



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>2014.004aP</b>	(to be completed by ICTV officers)
<b>Short title:</b> New species in the genus <i>Torradovirus</i> , <i>Lettuce necrotic leaf curl virus</i> (LNL CV) (e.g. 6 new species in the genus <i>Zetavirus</i> )		
<b>Modules attached</b> (modules 1 and 9 are required)	1 <input checked="" type="checkbox"/> 2 <input checked="" 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 checked="" type="checkbox"/>	

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

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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 Study Group

**ICTV-EC or Study Group comments and response of the proposer:**

**EC Comments:** This proposal was conditionally approved by the EC. The tree should be modified to properly show genetic distances. More specifically, it should be built using Neighbor joining or maximum likelihood methods rather than UPGMA

**SG Response:** The tree was corrected as requested

Date first submitted to ICTV:

June 2014

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	<b>2014.004aP</b>	(assigned by ICTV officers)
<b>To create 1 new species within:</b>		
Genus:	<i>Torradovirus</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:	<i>Secoviridae</i>	
Order:	<i>Picornavirales</i>	
<b>Name of new species:</b>	<b>Representative isolate:</b>	<b>GenBank sequence accession number(s)</b>
<i>Lettuce necrotic leaf curl virus</i>	5317015	KC855266 (RNA1) and KC855267 (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

Species demarcation criteria have not been defined specifically for the genus *Torradovirus*, but the demarcation criteria as set for the family *Secoviridae* have been used for the genera within this family. These criteria are:

- CP aa sequence with less than 75% identity (for viruses with two or three CPs, combined CP sequences are considered)
- Conserved Pro-Pol region aa sequence (as defined above) with less than 80% identity
- Differences in antigenic reactions
- Distinct host range
- Distinct vector specificity
- Absence of cross-protection
- For viruses with a bipartite genome, absence of re-assortment between RNA1 and RNA2

**Lettuce necrotic leaf curl virus**

Lettuce necrotic leaf curl virus (LNLCV) was isolated from lettuce grown in the Netherlands. A lettuce plant displayed symptoms of unknown etiology consisting of leaf necrosis and curling. Next generation sequencing revealed the presence of a virus with a typical torradovirus genome organization (including the typical ORF1 in RNA2), but with low identities in comparison to the known (tomato-infecting) torradoviruses. The entire sequences for RNA1 [7579 nucleotides (nts), excluding the poly(A) tail] and RNA2 [5290 nts, excluding the poly(A) tail] were elucidated. This virus was, as other torradoviruses, mechanically transmissible and

was able to infect different lettuce cultivars, experimental hosts (*Nicotiana* species), but not a tomato cultivar that is sensitive to *Tomato torrado virus* (ToTV), *Tomato marchitez virus* (ToMarV) and tomato chocolate virus ToChV. In a reciprocal test, it was verified that ToTV, ToMarV and ToChV did not infect lettuce. Electron microscopy of infected material identified spherical particles of approximately 30 nm in diameter.

LNLCV did not react with the ToMarV antiserum (annex 1).

Sequence identities for the two regions mentioned in the demarcation criteria are overall low: 38-40% for the CP region and 55-58% for the Pro-Pol region. Sequence identities for the RNA2-ORF1 region and 3' UTRs are 24-26% and 28-30%, respectively, see annex 2).

Taken together, these data clearly indicate that LNLCV should be regarded as a separate torradovirus species, which is illustrated by a phylogenetic analysis (based on the Pro-Pol region in RNA1) represented in annex 3.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

**References:**

- Alfaro-Fernández, A., Cebrián, M.C., Herrera-Vásquez, J.A., Córdoba-Sellés, M.C., Sánchez-Navarro, J.A. and Jordá, C. (2010) Molecular variability of Spanish and Hungarian isolates of *Tomato torrado virus*. *Plant Pathology* 59: 785-793
- Le Gall O, Sanfaçon H, Ikegami M, Iwanami T, Jones T, Karasev A, Lehto K, Wellink J, Wetzel T, Yoshikawa N (2007) Cheravirus and Sadwavirus: two unassigned genera of plant positive sense single-stranded RNA viruses formerly considered atypical members of the genus Nepovirus (family Comoviridae). *Archives of Virology* 152:1767–1774
- Sanfaçon, H., Wellink, J., Le Gall, O., Karasev, A., van der Vlugt, R., Wetzel, T., 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(5), 899-907.
- Verbeek, M., Dullemans, A.M., Van den Heuvel, J.F.J.M., Maris, P.C. and Van der Vlugt, R.A.A. (2010) Tomato chocolàte virus: a new plant virus infecting tomato and a proposed member of the genus *Torradovirus*. *Archives of Virology* 155: 751-755
- Verbeek, M., Dullemans, A.M., Van Raaij, H.M.G., Verhoeven, J.Th.J. and Van der Vlugt, R.A.A. (2013) Lettuce necrotic leaf curl virus, a new plant virus infecting lettuce and a proposed member of the genus *Torradovirus*. *Archives of Virology*, DOI : 10.1007/s00705-013-1835-z
- Verbeek, M. (2013) Characterization and epidemiology of members of the genus *Torradovirus*. Thesis Wageningen University, Wageningen, The Netherlands (<http://edepot.wur.nl/273098>)

