

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

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| **Code assigned:** | **2020.011P** |  |
| **Short title:** Create one new species in the genus *Cilevirus* (*Martellivirales*: *Kitaviridae*) | | |
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**Author(s) and email address(es)**

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

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| *Kitaviridae* SG |

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

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| The proposal has been approved by all member of the *Kitaviridae* Study Group. |

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

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

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| Date first submitted to SC Chair | July 29, 2020 |
| Date of this revision (if different to above) |  |

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

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

**Text of proposal**

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

**Name of accompanying Excel module**

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| 2020.011P.R.Kitaviridae\_1nsp.xlsx |

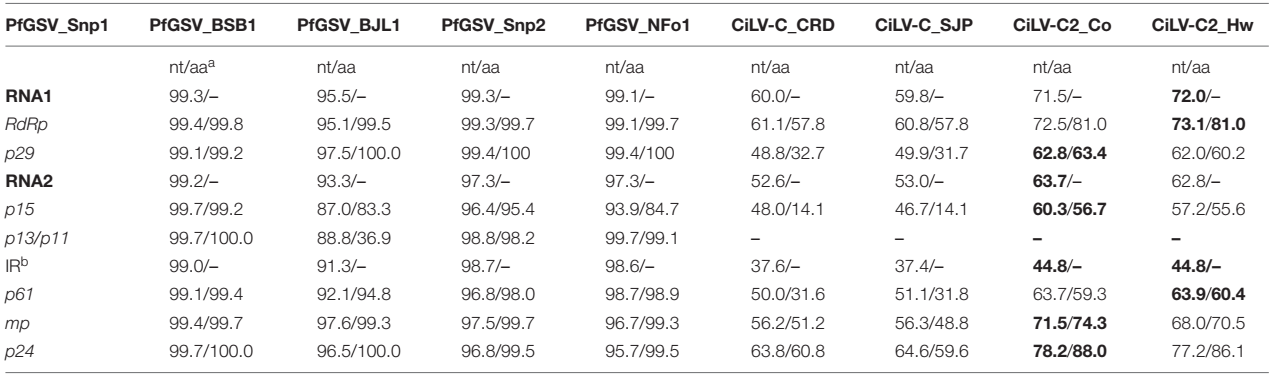
**Abstract**

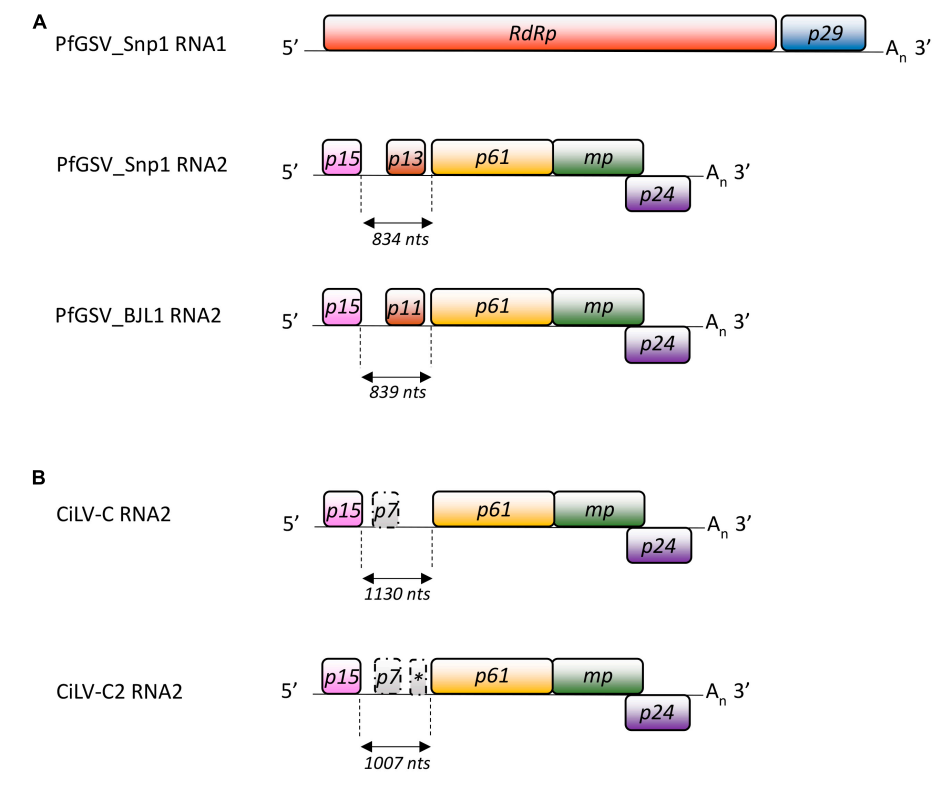
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| The current taxonomic proposal presents passion fruit green spot virus as a new species in the genus *Cilevirus* (family *Kitaviridae*), which currently comprises two members: the type species *Citrus leprosis virus C*, and *Citrus leprosis virus C2*. The proposal is based on the equivalency of biological features such as virion morphologic and size, general symptoms and absence of systemic infection in their host plants, transmission by *Brevipalpus* sp. mites, cytopathic effects on the infected plant cells, and also, the global genomic organization of members of the proposed species and those showed by the known cileviruses. At the same time, passion fruit green spot virus (PfGSV) shows a distinct natural host range and a sufficient nucleotide sequence divergence (32-50%) from definitive members of the genus *Cilevirus*. Phylogenetic analyses consistently indicate a common ancestor between PfGSV and recognized cileviruses. |

