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:	***2015.005aP***	(to be completed by ICTV officers)

Short title: Two new species in the genus *Nepovirus* (e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 10 are required)	**1** ☒	**2** ☒	**3** ☐	**4** ☐	**5** ☐
	6 ☐	**7** ☐	**8** ☐	**9** ☐	**10** ☒

Author(s):

Jeremy R. Thompson (Chair)	jrt36@cornell.edu
Alexander V. Karasev	akarasev@uidaho.edu
Hélène Sanfacon	Helene.Sanfacon@agr.gc.ca
Indranil Dasgupta	Indranil58@yahoo.co.in
Ioannis Tzanetakis	itzaneta@uark.edu
Karel Petrzik	petrzik@umbr.cas.cz
Marc Fuchs	mf13@cornell.edu
Nobu Yoshikawa	yoshikawa@iwate-u.ac.jp
René van der Vlugt	Rene.vanderVlugt@wur.nl
Thierry Wetzel	thierry.wetzel@dlr.rlp.de
Toru Iwanami	tiwsw37@affrc.go.jp
Luisa Rubino	l.rubino@ba.ivv.cnr.it

Corresponding author with e-mail address:

Jeremy R. Thompson	jrt36@cornell.edu

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)	Secoviridae

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

Date first submitted to ICTV: June 2015

Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

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	***2015.005aP***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Nepovirus***
Subfamily:	***Comovirinae***
Family:	***Secoviridae***
Order:	***Picornavirales***

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.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Aeonium ringspot virus	Scafati-2011	RNA1 (JX304792) full; RNA2 (JQ670669) full
Mulberry mosaic leaf roll associated virus	zj	RNA1 (KC904083) full; RNA2 (KC904084) full

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 in the genus *Nepovirus*, family *Secoviridae* are: aa sequence of CP with less than 75% identity, aa sequence of conserved Pro-Pol region (region between the conserved "GC" motif in the protease and "GDD" motif in the polymerase) with less than 80% identity, differences in antigenic reactions, host range and/or vector specificity.

Aeonium ringspot virus (AeRSV)

Chlorotic spots and rings on both leaf surfaces were observed on several potted plants of an unidentified Aeonium species growing in private gardens of the city of Scafati (Campania, Italy). Virions of 30 nm in diameter as determined by electron microscopy were readily purified from *Nicotiana benthamiana* plants inoculated with plant extracts from the infected *Aeonium* spp (Sorrentino et al 2013).

Antiserum produced against AeRSV virions was not cross-reactive with *Tobacco ringspot virus* (TRSV) In immunoelectron microscopy and this same antiserum decorated AeRSV particles but not TRSV particles. Conversely, an antiserum against TRSV decorated purified TRSV, but not AeRSV particles. Separation of the virions by denaturing polyacrylamide gel electrophoresis identified a single protein band with an estimated molecular weight of 54 kDa.

Cytopathological effects of infection as visualized by electron microscopy were consistent with nepovirus infections (Martelli and Russo, 1984; Ritzenthaler et al., 2002; Gokalp et al.,

2003).

Two RNA species (RNA1 and RNA2) extracted from purified virions were separated by gel electrophoresis. The full-length sequences of both RNAs were obtained. RNA1 (7549 nt) contains typical motifs for the putative viral protease cofactor, the NTP-binding domain, cysteine protease and the RNA-dependent RNA polymerase (RdRp). RNA2 (4010 nt) contains the "P" motif conserved in the movement proteins (MP) of nepoviruses and other plant viruses and a motif FWGR, similar to the nepovirus coat protein motif FYGR. A smaller RNA2 species (RNA2') (3472 nt) unique to *Aeonium* plants was found in addition to the 4010 nt RNA2. The smaller length of this RNA2' is the result of 537 nt deletion in the predicted MP region.

Amino acid sequence identities between the closest known relative of AeRSV (TRSV) were 84% for the RdRp Pro-Pol domain and 66% for the CP.

