



This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	2010.001a-qI	(to be completed by ICTV officers)			
Short title: Revision of the family Tetraviridae (e.g. 6 new species in the genus <i>Zetavirus</i>)					
Modules attached (modules 1 and 9 are required)	1 <input checked="" type="checkbox"/> 6 <input type="checkbox"/>	2 <input type="checkbox"/> 7 <input checked="" type="checkbox"/>	3 <input checked="" type="checkbox"/> 8 <input checked="" type="checkbox"/>	4 <input type="checkbox"/> 9 <input checked="" type="checkbox"/>	5 <input checked="" type="checkbox"/>

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List the ICTV study group(s) that have seen this proposal:

A list of study groups and contacts is provided at <http://www.ictvonline.org/subcommittees.asp> . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)

The proposers are members of the Tetravirus Study Group

ICTV-EC or Study Group comments and response of the proposer:

Date first submitted to ICTV:

August, 2011

Date of this revision (if different to above):

MODULE 3: **NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	2010.001aI	(assigned by ICTV officers)
To create a new genus within:		
Subfamily:		Fill in all that apply. • If the higher taxon has yet to be created (in a later module, below) write “(new)” after its proposed name. • If no family is specified, enter “unassigned” in the family box
Family:	<i>Permutotetraviridae</i> (new)	
Order:		

naming a new genus

Code	2010.001bI	(assigned by ICTV officers)
To name the new genus: <i>Alphapermutotetravirus</i>		

Assigning the type species and other species to a new genus

Code	2010.001cI	(assigned by ICTV officers)
To designate the following as the type species of the new genus		
<i>Thosea asigna virus</i>		Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered
The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). Please enter here the TOTAL number of species (including the type species) that the genus will contain:		
two		

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

To satisfy an ICTV rule that requires at least a single genus to be created in a family. This is the first genus proposed for the family and it accommodates two closely related species proposed for this family.

Origin of the new genus name:

Alphapermutotetravirus: *alpha* stands for the first (alpha- in Greek) genus of the family *Permutotetraviridae*

Reasons to justify the choice of type species:

Includes TaV that has been characterized using different techniques more extensively than EeV.

Species demarcation criteria in the new genus:

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

TaV and EeV were already assigned to separate species in the family *Tetraviridae* and this assignment is observed in this newly created family where they were moved. They are closely related viruses and a decision how to classify them – as a single or separate species – is not

straightforward, also given that the characterization of these viruses is very limited and no other viruses are currently assigned to this family. They share 84% and 82% of identical amino acid residues in the capsid and replicase proteins, respectively. The former number is lower than the 87% of identical residues between the closest species in the *Tetraviridae* that could be considered as the current de facto demarcation threshold for viruses of three families discussed in this proposal. This important parameter could be reviewed along with the advancement of characterization of these viruses and other viruses whose genomes have not yet been sequenced.

MODULE 5: **NEW FAMILY**

creating and naming a new family

Code	2010.001dI	(assigned by ICTV officers)
<p>To create a new family containing the subfamilies and/or genera listed below within the Order: <i>Unassigned</i></p> <p>If there is no Order, write "unassigned" here. If the Order has yet to be created (in Module 6) please write "(new)" after the proposed name.</p>		

Code	2010.001eI	(assigned by ICTV officers)
<p>To name the new family: <i>Permutotetraviridae</i></p>		

assigning subfamilies, genera and unassigned species to a new family

Code		(assigned by ICTV officers)
<p>To assign the following subfamilies (if any) to the new family: You may list several subfamilies here. For each subfamily, please state whether it is new or existing.</p> <ul style="list-style-type: none"> • If the subfamily is new, it must be created in Module 4 • If the subfamily already exists, please complete Module 7 to 'REMOVE' it from its existing family 		

Code	2010.001fI	(assigned by ICTV officers)
<p>To assign the following genera to the new family: You may list several genera here. For each genus, please state whether it is new or existing.</p> <ul style="list-style-type: none"> • If the genus is new, it must be created in Module 3 • If the genus already exists, please state whether it is currently unassigned or is to be removed from another family. If the latter, complete Module 7 to 'REMOVE' it from that family 		

Alphapermutotetravirus

The new family will also contain any other new species created and assigned to it (Module 3) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of unassigned species that the family will contain (those NOT within any of the genera or subfamilies listed above):**

none

Reasons to justify the creation of the new family:

[Additional material in support of this proposal may be presented in the Appendix, Module 9](#)

