



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:	2013.001aP	(to be completed by ICTV officers)			
Short title: <i>create Raspberry leaf blotch virus as new species in the genus Emaravirus</i> (e.g. 6 new species in the genus Zetavirus)					
Modules attached (modules 1 and 9 are required)	1 <input checked="" type="checkbox"/>	2 <input type="checkbox"/>	3 <input type="checkbox"/>	4 <input type="checkbox"/>	5 <input type="checkbox"/>
	6 <input type="checkbox"/>	7 <input type="checkbox"/>	8 <input type="checkbox"/>	9 <input checked="" type="checkbox"/>	

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

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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 (fungal, invertebrate, plant, prokaryote or vertebrate viruses)

Emaravirus study group

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	2013.001aP	(assigned by ICTV officers)
To create 1 new species within:		
Genus:	<i>Emaravirus</i>	Fill in all that apply. <ul style="list-style-type: none"> • 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:		
Order:		
And name the new species: Raspberry leaf blotch virus		GenBank sequence accession number(s) of reference isolate:
		FR823299-FR823303

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

Species demarcation criteria for the genus *Emaravirus* are:

1. Differences in relevant gene product sequences of more than 25%
2. Differences in host ranges
3. Differences in vector specificities

As outlined in the following section in detail, Raspberry leaf blotch virus meets all the species demarcation criteria.

A yellow blotch symptom of raspberry leaves associated with infestation of the raspberry leaf and bud mite (*Phyllocoptes gracilis*) was reported in the early part of the twentieth century (Masse, 1924). The blotching and twisting of affected leaves was similar to symptoms caused by virus infection but in a study of symptomatic tayberry plants (raspberry x blackberry hybrid) it was found that treatment of plants with an insecticide prevented reoccurrence of the symptoms (Jones *et al.*, 1984), apparently confirming that the disease was induced by mite feeding and not by virus infection. Nevertheless, recent investigations of leaf blotch-affected raspberry plants, using molecular methods to randomly amplify, clone and sequence double-stranded RNA isolated from these plants, identified negative-strand RNAs with similarities to those of other viruses including European mountain ash ringspot-associated virus (EMARAV) (McGavin *et al.*, 2012). This study identified five viral RNAs, although subsequent work has identified an additional three RNAs, giving eight in total at present (S. MacFarlane, unpublished).

The virus, for which the name Raspberry leaf blotch virus (RLBV) was proposed, could be transferred by mechanical inoculation to *Nicotiana benthamiana*, causing yellowing of

systemically-infected leaves, and was transmitted (most likely) by mites after laying a mite-infested, virus-infected raspberry leaf onto a leaf of a second, healthy raspberry plant. The recipient raspberry plants, which were maintained in an enclosed growth chamber, did not develop the full symptoms (extensive yellow blotching and development of necrotic patches) that are seen in the field suggesting that other factors, perhaps environmental, may be involved in the disease syndrome. However, both the recipient raspberry plants and *N. benthamiana* plants were shown by RT-PCR to be infected with the RLBV RNAs. Also, testing of yellow blotch-affected raspberry plants sampled in England, Scotland, Finland, Serbia, Montenegro and Bulgaria (Irena Mavrič Pleško, personal communication) has revealed the presence of RLBV in these countries.

RLBV properties

Virus particles: no EM studies done for this virus

(i) dsRNAs: not directly observed by gel electrophoresis but dsRNA purification protocol yielded amplifiable nucleic acid

(ii) RNA-dependent RNA-polymerase - 269 kDa; glycoprotein precursor - 75 kDa; Nucleocapsid protein - 32 kDa; P4 movement protein – 42 kDa; P5 (function unknown) 56 kDa; P6 (function unknown) 22 kDa; P7 (function unknown) 22 kDa; P8 (function unknown) 27 kDa; (determined from deduced sequence data; McGavin *et al.*, 2012 and unpublished)

(iii) Nucleic acid: (at least) eight molecules of negative sense ssRNA. RNA 1- 7062nt, RNA 2 – 2135nt, RNA 3-1356nt, RNA4-1675nt , RNA5-1718nt , RNA6-1095nt , RNA7-1089nt , RNA8-1271nt (Accession numbers FR823299-FR823303, RNA1-5, respectively)

(iv) Genome: octopartite, possibly larger, 8 ORFs, one in each complementary strand of the characterized negative-sense RNAs

Genome structure resembling that of members of the genus *Emaravirus*

(v) Phylogenetic relationships: RLBV groups with members of the genus *Emaravirus* in trees constructed with RdRp sequences and, thereafter, is related to plant and animal viruses in the family *Bunyaviridae*. At the amino acid level, the RLBV RDRP did not show amino acid identities higher than 68% to the RDRP of other emaraviruses (Mielke-Ehret & Mühlbach, 2012). (meets species demarcation criterion 1)

(vi) Mechanical transmission: Demonstrated from raspberry to *Nicotiana benthamiana* and intermittently from *N. benthamiana* to *N. benthamiana*