**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.

Annex 1:

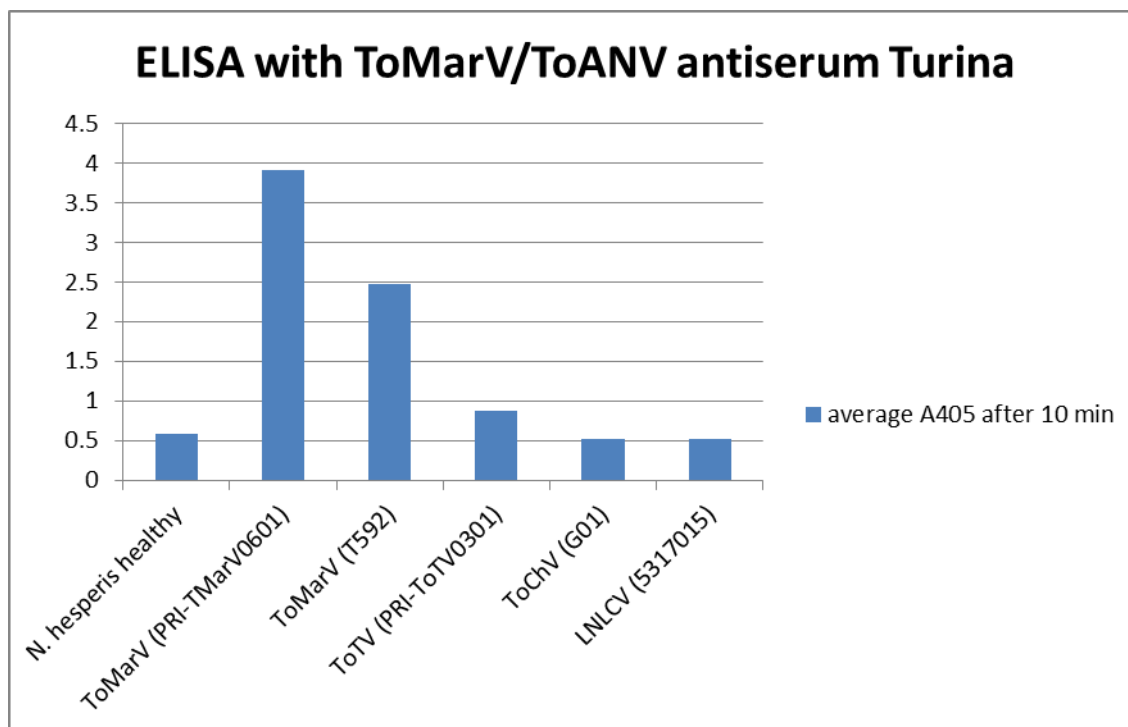
**Results of ELISA using the antiserum raised against tomato apex necrosis virus, belonging to the species *Tomato marchitez virus* (virus samples from test plants *N. hesperis* or *N. occidentalis*)**

Antiserum: anti-ToANV (tomato apex necrosis virus, an isolate of the species *Tomato marchitez virus*, with acknowledgement to dr. M.Turina, Istituto di Virologia Vegetale, CNR, Turino, Italy

	Buffer	sample	sample (duplo)	Buffer
Buffer	0.000625	-0.002	-0.000375	0.006
<i>N. hesperis</i> healthy	-0.003	0.587	0.596	-0.009
ToMarV (PRI-TMarV0601)	0.005	3.735	4.09	-0.002
ToMarV (T592)	-0.001	2.445	2.511	0.01
ToTV (PRI-ToTV0301)	-0.00038	0.866	0.908	-0.002
ToChV (G01)	0.003	0.529	0.515	0.00038
LNLCV (5317015)	-0.002	0.531	0.532	-0.003
Buffer	-0.00038	-0.002	-0.002	-0.006

average A405 after 10 min

<i>N. hesperis</i> healthy	0.5915
ToMarV (PRI-TMarV0601)	3.9125
ToMarV (T592)	2.478
ToTV (PRI-ToTV0301)	0.887
ToChV (G01)	0.522
LNLCV (5317015)	0.5315



Annex 2:

**Pairwise sequence comparisons**

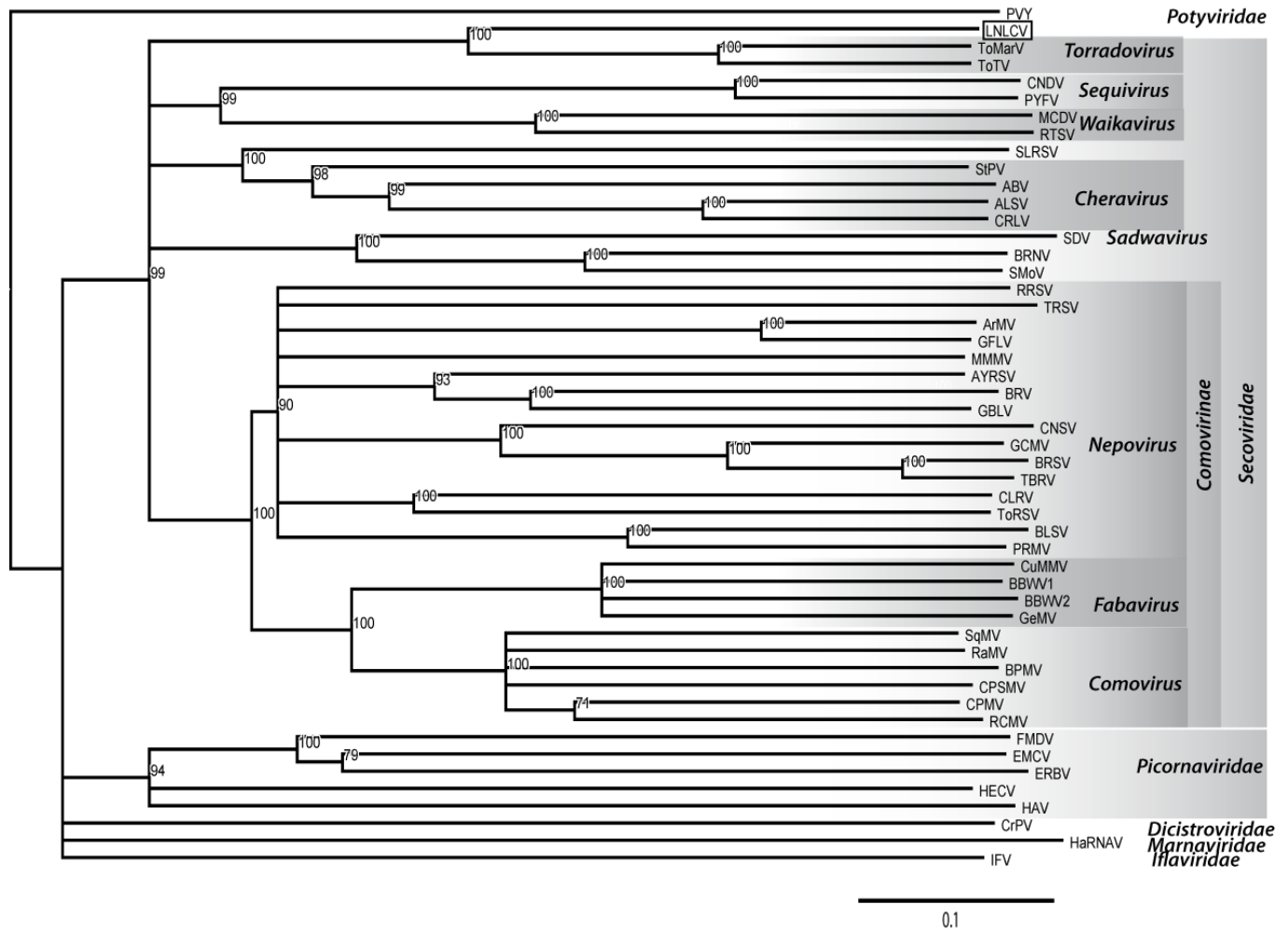
Figure 1: pairwise sequence comparison of the CP regions (combined three CPs) of torradoviruses

		1	2	3	4	5	6	7
ToTV-PRI-CP	1		99,58	74,86	75,87	75,84	73,78	39,32
ToTV-Wal'03 CP	2	99,58		75,00	76,01	75,98	73,92	39,18
ToMarV-CP	3	74,86	75,00		92,61	87,13	82,82	39,86
ToANV-CP	4	75,87	76,01	92,61		86,89	81,62	39,86
ToChSV-CP	5	75,84	75,98	87,13	86,89		82,68	40,00
ToChV-CP	6	73,78	73,92	82,82	81,62	82,68		38,22
LNL CV-CP	7	39,32	39,18	39,86	39,86	40,00	38,22	

Figure 2: pairwise sequence comparison of the RNA1 Pro-Pol regions of torradoviruses

		1	2	3	4	5	6	7
ToTV-PRI_ProPol	1		99,78	78,38	78,82	78,60	79,04	54,74
ToTV_Wal03_ProPol	2	99,78		78,38	78,82	78,60	79,04	54,53
ToMarV-PRI_ProPol	3	78,38	78,38		99,56	89,96	84,93	57,76
ToANV-ProPol	4	78,82	78,82	99,56		90,17	85,37	57,97
ToChSV_ProPol	5	78,60	78,60	89,96	90,17		82,10	55,82
ToChV_ProPol	6	79,04	79,04	84,93	85,37	82,10		56,68
LNL CV_ProPol	7	54,74	54,53	57,76	57,97	55,82	56,68	

Phylogenetic analysis based on the Pro-Pol region



Neighbor-joining tree of members of the order Picornvirales 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 newly described Lettuce necrotic leaf curl virus (LNLCV) is boxed. The alignment and the tree were generated using CLUSTALX (Thompson et al,1997) 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 Picornvirales 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 (RRSV, 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).

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