



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:	2014.001aP	(to be completed by ICTV officers)			
Short title: 2 new species in the genus <i>Tobamovirus</i> , family <i>Virgaviridae</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"/> 6 <input type="checkbox"/>	2 <input checked="" type="checkbox"/> 7 <input type="checkbox"/>	3 <input type="checkbox"/> 8 <input type="checkbox"/>	4 <input type="checkbox"/> 9 <input checked="" type="checkbox"/>	5 <input type="checkbox"/>

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

Mike Adams on behalf of the Virgaviridae SG (mike.adams.ictv@gmail.com) and in consultation with Steve Wylie (s.wylie@murdoch.edu.au)

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)

Virgaviridae

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

Date first submitted to ICTV:

May, 2014

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	2014.001aP	(assigned by ICTV officers)
To create 2 new species within:		
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>	
Order:		
Name of new species:	Representative isolate:	GenBank sequence accession number(s)
<i>Tomato mottle mosaic virus</i>	MX5	KF477193
<i>Yellow tailflower mild mottle virus</i>	Cervantes	KF495564

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

The genus *Tobamovirus* contains viruses with a monopartite ssRNA+ genome encapsidated in rigid rod-shaped particles. They have no known natural vectors but are readily transmitted by mechanical inoculation. Other genera in the family *Virgaviridae* have divided genomes with 2 or 3 components and have different modes of transmission (seed, nematodes, fungoid protists) but there are clear phylogenetic relationships between some of the major gene products. Genome organization is conserved within each of the genera. In members of the genus *Tobamovirus* the first major ORF is a replication protein of 126-130 kDa with a ‘leaky’ stop codon that, when suppressed, extends the protein into an RdRp domain and results in a product of about 185 kDa. A cell-to-cell movement protein of about 30 kDa and a single coat protein of about 18 kDa are encoded in ORFs located downstream of the replicase and are translated from separate subgenomic mRNAs.

Species discrimination criteria within the genus as listed in the 9th report 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: many of these viruses have wider and more overlapping host ranges in experimental rather than natural situations
- Antigenic relationships between the CPs

Tomato mottle mosaic virus (Li *et al.*, 2013)

A virus, provisionally named tomato mottle mosaic virus (ToMMV), was isolated from a greenhouse tomato sample collected in Mexico in 2009 and biologically purified through three passages on the local lesion host *Nicotiana rustica*. It was then maintained on tomato cv. Money-maker. After inoculation, the virus induced a rapid tissue necrosis on the upper leaves of tomato seedlings or mosaic and leaf distortion on mature plants. Preliminary identification was made by isolating total RNA from infected tomato leaves and sequencing the small RNAs in an Illumina HiSeq 2000. This indicated the presence of a tobamovirus. To confirm the identified sequence, overlapping reverse transcription-PCR (RT-PCR) products covering the entire virus genome were generated using virus-specific primers and sequenced by use of Sanger technology. Genome ends were confirmed by RACE. The sRNAs were reassembled based on the Sanger template to produce a full-length consensus ToMMV genome sequence. The two sequences were nearly identical, and BLASTn search using the full genome sequence (KF477193 = NC_022230) indicated that ToMMV was most closely related to tomato mosaic virus (FN985165), with an identity of 85%. Genome organisation is also typical of a member of the genus *Tobamovirus*. Phylogenetic analysis shows that ToMMV clusters with other tobamoviruses infecting solanaceous plants (Annex, Figure 1).

Yellow tailflower mild mottle virus (Wylie *et al.*, 2014)

Yellow tailflower (*Anthocercis littorea* Labill., family Solanaceae) is a shrub endemic to southern temperate Australia. Leaves from a yellow tailflower plant with mild mottling and chlorosis were collected from a sparse stand of them in sand dunes (GPS coordinates - 30.652238, 115.145273) south of the village of Cervantes in southwestern Australia. Sap from these leaves was inoculated to seedlings of *Nicotiana glutinosa*, *N. benthamiana* and *N. umbratica*, *Chenopodium quinoa* and *C. amaranticolor*. Systemic symptoms, sometimes severe, developed on *Nicotiana* plants and local lesions on *Chenopodium*. Total RNA was extracted from leaves of the original yellow tailflower plant, and from a symptomatic plant of *N. benthamiana* that was inoculated with sap of the original host plant. Illumina sequencing of small cDNAs and subsequent assembly yielded virtually identical sequences from the two samples, with characteristic size and genome organization of a tobamovirus. The virus was named yellow tailflower mild mottle virus (YTMMV). The entire genome (KF495564 = NC_022801) was most closely related to that of Obuda pepper virus (62% nucleotide identity) and to other tobamoviruses infecting solanaceous plants. Phylogenetic analysis shows that YTMMV clusters with other tobamoviruses infecting solanaceous plants (Figure 1).

Steve Wylie, the lead author of the paper has kindly provided the following additional information to support the existence of YTMMV as a real virus and agrees for it to be in the public domain in this proposal:

“We recently used Illumina sequencing to determine the near-complete genome sequence of a second isolate of YTMMV from another plant of *Anthocercis littorea*, the species from which we identified the first isolate at Cervantes (YTMMV-Cervantes). YTMMV-Kalbarri was isolated from a plant growing just south of the village of Kalbarri, located about 380 km north of Cervantes. The consensus sequence of YTMMV-Kalbarri was assembled without reference to the sequence of isolate Cervantes, but the two genomes are very similar in length (Isolate Cervantes 6382 nt, isolate Kalbarri 6351 nt) and nucleotide identity is high (96.9%). ORFs are identical in length and deduced proteins are almost identical in mass and aa identities (replicase 99.2%, MP 98.9%, CP 99.4%).

“Since publishing the Arch Virol paper we have inoculated YTMMV isolates to tomato, chilli, eggplant, and several new accessions of *Nicotiana benthamiana* and *N. occidentalis* collected from the wild. These species are all systemic hosts and display symptoms. These results are being prepared for publication.”

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Li R, Gao S, Fei Z, Ling K-S (2013). Complete Genome Sequence of a New Tobamovirus Naturally Infecting Tomatoes in Mexico. *Genome Announcements* **1**:e00794-13.

Wylie SJ, Li H, Jones MGK (2014). Yellow tailflower mild mottle virus: a new tobamovirus described from *Anthocercis littorea* (Solanaceae) in Western Australia. *Arch Virol* **159**:791-795.

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.

Figure 1. Phylogenetic (Maximum Likelihood) tree of the concatenated protein sequences (entire proteome) of tobamoviruses infecting solanaceous hosts. A single sequence has been chosen to represent each of the existing species for which a complete genome sequence is available. Tree prepared in MEGA5 with JTT amino acid substitutions. Bootstrap percentages from 1000 replicates are shown at the branches (where >60%). Viruses being used to propose new species are shown in red. BPMV, Bell pepper mottle virus; BrMMV, Brugmansia mild mottle virus; ObPV, Obuda pepper virus; ORSV, Odontoglossum ringspot virus (outgroup); PaMMV, Paprika mild mottle virus; PMMoV, Pepper mild mottle virus; RheMV, Rehmannia mosaic virus; TMGMV, Tobacco mild green mosaic virus; TMV, Tobacco mosaic virus; ToMV, Tomato mosaic virus; **ToMMV, Tomato mottle mosaic virus**; **YTMMV, Yellow tailflower mild mottle virus**.

