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:	***2010.006aP***	(to be completed by ICTV officers)

Short title: create species *Bombyx mori latent virus* to be unassigned in the family *Tymoviridae*, order *Tymovirales*
(e.g. 6 new species in the genus *Zetavirus*)

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

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

Edwards, Michael (michael.edwards@ars.usda.gov); Hammond, Rosemarie (rose.hammond@ars.usda.gov)
on behalf of the Tymoviridae Study Group

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)	Tymoviridae

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

Proposal 2010.006aP.v1 considered at EC42, Paris in 2010, was a proposal to create a species in the genus Maculavirus, family Tymoviridae.

The EC commented: assignment to family probably OK but is there sufficient evidence to place the species in this monotypic genus? Also, allow time for comments from insect virus SC.

The insect SC has no comments to add.

Response from SG: we have reconsidered this in the light of further phylogenetic analyses provided here and are now proposing this to be a species unassigned in the family. New phylogenetic trees are provided and additional comments are in red.

Date first submitted to ICTV:	27 May 2010
Date of this revision (if different to above):	10 August 2011

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	***2010.006aP***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	**Unassigned**
Subfamily:	
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.

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Bombyx mori latent virus	AB186123

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

Key genus demarcation criteria of the family *Tymoviridae* are (see 8th ICTV Report, Dreher et al. (2005):

- 30 nm non-enveloped icosohedral particles showing the presence of both full and empty (stain-penetrating) particles when observed by negative staining EM;
- 6 – 7.5 kb positive sense RNA genome

Within the genera of the *Tymoviridae*, species demarcation criteria include:

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

Members of the genus *Tymovirus:*

- Have genome sizes of 6.0 – 6.7 kb and a single 0.6 kb subgenomic RNA
- Have genomes encoding 3 proteins (1) RP (replicase), (2), OP, a 50-80 kDa proline-rich protein that serves as a movement protein and RNAi silencing suppressor, and (3) a 20 kDa CP.
- Have a 17 nt tymobox sequence involved in subgenomic RNA synthesis
- Mechanically transmissible
- Have a non-polyadenlyated genome, typically with a tRNA-like structure at the 3'end
- Spread to all main tissues of the host

Members of the genus *Marafivirus*:

- Have a 6.3 – 6.8 genome size and a 0.6 – 1.0 kb subgenomic RNA
- Have genomes with a large polyprotein that includes the MTR, PRO, HEL, POL, and CP domains; a second ORF may be present
- Have a 16 nt marafibox related to, but distinct, from the tymobox

- Have a polyadenylated genome
- Have particles composed of major and minor CP forms (21 kDa and 23-25 kDa, respectively)
- Are phloem restricted and not usually mechanically transmissible
- In some cases (OBDV, MRFV, BELV) are vectored by insects in a persistent-propagative manner with replication occurring in the insect.

Members of the genus *Maculavirus*:
There is only one species member of this genus: *Grapevine fleck virus* (GFkV) (Sabanadzovic et al., 2001). The *Maculavirus* genus (Martelli et al., 2002) is characterized by:

- A genome of 7.5 kb excluding the polyA tail and two putative subgenomic RNAs of 1.3 and 1.0 Kb.
- The genome contains four putative open reading frames (ORF): a large polyprotein ORF of 215 kDa (ORF1) with conserved motifs of replication-associated proteins of positive-strand RNA viruses, including MTR, PRO (papain-like proteinase), HEL, and POL domains, the coat protein (CP) (ORF2), and two proline-rich proteins of 31 kDa (ORF3) and 16 kDa (ORF4) located at the extreme 3' end of the viral genome, and with unknown function.
- CP ORF is separate from the replicase polyprotein ORF.
- Replication-associated proteins and CP are phylogenetically related to but distinct from genera *Tymovirus* and *Marafivirus*.
- Has a 3' polyadenylated genome.
- ORF 1 lacks the highly conserved 16 nt long subgenomic RNA promoter referred to as "tymobox" or "marafibox".
- Non-mechanically transmissible and phloem-limited; graft transmissible.

