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:	***2016.022a,bP***	(to be completed by ICTV officers)

Short title: Create 2 new species in the family *Tymoviridae* (1 in the genus *Tymovirus* and 1 in the genus *Marafivirus*)
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 11 are required)	**2** ☒ **3** ☐ **4** ☐ **5** ☐ **6** ☐ **7** ☐ **8** ☐ **9** ☐ **10** ☐

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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 (there are six virus subcommittees: animal DNA and retroviruses, animal ssRNA-, animal ssRNA+, fungal and protist, plant, bacterial and archaeal)	Tymoviridae SG

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

The SG members are authors of the proposal.

Date first submitted to ICTV: July 2016
Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

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	***2016.022aP***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Marafivirus***
Subfamily:	***N/A***
Family:	***Tymoviridae***
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)**
Nectarine marafivirus M	12C51	KT273411.1

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 11

Background and Justification for the proposal

According to the 9th ICTV Report (Dreher et al., 2012), the current criteria demarcating species in the genus *Marafivirus* (family *Tymoviridae*) are:

- Overall sequence identity less than 80%
- Coat protein sequences less than 90% identical
- Differences in the 3' terminal structure and number of ORFs
- Differential host range
- Vector specificity
- Serological specificity

Nectarine virus M (NeVM)

A novel virus with genome characteristics typical of marafiviruses, referred to as nectarine virus M (NeVM), was detected in several stem pitting-affected nectarine selections by applying Next Generation Sequencing (Illumina) (Villamor et al., 2016). Genome sequences were verified by multiple RT-PCR and RACE experiments.

The complete genome sequences were generated for three isolates of NeVM (12C51, SF04522E, and 12P42) resulted to be 6,421, 6,471, and 6,701 nt in length, respectively,

excluding the poly(A) tail (GenBank accessions KT273411, KT273412, and KT273413).

A single large ORF, potentially coding for a putative product of 2055 aa (isolates 12C51 and SF04522E) or 2,137 aa (isolate 12P42), was identified in the sense orientation (Fig. 1). The difference in length of putative product is due to a 246 nt insertion present in NeVM-12P42 that precedes an AUG codon in 12C51 and SF04522E, assumed to be starting codon for expression of the ORF in these two isolates. Taking into consideration that the genomic nucleotide sequence data for the NeVM-12C51 are used for generating Refseq file in the NCBI, in this proposal we refer to that particular virus as a reference isolate for this species.

Similar to recognized marafiviruses, the predicted NeVM protein contained the conserved motifs of viral methyltransferase (MTR, pfam 01660), tymovirus endopeptidase (Pep, pfam 05381), viral RNA helicase (Hel-1, pfam01443), RdRp (RdRp, pfam00978), and tymovirus CP (CP, pfam00983) (Fig. 1). The putative overlapping ORF, present in maize rayado fino virus (MRFV) (Hammond and Ramirez 2001), is lacking in all three sequenced NeVM isolates (Willamor et al., 2016).

The complete nucleotide sequence of the viruses from 12C51, SF04522E, and 12P42 accession mutually shared 85 to 97% nucleotide sequences, therefore representing isolates of the same virus. However, the three isolates of NeVM showed greater nucleotide identities to marafiviruses (45-62%) than to tymoviruses (45-46%) or a maculavirus, grapevine fleck virus (41-42%). Similar results were obtained in pairwise comparisons of the amino acid sequences of the viral replicases or coat proteins. In phylogenetic analyses, either using viral CP amino acid or full genome nucleotide sequences to generate trees, NeVM grouped within the genus *Marafivirus*, most closely with citrus sudden death associated virus - CSDaV (Maccheroni et al. 2005) (Fig. 2) with which it shares 62% overall nt and 72% aa identity in the CP.

However, the identity values at the whole genome and CP of NeVM and any of recognized marafiviruses are much lower than the species demarcation criteria proposed by the Tymoviridae Study Group for the genus *Marafivirus*, which is <80% nucleotide for whole genome and <90% amino acid for CP (Dreher et al. 2012).

The results of the original study (Villamor et al., 2016) indicate close association of NeVM with the stem pitting disease in nectarines, as all symptomatic plants contained the virus. Nevertheless, the involvement of NeVM in the disease needs to be further studied and clarified as it was present in several asymptomatic plants too.

