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:	***2012.005aP***	(to be completed by ICTV officers)

Short title: One new species in the genus *Nepovirus*
(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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and in consultation with Masamichi Isogai (isogai@iwate-u.ac.jp)

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-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	**2012.005aP**	(assigned by ICTV officers)

To create 1 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.

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Blueberry latent spherical virus	AB649296 (RNA1) AB649297 (RNA2)

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

A new virus was isolated from a variety of blueberry cultivars in Japan, none of which showed obvious symptoms (Isogai et al., 2012). Transmission by grafting to blueberry seedlings led to transmission of the virus as confirmed by RT-PCR. Similarly the virus was mechanically transmitted to herbaceous hosts; *Luffa cylindrical* and *Nicotiana benthamiana* showing symptoms upon infection. Sucrose gradient centrifugation of purified particles identified three components (Top (T), Middle (M), Bottom (B)) of uniform morphology, diameter 30nm. The M and B component particles were found to contain RNA of approximately 8 and 6.5kb, respectively, while the T component particles were empty. The structural protein component of the virus particles was resolved by SDS-PAGE to a single band of approximately 55kDa.

The genome was completely sequenced and consists of (excluding the poly(A) tail) RNA1 (B component) and RNA2 (M component) with 7,960 and 6,344 nts, respectively. RNA1 contains a single ORF encoding a 2,172aa protein with highest similarity (59%) to the subgroup C nepovirus *Peach rosette mosaic virus* (PRMV). Motifs consistent with the presence of a putative protease, helicase, RNA-dependent RNA polymerase (RdRp) were also identified. RNA2 contains a single ORF encoding a 1,631aa protein with motifs consistent with the presence of movement (MP) and coat (CP) proteins. Highest similarities are with the MP of the subgroup C nepovirus *Blackcurrant reversion virus* (BRV) and the CP of the subgroup C nepovirus *Apricot latent ringspot virus,* at 37% and 43%, respectively. The 5' non-coding regions contain a sequence similar to a consensus for nepoviruses (Fuchs et al., 1989). The 3'

non-coding regions [1,379nts (RNA1) and 1,392nts (RNA2)] are 97% identical to each other and share 65% identity with PRMV.

Phylogenetic analysis using the deduced amino acid sequence of the RdRp and CP grouped this new virus with members of the genus *Nepovirus* subgroup C (Isogai et al., 2012). To provide further evidence for the grouping of the virus with other nepoviruses, we have produced a phylogenetic tree by the neighbor-joining method of the MEGA5 software (Tamura et al., 2011) using the deduced amino acid sequence of the Pro-Pol region (region between the conserved CG motif of the proteinase and the conserved GDD motif of the polymerase) (Fig. 1). The Pro-Pol sequence was previously shown to be a useful indicator of taxonomy within the order *Picornavirales* (Le Gall et al., 2008) and within the family *Secoviridae* (Sanfacon et al., 2009). In this analysis, we included all available sequences from the type isolates of members of the family *Secoviridae*. We also included the corresponding sequences from representatives of other families in the order *Picornavirales* and from *Potato virus Y*, a member of the family *Potyviridae*, which was used as an outgroup. The analysis confirmed the grouping of the virus with other members of the genus *Nepovirus* (Fig. 1)

Current species demarcation criteria within the family *Secoviridae* (as defined in the Ninth Report) are: CP amino acid (aa) sequence with less than 75% identity and Pro-Pol region aa sequence with less than 80% identity. For this virus the highest sequence identities with the CP and Pro-Pol regions are 43% and 64%, respectively. Other useful criteria include antigenic reactions, host range and vector specificity. In inoculations to seven herbaceous hosts all were shown to be infected with the new virus, with two showing symptoms. Although not exhaustive these results are indicative of the virus having a broad host range; as described for other nepoviruses. The vector (if any) for this new virus is not known. Seed and/or pollen transmission is possible but has not been confirmed experimentally.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Fuchs, M., Pinck, M., Serghini, M.A., Ravelonandro, M., Walter, B., Pinck, L., 1989. The nucleotide sequence of satellite RNA in grapevine fanleaf virus, strain F13. J Gen Virol 70 (Pt 4), 955-962.

