This Word module should be used for all taxonomic proposals.

Please complete **Part 1** and:

either **Part 3** for proposals to create new taxa or change existing taxa

or **Part 2** for proposals of a general nature.

Submit the completed Word module, together with the accompanying Excel module named in Part 3, to the appropriate ICTV Subcommittee Chair.

For guidance, see the notes written in blue, below, and the help notes in file Taxonomic\_Proposals\_Help\_2018.

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

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| **Code assigned:** | ***2018.032P*** | | (to be completed by ICTV officers) |
| **Short title:** Reorganization of the family *Amalgaviridae* by recognizing five new species in the genus *Amalgavirus* and creating a new genus *Zybavirus* | | | |
|  | | | |
| **Author(s):** | | | |
| Sead Sabanadzovic (Chair *Amalgaviridae* SG)  Max L Nibert (Member *Amalgaviridae* SG)  Mart Krupovic (Member *Amalgaviridae* SG)  Ioannis E Tzanetakis (Member *Amalgaviridae* SG)  Rodrigo A Valverde (Member *Amalgaviridae* SG) | | | |
| **Corresponding author with e-mail address:** | | | |
| Sead Sabanadzovic, ss501@msstate.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) | | *Amalgaviridae* SG | |
| **ICTV Study Group comments (if any) and response of the proposer:** | | | |
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| Date first submitted to ICTV: | | | June 6th, 2018 |
| Date of this revision (if different to above): | | | September 23rd, 2018 |

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| **ICTV-EC comments and response of the proposer:** |
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**Part 2:** **NON-STANDARD**

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

| **Text of proposal:** |
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**Part 3:** **PROPOSED TAXONOMY**

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| **Name of accompanying Excel module: 2018.032P.N.v1.Amalgaviridae\_rev.xlsx** |

The taxonomic changes you are proposing should be presented on an accompanying Excel module, 2017\_TP\_Template\_Excel\_module. Please enter the file name of the completed module in this box.

