has an ORF coding for a 354 aa polypeptide, of which the C-terminal part, including residues 81 to 354, is capable of carrying out the nitrogen regulation function of Ure2p if overexpressed. The N-terminal 80 residues (the prion domain) is necessary for *in vivo* stability of Ure2p and for tight nitrogen regulation. The Ure2p prion domain can propagate [URE3] in the absence of the C-terminal domain and efficiently transmits the prion to the full-length molecule. Amyloid of the prion domain alone or fused to another unrelated protein is highly infectious, transmitting the [URE3] to yeast cells. The prion domain is required for induction of [URE3], for propagation of the prion, and without a covalently attached prion domain, a Ure2 molecule is not affected by the presence of [URE3]. Propagation of [URE3] requires the chaperones Hsp104 and Ssa2 and is blocked by overproduction of the Hsp40-group chaperone Ydj1p, and by the GTP-exchange protein Sse1p. Overexpression of Btn2p also cures [URE3], apparently by collecting [URE3] seeds to one cellular site. Deletion of Btn2 increases the number of [URE3] seeds per cell.

## Antigenic properties

Not studied.

## Biological properties

[URE3] makes cells able to take up ureidosuccinate from the media containing a good nitrogen source such as ammonia or glutamine. The *ure2* mutants have the same phenotype, and the propagation of [URE3] requires the *URE2* gene. Yeast cells turn off transcription of genes for enzymes involved in utilization of poor nitrogen sources when a good nitrogen source is available (nitrogen catabolite regulation). *DAL5* encodes the permease for allantoate, a poor, but usable, nitrogen source for yeast. *DAL5* is thus subject to nitrogen catabolite regulation. Allantoate is structurally similar to ureidosuccinate, and so Dal5p can take up ureidosuccinate. Ureidosuccinate is an intermediate in uracil biosynthesis, the product of the first step in the pathway, aspartate transcarbamylase (*URA2*). Thus, *ura2* mutants can grow on ureidosuccinate in place of uracil if the cell has either the [URE3] prion or a mutation in *ure2*. [URE3] can be cured by growth in the presence of 5 mM guanidine HCl.

[URE3] is not found in wild strains indicating that [URE3] is a disease of yeast rather than an adaptive mechanism. Indeed, one *Saccharomyces* species has an N-terminal domain similar to the *cerevisiae* Ure2 prion domain, but is unable to have the [URE3] prion, indicating that this domain is not present in order to allow cells to have a prion. The prion domain has a normal function in stabilizing the full-length protein against degradation, again consistent with [URE3] being a disease. The prion domain changes in evolution more rapidly than the C-terminal part of the molecule resulting in a species barrier to [URE3] transmission. This variation may be selected to protect cells from acquiring the prion. Finally, [URE3] cells generally grow slowly – not an advantageous trait.

### Prion variants

Even a single protein can form prions of distinct "strains", or "variants", apparently due to different self-propagating amyloid structures, much as a given protein can assume more than one crystal form. Variants are distinguished by the intensity of their phenotypes, by the stability of prion propagation, by the effect of overproduction or deficiency of various chaperones on prion propagation and by species barriers. Different yeast species can have the [URE3] prion, but their differing Ure2p sequences produce a "species barrier" to transmission. *Saccharomyces castellii* is apparently unable to have the [URE3] prion, while *S. bayanus*, *S. cariocanus* and *S. mikatae* can have it.

## Species [PSI+]

Host ***Saccharomyces cerevisiae***

### Distinguishing features

[PSI] is a self-propagating amyloid form (a prion) of the Sup35 protein, a subunit of the translation termination factor of the yeast *Saccharomyces cerevisiae*. The presence of the [PSI] prion results in a relative shortage of the soluble form of Sup35p, and a resulting decrease in the efficiency of translation termination. This makes readthrough of termination codons more frequent. The phenotype of [PSI] is ability to grow in the absence of adenine in spite of a premature stop mutation in *ADE1* or *ADE*2, combined in the latter case with a weak serine-inserting tRNA suppressor mutation. The prion domain of Sup35p (residues 1-123) also is necessary for normal mRNA turnover, interacting with the polyA binding protein and the polyA-degrading enzymes to favor shortening of the 3′ polyA structure of mRNAs, a first step in mRNA degradation.

### Prion properties

#### Morphology

Sup35p forms typical amyloid filaments *in vitro* (Figure 1), and well-ordered, laterally aligned arrays of filaments *in vivo*.

#### Physicochemical and physical properties

Amyloid filaments of the Sup35 protein are high in beta sheet structure, but are only slightly protease-resistant. They have an in-register parallel β-sheet structure based on solid-state NMR studies. The filaments have one molecule for about every 4.7 Å of filament length, consistent with the in-register parallel structure. Filaments formed under different conditions may have different extents of β-sheet structure in the prion domain.

#### Proteins

Sup35p (79 kDa) is a subunit of the translation termination factor, which also includes Sup45p. Amyloid of Sup35p isolated from [PSI]-containing cells also contains the Hsp70 group chaperone Ssa1/2 in large amounts and smaller amounts of Hsp104, Ssb1/2, Sis1, Sse1, Ydj1 and Sla2.

### Genome organization and replication

The N-terminal 123 residues of Sup35p are sufficient to propagate the [PSI] prion. This part of Sup35p is very high in glutamine and asparagine residues, and these are critical for prion formation

and propagation. Overexpression of this prion domain of Sup35p also induces the *de novo* appearance of [PSI] at a much higher frequency than does similar overexpression of the full-length Sup35p. Amyloid of full-length Sup35p or the prion domain (with or without fusion to another protein) is infectious for yeast transmitting the [PSI+] prion.

The level of the Hsp104 chaperone is critical for [PSI] propagation with either overexpression or under-expression leading to the loss of the prion. Hsp104, together with other chaperones, cleaves long filaments to make shorter ones that can serve as new seeds of amyloid formation. The Hsp70s in the Ssa group are also essential for [PSI] propagation.

### Antigenic properties

Not studied.

### Biological properties

[PSI] increases the efficiency with which ribosomes read through termination codons. The Sup35 protein is a subunit of the translation release factor that recognizes termination codons, and releases the peptidyl tRNA and cleaves the nascent peptide from the tRNA. Thus, *sup35* mutants have the same phenotype as do [PSI] strains. Furthermore, the *SUP35* gene is necessary for the propagation of [PSI]. [PSI], like [URE3] and [PIN], can be cured by guanidine inhibition of or deficiency of Hsp104, but from the cured strains, can again be isolated subclones carrying [PSI]. Hsp104 is a disaggregating chaperone necessary for the propagation of each of these prions. By breaking up amyloid filaments into smaller filaments, Hsp104 increases the number of "seeds" and so ensures inheritance of the amyloid by each of the daughter cells. Overproduction of Sup35p results in a 100-fold increase in the frequency with which [PSI] arises *de novo*.

Strains of [PSI], like the strains known for the scrapie agent and other transmissible spongiform encephalopathies, have been found for [PSI]. They are associated with different stabilities, curability by guanidine, and efficiency of suppression of nonsense mutations.

[PSI+] is not found in wild strains, suggesting that it is a disease of yeast, rather than an adaptive mechanism. The prion domain of Sup35p has a normal non-prion function in mRNA turnover, by facilitating the normal gradual shortening of the 3′ polyA structure. Thus, like the mammalian prion protein PrP, the Sup35 prion domain is not present simply to allow cells to have a prion. The Sup35 prion domain changes more rapidly in evolution than does the C-terminal domain, developing a species barrier to [PSI+] transmission. This variation may be selected to protect against acquisition of a prion. In one study, one-quarter of wild *S. cerevisiae* isolates had a large deletion in their prion domain so that they could no longer become [PSI+], indicating that prion formation is not conserved within this species.

### Prion variants

Even a single protein can form prions of distinct "strains", or "variants", apparently due to different self-propagating amyloid structures, much as a given protein can assume more than one crystal form.

## Species [PIN+]

Host ***Saccharomyces cerevisiae***

### Distinguishing features

[PIN+] is a self-propagating amyloid form of the Rnq1 protein, whose normal function is unknown. [PIN+] has the ability to dramatically increase the frequency with which the [PSI+] prion arises. [PIN+] also has a smaller effect on [URE3] prion generation.

## Prion properties

### Morphology

Rnq1p is aggregated in [PIN+] strains and recombinant Rnq1p forms amyloid filaments *in vitro.*

### Physicochemical and physical properties

Filaments of the Rnq1 protein formed *in vitro* are 11 nm in diameter, stain with Thioflavin T, and have high β-sheet content indicating amyloid structure. Solid state NMR studies of the Rnq1p prion domain indicate that the amyloid has an in-register parallel architecture.

### Proteins

Rnq1p is a 405 aa residue protein with a C-terminal domain rich in asparagine and glutamine residues (like the prion domains of Ure2p and Sup35p). This domain can act as a prion domain when fused to the C-terminal part of Sup35p. Amyloid of Rnq1p isolated from [PIN+] cells contains roughly equimolar amounts of the Hsp40 family chaperone Sis1p.

## Genome organization and replication

Amyloid formed *in vitro* from recombinant Rnq1p is infectious for yeast transmitting the [PIN+] prion. The Hsp104 chaperone is essential for [PIN] propagation. The Hsp40 Sis1p is also essential for [PIN] propagation.

## Antigenic properties

Not studied.

## Biological properties

[PIN] dramatically increases the frequency with which the [PSI] prion arises *de novo*, so much so that it is nearly essential for [PSI] generation. [PIN] also increases the frequency of [URE3] *de novo* generation, but only 10- to 100-fold. The evidence suggests that the amyloid formed by one asparagine–glutamine-rich protein can prime the formation of amyloid by another such protein. Overproduction of Swi1p or Cyc8p can mimic [PIN+] in stimulating generation of [PSI+], and now these proteins are known to form prions themselves, [SWI+] and [OCT+], respectively.

## Prion variants

Even a single protein can form prions of distinct "strains", or "variants", apparently due to different self-propagating amyloid structures, much as a given protein can assume more than one crystal form. [PIN+] is known to have several variants distinguished by their strength in inducing [PSI+] formation.

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# Species [Het-s]

Host ***Podospora anserina***, a filamentous fungus

## Distinguishing features

[Het-s] was found as a non-chromosomal gene necessary for heterokaryon incompatibility based on the chromosomal *het-s/S* locus. [Het-s] is a self-propagating amyloid of the HET-s protein. Heterokaryon incompatibility is a normal fungal function, and so the [Het-s] prion is the first prion known to be responsible for a normal function, a conclusion further confirmed by the fact that nearly all wild *het*-s strains carry this prion. Amyloid formed *in vitro* from recombinant HET-s protein is infectious to normal *Podospora* transmitting the [Het-s] prion.

## Prion properties

### Morphology

The HET-s protein shows self-seeding formation of amyloid filaments 15–20 nm in diameter.

### Physicochemical and physical properties

The amyloid of the HET-s prion domain is a two-turn β-helix with a very uniform structure. This is in contrast to the amyloids of the yeast prions which are heterogeneous in detail structure, although uniform in having in-register parallel architecture. There is only one known [Het-s] prion variant, and this may explain the uniform structure. The HET-s protein has evolved to be a prion with special properties, explaining the uniformity of filament structure.

### Proteins

The HET-s protein is the product of the het-s gene. The prion domain sufficient for amyloid formation *in vitro* and prion propagation *in vivo* is located at the C-terminal part of the molecule. Deletions of the N-terminal part destabilize the prion domain.

## Genome organization and replication

The prion domain of the HET-s protein is the C-terminal 72 aa residues. This region is very protease-sensitive in the soluble form and protease resistant in the amyloid form. Unlike the proteins that form the [URE3], [PSI] and [PIN] prions, the HET-s protein prion domain is not rich in asparagine or glutamine residues. Residues 23 and 33 are particularly critical in determining whether the protein can undergo the prion change or not.

