



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:	2012.020a-eP	(to be completed by ICTV officers)			
Short title: create 12 new species in the family <i>Betaflexiviridae</i> (e.g. 6 new species in the genus <i>Zetavirus</i>)					
Modules attached (modules 1 and 9 are required)	1 <input checked="" type="checkbox"/>	2 <input checked="" type="checkbox"/>	3 <input type="checkbox"/>	4 <input type="checkbox"/>	5 <input type="checkbox"/>
	6 <input type="checkbox"/>	7 <input type="checkbox"/>	8 <input type="checkbox"/>	9 <input checked="" type="checkbox"/>	

Author(s) with e-mail address(es) of the proposer:

Jan Kreuze (j.kreuze@cgiar.org) on behalf of the *Flexiviridae* SG

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

Date first submitted to ICTV:

Date of this revision (if different to above):

MODULE 2: 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	2012.020aP	(assigned by ICTV officers)
To create 2 new species within:		
Genus:		Fill in all that apply. <ul style="list-style-type: none"> • 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.
Subfamily:		
Family:	<i>Betaflexiviridae</i>	
Order:	<i>Tymovirales</i>	
And name the new species:		GenBank sequence accession number(s) of reference isolate:
<i>Banana virus X</i> <i>Hardenbergia virus A</i>		AY710267 HQ241409

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.
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The family *Betaflexiviridae* contains viruses with flexuous filamentous virions that infect plants. They share a distinct lineage of alphavirus-like replication proteins that is unusual in lacking any recognized protease domain. Throughout the family, isolates of different species should have less than about 72% nt identity (or 80% aa identity) between their respective CP or polymerase genes. Viruses from different genera usually have less than about 45% nt identity in these genes.

Table 1: Distinguishing properties of genera in the family *Betaflexiviridae*

Genus	Virion length (nm)	ORFs	Rep ^a	MP(s) ^b	CP ^c
<i>Capillovirus</i>	640–700	2	210–245	30K	25–27
<i>Carlavirus</i>	610–700	6	215–225	TGB	32–36
<i>Citivirus</i>	960	3	227	30K	41
<i>Foveavirus</i>	800+	5	230–250	TGB	28–44
<i>Trichovirus</i>	640–890	3 or 4	215–220	30K	21–24
<i>Vitivirus</i>	725–785	5	190–200	30K	18–22

^aRep, Replication protein size (kDa).

^bMP, Movement protein either of the “30K” superfamily or a triple gene block (TGB).

^cCP, Coat protein size (kDa).

Banana virus X (BanVX; Teycheney et al., 2005)

BVX was identified in symptomless banana plants in Guadeloupe and the 3' 2917 nts were determined including the partial RdRp, TGB and CP ORFs. Small scale survey found 7 out of 44 tested plants in Guadeloupe positive for this virus, all were symptomless, and little variation was found between the sequences. Sequence similarities with other betaflexiviruses are clearly below the species demarcation threshold. Its genome structure is similar to foveaviruses, nevertheless, it clearly groups outside the foveavirus clade (Teycheney et al., 2005).

Hardenbergia virus A (HarVA; Wylie & Jones, 2011)

Complete sequence of an isolate from *Hardenbergia comptoniana*. Genome structure is similar to capilloviruses, but groups between the genera *Capillovirus* and *Foveavirus* phylogenetically (Wylie & Jones, 2011). We propose to enter virus as an unassigned species in the family until further information is available to provide a definite classification.

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Code	2012.020bP	(assigned by ICTV officers)
To create 6 new species within:		
Genus:	<i>Carlavirus</i>	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.
Subfamily:		
Family:	<i>Betaflexiviridae</i>	
Order:	<i>Tymovirales</i>	
And name the new species:		GenBank sequence accession number(s) of reference isolate:
<i>Hippeastrum latent virus</i>		DQ098905
<i>Butterbur mosaic virus</i>		AB517596
<i>Cucumber vein-clearing virus</i>		JN591720
<i>Helleborus mosaic virus</i>		FJ196838
<i>Hydrangea chlorotic mottle virus</i>		EU754720
<i>Mirabilis jalapa mottle virus</i>		JN039374

