

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

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| **Code assigned:** | ***2023.002M*** |  |
| **Short title:** Create two new genera and two new species for viruses from freshwater mussels (*Mononegavirales*: *Rhabdoviridae*) |
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**List the ICTV Study Group(s) that have seen this proposal**

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| ICTV *Rhabdoviridae* Study Group |

**ICTV Study Group comments and response of proposer**

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| Minor corrections. Completed. |

**ICTV Study Group votes on proposal**

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| **Study Group** | **Number of members** |
| **Votes support** | **Votes against** | **No vote** |
| ICTV *Rhabdoviridae* Study Group | 14 | 0 | 0 |

**Authority to use the name of a living person**

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| **Is any taxon name used here derived from that of a living person (Y/N)** | N |

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| **Taxon name** | **Person from whom the name is derived** | **Permission attached (Y/N)** |
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**Submission dates**

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| Date first submitted to SC Chair | June 23, 2023 |
| Date of this revision (if different to above) |  |

**ICTV-EC comments and response of the proposer**

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**Part 2:** **NON-TAXONOMIC PROPOSAL**

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**Part 3:** **TAXONOMIC PROPOSAL**

**Name of accompanying Excel module**

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| 2023.002M.N.v1.Alpharhabdovirinae\_2ngen\_2nsp.xlsx |

**Abstract**

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| Two new genera in the family *Rhabdoviridae*,each represented by one new species,are proposed to accommodate two newly discovered viruses detected in freshwater mussels in the USA. One virus clusters phylogenetically within members of the *Alpharhabdovirinae* and the other is a close phylogenetic outgroup to members of the genus *Novirhabdovirus* within the *Gammarhabdovirinae*. Both viruses have genome organizations and transcriptional regulatory sequences highly similar to their close phylogenetic relatives, but with unique genome features not present in previously described rhabdoviruses. Based on coding complete genome sequence architecture, *L* gene phylogeny, and host type, both new viruses are sufficiently different from currently recognized rhabdoviruses to merit the creation of two new species, each in a new genus. |

