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:	***2015.011a-adP***	(to be completed by ICTV officers)

Short title: Revision of family *Betaflexiviridae*, order *Tymovirales*
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 10 are required)					
	1 ☒	**2** ☒	**3** ☒	**4** ☒	**5** ☐
	6 ☐	**7** ☒	**8** ☐	**9** ☐	**10** ☒

Author(s):

Mike Adams & Jan Kreuze on behalf of the *Flexiviridae* SG

Corresponding author with e-mail address:

Jan Kreuze, j.kreuze@cgiar.org

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)	*Flexiviridae* SG

ICTV Study Group comments (if any) and response of the proposer:

Date first submitted to ICTV:	June 2015
Date of this revision (if different to above):	July 28, 2015

ICTV-EC comments and response of the proposer:

EC comments: A scale needs to be added to the phylogenetic trees on Fig. 5 and Fig. 6. Wording was corrected on the top of p.2 (open reading frame encoding protein).

Response: Done.

SUMMARY and KEY

The family *Betaflexiviridae* contains plant viruses with a monopartite ssRNA+ polyadenylated genome (6.5-9.5 kb) and flexuous, filamentous virions. All have a short 5'-UTR followed by an open reading frame encoding a large (185-250 kDa) replication protein with conserved methyltranferase, helicase and RdRp domains. There are currently 7 genera distinguished mainly by the phylogeny of the replicase and the number and arrangement of other genes as shown in the diagram below. There are also 9 species currently unassigned in the family and several other candidate species.

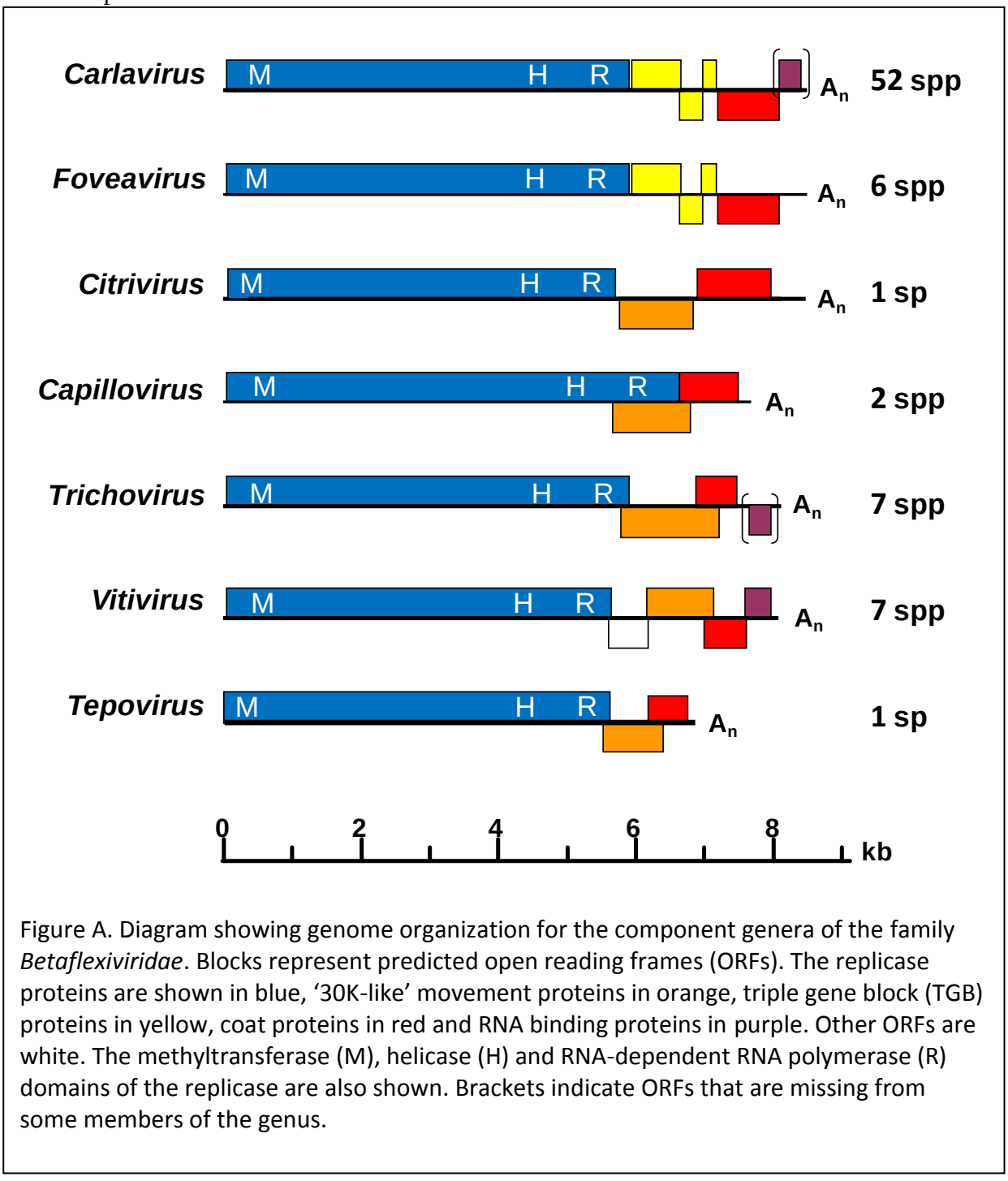


Figure A. Diagram showing genome organization for the component genera of the family *Betaflexiviridae*. Blocks represent predicted open reading frames (ORFs). The replicase proteins are shown in blue, '30K-like' movement proteins in orange, triple gene block (TGB) proteins in yellow, coat proteins in red and RNA binding proteins in purple. Other ORFs are white. The methyltransferase (M), helicase (H) and RNA-dependent RNA polymerase (R) domains of the replicase are also shown. Brackets indicate ORFs that are missing from some members of the genus.

In phylogenetic analyses of the replicase proteins, there are two major branches within the family and these correlate with the type of movement protein ('30K-like' or TGB). **We propose to use these distinctions to create two subfamilies and to create 4 new genera to accommodate most of the species currently unassigned in the family together with several new species. We also propose removing some species from the genus *Carlavirus*.**

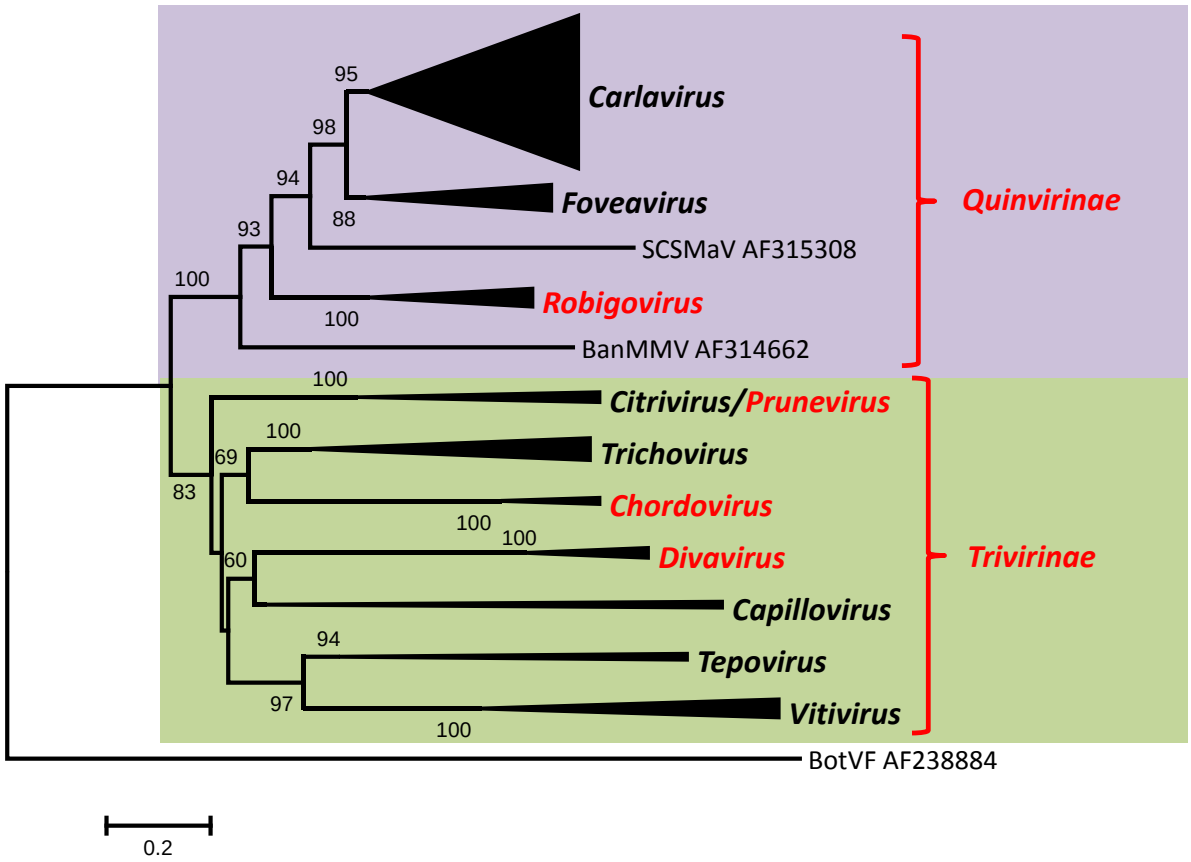


Figure B. Phylogenetic tree of the replicase amino acid sequences of members of the family *Betaflexiviridae* with Botrytis virus F (genus *Mycoflexivirus*, family *Gammaflexiviridae*) as the outgroup. Neighbor-Joining Tree (JTT substitution model) with 1000 bootstrap replicates produced in MEGA6. Percentage bootstrap support is shown at all branches if >60%. All existing and proposed new species are represented but the genus branches have been collapsed for this summary tree. The proposed new genera and subfamilies are shown in red. *Banana mild mosaic virus* (BanMMV) and *Sugarcane streak mosaic-associated virus* (SCSMaV) remain as unassigned species.

