



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**

<b>Code assigned:</b>	<b>2016.018aP</b>	(to be completed by ICTV officers)			
<b>Short title:</b> Create <i>Redbud yellow ringspot-associated emaravirus</i> as a new species in the genus <i>Emaravirus</i> (e.g. 6 new species in the genus <i>Zetavirus</i> )					
<b>Modules attached</b> (modules 1 and 10 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 type="checkbox"/>	10 <input checked="" type="checkbox"/>

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

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

**ICTV-EC comments and response of the proposer:**

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## 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	<b>2016.018aP</b>	(assigned by ICTV officers)
<b>To create 1 new species within:</b>		
Genus:	<i>Emaravirus</i>	Fill in all that apply. • If the higher taxon has yet to be created (in a later module, below) write “ <b>(new)</b> ” after its proposed name. • If no genus is specified, enter “ <b>unassigned</b> ” in the genus box.
Subfamily:		
Family:		
Order:		
<b>Name of new species:</b>	<b>Representative isolate: (only 1 per species please)</b>	<b>GenBank sequence accession number(s)</b>
<i>Redbud yellow ringspot-associated emaravirus</i>	Redbud yellow ringspot virus isolate Arkansas	JF795479-JF795482 and KU904300.

### 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

Redbud yellow ringspot-associated virus (RYRSaV) infects redbud (*Cercis* spp.) in nature (Kim and Martin, 1978; Kim *et al.*, 2001; Laney, 2010; Di Bello *et al.*, 2016). Based on molecular, morphological and biological characteristics, this virus was found to possess common features with members of the genus *Emaravirus*, which comprises the following species: *Fig mosaic virus* (Elbeaino *et al.*, 2009a, 2009b, 2012); *Rose rosette virus* (Laney *et al.*, 2011); *Raspberry leaf blotch virus* (McGavin *et al.*, 2012); *Pigeonpea sterility mosaic virus* (Elbeaino *et al.*, 2014; Kumar *et al.*, 2003), *High Plains wheat mosaic virus* (Tatineni *et al.*, 2014; Skare *et al.*, 2006) and *European mountain ash ringspot-associated virus* as the type species of the genus (Mielke-Ehret & Mühlbach, 2007). However, pigeonpea sterility mosaic virus 2 (PPSMV-2) (Elbeaino *et al.*, 2015), actinidia chlorotic ringspot-associated virus (AcCRaV) (Zheng *et al.*, 2016) and woolly burdock yellow vein virus (WBYVV) (Bi *et al.*, 2012) are yet putative emaraviruses awaiting their final classification.

Although the vector transmission of RYRSaV in redbud plants is highly plausible, its specific vector has not been yet identified. Possible vectoring role of the mite *Aculops cercidis* was excluded according to results of recent experiments (Di Bello *et al.*, 2016). The RNA-dependent RNA polymerase (RdRp), glycoprotein precursor (GP), nucleocapsid (NC), and movement protein (MP) putatively encoded by RYRSaV

exhibited different levels of sequence identity with ortholog proteins of other emaraviruses (Table 1). The P5 proteins showed higher homologies with the P4 of EMARaV and P6 of FMV and RRV.