## Antigenic properties

Not studied.

## Biological properties

[Het-s] makes *Podospora anserina* strains differing at the *het-s* locus able to carry out heterokaryon incompatibility. It is the first prion described that is responsible for a normal cellular function, rather than a disease of the organism. When two fungal colonies grow together, the hyphae of the two colonies fuse with each other. This process facilitates cooperation between the colonies in acquisition of nutrients, and perhaps other functions. One risk of this process is that viruses present in one colony will spread into the other colony. Apparently to prevent this, most fungi will only form heterokaryons with closely related strains, identical for alleles at each of 6 or more loci. In *Podospora anserina*, there are eight such loci called *Het* loci. The *het-s* locus has alleles *het-s* and *het-S*. The *het-s* strains show incompatibillity for heterokaryon formation with *het-S* strains only if the protein encoded by *het-s* is in a prion form. The presence of this prion is found as a non-Mendelian genetic element, called [Het-s]. [Het-s] is reversibly curable, the frequency of its appearance *de novo* is enhanced by overproduction of the protein encoded by the *het-s* gene, and the *het-s* gene is necessary for the propagation of [Het-s]. The protein encoded by the *het-s* gene is more protease-resistant in strains carrying [Het-s].

## Prion variants

At this time, only a single [Het-s] variant is known, perhaps reflecting its being evolved to be a prion with specific properties, unlike the known yeast prions which appear to be diseases.

# Species [Beta]

Host ***Saccharomyces cerevisiae***

## Distinguishing features

An enzyme that is necessary for its own activation (or maturation) will remain inactive if not initially activated or remain active in a self-propagating manner if initially active. This can be the basis for a prion (infectious protein) since transmission of the active enzyme from one individual to another lacking the active enzyme will change the self-propagating status of the recipient individual. In the absence of protease A, which normally can activate protease B, protease B acts as a prion of *Saccharomyces cerevisiae*.

## Prion properties

### Morphology

The beta prion is identical with the mature active form of the vacuolar protease B of yeast.

### Physicochemical and physical properties

Active protease B is a soluble protein, synthesized as a precursor protein encoded by *PRB1.* Its activation requires both N- and C-terminal cleavage, which either protease A or mature protease B can carry out.

### Proteins

The mature active form of Prb1p (vacuolar protease B) is a 284 aa residue protein which is derived by N- and C-terminal cleavages of a 635 residue precursor protein. Prb1p is a serine protease of the subtilisin group with roles in maturation of other vacuolar proteins, and in protein degradation by the vacuole which is critical for meiosis and spore formation and for survival of starvation.

## Genome organization and replication

Normally, protease A, as well as mature protease B, can carry out the cleavage-maturation of pro-protease B. In a *pep4* deletion mutant lacking protease A, protease B acts as a prion. Cells initially lacking mature active protease B give rise to progeny, nearly all of which lack the active enzyme. Cells initially carrying active mature protease B give rise to offspring, nearly all of which also have active protease B. Transmission of active protease B to a cell lacking it, converts the pro-protease B in the recipient to the active form. This activity is then passed on to progeny by continued auto-activation. About 1 in $10^5$ cells spontaneously develops protease B activity.

The protease B precursor protein requires removal of both N-terminal and C-terminal extension for maturation.

## Antigenic properties

Not studied.

## Biological properties

Like the amyloid-related prions, [BETA] is reversibly curable. Activity is lost by culturing cells on media that repress expression of Prb1p, but from a cured strain, [BETA] arises spontaneously in about 1 in $10^5$ progeny cells. Overexpression of pro-Prb1p increases the frequency with which [BETA] arises about 100- to 1000-fold. The propagation of [BETA] requires the *PRB1* gene, as expected. [BETA] is present in all wild-type cells, but is inapparent because protease A can activate proprotease B. Only in the absence of protease A does the prion character become evident.

[BETA] allows cells to go through meiosis and spore formation and to better survive starvation, a process that requires protein breakdown in the vacuole.

## Prion variants

None reported.

## Similarity with other taxa

The N-terminal prion domain of Sup35p includes repeat aa sequences similar to the octapeptide repeats in PrP, but the Sup35p repeats are not necessary for prion formation as shuffling the Sup35p prion domain does not prevent amyloid formation. The Sup35 prion domains of *Candida albicans*, *Kluyveromyces lactis* and *Pichia methanolica* can be prion domains in *S. cerevisiae* when fused to the *S. cerevisiae* Sup35MC (non-prion) domain. Full length Ure2p from *S. bayanus*, *S. uvarum*, *S. mikatae*, *S. cariocanus* and *S. paradoxus* can form the [URE3] prion in *S. cerevisiae*.

Overall, the yeast and fungal prions are analogous to the mammalian prion protein PrP, but not at all homologous.

## Derivation of names

[BETA]: from protease B and the Greek letter beta.
[Het-s] and het-s: from heterokaryon incompatibility.
[PIN]: from [PSI] inducibility.
[PSI] from the greek letter Psi.
[URE3] and URE2: from ureidosuccinate.
[SWI]: from the Swi1 protein.
[OCT]: from the Cyc8 protein.
[MOT3]: from the Mot3 protein.
[ISP]: from the phenotype being the inverse of that or [PSI].
Prion: sigla from infectious protein.

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# Vertebrate Prions

Prions are proteinaceous infectious particles that lack nucleic acids. In mammals, prions are composed largely, if not entirely, of a pathogenic isoform of the host-encoded cellular prion protein (PrP). Neither prion-specific nucleic acids nor virus-like particles have been detected in highly purified, infectious preparations. In fungi, evidence for nine different prions has been accumulated. Those studies are summarized in the section on Fungal Prions.

## General description of the mammalian prions

The mammalian prions cause scrapie and other related neurodegenerative diseases of animals and humans (Table 1). The prion diseases are also referred to as the transmissible spongiform encephalopathies (TSEs). TSEs are inevitable fatal and no treatment is available for the afflicted species.

Prions are composed of an abnormal, pathogenic PrP isoform, denoted $PrP^{Sc}$. The "Sc" superscript was initially derived from the term scrapie, because scrapie was the prototypic prion disease. Since all of the known prion diseases (Table 1) of mammals involve aberrant metabolism of PrP similar to that observed in scrapie, the "Sc" superscript was suggested for all pathogenic PrP isoforms. In this context, the "Sc" superscript is used to designate the scrapie-like isoform of PrP; for those who desire a more general derivation "Sc" can equally well be derived from the term "prion sickness" (see Table 2).

A post-translational process converts the normal cellular isoform of the protein, designated $PrP^{C}$, into $PrP^{Sc}$. Attempts to identify posttranslational modifications that distinguish $PrP^{Sc}$ from $PrP^{C}$ have not been successful; moreover, $PrP^{C}$ and $PrP^{Sc}$ are encoded by the same single copy chromosomal gene. The conformations of the two PrP isoforms are profoundly different; $PrP^{C}$ has little β-sheet while $PrP^{Sc}$ has a high β-sheet content.

Like viruses, prions are infectious because they stimulate a process by which more of the pathogen is produced. As prions or viruses accumulate in an infected host, they eventually cause disease. Both prions and viruses exist in different varieties or subtypes that are called strains. But many features of prion structure and replication distinguish them from viruses and all other known infectious pathogens.

Prions differ from viruses and viroids since they lack a nucleic acid genome that directs the synthesis of their progeny. Prions are composed of an abnormal isoform of a cellular protein whereas most viral proteins are encoded by the viral genome and viroids are devoid of protein. Prions can exist in multiple molecular forms, whereas viruses exist in a single form with a distinct ultrastructural morphology. Prions are non-immunogenic, in contrast to viruses, which almost always provoke an immune response. An enlarging body of evidence argues that strains of prions are enciphered in the conformation of $PrP^{Sc}$; in contrast, strains of viruses and viroids have distinct nucleic acid sequences that produce pathogens with different properties.

## Mammalian prion diseases

Scrapie was reported for the first time 250 years ago in Great Britain and it affects mainly sheep and goats. The most efficient proof for the transmissibility of the disease was demonstrated when more than 1,500 sheep came down after vaccination against looping-ill virus with a formalin-treated vaccine derived from ovine lymphoid tissue unknowingly infected with scrapie. More recently, there has also been a recrudescence of scrapie outbreaks among European sheep flocks. The mechanism on how the disease can be transmitted from one animal to another is still unclear. Chronic inflammation seems to alter the tropism of prion infectivity to organs hitherto believed prion-free, like mammary glands. This raised concerns about scrapie transmission through milk.

The commonest prion disease is the bovine spongiform encephalopathy, more commonly known as "mad cow disease", which appeared for the first time in Great Britain in 1986. Since then more than 280,000 cattle have died from this disease. The source of the disease was attributed to animal-derived

**Table 1:** The prion diseases

| Disease (Abbreviation) | Natural host | Prion | Pathogenic PrP isoforms |
|---|---|---|---|
| Scrapie | sheep and goats | Scrapie prion | $OvPrP^{Sc}$ |
| Transmissible mink encephalopathy (TME) | mink | TME prion | $MkPrP^{Sc}$ |
| Chronic wasting disease (CWD) | mule deer and elk | CWD prion | $MDePrP^{Sc}$ |
| Bovine spongiform encephalopathy (BSE) | cattle | BSE prion | $BoPrP^{Sc}$ |
| Feline spongiform encephalopathy (FSE) | cats | FSE prion | $FePrP^{Sc}$ |
| Exotic ungulate encephalopathy (EUE) | nyala, oryx and greater kudu | EUE prion | $UngPrP^{Sc}$ |
| Kuru | humans | Kuru prion | $HuPrP^{Sc}$ |
| Sporadic Creutzfeldt–Jakob disease (sCJD) | humans | sCJD prion | $HuPrP^{Sc}$ |
| Familial Creutzfeldt–Jakob disease (fCJD) | humans | fCJD prion | $HuPrP^{Sc}$ |
| Iatrogenic Creutzfeldt–Jakob disease (iCJD) | humans | iCJD prion | $HuPrP^{Sc}$ |
| Variant Creutzfeldt–Jakob disease (vCJD) | humans | vCJD prion | $HuPrP^{Sc}$ |
| Gerstmann–Sträussler Scheinker syndrome (GSS) | humans | GSS prion | $HuPrP^{Sc}$ |
| Sporadic fatal familial insomnia (iFFI) | humans | sFFI prion | $HuPrP^{Sc}$ |
| Familial fatal familial insomnia (fFFI) | humans | fFFI prion | $HuPrP^{Sc}$ |

food supplements that were fed as a nutritional supplement to cattle. The disease has become quite rare since 2003 and only 37 cases were reported in the UK in 2008 (http://www.oie.int/).

Another animal prion disease that has evolved in the United States of America is Chronic Wasting Disease (CWD) in captive deer, with the first case observed in 1967 in a research facility in Fort Collins. Since 1980, cases involving free-ranging animals in Colorado and Wyoming have been reported and to date CWD has been observed in 14 states and two Canadian province. The known natural hosts of CWD are mule deer (*Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), Rocky Mountain elk (*Cervus elaphus nelsoni*) and Shira's moose (*Alces alces Shirasi*). The disease is believed to be horizontally transmitted between cervids with high efficiency.

Transmissible mink encephalopathy (TME), feline spongiform encephalopathy (FSE) and exotic ungulate encephalopathy (EUE) are all thought to occur after the consumption of prion-infected materials.

## Human prion diseases

The human prion diseases comprise Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker (GSS) syndrome, Fatal Familial Insomnia (FFI) and Kuru. Epidemiologically, CJD is classified as sporadic (sCJD), familial (fCJD), iatrogenic (iCJD) and variant (vCJD). Even the most frequent form, sCJD, is very rare, with an incidence of 0.4–1.8 person in one million people each year worldwide. Typically, humans with an age between 55 and 70 years are affected. The source of the disease is still unknown, but might have its origin in a spontaneous conversion of $PrP^{C}$ into $PrP^{Sc}$ as a rare stochastic event or in somatic mutations in the *PRNP* gene that encodes the prion protein.

