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:	***2011.003aP***	(to be completed by ICTV officers)

Short title: create species *Melon mild mottle virus* in the genus *Nepovirus*
(e.g. 6 new species in the genus *Zetavirus*)

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

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

Members of the ICTV secoviridae committee:
Hélène Sanfaçon (Helene.Sanfacon@agr.gc.ca) (chair)
Toru Iwanami (tiwsw37@affrc.go.jp)
Jeremy R Thompson (jrt36@cornell.edu)
Joan Wellink (Joan.Wellink@wur.nl)
Thierry Wetzel (thierry.wetzel@agroscience.rlp.de)

and in consultation with Shinya Tsuda (shinyat@affrc.go.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	***2011.003aP***	(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:**
Melon mild mottle virus	AB518485 (RNA1) and AB518486 (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 melon in Japan (6). The virus induced a mild mottle in melon but symptoms were only visible early in infection. Virus particles are spherical and approximately 28 nm in diameter. Both empty and full virus particles are detected in purified virus preparations. A single CP (~55 kDa) was detected. The genome consists of two molecules of positive-sense single-stranded RNAs. RNA1 is 7,721 nts in length and RNA2 is 3,854 nts in length. These properties resembled those of members of the genus *Nepovirus*, within the family *Secoviridae* (4). Isolates from two nepovirus species (*Tomato ringspot virus* and *Tobacco ringspot virus*) have been reported to infect melon but induce different symptoms (mosaic).

The genome of the new virus was completely sequenced. Phylogenetic analysis using the deduced amino acid sequence of the entire RNA1-encoded polyprotein, the entire RNA2-encoded polyprotein or specific regions of these polyproteins (Pro-Pol region, see below and coat protein) grouped the new virus with members of the genus *Nepovirus* (6). Only nepovirus sequences were included in these published alignments. To provide further evidence for the grouping of the virus with other nepoviruses, we have produced a new sequence alignment 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) (see Fig. 1). The Pro-Pol sequence was previously shown to be a useful indicator of taxonomy within the order *Picornavirales* (1). 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. Other useful criteria include antigenic reactions, host range and vector specificity. Similarly to other nepoviruses, the host range of the new virus was wide. As mentioned above, symptoms induced in melon were distinct from those induced by other nepoviruses known to infect melon. The vector (if any) for this new virus is not known. Seed and/or pollen transmission is possible but has not been confirmed experimentally.

The new virus was only distantly related to other nepoviruses. Sequence identity when compared to other nepoviruses was between 31.3 and 52.2% for the Pro-Pol aa sequence and 21.8 to 26.9% for the CP aa sequence (6). Proteolytic cleavage between the movement protein and coat protein was experimentally demonstrated to occur at an M/A dipeptide (6). Although cleavage sites are known to be diverse among some nepoviruses (subgroups A and B), cleavage at M/A dipeptides had not been previously demonstrated for this group of virus. Similarly to nepoviruses of subgroups A and B, a leucine was present in the substrate-binding pocket of the proteinase (rather than the histidine present in subgroup C nepoviruses, comoviruses and fabaviruses).

Taken together, these observations suggest that the new virus should be classified as a new species in the genus *Nepovirus*. The virus has characteristics of a subgroup A nepovirus (size of RNA2 and presence of a leucine in the substrate-binding pocket of the protease) although phylogenetic analysis indicated that it was only distantly related to other subgroup A nepoviruses (6, Fig 1). The name *Melon mild mottle virus* was proposed for the new species (6). This is the first report of this virus and to our knowledge, no other names have been proposed for the virus.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

1. **Le Gall, O., P. Christian, C. M. Fauquet, A. M. King, N. J. Knowles, N. Nakashima, G. Stanway, and A. E. Gorbalenya.** 2008. Picornavirales, a proposed order of positive-sense single-stranded RNA viruses with a pseudo-T = 3 virion architecture. Archive of Virology **153:**715-727.
2. **Page, R. D.** 1996. TreeView: an application to display phylogenetic trees on personal computers. Computer applications in the biosciences : CABIOS **12:**357-8.
3. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular biology and evolution **4:**406-25.
4. **Sanfacon, H., J. Wellink, O. Le Gall, A. Karasev, R. van der Vlugt, and T. Wetzel.** 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.
5. **Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins.**

References:

1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic acids research **25:**4876-82.