In considering the demarcation criteria for species of the *Secoviridae* AeRSV complies with a less than 75% identity for the CP but exceeds the 80% cut-off for the Pro-Pol. In such a situation, if the identity is near the proposed cut-off (75-85% for the Pro-Pol) it is recommended that other factors are taken into consideration, namely – 1) differences in antigenic reactions, 2) distinct host range, 3) distinct vector specificity, 4) absence of cross-protection, and 5) for viruses with a bipartite genome, absence of re-assortment between RNA1 and RNA2 (Sanfacon et al, 2009). AeRSV complies with two of these criteria: it is shown to have distinct antigenic properties to TRSV and no clear evidence of reassortment. A neighbor-joining derived phylogenetic tree of the Pro-Pol region of all recognized members of the *Secoviridae* showed AeRSV to be monophyletic with *Tobacco ringspot virus* (TRSV) with approximately the same genetic distance of separation as for *Arabis mosaic virus* (ArMV) and *Grapevine fanleaf virus* (GFLV); and *Beet ringspot virus* (BRSV) and *Tomato black ring virus* (TBRV) (Fig. 1). Amino acid identities in the same Pro-Pol region showed percent identities between ArMV and GFLV, and BRSV and TBRV to be 82 and 90, respectively (Table 1). Amino acid identities of the full-length ORFs of RNA1 and RNA2 showed percent identities between AeRSV and TRSV to be 80 and 73, respectively; and between BRSV and TBRV to be 84 and 69, respectively (Table 2). (Primary reference: Sorrentino et al, 2013).

Mulberry mosaic leaf roll associated virus (MMLRaV)

Diseased mulberry (*Morus alba* L.) leaves were collected from orchards at Sericultural Research Institute, Chinese Academy of Agricultural Sciences (Zhenjiang, Jiangsu province) and virus infection confirmed by RT-PCR. Partially purified virus preparations were extracted from mulberry leaves (Xie et al, 1991) and viral RNA extracted to use as a template for sequence-independent amplification (Agindotan et al, 2010) while 5' and 3' RACE was used to obtain the sequence termini. The full-length sequences of two RNAs were obtained: RNA1 is 7183 nt long, and RNA2 is 3742 nt long. The predicted translation of the RNA1 ORF leads to a polyprotein composed of 2102 aa with a molecular mass of 235kDa which is phylogenetically most related to *Melon mild mottle virus* (MMMoV) of the genus *Nepovirus* (subgroup-A) of the subfamily *Comovirinae* with an identity of 61 % in the predicted RNA-dependent RNA polymerase domain. The predicted translation of the RNA2 ORF 1093 aa (120 kDa) shared highest identity (32 %) with the RNA2 polyprotein of GFLV (ACR46367), a subgroup-A nepovirus. Antiserum produced using a prokaryotic expression product of the partial predicted CP gene of MMLRaV identified an approximately 60 kDa protein in infected plants. This size corresponds with a CP size of 59kDa based on a predicted MP/CP cleavage at

553E/V554. The deduced CP amino acid sequence of MMLRaV shares <28 % identity with that of other nepoviruses. A neighbor-joining derived phylogenetic tree of the Pro-Pol region of all recognized members of the *Secoviridae* showed MMLRaV to be unambiguously monophyletic within the *Nepovirus* genus. (Fig. 1). (Primary reference: Lu et al, 2015).

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

-Sorrentino, R., De Stradis, A., Russo, M., Alioto, D. & Rubino, L. (2013) Characterization of a putative novel nepovirus from Aeonium sp. Virus Research 177, 217-221.

Martelli, G.P., Russo, M., 1984. Use of thin sectioning for the visualization and identification of plant viruses. In: Maramorosch, K., Koprowski, H. (Eds.), Methods in Virology, vol. 8. Academic Press, New York, USA, pp. 143–224.