**Supporting material:**

| additional material in support of this proposal |
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| 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.   **Background and current organization of the family *Amalgaviridae***  Family *Amalgaviridae* comprises viruses with relatively small non-segmented dsRNA genomes of approximately 3.5 kbp in size. The plus strand of the genomic RNA contains two partially overlapping ORFs, with the ORF2 expressed as a fusion protein via programed +1 ribosomal frameshifting mechanism. While the protein encoded by ORF2 contains conserved motifs of RNA-dependent RNA polymerases (RdRps), the function of ORF1-encoded product is yet to be fully clarified. Computer-assisted analyses of the protein encoded by the ORF1 of several amalgaviruses suggest that the amalgaviral p1 protein is evolutionary related to nucleocapsid proteins of some negative sense RNA viruses (Krupovic et al., 2015). However, despite experimental evidence of p1 expression in plants during infection of blueberry latent virus (BlLV; Isogai et al., 2011), an electron microscope-based evidence of true virion formation is still lacking (Martin et al. 2011, Sabanadzovic et al., 2009; 2010). Amalgaviruses display persistent lifestyles, apparently not associated with any visible alterations in infected hosts and are efficiently transmitted via seeds. At present, the family comprises a single genus *Amalgavirus* that embraces four recognized species of plant-infecting viruses (*Southern tomato virus*, *Blueberry latent virus*, *Rhododendron virus A* and *Vicia cryptic virus M*) (Table 1).  Currently valid species demarcation criteria in the genus *Amalgavirus* are:   * More than 25% amino acid sequence divergence in viral RdRp * Differences in natural host range   Recent studies involving advanced bioinformatic mining of the Transcriptome Shotgun Assembly (TSA) / Sequence Read Archive (SRA) resources resulted in the discovery of several complete or near-complete genome sequences of several plant viruses related to, but distinct from currently recognized members in the family. In addition, resequencing of Zygosaccharomyces bailii virus Z (ZbV-Z) genome, a virus infecting budding yeast, revealed several common features with amalgaviruses; however, the virus is distinct from the plant-infecting amalgaviruses and represents a new taxon in the family *Amalgaviridae*.  Therefore, in this proposal we seek to update the taxonomy of amalgaviruses by:   1. Creating five new species in the existing genus *Amalgavirus* to include several new viruses; 2. Establishing a new genus, *Zybavirus*, typified by a bicistronic dsRNA virus replicating in the budding yeast *Zygosaccharomyces bailii.* 3. **Proposal for creation of five new species in the genus *Amalgavirus***   Complete or near complete sequences (spanning complete coding region) of five putative new amalgaviruses: Allium cepa amalgaviruses 1 and 2 (AcAV1 and AcAV2; Nibert et al., 2016), Zoostera marina amalgaviruses 1 and 2 (ZmAV1 and ZmAV2; Park et al., 2018) and spinach amalgavirus 1 (SpAV1; Park and Hahn, 2017) have recently been discovered by mining TSAs/SRAs (Table 1, red font).  Genome size and organization of all these viruses are comparable to amalgaviruses (common scheme for all five new viruses is presented in Figure 1). They all have bicistronic genomes with the two partially overlapping genes. Additionally, these viruses contain a +1 ribosomal frameshifting motif (*UUU\_CGN*) which is conserved in the majority of studied amalgaviruses (Nibert et al., 2016).  Furthermore, RdRps encoded by the ORF2 in these viruses are evolutionarily related to those of recognized members in the genus *Amalgavirus* and cluster together in phylogenetic analyses (Figure 2).  Pairwise comparisons of amino acid sequences of viral RdRps of these five viruses with those of the currently recognized members of the genus *Amalgavirus* showed that SpAV1 shares the highest identity with VCVM (~52%), whereas ZmAV1 and ZmAV2, and AcAV1 and AcAV2 are most similar to each other (~66% and ~67%, respectively) (Table 2).  Therefore, based upon available data on the genome size and organization as well as results of phylogenetic analyses and pairwise comparisons, these five recently characterized viruses are clearly amalgaviruses distinct from currently recognized members of the genus. Accordingly, we propose that they represent five new homonymous species in the genus (see Table 3).   1. **Proposal for establishement of a new genus, *Zybavirus*, in the family *Amalgaviridae* typified by Zygosaccharomyces bailii virus Z**   Zygosaccharomyces bailii virus Z (ZbV-Z) has been originally reported from the budding yeast *Z. bailii* 25 years ago (Schmitt and Neuhausen, 1994). ZbV-Z genome sequences (GenBank Acc No AF224490) have been publicly available for some time. Nevertheless, the virus was not officially classified as the reported sequences (3,157 nt) were different from those of known viruses and contained two ORFs separated by a 190 nt-long intergenic region (IGR). The product of ORF2 showed some relationships with RdRps encoded by amalgaviruses. Nevertheless, the ZbV-Z genome organization appeared clearly distinct from that of known amalgaviruses (i.e., presence of an IGR vs partially overlapping ORFs), which prevented official classification of the virus.  In order to clarify its taxonomic position, Depierreux and colleagues re-sequenced the isolate 412 of ZbV-Z by applying classic cloning and Sanger sequencing approaches along with RACE experiments. Re-sequencing of ZbV-Z revealed at least one error in the originally deposited nucleotide sequence AF224490 (Depierreux et al., 2016). The corrected genome sequence of ZbV-Z (GenBank acc no KU200450) showed organization resembling that of amalgaviruses: presence of two partially overlapping genes, with ORF2 overlapping ORF1 in the +1 frame (Depierreux et al., 2016). Additionally, the conserved sequence motif (CUU UUU CGN), putatively involved in a +1 programed frameshift in several groups of viruses (Firth et al., 2012), including plant amalgaviruses (Nibert et al., 2016), was found in the overlapping region between the two ORFs. Finally, function of the protein encoded by the ORF2 (RdRp) is homologous to amalgavirus counterparts.  Nevertheless, despite the above-mentioned similarities with members of the *Amalgavirus* genus, ZbV-Z possesses peculiar features that distinguish it from amalgaviruses:   * All known amalgaviruses (including the ones proposed in this document) are characterized from plants, whereas ZbV-Z is a mycovirus infecting budding yeast. * ZbV-Z genome is smaller than that those of amalgaviruses (3.1 kbp vs 3.3-3.4 kbp). * RdRps of ZbV-Z and amalgaviruses share considerably lower pairwise amino acid sequence identity (20-22%) compared to the mutual amino acid identities between recognized and/or proposed amalgaviruses (43% to 67%; Table 2). * Phylogenetic analysis based on the RdRp suggests that ZbV-Z belongs to the family *Amalgaviridae,* but in a lineage distinct from members of the genus *Amalgavirus* (Figure 2)   Therefore, based on the above-mentioned data, the *Amalgaviridae* SG proposes the establishment of a new genus, *Zybavirus*, in the family *Amalgaviridae*. The proposed genus is typified by Zygosaccharomyces bailii virus Z (ZbV-Z) isolate 412 classified into a newly proposed species *Zygosaccharomyces bailii virus Z*.  Origin of the genus name is constructed from initial two letters of the virus host, *Zygosaccharomyces bailii*, followed by the ending *virus.* |