Bombyx mori macula-like latent virus (BmMLV) was described with some properties that might justify its designation as a new species of the genus *Maculavirus*
BmMLV was initially identified in *Bombyx mori* (silk moth) cultured cells (BmN cell line) by cDNA microarray (Katsuma et al., 2005). The full-length sequence of the latent virus was cloned and determined to be 6,513 bp in length, excluding the polyA tail. Putative isometric virions of 28 – 30 nm are infectious to SF-9 cells and tissues of *B. mori* larvae.

- The genome encodes three putative ORFs: ORF1 encodes 1,747 amino acid residues and contains the MTR, PRO (papain-like proteinase), HEL and POL conserved regions of the tymovirus replicase polyprotein. It shares 44% amino acid identity to the replicase of GFkV. ORF 2 encodes the CP of 237 amino acid residues (25 kDa), and shares 41% amino acid sequence identity to the CP of GFkV. The CP is expressed from a 1.25 kb subgenomic RNA. In addition, a third putative ORF (ORF3), located at the 3' terminus of the genome, encodes a putative 15 kDa protein.
- CP ORF is separate from the replicase polyprotein ORF.
- The genome is polyadenylated at the 3' end.
- ORF 1 lacks the highly conserved 16 nt long subgenomic RNA promoter referred to as "tymobox" or "marafibox".
- Phylogenetic analysis of the replicase polyprotein and CP sequences demonstrates that BmMLV belongs within the family *Tymoviridae*, is distinct from the tymoviruses and marafiviruses, and is most closely related to GFkV (Katsuma et al., 2005; Sabanadzovic et al., 2001).

Thus, virion size, genome organization and features, sequence identity, and phylogenetic analysis (Figure 1 – N.B. this figure in v1 has now been replaced) support the designation of BmMLV as a new member of the genus *Maculavirus*.

Note that the Study Group accepts that the term "macula-like" should be dropped from the official species name of the virus.

EXTRA COMMENTS added August 2011 for version 2 of this proposal.

Phylogenetic analyses of the replicase polyprotein and the coat protein confirm that BmLV should be a

member of the family *Tymoviridae*. While GFkV is its nearest relative, there is no bootstrap support for placing it in the genus *Maculavirus* (which is monotypic). In the absence of data on related viruses to justify the creation of a new genus, it is suggested that it should become a species unassigned in the family.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

1. Martelli, G. P., Sabanadzovic, S., Abou Ghanem-Sabanadzovic, N., and Saldarelli, P. Maculavirus, a new genus of plant viruses. Archives of Virology 147, 1847-1853, 2002.
2. Katsuma, S., Tanaka, S., Omuro, N., Takabuchi, L., Daimon, T., Imanishi, S., Yamashita, S., Iwanaga, M., Mita, K., Maeda, S., Kobayashi, M., and Shimada, T. Novel macula-like virus identified in Bombyx mori cultured cells. Journal of Virology 79, 5577-5584, 2005.
3. Sabanadzovic, S., Abou Ghanem-Sabanadzovic, N., Saldarelli, P., Martelli, G.P., 2001. Complete nucleotide sequence and genomic organization of Grapevine fleck virus. J. Gen. Virol. 82, 2009-2015.

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 (neighbor-joining) tree showing the relationship of BmMLV (sequences of two isolates shown in red) to other species and genera of the family *Tymoviridae.* The tree was constructed in MEGA5 using the maximum composite likelihood method and 10,000 bootstrap replicates (percentage values shown at branches where >60%) and was based on the codon-aligned **replication polyprotein** nucleotide sequence and rooted with Botrytis virus F, genus *Mycoflexivirus*, family *Gammaflexiviridae*, order *Tymovirales.* Colors are used to show approved members of the 3 genera and the unassigned species.

