

The International Committee on Taxonomy of Viruses

Taxonomy Proposal Form, 2024

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

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| **Title:** | Create eight new species in the family *Alphaflexiviridae* | |
| **Code assigned:** | 2024.002P.A.v1.Alphaflexiviridae\_7nsp |

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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:** |
| *Alphaflexiviridae* SG |

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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** |
| *Alphaflexiviridae* SG | 8 | 0 | 5 |
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| **Submission date:** | 14/06/2024 |

**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| W - Withdrawn |  |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:** |
| 2024.002P.A.v1.Alphaflexiviridae\_7nsp.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 affected*: Species.  *Description of current taxonomy*: The family *Alphaflexiviridae* currently includes 65 virus species in genera *Allexivirus* (13), *Botrexvirus* (1), *Lolavirus* (1), *Platypuvirus* (1), *Potexvirus* (48) and *Sclerodarnavirus* (1).  *Proposed* *taxonomic changes*: This taxonomic proposal considers the recognition of 7 new virus species belonging to genera *Allexivirus* (1), *Botrexvirus* (2) and *Potexvirus* (4) within the family *Alphaflexiviridae.*  *Justification*: Throughout the family, isolates of different species should have less than 72% nucleotide identity (or 80% amino acid identity) between their respective coat protein or polymerase genes (or proteins). Viruses from different genera usually have less than about 45% nucleotide identity in these genes. The nucleotide or amino acid sequences of viruses belonging to the seven newly proposed species fit well within these demarcation criteria. |