**Text of proposal**

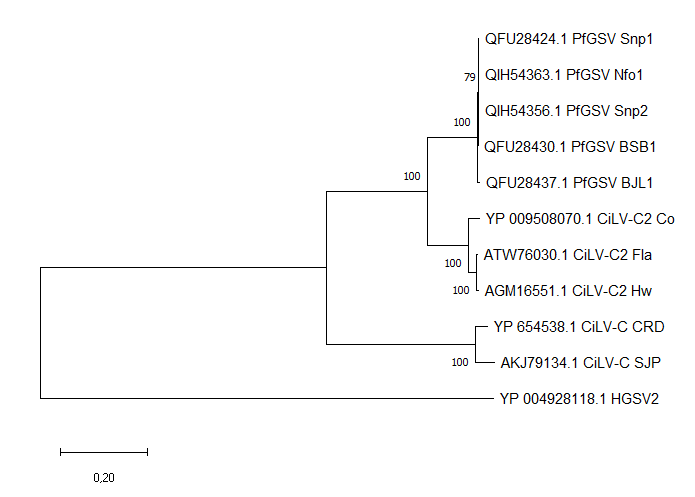
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| |  | | --- | | The genus *Cilevirus* groups single-stranded positive-sense bipartite RNA viruses [1]. The genus was created in 2012 to accommodate viruses with the unique biological and molecular features of its type species: *Citrus leprosis virus C* [2]. Besides the type species, the genus comprises another citrus-infecting virus clustered in the species *Citrus leprosis virus C2*.  Typically, a cilevirus infected plant cell contains both short, membrane-bound, bacilliform particles with 50–70 × 110–120 nm within the endoplasmic reticulum cisternae and an electron-dense, vacuolated viroplasm in the cytoplasm [3]. The RNA1 molecule of cileviruses, of ∼9 kb, has two open reading frames (ORFs) that encode the RNA-dependent RNA polymerase (RdRp) and the putative 29 kDa capsid protein. The ∼5 kb RNA2 has four ORFs encoding, beside the movement protein (3A superfamily, pfam00803), three proteins *i*.*e*. P61, P24, and P15, whose functions have not yet been determined. P61 and P24 seem to be virion structural components according to the role of their homologous proteins identified in arthropod-infecting viruses of the Negevirus and Nege-like virus groups. P15 protein is the more divergent among cileviruses, which is encoded by an orphan ORF.  The current demarcation criteria for species of the genus *Cilevirus* is based on:  • Extent of the 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  Passion fruit green spot virus (PfGSV)  Passion fruit green spot virus (PfGSV) was first identified in 1997 and transmitted by *Brevipalpus* mites after a severe outbreak at Vera Cruz municipality, state of São Paulo, Brazil [4].  Virions of PfGSV are short, bacilliform particles with 50–70 nm width and 100–120 nm length. In the infected plant cells they accumulate in the cisternae of the endoplasmic reticulum, and viroplasms are observed in the cytoplasm [5]. Sap extracts obtained from passion fruit (*Passiflora edulis* Sims. f. *flavicarpa*) plants infected with PfGSV do not react with polyclonal antibodies produced in rabbits using the P29 recombinant protein of CiLV-C [6]. PfGSV has been identified naturally infecting more than a dozen species of plants belonging to nine families, which are not naturally affected by either CiLV-C or CiLV-C2.  The virus has been successfully transmitted using viruliferous mites of the species *Brevipalpus* *yothersi* and *B. papayensis* [4].  The genomes of five isolates of PfGSV have been obtained using either high throughput sequencing or Sanger method [7]. They were annotated and are available in a public database identified with the following GenBank accession numbers: PfGSV\_Snp1 RNA1: MK804171 and RNA2: MK804172, PfGSV\_BSB1 RNA1 MK804173: and RNA2 MK804174, PfGSV\_BJL1 RNA1: MK804175 and RNA2: MK804176, PfGSV\_Snp2 RNA1: MN746810 and RNA2: MN746811, and PfGSV\_NFo1 RNA1: MN746812 and RNA2: MN746813.  