This form should be used for all taxonomic proposals. Please complete all those modules that are applicable.

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.

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

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| **Code assigned:** | ***2018.024P*** | | | | (to be completed by ICTV officers) |
| **Short title:** Two new species in the family *Closteroviridae* | | | | | |
| **Modules attached**  (Modules 1, 4 and either 2 or 3 are required. | | **1**  **2  3  4** | | | |
| **Author(s):** | | | | | |
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| |  |  | | --- | --- | | Marc Fuchs | [mf13@cornell.edu](mailto:mf13@cornell.edu) | | | | | | |
| **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 (there are six virus subcommittees: animal DNA and retroviruses, animal ssRNA-, animal ssRNA+, fungal and protist, plant, bacterial and archaeal) | | | ***Closteroviridae*** | | |
| **ICTV Study Group comments (if any) and response of the proposer:** | | | | | |
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| Date first submitted to ICTV: | | | | June 5th, 2018 | |
| Date of this revision (if different to above): | | | |  | |

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| **ICTV-EC comments and response of the proposer:** |
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**Part 2**: **PROPOSED TAXONOMY**

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| Present the proposed new taxonomy on accompanying spreadsheet |
| **Name of accompanying spreadsheet: 2018.024P.N.v1.Closteroviridae\_2sp.xlsx** |

Please display the taxonomic changes you are proposing on the accompanying spreadsheet module 2017\_TP\_Template\_Excel\_module. Submit both this and the spreadsheet to the appropriate ICTV Subcommittee Chair.

**Part 3:** **NON-STANDARD**

Template for any proposal regarding ICTV procedures, rules or policy, not involving the creation of new taxonomy.

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| non-standard proposal |
| **Title of proposal:** |
| **Text of proposal:** |
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**Part 4:** **APPENDIX**: supporting material

Current species demarcation criteria in the family *Closteroviridae* (Martelli et al., 2012b) and justification of the proposal.

Viruses of the family *Closteroviridae* have filamentous particles (650-2,200 nm in length) and monopartite or multipartite single-stranded RNA genomes with size varying from 12,000 to nearly 19,000 nucleotides (nt) in length. They are divided into four genera: *Closterovirus* (monopartite genome), *Ampelovirus* (monopartite genome), *Velarivirus* (monopartite genome) and *Crinivirus* (bipartite or tripartite genome) (Figure 1). Ampeloviruses are transmitted by pseudococcid mealybugs and soft scale insects, closteroviruses are transmitted by aphids, and criniviruses are transmitted by whiteflies. No vectors are known for velariviruses. Species demarcation criteria used for all genera in the family *Closteroviridae* are particle size, size of the coat protein, genome structure and organization [number and relative location of open reading frames (ORFs)], vector species and specificity, cytopathological features, host range, as well as amino acid (aa) sequence of relevant gene products, i.e. RNA-dependent RNA polymerase (RdRp), coat protein (CP), heat shock protein 70 homolog (HSP70h), differing by more than 25%.