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| * **Text of Taxonomy proposal:** |
| *Taxonomic rank affected*: Species.  *Description of current taxonomy*: The family *Alphaflexiviridae* currently includes 65 virus species in genera *Allexivirus* (13), *Botrexvirus* (1), *Lolavirus* (1), *Platypuvirus* (1), *Potexvirus* (48) and *Sclerodarnavirus* (1).  *Proposed* *taxonomic changes*: This taxonomic proposal considers the recognition of 7 new virus species belonging to genera *Allexivirus* (1), *Botrexvirus* (2) and *Potexvirus* (4) within the family *Alphaflexiviridae.*  *Demarcation criteria*:Throughout the family, isolates of different species should have less than 72% nucleotide identity (or 80% amino acid identity) between their respective coat protein or polymerase genes (or proteins). Viruses from different genera usually have less than about 45% nucleotide identity in these genes.  *Justification*:  **Creation of a first novel species in the genus *Allexivirus* of the family *Alphaflexiviridae*.** The complete genome sequence of Rehmannia allexivirus (ReAV) was determined using high throughput sequencing and sequencing of cloned cDNA amplicons (Qin et al. 2024). The complete genome sequence is 7,211 nucleotides (nt) long (accession number PP097219.1) excluding the 3’-terminal poly(A) tail. The 5' and 3' untranslated regions (UTRs) sequences were determined using rapid PCR amplification of complementary ends. The 5' and 3' UTRs are 146 and 207 nt long, respectively. The ReAV genome has five open reading frames (ORFs) encoding the replicase, triple gene block (TGB) proteins 1, 2 and 3, and the coat protein (CP). A blastp search using as query the ReAV replicase protein identified as closest homolog garlic virus A replicase, with amino acid (aa) identity of 64.1%. The CP protein gave a less than complete full query hit against garlic virus E at 55.8%. The ReAV CP protein of 584 aa is longer than those of most *Allexivirus* members, e.g., CP ( ~260 aa) of shallot virus X. ReAV appears to cluster with the non-allium infecting allexiviruses. Phylogenetic analyses based on the replicase (Figure 1) and CP (Figure 2) aa sequences placed ReAV in the genus *Allexivirus* of the family *Alphaflexiviridae*.  Proposed new species name – *Allexivirus rehmanniae*; species epithet derived from the host plant genus *Rehmannia*.  **Creation of a second novel species in the genus *Botrexvirus* of the family *Alphaflexiviridae*.** The complete genome sequence of Sclerotinia sclerotiorum alphaflexivirus 1 (SsAFV1) was determined by metatranscriptome analyses followed by cloning and sequencing cDNA amplicons from *Sclerotinia sclerotiorum* hypovirulent strain AHS31 (Ye at al., 2023). The 3' and 5' UTRs of SsAFV1 were cloned through sequence-independent cDNA amplification. The SsAFV1 genome comprises 6,939 nt (accession number ON993219) with four ORFs, a conserved 5'-UTR, and a poly (A) tail in the 3' UTR; ORF1 and ORF3 encode a replicase and a CP, respectively, while the functions of the proteins encoded by ORF2 and ORF4 remain unknown. The virion of SsAFV1 is a flexuous filament, 480–510 nm in length and 9–10 nm in diameter. A Blastp search using as query the SsAFV1 replicase and CP proteins identified as closest homologs the Sclerotinia sclerotiorum alphaflexivirus 2 (see below) replicase and CP proteins, respectively, with 53.5% and 43.5% of aa identity, respectively. Phylogenetic analyses with its replicase (Figure 1) and CP (Figure 2) aa sequences placed SsAFV1 in the genus *Botrexvirus* of the family *Alphaflexiviridae*.  Proposed new species name – *Botrexvirus unosclerotiniae*; species epithet derived from ‘uno’ (one) combined with the host genus *Sclerotinia*.  **Creation of a third novel species in the genus *Botrexvirus* of the family *Alphaflexiviridae*.** The complete genome sequence of Sclerotinia sclerotiorumalphaflexivirus 2 (SsAFV2) was determined by metatranscriptome analyses followed by cloning and sequencing cDNA amplicons from *S. sclerotiorum* hypovirulent strain 32–9 (Wu at al., 2023). The SsAFV2 genome contains 7,162 nt (accession number OQ865609), excluding the poly (A) tail, and is composed of four ORFs (ORF1–4). ORF1 encodes a protein that contains three conserved domains: methyltransferase, helicase, and RNA-dependent RNA polymerase (RdRp). The ORF3 encodes the CP, with ORF2 and ORF4 encoding hypothetical proteins of unknown functions. A Blastp search using as query the SsAFV2 replicase and CP proteins identified as closest homologs the SsAFV1 replicase and CP proteins, respectively, with 53.5% and 43.5% of aa identity, respectively. Phylogenetic analyses with its replicase (Figure 1) and CP (Figure 2) aa sequences placed SsAFV2 in the genus *Botrexvirus* of the family *Alphaflexiviridae*.  Proposed new species name – *Botrexvirus duosclerotiniae*; species epithet derived from ‘duo’ (two) combined with the host genus *Sclerotinia*.  **Creation of a fourth novel species in the genus *Potexvirus* of the family *Alphaflexiviridae*.** The complete genome sequence of Adenium obesum virus X (AobVX) was determined by RNAseq followed by 5’ and 3’ rapid amplification of cDNA ends with *A. obesum* RNA (Gauthier et al., 2023). The AobVX genome is 6,781 nt long (accession number OR039325) excluding the poly (A) tail and is predicted to encode conserved potexvirus proteins and sequence motifs across five open reading frames. The AobVX replicase shares the highest aa sequence similarity with that of nerine potexvirus 1 (58.7% identity) and nerine virus X (58.6% identity). A few weeks after the AobVX sequence publication, Bello et al. (2023) published the sequence of an isolate of this virus that they named desert rose mottle virus. Phylogenetic analyses with AobVX replicase (Figure 1) and CP (Figure 2) aa sequences placed this virus in the genus *Potexvirus* of the family *Alphaflexiviridae*.  Proposed new species name – *Potexvirus ecsadenii*; species epithet derived from ‘X’ of the common name, and the host genus *Adenium*.  **Creation of a fifth novel species in the genus *Potexvirus* of the family *Alphaflexiviridae*.** The complete genome sequence of Chaenostoma potexvirus (ChPV) was determined from *Chaenostoma* *cordatum* using high throughput sequencing and cloned cDNA amplicons (Doski et al. 2022). The 5' and 3' UTRs were determined by rapid amplification of cDNA ends followed by Sanger sequencing. The ChPV genome is 6,071 nt long (accession number OL979628) including the 5' and 3' UTRs. The 5' and 3' UTRs are 84 nt and 139 nt long. ChPV has five ORFs, ORF1 is predicted as a replicase, ORF2 through ORF4 encode the TGB, and ORF5 encodes the CP. The ChPV replicase and CP share it highest aa identity (68.1% and 65.7%) with the counterparts of Plantago asiatica mosaic virus, respectively. A similar GenBank entry named Sutera flower mottle virus, shares 100% aa identity with ChPV, and both were reported from the same plant host, as *Chaenostoma cordatum* and *Sutera cordata* are synonyms. Phylogenetic analyses with the ChPV replicase (Figure 1) and CP (Figure 2) aa sequences placed this virus in the genus *Potexvirus* of the family *Alphaflexiviridae*.  Proposed new species name – *Potexvirus chaenostomae*; species epithet derived from host genus  *Chaenostoma.*  **Creation of a sixth novel species in the genus *Potexvirus* of the family *Alphaflexiviridae*.** The coding-complete genome sequence of Hibiscus virus X (HiVX) was determined from *H. rosa-sinensis* plants by high-throughput sequencing (Roy et al., 2024). The assembled single long contig of HiVX was 6,406 nt long (accession number PP115950), excluding the poly (A) tail, with 93 and 131 nt long 5'- and 3'- UTRs, respectively, and predicted to encode the conserved potexvirus proteins and sequence motifs across its five open reading frames. The HiVX replicase shares its highest aa sequence identity (58.5%) with that of Sichuan alphaflexivirus X, and the CP with that of Yucca alphaflexivirus 1 (54.5%). Phylogenetic analyses with HiVX replicase (Figure 1) and CP (Figure 2) aa sequences placed this virus in the genus *Potexvirus* of the family *Alphaflexiviridae*.  Proposed new species name – *Potexvirus ecshibisci*; species epithet derived from ‘X’ of the common name, and the host genus *Hibiscus*.  **Creation of a seventh novel species in the genus *Potexvirus* of the family *Alphaflexiviridae*.** The coding-complete genome sequence of Papaya virus X (PapVX) was determined using high throughput sequencing from virus particles purified from *Carica papaya* plants (Cabrera Mederos et al. 2022). The PapVX genome is 6,667 nt long (accession number MN265368) excluding the poly (A) tail. PapVX genome has UTRs of 89 nt and 106 nt at the 5' and 3' ends, respectively. The genome is organized into five ORFs; ORF 1 encodes the replicase, ORFs 2-4 encode the TGB 1, 2 and 3, and ORF5 encodes the CP. The PapVX replicase shares 67.7% aa sequence identity with Opuntia potexvirus A, whereas the PapVX CP shares 70.8% aa sequence identity with Schlumbergera virus X. Phylogenetic analyses with the PapVX replicase (Figure 1) and CP (Figure 2) aa sequences placed the virus in the genus *Potexvirus* in the family *Alphaflexiviridae*.  Proposed new species name – *Potexvirus ecscaricae*; species epithet derived from ‘X’ of the common name, and the host genus *Carica* to differentiate from the long-known distinct species *Potexvirus papayae* from the same host. |

