

The International Committee on Taxonomy of Viruses

Taxonomy Proposal Form, 2024

**Part 1a: Details of taxonomy proposals**

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| **Title:** | Create a new species in the genus *Cilevirus* andtwo in the genus *Higrevirus*, family *Kitaviridae* (*Martellivirales*). | |
| **Code assigned:** | 2024.006P.A.v1.Kitaviridae\_3nsp |

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**Part 1b: Taxonomy Proposal Submission**

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| **ICTV Subcommittee:** | | | |
| Animal DNA Viruses and Retroviruses |  | Bacterial viruses |  |
| Animal minus-strand and dsRNA viruses |  | Fungal and protist viruses |  |
| Animal positive-strand RNA viruses |  | Plant viruses | X |
| Archaeal viruses |  | General - |  |

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| **List the ICTV Study Group(s) that have seen or have been involved in creating this proposal:** |
| *Kitaviridae* Study Group |

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| **Optional – complete only if formally voted on by an ICTV Study Group:** | | | |
| **Study Group** | **Number of members** | | |
| **Votes in support** | **Votes against** | **No vote** |
| *Kitaviridae* | 6 |  | 1 |
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| **Submission date:** | 11/06/2024 |

**Part 1c: Feedback from ICTV Executive Committee (EC) meeting**

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| **Executive Committee Meeting Decision code:** | **X** |
| A – Accept | **X** |
| Ac – Accept subject to revision by relevant subcommittee chair. No further vote required |  |
| U – Accept without revision but with re-evaluation and email vote by the EC |  |
| Uc – Accept subject to revision and re-evaluation and email vote by the EC |  |
| Ud – Deferred to the next EC meeting, with an invitation to revise based on EC comments |  |
| J - Reject |  |
| W - Withdrawn |  |

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| **Comments from the Executive Committee:** |
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**Part 1d: Revised Taxonomy Proposal Submission**

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| **Response of proposer:** |
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| **Revision date:** | DD/MM/YYYY |

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

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| **Name of accompanying Excel module:** |
| 2024.006P.A.v1.Kitaviridae\_3nsp.xlsx |

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| **Taxonomic changes proposed:** | | | |
| Establish new taxon | X | Split taxon |  |
| Abolish taxon |  | Merge taxon |  |
| Move taxon |  | Promote taxon |  |
| Rename taxon |  | Demote taxon |  |
| Move and rename |  |

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| **Is any taxon name used here derived from that of a living person:** | | **N** |
| **Taxon name** | **Person from whom the name is derived** | **Attached X** |
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| **Abstract of Taxonomy Proposal:** |
| *Taxonomic rank(s) affected*: Species  *Description of current taxonomy*:  Family *Kitaviridae*, order *Martellivirales*, includes plant-infecting viruses having linear single-stranded (ss) positive-sense (+) split RNA genomes. Viruses in this family are assigned to the genera *Cilevirus*, *Higrevirus*, or *Blunervirus* (Quito-Avila *et al.*, 2021; Ramos-González et al., 2023).  *Proposed* *taxonomic change(s):*  Create three new species in the family *Kitaviridae*; one in the genus *Cilevirus,* and two in the genus *Higrevirus*.    *Justification*:  The genomes of the three novel viruses show an arrangement that resembles that of kitavirids, and their core conserved proteins share relatively low amino acid (aa) sequence identities (<85%) with recognized members of the family *Kitaviridae*. In phylogenetic analyses, the three viruses grouped with characterized members of the genera *Cilevirus* and *Higrevirus*, but they are well-separated and supported by bootstrap values higher than 95%. All new species meet the already established or the demarcation criteria defined in this proposal. |

