

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

Code<sup>†</sup>  To create a new unassigned genus

Code<sup>†</sup>  To name the new genus\*

Code<sup>†</sup>  To create and designate the species  
As the type species of the new genus\*

Code<sup>†</sup>  To create the following as additional species of the new genus\*:

*Tomato marchitez virus*

<sup>†</sup> Assigned by ICTV officers

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

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## Old Taxonomic Order

Order  
Family  
Genus  
Type Species  
Species in the Genus  
Unassigned Species in the Genus  
Unassigned Species in the family

## New Taxonomic Order

Order *Picornavirales*  
Family *Secoviridae* (proposed new family, see 2007 proposal)  
Genus *Torradovirus*  
Type Species *Tomato torrado virus*  
Species in the Genus *Tomato marchitez virus*

## ICTV-EC comments and response of the SG

## Argumentation to choose the type species in the genus

Tomato torrado virus (ToTV) was the first virus for which the entire sequence is known. The 3 CPs have been characterized and the nature of the CPs has been confirmed experimentally by mass spectrometry analysis (Verbeek, 2007a). The sequence of Tomato marchitez virus (ToMarV) was recently elucidated (Verbeek, 2007b). Three CPs have been detected but have not been sequenced. A third virus, Tomato apex necrosis virus has only been partially sequenced (Turina, 2007).

## Species demarcation criteria in the genus

Type of biological vector

Host range

Absence of serological cross-reaction

Absence of cross-protection

Sequence similarity: Less than 75% amino-acid sequence identity in the CPs within a species

Less than 75% amino-acid sequence identity in the proteinase-polymerase region (by analogy with criteria established with other known genera within the proposed family *Secoviridae*. These criteria may need to be adjusted as more viruses become characterized).

Tomato marchitez virus (ToMarV) has 78% a.a. sequence identity with Tomato torrado virus (ToTV) in the Pro-Pol region. The two viruses share 75% a.a. sequence identity when all three CPs are considered together (72% for the first CP, 86% for the second CP and 71% for the third CP). Using the above criteria, this would suggest that the two viruses should be considered distinct species.

The genome of tomato apex necrosis virus (ToANV) has been partially sequenced. The amino acid sequence of the Pro-Pol region shares 99% identity with that of ToMarV. The amino acid sequences of the CPS share 93% identity between ToMarV and ToANV. This would suggest that the two viruses should be considered isolates of the same species.

## List of Species in the created genus

*Tomato torrado virus*

*Tomato marchitez virus*

## Argumentation to create a new genus:

Plant members of the order *Picornavirales* (Le Gall, 2008) although sharing common characteristics such as particle structure, polyprotein strategy, a common replication block, are also remarkably diverse. The plant picornavirales Study Group is proposing to group these viruses in a single family, termed *Secoviridae* and to further divide this family in sub-families and genera to reflect their diversity (see *Secoviridae* proposal for a complete discussion of the common characteristics and diversity of plant picorna-like viruses). Plant members of the order *Picornavirales* include the genera *Nepovirus*, *Comovirus* and *Fabavirus*, currently members of the family *Comoviridae* (Le Gall et al, 2005a) and now proposed members of the sub-family *Comovirinae*, the genera *Sequivirus* and *Waikavirus*, currently members of the family *Sequiviridae* (Le Gall et al, 2005b) and the genera *Cheravirus* and *Sadwavirus* (Le Gall et al, 2005c and 2005d). These genera are distinguished by the number of RNAs, the genomic organization, the number of coat proteins (CPs) and hierarchical clusterings (see Annexes).

