

Template for Taxonomic Proposal to the ICTV Executive Committee To create a new Family

Code [†]	2007.057P	To create a new family in the existing order	<i>Picornavirales</i>
Code [†]	2007.058P	To name the new family*	<i>Secoviridae</i>
Code [†]	2007.059P	To designate the following genera as part of the new family*: <i>Comovirus, Fabavirus and Nepovirus (proposed to form the sub-family Comovirinae), Sequivirus, Waikavirus, Cheravirus, Sadwavirus and the proposed genus Torradovirus (see proposal 2007.039-042P)</i>	

[†] Assigned by ICTV officers

[°] Leave blank is not appropriate

* repeat these lines and the corresponding arguments for each genus created in the family

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Old Taxonomic Order

Order: *Picornavirales*

Family: *Sequiviridae* including genera *Sequivirus* and *Waikavirus*

Family: *Comoviridae* including genera *Comovirus*, *Fabavirus* and *Nepovirus*

Unassigned Genera: *Sadwavirus* and *Cheravirus*

New Taxonomic Order

Order: *Picornavirales*

Family: *Secoviridae*

Sub-Family: *Comovirinae* including genera *Comovirus*, *Fabavirus* and *Nepovirus*

Genera: *Sequivirus*, *Waikavirus*, *Sadwavirus*, *Cheravirus* and *Torradovirus* (proposed new genus, see separate proposal)

ICTV-EC comments and response of the SG

The continuing critical examination of the taxonomy of this group is very welcome. With the impending acceptance of the order *Picornavirales*, it was felt that the level of family was the correct one for the proposed *Secoviridae*. Can the authors confirm how the new family differs from others in the order – is it only the fact that they infect plants?

SG response: Distinguishing characteristics of the family *Secoviridae*: they infect plants and either encode specialized protein(s) (domain) essential for short and long distance movement in plant (so called movement protein) or possess capsid protein(s) adapted for this function. In phylogenetic studies, the *Secoviridae* group together in a branch that is separated from other members of the order *Picornavirales* when the Pro-Pol conserved sequence is considered.

Argumentation to create a new family:

The family *Comoviridae* was for a long time the only known group of plant picorna-like viruses (Le Gall et al, 2005a). Members of the family *Comoviridae* are bipartite and contain either a single coat protein (genus *Nepovirus*) or two coat proteins (genera: *Comovirus* and *Fabavirus*, which are mainly distinguished on the basis of hierarchical clusterings, mode of transmission and host range size). The discovery of monopartite plant picorna-like viruses with three CP subunits resulted in the creation of a distinct family, the family *Sequiviridae* which currently includes two genera (*Sequivirus* and *Waikavirus*, distinguished by their genomic organization and their mode of transmission) (Le Gall et al, 2005b). The molecular characterization of a number of new viruses as well as viruses previously considered as members of the genus *Nepovirus*, revealed a surprising degree of diversity among plant picorna-like viruses. These viruses were bipartite and encoded either two or three CP subunits, distinguishing them from the nepoviruses. In phylogenetic studies, they were only distantly related to members of the families *Comoviridae* or *Sequiviridae*. Two new genera were created which are currently unassigned (Le Gall et al, 2007). The genus *Cheravirus* includes viruses with three CPs that branch together in phylogenetic studies (Le Gall et al, 2005c). The genus *Sadwavirus* includes viruses with two CPs that generally branch together in phylogenetic studies (Le Gall et al, 2005d). However, this classification is far from being perfect as some viruses are still difficult to assign to existing genera/families (Le Gall et al, 2007). For example, Strawberry latent ringspot virus (SLRSV), a founder member of the nepovirus “group” is currently considered a tentative sadwavirus because it encodes two CP subunits, but it groups with cheraviruses in phylogenetic studies and has other characteristics typical of cheraviruses (see Annexes, Fig. 1-3 and Table). Recently, the characterization of Tomato torrado virus (ToTV) also suggested that it does not belong to any of the currently accepted genera and that a new genus should be created for this virus (Verbeek et al, 2007; see also proposal on the creation of the genus *Torradovirus*).