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.**
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Hippeastrum latent virus (HLV; the flexiviridae study group)

In the taxonomic description of *Narcissus symptomless virus*, Chen et al (2006) provide Fig 2, which describes the phylogenetic relationships of the 13 carlavirus genomes available. Included is a sequence described as *Nerine latent virus* (NeLV) and the GenBank accession given is DQ098905. In fact, DQ098905 corresponds to HLV and no sequence of NeLV was available in 2006. The confusion was probably due to the fact that Maat et al. 1978 proposed, based on resemblances in serology and experimental host responses, that NeLV and HLV were probably the same virus. Subsequently Adams et al (2004) and also the Springer Index of Viruses 2nd Ed (Tidona C, & Darai G, eds, 2011) list HLV as a synonym of NeLV. Nucleotide pairwise alignment of the complete genomes of HLV and *Narcissus symptomless virus* [synonym of, and proposed to be renamed to *Nerine latent virus*; see also proposal 2012.015aP to abolish *Narcissus symptomless virus*] reveal they share 54% nt identity, below the species demarcation line for betaflexiviruses and thus HLV should be classified as a new virus species.

Butterbur mosaic virus (ButMV; Hashimoto et al., 2009)

Isolated from Japanese butterbur (*Petasites japonicus*) and maintained in *Chenopodium quinoa*

from where it was purified and its complete genome sequence determined. ButMV has flexuous filamentous virions measuring 670 nm in length and 13 nm in diameter, and reacting with antiserum raised against carnation latent virus (CLV) virions. Based on the serological relationship, virion morphology, and vector transmissibility by an aphid, *Myzus persicae*, ButMV has been tentatively assigned to the genus *Carlavirus* in the family *Flexiviridae* (Tochihara et al, 1979, King et al., 2012). The ButMV replicase and CP genes share 46.4–54.9 and 43.2–62.1% nucleotide and 38.6–46.6 and 31.3–65.0% amino acid sequence identities, respectively, with those of other carlaviruses.

Cucumber vein-clearing virus (CuVCV; Menzel et al., 2011)

This virus was discovered in 2009 by whitefly transmission from a sample collected in Arusha, Tanzania, using a *Bemisia tabaci* B biotype population in a semi persistent transmission test. Three weeks after the transmission experiments, cucumber plants showed vein clearing symptoms. By electron microscopy, carlavirus-like filamentous particles were observed. The presence of the carlavirus was also confirmed in watermelon plants which remained symptomless. DsRNA was extracted from cucumber plants, yielding three major dsRNAs typical for carlaviruses, which served as template for a random RT-PCR approach. A 3'-terminal part of the genome consisting of 5,218 nucleotides (JN591720), excluding the 3'-poly(A) tail, could be obtained, showing a genome organization typical for carlaviruses. This virus showed the highest overall nt sequence identity to cowpea mild mottle virus (CPMMV, 60.7%), another whitefly transmitted carlavirus. The partially sequenced replicase showed the highest aa sequence identity to CPMMV (64.7%). The CP shows an aa sequence identity of 63.8 % to CPMMV, which is, as for the replicase, clearly below the molecular criterion for carlavirus species demarcation. When an antiserum to a Sudanese CPMMV isolate from bean (PV-0907) was used in DAS-ELISA, a strong homologous reaction was observed (A405 reading value >1.7 after 2 h), but no cross reaction with CuVCV (A405 reading value <0.07), indicating that these viruses are serologically unrelated. Based on the symptomatology in single infected cucumber plants, the name cucumber vein-clearing virus is proposed. CuVCV is the only known carlavirus naturally occurring in cucurbits in Africa.

Helleborus mosaic virus (HeMV; Eastwell et al., 2009)

HeMV was first reported from a symptomless infection of *Helleborus niger* in Germany (Koenig, 1985; Mansour et al., 1998). HeMV is serologically related to other *Carlavirus* spp., including *Chrysanthemum virus B* (CVB; Brunt, 1996), *Helenium virus S* (HVS; Brunt, 1996), and *Cowpea mild mottle virus* (CPMMV; Mansour et al., 1998). The vast majority of *Carlavirus* spp. are transmitted nonpersistently by aphids, but there has been no evidence of spread of HeMV in Germany (Koenig, 1985). Eastwell et al. (2009) determined the 3' partial genomic sequence including partial RdRp, TGB, CP and NABP of the HeMV isolate from DSMZ from Germany, and revealed only 49% nucleotide identity with the closest related virus sequence: *Helleborus net necrosis virus*.