Tomato torrado virus (ToTV) has been recently characterized. The entire nucleotide sequence has been determined (DQ388879-80) allowing deducing the genomic organization (Verbeek et al, 2007). ToTV was isolated from diseased tomato plants in Spain. Transmission of the virus by greenhouse whitefly (*Trialeurodes vaporariorum*) has been reported (Pospieszny et al, 2007). The virus particles are icosahedral with a diameter of approximately 28 nm. The genome consists of two molecules of positive-strand RNA that are polyadenylated. Sequence comparisons revealed the domains for a type III helicase, a 3C-like proteinase and a type I RNA-dependent RNA polymerase, expressed from RNA1 using a polyprotein strategy. RNA2 also encodes a large polyprotein containing the domains for the CPs and a putative movement protein. The presence of the 3 CPs has been confirmed by SDS-polyacrylamide gel analysis of purified virus particles. Mass spectrometry analysis of the 3 CPs confirmed the position of the CP domains in the RNA2-encoded polyprotein. These features confirm that ToTV is a plant picorna-like virus. The bipartite genome and the 3 CPs are properties shared by members of the genus *Cheravirus*. However, other properties are unique to ToTV. In particular, a second partially overlapping reading frame was detected on RNA2. This is a novel feature for this group of viruses (see Fig. 3). In addition, phylogenetic studies using the region between the proteinase “CG” motif and the polymerase “GDD” motif or a conserved region of the helicase domain revealed that ToTV branches separately from members of the genera *Cheravirus*, *Sadwavirus*, and the current families *Sequiviridae* or *Comoviridae* (Verbeek et al, 2007 and Fig. 1-2 in Annexes). Based on this information, it is proposed to create a new genus, which we termed *Torradovirus*, and to assign ToTV, as the type species. Recently, the complete sequence of a second virus, Tomato marchitez virus (ToMarV) was characterized (EF681764/5; Verbeek, 2008). In addition, the partial sequence of another virus, Tomato apex necrosis virus (ToANV), was recently deposited in the database (EF063641/2; Turina, 2007). ToMarV and ToANV are closely related to each other, suggesting that they should be considered isolates of the same species (Verbeek, 2008 and see discussion above on species demarcation criteria). ToMarV shared many common properties with ToTV, including the presence of three CPs and of an additional open reading frame on RNA2. ToMarV is related to ToTV in phylogenetic studies (Fig. 2), suggesting that it should be included in the proposed genus *Torradovirus*. The sequence identity at the amino acid level between ToMarV and ToTV is 65% for the entire RNA1-encoded polyprotein and 78% in the Pro-Pol region. In the RNA2-encoded proteins, the first open reading frame share 63% sequence identity (at the a.a. level) between ToMarV and ToTV, while the second open reading frame share 66% sequence identity. The a.a. sequence identity for the three CPs combined is 75%. Based on criteria currently accepted to distinguish species in other genera of plant *Picornavirales* (corresponding to the proposed family *Secoviridae*), this suggests that ToMarV is likely a distinct species and that ToANV should be considered an isolate of ToMarV. In support of this suggestion, it should also be noted that the size of the two ToMarV RNAs differs from that of the two ToTV RNAs. In fact the length of the 3’NTRs is 628 and 655 nts for the ToMarV RNAs and 1210 and 1092 nts for the ToTV RNAs. Extensive sequence identity is found between the 3’ UTRs of RNA1 and 2 of each virus (90-91%), but not between the 3’ UTRs of ToTV and ToMarV (less than 50%).