This family is created to accommodate viruses of the two species, *Euprosterina elaeasa virus* and *Thosea asigna virus*, currently classified with the family *Tetraviridae*. Viruses of these species, EeV and TaV, respectively, form a close phylogenetic cluster across the genome. They are predicted to have terminal non-coding sequences arranged in unique secondary structure elements. In the replicase ORF, accounting for the largest part of the genome, they resemble most the double-stranded RNA family *Birnaviridae* rather than other members of the family *Tetraviridae*. The replicase similarity is extended to include an RdRp domain with the permuted active site and a VPg signal. Despite the sequence affinity between the family *Birnaviridae* and EeV and TaV, the evolutionary distances between viruses of the two groups grossly exceed those observed within the groups (intrafamily divergence). The difference in the type of RNA

genome – double-stranded and single-stranded – employed by viruses of these two groups is also of note. Because of this phylogenetic separation from other viruses in the replicase ORF, *Euprosterina elaeasa virus* and *Thosea asigna virus* species were proposed to be placed in the newly designated family *Permutotetraviridae* (Zeddiam *et al.*, 2010). The unique combination of characteristics that defines this family, including jelly-roll based capsid architecture, highly structured genome terminal sequences, conservation of permuted RdRp and VPg with sequence affinities to the *Birnaviridae* could be used as parameters to evaluate new viruses for the inclusion into the family. The characteristics list could be expanded along with the progress in molecular biology of these viruses.

Origin of the new family name:

The *Permutotetraviridae* name combines parts that refer to the unique type of RdRp (replicase) whose active site is permuted (“permute”) and the unique type of $T=4$ capsid architecture (“tetra”) that together define the family.

MODULE 3: NEW GENUS

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	2010.001gI	(assigned by ICTV officers)
To create a new genus within:		
Subfamily:		Fill in all that apply. • If the higher taxon has yet to be created (in a later module, below) write “ (new) ” after its proposed name. • If no family is specified, enter “ unassigned ” in the family box
Family:	<i>Carmotetraviridae</i>	
Order:		

naming a new genus

Code	2010.001hI	(assigned by ICTV officers)
To name the new genus: <i>Alphacarmotetravirus</i>		

Assigning the type species and other species to a new genus

Code	2010.001iI	(assigned by ICTV officers)
To designate the following as the type species of the new genus		
<i>Providence virus</i>	Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered	
The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). Please enter here the TOTAL number of species (including the type species) that the genus will contain:		
<i>one</i>		

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

To satisfy an ICTV rule that requires at least a single genus to be created in a family. This is the first genus proposed for the family and it accommodates a sole species proposed for this family.

Origin of the new genus name:

Alphacarmotetravirus: *alpha* stands for the first (alpha- in Greek) genus of the family *Carmotetraviridae*

Reasons to justify the choice of type species:

It is the only species proposed for this genus

Species demarcation criteria in the new genus:

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

No species demarcation criteria have been developed since the single proposed species includes only one virus.

MODULE 5: **NEW FAMILY**

creating and naming a new family

Code	2010.001jI	(assigned by ICTV officers)
<p>To create a new family containing the subfamilies and/or genera listed below within the Order: <i>Unassigned</i></p> <p>If there is no Order, write "unassigned" here. If the Order has yet to be created (in Module 6) please write "(new)" after the proposed name.</p>		

Code	2010.001kI	(assigned by ICTV officers)
<p>To name the new family: <i>Carmotetraviridae</i></p>		

assigning subfamilies, genera and unassigned species to a new family

Code		(assigned by ICTV officers)
<p>To assign the following subfamilies (if any) to the new family: You may list several subfamilies here. For each subfamily, please state whether it is new or existing.</p> <ul style="list-style-type: none"> • If the subfamily is new, it must be created in Module 4 • If the subfamily already exists, please complete Module 7 to 'REMOVE' it from its existing family 		

Code	2010.001II	(assigned by ICTV officers)
<p>To assign the following genera to the new family: You may list several genera here. For each genus, please state whether it is new or existing.</p> <ul style="list-style-type: none"> • If the genus is new, it must be created in Module 3 • If the genus already exists, please state whether it is currently unassigned or is to be removed from another family. If the latter, complete Module 7 to 'REMOVE' it from that family 		

Alphacarmotetravirus

The new family will also contain any other new species created and assigned to it (Module 3) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of unassigned species that the family will contain (those NOT within any of the genera or subfamilies listed above):**

none

Reasons to justify the creation of the new family:

[Additional material in support of this proposal may be presented in the Appendix, Module 9](#)

This family is created to accommodate viruses (PrV) of the *Providence virus* species currently classified with the family *Tetraviridae*. The genome terminal non-coding regions of PrV are not arranged in the predicted secondary structures observed in other tetraviruses (including EeV and TaV). PrV has no close viruses with respect to the replicase ORF, which is unique amongst the tetraviruses in that it encodes a functional read-through stop signal. The PrV genome encodes a third ORF of unknown function not present in any other tetraviruses. It is the largest ORF on the genome and it overlaps with the replicase ORF. The latter distantly clusters, as an outgroup, with ssRNA+ plant viruses comprising umbra- and tombusviruses and other carmo-like viruses rather than with other members of the *Tetraviridae* family. Because of this separation from other viruses, *Providence virus* species is proposed to be placed in the newly designated *Carmotetraviridae* family. The unique combination of characteristics that defines this family,

including jelly-roll based capsid architecture, unstructured genome terminal sequences, conservation of an RdRp of a particular type and the presence of the readthrough signal controlling RdRp expression could be used as parameters to evaluate new viruses for the inclusion into the family. The characteristics list could be expanded along with the progress in molecular biology of these viruses.

