



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:	2016.010aP	(to be completed by ICTV officers)
Short title: 1 new species in the genus <i>Cilevirus</i> (e.g. 6 new species in the genus <i>Zetavirus</i>)		
Modules attached (modules 1 and 11 are required)	6 <input type="checkbox"/> 7 <input type="checkbox"/> 8 <input type="checkbox"/> 9 <input type="checkbox"/> 10 <input type="checkbox"/>	
2 <input checked="" type="checkbox"/> 3 <input type="checkbox"/> 4 <input type="checkbox"/> 5 <input type="checkbox"/>		

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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)

Cilevirus

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

Date first submitted to ICTV: July 18, 2016
Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

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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	2016.010aP	(assigned by ICTV officers)
To create 1 new species within:		
Genus:	<i>Cilevirus</i>	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.
Subfamily:	<i>n/a</i>	
Family:	<i>unassigned</i>	
Order:	<i>unassigned</i>	
Name of new species:	Representative isolate: (only 1 per species please)	GenBank sequence accession number(s)
<i>Citrus leprosis virus C2</i>	L147V1	JX000024 JX000025

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 11

The genus *Cilevirus* was established in 2009 and is currently represented by a single member, *Citrus leprosis virus C* (CiLV-C). This genus has not been assigned to a family. CiLV-C is bacilliform with particles approximately 125x50nm located in the cytoplasm of infected cells. The genome is bipartite, composed of two linear, positive sense ssRNA molecules. RNA 1 encodes two open reading frames. The first encodes a large polyprotein involved with virus replication. The second encodes a 29 kDa protein of unknown function. RNA 2 encodes four ORFs, one of which (ORF 3) has homology to viral movement proteins. The other three are of unknown function, however both ORF 2 of RNA 1 and ORF 4 of RNA 2 have been implicated as structural or capsid proteins. Both RNAs have a 5’ cap structure and a 3’ poly(A) tail. CiLV-C is non-systemic, and the virus is transmitted by tenuipalpid mites of the genus *Brevipalpus*. At present, no species demarcation criteria exist for the genus *Cilevirus* since only one species has been characterized.

Citrus leprosis virus C2

In 2013, a virus from Colombia associated with symptoms similar to those caused by CiLV-C was characterized (Roy et al. 2013). Transmission electron microscopy, DAS-ELISA, arthropod transmission, and complete genome sequencing was used to characterize this virus. Based on the results of these experiments, this virus was proposed to be a novel cilevirus, designated Citrus leprosis virus cytoplasmic type 2. For consistency with the cilevirus type species, here we propose the name “Citrus leprosis virus C2” (CiLV-C2).

Virions approximately 105x45nm were observed in the cytoplasm of CiLV-C2-infected cells. CiLV-C2 was found have a bipartite genome similar in size to CiLV-C. RNA 1 of CiLV-C and

CiLV-C2 are 8730 and 8717 nt, respectively, and share 58% sequence homology. RNA 2 of CiLV-C and CiLV-C2 are 4975 and 4989 nt, respectively, and share 50% sequence homology. The ~2500aa polyprotein encoded by RNA 1 is 59% identical between CiLV-C and CiLV-C2, and the RNA-dependent RNA 2 (RdRp-2) polymerase domain present in this polyprotein is 75% identical between the two. CiLV-C2 does not react with the polyclonal antibody CREC-13 that was raised against CiLV-C. Similarly, RT-PCR assays are able to distinguish between these viruses.

A virus with a very similar genome organization and high sequence identity to CiLV-C2 was identified in hibiscus (Melzer et al 2013). The polyprotein of RNA 1 had 95% amino acid identity to its homolog in CiLV-C2 from citrus. Overall, the proteomes of the two viruses were 92% identical. We contend this virus represents a hibiscus strain of CiLV-C2.

Proposed species demarcation criteria for the genus *Cilevirus*

Based on the discovery of a second putative member of the genus *Cilevirus*, we propose the following species demarcation criteria for the genus:

- Extent of serological relationship as determined by immunodiffusion and/or ELISA
- Less than 85% aa sequence identity for the proteome
- Natural host range
- Artificial host range reactions
- Vector species and transmission

With the lack of a serological relationship and limited nucleotide and amino acid sequence similarity between CiLV-C and CiLV-C2, we propose that CiLV-C2 represents a new member of the genus *Cilevirus*.