-Ritzenthaler, C., Laporte, C., Gaire, F., Dunoyer, P., Schmitt, C., Duval, S., Piéquet, A., Loudes, A.M., Rohfritsch, O., Stussi-Garaud, C., Pfeiffer, P., 2002. Grapevine fanleaf virus replication occurs on endoplasmic reticulum-derived membranes.J. Virol. 76, 8808–8819.

-Gokalp, K., Digiaro, M., Cigsar, I., Abou Ghanem-Sabanadzovic, N., De Stradis, A., Boscia, D., Martelli, G.P., 2003. Properties of a previously undescribed nepovirus from South-East Anatolia. J. Plant Pathol. 85, 35–41.

-Sanfacon H, Wellink J, Le Gall O, Karasev A, van der Vlugt R, et al. (2009) Secoviridae: a proposed family of plant viruses within the order Picornavirales that combines the families Sequiviridae and Comoviridae, the unassigned genera Cheravirus and Sadwavirus, and the proposed genus Torradovirus. Arch Virol 154: 899–907.

-Xie LH, Zhou ZJ, Lin QY, Song XG, Xie LY (1991) Study on rice stripe disease: III. Pathogen of the disease. J Fujian Agric Coll 20:144–149

-Agindotan BO, Ahonsi MO, Domier LL, Gray ME, Bradley CA (2010) Application of sequence-independent amplification (SIA) for the identification of RNA viruses in bioenergy crops. J Virol Methods 169:119–128

-Lu, Q. Y., Wu, Z. J., Xia, Z. S. & Xie, L. H. (2015) A new nepovirus identified in mulberry (*Morus alba L.*) in China. Archives of Virology 160, 851-855.

-Tommaso, P., Moretti, S., Xenarios, I., Orobitg, M., Montanyola, A., Chang, J. M., Taly, J. F. & Notredame, C. (2011) T-Coffee: a web server for the multiple sequence alignment of protein and RNA sequences using structural information and homology extension. Nucleic Acids Res 39, W13-W17.

-Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25, 4876-4882.

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.

