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:** | ***2018.002F*** | (to be completed by ICTV officers) |
| **Short title:** Assigning 4 new and 2 unassigned genera to the family *Marnaviridae* |
|  |
| **Author(s):**  |
| Andrew S. Lang, Marli Vlok, and Curtis A. Suttle |
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| Andrew S. Lang, Department of Biology, Memorial University of Newfoundland, St. John’s, NL, Canada; aslang@mun.ca |
| **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) |  |
| **ICTV Study Group comments (if any) and response of the proposer:** |
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| Date first submitted to ICTV: | June 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 3:** **PROPOSED TAXONOMY**

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| **Name of accompanying Excel module: 2018.002F.N.v1.Marnaviridae\_3gen** |

**Supporting material:**

**Summary**

 Metagenomic studies have discovered many novel viruses, including at the level of complete viral genomes. We have applied a sequence-based framework for analysis of twenty marine RNA viruses that provides the justification for taxonomic classification of these viruses within the family *Marnaviridae*. The twenty viruses include the original and sole representative of the family, Heterosigma akashiwo RNA virus (HaRNAV), 7 additional viruses represented by isolates, and 12 viruses discovered using metagenomics. Using RNA-dependent RNA polymerase (RdRp) phylogeny-based analyses, we propose to assign the previously unassigned genera *Labyrnavirus* (n=1) and *Bacillarnavirus* (n=3) to the family, and to create four additional new genera in the family with the proposed names *Locarnavirus* (n=4), *Kusarnavirus* (n=1), *Salisharnavirus* (n=4) and *Sogarnavirus* (n=6). Capsid amino acid pairwise comparisons were used to delineate species within genera.

**Delineation of genera**

 Maximum-likelihood phylogenetic analysis of the RdRp domain sequences placed the 20 marine RNA virus sequences in a strongly supported monophyletic group relative to other *Picornavirales* sequences (Figure 1). The sole current *Marnaviridae* genus, *Marnavirus*, is basal within the clade (Figure 1). The analysis places the 20 viruses into seven clades that we propose as genera within the family *Marnaviridae*. These include the previously unassigned genera *Bacillarnavirus* and *Labyrnavirus*, and we propose the names *Kusarnavirus*, *Locarnavirus*, *Salisharnavirus* and *Sogarnavirus* for the four new genera.

 Among the 20 viruses and 7 genera, there is a mixture of mono- and dicistronic genome organizations (Table 1). Genome organization can be useful for comparing virus groups but is not a sufficient marker for either family- or genus-level demarcations. While the majority of the 20 viruses analyzed here have a dicistronic genome organization, HaRNAV and SF-3 have a single predicted polyprotein encoded in their genomes. HaRNAV is the only representative of the genus *Marnavirus*, but SF-3 is one of four viruses falling within the genus *Locarnavirus* in our analyses. There is also precedent in support of our argument for the inclusion of members with different genomic organization into the family *Marnaviridae*. In the *Secoviridae*, another family within the order *Picornavirales*, both mono- and bipartite members are found. Like the proposed additions to the *Marnaviridae*, the *Secoviridae* form a monophyletic clade based on RdRp sequences.

**Naming**

 For the novel genera, “rna” was incorporated into the names for consistency with the established genera. The name *Locarnavirus* is based on the name of Locarno beach, which is where the first marine RNA virus metagenomes originated from. *Salisharnavirus* is derived from the “Salish Sea”, the water mass around coastal southern British Columbia from which the first marine RNA virus sequences were amplified. *Sogarnavirus* refers to the Strait of Georgia (SOG), a major water body of the Salish Sea. The name *Kusarnavirus* is derived from the Afrikaans word for coastal.

**Delineation of species within genera**

 We propose a conservative cut-off of 75% pairwise amino acid identity for the capsid polyprotein for species demarcation (Figure 2). This is 15% higher than our lowest observed value for what we have defined as species within the genera here. This is less stringent than the 90% cut-off for species in the families *Iflaviridae* and *Dicistroviridae* (Chen et al. 2011ab), but in accordance with the parameters required for species classification in the *Secoviridae* (Sanfacon et al. 2009, 2011).

 For the proposed genus *Locarnavirus*, the *Marine RNA virus JP-B* was selected as the type species because it is located at the base of the clade and was the first virus discovered among those falling within the genus. For the proposed genus *Sogarnavirus*, the *Marine RNA virus BC-1* virus was selected as the type representative because it is the only virus in the clade for which the genome assembly could be fully verified and where it was certain that the ORFs were complete. *Chaetoceros tenuissimus RNA virus type II* was selected for the proposed genus *Salisharnavirus* because it is the better-studied of the two isolates in the clade. *Asterionellopsis glacialis RNA virus* is the only member of the proposed genus *Kusarnavirus*.