A novel virus of the family *Closteroviridae* was detected in air potato (*Dioscorea bulbifera*) showing virus-like symptoms. This virus was tentatively named air potato ampelovirus 1 (AiPoV1; Melzer et al., 2018). A high molecular weight double-stranded RNA was isolated from symptomatic air potato and used for high-throughput sequencing by MiSeq (Illumina). Sequence analysis using Geneious Pro software revealed a virus genome 13,398 nt in length (GenBank accession No. MH206615) possessing seven open reading frames (ORFs). The genome organization is typical of subgroup II ampeloviruses. Open reading frame (ORF) 1A encodes a protein of 2,191 aa in length with methytransferase and helicase domains. ORF1B is an RNA-dependent RNA polymerase putatively expressed by a +1 frameshift. ORF2 encodes a small 9kDa protein with a transmembrane domain but no homology with proteins currently deposited in GenBank. ORF3 codes for a HSP70h and ORF4 codes for a 62kDa protein orthologous to p61 of closteroviruses. The putative 28kDa CP of the virus is encoded by ORF5. The 3’ terminal ORF6 encodes a 24kDa protein possessing no considerable homology with current GenBank accessions. Phylogenetic analyses performed on RdRp, HSP70h, and CP aa sequences using both neighbor-joining and maximum likelihood algorithms placed AiPoV1 in a clade with members of the genus *Ampelovirus*, specifically in the subgroup II clade adjacent to plum bark necrosis stem pitting-associated virus (PBNSPaV) (Figure 2). Sequence comparisons for the taxonomically relevant RdRp, HSP70h and CP sequences revealed AiPoV1 is most closely related to PBNSPaV, with sequence identity values of 47, 50, and 48%, respectively (Table 1). Taken together, AiPoV1 is a virus distinct from any currently recognized member of the family *Closteroviridae* and we propose to recognize *Air potato ampelovirus 1* as a new species in the genus *Ampelovirus* of the family *Closteroviridae*.

Another novel putative virus of the family *Closteroviridae* was detected in *Actinidia chinensis*, a close relative of the kiwifruit, showing foliar chlorotic and necrotic rings. This virus was tentatively named actnidia virus 1 (AcV1; Blouin et al., 2018). Double-stranded RNAs was isolated from *A. chinensis*, subjected to RT-PCR and used for high throughput sequencing by Illumina HiSeq. Sequence analysis using Geneious Pro software revealed ORFs coding for a helicase, methyltransferase, HSP70h and CP. Overlapping RT-PCR followed by Sanger sequencing confirmed a virus genome 18,848 nt in length (GenBank accession No. KX857665) with a structure similar to the unclassified persimmon virus B (PeVB) with 12 ORFs greater than 6 KDa. ORF1A codes a multifunctional protein with conserved domains for two papain-like leader proteases (L-Pro) located more than 900 aa from the N-terminus, a methyltransferase and a helicase domain. Only PeVB has a similar feature within the family *Closteroviridae* with a single L-Pro located more than 800 aa from the N-terminus. ORF1B is 1,533 nt long and encodes an RdRp domain expressed through a +1 ribosomal frameshift and contains a unique 26 aa insert immediately after the GDD motif. ORF2 encodes a 14KDa protein, ORF3 a 25KDa protein and ORF4 a small 6kDa protein with a transmembrane domain. Additional ORFs code for homologs of HSP70 (64KDa), HSP90 (59kDa) and CP (27KDa). AcV1 encodes a thaumatin-like protein, a peculiarity unreported for other viruses, except for PeVB. Sequence analyses revealed that AcV1 is most closely related to PeVB with sequence identity values of 22, 42, and 32% for RdRp, HSP70h and CP sequences, respectively (Table 2). In phylogenetic analyses, AcV1 groups alongside PeVB but separately from closteroviruses and any other virus species within the family *Closteroviridae* (Figure 2). Therefore, AcV1 is a virus distinct from any currently recognized member of the family *Closteroviridae* and we propose to recognize *Actinidia virus 1* as a new unassigned species of the family *Closteroviridae*.

**Table 1.** Genetic relatedness of air potato ampelovirus 1 (AiPoV1), a proposed new species of the genus *Ampelovirus* within the family *Closteroviridae*, with plum bark necrotic stem pitting-associated virus (PBNSPaV), its closest relative.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Acronym** | **Name** | **Most closely related virus** | **% CP aa sequence identity** | **% RdRp aa sequence identity** | **% HSP70h aa sequence identity** |
| AiPoV1 | air potato ampelovirus 1 (AiPoV1) | plum bark necrotic stem pitting-associated virus (PBNSPaV) | 48 | 50 | 47 |

