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:	*2015.006aP*	(to be completed by ICTV officers)

Short title: Two new species in the genus *Torradovirus* (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 ☒

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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)	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:

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	*2015.006aP*		(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Torradovirus***	Fill in all that apply.
Subfamily:		• If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
Family:	***Secoviridae***	• If no genus is specified, enter "**unassigned**" in the genus box.
Order:	***Picornavirales***	

Name of new species:	Representative isolate: (only 1 per species please)	GenBank sequence accession number(s)
Carrot torradovirus 1	H6	RNA1 KF533719 (partial) RNA2 KF533720 (partial)
Motherwort yellow mottle virus	AD01	RNA1 KM229700 (full) RNA2 KM229701 (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 *Torradovirus*, family *Secoviridae* are: aa sequence of combined CPs 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.

Carrot torradovirus 1 (CaTV1)

Two carrot (*Daucus carota*) samples one symptomatic (necrotic) and one asymptomatic, taken from a field in York, U.K., and analyzed by deep sequencing (Illumina MiSeq) were identified with over 10,000 reads as containing 6.9 knt and 4.7 knt of a bipartite viral genome (Adams et al, 2014). Sequences from the two different samples were 99% identical suggesting that they were infected with the same virus. The 6.9 knt fragment (RNA1) contained one open reading frame (ORF) translating a predicted polypeptide of 2214 aa (249 kDa). BLAST analysis of this putative protein showed it to contain RNA helicase and RdRp domains and have the highest identity of 40% to the equivalent protein sequences from *Tomato marchitez virus* (Verbeek et al, 2008). The 4.7 knt fragment (RNA2) genome contains two open reading frames. ORF1 encoding a putative 202 amino acid (22 kDa) protein with 43% identity to the RNA2 ORF1 of the torradovirus *Lettuce necrotic leaf curl virus* (LNLCV). ORF2 encodes a putative 1167 amino acid (130 kDa) polyprotein containing movement and coat protein domains and has closest

identity (35%) to the RNA2 ORF2 of *Tomato torrado virus*. Preliminary studies using a qRT-PCR diagnostic assay for field-grown carrots at two separate locations in the U.K. found CaTV1 incidence at 14 and 25%. (Fox et al, 2015). A neighbor-joining derived phylogenetic tree of the Pro-Pol region of all recognized members of the *Secoviridae* showed CaTV1 to be monophyletic with LNLCV within the *Torradovirus* genus, but clearly distinct from the two tomato-infecting torradoviruses ToTV and ToMarV (Fig. 1). Highest percent identities for the Pro-Pol region are with LNLCV (68%) and the newly described Motherworth yellow mottle virus (see below) (71%) (Primary reference: Adams et al, 2014).

Motherwort yellow mottle virus (MYMoV)

Field-grown motherwort (*Leonurus sibiricus* L.) plants showing yellow mottle, mild mosaic, and stunting symptoms were collected near a pepper field in Andong, Korea. Extracted total RNA was used to generate a transcriptome library with which deep sequencing was performed (Illumina HiSeq2000). BLASTx analysis of two large virus-like contigs (7023 nt and 4906 nt in length) showed the first contig contained one large open reading frame (ORF) with a maximum aa identity of 53 % (93 % coverage) to a polyprotein encoded by the torradovirus *Lettuce necrotic leaf curl virus* (LNLCV) RNA1 (KC855266), and the second contig has an ORF with a maximum aa identity of 57 % (70 % coverage) to a polyprotein encoded by LNLCV RNA2 (KC855267). The sequences for both RNAs were confirmed by RT-PCR and Sanger sequencing and 5' and 3' RACE was used to obtain the sequence termini from which the assembled full-length sequences for each RNA were determined to be 7068 and 4963 nt long, respectively. The genome organization of MYMoV is analogous to those of other torradoviruses (Verbeek et al, 2014) containing two predicted ORFs encoded on RNA2. The highest aa identities found for the Pro-Pol and CP regions were with LNLCV at 70.4% and 68.6%, respectively. A neighbor-joining derived phylogenetic tree of the Pro-Pol region of all recognized members of the *Secoviridae* showed MYMoV to be monophyletic with LNLCV within the *Torradovirus* genus, but clearly distinct from the two tomato-infecting torradoviruses ToTV and ToMarV (Fig. 1). (Primary reference: Seo et al, 2015)

additional material in support of this proposal

References:

-Adams, I. P., Skelton, A., Macarthur, R., Hodges, T., Hinds, H., Flint, L., Nath, P. D., Boonham, N. & Fox, A. (2014) Carrot yellow leaf virus Is Associated with Carrot Internal Necrosis. Plos One 9

-Verbeek M, Dullemans AM, van den Heuvel JF, Maris PC, van der Vlugt RA (2008) Tomato marchitez virus, a new plant picorna-like virus from tomato related to tomato torrado virus. Archives of Virology 153: 127–134.

