MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	*2012.010aP*	(to be completed by ICTV officers)

Short title: create one new species in the genus *Benyvirus*
(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:

David Gilmer (gilmer@unistra.fr) Claudio Ratti (claudio.ratti@unibo.it) on behalf of the *Virgaviridae* and *Benyvirus* SG

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: 2 July 2012
Date of this revision (if different to above):   3 October 2012

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	*2012.010aP*	(assigned by ICTV officers)

To create 1 new species within:

		Fill in all that apply.
Genus:	*Benyvirus*	• If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
Subfamily:		
Family:	**Unassigned**	• If no genus is specified, enter "**unassigned**" in the genus box.
Order:		

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Rice stripe necrosis virus	RNA1: EU099844 ; RNA2: EU099845

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 *Benyvirus* contains viruses with multipartite ssRNA+ genomes encapsidated in rigid rod shaped particles. The viruses are vectored by fungoid protists in the genus *Polymyxa*. Although these properties are shared by some members of the family *Virgaviridae*, benyviruses are unique in having a single ORF on RNA1 that encodes the replication-associated protein (terminating in a Sindbis-like RdRp domain) and in having a capped and polyadenylated RNA genome. The RdRp (and the entire replication protein) is only distantly related to those possessed by members of the *Virgaviridae*. Benyvirus RNAs share sequence similarities within the last 70 nts preceding the polyA sequence. Benyvirus RNA2 components show similar genomic organization and contain 6 ORFs. The first is the major coat protein of about 21 kDa, the second corresponds to the minor coat protein component produced from a read-through of the first ORF stop codon leading to a CP-RT protein of about 75 kDa that possesses a KTER domain required for vector transmission. The three following ORFs constitute the "triple gene block" required for cell-to cell movement. The last ORF encodes a cysteine rich protein. RNA3, RNA4 and RNA5 (which is not present in all isolates) are smaller RNAs each encoding a single protein with roles in transmission and symptom development.

The genus currently contains two species that naturally infect sugar beet, *Beet necrotic yellow vein virus* (type species) and *Beet soil-borne mosaic virus*. These have less than 60% amino acid identity between their coat proteins and about 84% identity between their replication-associated proteins. The formal species demarcation criteria are that distinct species should have coat protein sequences less than 90% identical, distant serological relations and distinct host ranges.

The proposed new species has particle morphology and organization typical of a member of the genus *Benyvirus* but has a different (monocot) host. The complete sequences of RNA1 and 2 have been determined from an isolate collected on rice in Colombia (see genome organization in Annex Figure 1). The conserved RdRp domain of the replication protein has only about 67% aa identity to those of the two sugar-beet infecting benyviruses. Phylogenetic trees for the core RdRp domain and the CP of benyviruses are shown in Figure 2 and comparisons amongst the proteins encoded by RNA2 in Table 1. These all show that RSNV should be regarded as a member of the genus *Benyvirus*, but that it significantly exceeds the molecular demarcation criteria and should therefore be classified as a distinct species. Further phylogenetic trees showing the relationships of the CP and RdRp to those of other rod-shaped viruses are provided in Figure 4 of Lozano & Morales, 2009.

MODULE 9: **<u>APPENDIX</u>**: supporting material

additional material in support of this proposal

References:

Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J. F., Guindon, S., Lefort, V., Lescot, M., Claverie, J. M., and Gascuel, O. (2008). Phylogeny.fr: Robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 36:465-469.

Katoh, K. and Toh, H. (2008). Recent developments in the MAFFT multiple sequence alignment program. Brief. Bioinf. 9:286-298.

Lozano, I. and Morales, F. (2009). Molecular characterisation of Rice stripe necrosis virus as a new species of the genus *Benyvirus*. European Journal of Plant Pathology 124:673-680.

Ratti, C., Hleibieh, K., Bianchi, L., Schirmer, A., Autonell, C.R., and Gilmer, D. (2009). Beet soil-borne mosaic virus RNA-3 is replicated and encapsidated in the presence of BNYVV RNA-1 and -2 and allows long distance movement in *Beta macrocarpa*. Virology 385:392-399.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24:1596-1599.

Table 1. Comparative amino acid sequence identity analyses of the proteins encoded by RNA2 of current and proposed members of the genus *Benyvirus*.