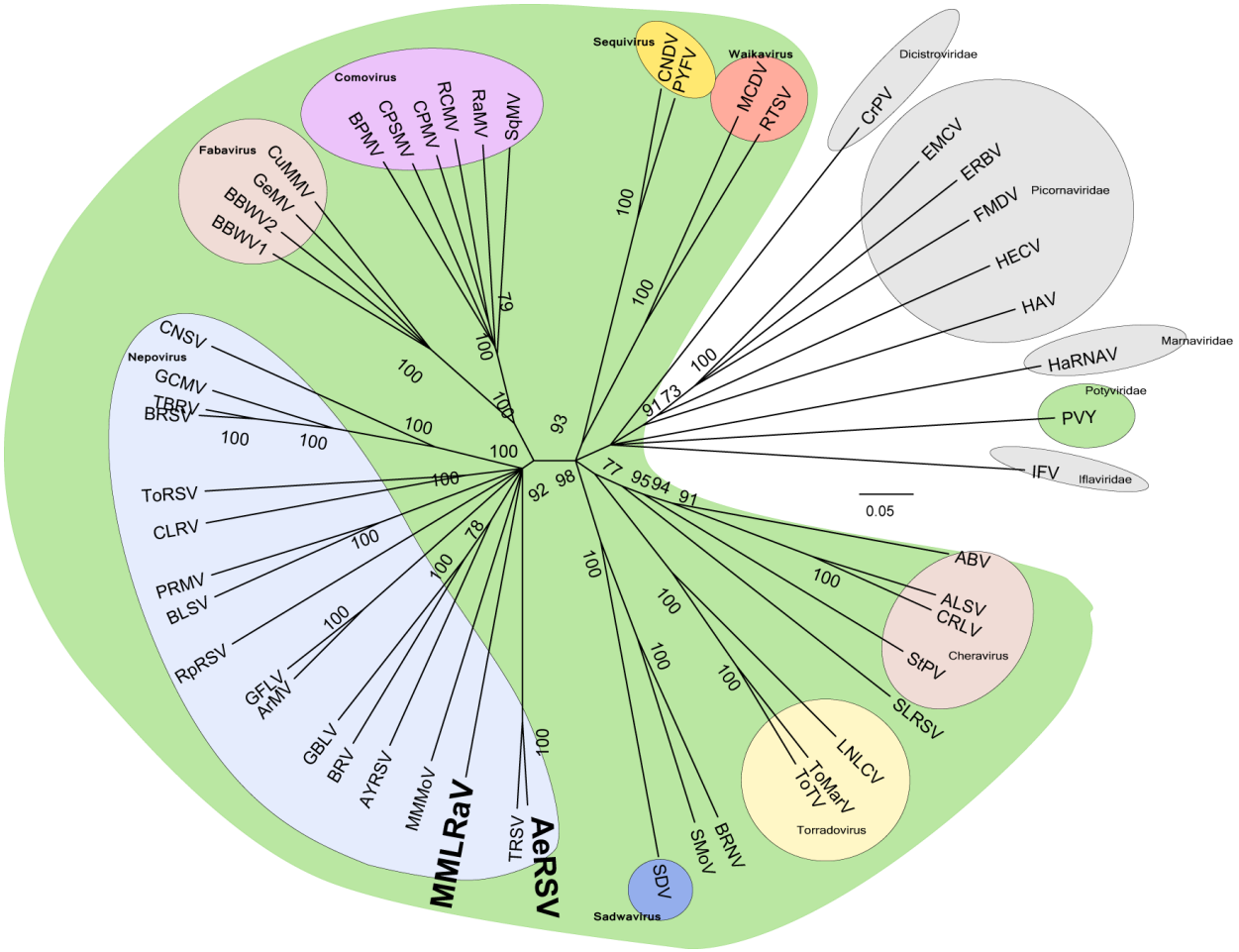

Fig1. Neighbor-joining tree of members of the order *Picornavirales* based on the amino acid sequences of the conserved domains between the "CG" motif of the 3C-like proteinase and the "GDD" motif of the polymerase (Pro-Pol region). The alignment was produced by the program T-Coffee (Tommaso et al, 2011) and the tree was generated using CLUSTALX (Thompson et al, 1997). Potato virus Y (PVY) a member of the family *Potyviridae* was used as an outgroup. Representative sequences were included for the family *Secoviridae* while only representative members of other families within the order *Picornavirales* were included. The families and genera are delineated on the right. Numbers on nodes show bootstrap values (1000 replicates) above 70%. The bar represents a P distance of 0.1. The GenBank accession numbers used for each virus are as follows: Aeonium rinspot virus (AeRSV, JX304792=AFR67086), Mulberry mosaic leaf roll associated virus (MMLRaV, KC904083 = AGY34703), Apple latent spherical virus (ALSV, NC_003787 = AB030940), Arabis mosaic virus (ArMV, NC_006057 = AY303786), Arracacha virus B (AVB, JQ437415), Artichoke yellow ringspot virus (AYRSV, AM087671), Bean pod mottle virus (BPMV, NC_003496 = U70866), Beet ringspot virus (BRSV, NC_003693 = D00322), Black raspberry necrosis virus (BRNV, NC_008182 = DQ344639), Blackcurrant reversion virus (BRV, NC_003509 = AF368272), Broad bean wilt virus 1 (BBWV1, NC_005289 = AB084450), Broad bean wilt virus 2 BBWV2 (NC_003003 = AF225953), Carrot necrotic dieback virus (CNDV, EU980442), Carrot torrado virus (CaTV, KF533719), Cassava torrado-like virus (CsTLV, KC_505250), Cherry leaf roll virus (CLRV, NC_015414 = FR851461), Cherry rasp leaf virus (CRLV, NC_006271 = AJ621357), Cowpea mosaic virus (CPMV, NC_003549 = X00206), Cowpea severe mosaic virus (CPSMV, NC_003545 = M83830), Cricket paralysis virus (CrPV, NC_003924 = AF218039), Cucurbit mild mosaic virus (CuMMV, FJ194941), Cycas necrosis stunt virus (CNSV, NC_003791 =

