



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:	2009.010a,bP	(to be completed by ICTV officers)			
Short title: create 2 new species in the genus Tobamovirus, family Virgaviridae (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:

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Has this proposal has been seen and agreed by the relevant study group(s)?
Please select answer in the box on the right

No

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

Date first submitted to ICTV: 2009-05-08
Date of this revision (if different to above):

MODULE 2: **NEW SPECIES**

Part (a) to create and name one or more new species.

If more than one, they should be a group of related species belonging to the same genus (see Part b)

Code	2009.010aP	(assigned by ICTV officers)
To create 2 new species with the name(s):		
<i>Brugmansia mild mottle virus</i>		
<i>Streptocarpus flower break virus</i>		

Part (b) assigning new species to higher taxa

All new species must be assigned to a higher taxon. This is usually a genus although it is also permissible for species to be “unassigned” within a subfamily or family.

Code	2009.010bP	(assigned by ICTV officers)
To assign the species listed in section 2(a) as follows:		
Genus:	<i>Tobamovirus</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>Virgaviridae</i> (awaiting ratification)	
Order:		

Species demarcation criteria in the genus *Tobamovirus* are:

- Sequence similarity; less than 10% overall nt sequence difference is considered to characterize strains of the same species, although most of the sequenced species have considerably less than 90% sequence identity,
- Host range; however many of these viruses have wider and more overlapping host ranges in experimental rather than natural situations,
- Antigenic relationships between the CPs.

Brugmansia mild mottle virus (BMMV) causes mild mottling in the ornamental *Brugmansia* (*Solanaceae*). This description is taken from Ilmberger et al (2006).

The symptoms caused by BMMV on additional experimental hosts differed slightly from those of TMV and *Turnip vein clearing virus* (TVCV). On *Nicotiana tabacum* L. cv. Xanthi, *Nicotiana glutinosa* L., *Datura stramonium* L., petunia (*Petunia hybrida* “colorama”) as well as *Chenopodium quinoa* Willd. and *C. amaranticolor* Coste & Reynier local lesion appeared when inoculated with the three viruses. On *Nicotiana benthamiana* Domin. and *Capsicum frutescens* Mill. cv. annum chloroses. On *Nicotiana rustica* L. a mosaic was induced after inoculation with the three viruses and on *Nicotiana clevelandii* Gray TMV and BMMV induced chloroses while TVCV a mosaic appeared. On *N. rustica* symptoms of BMMV differed significantly from those of the other two tobamovirus species. BMMV induced vein necrosis while TMV and TVCV caused a chlorosis.

BMMV is transmitted by mechanical inoculation. Electron micrograph of purified virus preparation revealed rod shaped particles with the size of about 300 x 18 nm. The A260/280 ratio was determined as 1,28.

Sequencing confirmed the results of the experimental determination of genome length as about 6380 nts (EMBL AM398436). Computer analysis of the sequence revealed the ORFs 1a, 1b, 2 and 3, flanked by 5' and 3' non-coding regions (NTR) typically found with tobamovirus genomes. The lengths of the 5' NTR with 60 nts and 3' NTR with 200 nts are comparable to the the respective regions of other tobamoviruses.

ORF1a contains 3348 nts and codes for a 125 kDa protein. Methyltransferase (ORF1, position amino acid (aa) 1-379) and helicase (ORF2, aa 812-1110) domains were detected. A leaky amber stop-codon which upon translation leads to a read-through protein with the size of approximately 182 kDa. This read-through protein shows highest homology to ToMV (74 % identical aa) and other subgroup 1 (solanaceous-infecting) viruses (67-73 % identical aa), while the identities to the brassica-infecting subgroup 3 (61-64 % identical aa) and cucurbit-infecting subgroup 2 (48 % identical aa) are lower.

ORF2 encodes the cell to cell movement protein with a size of about 30 kDa and overlaps with eight nucleotides of ORF1b, which is typical for subgroup 1 viruses. The highest identity was found with the respective proteins from *Tobacco mild green mosaic virus* (TMGMV), *Obuda pepper virus* (ObPV) and *Tobacco latent virus* (TLV) (67 to 71 % identical aa). In the phylogram based on the analysis of the complete genome (Fig 1a, Appendix) BMMV clusters together with all other species of subgroup 1, suggesting that this isolate belongs to this subgroup. This clustering was confirmed by phylogenetic analysis of the CP (ORF3) which clustered also in subgroup 1 (Fig 1b, Appendix). Within subgroup 1 the conservation of all isolates range from 62 to 88 % identical aa. The lowest conservation was found with TMV (63 % identical aa) whereas highest identities were obtained with Tropical soda apple mosaic virus (71 % identical aa).