Origin of the new family name:

The Carmotetraviridae name combines parts that refer to the type of replicase (RdRp) characteristic of viruses of Carmo-like plant viruses (“Carmo”) and the unique type of $T=4$ capsid architecture (“tetra”) that together define the family.

MODULE 7: **REMOVE and MOVE**

Use this module whenever an existing taxon needs to be removed:

- Either to abolish a taxon entirely (when only part (a) needs to be completed)
- Or to move a taxon and re-assign it e.g. when a species is moved from one genus to another (when BOTH parts (a) and (b) should be completed)

Part (a) taxon/taxa to be removed or moved

Code	2010.001mI	(assigned by ICTV officers)
To remove the following taxon (or taxa) from their present position:		
Species <i>Euprosterona elaeasa virus</i> , <i>Thosea asigna virus</i>		
The present taxonomic position of these taxon/taxa:		
Genus:	<i>Betatetravirus</i>	Fill in all that apply.
Subfamily:		
Family:	<i>Tetraviridae</i>	
Order:		
If the taxon/taxa are to be abolished (i.e. not reassigned to another taxon) write "yes" in the box on the right		

Reasons to justify the removal:

Explain why the taxon (or taxa) should be removed

Viruses of these two species differ profoundly from the prototypic tetraviruses and from PrV of the *Providencia virus* species. These differences include key genome characteristics outside the capsid gene, concerning genomic terminal sequences and replicase ORF.

Part (b) re-assign to a higher taxon

Code	2010.001nI	(assigned by ICTV officers)
To re-assign the taxon (or taxa) listed in Part (a) as follows:		
Genus:	<i>Alphapermutotetravirus</i> (new)	Fill in all that apply. <ul style="list-style-type: none"> • If the higher taxon has yet to be created write "(new)" after its proposed name and complete relevant module to create it. If no genus is specified, enter "unassigned" in the genus box.
Subfamily:		
Family:	<i>Permutotetraviridae</i> (new)	
Order:		

Reasons to justify the re-assignment:

- If it is proposed to re-assign species to an existing genus, please explain how the proposed species differ(s) from all existing species.
 - If species demarcation criteria (see module 3) have previously been defined for the genus, explain how the new species meet these criteria.
 - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Provide accession numbers for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

Viruses of the two species form a compact monophyletic cluster in every region of the

genome. In the replicase ORF, accounting for the largest part of the genome, they resemble most closely but still distantly the double-stranded RNA family *Birnaviridae*, which also feature an RdRp domain with the permuted active site and a VPg signal. The evolutionary distances between viruses of the two groups grossly exceed those observed within the groups (intrafamily divergence). Because of this phylogenetic separation from other viruses in the replicase ORF, *Euprosterna elaeasa virus* and *Thosea asigna virus* species were proposed to be placed in the newly designated family *Permutotetraviridae* (Zeddiam *et al.*, 2010).

Euprosterna elaeasa virus

Euprosterna elaeasa virus [AF461742] EeV

Thosea asigna virus

Thosea asigna virus [AF82930, AF062037] TaV

MODULE 7: **REMOVE and MOVE**

Use this module whenever an existing taxon needs to be removed:

- Either to abolish a taxon entirely (when only part (a) needs to be completed)
- Or to move a taxon and re-assign it e.g. when a species is moved from one genus to another (when BOTH parts (a) and (b) should be completed)

Part (a) taxon/taxa to be removed or moved

Code	2010.001oI	(assigned by ICTV officers)
To remove the following taxon (or taxa) from their present position:		
Species <i>Providencia virus</i>		
The present taxonomic position of these taxon/taxa:		
Genus:	<i>Betatetravirus</i>	Fill in all that apply.
Subfamily:		
Family:	<i>Tetraviridae</i>	
Order:		
If the taxon/taxa are to be abolished (i.e. not reassigned to another taxon) write "yes" in the box on the right		

Reasons to justify the removal:

Explain why the taxon (or taxa) should be removed

Viruses of the *Providencia virus* species differ profoundly from the prototypic tetraviruses and from *Euprosterna elaeasa virus* and *Thosea asigna virus* species including key genome features outside the capsid gene, concerning genomic terminal sequences, replicase ORF, and a third ORF unique to *Providencia virus*.