Iatrogenic CJD cases are rare. These cases originate from inoculation of prions through human pituitary-derived growth hormone, cornea transplants, duramater grafts, or cerebral electrode implants. vCJD was recently identified in humans and has affected ~200 victims worldwide since 2006. The histopathology of this disease is reminiscent of that of BSE indicating that this disease has originated by the transmission of BSE to humans. Kuru occurred among the people of the Fore Tribe in Papua New Guinea and was horizontally transmitted between humans by cannibalistic rituals. Since this practice was stopped by the government no new cases have been reported in individuals born after that date.

Familial forms of CJD as well as all cases of GSS and FFI are caused by mutations in *PRNP*.

**Table 2:** Glossary of prion terminology

| Term | Description |
|---|---|
| Prion | A proteinaceous infectious particle that lacks nucleic acid. Prions are composed largely, if not entirely, of $PrP^{Sc}$ molecules |
| $PrP^{Sc}$ | Abnormal, pathogenic isoform of the prion protein that causes sickness. This protein is the only identifiable macromolecule in purified preparations of prions |
| $PrP^{C}$ | Cellular isoform of the prion protein |
| $PrP^{27\text{-}30}$ | Digestion of $PrP^{Sc}$ with proteinase K generates $PrP^{27\text{-}30}$ by hydrolysis of the N-terminal part |
| $PrP^{res}$ | Alternative designation for $PrP^{Sc}$, that has been proposed to generalize the term for all types of TSEs and not only scrapie |
| $PrP^{sen}$ | Host-encoded $PrP^{C}$ that is sensitive to hydrolysis by Proteinase K |
| rPrP | Recombinant PrP generated in *E. coli* that lacks the two sugar moieties and the GPI anchor |
| *PRNP* | Human PrP gene located on chromosome 20 |
| *Prnp* | Mouse PrP gene located on syntenic chromosome 2. *Prnp* controls the length of the prion incubation time and is congruent with the incubation time genes *Sinc* and *Prn-i* |
| $Prnp^{o/o}$ | PrP-deficient mice that are resistant to prions |
| PrP amyloid | Fibril of PrP fragments derived from $PrP^{Sc}$ by proteolysis. Plaques containing PrP amyloid are found in the brains of some mammals with prion disease |
| Prion rod | An amyloid polymer composed of $PrP^{27\text{-}30}$ molecules. Created by detergent extraction and limited proteolysis of $PrP^{Sc}$ |
| Protein X | A hypothetical macromolecule that is thought to act like a molecular chaperone in facilitating the conversion of $PrP^{C}$ into $PrP^{Sc}$ |

## Nomenclature of the mammalian prions

The terminology for prions is still evolving (Table 2). While some terms are borrowed from infectious diseases caused by viruses, others are taken from genetics and still others from the biology of protein structure as well as neuropathology. This new area of biological investigation, which has such diverse roots, creates some unique problems with terminology.

## Prion properties

### Physicochemical and physical properties

The mature human prion protein consists of 208 amino acids. The N-terminal signal sequence that targets the protein into the endoplasmic reticulum is cleaved off in the mature form and a C-terminal polypeptide of 23 amino acids is replaced by the addition of a glycosylphospatidylinositol anchor (GPI) to the C-terminal serine residue. The protein also contains a single disulfide bridge which is formed between residues Cys-179 and Cys-214 and two N-linked glycosylation sites at positions Asn-181 and Asn-197 (Figure 1A).

The N-terminal part of the prion protein contains several Gly-Pro-rich tandem repeats, which vary in number, length and composition between species and are flanked by two positively charged clusters of amino acids 23–27 (CC1) and 95–110 (CC2). The center of the protein contains a hydrophobic stretch of amino acids 111–134 with the palindrome sequence AGAAAAGA. This region is highly conserved between species and is even present in fish and another member of the prion protein family named shadoo. This indicates that this region has gained an important, hitherto unknown physiological function during the evolution of the prion protein.

### $PrP^{Sc}$

Both $PrP^{Sc}$ and $PrP^{C}$ are encoded by the same host gene. Although the amino acid sequence of $PrP^{Sc}$ and $PrP^{C}$ are identical the proteins differ from each other in their tertiary structure and their biochemical and physicochemical properties. Low and high resolution structural methods showed that $PrP^{C}$ is rich in $\alpha$-helical secondary structure. To date, no structural data at the atomic level exist for

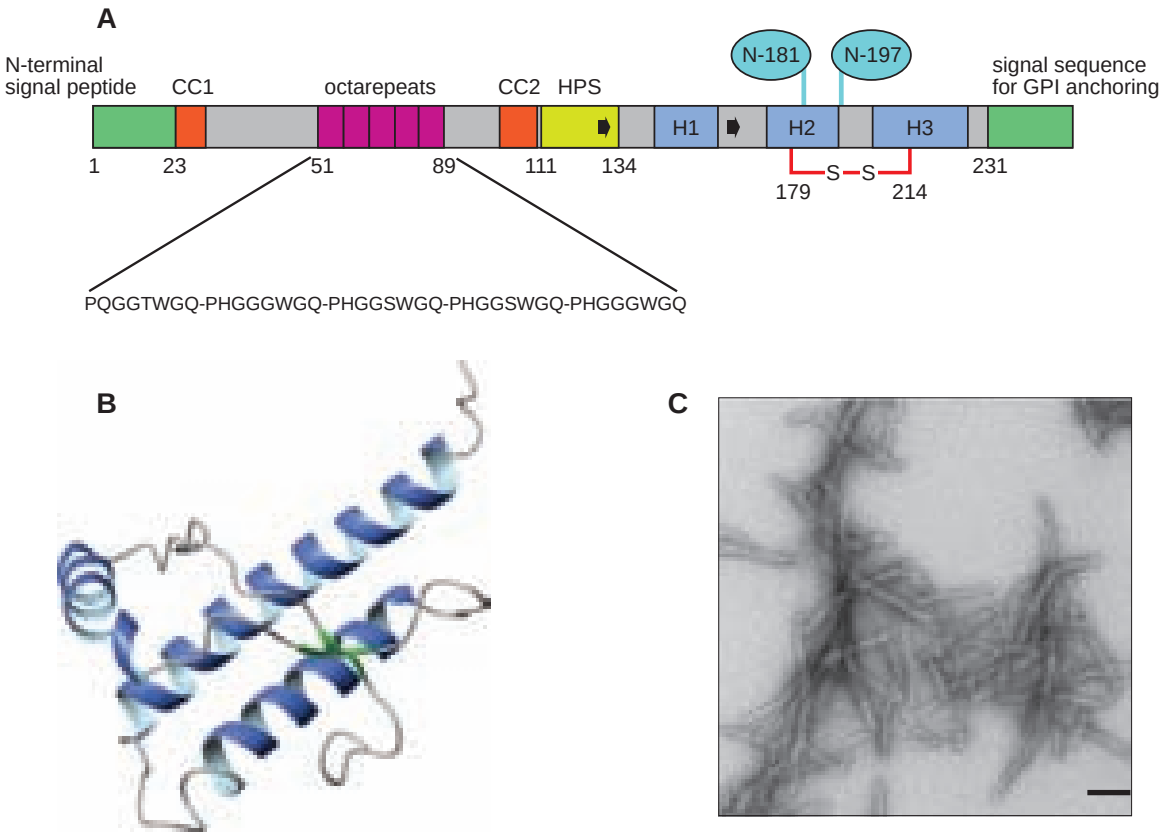


**Figure 1:** Organization and structural properties of the mouse prion protein. (A) Scheme of the organization of the mouse prion protein (numeration according to the human prion protein). In the mature form of the protein the N-terminal signal peptide (N-terminal green box) is cleaved off and the C-terminal peptide (C-terminal green box) is replaced by a GPI anchor at Ser-231. The N-terminal unstructured part of the protein contains five octarepeats (magenta boxes), two positively charged amino acid clusters CC1 and CC2 (orange boxes) and a highly conserved hydrophobic polypeptide segment comprising residues 111–134 (yellow box). The folded C-terminal domain contains three α-helices H1–H3 (blue boxes) and a short antiparallel β-sheet (indicated by two black arrows). Helices α2 and α3 are connected by a single disulfide bridge between Cys-179 and Cys-214. The protein also contains two potential glycosylation sites at amino acid positions 181 and 197 (light blue ellipses). (B) Three-dimensional structure of mPrP(121–231). The three α-helices are shown in blue and the antiparallel β-sheet in green. (C) Electron micrographs of negatively stained recombinant mouse fibrils. The bar represents 100 nm.

$PrP^{Sc}$, but optical measurements and Fourier-transform infrared spectroscopy showed an increase in β-sheet structure for $PrP^{Sc}$ when compared to $PrP^{C}$.

In view of their physicochemical differences, $PrP^{C}$ is monomeric and soluble in nondenaturing detergents, whereas $PrP^{Sc}$ forms oligomers and aggregates that cannot be dissolved in detergents. Another characteristic is that $PrP^{C}$ is sensitive to Proteinase K digestion, whilst $PrP^{Sc}$ can only be partially degraded by Proteinase K leading to an N-terminally truncated resistant core with a size of 27–30 kDa. This fragment is termed $PrP^{27\text{-}30}$. Diglycosylated, GPI-anchored $PrP^{Sc}$ has a size of 33–35 kDa as judged by its electrophoretic mobility on a SDS-PAGE.

### Nucleic acid

No prion-specific nucleic acid has been detected.

### Proteins

The three-dimensional structures of recombinant PrP from a wide variety of species have been solved by nuclear magnetic resonance spectroscopy in solution. The overall architecture of the

proteins is nearly identical containing an N-terminally flexible tail of residues 23–125 and a globular folded C-terminal domain of residues 126–231. The C-terminal domain is an autonomously folding unit and contains three α-helices and a short antiparallel β-sheet of residues 128–131 and 161–164 (Figure 1B). A comparison of the recombinant protein with $PrP^{C}$ purified from calf brain showed that the fold of the recombinant prion proteins is identical with the natural cellular protein and that the posttranslational modifications of $PrP^{C}$ do not affect the fold.

More recently, a special structural feature was identified in the loop β2–α2 between the second β-sheet and the second α-helix showing high conformational variability between species. Whereas the loop β2–α2 is flexible disordered in the prion proteins of most species, it is extremely well-ordered in the prion proteins from elk, bank vole and tammar wallaby.

In addition, crystal structures have been determined for the C-terminal domain in complex with monoclonal antibodies or FAB fragments, indicating a similar fold for $PrP^{C}$ as obtained by NMR spectroscopy. The only exception represents the crystal structure of a domain-swapped dimer in which the third α-helices are swapped and the disulfide bridge is newly arranged between the two molecules.

### Morphology

High resolution structural studies using X-ray crystallography and solution NMR spectroscopy for $PrP^{Sc}$ are more challenging due to the limited solubility of $PrP^{Sc}$. First morphological characterizations for PrP were obtained from rod-shaped particles that were isolated from detergent and proteinase K-treated microsomal fractions which were enriched for prion infectivity. These rod-shaped particles have a width of 10–20 nm and mean lengths of 100–200 nm. The rods are smooth, almost ribbon-like, and infrequently are twisted. The fine structure of these aggregates was originally designated by Mertz as scrapie-associated fibrils (SAF). These partially purified rods show the ultrastructural and tinctorial properties of amyloid, including Thioflavin T positivity and the green–gold birefringence obtained under cross-polarized light after Congo Red staining, indicating the presence of cross β-sheet secondary structure. The purified fractions of the rods also contained 2D crystals, which were used for crystallographic studies and structural models for $PrP^{Sc}$ based on these data were created.