The proposed names of the subfamilies reflect the number of conserved genes that all members have in common. Members of the *Trivirinae* have 3 (replicase, 30K movement protein, coat protein) and members of the *Quinvirinae* have 5 (replicase, 3 TGB proteins, coat protein).

Species and demarcation criteria in the family

Throughout the family *Betaflexiviridae*, different species are expected to have less than about 72% nucleotide identity (or 80% amino acid identity of encoded proteins) in the CP or replicase genes. Viruses from different genera usually have less than about 45% nucleotide identity in these genes (Adams et al., 2012).

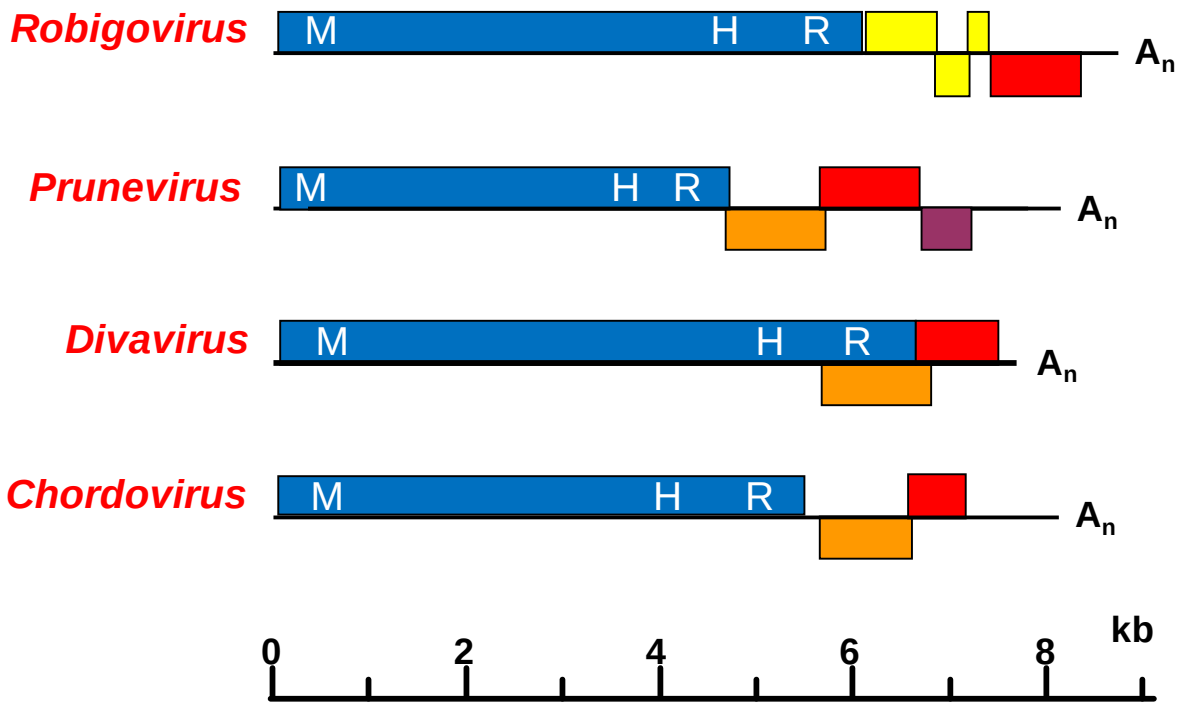


Figure C. Diagram showing genome organization for the proposed new genera of the family *Betaflexiviridae*. Blocks represent predicted open reading frames (ORFs). The replicase proteins are shown in blue, '30K-like' movement proteins in orange, triple gene block (TGB) proteins in yellow, coat proteins in red and RNA binding proteins in purple. The methyltransferase (M), helicase (H) and RNA-dependent RNA polymerase (R) domains of the replicase are also shown.

Revised structure of family (new taxa in red) and key to proposal modules:

Genus	New genus/ Subfamily module	Species change	Species module	No. of species
Subfamily: *Trivirinae*	4A			
Capillovirus		(none)		2
Chordovirus	3A	2 new species	2A	2
Citrivirus		(none)		1
Divavirus	3B	3 species moved from Unassigned	7A	3
Prunevirus	3C	2 new species	2B	2
Tepovirus		1 new species	2C	2
Trichovirus		(none)		7
Vitivirus		(none)		7
Subfamily: *Quinvirinae*	4B			
Carlavirus		2 new species; 7 species abolished	2D 7D	47
Foveavirus		(none)		6
Robigovirus	3D	2 new species; 3 species moved from Unassigned	2E 7B	5
Unassigned		3 species moved from Unassigned in family	7C	3

MODULE 2A: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

Code	***2015.011aP***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Chordovirus (new)***
Subfamily:	***Trivirinae (new)***
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

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 genus is specified, enter "**unassigned**" in the genus box.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Carrot Ch virus 1	CBV-1_S20	KF533711
Carrot Ch virus 2	CBV-2_S15	KF533710

Reasons to justify the creation and assignment of the new species:

- 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.
- Further material in support of this proposal may be presented in the Appendix, Module 9

Betaflexivirus sequences were obtained from carrots by high throughput sequencing of samples that also contained a number of other carrot viruses. The assembled sequences suggested the presence of two distinct viruses with a similar size (~8.5 kb) and genome organization, consisting of 3 ORFs: replicase, movement protein and coat protein (Adams et al., 2014). This is somewhat similar to trichoviruses but with no overlapping of the replicase and MP genes (compare Figs A and C). The two sequences had about 55% aa identity to one another in their replicase proteins, suggesting that they represented distinct species. In sequence comparisons and phylogenetic analyses, the two sequences clearly belonged within the *Betaflexiviridae* but as a distinct clade (replicase proteins <30% identical to other members of the family).

The assignment of the two species together in a new genus in the proposed subfamily is well supported by phylogenetic analyses of the replicase (Annex Fig. 1) and movement proteins (Annex Fig. 2). The coat proteins of viruses in the *Trivirinae* are fairly small and variable and do not always group well along genus lines in phylogenetic analyses but those of CChV-1 and CChV-2 clearly belong together (Annex Fig. 3), supporting the grouping of these two viruses in the same genus.

MODULE 2B: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

Code	***2015.011bP***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Prunevirus (new)***
Subfamily:	***Trivirinae (new)***
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

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 genus is specified, enter "**unassigned**" in the genus box.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Apricot vein clearing associated virus *Caucasus prunus virus*	VC Aze204	HG008921 KM507061

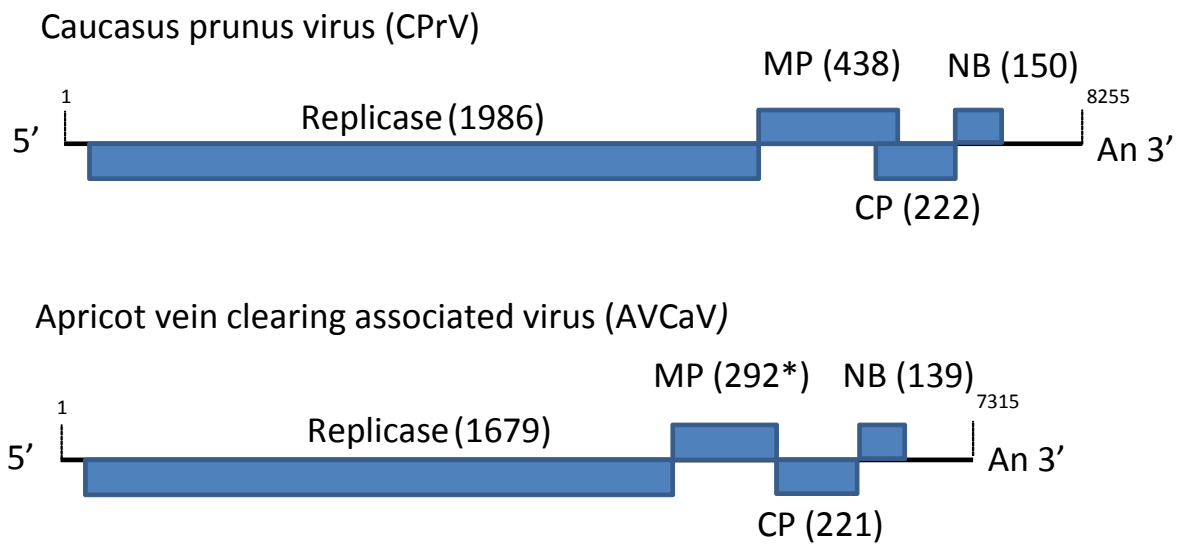
Reasons to justify the creation and assignment of the new species:

- 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.
- Further material in support of this proposal may be presented in the Appendix, Module 9

Analysis by deep-sequencing technology of double-stranded RNA recovered from an apricot tree with vein clearing symptoms allowed the identification of a novel virus with a single-stranded RNA genome, for which the provisional name *Apricot vein clearing-associated virus* (AVCaV) has been proposed (Elbeaino et al., 2014).