Part (b) re-assign to a higher taxon

Code	2010.001pI	(assigned by ICTV officers)
To re-assign the taxon (or taxa) listed in Part (a) as follows:		
Genus:	<i>Alphacarmotetravirus</i> (new)	Fill in all that apply. <ul style="list-style-type: none"> • If the higher taxon has yet to be created write "(new)" after its proposed name and complete relevant module to create it. If no genus is specified, enter " unassigned " in the genus box.
Subfamily:		
Family:	<i>Carmotetraviridae</i> (new)	
Order:		

Reasons to justify the re-assignment:

- If it is proposed to re-assign species to an existing genus, please explain how the proposed species differ(s) from all existing species.
 - If species demarcation criteria (see module 3) have previously been defined for the genus, explain how the new species meet these criteria.
 - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Provide accession numbers for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

In the replicase ORF, accounting for the largest part of the genome, *Providence virus* has no closely related viruses. It distantly clusters, as outgroup, with ssRNA+ plant viruses comprising umbra- and tombusviruses and other carmo-like viruses (Walter *et al.*, 2010). Because of this separation from other viruses, *Providence virus* species is proposed to be placed in the newly designated family *Carmotetraviridae*.

Providence virus

Providence virus [AF062037, GU991616]

PrV

MODULE 8: **NON-STANDARD**

Template for any proposal not covered by modules 2-7. This includes proposals to change the name of existing taxa (but note that stability of nomenclature is encouraged wherever possible).

non-standard proposal

Code	2010.001qI	(assigned by ICTV officers)
Title of proposal: Change the name of family <i>Tetraviridae</i> to <i>Alphatetraviridae</i>		

Text of proposal:

In the modules 2-7, we outlined a proposal to create two new families in which three established species of the family *Tetraviridae* are relocated. The family split is prompted by profound sequence dissimilarities in the replicase region of tetraviruses; the unique characteristics of replicase and genomic terminal regions and the presence of unique (large) ORFs that define these families. These dissimilarities contrast with capsid-based features – notably common $T=4$ quasi-symmetry of their capsid architecture - that unify viruses of three families and distinguish them from other viruses. To acknowledge the similarities and dissimilarities, the names of the two newly proposed virus families include parts that refer to the different affinities of replicase and capsid, respectively. Using this framework and for consistency reasons, the name of the original family *Tetraviridae* must also be revised to include an additional part that refers to replicase typical for these viruses. Since the prototypic tetraviruses cluster with viruses of Alpha-like supergroup in the replicase region, the virus name for this family is proposed to be changed into the *Alphatetraviridae*.

The above family name revision has no consequences for any other aspect of the family. Accordingly, the family retains two genera, *Betatetravirus* and *Omegatetravirus*, with all species being included except for those three that have been relocated to found two new families as it is detailed elsewhere in this proposal.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

- Dorrington, R.A., Short, J.R., 2010. The Tetraviruses. In Johnson, K., Asgari, S., (Eds), The Insect Viruses, Horizon Scientific Press, UK.
- Genty, P. (1976). Etude morphologique et biologique d'un lépidoptère défoliateur du palmier à huile en Amérique latine *Darna metaleuca*, Walker. *Oléagineux* 31, 99-107.
- Genty, P., Desmier de Chenon, R., Morin, J.-P., and Korytkowski, C. A. (1978). Oil palm pests in Latin America. *Oléagineux* 31, 325-419.
- Gorbalenya, A.E., Pringle, F.M., Zeddám, J.-L., Luke, B.T., Cameron, C.E., Kalmakoff, J., Hanzlik, T., Gordon, K., Ward, V.K., 2002. The palm subdomain-based active site is internally permuted in viral RNA-dependent RNA polymerases of an ancient lineage. *J Mol Biol*, 324, 47-62.
- Luke, G.A., de Felipe, P., Lukashev, A., Kallioinen, S.E., Bruno, E.A., Ryan, M.D., 2008. Occurrence, function and evolutionary origins of '2A-like' sequences in virus genomes. *J Gen Virol*, 89, 1036–1042.
- Pringle, F.M., Johnson, K.N., Goodman, C.L., McIntosh, A.H., Ball, L.A., 2003. Providence virus: a new member of the Tetraviridae that infects cultured insect cells. *Virology*, 306, 359-370.
- Speir, J.A., Taylor, D.J., Natarajana, P., Pringle, F.M., Ball, L.A., Johnson, J.E., 2010. Evolution in action: N and C termini of subunits in related T=4 viruses exchange roles as molecular switches. *Structure*, 18, 700-709.
- Walter, C.T., Pringle F.M., Nakayinga, R., de Felipe, P., Ryan, M., Ball, L.A. Dorrington, R.A. 2010. Genome organization and translation products of Providence virus: insight into a unique tetravirus. *J Gen Virol*, 91, 2826-2835.
- Zeddám, J.-L., Gordon, K.H.J., Lauber, C., Felipe Alves, C.A., Luke, B.T., Hanzlik, T.N., Ward, V.K., 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, 145-54.

