

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 genus Unirnavirus to accommodate 13 new species within family *Amalgaviridae* | |
| **Code assigned:** | 2024.006F.N.v1.Amalgaviridae\_newgen |

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| **Author(s), affiliation and email address(es):** | | | | |
| **Name** | **Affiliation** | **Email address** | **Corresponding author(s)** X |
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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 | **X** |
| Animal positive-strand RNA viruses |  | Plant viruses |  |
| Archaeal viruses |  | General - |  |

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| **List the ICTV Study Group(s) that have seen or have been involved in creating this proposal:** |
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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** |
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| **Submission date:** | 20/06/2024 |

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

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| **Executive Committee Meeting Decision code:** | **X** |
| A – Accept |  |
| 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| W - Withdrawn |  |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:** |
| 2024.006F.Amalgaviridae\_newgen.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:** | | **Y/N** |
| **Taxon name** | **Person from whom the name is derived** | **Attached X** |
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| **Abstract of Taxonomy Proposal:** |
| *Taxonomic rank(s) affected*: Family *Amalgaviridae*  *Description of current taxonomy*: Family *Amalgaviridae* accommodates 2 genera, *Amalgavirus* and *Zybavirus*  *Proposed* *taxonomic change(s):* Within family *Amalgaviridae*, establishing a new genus Unirnavirus to accommodate 13 new species  *Justification*: Sequence demarcation and phylogenetic analysis, genome organization and host range |

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| * **Text of Taxonomy proposal:** |
| *Taxonomic rank(s) affected*: The *Amalgaviridae* family accommodates viruses with non-segmented bicistronic double-stranded (ds) RNA genomes 3.0-3.5 kbp in size. The positive-sense strand of the dsRNA contains two partially overlapping open reading frames (ORFs), designated ORF1 and ORF2, that respectively encode a protein of unknown function and an RNA-dependent RNA polymerase (RdRP). Specifically, ORF2 overlaps ORF1 in the +1 frame (Depierreux *et al*., 2016), with the overlapping region between the two ORFs containing the conserved sequence motif (CUU UUU CGN), putatively involved in a +1 programed frameshift (Firth *et al*., 2012; Nibert *et al.*, 2016).  *Description of current taxonomy*: Since its last reorganization in 2018, the *Amalgaviridae* family comprises two genera, *Amalgavirus* and *Zybavirus*, that respectively comprise 9 and 1 species (Table 1). Genus *Zybavirus* and its sole species *Zybavirus bailii* were established to accommodate Zygosaccharomyces bailii virus Z (ZbV-Z), which was considered a member of *Amalgaviridae* but with ‘peculiar features that distinguish it from amalgaviruses’ (Sabanadzovic *et al*., 2018).  *Proposed* *taxonomic change(s)*: During the last decade, over 20 related viruses have been discovered (Table 2) that are similar to amalgaviruses and zybaviruses but with distinct features as described in the justification section below. These recent discoveries have led to the proposal for creation of novel taxa to accommodate these viruses:   1. Creation of a new genus Unirnavirus within the established family *Amalgaviridae*. 2. Creation and assignment of 13 new species to the proposed genus Unirnavirus. All proposed species are represented by viruses whose complete coding genome is publicly available and has been assigned an accession number.   The genus name was formed using the prefix *uni-* (from *unicus*, meaning ‘one’ or ‘single’ in Latin) to indicate a non-segmented genome, and the acronym RNA (the nucleic acid comprising the genome).  Most species names were formed by using the genus of the host as a Latinized epithet: for instance, Beauveria bassiana non-segmented RNA virus 1 was assigned to species ‘Unirnavirus beauveriae’.  In cases of unirnaviruses infecting more than one fungal species with the same fungal genus, the Latinized epithet is an amalgam of the binomial host name: Alternaria dianthicola dsRNA virus 1 and Alternaria longipes non-segmented mycovirus 1 were respectively assigned to species ‘Unirnavirus aldianthicolae’ and ‘Unirnavirus allongipei’, while Colletotrichum gloeosporioides RNA virus 1 and Colletotrichum higginsianum non-segmented dsRNA virus 1 were respectively assigned to species ‘Unirnavirus cogleosporioidei’ and ‘Unirnavirus cohigginsiani’.  Two distinct unirnaviruses infect the same fungal species *Ustilaginoidea virens.* In this case, ‘primo’ and ‘secundo’ were introduced into their Latinized epithets to distinguish them: Ustilaginoidea virens unassigned RNA virus HNND 1 was assigned to species ‘Unirnavirus prustilaginoideae’ and Ustilaginoidea virens RNA virus M-A was assigned to species ‘Unirnavirus secustilaginoideae’. Simillarly, Penicillium janczewskii Beauveria bassiana-like virus 1 was assigned to species ‘Unirnavirus pripenicillii’, while Penicillium citrinum non-segmented RNA virus 1 and Penicillium miczynskii RNA virus 1 were assigned to species ‘Unirnavirus secupenicillii’.  Finally, one species name was formed by using the geographical origin of the virus as a Latinized epithet: Combu double-strand RNA mycovirus was assigned to species ‘Unirnavirus combuense’, i.e., from the Combu island in Brazil.  The proposed classification is supported by phylogenetic analysis (Fig. 1) and a pairwise distance matrix (Fig. 2). The genomic organization of Beauveria bassiana non-segmented RNA virus (BbNV1), representative of the proposed species Unirnavirus beauveriae, is depicted in Fig. 3.  *Demarcation criteria:*  The species demarcation criteria in the proposed genus Unirnavirus are the same as those of the established genus *Amalgavirus* (Sabanadzovic *et al*., 2018), i.e., > 25% amino acid sequence divergence in RdRP and differences in natural host range.  *Justification*:  The ORF1 sequences are homologous across all proposed members of genus Unirnavirus but bear no similarity to those of genera *Amalgavirus* and *Zybavirus*.  The ORF2/RdRP sequences of the proposed genus Unirnavirus cluster together in the phylogenetic analysis (Fig. 1) and have 44%-99% pairwise similarity scores with each other but less than 30% to the RdRP sequences of the genera *Amalgavirus* and *Zybavirus* (Fig. 2).  In members of proposed genus Unirnavirus, translation of the RdRP encoded by ORF2 is mediated via a –1 frameshift, unlike members of genera *Amalgavirus* and *Zybavirus* (Depierreux *et al*., 2016).  Members of the genera *Amalgavirus* and *Zybavirus* infect respectively plants and budding yeast; members of the proposed genus Unirnavirus infect filamentous ascomycetes. |