**Table 2.** Genetic relatedness of actinidia virus 1 (AcV1), a proposed new species within thte family *Closteroviridae*, with persimmon virus B (PeVB), its closest relative.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Acronym** | **Name** | **Most closely related virus** | **% CP aa sequence identity** | **% RdRp aa sequence identity** | | **% HSP70h aa sequence identity** | |
| AcV1 | actinidia virus 1 (AcV1) | persimmon virus B (PeVB) | 22 | | 42 | | 32 |



**Figure 1.** Schematic representation of the genome organization of air potato ampelovirus 1 (AiPoV1) (panel A) and actinidia virus 1 (AcV1) (panel B) compared with representative members of four genera [beet yellows virus (BYV) from the genus *Closterovirus*; grapevine leafroll-associated virus 3 (GLRaV3) from the genus *Ampelovirus*; cordyline virus 1 (CoV1) from the genus *Velarivirus*; lettuce infectious yellows virus (LIYV) from the genus *Crinivirus*] within the family *Closteroviridae* (panel C). Blocks represent predicted open reading frames (ORFs). The replicase proteins are shown in grey with the papain-like protease (Pro), methyltransferase (Met), alkB domain (AlkB) helicase (HEL), and RNA-dependent RNA polymerase (RdRp) domains. Small transmembrane proteins (p4, p5 and/or p6) are shown in pink, the heat shock protein 70 homolog (HSP70h) in red, the coat protein (CP) in salmon, and the minor coat protein (CPm) in orange.



**Figure 2.** Maximum likelihood phylogenetic tree showing the relationships between air potato ampelovirus 1 (AiPoV1), actinidia virus 1 (AcV1) and recognized members of the family *Closteroviridae* based on an alignment of the complete amino acid sequence of the heat shock protein 70 homolog using MUSCLE (Edgar 2004). The maximum likelihood tree was inferred using RAxML (Stamatakis 2014) in the T-REX web server (Boc et al., 2012). Distances are proportional to branch lengths and the bar represents the genetic distance. The heat shock protein 70 from *Arabidopsis thaliana* (AEE75218) was used as outgroup. The sequenced AiPoV1 in the genus *Ampelovirus* is boxed in red and the sequence of AcV-1 is boxed in light purple. The GenBank accession number used for each virus is as follows: actinidia virus 1 (AcV1, KX857665), air potato ampelovirus 1 (AiPoV1, MH206615), areca palm velarivirus 1 (ArPV1, KR349464), bean yellow disorder virus (BYDV, EU191904), beet pseudoyellows virus (BPYV, AY330918), beet yellow stunt virus (BYSV, U51931), beet yellows virus (BYV, AF056575), blackberry vein banding-associated virus (BVBaV, KC904540), blueberry virus A (BVA, AB733585), carnation necrotic fleck virus (CNFV, GU234166), carrot yellow leaf virus (CYLV, FJ869862), citrus tristeza virus (CTV, U16304), cordyline virus 1 (CoV1, HM588723), cordyline virus 2 (CoV2, JQ599282), cordyline virus 3 (CoV3, JQ599283), cordyline virus 4 (CoV4, JQ599284), cucurbit yellow stunting disorder virus (CYSDV, AY242077), diodia vein chlorosis virus (DVCV, CQ376201), fig leaf mottle-associated virus 2 (FLMaV2, FJ473383), fig mild mottle-associated virus (FMMaV, FJ611959), grapevine leafroll-associated virus 1 (GLRaV1, JQ023131), grapevine leafroll-associated virus 2 (GLRaV2, JX513891), grapevine leafroll-associated virus 3 (GLRaV3, EU259806), grapevine leafroll-associated virus 4 (GLRaV4, FJ467503), grapevine leafroll-associated virus 7 (GLRaV7, HE588185), grapevine leafroll-associated virus 13 (GLRaV13, LC052212), lettuce chlorosis virus (LCV, FJ380118), lettuce infectious yellows virus (LIYV, U15440), little cherry virus 1 (LChV1, EU715989), little cherry virus 2 (LChV2, AF531505), mint vein banding-associated virus (MVBaV, KJ572575), mint virus 1 (MV1, AY792620), persimmon virus B (PeBV, AB923924), pineapple mealybug wilt-associated 1 (PMWaV1, AF414119), pineapple mealybug wilt-associated 2 (PMWaV2, AF283103), pineapple mealybug wilt-associated 3 (PMWaV3, DQ399259), plum bark necrosis stem pitting-associated virus (PBNSPaV, EF546442), raspberry leaf mottle virus (RLMoV, DQ357218), rose leaf rosette-associated virus (RLRaV, KJ7488003), strawberry chlorotic fleck-associated virus (SCFaV, DQ860839), potato yellow vein virus (PYVV, AJ557128), strawberry pallidosis-associated virus (SPaV, AY488138), sweet potato chlorotic stunt virus (SPCSV, AJ428554), tetterwort vein chlorosis virus (TwVSV, KR002687), tobacco virus 1 (TV1, KT203917), tomato chlorosis virus (ToCV, AY903447) and tomato infectious chlorosis virus (TICV, FJ815440).