- Fox. A, Rozado, Z., Adams, I.P., Skelton, A., Dickinson, M. & Boonham, N. (2015) Investigating the viral causes of carrot internal necrosis. Acta Horticulturae. *In press*

-Verbeek M, Dullemans AM, van Raaij HM, Verhoeven JT, van der Vlugt RA (2014) Lettuce necrotic leaf curl virus, a new plant virus infecting lettuce and a proposed member of the genus *Torradovirus*. Arch Virol 159:801–805.

-Seo, J. K., Kang, M., Kwak, H. R., Kim, M. K., Kim, C. S., Lee, S. H., Kim, J. S. & Choi, H. S. (2015) Complete genome sequence of motherwort yellow mottle virus, a novel putative member of the genus Torradovirus. Archives of Virology 160, 587-590.

-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.
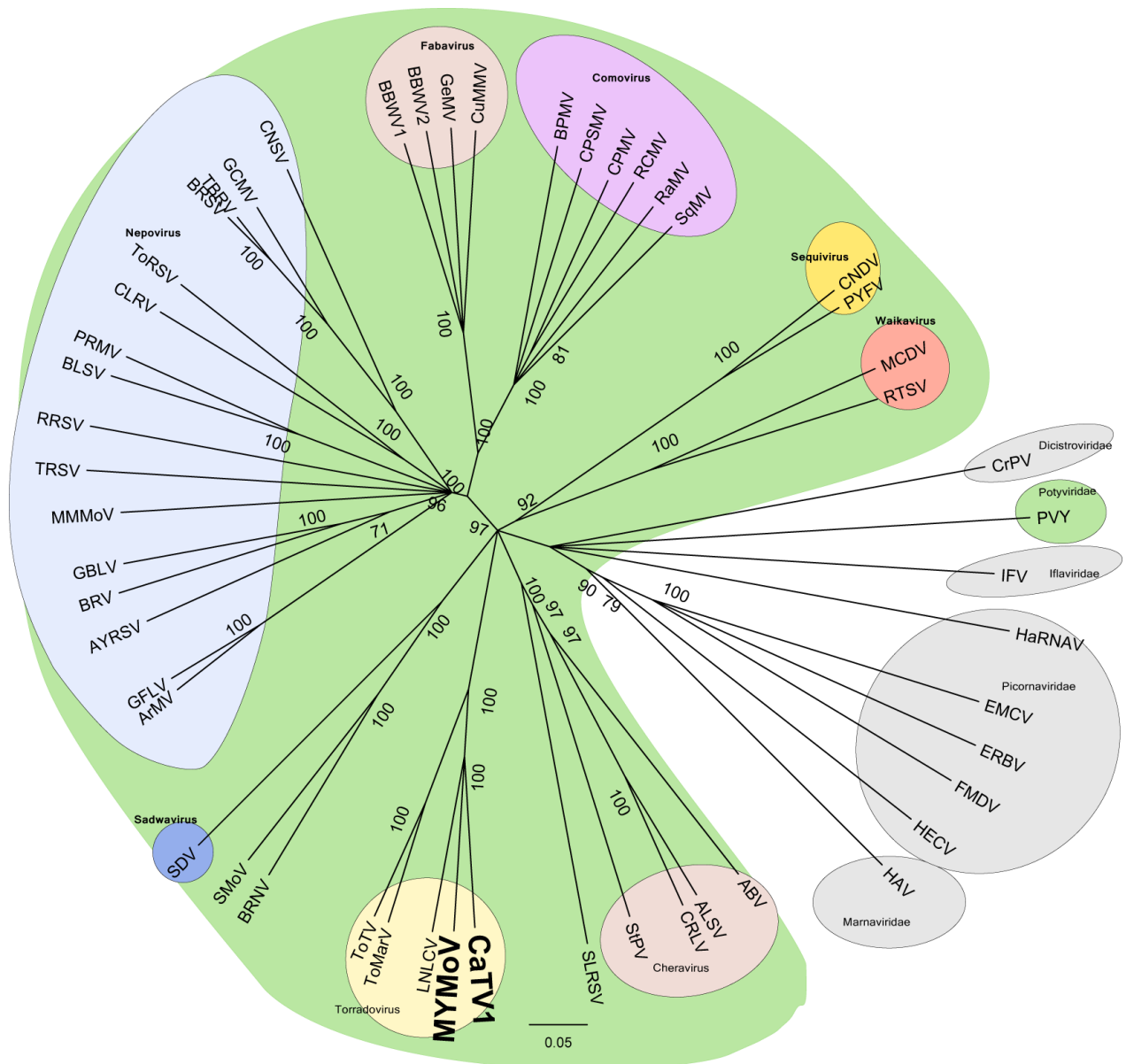
Fig1. Neighbor-joining tree of members of the order *Picornavirales* based on the amino acid sequences of the conserved domains between the "CG" motif of the 3C-like proteinase and the "GDD" motif of the polymerase (Pro-Pol region). The alignment was produced by the program T-Coffee (Tommaso et al, 2011) 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: Carrot torradovirus 1 (CaTV1, KF533719 = AHA85556), Motherwort yellow mottle virus (MYMoV, KM229700 = AIT59085), 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).