Several *in vitro* conversion model systems to obtain PrP fibers from prion proteins produced in *Escherichia coli* were also used to study their morphological and structural aspects. Amyloid fibers obtained with these assays under physiological conditions appear as long and unbranched fibers in negatively stained electron micrographs (Figure 1C). Low resolution structural data are also available for recombinant PrP and PrP fragments.

Soluble oligomers rather than insoluble fibrils have been hypothesized in other amyloid diseases such as Alzheimer`s disease to be the relevant toxic species and the formation of large aggregates might have a protective effect by sequestering the more toxic oligomers. Solubilization of $PrP^{27\text{-}30}$ into liposomes indicates that large fibrillar structures are not required for infectivity and that $PrP^{Sc}$ oligomers are the relevant molecules for infectivity. To investigate the relationship between size and infectivity in prion diseases, purified $PrP^{res}$ was partially disaggregated to obtain a spectrum of different-sized aggregates. It turned out that particles with a size of 17–27 nm which corresponds to a molecular weight of 300–600 kDa and an association of 14–28 PrP molecules are the most infectious particles. These particles are non-fibrillar and appear as small amorphous ellipsoidal and spherical particles with a diameter of 20–25 nm in transmission electron micrographs.

### Lipids

The prion protein is attached by a glycosylphosphatidylinositol anchor (GPI) in cholesterol rich lipid rafts in the outer leaflet of the cell membrane of neurons to its carboxy-terminal serine residue. The glycan core of the GPI-anchor consists of mannose-mannose-mannose-(N-acetyl neuraminic acid-galactose-N-acetylgalactosamine)-mannose-glucosamine-myo-inositol. As commonly found for GPI anchored proteins, this core is attached to the cell membrane by a phospholipid membrane and to the C-terminus of the GPI-anchored protein through a phosphoethanolamine bridge.

$PrP^{C}$ can be released from the cell surface by digestion with phosphoinositide-specific phospholipase C (PI-PLC), whereas $PrP^{Sc}$ cannot be released by the enzyme, which indicates that the cleavage

site is not accessible due to the conformational rearrangement of $PrP^{C}$. The importance of the GPI anchor for disease was recently demonstrated by the generation of transgenic mice that express a PrP construct lacking the GPI anchor. After infection with RML prions these heterozygous mice developed widely spread plaques in the brain, but without developing any clinical signs of disease. In a follow-up study the authors reported that infection of homozygous transgenic mice expressing anchorless PrP with RML induced a new fatal disease with unique clinical signs and altered neuropathology.

### Carbohydrates

PrP also contains two highly conserved Asn-Xaa-Ser/Thr sequons for N-linked glycosylation at Asn-181 and Asn-197 (numeration according to human PrP, resulting in di-, mono- and unglycosylated states of the protein. Mass spectrometry analysis of the two N-linked oligosaccharides of $PrP^{C}$ and $PrP^{Sc}$ from hamster and mouse showed that the oligosaccharides consist of different neutral and sialylated bi-, tri- and tetra-antennary oligosaccharides with more complex acidic glycan structures at Asn-196. In addition, a higher content of tri- and tetra-antennary glycans and reduced levels of glycans with bisecting GlcNAc residues were identified for $PrP^{Sc}$, leading to the suggestion that the activity of N-acetylglucosaminyltransferase III is reduced. No O-linked glycosylation has been identified.

## Genome organization and replication

### Genome organization

The PrP gene has been identified in many mammalian species, birds, reptiles and amphibians. PrP-like genes have also been identified in fish. The gene encoding the prion protein consists of three exons, from which exon 3 encodes the complete mature protein. The entire ORF of all known mammalian PrP genes is contained within a single exon. The gene encoding the human prion protein is located on chromosome 20 in a single exon, whereas the gene encoding the murine prion protein is located on the systemic chromosome 2.

In general, PrP genes are composed of three exons, as clearly demonstrated for mouse and sheep. The PrP genes of humans and Syrian hamsters (SHa) appear to have three exons but most HuPrP and SHaPrP mRNAs are spliced from only two exons that are separated by ~10 kb. Exon-1 of the SHaPrP gene encodes a portion of the 5′ untranslated leader sequence while exon-3 encodes the ORF and the 3′-UTR. The ORF of the HuPrP gene encodes a protein of 253 amino acids while the mouse and SHaPrP genes encode proteins of 254 residues. The promoters of the PrP genes of both animals contain three or two repeats, respectively, of G–C nonamers, but are devoid of TATA boxes. These nonamers represent a motif which may function as a canonical binding site for transcription factor Sp1.

The sequence similarity between the ORFs encoding different mammalian PrPs is ~90%: 30–50% identities and around 50% similarities are found between mammals, birds, reptiles and amphibians. The sequence homology between fish and tetrapod PrPs is less than 25%.

### Replication

Several hypotheses have been described to explain the unusual nature of the infectious prion agent. The most widely discussed hypotheses are (i) the "protein-only" hypothesis, (ii) the virino hypothesis and (iii) the hypothesis that stoichometric transformation of $PrP^{C}$ to $PrP^{Sc}$ *in vitro* requires specific RNA molecules.

Although the "protein-only" hypothesis has long been under debate, it is to date the most widely accepted theory to explain the unusual nature of the infectious agent. The "protein-only" hypothesis postulates that a protein rather than a virus or nucleic acid is the infectious agent which transfers the disease related information from one host to another. The "protein-only" hypothesis was first formulated by Alper and Griffith in 1967, renewed by Prusiner in 1982 and later refined by Weissmann. It postulates that the infectious agent consists solely of a host-encoded protein. The propagation is assumed to be a post-translational conformational process in which the infectious agent causes the refolding of the healthy form of the prion protein into the pathogenic isoform by acting as a template. An increasing number of experiments are in line with the "protein-only"

hypothesis. The first strong argument is that prions cannot be transmitted to PrP knockout mice in which the *Prnp* gene is removed and so these mice do not carry prion infectivity in the brain. Mice overexpressing $PrP^{C}$ were found to be highly susceptible to scrapie. Futhermore, familial human prion diseases are associated with mutations in the *PRNP* gene. This group of experiments strongly indicates that either $PrP^{C}$ or mutations in the gene encoding PrP are needed to develop a prion disease.

Over the past years many efforts have been undertaken to prove the "protein-only" hypothesis by the *de novo* generation of infectious prions from either noninfectious brain-derived $PrP^{C}$ or recombinant PrP produced in *E. coli*. The first evidence consisted of producing partially proteinase K resistant PrP ($PrP^{res}$) from radioactivity labeled $PrP^{C}$ in cell-free systems. However, the amount of the de novo produced $PrP^{Sc}$ was low compared to the starting material and was not infectious.

Further support for the hypothesis was obtained from several studies using the PMCA (protein misfolding cyclic amplification) technology. This assay in which $PrP^{res}$ is multiplied by serial amplification steps was developed by Soto and colleagues. In the PMCA, brain homogenate from healthy animals is mixed with small quantities of $PrP^{res}$. After several rounds of incubation, sonicating and dilution with fresh brain homogenate from healthy animals, $PrP^{res}$ can be amplified in large quantities. In one approach, PMCA generated hamster $PrP^{res}$ could induce scrapie in wild-type hamster. Modified protocols of the PMCA in which either purified $PrP^{C}$ or recombinant expressed PrP have been used as substrate were also applied. However, the sensitivity was much lower than in the PMCA performed with brain homogenate, suggesting that additional auxiliary factors might be necessary for successful amplification.

A systematic investigation for the minimal compounds needed for efficient amplification was performed by Supattapone and coworkers. In this approach $PrP^{res}$ was produced from purified $PrP^{C}$, copurified lipids and single stranded polyanions. Inoculation of these *de novo* prions caused TSE in wild-type hamster. The most convincing evidence for the "protein-only" hypothesis comes from a study that was recently published by Ma and coworkers. Here, infectious prions were obtained with the PMCA using $PrP^{C}$ purified from *E. coli* extracts, anionic phospholipids (POPG) and RNA obtained from mouse liver. These prions induced a neurologic disease in wild-type mice after 130 days with the classical signs of a transmissible prion disease. In other approaches, recombinant prion proteins without any cofactors were converted into cross-β-sheet aggregates that are capable of inducing a transmissible prion disease in transgenic mice or wild-type hamster.

Genetic evidence that supports the "protein-only" hypothesis was obtained from a study in which transgenic mice expressing two amino acids at positions 170 and 174 from the elk prion protein in the *Prnp* gene were generated. These two mutations lead to a well-structured loop that connects the second β-sheet and the second *a*-helix in the C-terminal domain of the protein. Mouse lines expressing these transgenes develop a spontaneous prion disease which is transmissible to transgenic mice overexpressing wild-type PrP and consecutively to wild-type mice.

## Antigenic properties

The generation of antibodies against $PrP^{C}$ and $PrP^{Sc}$ is difficult, because of the strong self-tolerance of $PrP^{C}$. These limitations have been overcome by using $Prnp^{0/0}$ mice, which are not immune tolerant to $PrP^{C}$, and show a strong immune response against $PrP^{C}$. These mice have been used for the production of highly efficient monoclonal antibodies, including the "POM" library, which contains antibodies directed against conformational epitopes in the folded C-terminal part of the protein as well as against linear epitopes in the unstructured N-terminal part. These monoclonal antibodies represent valuable high immunoaffinity reagents that can be applied in a variety of techniques for the detection of $PrP^{C}$ and $PrP^{Sc}$.

Several studies showed that anti-PrP antibodies have neutralizing effects by clearing $PrP^{Sc}$ levels in scrapie-infected cell lines, in cell-free systems and by recognition of $PrP^{C}$ in transgenic mice on intraperitoneal prion inoculation. Furthermore, passive transfer of anti-PrP monoclonal antibodies delays the onset of scrapie in mice infected with prions by the intraperitoneal route. However, this method requires high levels of the antibodies to cause a significant effect on the survival of

prion-infected mice and it has been ineffective in mice showing already clinical signs, which might be due to the blood–brain barrier (BBB) impermeability of the antibodies. Active immunization as antibody-based anti-prion prophylaxis is still difficult to achieve, although intense effort has been made to circumvent the tolerance of the humoral immune system to $PrP^{C}$ by using different antigens and various antibody retrieval techniques.

The production of antibodies directed against $PrP^{Sc}$ is more challenging as this requires binding of the antibodies against specific conformational epitopes that are only exposed in $PrP^{Sc}$. Antibodies that specifically bind $PrP^{Sc}$ without binding to $PrP^{C}$ have been reported, but their affinity seems to be poor and their diagnostic value has awaited confirmation for more than a decade.

## Biological properties

### Physiological function of $PrP^{C}$

Despite extensive research, the physiological function of $PrP^{C}$ is still unclear. A clarification of the function of $PrP^{C}$ was expected from the generation of $PrP^{C}$ knock out mice. However, these transgenic mouse lines show only a subtle phenotype. Since then several biological functions, some of which are controversial, have been attributed to $PrP^{C}$, including neuronal signal transduction, lymphocyte function, copper-binding, as well as pro- and anti-apoptotic functions.

In the search for intrinsic functional domains of $PrP^{C}$ several mouse lines were generated with specific deletions in the *PrnP* gene. These mice developed several prionopathies characterized by shortened life span and the development of white matter disease in the central nervous system (CNS) as well as neuronal death in the cerebellum.

More recently, $PrP^{C}$ was shown to play an important role in maintaining peripheral myelin in Schwann cells, indicating that $PrP^{C}$ acts as a signaling protein in axons necessary to keep peripheral nerves intact. The absence of $PrP^{C}$ causes otherwise demyelination in the Schwann cells.