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.

Viruses of the *Tetraviridae* family have mono- and bipartite genomes with open reading frames (ORFs) whose organizations vary grossly between viruses with four major layouts being recognized (Fig. 1)

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 capsid proteins (CPs) form a monophyletic group (Fig. 2). The jelly-roll fold β subunits of CPs, which

form the capsid shell, are most closely but still distantly related structurally to those of the CPs of noroviruses and dsRNA birnaviruses having $T=3$ and $T=13$ capsids, respectively.

Comparative analysis of currently available non-structural protein sequences splits tetraviruses into three distinct lineages, prototyped by NβV/HaSV (Fig. 3), TaV/EeV (Fig. 4) and PrV (Fig. 5), 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 (Fig. 1) and through phylogenetic clustering with viruses having these three domains (Fig. 3). The replicases of TaV and EeV lack both NMT and HEL1 domains (Fig. 1). 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 motif arrangement is also found in replicases of all dsRNA birnaviruses. The permuted TaV, EeV and birnavirus enzymes form a minor, deeply separated cluster in the RdRp tree that also includes viruses of the “picornavirus-like supercluster” and the order *Nidovirales* (Fig. 4). The pRdRp domains of these viruses are also flanked by the uniquely conserved VPg signal and another poorly characterized domain from the N-terminus (Fig. 1).

The RdRp of PrV also lacks the NMT and HEL1 domains (Fig. 1), 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 (Fig. 5), which belong to the third virus supergroup, the carmo-like viruses of other families (Walter *et al.*, 2010) and therefore appears to belong to a third lineage. The PrV replicase encodes a functional read-through stop codon, which is conserved in the distantly related replicases of tombusviruses, but not observed in replicases of other tetraviruses.

These complex and incongruent relationships of CP and replicase proteins imply that viruses currently classified as tetraviruses on the basis of their CP are likely to form a polyphyletic group based upon the properties of their replicase. According to a recently developed evolutionary model (Zeddarn *et al.*, 2010), 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 PrV, are proposed to be placed in two new families, *Permutotetraviridae* and *Carmotetraviridae*, respectively. For consistency reasons, the original *Tetraviridae* is proposed to be renamed into the family *Alphatetraviridae*.

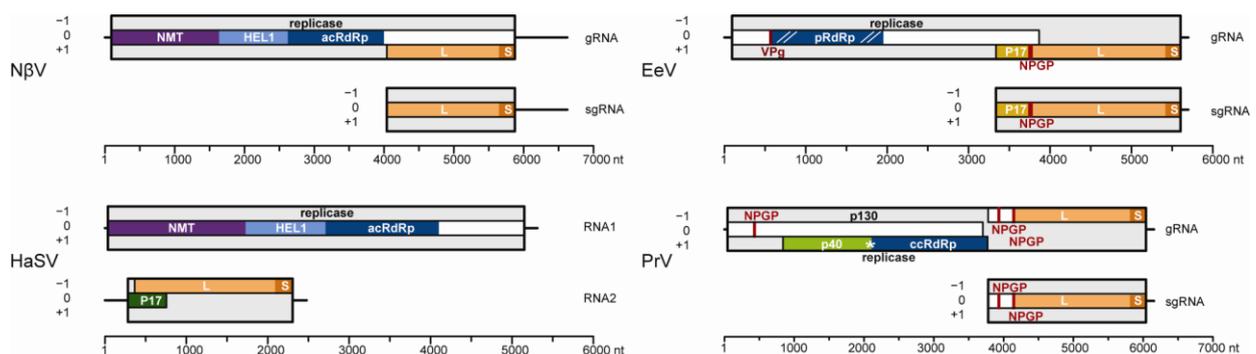


Figure 1. Genome comparisons of viruses of the *Tetraviridae* family that represent three different lineages. The genome organization, including genome segments, ORFs and selected domains, is depicted for four tetraviruses EeV, NβV, HaSV and PrV, using virus-specific scales. EeV, NβV and PrV have monopartite 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. The figure is a modified version of Figure 1 of Zeddiam *et al.*, 2010.