MODULE 11: **APPENDIX**: supporting material

additional material in support of this proposal

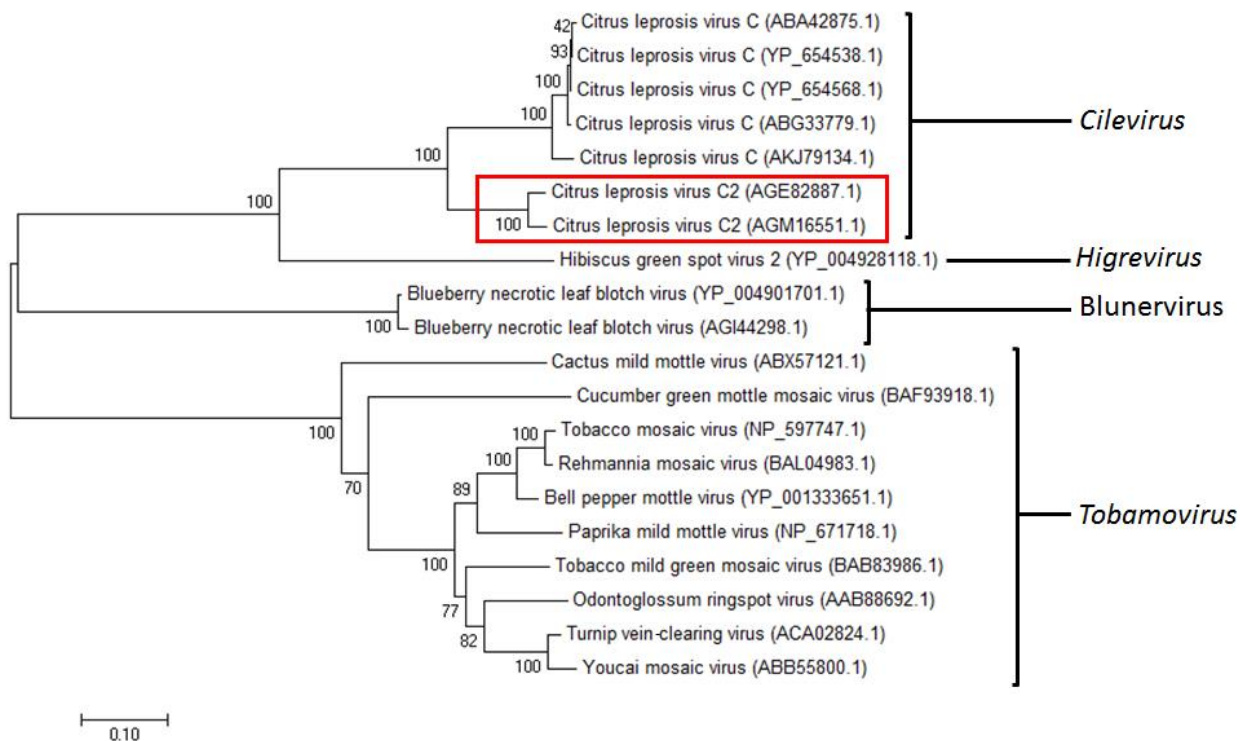
References:

Melzer, M, Simbajon, N, Carillo, J, Borth, WB, Freitas-Astua, J, Kitajima, EW, Neupane, KR, and Hu, JS. 2013. A cilevirus infects ornamental hibiscus in Hawaii. Archives of Virology 158:2421-2424.

Roy, A, Choudhary, N, Guillermo, LM, Shao, J, Govindarajulu, A, Achor, D, Wei, G, Picton, DD, Levy, L, Nakhla, MK, Hartung, JS, and Brlansky, RH. 2013. A novel virus of the genus Cilevirus causing symptoms similar to citrus leprosis. Phytopathology 103:488-500.

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.



Phylogenetic tree generated using a neighbour joining algorithm depicting the relationship of the proposed species Citrus leprosis virus C2 (highlighted in the red box) to *Citrus leprosis virus C* and related viruses from other genera based on their RdRp-2 protein sequence. Citrus leprosis virus C2 is represented by strains from citrus (AGE82887.1) and hibiscus (AGM16551.1). Included are Blueberry necrotic ring blotch virus and the genus Blunervirus, which are being proposed as a new species and genus, respectively, in a separate ICTV proposal. GenBank accession numbers are provided for each virus in parentheses. The scale in the bottom left represent the number of substitutions for the indicated branch length. Numbers indicate branch support following 1000 bootstrap replications.