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:	***2010.007aP***	(to be completed by ICTV officers)

Short title: To create a new species in the genus Marafivirus, fam. Tymoviridae
(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:

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on behalf of the Tymovirdae 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:

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	***2010.007aP***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Marafivirus***
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:**
Blackberry virus S	FJ915122

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

According to the 8th ICTV Report (Dreher et al., 2005), the current species demarcation criteria in the genus *Marafivirus* 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
- Presence of a marafibox
- Cytopathological features

Blackberry virus S (BlVS) was originally identified in diseased samples of wild blackberries during a study on viruses of native *Rubus* spp in the Great Smoky Mountains National Park (Tennessee and North Carolina). It was later detected in production fields in Mississippi (Fig. 1A), indicating that it may be relatively widespread in Southeastern United States. The virus was partially purified, molecularly characterized and diagnostics were developed (Sabanadzovic and Abou Ghanem-Sabanadzovic, 2009).

BlVS properties

- **Virus particles**: isometric, ~ 30 nm in diameter. Two types of virus particles observed in partially purified preparations by electron microscopy: empty and intact particles with prominent surface structures resembling members of the family *Tymoviridae* (Fig. 1B).
- **Genome:** 6,463 nt-long (excluding 3' polyA tail) polyadenylated, cytosine rich (38%), single stranded RNA (full genomic sequence is available under accession number FJ915122) containing a single open reading frame (ORF) coding for a 222.5 kDa putative product (p223). The N-terminal portion of p223, identified as replication-associated polyprotein, contains conserved motifs of methyltransferase (MTR), endopeptidase/protease (PRO), helicase (Hel) and RNA-dependent RNA polymerase (RdRp or Pol). The C-terminal portion is involved in the formation of two viral coat protein (CP) subunits with deduced molecular masses of ~ 23 and 21 kDa (by proteolytic processing and via subgenomic RNA expression, respectively, based on the properties of other marafiviruses). The genome contains

conserved “marafibox” sequence that serves as a sub-genomic RNA (sgRNA) promoter (Fig. 2). Northern hybridization, applying 3’ end-proximal DIG-labled probe, detected a putative subgenomic RNA of ~ 0.8 kb in BlVS-infected tissue (Fig. 1C).

- **Relationships with closely related species:** Overall nucleotide identity with other members of the genus *Marafivirus* ranges from 56% (GSyV-1/GVQ) to 61% (OBDV). The replication-associated polyprotein shares 60% common amino acids with *Citrus sudden death-associated virus* (CSDaV), 59% with *Oat blue dwarf virus* (OBDV) and 56% with *Maize rayado fino virus* (MRFV). The same polyprotein shares similar levels of overall identity with replicases of the maculavirus *Grapevine fleck virus* (43%) and various tymoviruses (43-45%). Conservation of amino acid content for different domains in BlVS replicase is presented in Table 1. The two viral coat proteins, shared identities with marafiviruses ranging from 44-62% (for details see Table 1), 28-29% with maculaviruses and 21-24% with tymoviruses. These comparisons indicate that BlVS is distinct from all recognized marafiviruses. The BlVS genome contains a marafibox sequence identical (100%) to the corresponding region in GAMaV, OBDV and BELV. Furthermore, BlVS coat protein contains a stretch of 45 aa highly conserved in CSDaV and some other marafiviruses (Fig. 3). These properties support membership in the *Marafivirus* genus.
- **Phylogeny:** In phylogenetic trees constructed for viral polymerases or coat proteins BlVS grouped with other members of the genus *Marafivirus*. In both scenarios, BlVS appeared closer to OBDV and CSDaV (as well as with GAMaV in available genomic portions) than with monocot-infecting marafiviruses (MRFV and BELV) (Fig. 4 and not shown).
- **Serology**: no information.
- **Mechanical transmission:** negative (either from sap or partially purified preparations).
- **Vector transmission**: no information.
- **Cytopathology**: no information.
- **Natural host range**: cultivated and wild *Rubus* spp.