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| **References:** |
| Campo S, Gilbert KB, Carrington JC (2016) Small RNA-based antiviral defense in the phytopathogenic fungus *Colletotrichum higginsianum*. *PLoS Pathog* 12:1005640. doi: 10.1371/journal.ppat.1005640.  Chiba Y, Oiki S, Yaguchi T, Urayama SI, Hagiwara D (2020) Discovery of divided RdRp sequences and a hitherto unknown genomic complexity in fungal viruses. *Virus Evol* 7:veaa101. doi: 10.1093/ve/veaa101.  Depierreux D, Vong M, Nibert ML (2016) Nucleotide sequence of Zygosaccharomyces bailii virus Z: Evidence for +1 programmed ribosomal frameshifting and for assignment to family *Amalgaviridae*. *Virus Res* 217:115-124. doi: 10.1016/j.virusres.2016.02.008.  Firth AE, Jagger BW, Wise HM, Nelson CC, Parsawar K, Wills NM, Napthine S, Taubenberger JK, Digard P, Atkins JF (2012). Ribosomal frameshifting used in influenza A virus expression occurs within the sequence UCC\_UUU\_CGU and is in the +1 direction. *Open Biol* 2:120109. doi: 10.1098/rsob.120109.  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Nibert ML, Pyle JD, Firth AE (2016) A +1 ribosomal frameshifting motif prevalent among plant amalgaviruses. *Virology* 498:201-208. doi: 10.1016/j.virol.2016.07.002.  Sabanadzovic S, Nibert ML, Krupovic M, Tzanetakis IE, Valverde RA (2018) Reorganization of the family *Amalgaviridae* by recognizing five new species in the genus *Amalgavirus* and creating a new genus *Zybavirus*. *ICTV Taxonomic Proposal 2018.032P.A.v1.Amalgaviridae\_rev.*  Suharto AR, Jirakkakul J, Eusebio-Cope A, Salaipeth L (2022) Hypovirulence of *Colletotrichum gloesporioides* associated with dsRNA mycovirus isolated from a mango orchard in Thailand. *Viruses* 14:1921. doi: 10.3390/v14091921.  Tamura K., Stecher G., Kumar S. (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Mol Biol Evol* 38:3022-3027. doi: 10.1093/molbev/msab120.  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| **Tables, Figures:** |