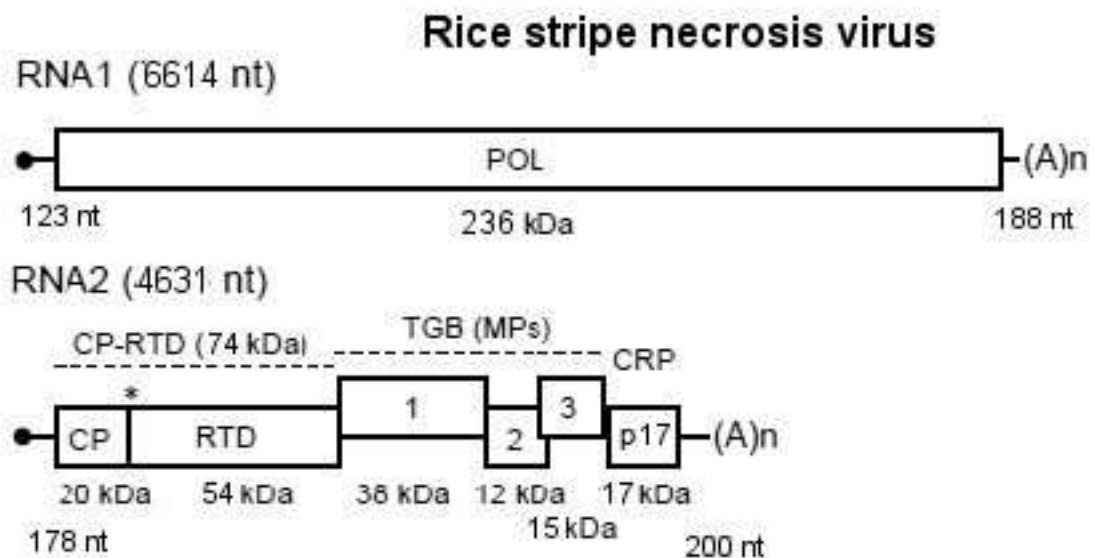| Viral protein	Identity (%)		
	RSNV v BNYVV	RSNV v BSBMV	BNYVV v BSBMV
CP	27	25	58
RTD	23	25	58
TGB1	37	37	75
TGB2	61	60	82
TGB3	58	31	66
CRP	20	19	38

Figure 1. Genome organization of RSNV RNAs 1 and 2, and their open-reading frames (ORFs, shown as rectangles), as deduced by cDNA sequence analysis.
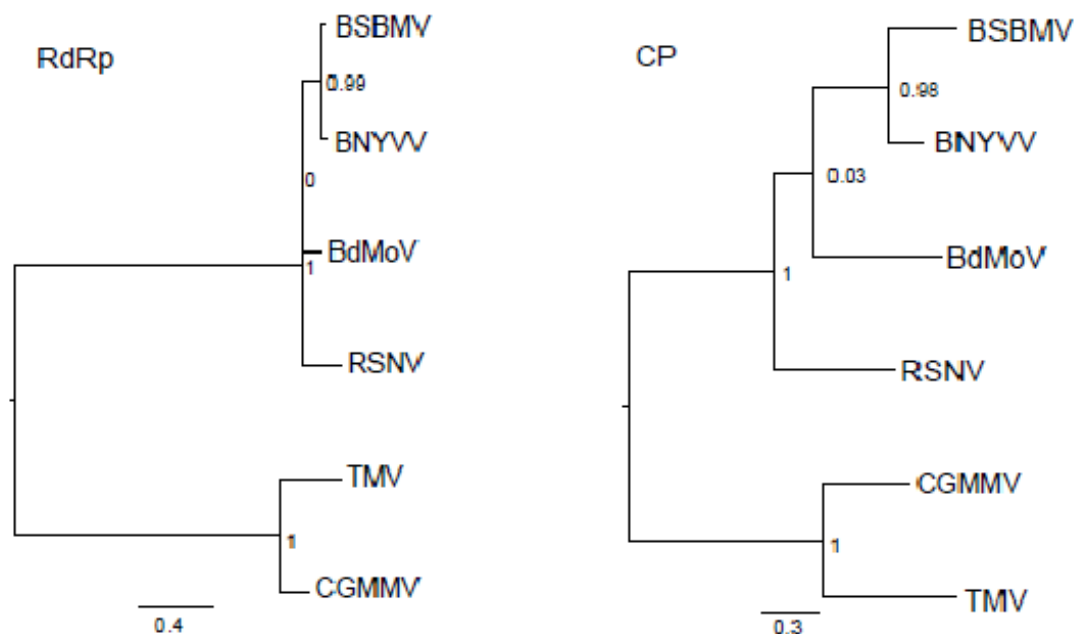
Figure 2. Phylogenetic analyses of Benyviruses based on amino acid sequences of RdRp motif and CP. Amino acid sequences of the core RdRp motif and CP from isolates of *Beet necrotic yellow vein virus* (BNYVV; respective accessions D84410 and D84411) and *Beet soil-borne mosaic virus* (BSBMV; AF280539 and AF061869) and the proposed species *Rice stripe necrosis virus* (RSNV; EU099844 and EU099845) were aligned with those of tobamoviruses *Tobacco mosaic virus* (TMV; V01408) and *Cucumber green mottle mosaic virus* (CGMMV; D12505) using MAFFT version 6 under the default parameters (Katoh and Toh 2008). Sequences of Burdock mottle virus (BdMoV) also included in the analysis are not yet publically available and this virus will the subject of a later taxonomic proposal. Alignments were edited for removing gaps by using MEGA4 version 4.02 software (Tamura *et al*. 2007). ML tree inferences were carried out with PhyML 3.0 (Dereeper *et al*. 2008), employing the general time reversible substitution model, gamma parameter estimated, number of substitution categories = 4, initial tree BIONJ and type of tree improvement NNI. The ML bootstrap support was calculated from 100 bootstrap replicates and shown on the nodes as 0~1. The obtained tree was visualized using FigTree v1.3.1 software. Both the trees clearly indicate the association of RSNV (and BdMoV) to the genus *Benyvirus*.