(vii) Possible transmission with *Phyllocoptes gracilis* (McGavin *et al.*, 2012) (meets species demarcation criterion 3) but not demonstrated using individual mites

(viii) Natural host range: Detected in red raspberry (*Rubus idaeus*), not yet surveyed in other *Rubus* species (meets species demarcation criterion 2)

The above data support the notion that RLBV is a distinct species in the unassigned genus *Emaravirus*. The genus *Emaravirus* currently consists of three species *European mountain ash ringspot-associated virus*, *Fig mosaic virus* and *Rose rosette virus*.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Jones, A.T., Gordon, S.C & Jennings, D.L. (1984). A leaf-blotch disorder of tayberry associated with the leaf and bud (*Phyllocoptes gracilis*) and some effects of three aphid-borne viruses. *J. Hort. Sci.* 59, 523-528.

Massee, A.M (1924). The leaf and bud mite of raspberry (*Eriophyes gracilis* Nal.). *J. Pomol. Hort. Sci.* 4, 59-61.

McGavin, W.J., Mitchell, C., Cock, P.J.A, Wright, K.M. & MacFarlane, S.A.(2012). Raspberry leaf blotch virus, a putative new member of the genus *Emaravirus*, encodes a novel genomic RNA. *J. Gen. Virol.* 93, 430 – 437.

Mielke-Ehret, N. & Mühlbach, H.P. (2012). *Emaravirus*: a novel genus of multipartite, negative strand RNA plant viruses. *Viruses* 4, 1515-1536.

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.