In summary, despite the lack of serological or epidemiological data (e.g. vector), several criteria (hosts, genome organization and expression strategy, presence of characteristic protein domains, phylogenetic relatedness, etc), suggest that NeVM represents a novel species in the genus *Marafivirus* (family *Tymoviridae*, order *Tymovirales*) for which the name *Nectarine marafivirus M* is proposed.

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	***2016.022bP***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Tymovirus***
Subfamily:	***N/A***
Family:	***Tymoviridae***
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)**
Tomato blistering mosaic tymovirus	SC50	KC 840043

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 11

Background and Justification:

According to the 9th ICTV Report (Dreher et al., 2012), currently applied criteria for species demarcation in the genus *Tymovirus* (family *Tymoviridae*) are:

- Overall sequence identity less than 80%
- Coat protein sequences less than 90% identical
- Differences in the 3' terminal structure
- Differential host range
- Serological specificity

Tomato blistering mosaic virus (ToBMV)

A new virus with properties characteristic of tymoviruses was reported from Brazil recently (De Oliveira et al., 2013). The virus was originally isolated from tomato plants with severe symptoms of leaf mosaic and blistering collected in Santa Catarina state (Fig. 3A), and referred to as tomato blistering mosaic virus (ToBMV).

ToBMV was successfully mechanically transmitted and induced systemic infections in several herbaceous plants belonging to families Solanaceae and Chenopodiaceae (Fig. 3B). The

infected plants contained icosahedral particles and chloroplasts with membrane deformations resembling cytopathic effects caused by tymoviruses (Fig 3C-E).

Full genome sequences of three distinct isolates of ToBMV are available in GenBank. These isolates were reported from tomato (SC50), tobacco (BR-001) and *Solanum violaefolium* Schott (SP-01).

The genome of the type isolate SC50 is 6277 nt long and contains three ORFs (Fig. 4). ORF1 (nt 152-5578) codes for a polyprotein of molecular mass of 201kDa and contains the hallmark motifs of methyltransferase (MTR), protease (PRO), NTPase/Helicase (Hel) and RNA-dependent RNA polymerase (RdRp). ORF2, with an UAG codon at position 145, overlaps with ORF1 and codes for proline rich protein of c. 70kDa presumed to be involved in cell-to-cell movement and RNAi suppression. ORF3 codes for coat protein (CP) of c. 20kDa. The ToBMV genome contains 16 nt long "tymobox" sequence characterized by single nucleotide substitution (U□G) in nt position 6 compared to other tymoviruses (de Oliveira et al., 2013).

Its coat protein amino acid sequence shares the maximum of 64 % identity with the chiltepin yellow mosaic virus (ChiYMV, Pagan et al., 2010), which is below the species demarcation threshold for the genus *Tymovirius* (Table 1).

In a phylogenetic tree, ToBMV clustered with other solanaceous-infecting tymoviruses (Fig. 5).

ToBMV is serologically related, but distinct from eggplant mosaic virus (EMV). Serological cross-reactivity is not unusual among tymoviruses.

In summary, data on biological, morphological, cytopathic and molecular properties of ToBMV, accumulated in several independent studies (de Oliveira et a., 2013, Blawid et al., 2016, Nicolini et al., 2014), strongly indicate that this virus represents a novel species in the genus *Tymovirus* (family *Tymoviridae*, order *Tymovirales*) for which the name *Tomato blistering mosaic tymovirus* is proposed.

MODULE 11: **APPENDIX**: supporting material

additional material in support of this proposal

References:

De Oliveira V.C., Nagata T., Guimarães F.C., Ferreira F.A., Kitajima E.W., Nicolini C., Resende R.O., Inoue-Nagata A.K., 2013. Characterization of a novel tymovirus on tomato plants in Brazil. Virus Genes 46:190–194.

Dreher T.W., M.C. Edwards, A-L. Haenni, R.W. Hammond, I. Jupin, R. Koenig, S. Sabanadzovic, and G.P. Martelli, 2012. Family *Tymoviridae*. In: A.M.Q. King, E. Lefkowitz, M.J. Adams, E.B. Carstens (Eds.) *Virus Taxonomy* (9th Report of the ICTV), Elsevier Academic Press, San Diego, 913-921.

Hammond R., Ramirez P., 2001. Molecular characterization of the genome of Maize rayado fino virus, the type member of the genus *Marafivirus*. Virology 282: 338-347.