**Table 1:** Current and proposed organization of the family *Amalgaviridae*.

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| **CURRENT ORGANISATION** | **PROPOSED ORGANISATION** |
| **Genus: *Amalgavirus*** | **Genus: *Amalgavirus*** |
| *Amalgavirus allii* | *Amalgavirus allii* |
| *Amalgavirus cepae* | *Amalgavirus cepae* |
| *Amalgavirus lycopersici* | *Amalgavirus lycopersici* |
| *Amalgavirus marinae* | *Amalgavirus marinae* |
| *Amalgavirus rhododendri* | *Amalgavirus rhododendri* |
| *Amalgavirus spinaciae* | *Amalgavirus spinaciae* |
| *Amalgavirus vaccinii* | *Amalgavirus vaccinii* |
| *Amalgavirus viciae* | *Amalgavirus viciae* |
| *Amalgavirus zosterae* | *Amalgavirus zosterae* |
|  | **Genus: Unirnavirus** |
|  | Unirnavirus aldianthicolae |
|  | Unirnavirus allongipei |
|  | Unirnavirus aspergilli |
|  | Unirnavirus beauveriae |
|  | Unirnavirus cogleosporioidei |
|  | Unirnavirus cohigginsiani |
|  | Unirnavirus combuense |
|  | Unirnavirus fusarii |
|  | Unirnavirus pripenicillii |
|  | Unirnavirus prustilaginoideae |
|  | Unirnavirus secupenicillii |
|  | Unirnavirus secustilaginoideae |
|  | Unirnavirus trichodermae |
| **Genus: *Zybavirus*** | **Genus: *Zybavirus*** |
| *Zybavirus bailii* | *Zybavirus bailii* |