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| **Text of Taxonomy proposal:** |
| *Taxonomic rank(s) affected*: Species  *Description of current taxonomy*:  Viruses classified in the family *Kitaviridae*, order *Martellivirales*, infect plants and have linear single-stranded (ss) positive-sense (+) split RNA genomes. They are assigned to the genera *Cilevirus*, *Higrevirus*, or *Blunervirus* (Quito-Avila et al., 2021; Ramos-González et al., 2023). Currently, the genus *Cilevirus* has seven species, and the genera *Higrevirus* and *Blunervirus* group one and three species, respectively (https://ictv.global/taxonomy). The genome of the typical cileviruses is divided into two RNA segments with 3’-poly(A) tails. The RNA1 comprises two open reading frames (ORFs) encoding RNA-dependent RNA polymerase (RdRp) and a putative coat protein, whereas RNA2, possesses three or four ORFs including the movement protein, and likely two virion structural proteins: the P24 transmembrane (TM) protein, and a putative glycoprotein. The genome of hibiscus green spot virus 2 (HGSV2), representing the only recognized higrevirus species (*Higrevirus waimanalo*), which was associated with leprosis-like symptoms in citrus and green spots on leaves in hibiscus in Hawaii, has three genomic RNA segments with a polyadenylated tail at the 3’-terminus (Ramos-González et al., 2023).  *Proposed taxonomic change(s)*:  This taxonomic proposal aims to classify Phellodendron-associated higre-like virus (PaHLV) and Pistachio virus X (PisVX) into two new species in the genus *Higrevirus*, and Pistachio virus Y (PisVY) into a new species in the genus *Cilevirus;* both genera are part of the family *Kitaviridae,* order *Martellivirales*. The PaHLV and PisVX genomes are divided into three single-strand (+) RNA molecules. RNA1 segments contain a large open reading frame (ORF) encoding a polyprotein with the conserved domains of Viral methyltransferase (VMT), RNA helicase (HEL), and RNA-dependent RNA polymerase (RdRp). The RNA2 segments of PaHLV and PisVX consist of two and three major ORFs, respectively, whereas the RNA3 segments have three putative ORFs. Out of there, one of the ORFs encodes a putative transmembrane protein having an SP24 domain. The genome of PisVY is divided into two single-strand (+) RNA molecules. PisVY-RNA1 has two ORFs, the largest one encoding a polyprotein with the VMT, HEL, and RdRp domains, whereas PisVY-RNA2 contains three putative ORFs, including a protein with an SP24 domain. The genomes of the novel viruses show a genomic arrangement that resembles those of kitavirids, whereas deduced encoded proteins from each ORF share relatively low aa sequence identities with the existing members of the family *Kitaviridae*. In phylogenetic analyses, PaHLV and PisVX occupy the existing higreviruses clade, while PisVY groups with cileviruses.   1. **Phellodendron-associated higre-like virus** (PaHLV) was first identified in *Phellodendron amurense* Rupr. plants in China (Li *et al.*, 2023). The full viral genome was determined by RNA-seq, RT-PCR, 5′-RACE, and 3′-RACE, and comprises three RNA segments (GenBank accession numbers OP324809-OP324811) (Figure 1A). PaHLV RNA1 (8,211nt, including non-coding regions and the poly-A tail) has an ORF encoding the polyprotein RNA-dependent RNA polymerase (RdRP) with the VMT, HEL, and RdRp domains. RNA2 (3,092 nt) has three ORFs: *p8* (hypothetical protein of 8 kDa), *p40* (encoding a protein with the conserved Viral\_helicase 1 superfamily), and *p10* (hypothetical protein of 10 kDa). RNA3 (4,036 nt) also has three ORFs encoding: *p73*, *p32* [a protein with the motifs SP24 and PspC (PspC\_subgroup\_2 superfamily domain pneumococcal surface protein), and three TMDs], and *p23* (hypothetical protein of 23 kDa). The deduced aa sequence of protein encoded by each ORF shows the highest aa identity (≤78.1%) with the respective homolog of pistachio virus X (PisVX), a tentative member of the genus *Higrevirus*. Amino acid identity values in the comparison with HGSV2 were below 43%. Based on the Maximum likelihood (ML) tree generated from RdRP aa sequences, PaHLV is placed in an independent branch with HGSV2 and PisVX (Figure 2).      1. **Pistachio virus X** (PisVX) was identified by transcriptomic dataset analysis of *Pistacia vera* L. (pistachio) plants, Ohadi cultivar, under salinity stress in Rafsanjan, Iran, from 2015 to 2017 (Mohammadi *et al.*, 2021). The complete coding genome (CCG) of PisVX includes three RNA molecules (GenBank accession numbers MT334618-MT334620) (Figure 1B). PisVX RNA1 (8,196 nt) has a large ORF encoding a polyprotein with three conserved domains VMT, HEL, and RdRp, while PisVX RNA2 (2,419 nt) has two ORFs encoding putative movement proteins (*p39* and *p9*). RNA3 (3,869 nt) encodes three proteins: two hypothetical proteins of 66 and 23 kDA (*p66* and *p23*), respectively, and a putative transmembrane protein of 31 kDa with the SP24 motif. The deduced aa sequences of proteins encoded by these ORFs share <41% aa sequence identity with proteins of HGSV2, a member of the only recognized species (*Higrevirus waimanalo*) in the genus. PisVX-deduced proteins show the highest identities (≤ 78,01%) to those of PaHLV, an unclassified member of the genus. Based on an ML tree generated from RdRp aa sequences, PisVX is placed within the clade of the genus *Higrevirus* with HGSV2 (Figure 2). 2. **Pistachio virus Y** (PisVY) was also identified by the analysis of a transcriptomic dataset from *Pistacia vera* L. (pistachio) plants, Ohadi cultivar, under salinity stress in Rafsanjan, Iran, during 2015–2017 (Mohammadi *et al.*, 2021). The CCG of PisVY comprises two RNA molecules (MT362605-MT362606) (Figure 1C). PisVY RNA1 (8,716 nt) has two ORFs; the larger one encodes a polymerase with the VMT, HEL, and RdRp domains, and the smaller one encodes *p33*, the putative coat protein. PisVY-RNA2 (3,871 nt) encodes three putative proteins: a 69-kDa hypothetical protein (*p69*) with a signal peptide and potential glycosylation sites, a protein with the 3A conserved movement domain, and a protein with the SP24 conserved domain. The deduced aa sequences of proteins encoded by the PisVY ORFs show the highest identities ≤56.91% with the counterparts of other classified cileviruses. Based on an ML tree generated from RdRp aa sequences, PisVY is placed in an independent branch, close to that of hibiscus yellow blotch virus, in a basal position of the clade comprising members of the genus *Cilevirus* (Figure 2).   We propose classifying PaHLV and PisVX into the new species *Higrevirus amurense* and *Higrevirus pistaciae,* respectively*,* in the genus *Higrevirus,* family *Kitaviridae*. We also recommend classifying PisVY into the new species *Cilevirus pistaciae*, in the genus *Cilevirus,* family *Kitaviridae*. The epithets in the binomial species names used for each of the three viruses refer to the plant hosts where these viruses were first detected.  *Demarcation criteria*:  The current demarcation criteria for species of the genus *Cilevirus* are based on:   1. The extent of the serological relationship as determined by immunodiffusion and/or ELISA 2. Less than 85% aa sequence identity for the proteome 3. Natural host range 4. Artificial host range reactions 5. Vector species and transmission   While neither the information on the serological relationship between PisVY and other viruses nor the vector transmission features are available, PisVY meets the criteria B and C.  Since only one species of higrevirus has been described so far, demarcation criteria for species of this genus were unavailable.Based on the demarcation criteria for viruses of the genus *Cilevirus*, we propose the following rules as criteria for the definition of new species in the genus *Higrevirus*.   1. The extent of the serological relationship as determined by immunodiffusion and/or ELISA 2. Less than 85% aa sequence identity for the proteome 3. Natural host range 4. Artificial host range reactions 5. Vector species and transmission   No information on vector species, transmission and the serological relationship between PaHLV, PisVX, and HiGSV2 is available, however, PaHLV and PisVX meet the criteria B and C. |