Although the level of diversity in the genomic organization of plant picorna-like viruses is apparent, these viruses share many common features (see Annexes, Fig. 3 and Table 1). The newly created order *Picornavirales* was recently ratified by the ICTV membership (Le Gall et al, 2008). The plant picorna-like viruses discussed in this proposal have all been included in the order *Picornavirales* and share many common properties with other members of this order. They all have small spherical particles (25-30 nm). They have similar particle symmetries (icosahedral with a pseudo T=3 symmetry) and the coat protein(s) structure is similar with three jelly-roll that can all be present in one large CP, or divided among two or three smaller CPs. They all have a single-stranded (+) strand genome that encodes one or two large polyproteins which are cleaved by a 3C-like proteinase. The replication block is conserved and includes a type III helicase, a 3C-like proteinase and a type I RNA-dependent RNA-polymerase in that order.

In addition to the characteristics in common with other members of the order *Picornavirales* mentioned above, the viruses discussed in this proposal share other common characteristics that differentiate them from other members of the order *Picornavirales*. They infect plants and use specialized protein(s) or protein domain(s) to potentiate cell-to-cell and long distance movement within the plant host. Members of the family *Comoviridae*, and several definite or tentative members of the genera *Cheravirus* and *Sadwavirus* have been shown to induce similar cytopathological effects in their plant hosts (proliferation of membrane vesicles which are associated with viral replication and formation of tubular structures traversing the plant cell wall that contain virus-like particles and potentiate cell-to-cell movement of the virus). The viral movement protein was shown to be a structural component of these tubular structures. The mechanism of cell-to-cell movement has not yet been elucidated for other viruses (*Sequiviridae* and *Torradovirus*). A putative movement protein was identified in torradoviruses, but has no homology with the *Comoviridae* movement protein, except for a very small motif. It is not known whether these viruses induce tubular structures to potentiate their movement. One sequivirus (PYFV) was reported to induce tubules, but the origin of these tubules has not been clarified and it is not known whether they are required for cell-to-cell movement of the virus. A movement protein has not (yet) been identified in the genome of *Sequiviridae*.

Phylogenetic studies using the conserved domains situated between the “CG” motif of the 3C-proteinase and the “GDD” motif of the polymerase revealed that all plant picorna-like viruses group as a single branch when compared to other members of the order *Picornavirales*. Although the level of diversity within this branch is large, it is in the same range as that currently accepted for the family *Picornaviridae* (see Annex, Fig 4). Considering the above, we propose to create a new family, termed “*Secoviridae*” to include the genera, *Comovirus*, *Fabavirus*, *Nepovirus*, *Sequivirus*, *Waikavirus*, *Cheravirus* and

Sadwavirus. We hope that this proposal will more clearly define the taxonomical status of currently unassigned plant picorna-like genera and viruses.

In a separate proposal, we also suggest to create the genus "*Torradovirus*" with the type species ToTV and to include this genus in the family "*Secoviridae*". We also considered the tight grouping of the currently recognized family *Comoviridae* (see Fig. 1) and felt that this should also still be recognized at the taxonomical level. One possibility was to create a sub-order *Secoviridae* to be included in a future order *Picornavirales* and keep the existing family *Comoviridae* and genera within that sub-order. The other possibility is to create the sub-family "*Comovirinae*" within the family "*Secoviridae*". Considering the level of diversity observed in other families, such as *Picornaviridae*, (see Fig. 4) we feel that all plant picorna-like viruses would probably better be included in a single family with one suggested sub-family and possibly more to be created.

Below are the defining characteristics of the proposed family "*Secoviridae*":

Common properties of viruses within the family *Secoviridae*:

1. Infect plants using specialized protein(s) or domain(s), so called movement protein, and/or capsid protein(s) adapted for this function
2. Common particle structure (icosahedral with pseudo T=3)
3. Positive-strand RNA with polyprotein expression strategy
4. Common replication block including type III helicase, 3C-like cysteine proteinase, type I polymerase
5. Clustering as a single branch in Pro-Pol dendrogram, when compared to picorna-like viruses infecting other kingdoms

Examples of criteria defining genera within the family *Secoviridae*:

1. Number of RNAs
2. Number of protein domains and/or processing sites
2. Number of CPs
3. Presence of additional ORFs and/or subgenomic RNAs
4. Clustering as a single branch in Pro-Pol dendrogram when compared to other genera of the family *Secoviridae*
5. Characteristics of protein(s) or protein domain(s) involved in transport through the plant.