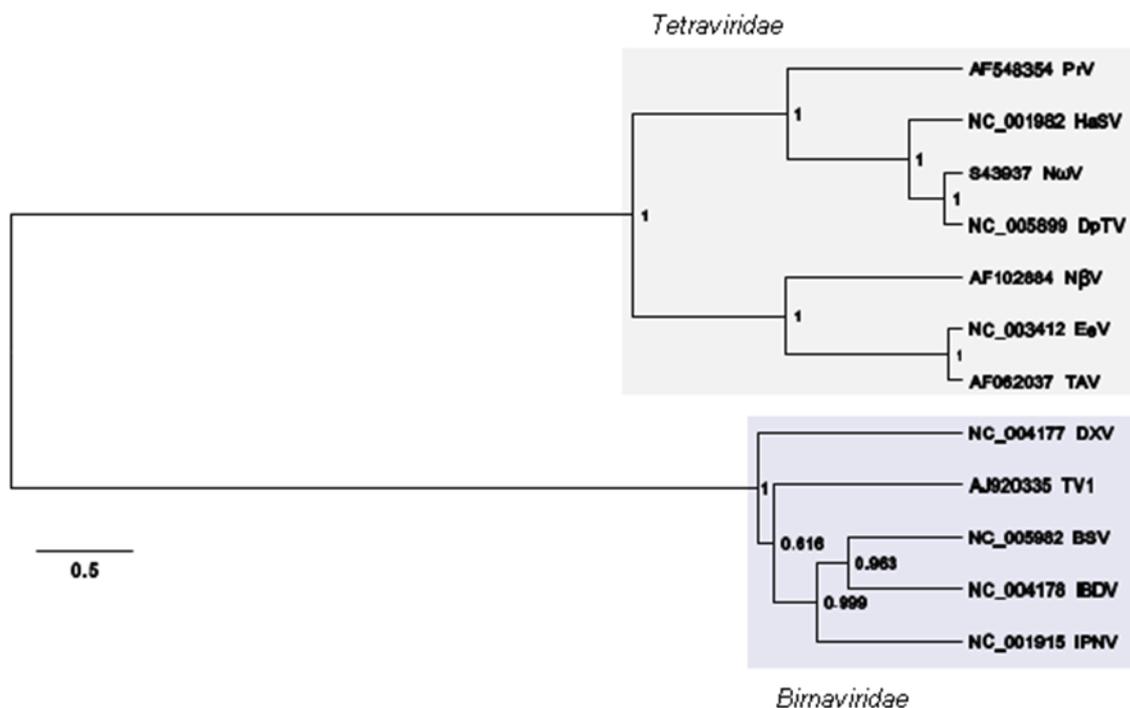


Figure 2. Phylogeny of tetravirus capsids. The tree for seven tetraviruses is based on an amino acid alignment of the jelly-roll domain of L protein (661 positions) and was rooted using the jelly-roll domain of S proteins of five birnaviruses as an outgroup. Numbers at branch points provide Bayesian posterior probability support values and the evolutionary scale is indicated by the bar of 0.5 amino acid substitutions per site on average. The figure was adapted from Figure 5b (left part) of Zeddiam *et al.*, 2010. GenBank/RefSeq accession numbers of virus sequences analysed were: genus *Omeгатetravirus* - Helicoverpa armigera stunt virus (HaSV): [NC_001982], Nudaurelia capensis ω virus (NωV): [S43937], Dendrolimus punctatus tetravirus (DpTV): [NC_005899]; genus *Betatetravirus* - Nudaurelia capensis β virus (NβV): [AF102884], Providence virus (PrV): [AF548354], Thosea asigna virus (TaV): [AF062037], Euprosterina elaeasa virus (EeV): [NC_003412]; family *Birnaviridae* - Infectious bursal disease virus (IBDV): [NC_004178], Infectious pancreatic necrosis virus (IPNV): [NC_001915], Blotched snakehead virus (BSV): [NC_005982], Tellina virus 1 (TV1): [AJ920335], Drosophila X virus (DXV): [NC_004177].

Sequence names used in the alignment and trees were prepared using SNAD (Sidorov et al., 2009) at <http://veb.lumc.nl/SNAD/>