## Origin of the proposed genus name

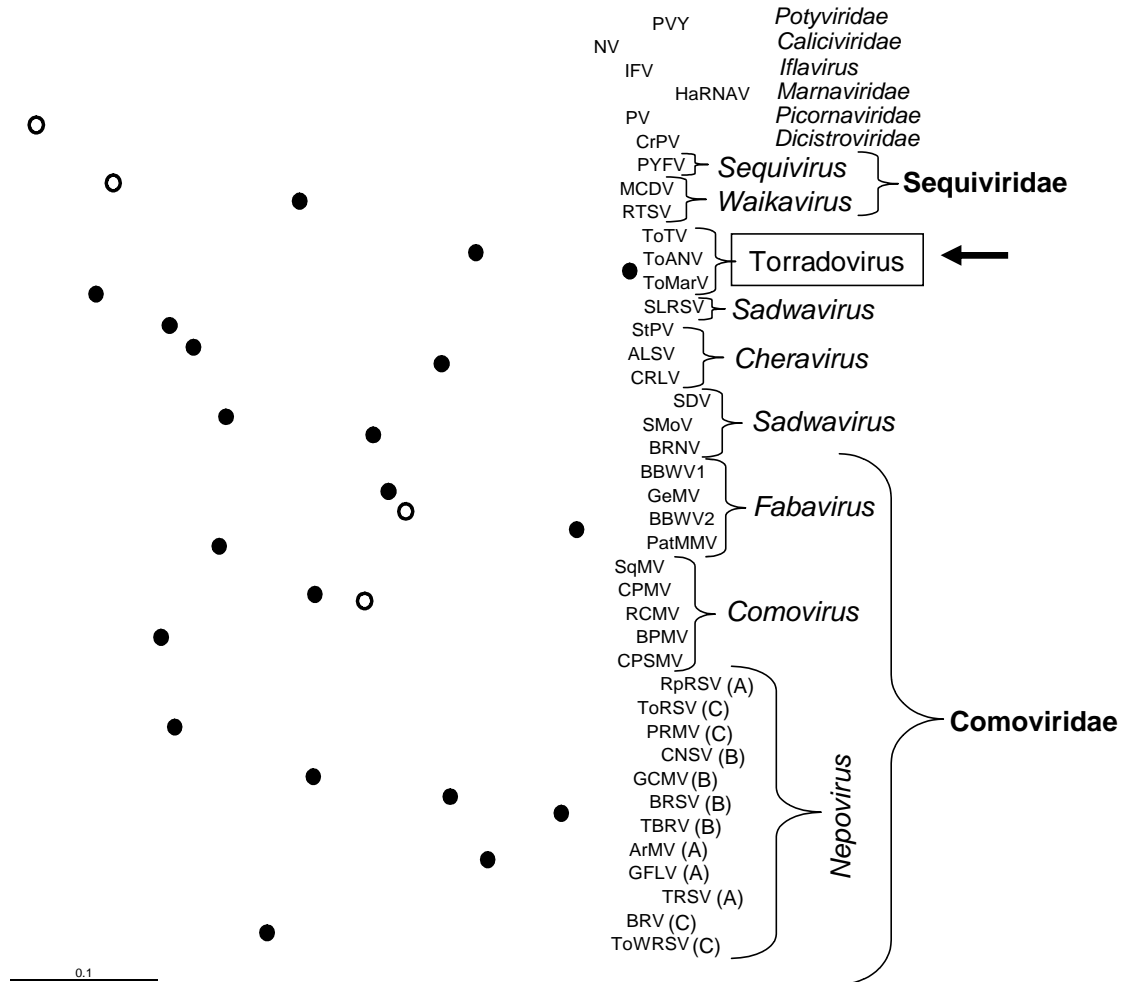
*Torradovirus* is derived from Tomato torrado virus. In Spanish, “torrado” means “toasted” to refer to the severe necrosis (burnt-like phenotype) observed in the disease induced by ToTV.

## References

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## Annexes:

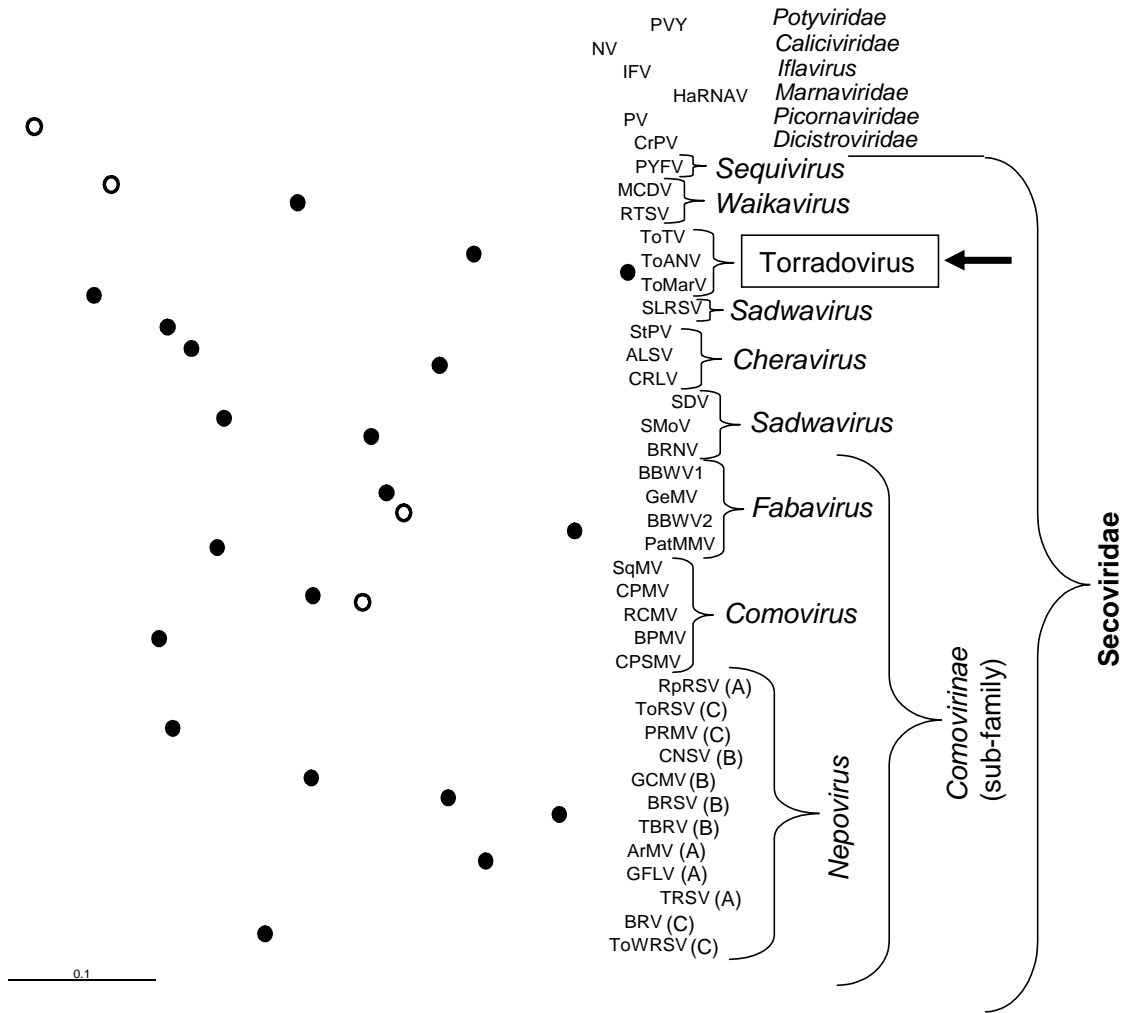
**Fig. 1. Phylogenetic tree showing the currently accepted taxonomy of plant picorna-like viruses. ToTV, ToMarV, and ToANV branch separately from other plant picorna-like viruses and are currently unassigned.**