The criteria used to define genera within the proposed family will continue to be considered by the Study Group. At this time, we propose that some but maybe not all criteria may need to be met to define a genus. For example, members of the genus *Nepovirus* vary in the number of processing sites (subgroup C nepoviruses have an additional protein domain on RNA-2), suggesting that this genus could be further divided. However, clustering in the Pro-Pol dendrogram does not support the current nepovirus subgroups (see Annexes). The possibility that the genus *Nepovirus* is split in several genera will be carefully considered by the Study Group in the coming year(s). Similarly, the genera *Sadwavirus* and *Cheravirus* will need to be further considered. The genus *Cheravirus* is well supported by the number of CPs, genomic organization and clustering in the Pro-Pol dendrogram [with the inclusion of *Stocky prune virus* (StPV) as a definite species, proposal recently ratified by the ICTV membership]. However, the genus *Sadwavirus* may need to be further defined. The genus is currently defined by the presence of two CPs. SLRSV is currently a tentative member of the genus because it encodes 2 CPs. However, it groups with cheraviruses in the Pro-Pol dendrogram and has a genomic organization which is similar to that of cheraviruses (with the exception of the number of CPs, see Annexes). The study group will therefore need to carefully consider the position of SLRSV in relation to the two genera and may need to redefine the criteria for the two genera. Similarly, the taxonomic position of Strawberry mottle virus and Black raspberry necrosis virus within the family *Secoviridae* will need to be redefined once more information is available regarding the genomic organization of RNA2 and the number of CPs (Table 1). For the time being, we suggest that they should be considered tentative members of the genus *Sadwavirus*, based on their grouping in the Pro-Pol dendrogram (Fig. 2).

Finally, although the proposed sub-family *Comovirinae* is well supported by the Pro-Pol dendrogram, we felt that more information is required (as additional sequences become available) before other sub-families can be suggested. For example, although the genera *Sequivirus* and *Waikavirus* share some common characteristics (number of RNAs and number of CPs), they also differ in other characteristics (presence of a polyA tail or not, presence of sub-genomic RNAs or not) and they do not branch together in the Pro-Pol dendrogram to the same level as that seen for the proposed sub-family *Comovirinae* (see

Annexes). The creation of other sub-families within the family *Secoviridae* will be considered by the Study Group in the coming years along with the redefinition of some of the genera.