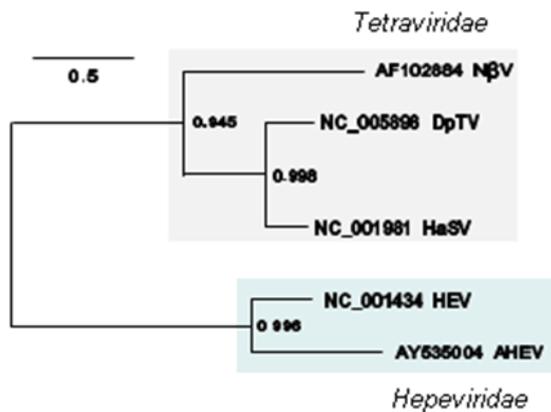


Figure 3. Phylogeny of prototypic tetravirus replicases. The tree for prototypic tetraviruses and DpTV is based on an amino acid alignment of the HEL1 and acRdRp domains (832 positions) and was rooted using avian and human Hepatitis E viruses as an outgroup. Numbers at branch points provide Bayesian posterior probability support values and the evolutionary scale is indicated by the bar of 0.5 amino acid substitutions per site on average. The figure was adapted from Figure 5b (right part) of Zeddiam *et al.*, 2010. GenBank/RefSeq accession numbers of virus sequences analysed were: genus *Omegatetravirus* - HaSV: [NC_001981], DpTV: [NC_005898]; genus *Betatetravirus* - NβV: [AF102884]; Human and avian Hepatitis E viruses (HEV, AHEV): [NC_001434, AY535004].