Figure 1. Maximum-likelihood phylogeny of the *Picornavirales* RNA-dependent RNA polymerase amino acid sequences rooted with sequences from the *Potyviridae*. Branch colours denote virus families: *Potyviridae* (dark green), *Picornaviridae* (red), *Iflaviridae* (yellow), *Dicistroviridae* (orange), *Secoviridae* (lime green) and *Marnaviridae* (blue). Existing and proposed genera within the *Marnaviridae* are indicated by coloured boxes. SH-like branch support values are indicated at the nodes when >0.7 and the maximum-likelihood scale bar is shown.



Figure 2. Pairwise comparisons of *Marnaviridae* member sequences. A. Pairwise identities calculated for the entire capsid polyprotein region quantified in bins. B. Pairwise identities calculated for the RdRp domain quantified in bins. C. Values for pairwise comparisons among genera.

Table 1. Summary of information for 20 viruses related to this proposal.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Genus | Species1 | Accession number(s) | Genome | Host or source | Country | References |
| **Nucleotide** | **Amino acid** | **Size (nt)** | **# ORFs** | **Architecture** |
| *Marnavirus* | ***Heterosigma akashiwo* *RNA virus*** | AY337486 | AAP97137 | 8587 | One |  | *Heterosigma akashiwo* | Canada | Lang *et al.*, 2004; Tai *et al.*, 2003 |
| *Labyrnavirus* | ***Aurantiochytrium single stranded RNA virus*** | AB193726 | BAE47143 | 9035 | Two |  | *Aurantiochytrium* sp. | Japan | Takao *et al.*, 2006 |
| *Locarnavirus* | ***Marine RNA virus JP-B*** | EF198242 | ABQ50601 ABQ50602 | 8926 | Two |  | Coastal marine | Canada | Culley *et al.*, 2007 |
| *Marine RNA virus SF-2* | KF412901 | AGZ83339 AGZ83340 | 9321 | Two |  | Coastal wastewater | USA | Greninger and DeRisi, 2015 |
| *Marine RNA virus SF-1* | JN661160 | AFM44930 AFM44929 | 8970 | Two |  | Coastal wastewater | USA | Greninger and DeRisi, 2015 |
| *Marine RNA virus SF-3* | KF478836 | AHA44480 | 8648 | One |  | Coastal wastewater | USA | Greninger and DeRisi, 2015 |
| *Kusarnavirus* | ***Asterionellopsis glacialis* *RNA virus*** | AB973945 | BAP16719 BAP16720 | 8842 | Two |   | *Asterionellopsis glacialis* | Japan | Tomaru *et al.*, 2012 |
| *Bacillarnavirus* | *Chaetoceros tenuissimus* *RNA virus 01* | AB375474 | BAG30951 BAG30952 | 9431 | Two |  | *Chaetoceros tenuissimus* | Japan | Shirai *et al.*, 2008 |
| ***Rhizosolenia setigera RNA virus*** | AB243297 | BAE79742 BAE79743 | 8877 | Two |  | *Rhizosolenia setigera* | Japan | Nagasaki *et al.*, 2004 |
| *Chaetoceros socialis f. radians RNA virus 01* | NC\_012212 | BAH22517 BAH22518 | 9467 | Two |  | *Chaetoceros socialis f. radians* | Japan | Tomaru *et al.*, 2009 |
| *Salisharnavirus* | ***Marine RNA virus BC-4*** | MH171300 |  | 8593 | Two |  | Coastal/oceanic marine | Canada | Vlok *et al*. unpublished |
| *Marine RNA virus PAL473* | KT727026 | AMK49159 AMK49160 | 6360 | Two |  | Coastal marine | USA | Miranda *et al.*, 2016 |
| *Marine RNA virus BC-1* | MG584187 |  | 8638 | Two |  | Coastal marine | Canada | Vlok *et al*. unpublished |
| *Marine RNA virus PAL128* | KT727023 | AMK49153 AMK49154 | 8660 | Two |  | Coastal marine | USA | Miranda *et al.*, 2016 |
| *Sogarnavirus* | *Marine RNA virus BC-2* | MG584188 |  | 8843 | Two |  | Coastal marine | Canada | Vlok *et al*. unpublished |
| *Marine RNA virus PAL156* | KT727024 | AMK49155 AMK49156 | 7897 | Two |  | Coastal marine | USA | Miranda *et al.*, 2016 |
| *Marine RNA virus BC-3* | MG584189 |  | 8496 | Two |  | Coastal marine | Canada | Vlok *et al*. unpublished |
| *Marine RNA virus JP-A* | EF198241 | ABQ50599 ABQ50600 | 9236 | Two |  | Coastal marine | Canada | Culley *et al.*, 2007 |
| ***Chaetoceros tenuissimus* *RNA virus type II*** | AB971661 | BAP99818 BAP99819 | 9562 | Two |  | *Chaetoceros tenuissimus* | Japan | Kimura and Tomaru, 2015 |
| *Chaetoceros* species *RNA virus01* | AB639040 | BAK40203 BAK40204 | 9417 | Two |  | *Chaetoceros* sp. | Japan | Tomaru *et al.*, 2013 |

1Assigned type species in existing genera and proposed type species in proposed genera are in bold.

| **References:** |
| --- |
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