Origin of the proposed family name

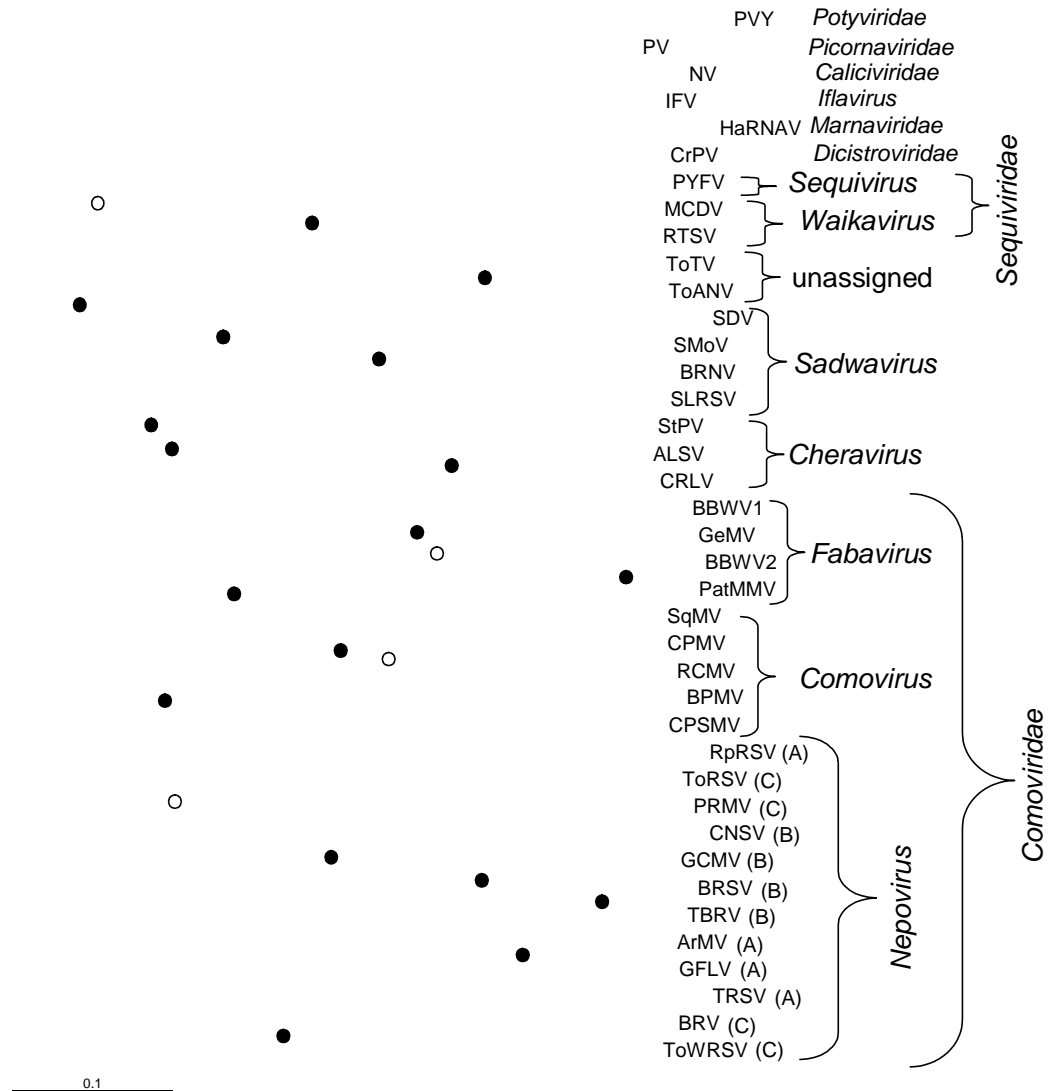
Secoviridae to reflect the amalgamation of the families *Sequiviridae* and *Comoviridae*

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Annexes:

Fig. 1. Phylogenetic tree using the Pro-Pol sequence showing the currently accepted taxonomy of plant picorna-like viruses.



Hierarchical clustering of plant picorna-like viruses based on the Pro-Pol sequence (region between the CG motif within the proteinase and the GDD motif with the polymerase). The families and genera are delineated on the right. The letter in parenthesis after the nepovirus acronyms refer to the subgroup in which they have been classified (A, B or C). Circles indicate nodes supported by bootstrap values above 80% (closed circles) or 60% (open circles); nodes without circles are not supported to these levels. The bar represents a p-distance of 0.1. The amino-acid sequence clustering is based on the region between the Pro (CG) and the Pol (GDD) sequence motifs. The GenBank accession numbers for each virus were as follows: infectious flacherie virus (IFV, AB000906), cricket paralysis virus (CrPV, AF318039), poliovirus (PV, V01149), Norwalk virus (NV, M87661), Heterosigma akashiwo RNA virus (HaRNV, AY337486), potato virus Y (PVY, X12456), maize chlorotic dwarf virus (MCDV, U67839), parsnip yellow fleck virus (PYFV, D14066), rice tungro spherical virus (RTSV, M95497), gentian mosaic virus (GeMV, BAD99001), broad bean wilt virus 1 (BBWV1, AB084450), broad bean wilt virus 2 (BBWV2, AF225953), patchouli mild mosaic virus, an isolate of BBWV2 (PatMMV, AB050782), squash mosaic virus (SqMV, AB054688), bean poddle mosaic virus (BPMV, U70866), red clover mosaic virus (RCMV, X64886), cowpea severe mosaic virus (CPSMV, M83830), cowpea mosaic virus (CPMV, X00206), tomato torrado virus (ToTV, DQ388879), tomato apex necrosis virus (ToANV, EF063641), stocky prune mosaic virus (StPV, AAZ76594), black raspberry mosaic virus (BRNV, DQ344639), strawberry mottle virus (SMoV, AJ311875), strawberry latent ringspot virus (SLRSV, AY860978), satsuma dwarf virus (SDV, AB009958), apple latent spherical virus (ALSV, AB030940), cherry rasp leaf virus (CRLV, AJ621357), tomato white ringspot virus (ToWRSV which is probably an isolate of artichoke ringspot virus, ABM65096), peach rosette mosaic virus (PRMV, AAB69867), blackcurrant reversion virus (BRV, AF3682772), tomato ringspot virus (ToRSV, L19655), tomato black ring virus (TBRV, AY157993), grapevine chrome mosaic virus (GCMV, X15346), cycas necrosis stunt virus (CNSV, AB073147), beet ringspot virus (BRSV, D00322), tobacco ringspot virus (TRSV, U50869), raspberry ringspot virus (RpRSV, AY303787), arabis mosaic virus (ArMV, AY303786), grapevine fanleaf virus (GFLV, D00915)