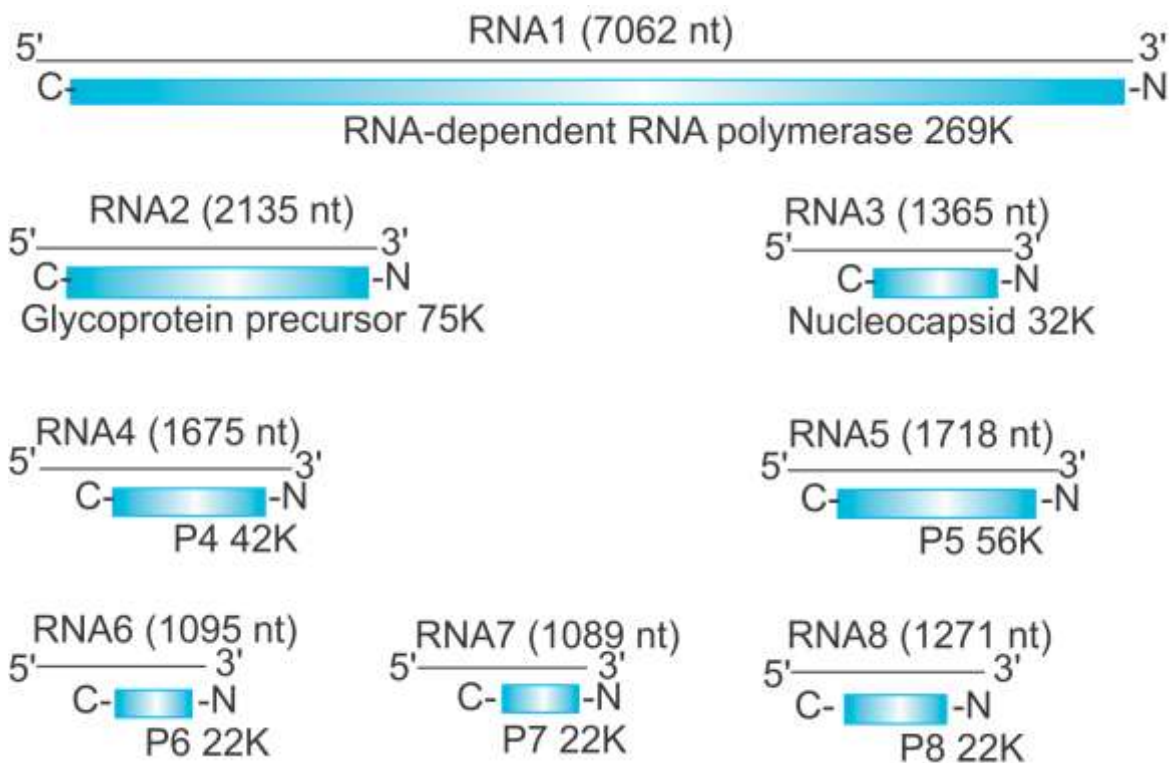


Fig 1. Genome organization of RLBV

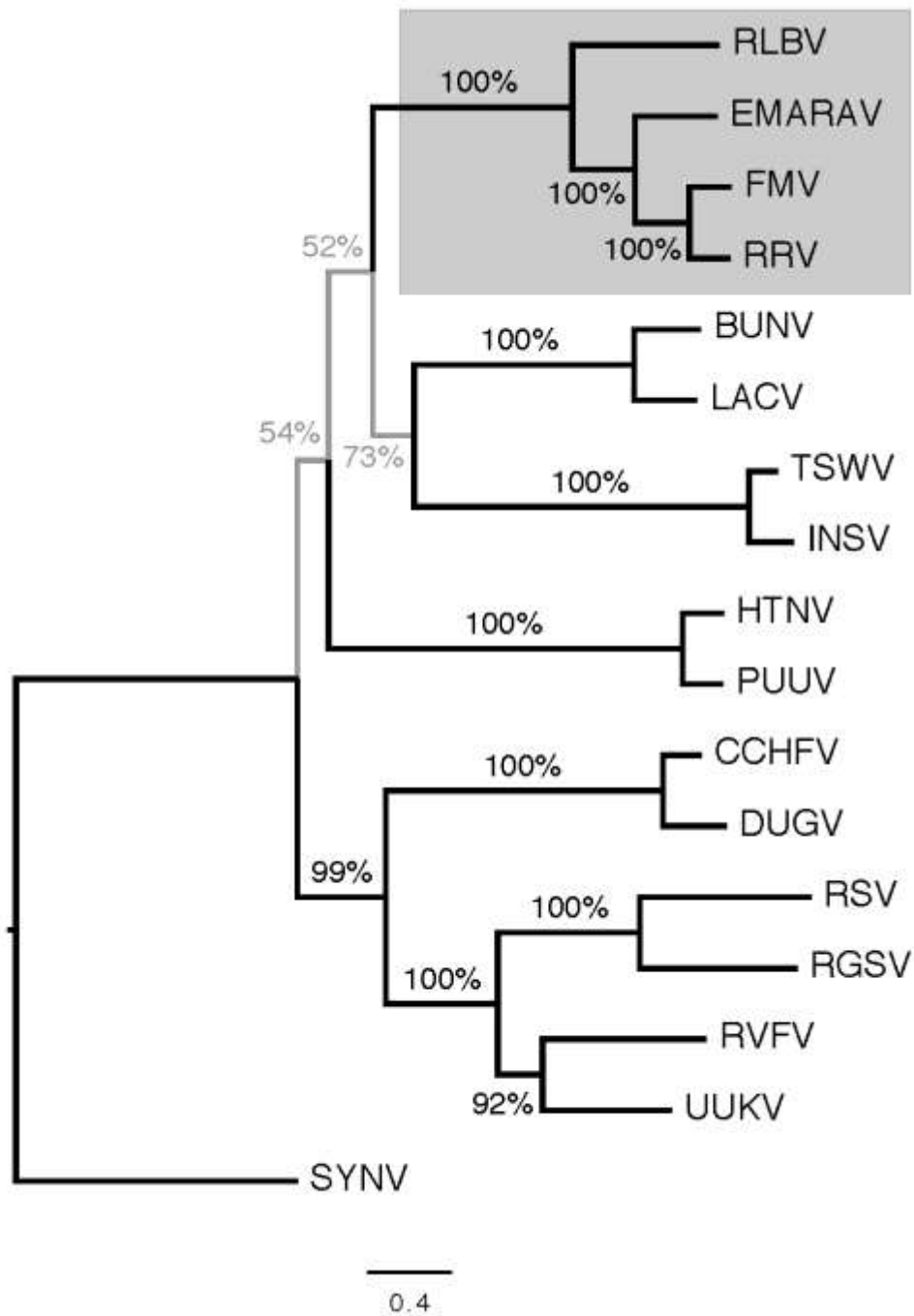


Figure 2. Phylogenetic trees showing the sequence relationships between the RDRP proteins of RLBV and other negative-strand RNA viruses of plants and animals. Bootstrapped maximum-likelihood tree using RAxML with 1000 replicates, Gamma model of rate heterogeneity, BLOSUM62 matrix, and estimate of proportion of invariable sites. The grey-boxed region highlights RLBV within the *Emaravirus* genus. Branches with less than 75% bootstrap support are in grey. Branch lengths are proportional to the genetic distance between sequences, and the bar represents 0.4 expected amino acid changes per site. The tree is rooted with SYN as an outgroup. The Bayesian tree for this alignment showed the same branch structure (not shown). Viruses are RLBV, RRV, FMV, EMARAV (all *Emaravirus* genus); *Tomato spotted wilt virus* (TSWV), *Impatiens necrotic spot virus* (INSV), both family *Bunyaviridae*/ genus *Tospovirus*;

Crimean-Congo hemorrhagic fever virus (CCHFV), Dugbe Virus (DUGV), both Bunyaviridae/Nairovirus; Hantaan virus (HTNV), Puumala virus (PUUV), both Bunyaviridae/Hantavirus; Rift valley fever virus (RVFV), Uukuniemi virus (UUKV), both Bunyaviridae/Phlebovirus; Bunyamwera virus (BUNV), La Crosse virus (LACV), both Bunyaviridae/Orthobunyavirus; Rice grassy stunt virus (RGSV), Rice stripe virus (RSV), both unassigned genus Tenuivirus; Sonchus yellow net virus (SYNV), Rhabdoviridae/Cytorhabdovirus.

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