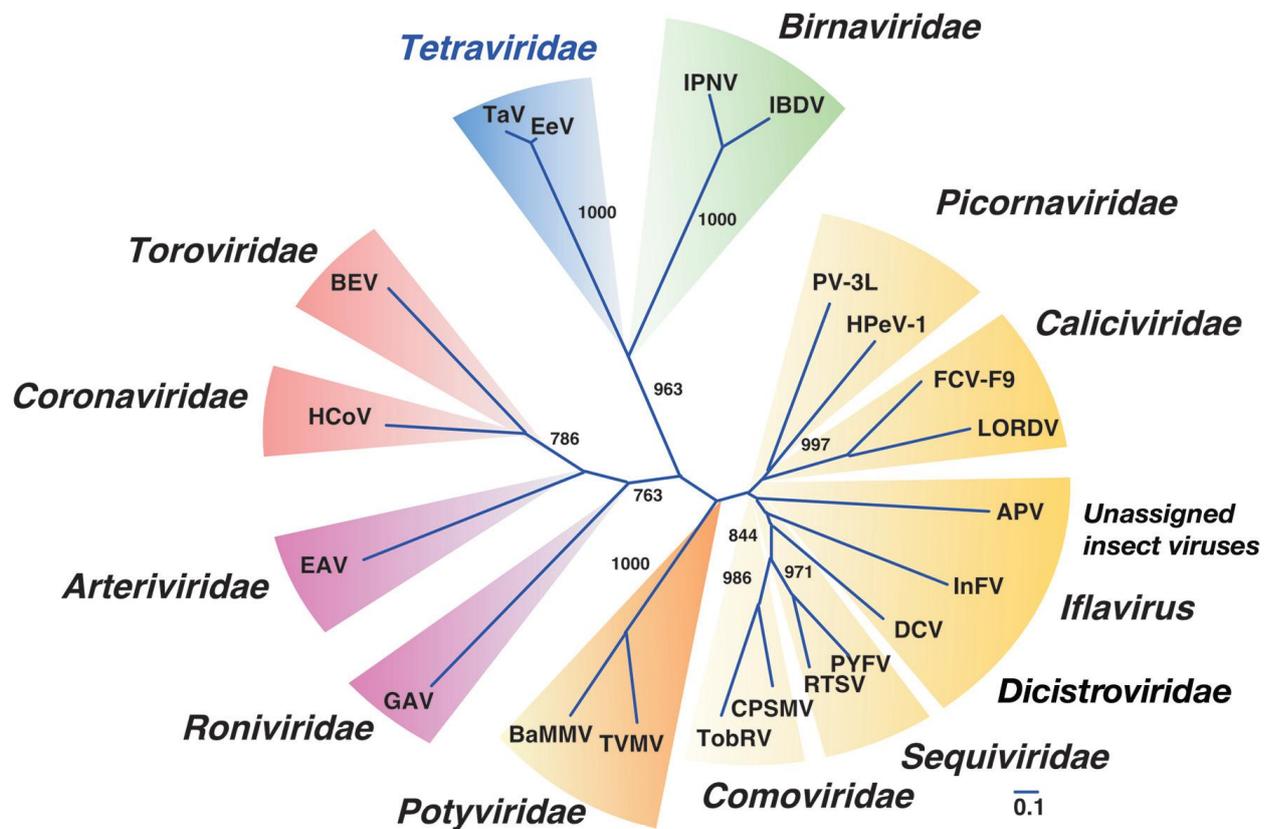


Figure 4. Unrooted phenogram showing the relationships of the RdRps of the tetraviruses TaV and EeV to other virus families and viruses in the “picornavirus-like supercluster”. The pRdRps of TaV, EeV and the birnaviruses were converted into the canonical form by relocating the motif C sequence (18-20 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*, *Sequiviridae*, *Comoviridae*, *Caliciviridae*, *Potyviridae*, *Coronaviridae*, *Roniviridae*, *Arteriviridae*, the genus *Iflavirus* 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 other specified) IDs [] are as follows: *Picornaviridae*: Human poliovirus type 3 Leon strain (PV-3L) [130503] and Human parechovirus 1 (HpeV-1) [6174922]; *Iflavirus*: Infectious flacherie virus (InFV) [3025415]; unclassified insect virus *Acyrtosiphon pisum* virus (APV) [7520835]; *Dicistroviridae*: *Drosophila C* virus (DCV) [2388673]; *Sequiviridae*: Rice tungro spherical virus (RTSV) [9627951] and Parsnip yellow fleck virus (PYFV) [464431]; *Comoviridae*: 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]; *Coronaviridae*: Human coronavirus 229E (HCoV) [12175747] and 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 *Euprosterina 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*. The figure is reproduced from the VIII Report of the ICTV and was adapted from Gorbalenya *et al.*, 2002.

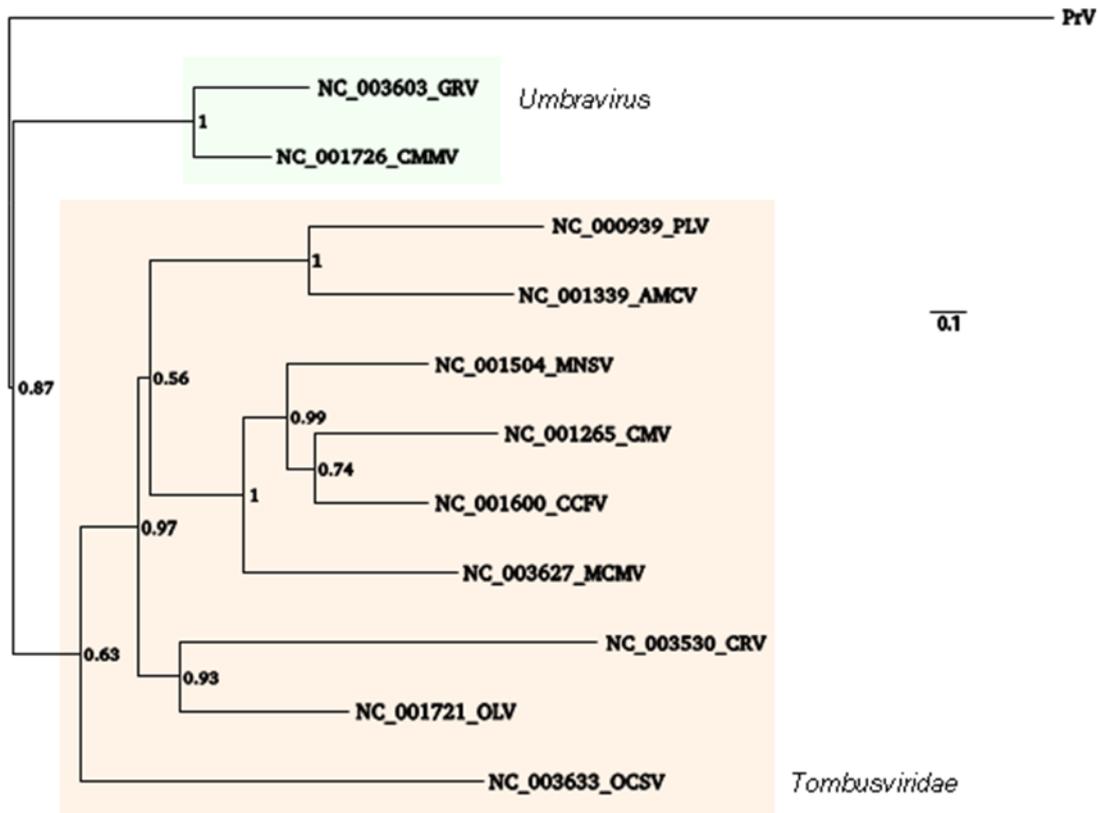


Figure 5. Phylogeny of PrV and related tombus- and umbravirus RdRps. The tree is based on an amino acid alignment of the C-terminal half of the replicase starting after the read-through stop codon (578 positions) encoding putative RdRp and was midpoint pseudo-rooted. Poorly conserved alignment termini 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 Zeddiam *et al.*, 2010 (see Figures 2 and 3). 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: CMMV, Carrot mottle mimic virus; GRV, Groundnut rosette virus; OCSV, Oat chlorotic stunt virus; CRV, Carnation ringspot virus; OLV, Olive latent virus 1; CCFV, Cardamine chlorotic fleck virus; CMV, Carnation mottle virus; MNSV, Melon necrotic spot virus; MCMV, Maize chlorotic mottle virus; AMCV, Artichoke mottled crinkle virus; PLV, Pothos latent virus. This tree is by Lauber and Gorbalenya (unpublished) using the PrV sequence (GenBank accession no: GU991616).