**Table 1.** Names, acronyms, genome size and GenBank accession numbers of six viruses proposed to represent five new species in the genus *Amalgavirus* (in red) and a type species in the newly proposed genus Zybavirus (in blue).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Virus name** | **Acronym** | **Exemplar isolate** | **Genome size**  **(bp)** | **GenBank** |
| Allium cepa amalgavirus 1 | AcAV1 | OH1 | 3,453 | BK010347 |
| Allium cepa amalgavirus 2 | AcAV2 | DH5225 | 3,453 | BK010348 |
| Spinach amalgavirus 1 | SpAV1 | SRP059420 | 3,420 | KY695011 |
| Zoostera marina amalgavirus 1 | ZmAV1 | SRP035489-1 | 3,383 | KY783316 |
| Zoostera marina amalgavirus 2 | ZmAV2 | SRP035489-2 | 3,316 | KY783317 |
| **Zygosaccharomyces bailii virus Z** | ZbV-Z | 412 | 3,160 | KU200450 |

**Table 2.** Percent (%) pairwise identities of the amino acid content of RdRps encoded by recognized amalgaviruses and newly proposed members of the genera *Amalgavirus* and Zybavirus (reported in red and blue font, respectively).

**ZbV-Z** VCVM **SpAV1** RhVASTV **AcAV1 AcAV2** BLV  **ZmAV1 ZmAV2**

**ZbV-Z** 100.00 20.42 20.26 21.56 21.56 **22.20** 20.10 22.20 20.91 20.58

VCVM\_ 20.42 100.00 52.18 49.08 48.88 47.10 48.83 42.74 43.40 43.42

**SpAV1** 20.26 52.18 100.00 53.79 48.02 50.19 51.72 47.50 46.74 46.95

RhVA 21.56 49.08 **53.79** 100.00 46.59 49.04 49.11 44.99 44.81 46.82

STV 21.56 48.88 48.02 46.59 100.00 49.80 51.92 44.62 43.55 42.95

**AcAV1** **22.20** 47.10 50.19 49.04 49.80 100.00 **67.36** 46.73 44.86 45.66

**AcAV2** 20.10 48.83 51.72 49.11 51.92 **67.36** 100.00 47.47 47.87 48.28

BLV 22.20 42.74 47.50 44.99 44.62 46.73 47.47 100.00 48.05 49.12

**ZmAV1** 20.91 43.40 46.74 44.81 43.55 44.86 47.87 48.05 100.00 **65.63**

**ZmAV2** 20.58 43.42 46.95 46.82 42.95 45.66 48.28 49.12 **65.63** 100.00

**Table 3.** Current status and proposed changes in the organization of the family *Amalgaviridae.*

|  |  |  |
| --- | --- | --- |
| **Current organization** |  | **Proposed changes** |
| **Genus *Amalgavirus*** |  | **Genus *Amalgavirus*** |
| Species *Southern tomato virus* |  | Species *Southern tomato virus* |
| Species *Blueberry latent virus* |  | Species *Blueberry latent virus* |
| Species *Rhododendron virus A* |  | Species *Rhododendron virus A* |
| Species *Vicia cryptic virus M* |  | Species *Vicia cryptic virus M* |
|  |  | Species *Allium cepa amalgavirus 1* |
|  |  | Species *Allium cepa amalgavirus 2* |
|  |  | Species *Zoostera marina amalgavirus 1* |
|  |  | Species *Zoostera marina amalgavirus 2* |
|  |  | Species *Spinach amalgavirus 1* |
|  |  |  |
|  |  | **Genus *Zybavirus*** |
|  |  | Species *Zygosaccharomyces bailii virus Z* |