Characterization of new *Prunus*-infecting viruses has provided information about a previously uncharacterized virus in *Prunus amygdalus* (almond) originating from Azerbaijan (Marais et al., 2015b). The complete genome sequence of an isolate has been determined and phylogenetic relationships of the new agent with members of family *Betaflexiviridae* analyzed. Caucasus prunus virus (CPrV) is proposed as a name for this new viral agent.

Genome structure (see below and compare Figs A and C) and phylogenetic analyses place AVCaV and CPrV in the family *Betaflexiviridae* (presence of a 30K-type movement protein, clear phylogenetic affinities for the replicase, the coat protein, the whole genome). They are most closely related to Citrus leaf blotch virus (CLBV; genus *Citrivirus*) in the replicase (Annex Fig. 1) and movement protein (Annex Fig. 2) but CLBV has a very different coat protein (related to members of the proposed subfamily *Quinvirinae*: see Annex Fig. 3) and has only three genes. In contrast, like members of genera *Carlavirus* and *Vitivirus*, as well as *Cherry mottle leaf virus* and *Peach mosaic virus* (ChMLV & PcMV, *Trichovirus*), AVCaV and CPrV contain a fourth open reading frame (ORF) encoding a putative nucleic acid binding protein (NB).



Schematic representation of the genome organization of Caucasus prunus virus and of Apricot vein clearing associated virus. The size (in amino acids) of the encoded proteins is indicated between brackets. MP, Movement protein; CP, Coat protein; NB, Nucleic acid binding protein; An, polyA stretch. *, further sequences of this virus have a MP similar in size to CPrV (Marais et al., 2015b)

Molecular comparisons of AVCaV and CPrV with other sequenced *Betaflexiviridae* members showed that whatever the ORF considered, only distant relationships and identity levels are observed. The closest affinity was found between AVCaV and CPrV which show 47.1-68.3% nucleotide identity (36.5-73% amino acid identity) for the four genes. Nevertheless, the levels of identity between the replicase (44% amino acid identity) and the CP (36.5% amino acid identity) are clearly outside the species demarcation criteria of 80%:

Percentage of identity between proteins encoded by the genome of the Caucasus prunus virus isolate Aze204 and the corresponding proteins of representative members of the family *Betaflexiviridae* with a 30K-type movement protein. The nucleic acid identities between corresponding genes are indicated between brackets.

	Replicase	MP	CP	NB
AVCaV HG008921	44 (51.6)	42.1 (50.2)	36.5 (47.1)	73 (68.3)
GVA NC 003604 (*Vitivirus*)	27 (40.1)	9.2 (30.2)	24.9 (38.3)	11.1 (34.4)
ChMLV NC 002500 (*Trichovirus*)	26.4 (40.9)	13.2 (33.4)	30.4 (41.1)	13.6 (32.4)
ASGV NC 001749 (*Capillovirus*)	26.5 (40.2)	13.5 (30.9)	25.9 (36.7)	na
CLBV NC 003877 (*Citrivirus*)	40.7 (49)	53 (56.7)	11.2 (30.4)	na
PVT NC 011062 (*Tepovirus*)	29.2 (42.9)	13.4 (29.9)	21.6 (38.7)	na
ACLSV NC 001409 (*Trichovirus*)	26.8 (40.8)	10.2 (31.1)	24.6 (39.1)	na

na, not applicable. Abbreviations followed by the accession numbers are: AVCaV, *Apricot vein clearing associated virus*; CLBV, *Citrus leaf blotch virus*; ASGV, *Apple stem grooving virus*; ACLSV, *Apple chlorotic leaf spot virus*; ChMLV, *Cherry mottle leaf virus*; PVT, *Potato virus T*; GVA, *Grapevine virus A*. The genus to which particular viruses belong is indicated between brackets.

MODULE 2C: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

Code	***2015.011cP***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Tepovirus***
Subfamily:	***Trivirinae (new)***
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

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 genus is specified, enter "**unassigned**" in the genus box.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Prunus virus T	C21	KF700262

Reasons to justify the creation and assignment of the new species:

- 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.
- Further material in support of this proposal may be presented in the Appendix, Module 9

Double-stranded RNAs purified from a cherry tree collected in Italy and a plum tree collected in Azerbaijan were submitted to deep sequencing. Contigs showing weak but significant identity with various members of the family *Betaflexiviridae* were reconstructed. Sequence comparisons led to the conclusion that the viral isolates identified in the analyzed Prunus plants belong to the same viral species. The two complete genome sequences were determined (KF700262 Italian isolate C21; KF700263 Azerbaijan isolate Aze239; Marais et al., 2015a). Genomes had three overlapping open reading frames (RNA polymerase 206 kDa, movement protein 43 kDa, and capsid protein 25 kDa with aa identities between the isolates of 79%, 78 and 89% respectively). Phylogenetic analyses of the deduced encoded proteins showed a significant, but distant, clustering with the sole member of the genus *Tepovirus*, Potato virus T (PVT), which has a similar genome organization. The assignment of the two species together in a new genus in the proposed subfamily is well supported by phylogenetic analyses of the replicase (Annex Fig. 1) and movement proteins (Annex Fig. 2). The two viruses are only distantly related (about 45% nt identity over the entire genome with aa identities for the three ORFs of 30, 20 and 27% respectively – although there are conserved regions with higher values. The name *Prunus virus T* (PrVT) has been proposed for what is clearly a distinct species. A reverse-transcription polymerase chain reaction detection assay was developed and allowed the identification of two other PrVT isolates and an estimate of 1% prevalence in the large Prunus collection screened.

MODULE 2D: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

Code	***2015.011dP***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Carlavirus***
Subfamily:	***Quinvirinae (new)***
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

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 genus is specified, enter "**unassigned**" in the genus box.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Gaillardia latent virus	5/18-05-2010	KJ415259
Potato virus H	Huhhot	HM584819

Reasons to justify the creation and assignment of the new species:

- 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.
- Further material in support of this proposal may be presented in the Appendix, Module 9

According to the ICTV 9th Report:

Each distinct species in the genus *Carlavirus* usually has a specific natural host range. Distinct species do not cross-protect in infected common host plant species. Distinct species are usually readily differentiated by serological procedures. Distinct species have less than about 72% nt identity (or 80% aa identity) between their CP or replicase genes.

The following viruses meet the criteria to justify the creation of new species, which is supported by phylogenetic analysis of the proteome (Annex Fig. 4).

Gaillardia latent virus (GalLV)

During investigation of *Gaillardia aristata* breeding material several plants reacted strongly with an antiserum to Chrysanthemum virus B (CVB), a member of the genus *Carlavirus*. In order to confirm the identity of the virus, a part of its replicase gene was sequenced, showing just 26% amino acid sequence identity to CVB. The entire genome of this virus isolate (8659 nt excluding the polyA tail) was then determined by PCR and conventional sequencing (KJ415259; Menzel et al., 2014). The complete genome was 8659 nt in length (excluding poly-A tail) and contained six open reading frames. The genome organisation resembled that of typical carlaviruses. The replicase (70%) and CP (71%) showed the highest aa sequence identities to Phlox virus S (PhVS), being well below the species demarcation threshold of 80%.

Potato virus H (PVH)
The virus was obtained from potatoes showing mild virus symptoms and was sequenced from RT-PCR products. Virus particles typical of a carlavirus were observed. In inoculation experiments, PVH produced mild symptoms in *Nicotiana glutinosa*, *Solanum lycopersicum*, and potato, but did not infect *Chenopodium amaranticolor*, *C. quinoa*, *Tetragonia expansa*, *Nicotiana tabacum* var. Xanthi-nc or *N. benthamiana*. Most PVH-infected plants remained symptomless or latent. The complete genome sequences of two isolates were determined (Huhot isolate, HM584819, 8417 nt and YN isolate, JQ904630, 8410 nt; Li et al, 2013). They have the predicted ORFs expected for a member of the genus *Carlavirus*. There is <48% aa identity to other carlaviruses in the replicase and <57% aa identity in the CP.

MODULE 2E: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

Code	***2015.011eP***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Robigovirus (new)***
Subfamily:	***Quinvirinae (new)***
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

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 genus is specified, enter "**unassigned**" in the genus box.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Cherry rusty mottle associated virus	95CI192R3	KC218926
Cherry twisted leaf associated virus	95CI205R1 (s1)	KF030846

Reasons to justify the creation and assignment of the new species:

- 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.
- Further material in support of this proposal may be presented in the Appendix, Module 9

Four betaflexiviruses infecting cherry have been described and there are several complete genomes of each of them. These are discussed in detail by Villamor et al., 2015. Two have already been classified and they proposed that *Cherry green ring mottle virus* and *Cherry necrotic rusty mottle virus* (two species currently unassigned in the family *Betaflexiviridae*) should be classified in a new genus, together with two new species.

A total of 24 complete genome sequences of the four viruses were analysed in detail. They have identical genome organisation (replicase, TGB and coat protein) and fall into 4 distinct clades that correspond with distinct symptoms in cherry and woody indicator hosts (Villamor & Eastwell, 2013). The only genus in the family in which the viruses have a similar genome organization is *Foveavirus*, but the proteins of those viruses have <40% aa identity to their homologues in the four cherry viruses. Moreover, phylogenetic analyses of the replicase genes of viruses in the family do not justify placing the cherry viruses in genus *Foveavirus* (see Annex Fig 5).