6. **Tomitaka, Y., T. Usugi, F. Yasuda, H. Okayama, and S. Tsuda.** 2011. A novel member of the genus Nepovirus isolated from Cucumis melo in Japan. Phytopathology **101:**316-22.

Fig. 1. Phylogram using Pro-Pol aa sequence

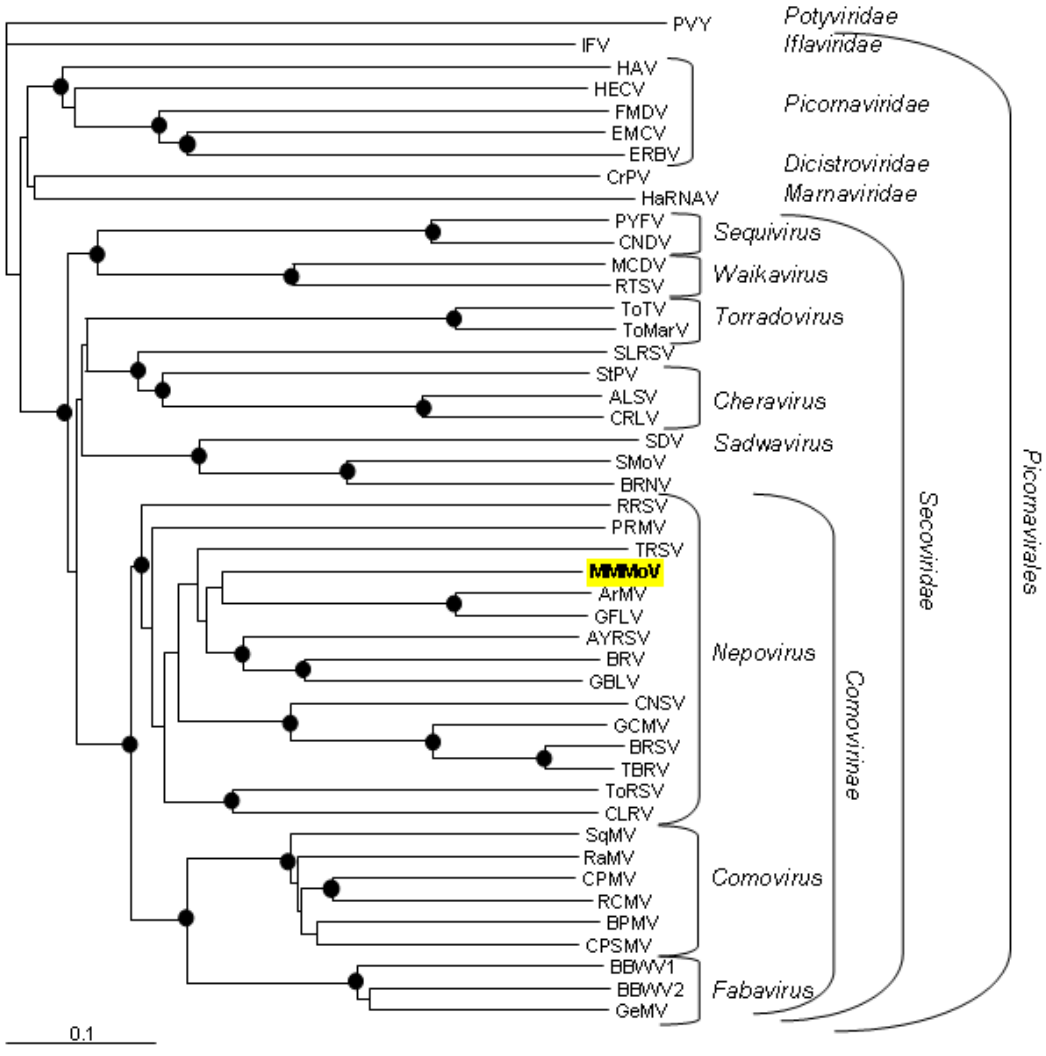

Figure legend: Hierarchical clustering of members of the order *Picornavirales* based on the amino acid sequences of the conserved domains between the "CG" motif of the 3C-like proteinase and the "GDD" motif of the polymerase (Pro-Pol region). The alignment was produced using the neighbour-joining method implemented in ClustalX (3, 5) and the tree (distance dendogram) was drawn using Treeview (2). Potato virus Y (PVY) a member of the family *Potyviridae* was used as an outgroup. All available 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. Black circles indicate nodes supported by bootstrap values (10,000 replicates) above 80% (closed circles); nodes without circles are not supported to this level. 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 entorovirus 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).