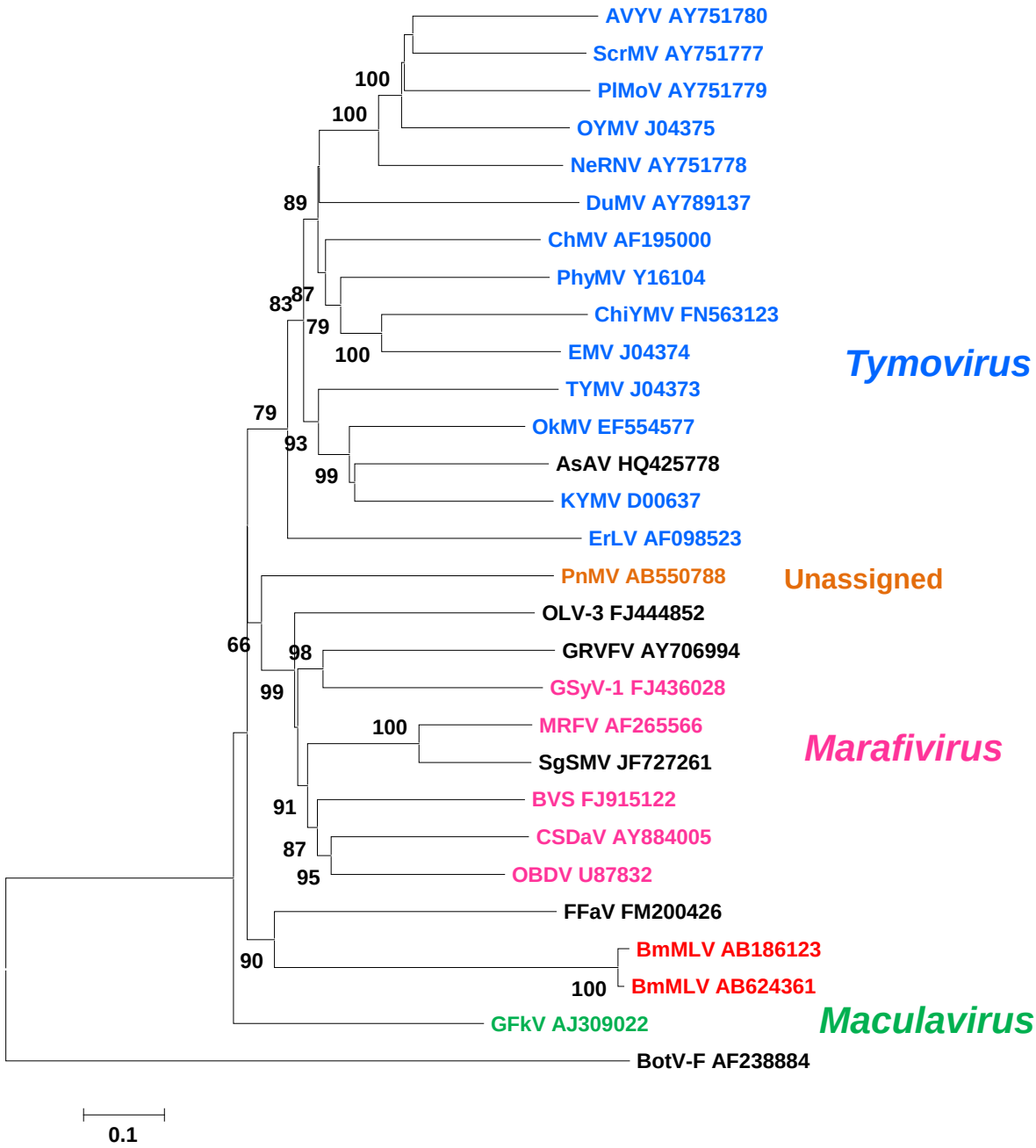

Figure 2. Phylogenetic (neighbor-joining) tree of the codon-aligned **coat protein** nucleotide sequences of members of the family *Tymoviridae* showing their relationship to BmMLV (sequences of two isolates shown in red). The unrooted tree was constructed in MEGA5 using the maximum composite likelihood method and 10,000 bootstrap replicates (percentage values shown at branches where >60%).