Amino acid and nucleotide identities within and between the four cherry viruses are shown in Annex Table 1. While these are clearly closely-related viruses, as a general rule, they satisfy the molecular criteria used in the family that different species should have less than about 72% nt identity (or 80% aa identity) between their respective CP or replicase genes.

Phylogenetic analyses of the entire proteome (Annex Fig 6) or the replicase or CP amino acid sequences (see Villamor et al., 2015) all show four distinct clades corresponding to the four viruses.

The two new species proposed here are:

Cherry rusty mottle associated virus

Isolate	Accession number	Reference
95CI192R3	KC218926	Villamor et al., 2013
B48-C	KC218927	Villamor et al., 2013
98CI73R1 (s1)	KF030849	Villamor & Eastwell, 2013
98CI194 (s1)	KF030850	Villamor & Eastwell, 2013
8099-5 (s1)	KF030869	Villamor & Eastwell, 2013
8241-2 (s1)	KF030870	Villamor & Eastwell, 2013
8804	KF356396	Villamor et al., 2014

Cherry twisted leaf associated virus

Isolate	Accession number	Reference
95CI205R1 (s1)	KF030846	Villamor & Eastwell, 2013
95CI206 (s1)	KF030848	Villamor & Eastwell, 2013
101-13 (s2)	KF030859	Villamor & Eastwell, 2013
103-15 (s3)	KF030865	Villamor & Eastwell, 2013
8242-3 (s1)	KF030873	Villamor & Eastwell, 2013
8265 (s3)	KF030878	Villamor & Eastwell, 2013
C3 (s1)	KF030880	Villamor & Eastwell, 2013
CTLV_8431	KF958838	James et al., 2014

MODULE 3A: **NEW GENUS**

creating a new genus

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

Code	***2015.011fP***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	***Trivirinae (new)***
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

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

naming a new genus

Code	***2015.011gP***	(assigned by ICTV officers)

To name the new genus: *Chordovirus*

Assigning the type species and other species to a new genus

Code	***2015.011hP***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Carrot Ch virus 1	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:**

2

Reasons to justify the creation of a new genus:

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

See module 2A and phylogenetic trees (Annex Figs 1-3).

Origin of the new genus name:

'Chord' as the root of the name is the name of the strings on a harp or other stringed instrument. This is a reference to the (presumed) thread-like virions.

Reasons to justify the choice of type species:

First sequenced.

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.

As in other genera of the family, distinct species have less than about 72% nt identity (or 80% aa identity) between their CP or polymerase genes.

MODULE 3B: **NEW GENUS**

creating a new genus

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

Code	***2015.011iP***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	***Trivirinae (new)***
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

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

naming a new genus

Code	***2015.011jP***	(assigned by ICTV officers)

To name the new genus: *Divavirus*

Assigning the type species and other species to a new genus

Code	***2015.011kP***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Diuris virus A	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:**

3

Reasons to justify the creation of a new genus:

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

It is proposed to create this genus for three existing species that are currently unassigned in the family *Betaflexiviridae.*

Hardenbergia virus A was created by proposal 2012.020aP on the basis of results reported by Wylie & Jones, 2011.

Diuris virus A and *Diuris virus B* were created by proposal 2013.002aP based on Wylie et al., 2013.

The three viruses have a similar genome organization to those in the genus *Capillovirus*, with the coat protein at the C-terminus of the replication protein. However, phylogenetic analyses of the replicase (Annex Fig. 1), movement protein (Annex Fig. 2) and coat protein (Annex Fig. 3) show that the three viruses cannot be classified with capilloviruses. As they are clearly related to one another, it seems appropriate to place them in a separate genus.

Origin of the new genus name:

From the type species ***Di****uris* ***v****irus* ***A***

Reasons to justify the choice of type species:

This is mostly an arbitrary choice between very similar viruses, but there is a slightly greater level of information about the two Diuris viruses.

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.

As in other genera of the family, distinct species have less than about 72% nt identity (or 80% aa identity) between their CP or polymerase genes.

MODULE 3C: **NEW GENUS**

creating a new genus

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

Code	***2015.011lP***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	***Trivirinae (new)***
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

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

naming a new genus

Code	***2015.011mP***	(assigned by ICTV officers)

To name the new genus: *Prunevirus*

Assigning the type species and other species to a new genus

Code	***2015.011nP***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Apricot vein clearing associated virus

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:**

2

Reasons to justify the creation of a new genus:

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

See Module 2B and phylogenetic trees (Annex Figs 1-3).

Apricot vein clearing-associated virus (AVCaV) (Elbeaino et al., 2014) and Caucasus prunus virus (CPrV) (Marais et al., 2015b) have a similar genomic organization encoding four proteins: the viral replicase, a 30K-type movement protein, the coat protein, and a nucleic acid binding protein. The sizes of the encoded proteins are similar (the comparison of three AVCaV isolates reveals that the MP of the reference AVCaV isolate is shorter due to a frameshift mutation which is absent from the other two isolates sequenced; Marais et al., 2015b). The two viruses cluster together in phylogenetic analyses of all the viral proteins. No *Betaflexiviridae* member has this kind of genomic organization, except *Cherry mottle leaf virus* (ChMV) and *Peach mosaic virus* (PcMV), which belong to the genus *Trichovirus* (James et al., 2000; 2006) and with which AVCaV and CPrV have no strong phylogenetic affinities.

As discussed in Module 2B, there are affinities with Citrus leaf blotch virus (CLBV, type member of the genus *Citrivirus*; Vives et al., 2001) in the replicase and the movement protein. However, the genetic distances between the replicases, the differences in genome organization and the very

different coat proteins (about 30% nt or 11% aa identity), that is more similar to those of the TGB containing *Quinvirinae* (see Annex Fig. 3) suggest that the viruses should not be placed in the same genus.

Origin of the new genus name:

Both viral species defining the genus *Prunevirus* (AVCaV and CPrV) were found to infect *Prunus* species (Apricot and almond, respectively) (Elbeinao et al., 2014; Marais et al., 2015b). Moreover, six additional AVCaV isolates have been characterized (Marais et al., 2015b), all of them infecting various *Prunus* species (*Prunus salicina*, *Prunus mume*, *Prunus domestica*, *Prunus persica*, and *Prunus armeniaca*). The name Prunevirus refers to the infected host (*Prunus*, Proune in Ancient Greek) and the viral origin of the infecting agent.

Reasons to justify the choice of type species:

AVCaV is the better characterized virus. In addition to the Italian isolate initially described by Elbeinao et al. (2014), two French AVCaV isolates from *Prunus salicina* have been fully sequenced (Marais et al., 2015b; Accession numbers KM507062 and KM507063), a peach isolate from Iran has been partially sequenced (KM507070 and three further isolates have been detected in *Prunus* species in China. In contrast, the Aze204 isolate is so far the only member of CPrV identified to date.

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.

Members of the genus *Prunevirus* should have the same genomic organization (gene number and gene order). As for other genera in the family *Betaflexiviridae*, we proposed that isolates of different species should have less than about 72% nucleotide identity (or 80% amino acid identity) between their respective CP or replicase genes.

MODULE 3D: **NEW GENUS**

creating a new genus

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

Code	***2015.011oP***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	***Quinvirinae (new)***
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

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

naming a new genus

Code	***2015.011pP***	(assigned by ICTV officers)

To name the new genus: *Robigovirus*

Assigning the type species and other species to a new genus

Code	***2015.011qP***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Cherry necrotic rusty mottle virus

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:**

5

Reasons to justify the creation of a new genus:

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

See module 2E.

Cherry green ring mottle virus and *Cherry necrotic rusty mottle virus* (two species currently unassigned in the family *Betaflexiviridae*) and the two new species proposed in module 2E (*Cherry rusty mottle associated virus* and *Cherry twisted leaf associated virus*) are clearly closely related and cannot be classified within any of the existing genera (Annex Figs 5 and 6).

As shown by the phylogenetic trees, the species *African oil palm ringspot virus* (currently unassigned in the family *Betaflexiviridae*) is a strong candidate for inclusion in the genus. Villamor et al., 2015 suggest that it should be excluded because an AlkB-like domain is present in the replicases of CTLaV, CNRMV, CRMaV, and CGRMV, but is absent in AOPRV. However, this domain is not a good marker for genus differentiation (as witnessed by its presence or absence within members of other genera in the family).

Origin of the new genus name:

From the Latin “robigo”, meaning rust, for the rusty symptoms associated with some of the viruses, including *Cherry necrotic rusty mottle virus*, the proposed type species

Reasons to justify the choice of type species:

Best characterized virus

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.

- Distinctive symptoms on indicator hosts
- As in other genera of the family, isolates of different species have less than about 72% nt identity (or 80% aa identity) between their CP or polymerase genes

MODULE 4A: **NEW SUBFAMILY**

creating a new subfamily

A subfamily can only be created within a family.

Code	***2015.011rP***	(assigned by ICTV officers)

To create a new subfamily within:

Family:	***Betaflexiviridae***
Order:	***Tymovirales***

If the family has yet to be created (in Module 5) please write "**(new)**" after the proposed name.

- If there is no Order, write "**unassigned**" here.

naming a new subfamily

Code	***2015.011sP***	(assigned by ICTV officers)

To name the new subfamily: *Trivirinae*

genera and species assigned to the new subfamily

Code	***2015.011tP***	(assigned by ICTV officers)

To assign the following genera to the new subfamily:

You may list several genera here. For each genus, please state whether it is new or existing.

