

**Part 1:** **TITLE, AUTHORS, APPROVALS, etc**

|  |  |  |
| --- | --- | --- |
| **Code assigned:** | **2021.008P** |  |
| **Short title:** Create one new species in the genus *Cilevirus* (*Martellivirales*: *Kitaviridae*) | | |
|  | | |

**Author(s) and email address(es)**

|  |  |
| --- | --- |
|  |  |
| Melzer MJ, Freitas-Astúa J, Li JM, Peters J, Ramos-González PL, Rodrigues JVC, Roy A | [melzer@hawaii.edu](mailto:melzer@hawaii.edu); [juliana.astua@embrapa.br](mailto:juliana.astua@embrapa.br); [lijunmin@nbu.edu.cn](mailto:lijunmin@nbu.edu.cn); [j.peters2@ug.edu.au](mailto:j.peters2@ug.edu.au); [plrg1970@gmail.com](mailto:plrg1970@gmail.com); [jose\_carlos@mac.com](mailto:jose_carlos@mac.com); [avijit.roy@usda.gov](mailto:avijit.roy@usda.gov) |

**Author(s) institutional address(es) (optional)**

|  |
| --- |
|  |

**Corresponding author**

|  |
| --- |
| Michael J Melzer |

**List the ICTV Study Group(s) that have seen this proposal**

|  |
| --- |
| Family *Kitaviridae* |

**ICTV study group comments and response of proposer**

|  |
| --- |
|  |

**Authority to use the name of a living person**

|  |  |
| --- | --- |
| **Is any taxon name used here derived from that of a living person (Y/N)** | N |

|  |  |  |
| --- | --- | --- |
| **Taxon name** | **Person from whom the name is derived** | **Permission attached (Y/N)** |
|  |  |  |
|  |  |  |
|  |  |  |

**Submission dates**

|  |  |
| --- | --- |
| Date first submitted to SC Chair | May 21, 2021 |
| Date of this revision (if different to above) | August 17, 2021 |

**ICTV-EC comments and response of the proposer**

|  |
| --- |
|  |

**Part 2:** **NON-TAXONOMIC PROPOSAL**

**Text of proposal**

|  |
| --- |
|  |

**Part 3:** **TAXONOMIC PROPOSAL**

**Name of accompanying Excel module**

|  |
| --- |
| 2021.008P.A.v1.Cilevirus\_1ns.xlsx |

**Abstract**

|  |
| --- |
| This taxonomic proposal justifies the creation of *Cilevirus oahuense* as a new species in the genus *Cilevirus* (family *Kitaviridae*), which currently consists of three members: *Citrus leprosis virus C*, *Citrus leprosis virus C2*, and *Passion fruit green spot virus*. The genome organization of the exemplar isolate hibiscus yellow blotch virus is distinct, and it shares low amino acid sequence identities with established members of the genus *Cilevirus*. Phylogenetic analyses place hibiscus yellow blotch virus and the species *Cilevirus oahuense* within the family *Kitaviridae* as a basal member of the *Cilevirus* genus. |