The solanaceous host and sequence comparisons suggest that BMMV is a new tobamovirus, which belongs to subgroup 1. The criteria for the demarcation of tobamovirus species require more than 10 % overall sequence divergence, a specific host range and a specific antigenic relationship. The two first criteria are fulfilled and it should therefore be regarded as a new species within this genus. The motif (position 4404-4450) for species demarcation proposed by Gibbs et al. (2004) was also present in the BMMV genome and according to this isolate BMMV should be regarded as a separate species. Due to the symptoms in *Brugmansia* sp. we propose the name *Brugmansia mild mottle virus* (BMMV).

Streptocarpus flower break virus (SFBV) causes flower break symptom in the ornamental *Streptocarpus* (*Gesneriaceae*). Before, only TMGMV has been found in that plant family. The proposed virus SFBV is different from all so far known tobamoviruses and its characteristics have been described by Verhoeven et al., 1995 and Heinze et al., 2006.

In addition to its original host, SFBV can infect at least 8 plant species in 3 families. Local symptoms arise in *Chenopodium quinoa*, *Datura stramonium*, *Cucumis sativus*, *Nicotiana glutinosa* and *Nicotiana tabacum* "White burley". Systemic symptoms occur on *N. benthamiana*, *N. clevelandi* and *N. occidentalis*. No symptoms are visible on *N. tabacum* cv. Samsun, *Lycopersicon esculentum* and *Capsicum annuum*.

SFBV is transmitted by mechanical inoculation. The thermal inactivation in *N. benthamiana* plant sap was 10 min with 90 °C. Dilution end point is 10⁻⁸. Electron micrograph of purified virus preparation revealed rod shaped particles with the size of about 300 x 18 nm.

The SFBV genome (EMBL AM040955) contained c. 6280 nt (the extreme 3' end remained incomplete). Four open reading frames were detected. ORF 1 contains 3317 nt and encodes a polypeptide of about 124,5 kDa. Methyltransferase (position 1-376 aa), and helicase (position 800-1100 aa) domains were detected. There is probably a leaky stop-codon which may lead together with ORF 1 to a read-through protein with the size of approximately 180 kDa (ORF1a). The read-through protein shows highest similarity to ORSV (67 % identical aa). Other tobamoviruses from subgroup 1 and 3 show similarities between 60 and 65 % identical aa whereas the similarity to *Cucumber green mottle mosaic virus* (CGMMV) is less (43,5 % identical aa). ORF 2 encodes the putative cell to cell movement protein (MP) with a size of c. 31,4 and has highest similarity (up to 50 % identical aa) to the MP of subgroup 3 viruses, whereas subgroup 1 viruses (31 – 37 % identical aa) and subgroup 2 (25 % identical aa) were less conserved. ORF 3 encodes the coat protein with a size of 17,5 kDa and shows highest similarities (58 % identical aa) to subgroup 3 viruses. Again, the CPs of subgroup 1 (up to 48 % identical aa) and subgroup 2 (38 % identical aa) viruses are less conserved. According to the demarcation criteria of the ICTV (less than 10 % identical nucleotides over the genome) SFBV is a new species in the genus *Tobamovirus*. This is supported by the trees in the Appendix (Fig. 1).

In addition the pattern of overlapping of the three open reading frames differs from that in all other tobamoviruses so far investigated. The SFBV MP and CP ORFs overlap by 127 nt (similar to subgroup 3 viruses which overlap by 77 nt). The overlap between RdRp and MP ORFs of 19 nt is similar to that of subgroup 1 viruses.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Gibbs AJ, Armstrong JS, Gibbs MJ (2004) A type of nucleotide motif that distinguishes Tobamovirus species more efficiently than nucleotide signatures. Arch Virol 149:1941–1954
Heinze, D.-E. Lesemann, N. Ilmberger, P. Willingmann and G. Adam (2006). Arch Virol 151, 763
Ilmberger N, Willingmann P, Adam G, Heinze C (2006) A subgroup 1 Tobamovirus isolated from Brugmansia sp. and its detection by RT-PCR. J Phytopath 55: 326-332
Verhoeven, J.Th.J., Bouwen, I., Roenhorst, J.W. (1995). Eur J Plant Pathol 101, 311-318

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.