- 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

Capillovirus (existing)
Citrivirus (existing)
Tepovirus (existing)
Trichovirus (existing)
Vitivirus (existing)
Chordovirus (new)
Divavirus (new)
Prunevirus (new)

The new subfamily 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 unassigned species that the subfamily will contain (those NOT within any of the genera listed above):**

0

Reasons to justify the creation of the new subfamily:

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

See the summary page 3 and Figure B there. The subfamily *Trivirinae* is proposed to contain those viruses with a 30K-like movement protein. In phylogenetic analyses of the replicase proteins, these form one of the two major branches within the family.

Origin of the new subfamily name:

Tri- signifies the three conserved genes that all members of the subfamily share (replicase, movement protein, coat protein).

MODULE 4B: **NEW SUBFAMILY**

creating a new subfamily

A subfamily can only be created within a family.

Code	***2015.011uP***	(assigned by ICTV officers)

To create a new subfamily within:

Family:	***Betaflexiviridae***
Order:	***Tymovirales***

If the family has yet to be created (in Module 5) please write "**(new)**" after the proposed name.

- If there is no Order, write "**unassigned**" here.

naming a new subfamily

Code	***2015.011vP***	(assigned by ICTV officers)

To name the new subfamily: *Quinvirinae*

genera and species assigned to the new subfamily

Code	***2015.011wP***	(assigned by ICTV officers)

To assign the following genera to the new subfamily:

You may list several genera here. For each genus, please state whether it is new or existing.

- 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

Carlavirus (existing)
Foveavirus (existing)
Robigovirus (new)

The new subfamily 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 unassigned species that the subfamily will contain (those NOT within any of the genera listed above):**

2

Reasons to justify the creation of the new subfamily:

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

See the summary page 3 and Figure B there. The subfamily *Quinvirinae* is proposed to contain those viruses with a Triple Gene Block associated with virus cell-to-cell movement. In phylogenetic analyses of the replicase proteins, these form one of the two major branches within the family.

Origin of the new subfamily name:

Quin- signifies the five conserved genes that all members of the subfamily share (replicase, Triple Gene Block, coat protein).

MODULE 7A: **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	***2015.011xP***	(assigned by ICTV officers)

To remove the following taxon (or taxa) from their present position:

Diuris virus A
Diuris virus B
Hardenbergia virus A

The present taxonomic position of these taxon/taxa:

Genus:	***Unassigned***
Subfamily:	***Unassigned***
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

Fill in all that apply.

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

To be classified within a new genus (see below)

Part (b) re-assign to a higher taxon

Code	***2015.011yP***	(assigned by ICTV officers)

To re-assign the taxon (or taxa) listed in Part (a) as follows:

Genus:	***Divavirus (new)***
Subfamily:	***Trivirinae (new)***
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

Fill in all that apply.
- 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.

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

Creation of a new genus. See module 3B.

MODULE 7B: **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	***2015.011zP***	(assigned by ICTV officers)

To remove the following taxon (or taxa) from their present position:

African oil palm ringspot virus
Cherry green ring mottle virus
Cherry necrotic rusty mottle virus

The present taxonomic position of these taxon/taxa:

Genus:	***Unassigned***
Subfamily:	***Unassigned***
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

Fill in all that apply.

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

To be classified within a new genus (see below)

Part (b) re-assign to a higher taxon

Code	***2015.011aaP***	(assigned by ICTV officers)

To re-assign the taxon (or taxa) listed in Part (a) as follows:

Genus:	***Robigovirus (new)***
Subfamily:	***Quinvirinae (new)***
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

Fill in all that apply.
- 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.

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

Creation of a new genus. See modules 2E and 3D.

MODULE 7C: **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	***2015.011abP***	(assigned by ICTV officers)

To remove the following taxon (or taxa) from their present position:

Banana mild mosaic virus
Banana virus X
Sugarcane striate mosaic-associated virus

The present taxonomic position of these taxon/taxa:

Genus:	***Unassigned***
Subfamily:	***Unassigned***
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

Fill in all that apply.

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

To be classified within a new subfamily (see below)

Part (b) re-assign to a higher taxon

Code	***2015.011acP***	(assigned by ICTV officers)

To re-assign the taxon (or taxa) listed in Part (a) as follows:

Genus:	***Unassigned***
Subfamily:	***Quinvirinae (new)***
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

Fill in all that apply.
- 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.

Reasons to justify the re-assignment:

Creation of a new subfamily (see module 4B). The three viruses represented have the characteristic TGB proteins and in analyses clearly belong in this subfamily. The only sequence of Banana virus X is incomplete and it does not therefore appear in the trees presented here.

MODULE 7D: **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	***2015.011adP***	(assigned by ICTV officers)

To remove the following taxon (or taxa) from their present position:

Dandelion latent virus
Elderberry symptomless virus
Honeysuckle latent virus
Hydrangea latent virus
Lilac mottle virus
Mulberry latent virus
Muskmelon vein necrosis virus

The present taxonomic position of these taxon/taxa:

Genus:	***Carlavirus***
Subfamily:	
Family:	***Betaflexiviridae***
Order:	***Tymovirales***

Fill in all that apply.

If the taxon/taxa are to be abolished (i.e. not reassigned to another taxon) write "yes" in the box on the right	**YES**

Reasons to justify the removal:
Explain why the taxon (or taxa) should be removed

These species have been included in the Master Species List for many years but there are no sequences (even genome fragments) and most were created on the basis of a single research report 30-40 years ago. Genus assignment was done on the basis of particle morphology and sometimes serological affinities. There has been no published work on any of these viruses for over 30 years and the viruses are not represented in any culture collection. In the absence of authentic material associated with the viruses it seems inappropriate to retain the species on the ICTV list. Names will of course remain in the historical records and the Study Group will include them in lists of viruses that are possible members of the genus.

In particular, the species *Hydrangea chlorotic mottle virus* was created in 2012 (proposal 2012.020bP) on the basis of a study and genome sequences determined by Caballero et al. (2009) and Tang et al. (2010). Both sets of authors acknowledge that it is impossible to know whether their virus differs from *Hydrangea latent virus* (described by Allen et al., 1985).

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Adams I.P., Skelton A., Macarthur R., Hodges T., Hinds H., Flint L., Nath P.D., Boonham N., Fox A (2014). Carrot yellow leaf virus Is Associated with Carrot Internal Necrosis. PLoS One 9(11):E109125-E109125.

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. 2012. Family Betaflexiviridae. Pages 920-941 in : Virus Taxonomy – Ninth Report on the International Committee on Taxonomy of Viruses. A. M. Q. King, M. J. Adams, E. B. Carstens, and E.J. Lefkowitz, eds. Elsevier Academic Press, London.

Allen TC, McMorran JP, Lawson RH (1985) Detection and identification of viruses in Hydrangea. Acta Hortic 164:85–89

Elbeaino, T., Giampetruzzi, A., De Stradis, A., Digiaro, M. 2014. Deep sequencing analysis of an apricot tree with vein clearing symptoms reveals the presence of a novel betaflexivirus. Virus Research, 181, 1-5.

James, D., Jelkmann, W., Upton, C. 2000. Nucleotide sequence and genome organisation of Cherry mottle leaf virus and its relationship to members of the Trichovirus genus. Archives of Virology, 145, 995-1007.

James, D., Varga, A., Croft, H., Rast, H., Thompson, D., Hayes, S. 2006. Molecular characterization, phylogenetic relationships, and specific detection of Peach mosaic virus. Phytopathology, 96, 137-144.

James D., Varga A., Lye D. (2014) Analysis of the complete genome of a virus associated with twisted leaf disease of cherry reveals evidence of a close relationship to unassigned viruses in the family *Betaflexiviridae*. Arch Virol 159:2463–2468

Li Y-Y, Zhang R-N, Xiang H-Y, Abouelnasr H, Li D-W, et al. (2013) Discovery and Characterization of a Novel Carlavirus Infecting Potatoes in China. PLoS ONE 8(6): e69255. doi: 10.1371/journal.pone.0069255

Machado Caballero, J. E., Lockhart, B. E., Mason, S. L., and Daughtrey, M. 2009. Identification and properties of a carlavirus causing chlorotic mottle of florists' hydrangea (*H. macrophylla*) in the United States. Plant Dis. 93:891-895.

Marais A., Faure C., Mustafayev E., Barone M., Alioto D., Candresse T (2015a). Characterization by deep sequencing of Prunus virus T, a novel Tepovirus infecting *Prunus* species. Phytopathology 105:135-140 http://dx.doi.org/10.1094/PHYTO-04-14-0125-R

Marais, A., Faure, C., Mustafayev, E., Candresse T. 2015b. Characterization of new isolates of Apricot vein clearing-associated virus and of a new Prunus-infecting virus: evidence for recombination as a driving force in *Betaflexiviridae* evolution. PLoS One, in press.

Menzel W., Hamacher J., Winter S. (2014). Characterization of a New Carlavirus from Gaillardia aristata. Acta Hortic. 1072 :129-133.