Maccheroni, W., Alegria, M. C., Greggio, C. C., Piazza, J. P., Kamla, R. F., Zacharias, P. R., Bar-Joseph, M., Kitajima, E. W., Assumpcao, L. C., Camarotte, G., Cardozo, J., Casagrande, E. C., Ferrari, F., Franco, S. F., Giachetto, P. F., Girasol, A., Jordao, H., Jr., Silva, V. H., Souza, L. C., Aguilar-Vildoso, C. I., Zanca, A. S., Arruda, P., Kitajima, J. P., Reinach, F. C., Ferro, J. A., and da Silva, A. C. 2005. Identification and genomic characterization of a new virus (*Tymoviridae* family) associated with citrus sudden death disease. J. Virol. 79:3028-3037.

Martelli, G.P., Sabanadzovic, S., Abou Ghanem-Sabanadzovic, N., Edwards, M. C., and Dreher, T. 2002. Virology division news: The family *Tymoviridae*. Arch. Virol. 147:1838-1846.

Nicolini, C., Inoue-Nagata A.K., Nagata T., 2015. Complete genome sequence of a proposed new tymovirus, tomato blistering mosaic virus. Arch. Virol. 160, 609–612.

Pagan, I, Betancourt, M., de Miguel, J., Pinero, D., Fraile, A and Garcia-Arenal, F. 2010. Genomic and biological characterization of chiltepin yellow mosaic virus, a new tymovirus infecting *Capsicum annuum* var. aviculare in Mexico. Arch Virol 155:675-684.

Villamor, D. E. V., Mekuria, T. A., Pillai, S. S., and Eastwell, K. C. 2016. High-throughput sequencing identifies novel viruses in nectarine: Insights to the etiology of stem-pitting disease. Phytopathology 106:519-527.

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. Schematic representation of the nectarine virus M (isolate 15C2) genome organization with key nucleotide coordinates. Grey shaded box depicts large ORF and corresponding putative polyprotein, lines represent untranslated genomic regions at the genome extremes. Abbreviations: MTR = methyltransferase, PRO = endopeptidase/protease, Hel = helicase, RdRp = RNA-dependent RNA polymerase, CP = coat protein, box = "marafibox".

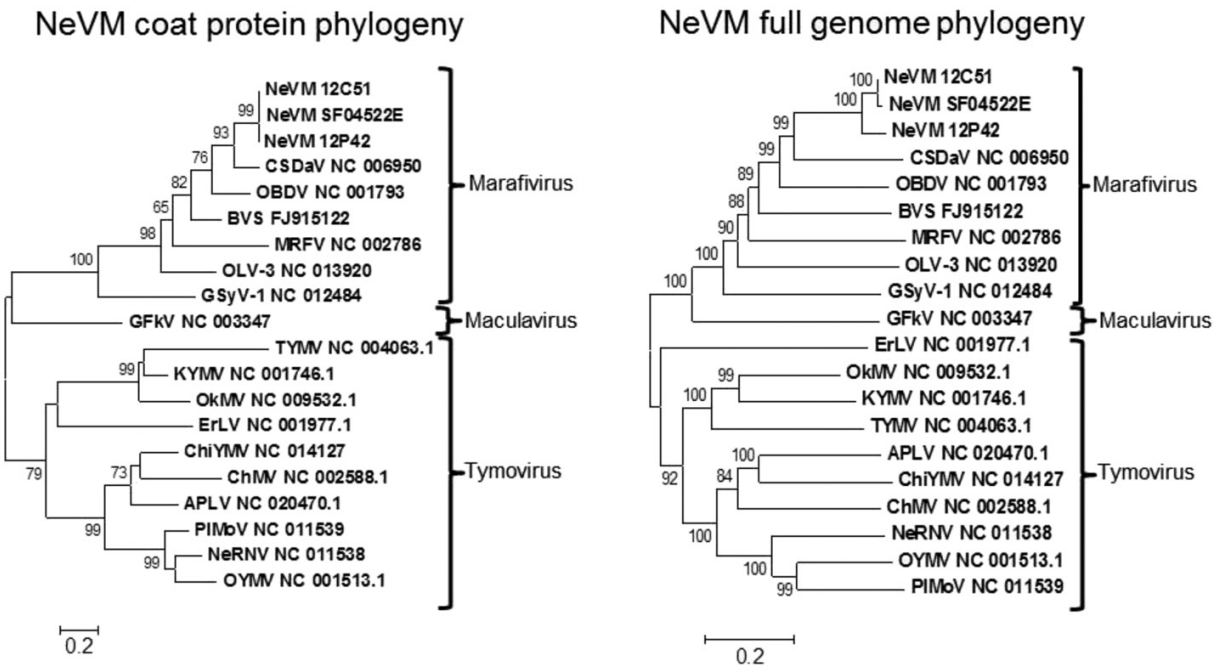

Figure 2. Maximum Likelihood phylogenetic trees constructed on the coat protein (CP) amino acids and full genomic sequences of the nectarine virus M (NaVM) and viruses classified in the family *Tymoviridae*. Trees were constructed with MEGA 6 software by applying JTT (CP) and Tamura Nei (full genome) models. Branching nodes with >60% bootstrap support out of 1000 replicates are shown. Figure taken from Villamor et al., 2016 (Phytopathology)