Fig. 2 Phylogenetic tree using the Pro-Pol sequence showing the proposed new taxonomy for plant picorna-like viruses

Hierarchical clustering was conducted using the alignments described in Fig 1

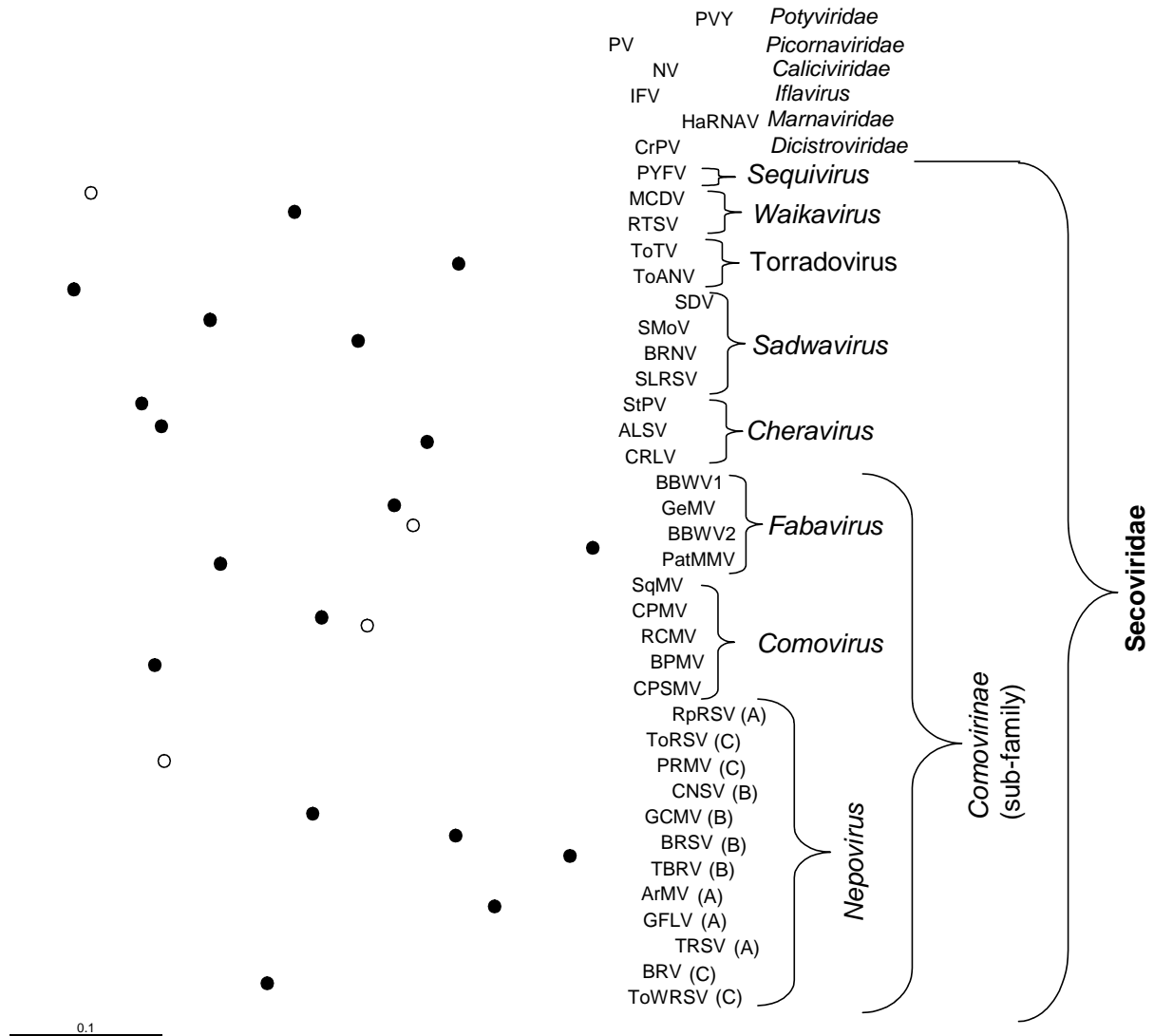
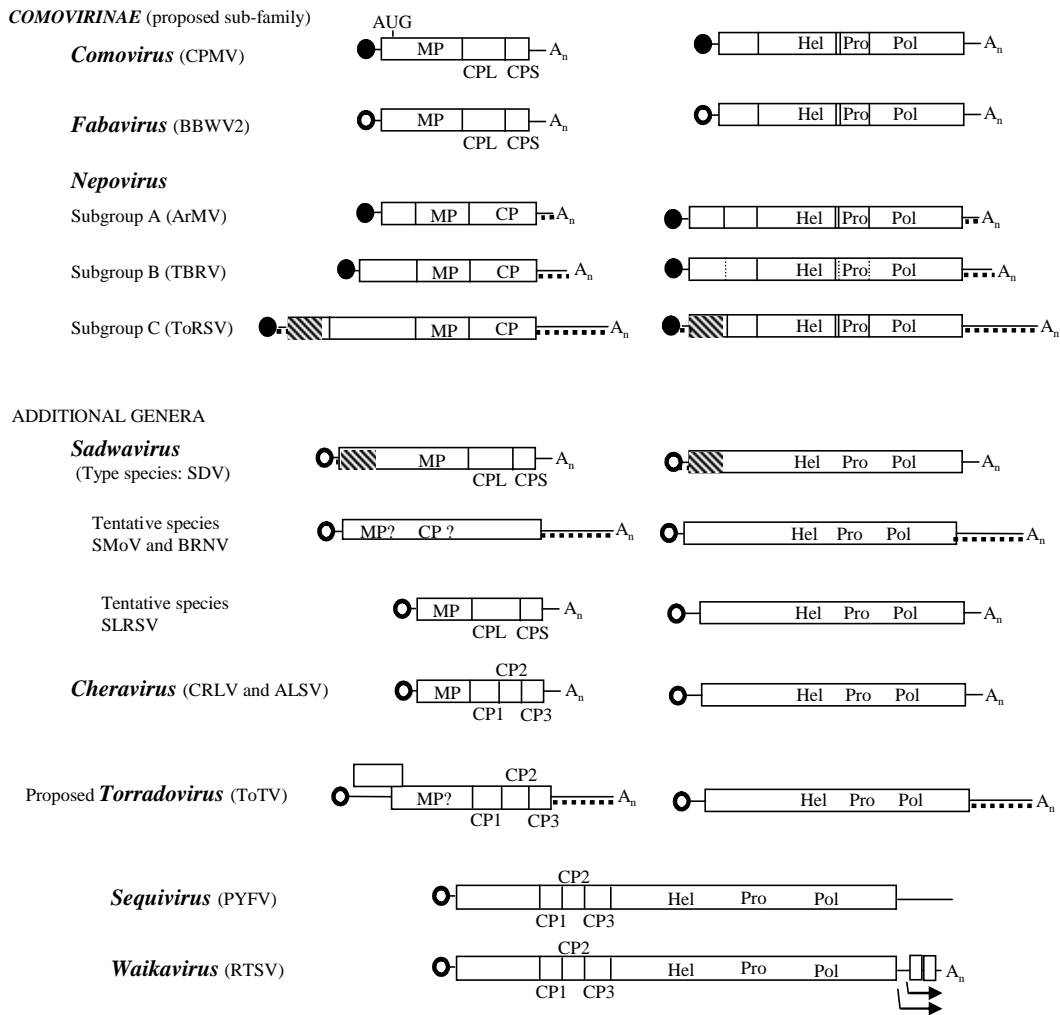