Tang J, SHarper SJ, Wei T, Clover GRG (2010). Characterization of hydrangea chlorotic mottle virus, a new member of the genus *Carlavirus* Arch. Virol. 155(1):7-12

Villamor D.E., Eastwell K.C. (2013). Viruses associated with rusty mottle and twisted leaf diseases of sweet cherry are distinct species. Phytopathology 103(12):1287-1295

Villamor D.E.V., Susaimuthu, J., Eastwell K.C (2015). Genomic Analyses of Cherry Rusty Mottle Group and Cherry Twisted Leaf-Associated Viruses Reveal a Possible New Genus Within the Family Betaflexiviridae. Phytopathology 105(3): 399-408

Villamor D.E.V., Ward K.F., Collman S.J., Eastwell K.C. (2014). First Report of Infection of

References:

Cherry Rusty Mottle Associated Virus in Portuguese Laurel (*Prunus lusitanica*) in Washington State. Plant Dis. 98(5):699-699.

Villamor D.V., Druffel K.L., Eastwell K.C. (2013). Complete nucleotide sequence of a virus associated with rusty mottle disease of sweet cherry (*Prunus avium*). Arch. Virol. 158(8):1805-1810.

Vives, MC., Galipienso, L., Navarro, L., Moreno, P., 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.

Wylie S, Jones M. (2011). Hardenbergia virus A, a novel member of the family *Betaflexiviridae* from a wild legume in Southwest Australia Arch. Virol. 156(7):1245-1250

Wylie SJ, Li H, Dixon KW, Richards H, Jones MG (2013). Exotic and indigenous viruses infect wild populations and captive collections of temperate terrestrial orchids (Diuris species) in Australia. Virus Res. 171(1):22-32.

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.

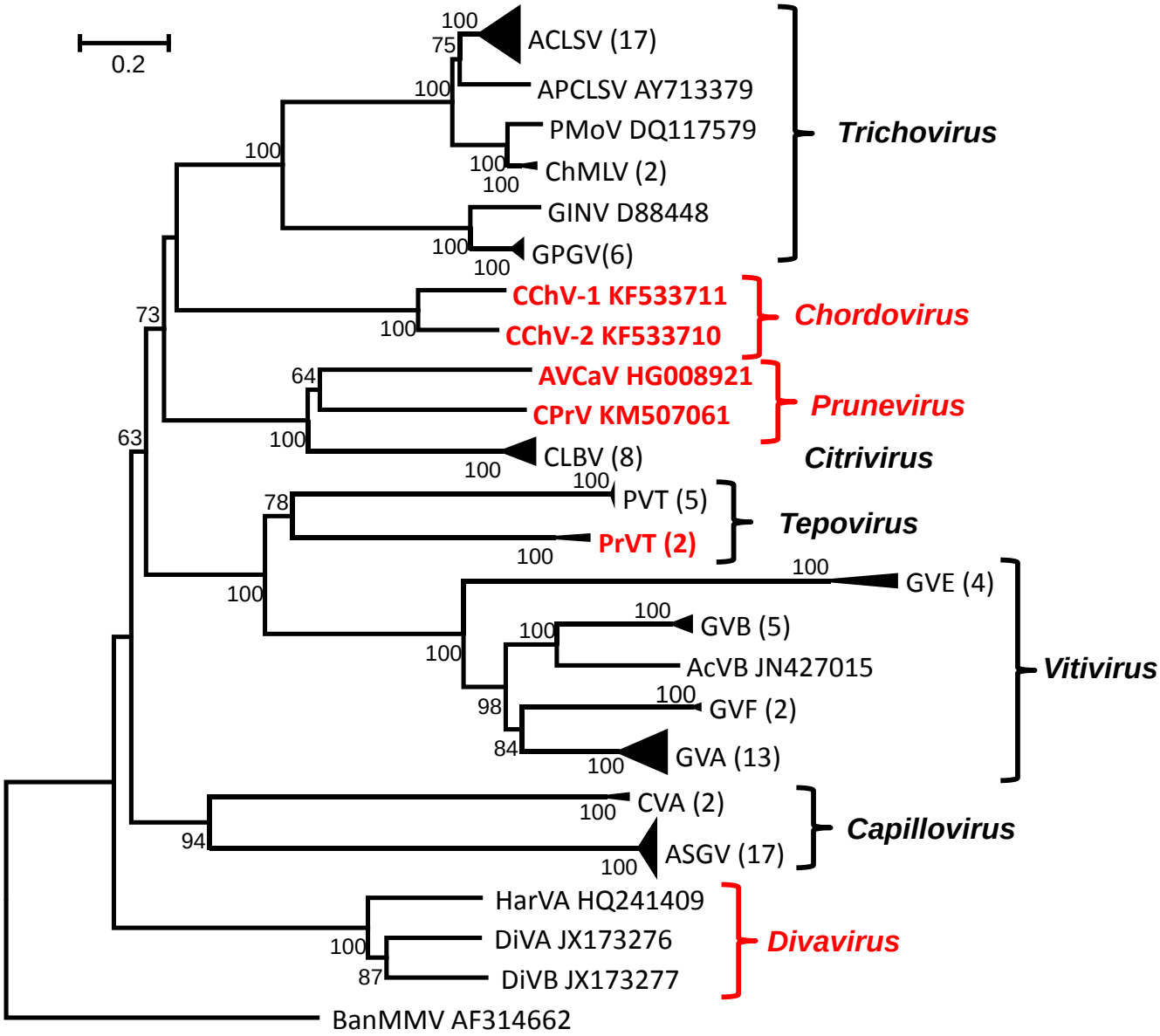


Fig. 1. Phylogenetic tree of the replicase amino acid sequences of all complete sequences of members of the proposed subfamily *Trivirinae*, with Banana mild mosaic virus (BanMMV – unassigned species in the proposed subfamily *Quinvirinae*) as the outgroup. Neighbor-Joining Tree (JTT substitution model) with 1000 bootstrap replicates produced in MEGA6. Percentage bootstrap support is shown at all branches if >60%. Genus names are shown on the right; those in red are the new genera proposed here. Viruses proposed here as members of new species are also shown in red. Branches representing a single species have been collapsed (with triangles denoting the range of variation within the species) and the number of sequences used are shown in brackets after the abbreviation. Abbreviations: ACLSV, Apple chlorotic leaf spot virus; AcVB, Actinidia virus B; APCLSV, Apricot pseudo-chlorotic leaf spot virus; ASGV, Apple stem grooving virus; AVCaV, Apricot vein clearing associated virus; CChV-1, Carrot Ch virus 1; CChV-2, Carrot Ch virus 2; ChMLV, Cherry mottle leaf virus; CLBV, Citrus leaf blotch virus; CPrV, Caucasus prunus virus; CVA, Cherry virus A; DiVA, Diuris virus A; DiVB, Diuris virus B; GINV, Grapevine berry inner necrosis virus; GPGV, Grapevine Pinot gris virus; GVA, Grapevine virus A; GVB, Grapevine virus B; GVE, Grapevine virus E; GVF, Grapevine virus F; HarVA, Hardenbergia virus A; PMoV, Peach mosaic virus; PrVT, Prunus virus T; PVT, Potato virus T.