| **Figure 1**. General depiction of genome organization of five viruses proposed to represent novel species in the genus *Amalgavirus*.    **Figure 2.** Maximum likelihood phylogenetic tree based on amino acid sequences of viral RNA-dependent RNA polymerases (RdRps) of currently recognized and proposed members of the family *Amalgaviridae*. Representatives of four new species in the genus *Amalgavirus* are reported in red, whereas the type species of the new genus ‘*Zybavirus*’is in blue letters. The evolutionary history was inferred using the maximum likelihood method based on the best substitution model (LG+G+I) (Le and Gascuel, 2008). The tree with the highest log likelihood is shown. Percentage bootstrap supports for the family *Amalgaviridae* and new genus are shown next to the branches. All positions containing gaps and missing data were eliminated. The final dataset contained 446 positions. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016). RdRp sequence of white clover cryptic virus 2 (WCCV-2), a betapartitivirus (family *Partitiviridae*) was used as an outgroup.    **References:** |
| --- |
| 1. Depierreux, D., Vong, M. and Nibert, M.L. 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. 2. Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792-1797. 3. Firth, A. E., Jagger, B. W., Wise, H. M., Nelson, C. C., Parsawar, K., Wills, N. M., Napthine, S., Taubenberger, J. K., Digard, P. and Atkins, J. F. 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. 4. Isogai, M., Nakamura, T., Ishii, K., Watanabe, M., Yamagishi, N., Yoshikawa, N., 2011.Histochemical detection of Blueberry latent virus in highbush blueberry plant. J Gen Plant Pathol 77:304–306. 5. Krupovic, M., Dolja, V. V. and Koonin, E. V. 2015. Plant viruses of the *Amalgaviridae* family evolved via recombination between viruses with double-stranded and negative-strand RNA genomes. Biol.Direct 10:12. 6. Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870-1874. 7. Le, S.Q. and Gascuel, O. 2008. An improved general amino acid replacement matrix. Mol Biol Evol 25:1307-1320. 8. Liu, W. and Chen, J. 2009. A double-stranded RNA as the genome of a potential virus infecting *Vicia faba*. Virus Genes 39:126-131. 9. Martin, R.R., Zhou, J. and Tzanetakis, I.E. 2011. Blueberry latent virus: an amalgam of the *Partitiviridae* and *Totiviridae*. Virus Res 155:175-180. 10. Nibert, M. L., Pyle, J. D. and Firth, A. E. 2016. A +1 ribosomal frameshifting motif prevalent among plant amalgaviruses. Virology 498:201-208. 11. Park, D., Goh, C.J., Kim J. and Hahn, Y. 2018. Identification of two novel amalgaviruses in the common eelgrass (Zostera marina) and in silico analysis of the amalgavirus +1 programmed ribosomal frameshifting sites. Plant Path J 34:150-156. 12. Park, D. and Hahn, Y. 2017. Genome sequences of spinach deltapartitivirus 1, spinach amalgavirus 1, and spinach latent virus identified in spinach transcriptome. J. Microbiol. Biotechnol 27:1324-1330. 13. Sabanadzovic, S., Abou Ghanem-Sabanadzovic, N. and Valverde, R.A. 2010. A novel monopartite dsRNA virus from rhododendron. Arch Virol 155:1859-1863. 14. Sabanadzovic, S., Valverde, R.A., Brown, J.K., Martin, R.R. and Tzanetakis, I.E. 2009. Southern tomato virus: The link between the families *Totiviridae* and *Partitiviridae*. Virus Res 140:130-137. |