### Mutations in the human PrP genes

About 10–15% of the CJD cases and virtually all cases of GSS and FFI are autosomal dominantly inherited prion diseases with point mutations, amino acid insertions or deletions in the *PRNP* gene (Table 3). There is variability in the clinical and pathological findings, the age of onset and the duration depending on the particular *PRNP* mutation involved. Some *PRNP* mutations might cause neurodegenerative diseases that are not transmissible and therefore represent a proteinopathy rather than a prion disease.

Most of the point mutations are located in the well-structured C-terminal domain in $PrP^{C}$ (Table 3; Figure 2A–C) and in the immediate preceding fragment of residues 102–125. Amino acid insertions and deletions are found in the N-terminal part of the protein affecting the length of the octapeptide repeat region.

### Polymorphisms in PrP genes

The M/V polymorphism at codon 129 in HuPrP influences the incidence of sporadic and infectious CJD as well as the age of onset of inherited prion diseases. Homozygosity at codon 129 is more frequent in both sporadic and infectious CJD; it also heralds an earlier age of clinical disease in people carrying pathologic mutations. Those individuals who carry the DI78N mutation develop FFI manifested by insomnia and dysautonomia if they encode M129 on the mutant allele, while those that encode V129 present with fCJD are characterized by dementia (Table 3).

The E/K polymorphism at codon 219 in HuPrP seems to influence resistance to CJD. Individuals with wild-type PrP sequences who are heterozygous for K219 appear to be resistant to CJD. This dominant negative effect is likely to be the results of increased affinity of $PrP^{C}$ (K219) for protein X which is thought to function like a molecular chaperone in facilitating the conversion of $PrP^{C}$ into $PrP^{Sc}$.

The Q/R polymorphism at codon 171 in OvPrP seems to govern the resistance of sheep to natural scrapie. Sheep with wild-type PrP sequences that are heterozygous for R171 appear to be resistant

**Table 3:** Human PrP gene mutations found in the inherited prion diseases

| Inherited prion disease | PrP gene mutation |
|---|---|
| Familial Creutzfeldt–Jakob disease | D178N–129V, V180I, V180I–M232R, T183A, T188A, E196K, E200K, V203I, E208H, V210I, E211Q, M232R |
| Gerstmann–Sträussler–Scheinker syndrome | P102L-129M, P105L–129V, A117V–129V, G131V–129M, F198S–129V, D202N–129V, Q212P, Q217R–129M, M232T |
| Fatal familial insomnia | D178N-129M |
| Mutations in *PRNP* associated with familial dementia and/or neuropsychiatric symptoms PrP (Not further classified) | I138M, G142S, Q160Stop–129M, T188K, T188R, M232R, P238S |
| Vascular amyloid depositions | Y145STOP-129M |
| Base pair insertion/deletion | Insertions: 24 bp, 48 bp, 96 bp, 120 bp, 144 bp, 168 bp, 192 bp, 216 bp; Deletion: 24 bp |
| Proven, but unclassified prion disease | H187R |
| Polymorphisms | N171S, E219K |
| Silent polymorphisms | P68P, A117A, G124G, V161V, N173N, H177H, T188T, D202D, Q212Q, R228R, S230S |

Point mutations, insertions and deletions identified in the human *PRND* gene in patients. The polymorphism at amino acid position 129 that is associated with disease is also indicated. Octarepeat inserts are designated as bpi (base pair insertion); Octarepeat deletions as bpd (base pair deletion).
Table based on Aguzzi and Calella (2009). *Physiol. Rev.*, **89**, 1105-1152; Aguzzi, Baumann and Bremer (2008). *Annu. Rev. Neurosci.*, **31**, 439–477.

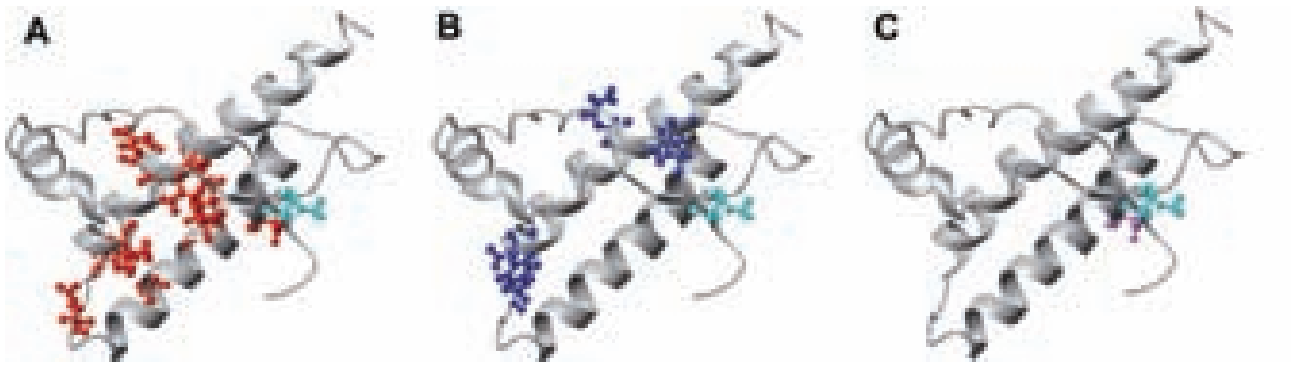


**Figure 2:** Location of point mutations in the three-dimensional structure of hPrP(121–230) that are associated with familial prion diseases. The backbone is shown in grey and the side chains of the residues associated with the mutations for inherited fCJD are shown in red (A), for GSS in blue (B) and fFFI in magenta (C). The polymorphism at position 129 is indicated in green. The molecules were generated with the program MOLMOL.

to natural scrapie, but scrapie can be transmitted to some of these animals by inoculation of prions. This dominant negative effect is also likely to be the result of increased affinity of $PrP^C$ (R171) for protein X. A polymorphism at codon 136 is also thought to influence the susceptibility of some breeds of sheep to scrapie.

The S/F polymorphism at codon 225 in mule deer influences susceptibility to CWD. In addition, elk have a polymorphism at codon 132 (M/L) of *PrnP* corresponding to the polymorphic codon 129 (M/V) in humans. Elk with codon 132LL experimentally infected with CWD were resistant to infection for at least 4 years, whereas 132MM or 132ML elk developed terminally clinical disease after 23 or 40 months. A reduced susceptibility to CWD was reported in white-tailed deer with G/S and Q/H polymorphisms at codons 96 and 95, respectively.

## Prion strains

One of the most intriguing phenomena in prion diseases is the appearance of different strains. Prion strains are defined as infectious isolates that, when transmitted to identical hosts, exhibit distinct disease phenotypes. Prion strains can be discriminated by specific incubation times, histopathological lesion profiling and clinical signs. Most phenotypes of strains are relatively stable during serial transmission. Often distinct new strains are observed upon transmission of prions across a transmission barrier or into animals that express specific polymorphisms of the prion gene, a phenomenon sometimes referred to as a "strain mutation". The transmission barrier is explained by differences in the amino acid sequence between two species whereby single amino acid changes can lead to significant changes in the incubation times.

To explain the appearance of different prion strains within the framework of the "protein-only" hypothesis it was suggested that the information for the strain-specific characteristics and phenotypes must be enciphered in the $PrP^{Sc}$ conformation itself or in other words $PrP^{Sc}$ of each strain exhibits a different conformation. A possible molecular explanation for the appearance of different conformations was recently provided from a systematic structural study on fibril-forming peptides derived from prion and other amyloid proteins. These peptides form small β-sheets and adopt distinct crystalline polymorphisms according to their relative orientation and packing arrangements. Each different crystalline polymorph could thereby retain and transfer the strain-specific information from one species to another. Experimental evidence for strain-specific $PrP^{Sc}$ conformations include the different electrophoretic mobilities of different strains, the immunological reactivity to amino-proximal antibodies upon proteinase K digestion and the different susceptibilities of $PrP^{Sc}$ derived from different strains to chaotropic salts. In addition, strains can be discriminated by their accessibility of specific epitopes to monoclonal antibodies. These epitopes are fully accessible in $PrP^{C}$, but buried in $PrP^{Sc}$ and only become exposed after treatment with defined concentrations of chaotropic salts.

Optical tools to classify cross-β-sheet amyloid deposits are historically small amyloidotropic dyes such as Congo red and Thioflavin T. However, the use of these dyes is limited to the detection of total amyloid per se and different amyloid morphologies as present in prion strains cannot be separated with these dyes. More sophisticated tools would therefore be of high interest for the diagnosis of amyloid deposits with distinct morphologies. Luminescent conjugated polymers (LCPs) are a class of novel amyloidotropic dyes that have recently been proven to overcome these limitations. LCPs are small fluorescent probes that were originally synthesized for use in electrical devices. These dyes exhibit a highly flexible backbone that adopts a distinct conformation upon binding to the ordered structure of amyloid fibrils and generates a fluorescent signature that is specific for the structural symmetry of these fibrils. The spectral properties of the LCPs were used for the analysis of brain sections from mice that were infected with distinct prion strains (Figure 3). The LCPs could discriminate different prion strains due to individual staining patterns and could even detect prion deposits that were negative for Congo red and Thioflavin T. These findings provide additional evidence that $PrP^{Sc}$ strains might be enciphered in the conformation of the prion aggregate.

## Taxonomic considerations of mammalian prions

A listing of the different mammalian prions is given in Table 1. Although the prions that cause TME and BSE are referred to as TME prions and BSE prions; this may be unjustified, because both are thought to originate from the oral consumption of scrapie prions in sheep-derived foodstuffs and because many lines of evidence argue that the only difference among the various prions is the sequence of PrP which is dictated by the host and not the prion itself. The human prions present a similar semantic conundrum. Transmission of human prions to laboratory animals produces prions carrying PrP molecules with sequences dictated by the PrP gene of the host, not that of the inoculum.

To simplify the terminology, the generic term $PrP^{Sc}$ is suggested in place of such terms as $PrP^{CJD}$, $PrP^{BSE}$ and $PrP^{res}$. To distinguish $PrP^{Sc}$ found in humans or cattle from that found in other animals, we suggest $HuPrP^{Sc}$ or $BoPrP^{Sc}$ instead of $PrP^{CJD}$ or $PrP^{BSE}$, respectively (Table 1). Once human prions and thus $HuPrP^{Sc}$ molecules have been passaged into animals, then the prions and $PrP^{Sc}$ are no longer of the human species unless they were formed in an animal expressing a HuPrP transgene. In the case of mutant PrPs, the mutation and any important polymorphism can be denoted in