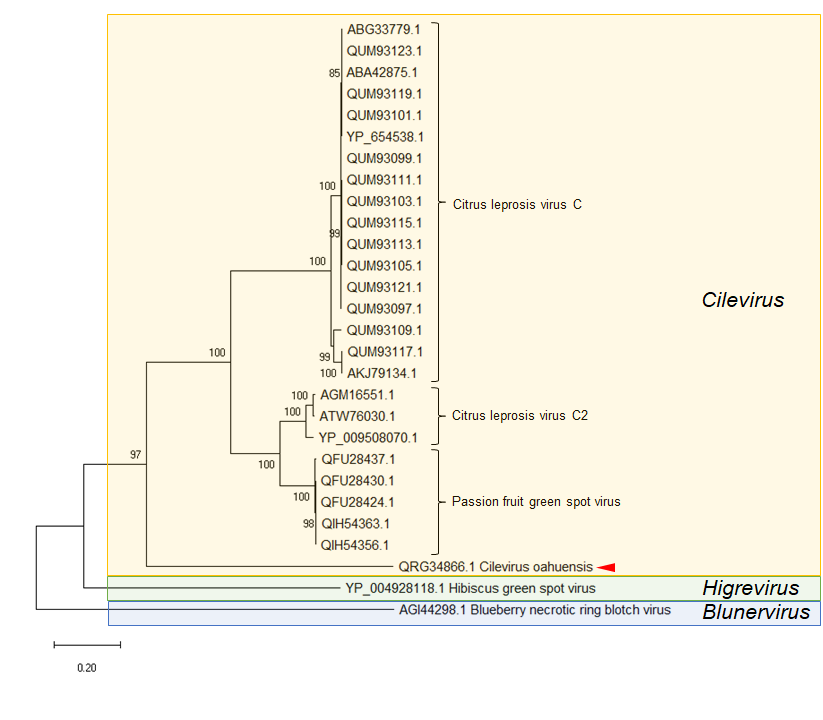
**Text of proposal**

|  |  |
| --- | --- |
| |  | | --- | | The genus *Cilevirus* was established in 2009 to accommodate a monophyly of plant viruses possessing positive sense, single-stranded RNA genomes consisting of two RNA molecules (Carstens 2010, Locali-Fabris et al. 2012). Both RNA molecules are polyadenylated at their 3’-terminus. RNA1 is approximately 9 kb in size and encodes a large replication-associated polyprotein and the putative coat protein (CP) (Locali-Fabris et al. 2012, Leastro et al. 2018). RNA2 is approximately 5 kb in size and encodes a ~15 kDa protein (p15) with RNA silencing suppression activity, a putative glycoprotein (p61), a putative movement protein (MP), and a membrane bound-protein (p24) (Kuchibhatla et al. 2014, Leastro et al. 2018, 2020). *Citrus leprosis virus C2* and *Passion fruit green spot virus* also encode ~7 kDa (p7) and ~13 kDa (p13) proteins of unknown function in the intergenic region between p15 and p61, respectively (Roy et al. 2013 and Ramos-González et al. 2020). Cileviruses are non-systemic in their plant host and typically produce chlorotic and/or necrotic lesions on foliage and stem tissue. Symptomatic host cells often contain bacilliform particles approximately 60 x 115 nm that are usually localized near the endoplasmic reticulum, as well as electron-dense viroplasm (Colariccio et al. 2000, Kitajima et al. 1974). Cileviruses are transmitted by mite species within the genus *Brevipalpus* (Rodrigues et al. 2003).  The current demarcation criteria for species of the genus *Cilevirus* are based on:  • Extent of the serological relationship as determined by immunodiffusion and/or ELISA  • Less than 85% amino acid (aa) sequence identity for the proteome  • Natural host range  • Artificial host range reactions  • Vector species and transmission  Hibiscus yellow blotch virus, proposed species *Cilevirus oahuense*  In 2019-2020, multiple hibiscus plants with symptoms consistent with cilevirus infection were observed by Olmedo-Velarde et al. (2021) growing on the island of Oahu, USA. Symptoms included localized chlorotic spots, often with a dark green perimeter on the leaves of affected plants. In some leaves, these lesions were necrotic. Data from high throughput sequencing (HTS) and Rapid Amplification of cDNA Ends (RACE) experiments indicated the presence of two RNA molecules, which comprised the genome of a novel virus with similarity to cileviruses (Figure 1). RNA1 (MT472637) was found to be 8382 nucleotide (nt) in length, excluding the poly(A) tail, and encoded a polyprotein with a methyltransferase, cysteine-like protease, FtsJ-like methyltransferase, helicase, and RNA-dependent RNA polymerase domains. RNA1 also possessed a second open reading frame (ORF) putatively encoding a ~10kDa protein of unknown function. Homologs of this putative protein have not been reported in other cileviruses, but Olmedo-Velarde et al. (2021) detected an ORF of similar size and position in the genome of hibiscus green spot virus 2, the lone member of the closely related genus *Higrevirus* (*Martellivirales*: *Kitaviridae*). RNA2 (MT472638) was 4411 nt in length, excluding the poly(A) tail, and lacked the p15 ORF and intergenic region found in cileviruses. RNA2 encoded four proteins: the p61 homolog, MP, and p24 homolog, which are typically found on RNA2 of cileviruses, and the putative CP (which is typically encoded by RNA1 of cileviruses).  When amino acid identity comparisons were made between hibiscus yellow blotch virus and cileviruses for the polyprotein, p61, MP, CP, and p24 proteins, no values exceeded 46% (Olmedo-Velarde et al. 2021).  Transmission electron microscopy of both ultrathin sections of infected leaf tissue and partial virion purification preparations revealed the presence of spherical structures approximately 60 nm in diameter (Olmedo-Velarde et al. 2021). It is unclear if these structures represent the virion of hibiscus yellow blotch virus. No viroplasms in cells of symptomatic tissue were reported.  Hibiscus yellow blotch virussequences were detected in *Brevipalpus* sp. specimens collected from symptomatic hibiscus plants, and these mites represent a potential vector of the virus (Olmedo-Velarde, 2021).  Phylogenetic analyses of the replication-associated polyprotein using distance- and character-based algorithms place hibiscus yellow blotch virus as a basal member of the cilevirus clade (Olmedo-Velarde et al. 2021 and Figure 2).  Considering the unique genome organization and low sequence identity between homologous proteins, it is clear that hibiscus yellow blotch virus represents the exemplar isolate of a distinct virus species. Phylogenetic analyses indicate the genus *Cilevirus* is the most appropriate current taxon for hibiscus yellow blotch virusplacement. Therefore, we propose to create the species *Cilevirus oahuense* as a new member of the genus *Cilevirus* of the family *Kitaviridae*. | |

**Supporting evidence**

****

**Figure 1.** Genome organization of hibiscus yellow blotch virus (*Cilevirus oahuense*) compared to members of the genus *Cilevirus*. Boxes represent open reading frames, and those with similar fill colors are orthologs-based. Unshaded boxes are of unknown function, and those with a dashed perimeter are not present in all virus isolates. The legend provides the predicted or putative function. MET, methyltransferase; C-PRO, cysteine-like protease; Fts-MET, FtsJ-like methyltransferase; HEL, helicase; RdRp, RNA-dependent RNA polymerase; AAAAA, poly(A) tail at 3’ end of RNA.