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| **References:** |
| Mohammadi M, Hosseini A, Nasrollanejad S, 2021. In silico identification of two novel viruses on Iranian pistachio. Iran.J. Plant Path., 57(1):81–85.  Li C, An W, Zhang S, *et al.*, 2023. Characterization of a putative novel higrevirus infecting *Phellodendron amurense* Rupr. in China. *Arch. Virol.*,168(2):58.  Quito-Avila DF, Freitas-Astúa J, Melzer MJ, 2021. Bluner-, Cile-, and Higreviruses (*Kitaviridae*). *Encyclopedia of Virology*, 247*–*251.  Ramos-González PL, Dias Arena G, Tassi AD, *et al*., 2023. Kitaviruses: A Window to Atypical Plant Viruses Causing Non-systemic Diseases. *Annu Rev Phytopathol*, 61:97–118.  Criscuolo, A.; Gribaldo, S. BMGE (Block Mapping and Gathering with Entropy): A New Software for Selection of Phylogenetic Informative Regions from Multiple Sequence Alignments. BMC Evol. Biol. 2010, 10, 210, doi:10.1186/1471-2148-10-210. |

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| **Tables, Figures:** |

1. Phellodendron-associated higre-like virus (PaHLV)

3’

RNA1

8,183 nts

5’

*RdRp* (297 kDa)

*p8*

RNA2

3,062 nts

5’

3’

*p40*

*p10*

RNA3

3,998 nts

*p73*

5’

*p32*

*p23*

3’

1. Pistachio virus X (PisVX)

3’

RNA1

8,153 nts

5’

*RdRp* (295 kDa)

RNA2

2,382 nts

5’

3’

*p40*

*p10*

RNA3

3,869 nts

*p66*

5’

*p31*

*p23*

3’

1. Pistachio virus Y (PisVY)

3’

RNA1

8,967 nts

5’

*RdRp* (284 kDa)

RNA2

3,851 nts

*p68*

5’