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| References: |
| Cabrera Mederos D, Debat H, Torres C, Portal O, Jaramillo Zapata M, Trucco V, Flores C, Ortiz C, Badaracco A, Acuña L, Nome C. An unwanted association: the threat to papaya crops by a novel Potexvirus in Northwest Argentina. Viruses. 2022;14(10):2297. https://www.mdpi.com/1999-4915/14/10/2297  Doski S, Bolus S, Grinstead S, Juszczak S, Groth-Helms D, Mollov D. Complete sequence and genome characterization of a new potexvirus isolated from Chaenostoma cordatum. Archives of Virology. 2022;167:2089-92. <https://doi.org/10.1007/s00705-022-05520-9>  Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research, 32(5), 1792–1797. https://doi.org/10.1093/nar/gkh340  Gauthier ME, Abeynayake SW, Lelwala RV, McMaster CA, Eichner R, Morrison J, Elliott CE, Fiorito S, Dinsdale A, Pattemore J, Barrero RA. First detection and complete genome sequence of a new potexvirus naturally infecting Adenium obesum. Archives of Virology. 2023;168:244. <https://link.springer.com/article/10.1007/s00705-023-05871-x>  Kozlov, A. M., Darriba, D., Flouri, T., Morel, B., & Stamatakis, A. (2019). RAxML-NG: A fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics, 35(21), 4453–4455. https://doi.org/10.1093/bioinformatics/btz305  Qin Y, Lu S, Wen Y, Li S, Gao S, Liu Y, Li X, Yang J, Wang F, Wang F, Lu C. Genomic Characterization and Molecular Detection of Rehmannia Allexivirus Virus, a Novel Allexivirus Infecting Rehmannia glutinosa. Microorganisms. 2024;12:844. https://www.mdpi.com/2076-2607/12/5/844  Roy A, Grinstead S, Leon Martínez G, Pinzón JC, Nunziata SO, Padmanabhan C, Hammond J. Meta-transcriptomic analysis uncovers the presence of four novel viruses and multiple known virus genera in a single Hibiscus rosa-sinensis plant in Colombia. Viruses. 2024;16:267. <https://doi.org/10.3390/v16020267>  Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution, 38(7), 3022–3027. https://doi.org/10.1093/molbev/msab120  Wu T, Mao H, Hai D, Cheng J, Fu Y, Lin Y, Jiang D, Xie J. Molecular characterization of a novel fungal alphaflexivirus reveals potential inter-species horizontal gene transfer. Virus Research. 2023;334:199151.  Ye T, Lu Z, Li H, et al (2023) Characterization of a Fungal Virus Representing a Novel Genus in the Family Alphaflexiviridae. Viruses 15:1–12. <https://doi.org/10.3390/v15020339> |