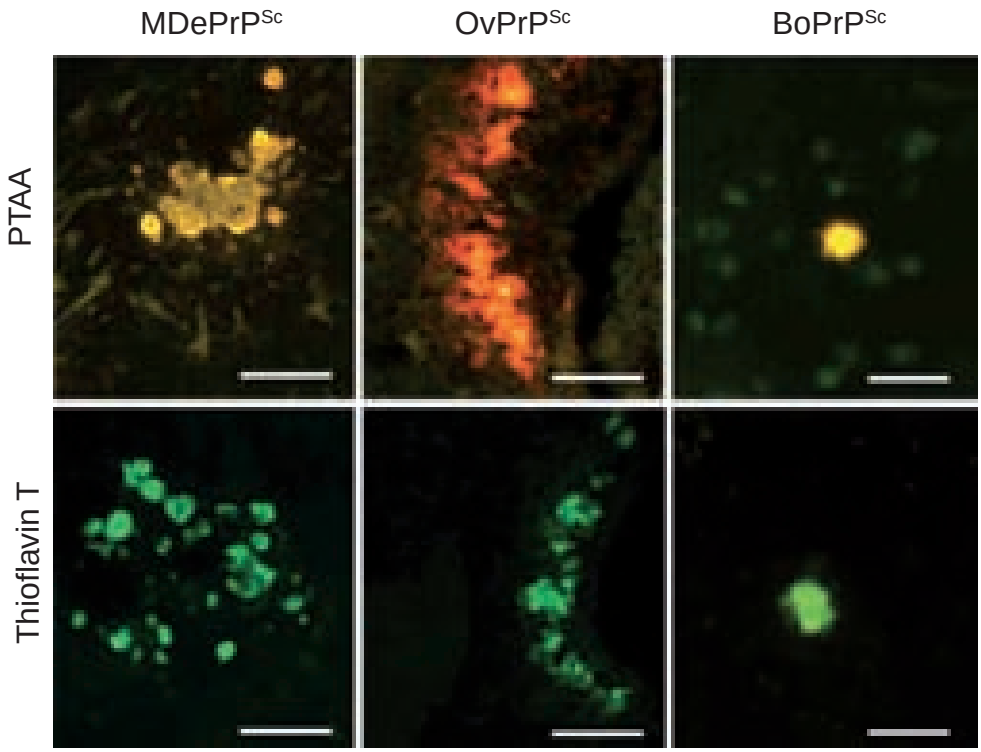


**Figure 3:** Comparison of images showing different prion strains stained with PTAA and Thioflavin T. (A) MDePrP$^{Sc}$, (B) OvPrP$^{Sc}$ and (C) BoPrP$^{Sc}$ prion deposits. PTAA-stained deposits appear in yellow-red. Scale bars represent 500 m. (Adapted from Sigurdson, C.J., Nilsson, K.P., Hornemann, S. *et al.* (2009). *Proc. Natl Acad. Sci., U S A*, **106**, 304–309.)

parentheses following the particular PrP isoform. For example, in FFI, the pathogenic PrP isoform would be referred to as PrP$^{Sc}$ or HuPrP$^{Sc}$; alternatively, if it were important to identify the mutation, then it would be written as HuPrP$^{Sc}$ (D178N, M129) (Table 3). The term PrP$^{res}$ or PrP-res is derived from the protease-*res*istance of PrP$^{Sc}$ but protease-resistance, insolubility, and high β-sheet content should be only considered as surrogate markers of PrP$^{Sc}$ since one or more of these may not always be present. Whether PrP$^{res}$ is useful in denoting PrP molecules that have been subjected to procedures that modify their resistance to proteolysis, but have not been demonstrated to convey infectivity or to cause disease remains debatable.

The term PrP* has been used in two different ways. First, it has been used to identify a fraction of PrP$^{Sc}$ molecules that are infectious. Such a designation is thought to be useful since there are ~$10^5$ PrP$^{Sc}$ molecules per infectious unit. Second, PrP* has been used to designate a metastable intermediate of PrP$^{C}$ that is bound to protein X. It is noteworthy that neither a subset of biologically active PrP$^{Sc}$ molecules nor a metastable intermediate of PrP$^{C}$ has been identified, to date.

In mice, the PrP gene denoted *Prnp* is now known to be identical with two genes denoted *Sinc* and *Prn-i* that control the length of the incubation time in mice inoculated with prions. These findings permit a welcome simplification. A gene designated *Pid-1* on mouse chromosome 17 also appears to influence experimental CJD and scrapie incubation times but information on this locus is limited.

Distinguishing among CJD, GSS and FFI has grown increasingly difficult with the recognition that CJD, GSS and FFI are autosomal dominant diseases caused by mutations in the *PRNP* gene. Initially, it was thought that a specific PrP mutation was associated with a particular clinico-neuropathologic phenotype but an increasing number of exceptions are being recognized. Multiple examples of variations in the clinico-neuropathologic phenotype within a single family where all affected members carry the same PrP mutation have been recorded. Most patients with a PrP

mutation at codon 102 present with ataxia and have PrP amyloid plaques; such patients are generally given the diagnosis of GSS, but some individuals within these families present with dementia, a clinical characteristic that is usually associated with CJD. One suggestion is to label these inherited disorders as "prion disease" followed by the mutation in parenthesis while another is the use the terms fCJD and GSS followed by the mutation. In the case of FFI, describing the Dl78N mutation and M129 polymorphism seems unnecessary since this is the only known mutation-polymorphism combination that gives the FFI phenotype.

## Derivation of name

*Prion*: sigla from *pro*teinaceous and *in*fectious particle.

## PrP orthologs: Doppel and Shadoo

Two other proteins, the Doppel and Shadoo, have been identified that belong to the PrP family. In contrast to PrP, these two proteins are not infectious. The Doppel protein (a German synonym of double; Dpl) was originally identified by the observation of phenotypic discrepancies in PrP0/0 mice. Two mouse lines, *ZHI-Prnp*$^{0/0}$ mice and Edbg *Prnp*$^{-/-}$ mice, showed no obvious phenotype, whereas *ZHII-Prnp*$^{0/0}$, *Ngsk-Prnp*$^{0/0}$ and *Rcm0-Prnp*$^{0/0}$ developed late onset ataxia caused by cerebellar Purkinje cell degeneration. The identification of a novel gene locus (*Prnd*) 16 kb downstream of the *Prnp* gene and its product, Dpl, provided an explanation for the different phenotypes.

The amino acid identity between PrP and Dpl is about 20%. The human Dpl consists of 179 amino acids and contains two glycosylation sites. Compared to PrP, Dpl lacks the octarepeats, the charged clusters and the hydrophobic stretch and contains an additional disulfide bridge. The N-terminal tail, residues 24–52, of Dpl is significantly shorter due to the lack of the octarepeats, but the C-terminal domain of residues 52–149 has a similar structural organization as seen for PrP. The well-structured C-terminal domain of the human Dpl contains four α-helices comprising residues 72–80 (α1), 101–115 (α2a), 117–121 (α2b) and 127–141 (α3) and a short antiparallel β-sheet of residues 58–60 and 88–90. The two disulfide bridges in the protein are formed between Cy109–Cys143, connecting helices α2 and α3, and between Cys95–Cys148, connecting the loop β2–α2 with the C-terminal end of Dpl.

Dpl is mainly expressed in testis and heart, to some smaller extent in other peripheral organs and at a very low expression rate in the brains of adult wild-type mice. Due to the high biochemical and structurally similarities between PrP and Dpl it was assumed that the proteins also exhibit similar physiological functions.

To elucidate the biological function of Dpl, *Prnd* knockout mice were generated by homozygous targeted disruption of the *Prnd* gene. The *Prnd*$^{0/0}$ mice survive until adulthood like the *Prnp* knockout mice. In contrast to the PrP$^{0/0}$, the *Prnd*$^{0/0}$mice show a specific phenotype. Whereas females lacking Dpl were viable and fertile, males without Dpl suffer from male sterility. In addition, the *Prnd gene* was disrupted in the ataxic Zurich II mice by using transallelic targeted meiotic recombination to generate *Prnp/Prnd* double deletion mice. These mice did not show any ataxia, proving the causal role for Dpl overexpression in the Zurich II phenotype.

The PrP-like protein Shadoo (Sho) (for shadow of the prion protein) was identified in 2003 by intense database search for nucleotide sequences that are similar to *PrnP*. Shadoo has been shown to present in many mammals and fish. The related gene *Sprn* is located on chromosomes 7 and 10 in mice and humans, respectively, which are distinct from *Prnp* and *Prnd*, indicating that *Sprn* has evolved separately. The entire open reading frame of *Sprn* is contained in a single exon. Sho seems to be expressed in the central nervous system, and to be neuroprotective by preventing cerebellar degeneration mediated by CNS-expressed Dpl and N-terminal deletion mutants of PrP.

The mature protein consists of 98 amino acids and is presumably gycosylated at one site. Similarly to PrP, Sho contains the highly conserved valine- and alanine-rich hydrophobic stretch, the N-terminal signal sequence for targeting the protein into the endoplasmatic reticulum, and the GPI

anchor. On the other hand, it lacks the octarepeats and has no cysteine residues. In addition, Shadoo does not contain a C-terminal domain and far-UV CD spectra indicate that the protein is unfolded.

## Further reading

Note: A version of this text with full cited references is available online on ScienceDirect®, www.sciencedirect.com


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# *The Statutes of the ICTV*

August 2008

Article 1
**Official name**

1.1 The official name is the International Committee on Taxonomy of Viruses (ICTV).

Article 2
**Status**

2.1 The ICTV is a Committee of the Virology Division of the International Union of Microbiology Societies (IUMS).

Article 3
**Objectives**

The objects of the Committee shall be for the public benefit and in particular to advance education in the taxonomy of viruses and in furtherance thereof. The objectives are:

3.1 To develop an internationally agreed taxonomy for viruses.
3.2 To establish internationally agreed names for virus taxa, virus species and subviral agents.
3.3 To communicate the decisions reached concerning the classification and nomenclature of viruses and subviral agents to virologists by holding meetings and publishing reports.
3.4 To maintain an Official Index of virus names.

Article 4
**Membership**

4.1 Membership of the ICTV shall be:
- A. President and Vice-President
- B. Secretaries
- C. Other members of the Executive Committee
- D. National Members
- E. Life Members
- F. Members of the Prokaryote Virus, Fungal Virus, Invertebrate Virus, Plant Virus, Vertebrate Virus, and Virus Data Subcommittees.

4.2 The composition of the ICTV Executive Committee shall be:
- (i) The Officers of ICTV, namely President, Vice-president and the Secretaries
- (ii) The Chairs of the Executive Committee Subcommittees, namely one each for the Subcommittees on viruses of prokaryotes (including Archaea), fungi (including algae), invertebrates, plants and vertebrates, and one for the Virus Data Subcommittee
- (iii) eight Elected Members.

4.3 Election or appointment procedures for positions on ICTV and its Executive Committee:
- A. President and Vice-President shall be nominated and seconded by any members of the ICTV and elected at a plenary meeting of the full ICTV membership. They shall be elected for a term of three years and may not serve for more than two consecutive terms of three years.
- B. Two Secretaries shall be nominated by the Executive Committee and elected at a plenary meeting of the full ICTV membership. The Secretaries shall be elected for a term of six years and may be re-elected.
- C. The Chairs of the Subcommittees shall be elected by the Executive Committee, normally at an interim meeting preceding a Plenary Meeting of the full ICTV membership. Nominations shall be made to the President at least one week before the election and must be accompanied by indications from each of the nominees that they are willing to serve. The term of office shall be for three years and may not exceed two consecutive terms of three years each.
- D. No person shall serve on the Executive Committee for more than four successive complete terms other than as Officers subject to the limitations set out in 4.3.A and 4.3.B.
- E. Nominees for each vacant Elected Member seat shall be proposed to an ICTV Plenary Meeting by a Nominations Subcommittee comprising the President, Vice-President, both Secretaries,

and the Chairs of the Subcommittees. The Nominations Subcommittee will be chaired by the President and a quorum will consist of 5 members. The Nominations Subcommittee will seek to ensure the widest possible ranges of virological expertise and geographical representation on the EC.

In addition, nominations for an Elected Member vacant seat may be submitted to the Nominations Subcommittee by any virologist and will be allowed up to 2 days prior to the Plenary Meeting at which elections will be held. All nominations must be accompanied by a brief biographical sketch and an indication that the nominee is willing to stand for election.

Elected Members shall be nominated, seconded, and elected at a Plenary Meeting of the ICTV for a term of three years and may not serve for more than two consecutive terms of three years each. Generally, three or four of the eight Elected Members shall be replaced every three years.

F. National Members shall be nominated by Member Societies of the Virology Division of the IUMS. Societies belonging to the IUMS are considered to be Member Societies of the Division if they have members actively interested in virology. Wherever practicable, each country shall be represented by at least one National Member and no country shall be represented by more than five National Members. Nominations of virologists as National Members shall not require approval by the ICTV. However, it is the responsibility of a Member Society to inform one of the ICTV Secretaries in writing of the selection of their National Member before the start of the Plenary Meeting at which that National Member is to participate. National Members of the ICTV shall be appointed for 3-year terms soon after each ICV. There is no limit to the number of terms that a National Member may serve but their appointment will be formally reviewed by the appointing National Society every three years.