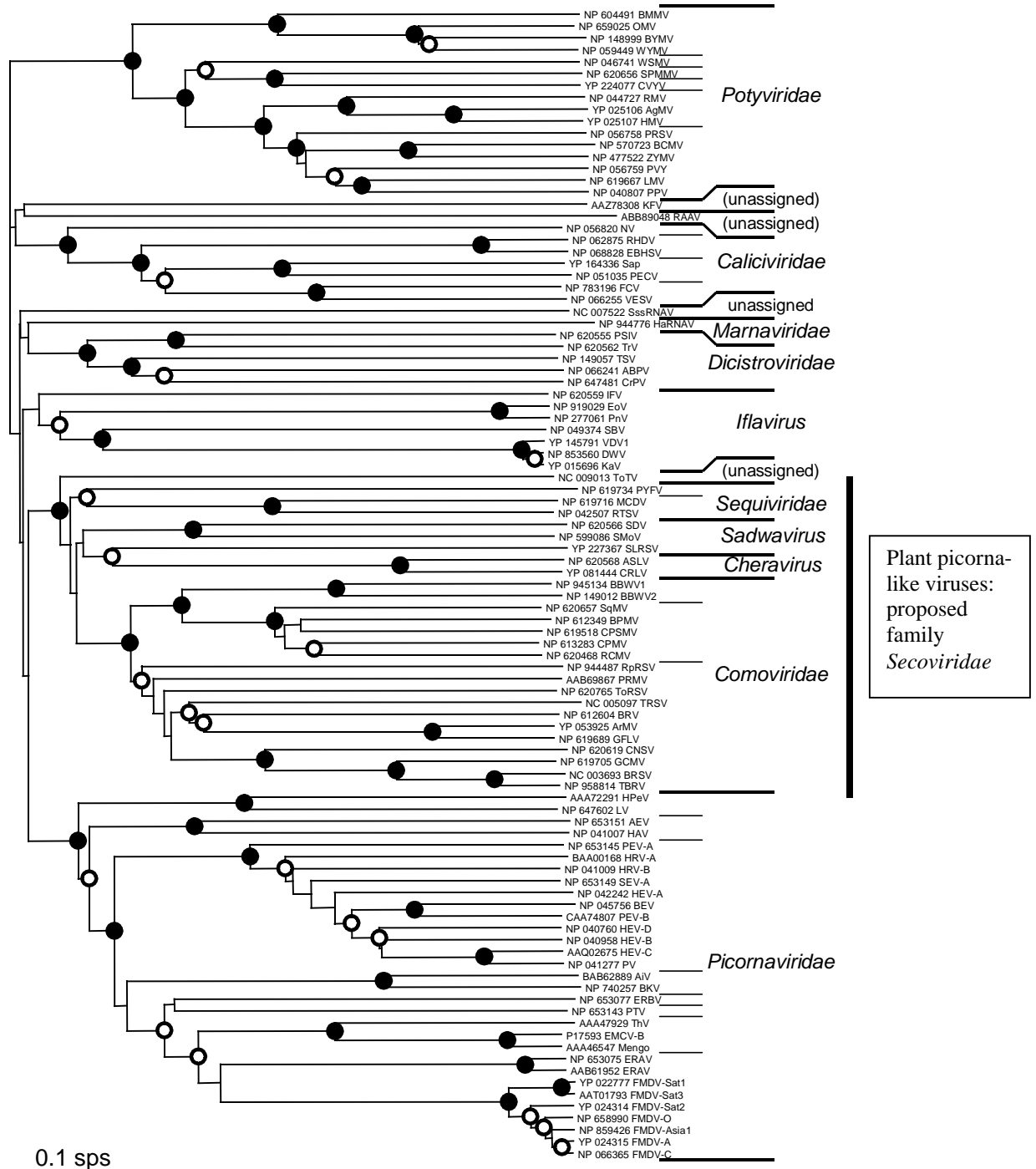
Fig. 3. Genomic organization of plant picorna-like viruses: the proposed members for the family *Secoviridae*.