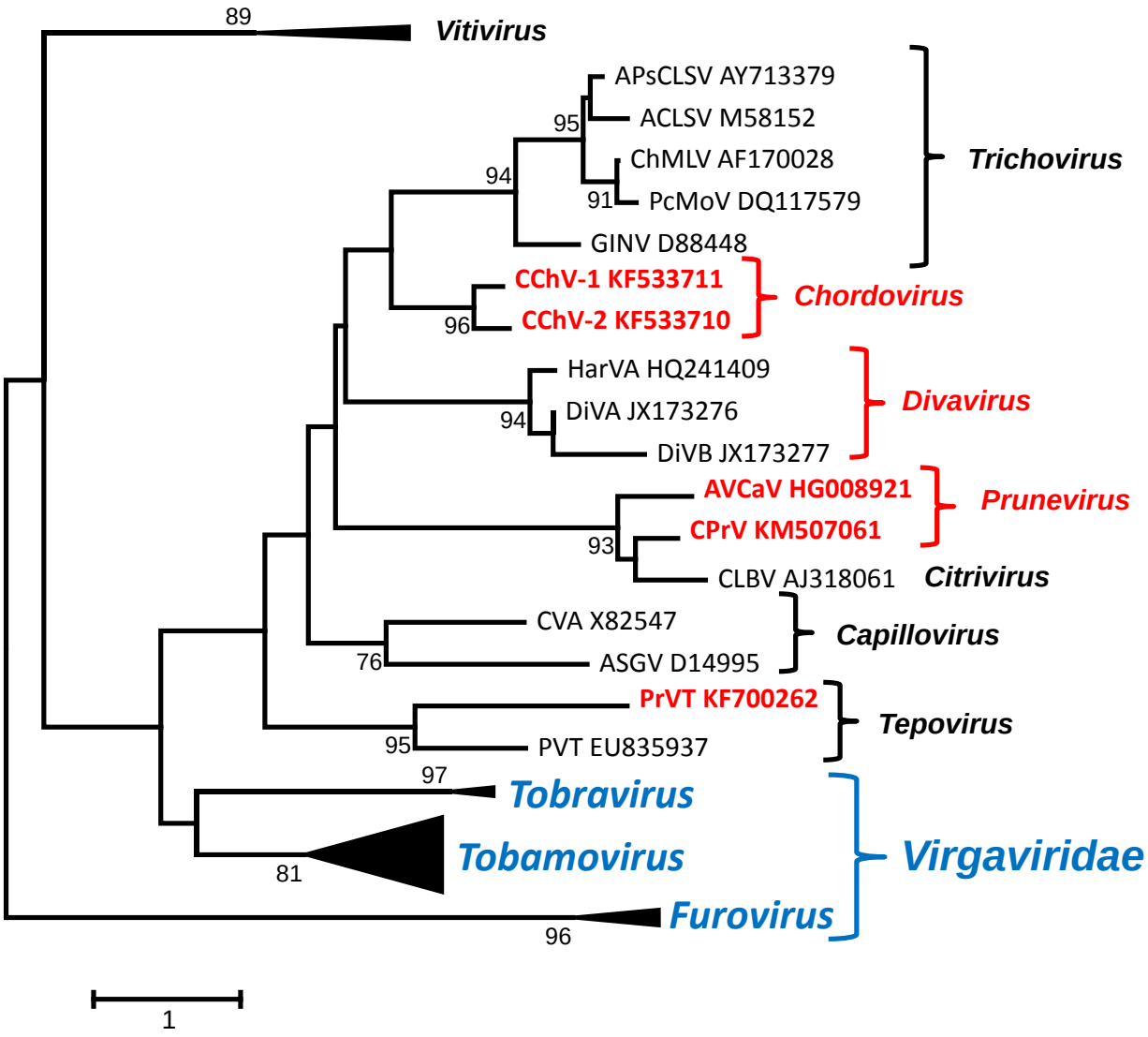


Fig. 2. Unrooted phylogenetic tree of the Movement Protein amino acid sequences of members of the proposed subfamily *Trivirinae*. Neighbor-Joining Tree (JTT substitution model) with 1000 bootstrap replicates produced in MEGA6. Percentage bootstrap support is shown at all branches if >60%. Genus names are shown on the right; those in red are the new genera proposed here. Viruses proposed here as members of new species are also shown in red. For abbreviations, see legend to Fig. 1. The related '30K' family MPs of members of the family *Virgaviridae* are also included (with genus branches collapsed).

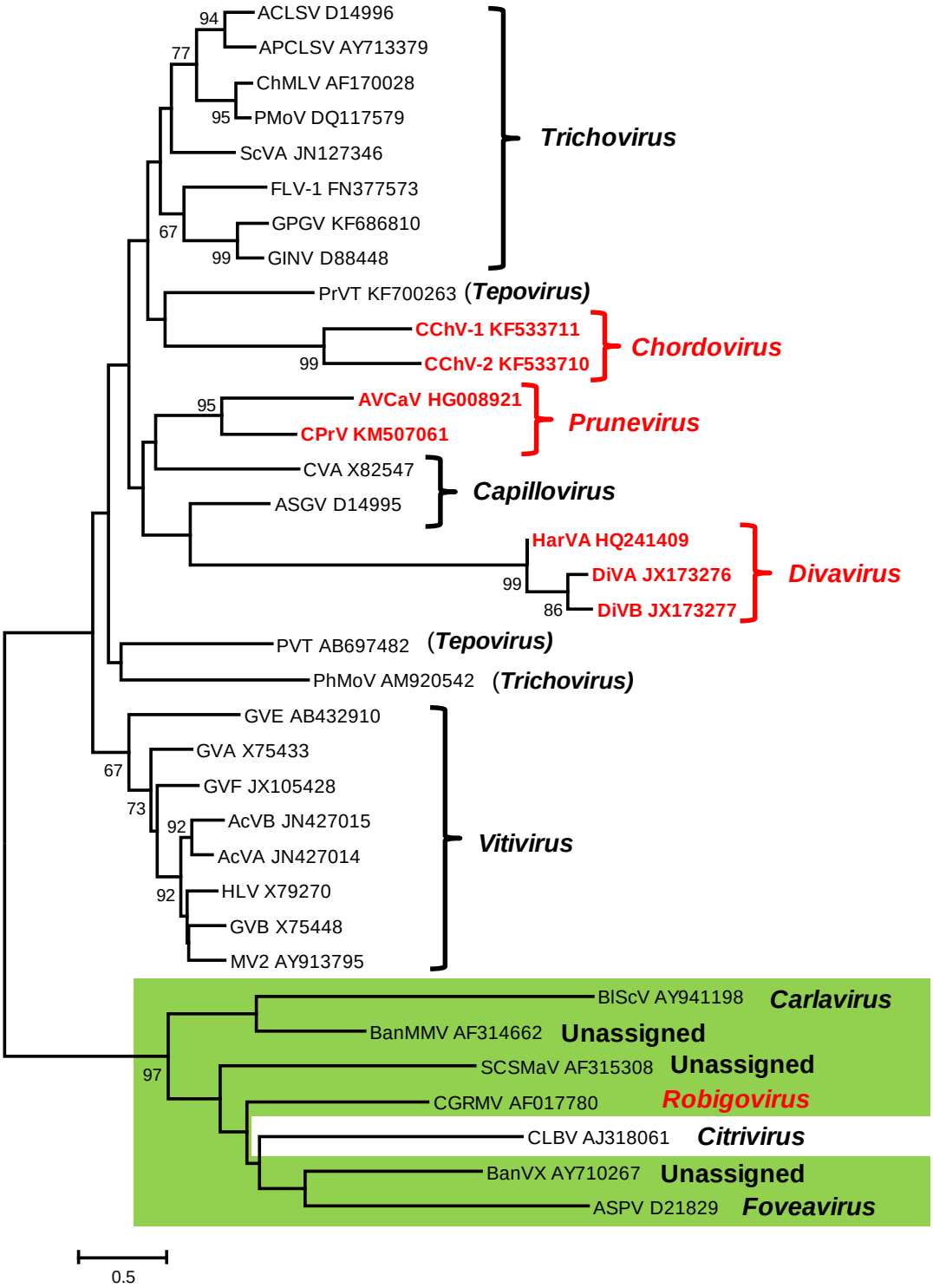


Fig. 3. Phylogenetic tree of the coat protein amino acid sequences of members of the proposed subfamily *Trivirinae* and selected members of the proposed subfamily *Quinvirinae* (shaded green). Neighbor-Joining Tree (JTT substitution model) with 1000 bootstrap replicates produced in MEGA6. Percentage bootstrap support is shown at all branches if >60%. Genus names are shown on the right; those in red are the new genera proposed here.

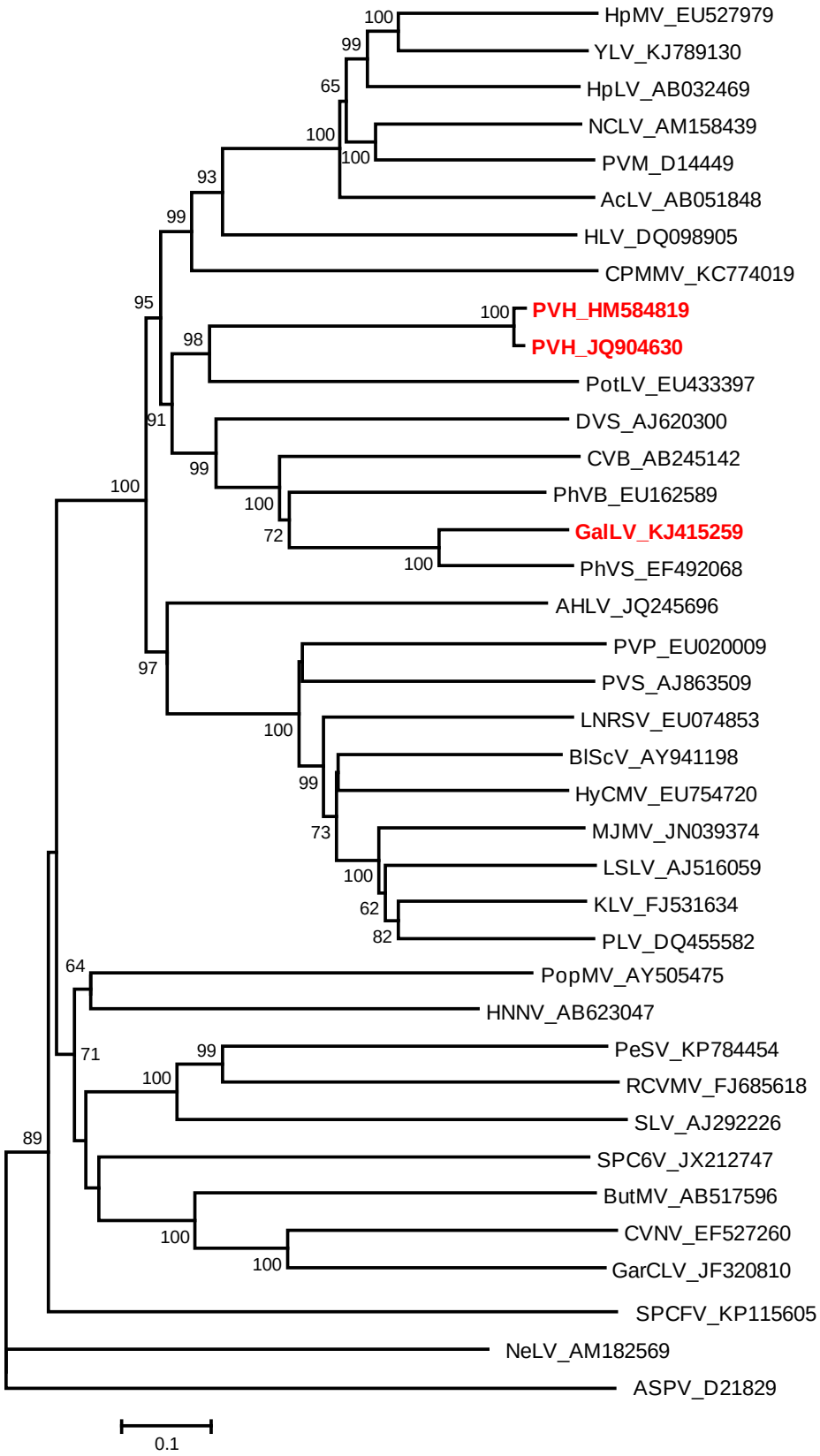


Fig. 4. Phylogenetic tree of the proteome amino acid sequences (concatenated replicase, TGB proteins and coat protein) of members of the genus *Carlavirus*, with Apple stem pitting virus (ASPV) genus *Foveavirus* as the outgroup. Neighbor-Joining Tree (JTT substitution model) with 1000 bootstrap replicates produced in MEGA6. Percentage bootstrap support is shown at all branches if >60%. Viruses proposed here as members of new species are shown in red.