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| **References:** |
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| Blouin, A., Biccheri, R., Khalifa, M.E., Pearson, M.N., Pollini, C.P, Hamiaux, C., Cohen, D. and Ratti, C. (2018). Characterization of Actinidia virus 1, a new virus within the family *Closteroviridae* encoding a thaumatin-like protein. Archives of Virology 163:229-234. Boc, A., Diallo, A.B. and Makarenkov, V. (2012). T-REX: a web server for inferring, validating and visualizing phylogenetic trees and networks. Nucleic Acids Research 40:573-579.  Dey, K.K., Sugikawa, J., Kerr, C. and Melzer, M.J. (2018). Air potato (*Dioscorea bulbifera*) plants displaying virus-like symptoms are co-infected with a novel potyvirus and a novel ampelovirus, submitted.  Edgar, R.C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792-1797.  Martelli, G.P., Abou Ghanem-Sabanadzovic, N., Agranowsky, A.A, Al Rawhanih, M., Dolja, V.V., Dovas, C.I., Fuchs, M., Gugerli, P., Hu, J.S., Jelkmann, W., Katis, N., Maliogka, V.I., Melzer, M.J., Menzel, W., Minafra, A., Rott, M.E., Rowhani, A., Sabanadzovic, S. and Saldarelli, P. (2012a). Taxonomic revision of the family *Closteroviridae* with special reference to the grapevine leafroll-associated members of the genus *Ampelovirus* and the putative species unassigned to the family. Journal of Plant Pathology 94:7-19.  Martelli, G.P., Agranowski, A.A., Bar-Joseph, M., Boscia, D., Candresse, T., Couts, R.H.A., Dolja, V.V., Hu, J.S., Jelkmann, W., Karasev, A.V., Martin R.R., Minafra, A., Namba, S. and Vetten H.J. (2012b). Family *Closteroviridae*. In: King A., Adams, M.J., Carstens, E.B., Lefkowitz, E. (Eds.). Virus Taxonomy: Ninth report of the International Committee on Taxonomy of Viruses. Elsevier-Academic Press, San Diego, pp. 987-1001.  Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312-1313. |

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| **Annex:**  Please explain the reasons for the taxonomic changes you are proposing and provide evidence to support them. The following information should be provided, where relevant:   * **Species demarcation criteria**: Explain how new species differ from others in the genus and demonstrate that these differences meet the criteria previously established for demarcating between species. If no criteriahave previously been established, and if there will now be more than one species in the genus, please state the demarcation criteria you are proposing. * **Higher taxa**:   + There is no formal requirement to state demarcation criteria when proposing new genera or other higher taxa. However, a similar concept should apply in pursuit of a rational and consistent virus taxonomy.   + Please indicate the **origin of names** assigned to new taxa at genus level and above.   + For each new genus a **type species** must be designated to represent it. Please explain your choice. * **Supporting evidence**: The use of Figures and Tables is strongly recommended (note that copying from publications will require permission from the copyright holder). For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance. |