### **Virus properties**

- (i) Virus particles: Quasi-spherical, double membrane-bound particles (DMBs) of 120-200 nm in diameter, were found in the cytoplasm of cell types of symptomatic leaves of infected plants (Ahn *et al.*, 1996).
- (ii) Genome: composed of five segments of negative sense ssRNA. RNA-1: 7,049 nt, RNA-2: 2220 nt, RNA-3: 1414 nt, RNA-4: 1513 nt, and RNA-5: 1272 nt (Fig.1) (in order from RNA-1 to RNA-5, accession numbers are: JF795479-JF795482 and KU904300) (Di Bello *et al.*, 2016). Each molecule is monocistronic, encoding a single protein translated from the complementary strand (Fig. 1). Untranslated regions (UTRs) at the 5' and 3' termini of all RNA segments extending from 110 to 515 nt and from 40 to 100 nt, respectively, and exhibiting a conserved stretch of 13 nt at their termini.
- (iii) Virus-encoded proteins: RNA-dependent RNA-polymerase (p1): 267 kDa; putative glycoprotein precursor (p2): 74 kDa; putative nucleocapsid protein (p3): 35 kDa; putative movement protein (p4): 42 kDa; p5 (function unknown): 26 kDa (Fig. 1).
- (iv) Phylogenetic relationships: Phylogenetic trees constructed with RdRp amino acid sequences grouped RYRSaV together with emaraviruses. In particular, RYRSaV and EMARaV formed a distinct clade (Figure 2). Sequences of these viruses share only 53%, 41%, and 44% amino acid identity between the RdRp, GP, and NC proteins, respectively (Table 1) (This meets species demarcation criterion 1).
- (v) Experimental transmission: RYRSaV was experimentally transmitted by cleft grafting to alternative hosts belonging to *Glycine max*, *Phaseolus vulgaris*, *Robinia pseudoacacia* and *Vigna unguiculata* (Di Bello *et al.*, 2016).
- (vi) Natural host range: *Cercis* spp.

In summary, we propose creation of a new species in the genus Emaravirus, named *Redbud yellow ringspot-associated emaravirus*. This species is typified by a recently characterized redbud yellow ringspot virus (RYRSaV, DiBello *et al.*, 2016).

### MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

### **References:**

- Ahn K.K., Kim K.S., Gergerich R.C., Anderson E.J., 1996. Comparative ultrastructure of double membrane-bound particles and inclusions associated with eriophyid mite-borne plant diseases of unknown etiology: A potentially new group of plant viruses. *Journal of Submicroscopic Cytology and Pathology* 28(3): 345-355.
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- Di Bello P.L., Laney A.G., Druciarek T., Ho T., Gergerich R.C., Keller K.E., Martin R.R., Tzanetakis I.E., 2016. A novel emaravirus is associated with redbud yellow ringspot disease. *Virus Research* 222: 41-47.
- Elbeaino T., Digiario M., Alabdullah A.K., De Stradis A., Minafra A., Mielke N., Castellano M.A., Martelli G.P., 2009a. A multipartite negative-sense single-stranded RNA virus is the putative agent of fig mosaic disease. *Journal of General Virology*, 90 (5): 1281-1288.
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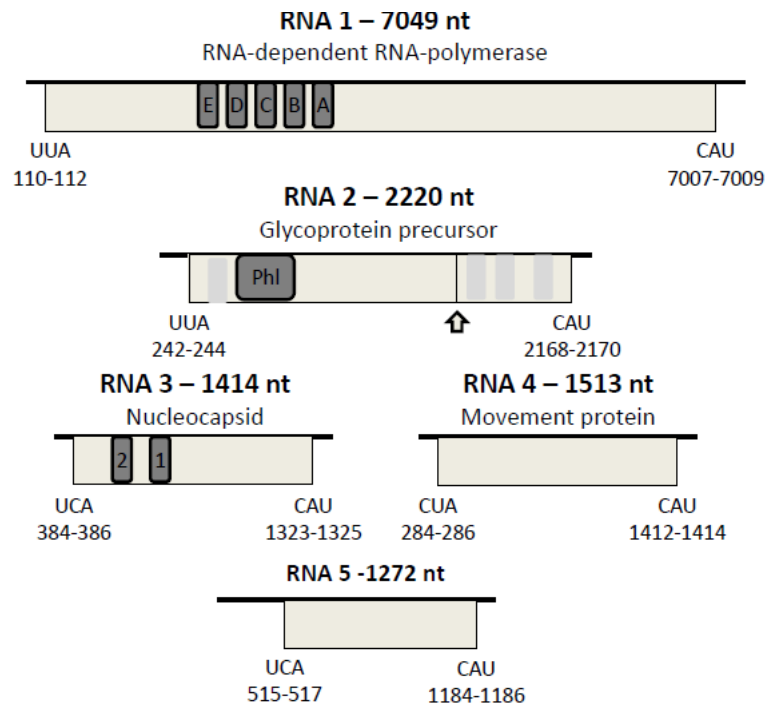
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- Kim, K., Martin E., 1978. Virus-like particles associated with yellow ringspot of redbud. *Phytopathol News* 12, 119.
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- Laney A.G., Keller K.E., Martin R.R., Tzanetakis I.E., 2011. A discovery 70 years in the making: characterization of the Rose rosette virus. *Journal of General Virology*, 92: 1727–1732.
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- Mielke N., Muehlbach, H.P., 2007. A novel, multipartite, negative-strand RNA virus is associated with the ringspot disease of European mountain ash (*Sorbus aucuparia* L.). *Journal of General Virology*, 88: 1337–1346.
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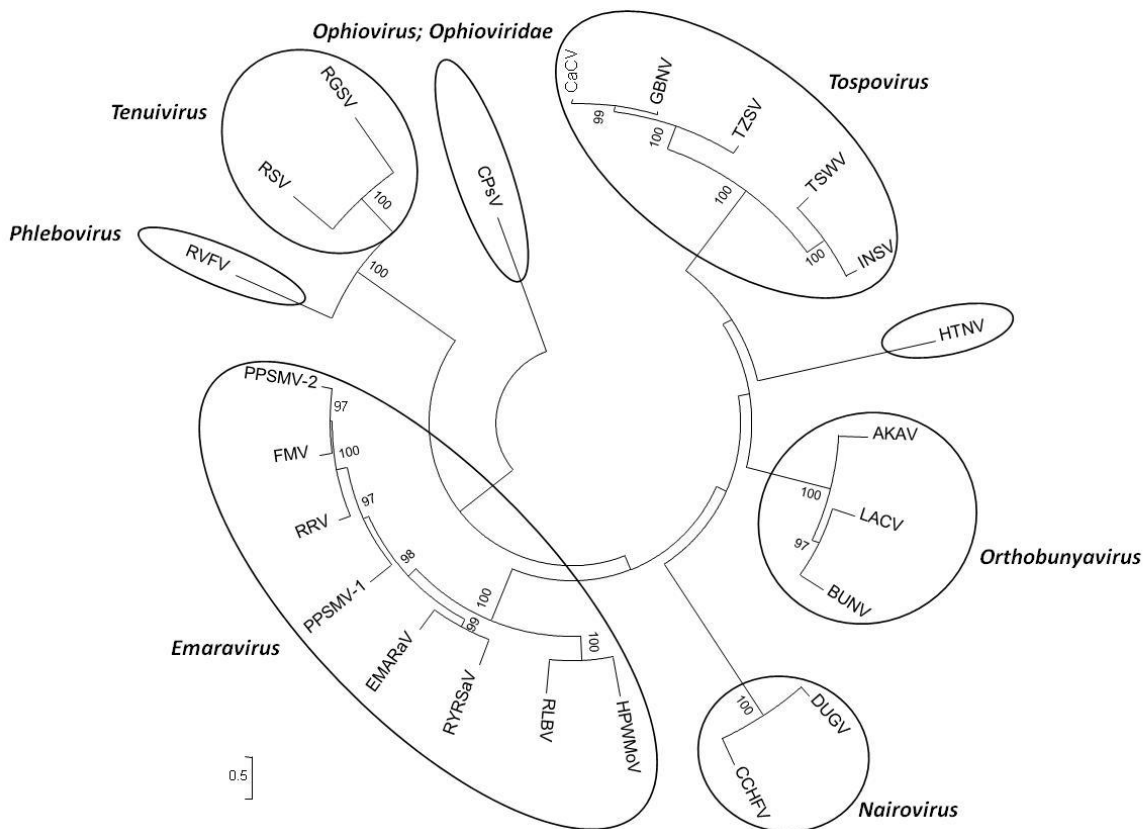
## Annex:

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** Redbud yellow ringspot-associated virus genome organization with signature motifs of the predicted proteins; RNA-1: Bunyaviridae RdRp motifs A–E, RNA-2: the phlebovirus glycoprotein motif (Phl) , the four transmembrane domains (gray boxes), and the putative cleavage site (arrow), RNA 3: emaravirus nucleocapsid motifs 1 and 2.