G. Life Members shall be nominated by the Executive Committee, normally in recognition of outstanding services to virus taxonomy. Currently serving members of the Executive Committee and virologists within 6 months of their retirement from the Executive Committee will not be eligible for election. Life Members shall be elected by the full ICTV.

H. Virologists shall be appointed to Subcommittees by the Chairs of the Subcommittees and the appointments will not require approval by the ICTV EC.

4.4 EC Finance Sub-Committee:
The Finance Sub-Committee will consist of the Officers of ICTV.

4.5 Study Groups:
Study Groups shall be formed by Subcommittee Chairs to examine the classification and nomenclature of particular groups of viruses. A Chair of a Study Group shall be appointed by the Chair of the appropriate Subcommittee and shall be a member of that Subcommittee ex officio.

Chairs of Study Groups shall appoint the members of their Study Groups. Members of Study Groups, other than Chairs, shall not be members of the ICTV, but their names shall be published in the minutes and reports of the ICTV to recognize their valuable contribution to the taxonomy of viruses. Study Group Chairs and Study Group members will normally be appointed immediately after an ICTV Plenary Session and will serve until the following Plenary Session, which is normally a period of three years. Except in unusual circumstances, the term of office of Study Group Chairs will be limited to two consecutive periods of three years.

Article 5
**Meetings**

5.1 Plenary meetings of the full ICTV membership shall be held in conjunction with the International Congresses of Virology. Meetings of the ICTV Executive Committee shall be held in conjunction with the International Congresses of Virology as well as at least once between International Congresses.

Article 6
**Taxonomic Proposals**

6.1 Taxonomic proposals may be initiated by an individual member of the ICTV, by a Study Group or by a Subcommittee member. In addition, any virologist may submit a taxonomic proposal or suggestion to the appropriate ICTV Subcommittee Chair following the procedures described below.

Proposals will be sent to the Chair of the appropriate Subcommittee for consideration by that Subcommittee and/or its Study Groups. Proposals will also be sent for consideration to all Study Groups and Subcommittees whose interests might be affected by the taxonomic or nomenclatural changes proposed. Taxonomic proposals approved by any Subcommittee, usually in consultation with

a Study Group, shall be submitted to the Executive Committee by the Subcommittee Chair. Proposals approved by the Executive Committee shall be presented for ratification to the full ICTV membership either at the subsequent Plenary meeting or by circulation of proposals by mail followed by a Ballot.

Separate proposals shall be required (1) to establish a new taxon, (2) to name a taxon, (3) to designate the type species of a genus and (4) to establish a list of members of a taxon.

Article 7
**Voting**

7.1 Decisions will be made by a simple majority of either those eligible to vote and present at a meeting or that reply to a call for a vote within 1 month of a proposition being circulated. A quorum for the ICTV Plenary Session decisions will consist of the President or Vice-President together with 15 voting members. In the event of a tied vote the proposal will not be approved.

7.2 The members of the full ICTV who are entitled to vote are the members of the EC, members of Subcommittees, the National Members and the Life Members. No member may vote more than once on any particular proposal.

7.3 Proposals for changes to taxonomy, nomenclature or the ICTV constitution will be voted on in two stages: (1) the EC will vote either at a meeting or by circulation of a Ballot if a proposal should be presented to the ICTV for a decision, and (2) the ICTV will decide either at a Plenary meeting or by circulation of a Ballot.

Decisions of the EC concerned with changes to taxonomy, nomenclature or the constitution will be by simple majority of members of the EC voting. The quorum shall be the President or Vice-President together with 1 Secretary and 7 members of the EC. Only EC members are entitled to vote on such matters.

7.4 Matters of EC business not directly concerned with changes to taxonomy, nomenclature or the constitution may be decided by consensus under the President's chairmanship. Any EC member may call for a vote on such matters.

Article 8
**The Code of Virus Nomenclature**

8.1 Classification and nomenclature of viruses and related agents will be subject to Rules formalized into a Code. The Code, and substantive modifications to it, are subject to the approval of ICTV Executive Committee and ICTV as under Article 7.

Article 9
**Duties of Officers**

9.1 Duties of the President shall be:

A. To preside at meetings of the Executive Committee and plenary meetings of the full ICTV membership.

B. To prepare with the Secretaries the agendas for meetings of the Executive Committee and the plenary meetings of the full ICTV membership.

C. To act as editor-in-chief for ICTV Reports.

9.2 Duties of the Vice-President shall be:

A. To carry out the duties of the President in the absence of the President.

B. To attend meetings of the Executive Committee and plenary meetings of the ICTV.

C. To act as Treasurer of the ICTV. To handle any funds that may be allocated to the ICTV by the Virology Division of the IUMS or other sources.

9.3 Duties of the Secretaries shall be:

A. To attend meetings of the Executive Committee and plenary meetings of the ICTV.

B. To prepare with the President the agendas for meetings of the EC and the plenary meetings of the ICTV.

C. To prepare the Minutes of meetings of the Executive Committee and plenary meetings of the ICTV and circulate them to all ICTV members.

D. To keep an up-to-date record of ICTV membership.

9.4 Duties of the Subcommittee Chairs shall be:

A. To attend meetings of the Executive Committee.

B. To appoint members of the Subcommittee.

C. To appoint Study Group Chairs.

D. To organize the Subcommittee and Study Groups to study taxonomic problems and to bring forward proposals.
E. To present taxonomic proposals to the Executive Committee for voting.
F. To co-ordinate the preparation of up-dates of the ICTV Reports.
G. To report to the plenary meetings of ICTV on taxonomic changes since the preceding ICTV Report and to present any current proposals for further change.

Article 10
**Publications**

10.1 Changes to taxonomy, nomenclature or the International Code approved by ICTV will be communicated to the virological community by publication in rapid short form, for example in Virology Division News, and as part of the formal triennial Report of ICTV.
10.2 Whenever feasible, taxonomic information will be published in a database form under the guidance of the Virus Data Subcommittee.
10.3 No publication of the ICTV shall bear any indication of sponsorship by a commercial agency, or institution connected in any way with a commercial company, except as an acceptable acknowledgement of financial assistance, unless approved by the Executive Committee on a case-by-case basis.
10.4 Publications may bear the name of the ICTV only if all the material contained has been authorized, prepared, or edited by the ICTV, or a committee or subcommittee of the ICTV.
10.5 Publications containing translations of ICTV-approved material may only bear the name of ICTV if they are approved by the ICTV Executive Committee.

Article 11
**ICTV Statutes**

11.1 The Statutes of the ICTV, and any subsequent changes, shall be approved by votes of the ICTV EC and the full ICTV membership as under Statute 7 and then approved by the Virology Division of the IUMS.

Article 12
**Disposition of Funds**

12.1 In the event of dissolution of the ICTV, any remaining funds shall be turned back to the Secretary-Treasurer of the Virology Division of the IUMS.
12.2 Any surplus assets/funds must be used for the charitable purposes set out at Article 3 of this constitution or for purposes that are charitable within the context of Section 505 of the Income and Corporation Taxes Act 1988 (or statutory re-enactment thereof).

# The International Code of Virus Classification and Nomenclature

August 2002

## 1. Statutory basis for the International Committee on Taxonomy of Viruses (ICTV)

1.1 The International Committee on Taxonomy of Viruses (ICTV) is a committee of the Virology Division of the International Union of Microbiological Societies. ICTV activities are governed by Statutes agreed with the Virology Division.

1.2 The Statutes define the objectives of the ICTV. These are:

(i) to develop an internationally agreed taxonomy for viruses
(ii) to develop internationally agreed names for virus taxa, including species and subviral agents.
(iii) to communicate taxonomic decisions to the international community of virologists.
(iv) to maintain an Index of virus names.

1.3 The Statutes also state that classification and nomenclature will be subject to Rules set out in an International Code.

**Comment:** Ratified changes will be published in Virology Division News in *Archives of Virology*, and in subsequent ICTV Reports.

## 2. Principles of Nomenclature

2.1 The essential principles of virus nomenclature are: (i) to aim for stability; (ii) to avoid or reject the use of names which might cause error or confusion; (iii) to avoid the unnecessary creation of names.

2.2 Nomenclature of viruses and subviral agents is independent of other biological nomenclature. Virus and virus taxon nomenclature are recognized to have the status of exceptions in the proposed International Code of Bionomenclature (BioCode).

2.3 The primary purpose of naming a taxon is to supply a means of referring to the taxon, rather than to indicate the characters or history of the taxon.

2.4 The application of names of taxa is determined, explicitly or implicitly, by means of nomenclatural types.

2.5 The name of a taxon has no official status until it has been approved by ICTV.

**Comment:** see section 3.8.

## 3. Rules of Classification and Nomenclature

### I – General Rules

*The universal scheme*

3.1 Virus classification and nomenclature shall be international and shall be universally applied to all viruses.

3.2 The universal virus classification system shall employ the hierarchical levels of Order, Family, Subfamily, Genus, and Species.

**Comments:** It is not obligatory to use all levels of the taxonomic hierarchy. The primary classification is of viruses into species. Most species are classified into genera and most genera are classified into families. Species not assigned to a genus will be "unassigned" in a family (see Rule 3.6) and genera not classified in families have the status of "unassigned" (sometimes referred to as "floating"). Some families are classified together into Orders, but for many, the family is the highest level taxon in use. Also, families are not necessarily divided into subfamilies. This taxon is to be used only when it is needed to solve a complex hierarchical problem (see Rule 3.29).

Contrasting examples of full classifications of some negative strand RNA viruses are: (1) species *Mumps virus*; genus *Rubulavirus*; subfamily *Paramyxovirinae*; family *Paramyxoviridae*; order *Mononegavirales*, and (2) species *Rice stripe virus*; genus *Tenuivirus* (see also Rule 3.41).

*Scope of the classification*

3.3 The ICTV is not responsible for classification and nomenclature of virus taxa below the rank of species. The classification and naming of serotypes, genotypes, strains, variants and isolates of virus species is the responsibility of acknowledged international specialist groups.

**Comments:** Particular virus isolates may be regarded as strains, variants, clusters or other subspecific entities that, together with other entities, constitute a species. Classification of such isolates is not the responsibility of the ICTV but is the responsibility of international specialty groups. It is the responsibility of ICTV Study Groups to decide if an isolate or a group of isolates should constitute a species.

Deciding the names of serotypes, genotypes, strains, variants or isolates of virus species is not the responsibility of the ICTV. However, it is recommended that new names not be the same as, or closely similar to, names already in use (Rule 3.14 for taxa). When a particular virus isolate is designated to represent a species, the decision as to which name will be adopted for the species for formal taxonomic purposes will be the responsibility of the ICTV, usually based on recommendations of a particular Study Group working on behalf of the ICTV. The Study Group will be expected to consult widely so as to ensure the acceptability of names, subject to the Rules in the Code. The policy of the ICTV is that as far as is possible, decisions on questions of taxonomy and nomenclature should reflect the majority view of the appropriate virological constituency.

3.4 Artificially created viruses and laboratory hybrid viruses will not be given taxonomic consideration. Their classification will be the responsibility of acknowledged international specialist groups.

**Comments:** Naturally occurring isolates that have genomes formed from parts of the genomes of different strains of a virus, either by recombination between genome nucleic acids or by re-assortment of separate genome parts, will be classified either as species or subspecific entities in the same way that other isolates are classified. Neither artificial variants made by recombination or re-assortment nor mutant viruses are subject to the Rules in the Code.

***Limitations***

3.5 Taxa will be established only when representative member viruses are sufficiently well characterized and described in the published literature so as to allow them to be identified unambiguously and the taxon to be distinguished from other similar taxa.