Hydrangea chlorotic mottle virus (HdCMV; Machado Caballero et al., 2009, Tang et al., 2010)

HdCMV was first described in a study by Machado Caballero et al. (2009) in which a partial sequence of a strain from *Hydrangea macrophylla* (an ornamental plant) from the USA was described. Members of this proposed species produce leaf blistering, reddening and chlorotic netting on *H. macrophylla* var. 'Endless Summer' and readily infect other *H. macrophylla* varieties. Tang et al. (2010) determined the complete nucleotide sequence of another isolate from *H. macrophylla* growing in Auckland New Zealand, and showed it could be mechanically transmitted to *Chenopodium quinoa*, *C. amaranticolor*, *Nicotiana occidentalis* and *N. occidentalis*. The HdCMV-NZ genome sequence of 8,433 nt possessed a typical carlavirus

organisation with six open reading frames. HdCMV is most closely related (60.4% nt identity) to *Blueberry scorch virus*, a relationship also suggested by serology, whereas it shows 98% identity with the isolate described by Machado Caballero et al from the USA.

Mirabilis jalapa mottle virus (MjMV; Hatlestad et al., 2011)

This virus was discovered fortuitously when analyzing next-generation sequence data of transcripts from flower buds of *Mirabilis jalapa* (an ornamental) growing in Austin, Texas, during an analysis of genes expressed in the flower pigmentation pathway. The complete genome of MjMV was determined to consist of 8315 nucleotides (nt), with the six open reading frames indicative of carlaviruses. MjMV is most similar to *Kalanchoe latent virus* (60% identity) and *Lily symptomless virus* (59% identity). The virus can be transmitted mechanically to *Mirabilis*, but thus far MjMV has only been shown to infect *M. jalapa*, causing a slight leaf mottling and leaf wrinkling phenotype.

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Code	2012.020cP	(assigned by ICTV officers)
To create 1 new species within:		
Genus:	<i>Foveavirus</i>	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.
Subfamily:		
Family:	<i>Betaflexiviridae</i>	
Order:	<i>Tymovirales</i>	
And name the new species:		GenBank sequence accession number(s) of reference isolate:
<i>Asian prunus virus 1</i>		FJ824737

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.**
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Asian prunus virus 1 (APV 1; Marini et al., 2009)

This virus was transmitted from peach to *Nicotiana occidentalis* in which necrotic local lesions and systemic chlorotic mottle were observed. The complete genome sequences was determined from partially purified virions from *N. occidentalis*. The complete nt sequence showed 90, 83 and 82% identity with the partial sequences present in GenBank of APV1 (DQ205236), APV2 (DQ205238), and APV 3 (DQ205237), respectively. The virus shows a typical genome structure and phylogenetic grouping consistent with members of the genus *Foveavirus* and shows sequence identity values below the demarcation criteria with other foveaviruses.

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Code	2012.020dP	(assigned by ICTV officers)
To create one new specie within:		
Genus:	<i>Trichovirus</i>	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.
Subfamily:		
Family:	<i>Betaflexiviridae</i>	
Order:	<i>Tymovirales</i>	
And name the new species:		GenBank sequence accession number(s) of reference isolate:
<i>Phlomis mottle virus</i>		AM920542

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.**
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Phlomis mottle virus (PhMV; Salderelli et al., 2008)

Isolated from *Phlomis fruticosa* by transmission to *N. occidentalis*, from which virions were isolated and the partial 3035 nucleotides of the 3’ region determined. It has flexuous particles, a typical genome structure and phylogenetic grouping for Trichovirus and exceeds species demarcation limit with available sequences.