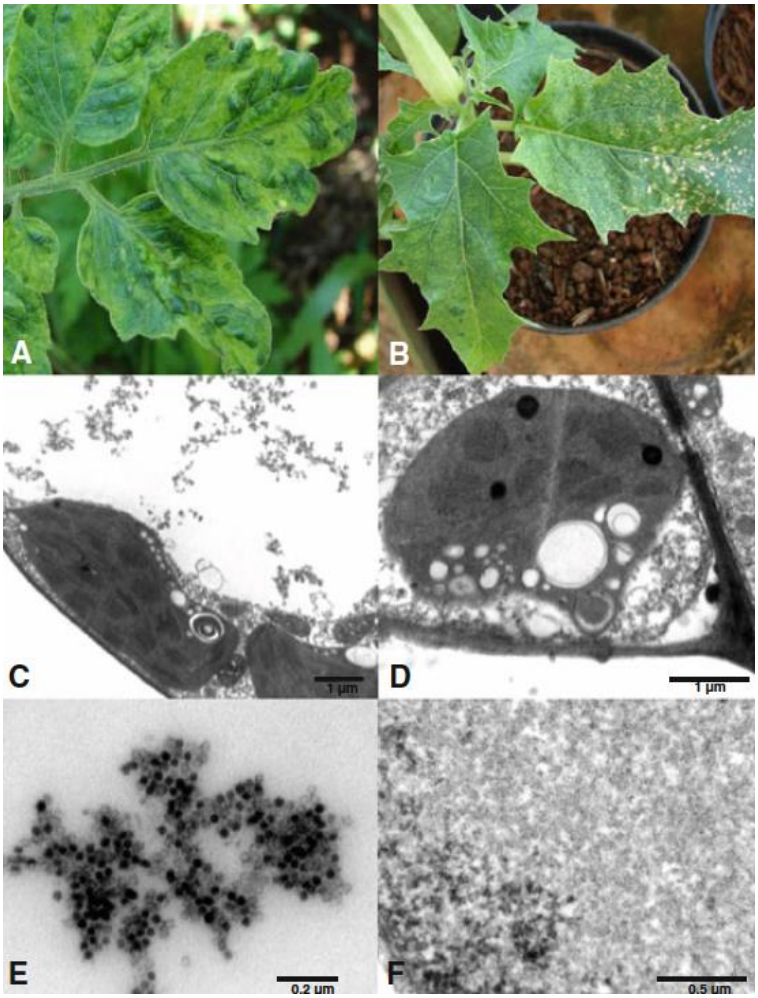

Figure 3. Symptoms caused by infections by an isolate of tomato blistering mosaic virus on naturally infected tomato (panel A) and artificially inoculated Jimson weed (*Datura stramonium*) (B). Negative stained electron micrographs of thin-sections of spongy parenchyma cells of *Nicotiana benthamiana* plant infected by ToBMV showing presence of putative virions in the vacuole (C and enlarged detail in E) or chloroplasts with intense vesicularization (panel D). Panel F shows particles, interpreted as virions, in a nucleus of palisade parenchyma. Figure taken from De Oliveira et al., 2013 (Virus Genes 46:190-194)

Figure 4. Schematic representation of the ToBMV (isolate SC-05) genome organization closely resembling those of recognized tymoviruses. The three putative ORFs with corresponding products are illustrated. The figure is not to scale. Modified from Nicolini et al., 2015 (Arch Virol 160: 609-612).