AB073147), Encephalomyocarditis virus (EMCV, NC_001479 = M81861), Equine rhinitis B virus 1 (ERBV, NC_003983 = X96871), Foot-and-mouth disease virus- type C (FMDV, NC_002554 = AF274010), Gentian mosaic virus (GeMV, BAD99001), Grapevine Bulgarian latent virus (GBLV, NC_015492 = FN691934), Grapevine chrome mosaic virus (GCMV, NC 003622 = X15346), Grapevine fanleaf virus (GFLV, NC_003615 = D00915), Hepatitis A virus (HAV, NC_001489 = M14707), Heterosigma akashiwo RNA virus (HaRNAV, NC_005281 = AY337486), Human enterovirus C (HECV, NC_002058.3 = V01149), Infectious flacherie virus (IFV, NC_003781 = AB000906), Lettuce necrotic leaf curl virus (LNLCV, KC8552566), Maize chlorotic dwarf virus (MCDV, NC_003626 = U67839), Melon mild mottle virus (MMMoV, AB518485), Motherworth yellow mottle virus (MYMoV, KM229700), Parsnip yellow fleck virus (PYFV, NC_003628 = D14066), Peach rosette mosaic virus (PRMV, AF016626), Radish mosaic virus (RaMV, NC_010709 = AB295643), Raspberry ringspot virus (RpRSV, NC_005266 = AY303787), Red clover mottle virus (RCMV, NC_003741 = X64886), Rice tungro spherical virus (RTSV, NC_001632 = M95497), Satsuma dwarf virus (SDV, NC_003785 = AB009958), Squash mosaic virus (SqMV, NC_003799 = AB054688), Stocky prune virus (StPV, DQ143874), Strawberry latent ringspot virus (SLRSV, NC_006964 = AY860978), Strawberry mottle virus (SMoV, NC_003445 = AJ311875), Tobacco ringspot virus (TRSV, NC_005097 = U50869), Tomato black ring virus (TBRV, NC_004439 = AY157993), Tomato marchitez virus (ToMarV, NC_010987 = EF681764), Tomato ringspot virus (ToRSV, NC_003840 = L19655), Tomato torrado virus (ToTV, NC_009013 = DQ388879).

Table 1 – Percent amino acid identities of the Pro-Pol region of the RNA1 ORF between the closely related nepoviruses: *Arabis mosaic virus* (ArMV), *Grapevine fanleaf virus* (GFLV), *Beet ringspot virus* (BRSV), *Tomato black ring virus* (TBRV), *Tobacco ringspot virus* (TRSV) and Aeonium ringspot virus (AeRSV).

AeRSV	ArMV	BRSV	GFLV	TBRV	TRSV	
	45	38	44	39	**84**	AeRSV
		40	82	41	42	ArMV
			40	90	38	BRSV
				41	41	GFLV
					39	TBRV
						TRSV

Table 2 – Percent amino acid identities of the complete ORFs for RNA1 and RNA2 between the closely related nepoviruses: *Beet ringspot virus* (BRSV), *Tomato black ring virus* (TBRV), *Tobacco ringspot virus* (TRSV) and Aeonium ringspot virus (AeRSV).

		AeRSV	BRSV	TBRV	TRSV
ORF1	AeRSV		30	31	80
	BRSV			84	30
	TBRV				31
	TRSV				
ORF2	AeRSV		23	26	73
	BRSV			69	25
	TBRV				26
	TRSV				