**Table 2:** Exemplar and additional isolates of the proposed species in genus Unirnavirus, family *Amalgaviridae*.

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| **Virus name & abbreviation** | **Accession number & size** | **Reference** |
| Alternaria dianthicola dsRNA virus 1  (AdRV1) | MT241326 (3,014 bp) | Hu *et al*., 2020 |
| Alternaria longipes non-segmented mycovirus 1  (AlRV1) strain HN28 | KJ817371 (3,415 bp) | Lin *et al*., 2015 |
| Alternaria longipes non-segmented mycovirus 1  (AlRV1) strain AT1 | MW656209 (3,417 bp) | unpublished |
| Aspergillus lentulus non-segmented dsRNA virus 1  (AlenNdsRV1) strain J-YC-1 | LC553704 (2,907 bp) | Chiba *et al*., 2020 |
| Aspergillus lentulus non-segmented dsRNA virus 1  (AlenNdsRV1) strain J-YC-2 | LC553705 (2,907 bp) | Chiba *et al*., 2020 |
| Beauveria bassiana non-segmented RNA virus 1  (BbNV1) | LN610699 (3,218 bp) | Kotta-Loizou *et al*., 2015 |
| Beauveria bassiana non-segmented RNA virus 1  (BbNV1) strain A24 | KM233415 (3,173 bp) | Koloniuk *et al*., 2015 |
| Beauveria bassiana non-segmented RNA virus 1  (BbNV1) strain ARSEF 8028 | MK279499 (3,170 bp) | Gilbert *et al*., 2019 |
| Colletotrichum gloeosporioides RNA virus 1  (CgRV1) strain Ssa-44.1 | ON887156 (2,939 bp) | Suharto *et al*., 2022 |
| Colletotrichum gloeosporioides RNA virus 1  (CgRV1) strain LS-1-2 | MK926570 (2,975 bp) | Wang *et al*., 2019 |
| Colletotrichum higginsianum non-segmented dsRNA virus 1  (ChNRV1) | KM923925 (2,993 bp) | Campo *et al*., 2016 |
| Combu double-strand RNA mycovirus  (CDSRMV) | MH990637 (2,992 bp) | unpublished |
| Erysiphe necator associated non-segmented virus 1  (EnANSV1) | MN617774 (2,173 bp) | unpublished |
| Fusarium culmorum virus 1  (FcV1) | MN187541 (2,898 bp) | Mahillon *et al*., 2020 |
| Fusarium oxysporum virus 1  (FoV1) | OR372790 (2,944 bp) | Wang *et al*., 2024 |
| Penicillium citrinum non-segmented RNA virus 1  (PcNRV1) | OP103962 (2,895 bp) | Huang *et al*., 2022 |
| Penicillium janczewskii Beauveria bassiana-like virus 1  (PjBIV1) | KT601106 (2,890 bp) | Nerva *et al*., 2016 |
| Penicillium miczynskii RNA virus 1  (PmRV1) | MK584820 (2,891 bp) | Nerva *et al*., 2019 |
| Trichoderma harzianum mycovirus 1  (ThMV1) | MH155602 (3,160 bp) | Liu *et al*., 2019 |
| Ustilaginoidea virens RNA virus M  (UvRVM) | KJ101567 (2,714 bp) | Jiang *et al*., 2014 |
| Ustilaginoidea virens RNA virus M-A  (UvRVM-A) | ON791647 (3,028 bp) | He *et al*., 2022 |
| Ustilaginoidea virens unassigned RNA virus HNND 1  (UvURVHNND-1) | KR106133 (2,903 bp) | Zhu *et al*., 2015 |
| Ustilaginoidea virens unassigned RNA virus HNND 1-A  (UvURVHNND-1-A) | ON791651 (2,912 bp) | He *et al*., 2022 |



**Figure 1:** Maximum likelihood phylogenetic tree created based on the RdRP sequences of unirnaviruses and related viruses within the established family *Amalgaviridae*. The sequences were aligned with MUSCLE as implemented by MEGA 11 (Tamura *et al*., 2021), all positions with less than 90% site coverage were eliminated and the LG+G substitution model was used. At the end of the branches, red circles indicate members of the proposed genus Unirnavirus and full red circles indicate that the virus is an exemplar of the proposed species. Squares indicate members of related established genera, green for *Amalgavirus* and blue for *Zybavirus*.

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**Figure 2:** Pairwise similarity matrix created based on the RdRP sequences of unirnaviruses and related viruses within the established family *Amalgaviridae*. The sequences were aligned with MUSCLE as implemented by SDT v1.3 (Muhire *et al*., 2014) and the pairwise similarity scores were calculated and displayed in a colour coded matrix. The RdRp sequence of white clover cryptic virus 2, a member of the genus *Betapartitivirus*, family *Partitiviridae*, was also included in the analysis.

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**Figure 3:** Schematic representation of the genomic organization of Beauveria bassiana non-segmented RNA virus 1 (BbNV1; Kotta-Loizou *et al*., 2015), exemplar virus for proposed species Unirnavirus beauveriae, proposed genus Unirnavirus. The genome consists of a single bicistronic dsRNA segment. The proteins encoded are represented by rectangular boxes, flanked by 5’- and 3’-UTRs represented by double lines. The light-coloured box in ORF2 represents the RdRP\_1 domain (PF00680, Pfam database). A typical motif G\_GAU\_UUUU for –1 ribosomal frameshifting is located at the 3’ end of ORF1 resulting in the expression of a ORF1-ORF2 fusion protein for which there is experimental evidence (Campo *et al*., 2016).