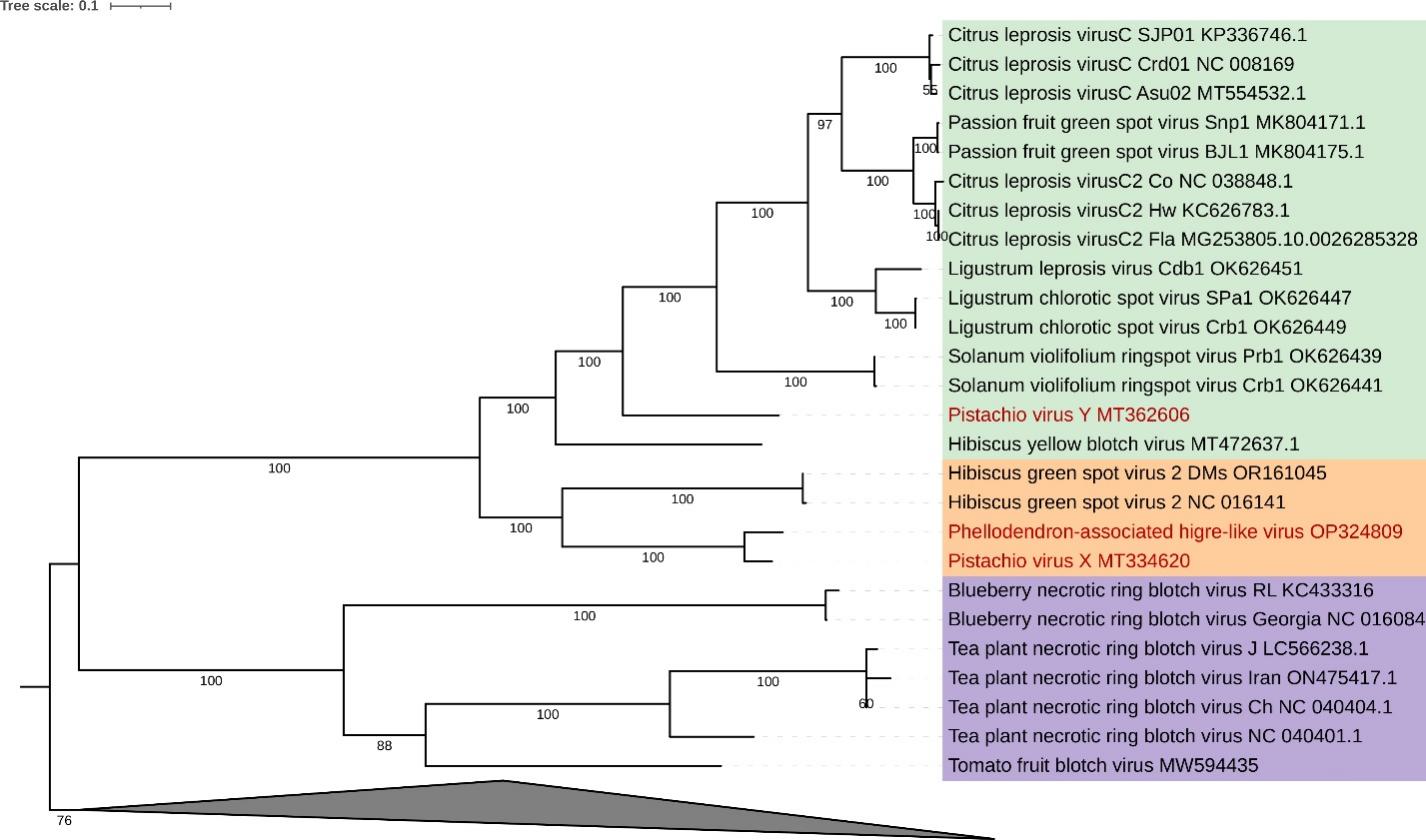
*p35*

*p23*

3’

*p33*

**Figure 1**. Genome organization of (**A**) Phellodendron-associated higre-like virus (PaHLV), (**B**) Pistachio virus Y (PisVY), and (**C**) Pistachio virus X (PisVX).



Genera of the family *Kitaviridae*

Genus *Cilevirus*

Genus *Higrevirus*

Genus *Blunervirus*

**Figure 2.** Maximum-likelihood phylogenetic tree constructed based on the aa sequences of the RNA-dependent RNA polymerase encoded by kitaviruses. Leaves comprising Phellodendron-associated higre-like virus, pistachio virus Y, and pistachio virus X are highlighted in red. The tree was rooted using RdRp sequences of nege/kita-like viruses (collapsed branch depicted by a grey triangle) as an external group. Phylogenetic informative regions of the multiple sequence alignment included 704 residues that were selected using BMGE software (Criscuolo et al., 2010). The evolutionary history was inferred based on the model LG+F+I+G4. The bootstrap support values (1,000 replications) of branches greater than 70% are indicated next to the corresponding nodes. The scale bar specifies the average number of aa substitutions per site. Sequences of the methyltransferase and helicase domains, and RdRp encoded by the RNA1 and RNA2 molecules, respectively, of the blunerviruses were concatenated before the analyses.