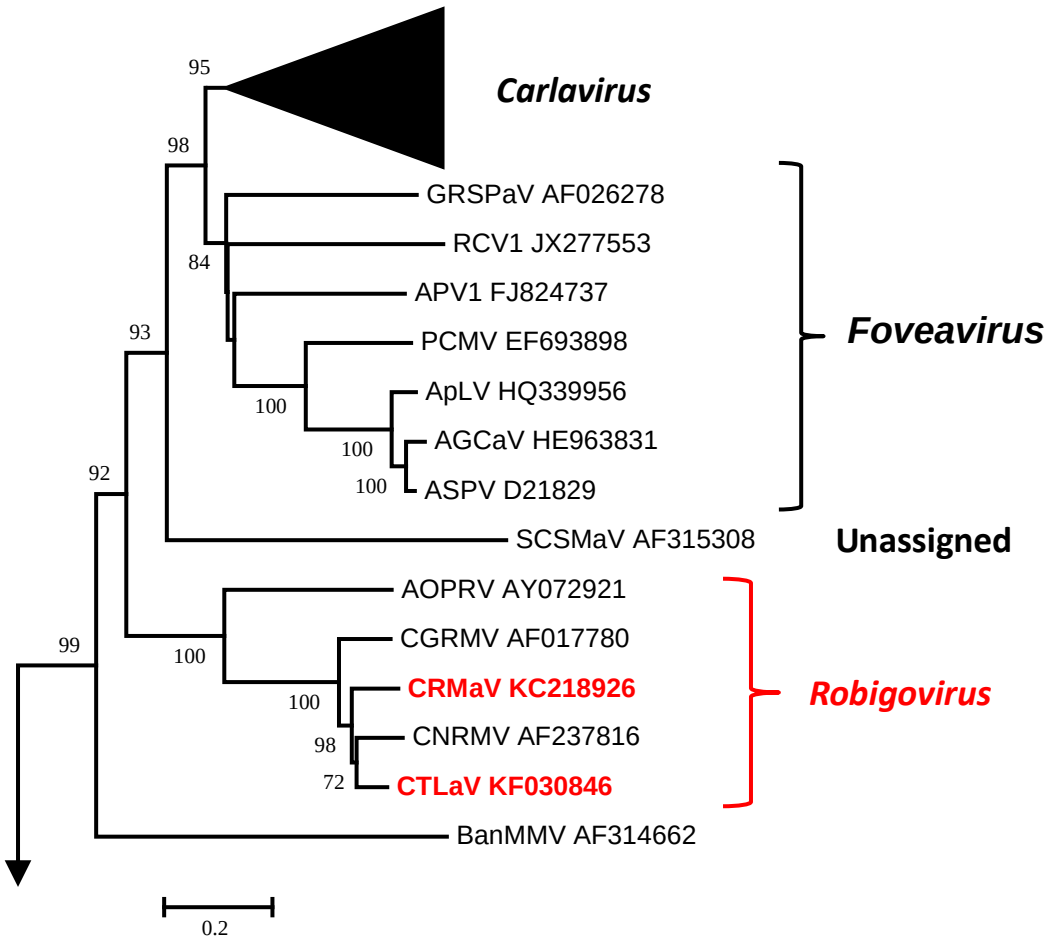


Fig. 5. Extract from phylogenetic tree of the replicase amino acid sequences of members of the family *Betaflexiviridae* showing members of the proposed subfamily *Quinvirinae*. Neighbor-Joining Tree (JTT substitution model) with 1000 bootstrap replicates produced in MEGA6. Percentage bootstrap support is shown at all branches if >60%. Viruses proposed here as members of new species and the proposed new genus *Robigovirus* are shown in red. The branch to members of the genus *Carlavirus* has been collapsed for clarity. AOPRV, *African oil palm ringspot virus* ; BanMMV, *Banana mild mosaic virus*; CGRMV, *Cherry green ring mottle virus*; CNRMV *Cherry necrotic rusty mottle virus*; CRMaV, Cherry rusty mottle associated virus; CTLaV, Cherry twisted leaf associated virus.

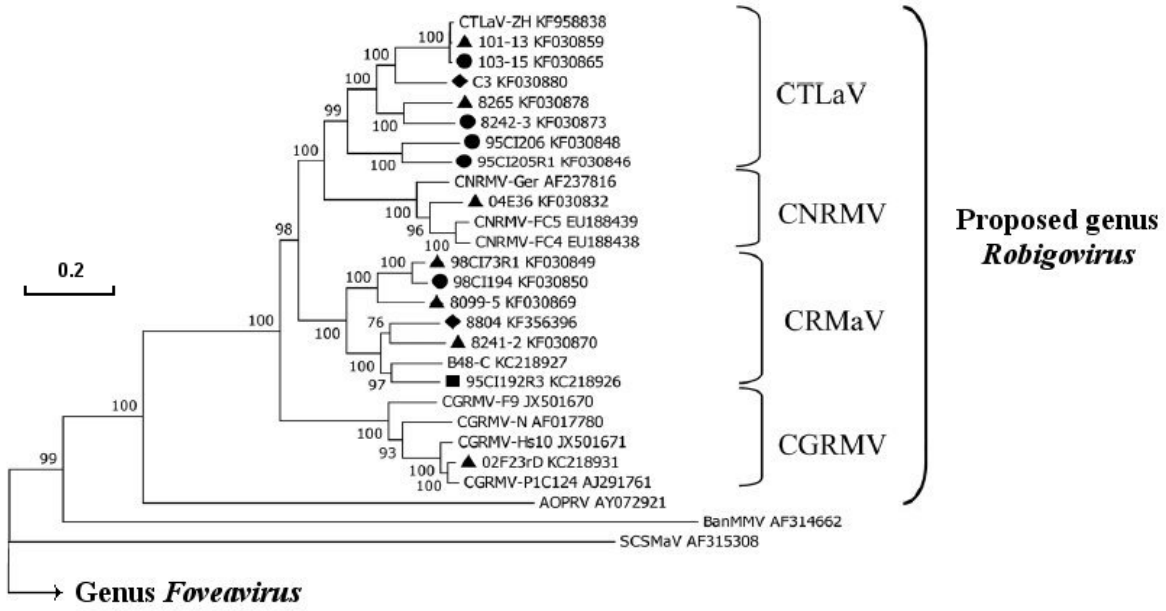


Fig. 6. Phylogenetic analysis of the entire proteome amino acid sequences of isolates of the four proposed members of the new genus *Robigovirus*. Extracted and modified from a larger tree in Villamor et al., 2015. Maximum likelihood tree with 1000 bootstrap replicates produced in MEGA6. Isolate designations and accession numbers are shown. Triangles denote sequences obtained by RT-PCR alone, circles are sequences determined by high-throughput sequencing and RT-PCR and diamonds are those from high-throughput sequencing alone. AOPRV, *African oil palm ringspot virus* ; BanMMV, *Banana mild mosaic virus*; CGRMV, *Cherry green ring mottle virus*; CNRMV *Cherry necrotic rusty mottle virus*; CRMaV, Cherry rusty mottle associated virus; CTLaV, Cherry twisted leaf associated virus.

Table 1. Range in percentage identities between genomes of the four viruses in the proposed genus *Robigovirus* (adapted from Villamor et al., 2015)

	Genome				ORF1 (replicase)				ORF5 (coat protein)			
	CTLaV	CNRMV	CRMaV	CGRMV	CTLaV	CNRMV	CRMaV	CGRMV	CTLaV	CNRMV	CRMaV	CGRMV
CTLaV	74–99	…	…	…	80–100 72–99	75–78	73–75	69–71	84–100 81–100	77–82	71–77	69–75
CNRMV	70–72	86–95	…	…	69–71	91–96 84–94	72–74	68–69	75–80	96–98 90–97	77–80	72–75
CRMaV	69–71	68–69	77–95	…	68–70	67–69	84–97 75–94	70–71	71–76	73–76	89–100 86–97	70–74
CGRMV	66–68	65–66	66–68	82–95	65–67	64–65	65–67	87–97 80–95	69–74	70–71	70–75	96–99 88–98

CTLaV = Cherry twisted leaf-associated virus, CNRMV = *Cherry necrotic rusty mottle virus*, CRMaV = Cherry rusty mottle-associated virus and CGRMV = *Cherry green ring mottle virus*. Numbers in red are % amino acid identity and those in black are % nucleotide sequence identity