Each RNA is shown with a horizontal line with the boxes representing the open reading frames. The presence of a Poly A tail (A_n) and confirmed (solid circle) or putative (open circle) presence of a VPg proteins are also indicated. Identified cleavage sites within the polyproteins are shown by the vertical bars. Conserved domains for the replication block are Hel: type III helicase, Pro: 3C-like proteinase and Pol: type I RNA-dependent RNA polymerase. Other conserved domains include the CP: coat proteins and MP: movement protein. The arrows represent identified sub-genomic RNAs. The hatched regions represent areas of the polyproteins that have extensive sequence similarity between the two RNAs. The dotted lines represent conserved regions shared by the 3'UTR of both RNAs within a virus. The AUG denotes an alternative start codon in the *como/faba* RNA2.

Fig. 4. Hierarchical clustering of proposed member of the order *Picornavirales* and of members of the families *Potyviridae* and *Caliciviridae*.

An alignment of the sequences between the CG proteinase motif and the GDD polymerase motif has been used to produce the dendrogram. Filled circle: node supported by a bootstrap value higher than 95%, empty circles: node supported by a bootstrap value higher than 66%. The database accession numbers are indicated alongside the name of each virus. Please note that the degree of diversity within the proposed family *Secoviridae* is similar to that observed within the family *Picornaviridae*



Summary of plant picorna-like viruses properties

Genus or Species	RNA	Size of RNA2 poly-protein	Large 3' UTR?	Proteinase substrate binding pocket and specificity	CP	vector	Special features
sequivirus	1	NA	yes	Leu (cleaves N, S or Q at -1 position)	3	Aphid (requires helper virus)	No Poly(A) tail at the 3' end of RNA
waikavirus	1	NA	yes	His (Q at -1)	3	Aphid or leafhopper	Two C-terminal subgenomic RNAs. Two small ORFs at 3' end of genomic RNA
comovirus	2	130 K	no	His (Q at -1)	2	beetle	
fabavirus	2	130 K	no	His (Q at -1)	2	aphid	
Nepovirus (subgroup A)	2	135 K	no	Leu (R, C, A or G at -1)	1	Nematodes Xiphinema Longidorus	3' end of RNA1 and RNA2 share sequence identity over 150 nts
Nepovirus (subgroup B)	2	160 K	no	Leu (K or R at -1)	1	Nematodes Longidorus	3' ends of RNA1 and 2 share sequence identity over 300 nts
Nepovirus (subgroup C)	2	190 K	yes	His (Q, N or D at -1)	1	Nematode Xiphinema or Mite (BRV)	extra protein domain in RNA2 polyprotein; RNA1 and 2 share sequence identity over 1000 nts at 5' and 3' ends
Sadwavirus (SDV)	2	174 K	no	Cys (To or A at -1)	2	Maybe nematode	
SMoV and BRNV (tentative sadwavirus)	2	160 K	yes	His (Q or E at -1)	?	aphid	Number of CPs unknown, possible additional protein domain at C-terminus of RNA2 polyprotein
SLRSV (tentative sadwavirus)	2	100 K	no	His (S at -1)	2	nematode	
Cheravirus (CRLV, ALSV)	2	100-110 K	no	His (Q or E at -1)	3	nematode	
StPV (proposed cheravirus)	2	?	no	Gly ? (cleavage sites unknown)	3	Maybe nematode	
ToTV (proposed Torradovirus)	2	134 K	yes	His (cleavage sites unknown)	3	Maybe whitefly	Extra partially overlapping ORF at 5' end of RNA2