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Code	2012.020eP	(assigned by ICTV officers)
To create 2 new species within:		
Genus:	<i>Vitivirus</i>	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.
Subfamily:		
Family:	<i>Betaflexiviridae</i>	
Order:	<i>Tymovirales</i>	
And name the new species:		GenBank sequence accession number(s) of reference isolate:
<i>Actinidia virus A</i>		JN427014
<i>Actinidia virus B</i>		JN427015

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

- Explain how the proposed species differ(s) from all existing species.
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Actinidia virus A and *Actinidia virus B* (AcVA & AcVB; Blouin et al., 2012)

Two viruses isolated from kiwi-fruit (*Actinidia chinensis*), they are mechanically transmissible to *N. occidentalis*, and their genome structure and phylogenetic grouping is characteristic of vitiviruses. Both virus genomes were sequenced and share 64% nucleotide identity to each-other, and less than that to other viruses.

additional material in support of this proposal

References:

- Adams MJ, Antinow JF, Bar-Joseph M, Brunt AA, Candresse T, Foster GD, Martelli GP, Milne RG, Fauquet CM (2004) The new plant family Flexiviridae and assessment of molecular criteria for species demarcation. *Arch Virol* 149:1045–1060
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- Giampetruzzi A., Roumi V., Roberto R., Malossini U., Yoshikawa N., La Notte P., Terlizzi F., Credi R., Saldarelli P. (2012). A new grapevine virus discovered by deep sequencing of virus- and viroid-derived small RNAs in Cv Pinot gris. *Virus Res.* 163(1):262-268.
- Hashimoto M, Komatsu K, Maejima K, Yamaji Y, Okano Y, Shiraishi T, Takahashi S, Kagiwada S, Namba S (2009). Complete nucleotide sequence and genome organization of butterbur mosaic virus. *Arch. Virol.* 154(12): 1955-1958.
- Hatlestad G.J., Elam L., Gonzalez A., Lloyd A.M. (2011). Mirabilis jalapa mottle virus: a new carlavirus infecting four o'clocks. *Arch. Virol.* 156(11):2109-2111.
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- Machado Caballero JE, Lockhart BE, Mason SL, 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
- Mansour, A., Al-Musa, A., Vetten, H. J., and Lesemann, D. E. 1998. Properties of a cowpea mild mottle virus (CPMMV) isolate from eggplant in Jordan and evidence for biological serological differences between CPMMV isolates from leguminous and solanaceous hosts. *J. Phytopathol. (Berl.)* 146:539-547.
- Marini DB, Gibson PG, Scott SW (2009). The complete nucleotide sequence of an isolate of Asian prunus virus 1 from peach [*Prunus persica* (L) Batch] . *Arch. Virol.* 154(8):1375-1377
- Menzel W, Vetten HJ. (2008). Partial nucleotide sequence of a carlavirus from carrot. *Virus Genes* 37:432-433.
- Menzel W., Abang M.M., Winter S. (2011). Characterization of cucumber vein-clearing virus, a whitefly (*Bemisia tabaci* G.)-transmitted carlavirus. *Arch Virol.* 156(12):2309-2311.
- Salderelli P., Boscia D., De Stradis A., Vovlas C. (2008). A new member of the family Flexiviridae from *Phlomis fruticosa*. *J. Plant Pathol* 90(2):281-286.
- Tang J, SHarper SJ, Wei T, Clover GRG (2010). Characterization of hydrangea chlorotic

additional material in support of this proposal

References:

- mottle virus, a new member of the genus Carlavirus Arch. Virol. 155(1):7-12
- Teycheney PY, Marais A, Svanella-Dumas L, Dulucq MJ, Candresse T. (2005). Molecular characterization of banana virus X (BVX), a novel member of the Flexiviridae family. Arch. Virol. 150:1715-1727.
- Tochihara H, Tamura M (1976) Viruses in Japanese Buterbur (*Petasites japonicum* Miq.). Ann Phytopath Soc Japan 42:533–539
- 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

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 (next page). Phylogenetic tree of Betaflexiviridae based on ClustalW alignment of RdRp domain amino acid sequences. Proposed new viruses from this proposal are indicated with red font. Putative new viruses not considered in the current proposal are indicated in blue font.