****

**Figure 2.** Phylogenetic placement of hibiscus yellow blotch virus (*Cilevirus oahuense*) with members of the genus *Cilevirus* and representative members of the genera *Higrevirus* and *Blunervirus*, which together comprise the family *Kitaviridae*. The replication-associate polyprotein was used in a Neighbor-Joining algorithm to estimate phylogenetic placement. Node numbers represent branch support following 1000 bootstrap replications. The scale bar represents the number of substitutions for the depicted branch length.

**References**

Carstens EB (2010) Ratification vote on taxonomic proposals to the International Committee on Taxonomy of Viruses (2009). Arch Virol 155**:**133–146. <https://doi.org/10.1007/s00705-009-0547-x>. PMID: 19960211

Colariccio A, Lovisolo O, Boccardo G, Chagas CM, d’Aquilio M, Rossetti V (2000) Preliminary purification and double stranded RNA analysis of citrus leprosis virus. In: da Graca JV, Lee RF, Yokomi RK (eds) Proceedings of the 14th Conference of the International Organization of Citrus Virologists. UC Riverside, Riverside California, USA, pp 159-163

Kitajima EW, Rosillo MA, Portillo MM, Müller GW, Costa AS (1974) Microscopia electronica de tecidos foliares de laranjeiras infectadas pela lepra explosive da Argentina. *Fitopatologia (Lima)* 9:254-258.

Kuchibhatla DB, Sherman W A, Chung BY, Cook S, Schneider G, Eisenhaber B, et al. (2014) Powerful sequence similarity search methods and in-depth manual analyses can identify remote homologs in many apparently “orphan” viral proteins. J Virol 88:10–20. 10.1128/JVI.02595-13. PMID: **24155369**

Leastro MO, Castro D, Freitas-Astúa J, Kitajima EW, Pallás V, Sánchez-Navarro J Á (2020) Citrus Leprosis Virus C Encodes Three Proteins With Gene Silencing Suppression Activity. Front Microbiol 11*:*1231. <https://doi.org/10.3389/fmicb.2020.01231>. PMID: **32655520**

Leastro MO, Kitajima EW, Silva MS, Resende RO, Freitas-Astua J. (2018) Dissecting the Subcellular Localization, Intracellular Trafficking, Interactions, Membrane Association, and Topology of Citrus Leprosis Virus C Proteins. Front Plant Sci9:1299. doi: 10.3389/fpls.2018.01299. PMID: **30254655**

Locali-Fabris E.C., Freitas-Astúa J, Machado MA (2012) Genus *Cilevirus*. InternationaCommittee on Taxonomy of Viruses. In: King A, Adams M, Carstens E, Lefkowitz E (eds) Virus Taxonomy, IX Report. Elsevier/Academic Press, London, United Kingdom, pp 1139–1142

Olmedo-Velarde A, Hu JS, Melzer MJ (2021) A Virus Infecting *Hibiscus rosa-sinensis* Represents an Evolutionary Link Between Cileviruses and Higreviruses. Front Microbiol 12:660237. doi: 10.3389/fmicb.2021.660237

Ramos-González PL, Santos GF dos, Chabi-Jesus C, Harakava R, Kitajima EW, Freitas-Astúa J (2020) Passion Fruit Green Spot Virus Genome Harbors a New Orphan ORF and Highlights the Flexibility of the 5′-End of the RNA2 Segment Across Cileviruses. Front Microbiol 11:206. <https://doi.org/10.3389/fmicb.2020.00206>. PMID: **32117189**

Rodrigues JVC, Kitajima EW, Childers CC, Chagas CM (2003) Citrus leprosis virus vectored by Brevipalpus phoenicis (Acari: Tenuipalpidae) on citrus in Brazil. Exp Appl Acarol 30:161-179. doi: [10.1023/b:appa.0000006547.76802.6e](https://doi.org/10.1023/b:appa.0000006547.76802.6e). PMID: 14756415

Roy A, Choudhary N, Guillermo LM, Shao J, Govindarajulu A, Achor D, Wei G, Picton DD, Levy L, Nakhla MK, Hartung JS, Brlansky RH (2013) A novel virus of the genus Cilevirus causing symptoms similar to citrus leprosis. Phytopathology. 103: 488-500. doi: 10.1094/PHYTO-07-12-0177-R. PMID: **23268581**