**Figure 1.** Phylogenetic analysis of viruses in the family *Alphaflexiviridae* inferred using replicase amino acid sequences. Multiple sequence alignment of 73 replicase sequences was done using MUSCLE (Edgar, 2004). Best amino acid substitution method was inferred using MEGA 11 (Tamura et al., 2021). Maximum likelihood trees were inferred using RAxML-NG software (Kozlov et al., 2019) using the LG method considering the proportion of invariable sites (+I) and the variation of the substitution rate among sites according to a gamma distribution (+G). Best ML tree with bootstrap support values (1000 replicates) is shown. Only bootstrap values higher than 50% are displayed. Carnation latent virus (QJX15400.1) (genus *Carlavirus*, family *Betaflexiviridae*) was used as an outgroup. Sequences from viruses in newly proposed *Alphaflexiviridae* species are marked with red arrows.



**Figure 2.** Phylogenetic analysis of viruses in the family *Alphaflexiviridae* inferred using coat protein (CP) amino acid sequences. Multiple sequence alignment of 73 CP sequences was done using MUSCLE (Edgar, 2004). Best amino acid substitution method was inferred using MEGA 11 (Tamura et al., 2021). Maximum likelihood trees were inferred using RAxML-NG software (Kozlov et al., 2019) using the LG method considering the variation of the substitution rate among sites according to a gamma distribution (+G). Best ML tree with bootstrap support values (1000 replicates) is shown. Only bootstrap values higher than 50% are displayed. Carnation latent virus (QJX153999.1) (genus *Carlavirus*, family *Betaflexiviridae*) was used as an outgroup. Sequences from viruses in newly proposed *Alphaflexiviridae* species are marked with red arrows.