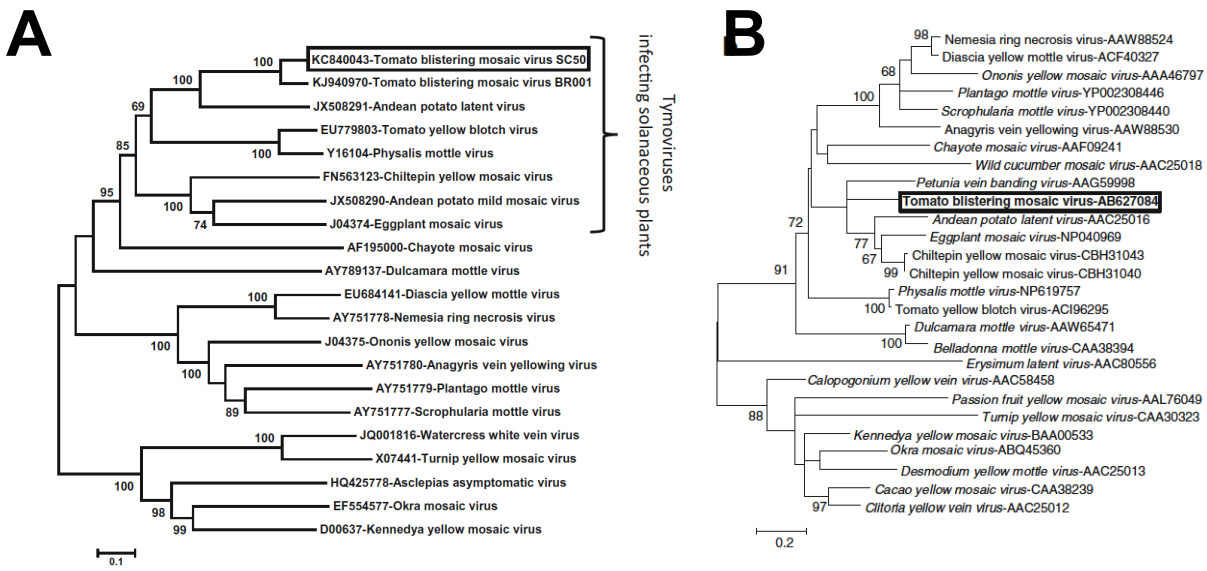

Figure 5. **A.** Phylogenetic tree of tymoviruses based on the complete genome sequences. Tree was constructed with MEGA 6 program applying ML method and General Time-Reversible (GTR) model. **B.** Phylogenetic tree based on the amino acid sequences of viral CPs of tymoviruses. Position of ToBMV isolate SC50 is presented by box. Numbers on branch nodes represent bootstrap values out of 1000 replicates. Panels A and B taken from Nicolini et al., 2015 and De Oliveira et al., 2013, respectively

Table 1. Percent (%) nucleotide and amino acid identities of ToBMV-SP01 with other two isolates of the same virus and closely related tymoviruses. Notice that aa identity more than 95% aa identity among the three ToBMV isolates and values lower than 70% with closest tymoviruses. (modified, from Blawid et al., 2016)

Virus[a]	Complete genome (%)	5′-UTR (%)	ORF1 nt % (PP aa %)	ORF2 nt % (MP aa %)	ORF3 nt % (CP aa %)	3′-UTR (%)
ToBMV tomato	76.6	72.6	76.1 (85)	78.2 (54.2)	79.2 (95.3)	95.1
ToBMV tobacco	76.3	60.9	76 (84)	77.9 (53.1)	80.5 (95.3)	88.6
APLV	67.6	68.3	67 (69.6)	67.1 (40.4)	68.4 (68.9)	85.8
ToYBV	61.3	50.7	61.1 (57.4)	60.5 (30.9)	66 (61.1)	63.3
PhyMoV	61.1	49.4	61.1 (57.6)	60.6 (31.5)	63 (62.6)	64.7
ChiYMV	61.1	54.2	61 (56.1)	59.6 (30.9)	65 (64.7)	56.1
APMMV	62.6	50.3	62.8 (62.9)	60.9 (30.5)	64.2 (63.2)	62.2
EMV	62.8	52.9	62.9 (62.2)	62.7 (33.6)	42.1 (64.7)	64.7

PP polyprotein; *MP* movement protein; *CP* coat protein

[a] Tomato blistering mosaic virus, ToBMV Tomato (SC50), KC840043; ToBMV Tobacco (BR001), KJ940970; Andean potato latent virus, APLV, JX508291; Tomato yellow blotch virus, ToYBV, EU779803; Physalis mottle virus, PhyMoV, Y16104; Chiltepin yellow mosaic virus, ChiYMV, FN563123; Andean potato mild mosaic virus, APMMV, JX508290, Eggplant mosaic virus, EMV, J04374