**Figure 2** Phylogenetic relationships of redbud yellow ringspot-associated virus (RYRSaV; JF795479) and members of the family *Bunyaviridae*, using the RdRp amino acids sequences, with representatives from the genera *Emaravirus*, *Hantavirus*, *Nairovirus*, *Orthobunyavirus*, *Phlebovirus*, *Tospovirus*, and *Tenuivirus*. Virus names and GenBank/Refseq accession numbers: tomato spotted wilt virus (TSWV; NP\_049362), capsicum chlorosis virus (CaCV; YP\_717924.1), impatiens necrotic spot virus, (INSV; NP\_619710.1), groundnut bud necrosis virus (GBNV; NP\_619688.1), tomato zonate spot virus (TZSV; YP\_001740047.1), rice stripe virus (RSV; NC\_003755), rice grassy stunt virus (RGSV; NC\_003755.1), Dugbe virus, (DUGV; NP\_690576.1), Crimean-Congo hemorrhagic fever virus (CCHFV; YP\_325663.1), Bunyamwera virus (BUNV; NP\_047211.1), Akabane virus (AKAV; YP\_001497159.1), California encephalitis virus [isolate La Crosse virus, LACV; NP\_671968.1), Rift Valley fever virus (RVFV; YP\_003848704.1), European mountain ash ringspot-associated virus (EMARaV; YP\_003104764.1), fig mosaic virus (FMV; CAQ03479.7), pigeonpea sterility mosaic virus (proposed to ICTV as PPSMV-1; CCP46989), pigeonpea sterility mosaic virus 2 (PPSMV-2; HF912243), rose rosette virus, (RRV; ADZ54688.1), raspberry leaf blotch virus (RLBV; CBZ42024.1), High Plains wheat mosaic virus (HPWMoV; AIK23031.1), and Hantaan virus (HTNV; NP\_941982.1). Citrus psorosis virus (CPsV; YP\_089661.1) was used as an outgroup. Analysis was performed on the alignment performed with MUSCLE in MEGA5.2, using the neighbor-joining algorithm, and bootstrapping consisting of 1000 pseudo replicates (Tamura *et al.*, 2013).



**Table 1** Amino acid identities among emaravirus polyproteins: p1 (RNA-dependent RNA polymerase), p2 (glycoprotein precursor), p3 (nucleocapsid protein) and p4 (movement protein). Percent of amino acid identity was calculated using MUSCLE.

		<b>PPSMV-1</b>	<b>RRV</b>	<b>PPSMV- 2</b>	<b>FMV</b>	<b>RYRSaV</b>	<b>EMARaV</b>	<b>RLBV</b>
<i>p1</i>	<b>RRV</b>	53						
<i>p2</i>		43						
<i>p3</i>		41						
<i>p4</i>		38						
<i>p1</i>	<b>PPSMV-2</b>	53	67					
<i>p2</i>		43	52					
<i>p3</i>		40	62					
<i>p4</i>		40	60					
<i>p1</i>	<b>FMV</b>	51	67	72				
<i>p2</i>		44	49	57				
<i>p3</i>		39	60	80				
<i>p4</i>		38	58	74				
<i>p1</i>	<b>RYRSaV</b>	47	46	46	46			
<i>p2</i>		39	39	39	39			
<i>p3</i>		36	36	35	37			
<i>p4</i>		27	29	32	30			
<i>p1</i>	<b>EMARaV</b>	47	48	49	48	53		
<i>p2</i>		39	36	35	37	41		
<i>p3</i>		33	31	36	38	44		
<i>p4</i>								
<i>p1</i>	<b>RLBV</b>	33	33	33	33	34	34	
<i>p2</i>		27	24	23	24	24	25	
<i>p3</i>		24	23	21	22	19	22	
<i>p4</i>		23	20	20	19	20		
<i>p1</i>	<b>HPWMoV</b>	31	32	32	32	32	32	41
<i>p2</i>		24	25	24	24	25	23	33
<i>p3</i>		18	18	19	19	20	16	29
<i>p4</i>		21	20	21	19	20		43