Fig. 1a

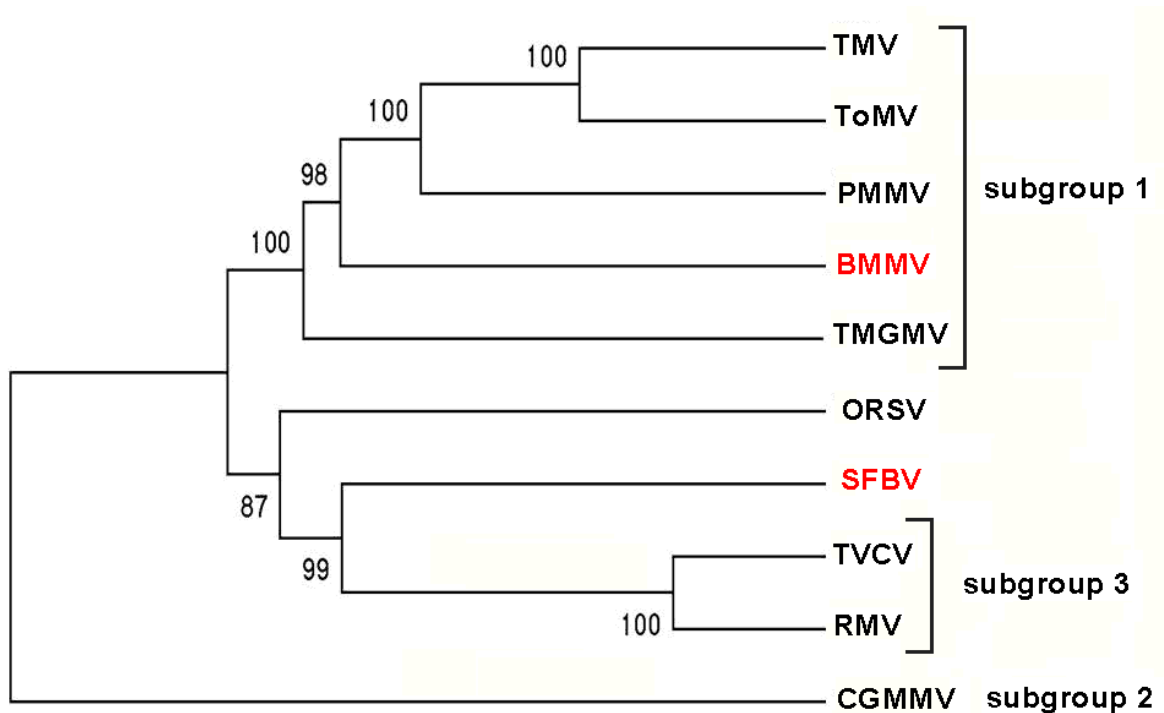
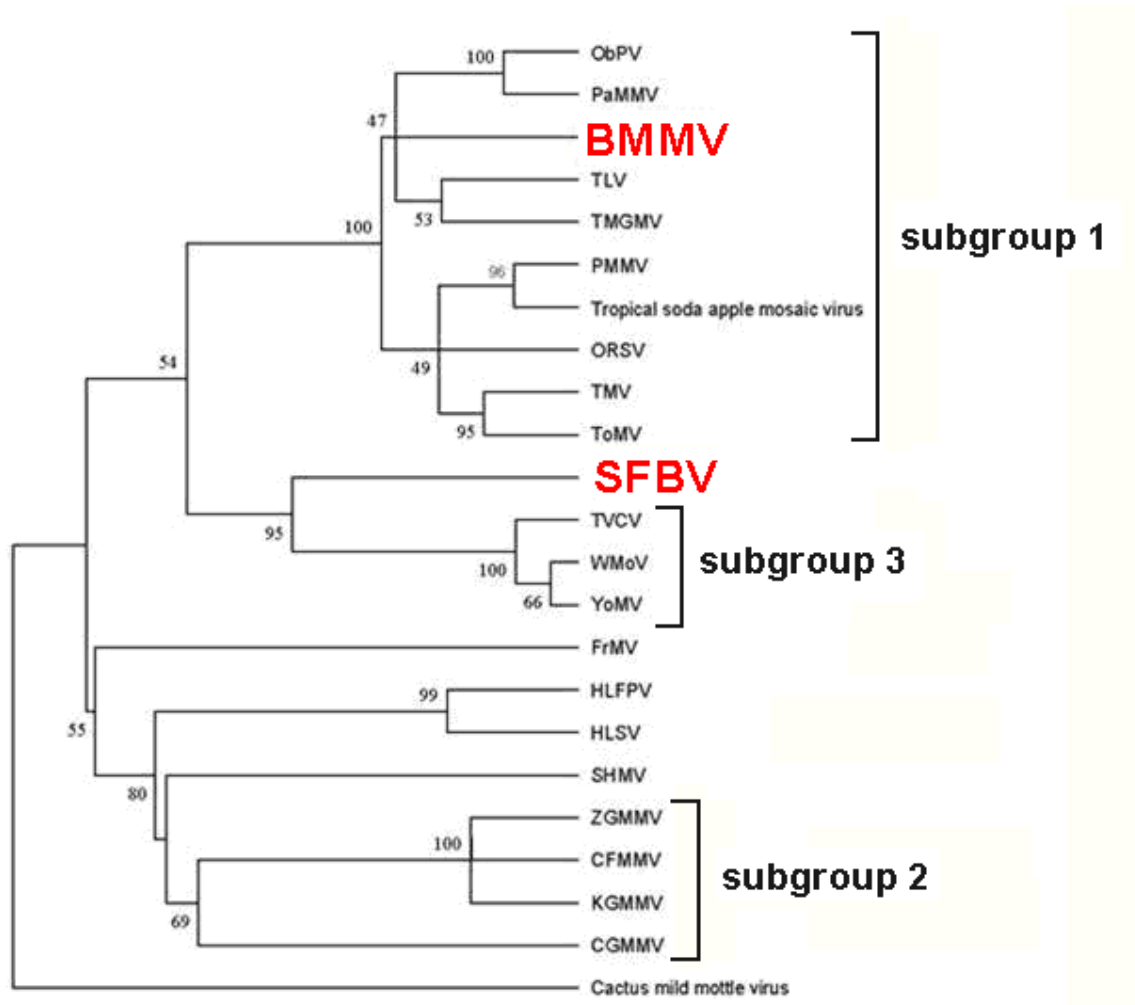