PfGSV isolate Snp1 is considered the exemplar member of the proposed species *Passion fruit green spot virus*. Excluding the poly-A tails at the 3’-ends, its genome consists of 13,523 nts split into two molecules designated as RNA1 and RNA2 with 8,740 and 4,783 nts, respectively. Pairwise comparisons of the genomic molecules of the isolate Snp1 with those from Snp2, NFo1, and BSB1 revealed a high nucleotide sequence identity (RNA1 > 99.1% and RNA2 > 97.3), whilst with the BJL1 isolate the values were slightly lower (RNA1: 95.5% and RNA2: 93.3%) (Table 1). In this context, PfGSV shows less intraspecies variability than that observed among the strains of CiLV-C and CiLV-C2.  The PfGSV genome contains seven putative ORFs with more than 200 nts, two in RNA1, and five in RNA2 (Figure 1). Comparisons of PfGSV\_Snp1 with known cileviruses revealed the highest nucleotide and deduced amino acid sequence identities with CiLV-C2 isolates Hw and Co (Table 1). At the genomic level, the highest identity values corresponded to RNA1 molecules (∼72.0%); while the RNA2 segments are more divergent (∼64.0%), even though they harbor the *p24* ORF that is the most conserved ORF across cileviruses. In the comparisons with the CiLV-C2 isolates, P29, P15, and P61 proteins accounted for the lower levels of amino acid identities. In general, regardless of the evaluated gene, deduced protein, or virus, the lowest values of identity always corresponded to the comparisons between PfGSV and CiLV-C. Particularly, the RNA2 of PfGSV possesses an orphan ORF, described as *p13*, not detected in the genome of known cileviruses (Figure 1).  Phylogenetic analyses of the RdRp sequences reveal a closer relationship between PfGSV and strains of CiLV-C2, CiLV-C, and, although to a lesser extent, with other viruses in the family *Kitaviridae* (Figure 2). Trees constructed using the amino acid sequences of P24 and MP also show the same topology (not shown).  Considering that PfGSV lacks a serological relationship with CiLV-C, shows a limited nucleotide and amino acid sequence identity with CiLV-C and CiLV-C2, and notably different natural host range with known definitive members of the genus, we propose to create the new species *Passion fruit green spot virus* in the genus *Cilevirus.* | |

**Supporting evidence**

**Table 1.** Nucleotide (nt) and deduced amino acid (aa) identities of passion fruit green spot virus (PfGSV).



**Figure 1.** Genome organization of passion fruit green spot virus (A) and the RNA2 of definitive members of the genus *Cilevirus* (B). Boxes indicate the ORFs. RdRp: RNA-dependent RNA polymerase; p29: putative coat protein; MP: putative movement protein; p15, p13, p11, p61, and p24: proteins with unknown functions. ORFs p7 and that identified with an asterisk in the IR of CiLV-C and CiLV-C2 have been poorly described. An: poly(A) tail.



**Figure 2.** Molecular phylogenetic analysis of plant-infecting viruses of the genera *Cilevirus* and *Higrevirus*, family *Kitaviridae* using the amino acid sequences of their RdRp proteins. The evolutionary history was inferred by using the Maximum Likelihood method and Le\_Gascuel\_2008 model [8]. The tree with the highest log likelihood (-21666,06) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (4 categories (+G, parameter = 2,3850)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 11 amino acid sequences. There were a total of 2692 positions in the final dataset. The sequence of hibiscus green spot virus 2 was used as an outgroup. Evolutionary analyses were conducted in MEGA X [9].

**References**

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