3.6 When it is uncertain how to classify a species into a genus but its classification in a family is clear, it will be classified as an unassigned species of that family.

**Comments:** A species can be classified as an unassigned member of a family when no genus has been devised. For example, *Suid herpesvirus 2* is a herpesvirus of vertebrates but is not a member of any of the currently recognized genera in the family *Herpesviridae*. Likewise, *Groundnut rosette assistor virus* resembles viruses in the family *Luteoviridae* but is not classified in any of the genera in that family. These viruses are each classified as an unassigned member of their respective families.

3.7 Names will only be accepted if they are linked to taxa at the hierarchical levels described in Rule 3.2 and which have been approved by the ICTV.

**Comments:** Taxa above the rank of species must be approved before a name is assigned to them. Proposals for the creation of taxa shall be accompanied by proposals for names. A decision to create a taxon can thus be followed immediately by a decision about the name for the taxon. Species will be approved together with their names as a single taxonomic act.

The following example is of a proposal concerning an imaginary virus with the vernacular name of "beta gamma virus" that is related to another virus, "alpha beta virus".

- Proposal 1. Approve *Beta gamma virus* as a species containing strains known as "beta gamma virus" and "alpha beta virus".
- Proposal 2. Create a genus to contain species resembling *Beta gamma virus*.
- Proposal 3. Name the genus created by Proposal 2, *Betavirus*.
- Proposal 4. Nominate *Beta gamma virus* as the type species of the genus *Betavirus*.
- Proposal 5. Create a family to contain genus *Betavirus* and similar genera.
- Proposal 6. Name the family created by Proposal 5, *Betaviridae*.
- Proposal 7. Assign species X, Y and Z to genus *Betavirus* (such a proposal should include a listing of the parameters for discriminating between species in the genus *Betavirus*).

**II – Rules about naming Taxa**

***Status of Names***

3.8 Names proposed for taxa are "valid names" if they conform to the Rules set out in the Code and they pertain to established taxa. Valid names are "accepted names" if they are recorded as approved International Names in the 8th ICTV Report or have subsequently become "accepted names" by an ICTV vote of approval for a taxonomic proposal.

**Comments:** A valid name is one that has been published, one that is associated with descriptive material, and one that is acceptable in that it conforms to the Rules in the Code. Accepted names will be kept in an "Index" by the ICTV.

3.9 Existing names of taxa and viruses shall be retained whenever feasible.
**Comment:** A stable nomenclature is one of the principal aims of taxonomy and therefore changes to names that have been accepted will only be considered in exceptional circumstances, and then only because of serious conflict with the Rules.

3.10 The rule of priority in naming taxa and viruses shall not be observed.
**Comments:** The earlier of candidate names for a taxon may be chosen as a convenience to virologists, but the Rule ensures that it is not possible to invalidate a name in current use by claiming priority for an older name that has been superseded.

3.11 No person's name shall be used when devising names for new taxa.
**Comments:** New taxon names shall not be made by adopting a person's name, by adding a formal ending to a person's name or by using part of a person's name to create a stem for a name. When existing names of species incorporate a person's name (for example, Mason–Pfizer monkey virus) continued usage of this name, in agreement with Rule 2.3 and 3.9, is in general preferable to the creation of a new name.

3.12 Names for taxa shall be easy to use and easy to remember. Euphonious names are preferred.
**Comments:** In general, short names are desirable and the number of syllables should be kept to a minimum.

3.13 Subscripts, superscripts, hyphens, oblique bars and Greek letters may not be used in devising new names.
**Comments:** The Rule is intended to make text unambiguous and easy to manipulate and its application should often make names more pronounceable, in agreement with Rule 3.12.

3.14 New names shall not duplicate approved names. New names shall be chosen such that they are not closely similar to names that are in use currently or have been in use in the recent past.
**Comments:** The name selected for a new taxon should not sound indistinguishable from the name of another taxon at any rank or from any taxon. For example, the existence of the genus *Iridovirus* means that forms of new name such as "irodovirus" or "iridivirus" are unacceptable as they are too easily confused with an approved name. Confusion can also be between species and genus names as both end in "virus". Thus, for example, the name selected for a genus typified by a species "Omega virus" would not be named "Omegavirus" because species and genus would then be too readily confused.

3.15 Sigla may be accepted as names of taxa, provided that they are meaningful to virologists in the field, normally as represented by Study Groups.
**Comments:** Sigla are names comprising letters and/or letter combinations taken from words in a compound term. The name of the genus *Comovirus* has the sigla stem "*co-*" from cowpea and "*mo*" from mosaic; the name of the family *Reoviridae* has the sigla stem "*r*" from "respiratory, "*e*" from "enteric" and "*o*" from "orphan".

***Decision making***

3.16 In the event of more than one candidate name being proposed, the relevant Subcommittee will make a recommendation to the Executive Committee of the ICTV, which will then decide among the candidates as to which to recommend to ICTV for acceptance.
**Comments:** When there is more than one candidate name for the same taxon, the decision as to which will be accepted shall be made on the basis of the Code and, if necessary, thereafter on the basis of likely acceptability to the majority of virologists.

3.17 If no suitable name is proposed for a taxon, the taxon may be approved and the name will be left undecided until the adoption of an acceptable international name when one is proposed to and accepted by ICTV.
**Comments:** Occasionally the names proposed for taxa are judged unsuitable and revisions are requested. To facilitate the classification into appropriate taxa, it is preferable to accept the creation of a taxon with a temporary name rather than delay. Temporary names are indicated by quotation marks and the type species name is used. For example, *Enterobacteria phage Mu* is the type species in an as yet un-named genus in the family *Myoviridae*. Until the genus is named, it is designated as "Mu-like viruses". This designation is regarded as temporary.

3.18 New names shall be selected such that they, or parts of them, do not convey a meaning for the taxon which would either (1) seem to exclude viruses which lack the character described by the name but which are members of the taxon being named, or (2) seem to exclude viruses which are as yet

undescribed but which might belong to the taxon being named, or (3) appear to include within the taxon viruses which are members of different taxa.

3.19 New names shall be chosen with due regard to national and/or local sensitivities. When names are universally used by virologists in published work, these or derivatives shall be the preferred basis for creating names, irrespective of national origin.

*Procedures for naming taxa*

3.20 Proposals for new names, name changes, establishment of taxa and taxonomic placement of taxa shall be submitted to the Executive Committee of the ICTV in the form of taxonomic proposals. All relevant ICTV subcommittees and study groups will be consulted prior to a decision being taken.
**Comments:** Proposals concerning a family containing genera of viruses that infect diverse types of host (e.g. plants and vertebrates, fungi and plants, and so on) must be considered by the Subcommittees responsible for viruses of each host type (i.e. Plant viruses, Vertebrate viruses, and so on). For example, taxonomic proposals concerned with the family *Partitiviridae* would be considered by the Fungal Virus Subcommittee and one of its Study Groups but because some genera in the family contain viruses of plants, proposals affecting the family would also be considered by the Plant Virus Subcommittee.

**III – Rules about Species**

*Definition of a virus species*

3.21 A virus species is defined as a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche.

*Definition of "tentative" status*

3.22 When an ICTV Subcommittee is uncertain about the taxonomic status of a new species or about assignment of a new species to an established genus, the new species will be listed as a tentative species in the appropriate genus or family. Names of tentative species, as of taxa generally (Rule 3.14), shall not duplicate approved names and shall be chosen such that they are not closely similar to names that are in use currently, names that have been in use in the recent past, or names of definitive species.
**Comments:** Species classified as tentative are candidates for taxonomic decision by the appropriate Study Groups to resolve their tentative status.

*Construction of a name*

3.23 A species name shall consist of as few words as practicable but be distinct from names of other taxa. Species names shall not consist only of a host name and the word "virus".
**Comments:** Species names normally comprise more than one word. The styles used when virus names are devised differ according to the traditions of the particular fields of virology. For example, plant virus names are usually constructed as host + symptom + "virus" (e.g. tobacco necrosis virus) whereas, in contrast, viruses in the family *Bunyaviridae* are usually named after the location at which the virus was found + "virus" (e.g. *Bunyamwera virus*).

3.24 A species name must provide an appropriately unambiguous identification of the species.
**Comments:** Species names should be distinctive. They should not be in a form that could be easily confused with the names of other taxa.

3.25 Numbers, letters, or combinations thereof may be used as species epithets where such numbers and letters are already widely used. However, newly designated serial numbers, letters or combinations thereof are not acceptable alone as species epithets. If a number or letter series is in existence it may be continued.
**Comments:** The existence of species names such as *Bovine adenovirus A*, *Bovine adenovirus B*, and so on, and *Human herpesvirus 1*, *Human herpesvirus 2*, *Human herpesvirus 3*, and so on justify the use of the next number in the series. A name such as "22" or "A7" is not acceptable.

**IV – Rules about Genera**

3.26 A genus is a group of species sharing certain common characters.

3.27 A genus name shall be a single word ending in ...*virus*.

3.28 Approval of a new genus must be accompanied by the approval of a type species.

**V – Rules about Subfamilies**

3.29 A subfamily is a group of genera sharing certain common characters. The taxon shall be used only when it is needed to solve a complex hierarchical problem.

3.30 A subfamily name shall be a single word ending in ...*virinae*.

**VI – Rules about Families**

3.31 A family is a group of genera (whether or not these are organized into subfamilies) sharing certain common characters.

3.32 A family name shall be a single word ending in ...*viridae*.

**VII – Rules about Orders**

3.33 An order is a group of families sharing certain common characters.

3.34 An order name shall be a single word ending in ...*virales*.

**VIII – Rules about Subviral Agents**

***Viroids***

3.35 Rules concerned with the classification of viruses shall also apply to the classification of viroids.

3.36 The formal endings for taxa of viroids are the word "*viroid*" for species, the suffix "*-viroid*" for genera, the suffix "*-viroinae*" for subfamilies (should this taxon be needed) and "*-viroidae*" for families.

**Comments:** For example, the species *Potato spindle tuber viroid* is classified in genus *Pospiviroid*, and the family *Pospiviroidae*.

***Other Subviral Agents***

3.37 Retrotransposons are considered to be viruses in classification and nomenclature

3.38 Satellites and prions are not classified as viruses but are assigned an arbitrary classification as seems useful to workers in the particular fields.

**IX – Rules for Orthography**

3.39 In formal taxonomic usage, the accepted names of virus orders, families, subfamilies, and genera are printed in italics and the first letters of the names are capitalized.

**Comments:** See Rule 3.8 for the definition of an "accepted" name.

3.40 Species names are printed in italics and have the first letter of the first word capitalized. Other words are not capitalized unless they are proper nouns, or parts of proper nouns.

**Comments:** When used formally, as labels for taxonomic entities, the names *Tobacco mosaic virus* and *Murray Valley encephalitis virus* are in the correct form and typographical style. Examples of incorrect forms are Ustilago maydis virus H (not italic), *Murray valley encephalitis virus* (Valley is a proper noun) or tobacco mosaic virus (not capitalized or italic).

Taxa are abstractions and thus when their names are used formally, these are written distinctively using italicization and capitalization. In other senses, such as an adjectival form (e.g. the tobacco mosaic virus polymerase) italics and capital initial letters are not needed. Equally, these are not needed when referring to physical entities such as virions (e, g. a preparation or a micrograph of tobacco mosaic virus).

This Rule was introduced in 1998 and is in contradistinction to Rules in the Code published in the 6th ICTV Report.

3.41 In formal usage, the name of the taxon shall precede the term for the taxonomic unit.

**Comments:** For example, the correct formal descriptions of various taxa are ... the family *Herpesviridae*, ... the genus *Morbillivirus*, .... the genus *Rhinovirus*, .... the species *Tobacco necrosis virus D*, and so on.

# Part IV
# The Indexes

# Virus Index

# Taxonomic Index

# Virus Index

## A

# B

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## Taxonomic Index