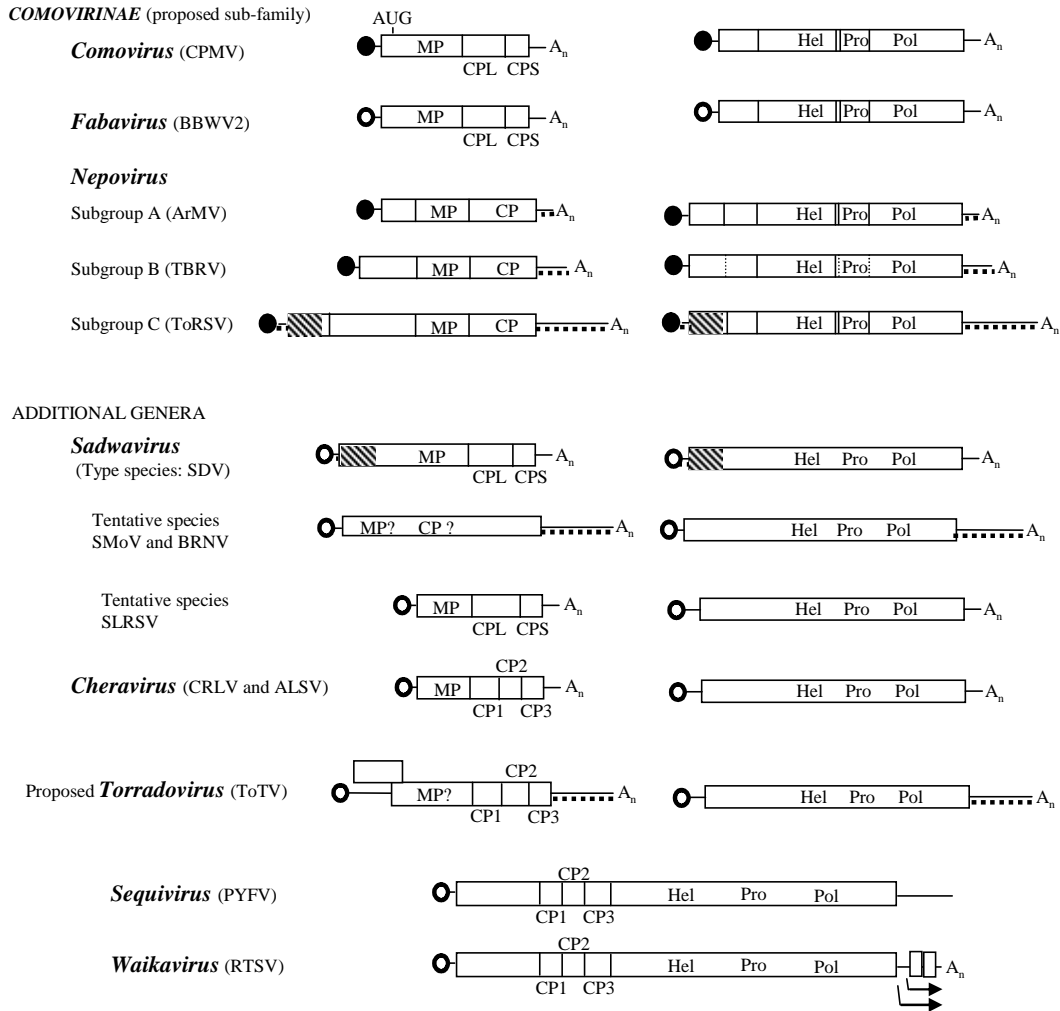
Hierarchical clustering of plant picorna-like viruses based on the Pro-Pol sequence (region between the CG motif within the proteinase and the GDD motif with the polymerase). The families and genera are delineated on the right. The letter in parenthesis after the nepovirus acronyms refer to the subgroup 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 (HaRNAV, 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 (CPMSV, M83830), cowpea mosaic virus (CPMV, X00206), tomato torrado virus (ToTV, DQ388879), tomato marchitez virus (ToMarV, EF681764), tomato apex necrosis virus (ToANV, EF063641), stocky prune mosaic virus (StPV, AAZ76594), black raspberry necrosis 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 showing the proposed new taxonomy for plant picorna-like viruses

The position of the proposed genus *Torradovirus* is shown in comparison to other members of the proposed family *Secoviridae*. 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*.** The proposed genus *Torradovirus* distinguishes itself by the presence of an additional overlapping reading frame upstream of the RNA-2 encoded large polyprotein, this property is unique among plant picorna-like viruses.



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.

## 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