Fig 1b



Neighbour Joining analyses of the complete tobamovirus genome (a) and coat proteins (b). Twenty four sequences from the following virus species were used: ObPV (Obuda pepper virus, D13438), PaMMV (Paprika mild mottle virus, X72586), **BMMV (Brugmansia mild mottle virus, AM398436)**, TLV (Tobacco latent virus, AY137775), TMGMV (Tobacco mild green mosaic virus, AB078435), PMMV (Pepper mild mottle virus, AB000709), Tropical soda apple mosaic virus (AY956381), ORSV (Odontoglossum ringspot virus, AF141927), TMV (Tobacco mosaic virus, X68110), ToMV (Tomato mosaic virus, X02144), **SFBV (Streptocarpus flower-break virus, AM040955)**, TVCV (Turnip vein-clearing virus, Z29370), WMoV (Wasabi mottle virus, AB017503), RMV (Ribgrass mosaic virus, U69271), YoMV (Youcai mosaic virus, U30944), FrMV (Frangipani mosaic virus, AF165884), HLFPV (Hibiscus latent Fort Pierce Virus, AY250831), HLSV (Hibiscus latent Singapore virus, AF395898), SHMV (Sunn-hemp mosaic virus, J02413), ZGMMV (Zucchini green mottle mosaic virus, AJ252189), CFMMV (Cucumber fruit mottle mosaic virus, AF321057), KGMMV (Kyuri green mottle mosaic virus, AB11084), CGMMV (Cucumber green mottle mosaic virus, AB015146), Cactus mild mottle virus (AJ557824). Bootstrap values are provided at the nodes of the branches.