Available information on particle morphology, together with genomic and phylogenetic data, suggest that BlVS belongs to a novel species in the genus *Marafivirus*.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Dreher T.W., Edwards M.C., Gibbs A.J., Haenni A-L., Hammond R.W., Jupin I., Koenig R., Sabanadzovic S., Abou Ghanem-Sabanadzovic N., Martelli G.P. (2005). Family Tymoviridae. In Fauquet C.M., Mayo M., Maniloff J., Desselberger U., Ball L.A. (Eds.): Virus Taxonomy (Eight Report of the ICTV). Elsevier/Academic Press, London pp 1061-1074.

Sabanadzovic S., Abou Ghanem-Sabanadzovic N. (2009). Identification and molecular characterization of a marafivirus in Rubus spp. Archives of Virology 154, 1729-1735.

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.

Table 1. Amino acid identity levels (%) between BlVS and some members of the fam. *Tymoviridae.* The highest value for each domain is indicated in red.

	Percentage (%) identity			
	MTR	HEL	RdRp	CP
OBDV	**68**	66	**85**	**62**
MRFV	61	62	77	51
CSDaV	65	**68**	81	**62**
GSyV-1 (GVQ)	50	66	65	39
GAMaV	67*	NA	80	57
GRVFV	55	64	74	44
GFkV (maculavirus)	47	55	68	28
tymoviruses	51-55	49-53	63-68	21-27

* - only partial sequences available for GAMaV.

Figure 1. A: Vein-clearing symptoms observed in a BlVS infected blackberry specimen. **B:** Partially purified preparation showing the presence of putative empty and intact (arrow) BlVS particles (negative staining EM). **C:** Northern blot hybridization with a 3' end-proximal probe showing two signals in nucleic acid extracts from BlVS-infected plants (lanes 1 and 2) assumed to represent full-genomic and subgenomic RNAs. Their relative sizes were estimated by comparison with DIG-labeled Molecular Weight Marker (Roche, USA) used as a reference (not shown). No signal present in controls (lane 3).

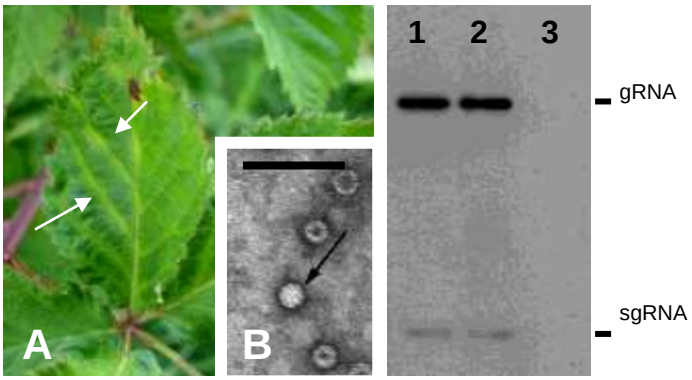

Figure 2. Diagrammatic representation of the monocistronic BlVS genome with nucleotide coordinates.

Figure 3. Highly conserved stretch of 45 aminoacids present in coat proteins of BlVS and several other marafiviruses.

```
BlVS   113PLAPSFSKPISVGAVWTIASISPASAHEQSYYGGRLLTLGG
CSDaV  119 PLAAAFSKPISVSAVWTIASISPASASETSYYGGRLFTVC
OBDV     134 PLAAAFAKPISVTAVWTIASIAPATTTELQYYGGRLLT
GAMaV    120 PLAGSFSKPITLSAVWTVGSITPATTTETSYYGGRVIT
             *** :*:***:: ****:.**:**:: * .******:*
```

Figure 4. Unrooted phylogenetic tree showing the relationships between BlVS and some members of the family *Tymoviridae*. Trees were generated by the neighbor-joining method on coat protein sequences. Bootstrap values are indicated at main branch points.