Isogai, M., Tatuto, N., Ujiie, C., Watanabe, M., Yoshikawa, N., 2012. Identification and characterization of blueberry latent spherical virus, a new member of subgroup C in the genus Nepovirus. Arch Virol 157(2), 297-303.

Le Gall, O., Christian, P., Fauquet, C.M., King, A.M., Knowles, N.J., Nakashima, N., Stanway, G., Gorbalenya, A.E., 2008. Picornavirales, a proposed order of positive-sense single-stranded RNA viruses with a pseudo-T = 3 virion architecture. Arch Virol 153(4), 715-727.

Maddison, W.P., Maddison, D.R., 2010. Mesquite: a modular system for evolutionary analysis. Version 2.73.

Sanfacon, H., Wellink, J., Le Gall, O., Karasev, A., van der Vlugt, R., Wetzel, T., 2009.

References:

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(5), 899-907.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S., 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28(10), 2731-2739.

Figure 1. Neighbor-joining tree of the Pro-Pol amino acid sequences of members of the Picornavirales

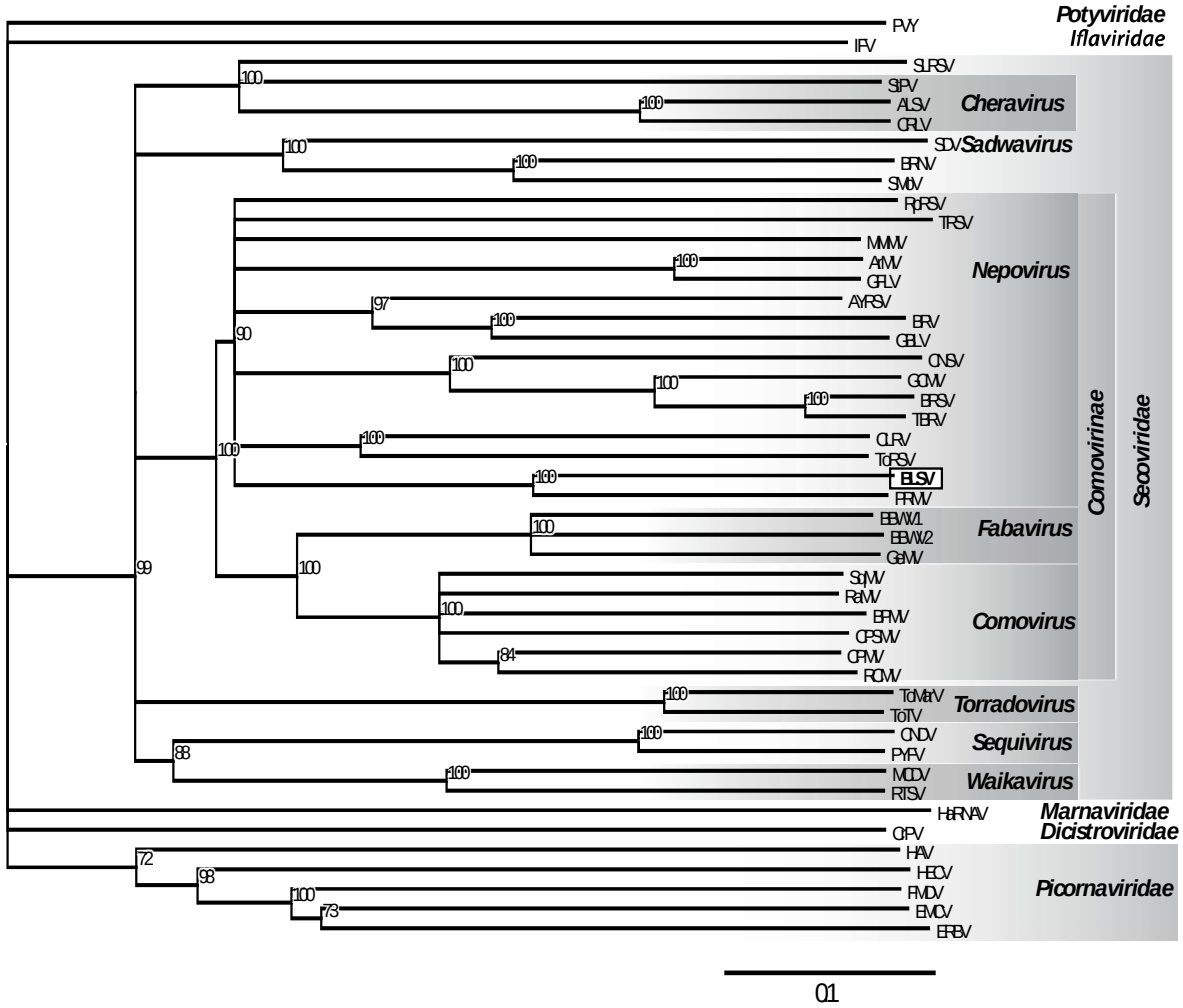

Fig1. Neighbor-joining tree of members of the order *Picornavirales* based on the amino acid sequences of the conserved domains between the "CG" motif of the 3C-like proteinase and the "GDD" motif of the polymerase (Pro-Pol region). The alignment and the tree were generated using MEGA5 (Tamura et al., 2011) and Mesquite (Maddison and Maddison, 2010). 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: potato virus Y (PVY, NC_001616 = X12456), infectious flacherie virus (IFV, NC_003781 = AB000906), hepatitis A virus (HAV, NC_001489 = M14707), human enterovirus C (HECV, NC_002058.3 = V01149), foot-and-mouth disease virus-type C (FMDV, NC_002554 = AF274010), encephalomyocarditis virus (EMCV, NC_001479 = M81861), equine rhinitis B virus 1 (ERBV, NC_003983 = X96871), cricket paralysis virus (CrPV, NC_003924 = AF218039), *Heterosigma akashiwo* RNA virus (HaRNV, NC_005281 = AY337486), parsnip yellow fleck virus (PYFV, NC_003628 = D14066), carrot necrotic dieback virus (CNDV, EU980442), maize chlorotic dwarf virus (MCDV, NC_003626 = U67839), rice tungro spherical virus (RTSV, NC_001632 = M95497), tomato torrado virus (ToTV, NC_009013 = DQ388879), tomato marchitez virus (ToMarV, NC_010987 = EF681764), strawberry latent ringspot virus (SLRSV, NC_006964 = AY860978), stocky prune virus (StPV, DQ143874), apple latent spherical virus (ALSV, NC_003787 = AB030940), cherry rasp leaf virus (CRLV, NC_006271 = AJ621357), satsuma dwarf virus (SDV, NC_003785 = AB009958), strawberry mottle virus (SMoV, NC_003445 = AJ311875), black raspberry necrosis virus (BRNV, NC_008182 = DQ344639), raspberry ringspot virus (RpRSV, NC_005266 = AY303787), peach rosette mosaic virus (PRMV, AF016626), tobacco ringspot virus (TRSV, NC_005097 = U50869), melon mild mottle virus (MMMoV, AB518485), arabis mosaic virus (ArMV, NC_006057 = AY303786), grapevine fanleaf virus (GFLV, NC_003615 = D00915), artichoke yellow ringspot virus (AYRSV, AM087671), blackcurrant reversion virus (BRV, NC_003509 = AF368272), grapevine Bulgarian latent virus (GBLV, NC_015492 = FN691934), cycas necrosis stunt virus (CNSV, NC_003791 = AB073147), grapevine chrome mosaic virus (GCMV, NC 003622 = X15346), beet ringspot virus (BRSV, NC_003693 = D00322), tomato black ring virus (TBRV, NC_004439 = AY157993), tomato ringspot virus (ToRSV, NC_003840 = L19655), cherry leaf roll virus (CLRV, NC_015414 = FR851461), squash mosaic virus (SqMV, NC_003799 = AB054688), radish mosaic virus (RaMV, NC_010709 = AB295643), cowpea mosaic virus (CPMV, NC_003549 = X00206), red clover mottle virus (RCMV, NC_003741 = X64886), bean pod mottle virus (BPMV, NC_003496 = U70866), cowpea severe mosaic virus (CPSMV, NC_003545 = M83830), broad bean wilt virus 1 (BBWV1, NC_005289 = AB084450), BBWV2 (NC_003003 = AF225953), gentian mosaic virus (GeMV, BAD99001).