**Text of proposal**

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| **Origins of new viruses and virus names**Killamcar virus 1 (KILLV-1) and chemarfal virus 1 (CHMFV-1) were detected by high throughput sequencing of hemolymph collected from two freshwater mussels in the USA. KILLV-1 was detected in a plain pocketbook (*Lampsilis cardium*) collected from Kilmore Creek in Indiana during a survey of mussel viruses in 2019. CHMFV-1 was detected in a western pearlshell (*Margaritifera falcata*) collected from the Chehalis River in Washington during an investigation into mussel mass mortality events in 2018. The virus names are combined sigla from the locations and taxonomy of the mussels from which they were collected.**Proposed new species and genera**We propose that a new genus, *Uniorhavirus*, be created to accommodate KILLV-1, to be assigned to the new species *Uniorhavirus killamcar.* We propose that a new genus, *Margarhavirus*, be created to accommodate CHMFV-1, to be assigned to the new species *Margarhavirus chemarfal.* Names for the new genera are derived as follows:*Uniorhavirus*: Derived from the freshwater mussel family Unionidae, to which *L. cardium* (host of KILLV-1) belongs.*Margarhavirus*: Derived from the freshwater mussel family Margaritiferidae, to which *M. falcata* (host of CHMFV-1) belongs.No isolates are yet available for these viruses, as virus isolation attempts have heretofore been unsuccessful.**Genome organizations**Coding-complete genome sequences of KILLV-1 (11,891 nt) and CHMFV-1 (15,402), plus partial 3' and 5' termini, are available (GenBank OQ368743 and OQ368744, respectively). Each virus contains the five canonical rhabdoviral genes (*N*, *P*, *M*, *G* and *L*) and additional accessory protein genes.KILLV-1 *N*, *P*, *M*, *G* and *L* genes are similar in length and GC content to their relatives in the *Alpharhabdovirinae* (**Figure 2; Table S3**). KILLV-1 also contains a 1389 nt duplicated glycoprotein gene, *GNS*, between *G* and *L*, which is not a feature shared with its close relatives [1]. Unique from other rhabdoviruses with duplicated glycoprotein genes, the *G* and *GNS* of KILLV-1 overlap, with the AUG start codon of the GNS ORF 40 nt upstream of the TAA termination codon of the G ORF.CHMFV-1 *N*, *P*, *M*, and *G* genes and all non-coding regions are longer than those of viruses in the genus *Novirhabdovirus* (**Figure 4**). Furthermore, the GC content of all CHMFV-1 genes and non-coding regions is lower than the GC content of corresponding genes in the novirhabdoviruses (**Table S4**). CHMFV-1 contains two accessory genes, *U1* and *U2*, between *M* and *G*, which is not a feature shared with its close relatives [1]. However, CHMFV-1 contains a pseudogene, *NVP*, between *G* and *L* that, if made functional by a single nucleotide substitution at position 385 (ATT 🡪 ATG) would create an intact putative ORF of 369 nucleotides, which is the same length as the *NV* genes of viral hemorrhagic septicemia virus (VHSV) and snakehead rhabdovirus (SHRV). This feature allies CHMFV-1 with the novirhabdoviruses, for which the presence of *NV* is a defining feature.**Phylogenetic analyses**A phylogenetic tree inferred using maximum likelihood from an alignment of complete L protein sequences places KILLV-1 within the *Alpharhabdovirinae* as a sister taxon to the genus *Scophrhavirus* with 100% bootstrap support (**Figure 1**). The same tree places CHMFV-1 as a sister taxon to viruses in the genus *Novirhabdovirus*, the sole recognized genus within the *Gammarhabdovirinae* (**Figure 1**). The branch linking CHMFV-1 to the novirhabdoviruses is shorter than many branches connecting genera within other rhabdoviral subfamilies, suggesting that CHMFV-1 should be placed within the *Gammarhabdovirinae*.**Nucleotide and amino acid sequence identities**The KILLV-1 *L* gene is 50.3% identical to the scophraviruses at the nucleotide level and the KILLV-1 L protein is 76.4% identical to the scophraviruses at the amino acid level (**Table S2**). The percent *L* gene nucleotide identity between the two currently recognized scophraviruses is 47.6%, and the percent L protein amino acid identity between the two currently recognized scophraviruses is 78.4%. Thus, at the amino acid level, but not at the nucleotide level, KILLV-1 L is slightly more distantly related to the scophraviruses than the scophraviruses are to each other.The CHMFV-1 L gene is 50.3% identical to the novirhabdoviruses at the nucleotide level and the CHMFV-1 L protein is 76.4% identical to the novirhabdoviruses at the amino acid level (**Table S2**). The percent *L* gene nucleotide identity among currently recognized novirhabdoviruses is an average of 62.6%, and the percent L protein amino acid identity among currently recognized novirhabdoviruses is 89.4%. Thus, at both the amino acid level and the nucleotide level, CHMFV-1 L is more distantly related to the novirhabdoviruses than the novirhabdoviruses are to each other.**Active transcription and transcriptional regulatory sequences**Sequence coverage maps for KILLV-1 show up to 2,343-fold coverage in the coding regions and down to 7-fold coverage in the non-coding regions, with a consistent pattern that shows active transcription of ORFs (**Figure 2a**). Sequence coverage maps for CHMFV-1 show up to 20,846-fold coverage in the coding regions and down to 5-fold coverage in the non-coding regions, again consistently showing active transcription of ORFs (**Figure 4a**).Transcription termination/polyadenylation (TTP) and transcription initiation (TI) consensus sequences are identical for all KILLV-1 genes to those of alpharhabdoviruses in the genera *Vesiculovirus*, *Sprivivirus*, *Perhabdovirus*, *Siniperhavirus* and *Cetarhavirus* and differ by only single nucleotides from those of viruses in the genera *Ledantevirus* and *Scophravirus* (**Figure 3**).Transcription termination/polyadenylation (TTP) and transcription initiation (TI) consensus sequences are highly similar between CHMFV-1 and novirhabdoviruses (**Figure 5**). The CHMFV-1 TTP consensus sequence is identical to that in 3 of 5 novirhabdoviruses and differs from the other 2 novirhabdoviruses by only a single nucleotide (**Figure 5**). The CHMFV-1 TI consensus sequence is identical to that of all novirhabdoviruses.**Host association with freshwater mussels**Despite the fact that virus isolation was not successful using the biological samples from which the KILLV-1 and CHMFV-1 coding-complete genomes were derived, the probability that these genome sequences represent rhabdoviruses that use the mussels as hosts is very high based on several factors. First, the completely consistent indication of active transcription of ORFs in the coverage maps for both viruses (Figures 2 and 3 in supporting reference) strongly supports active virus replication. Second, nucleic acids were extracted from mussel hemolymph, which is contained within the mussel circulatory system (i.e. is analogous to vertebrate blood). This fact, in combination with the aforementioned high sequence coverage, effectively excludes the possibility that these viruses could have been concentrated from the environment by filter feeding (in which case they would have appeared in the digestive system, rather than the circulatory system). Finally, not all mussels analyzed contained these sequences, even though in some cases sequence-negative mussels were collected from the same locations at the same times as sequence-positive mussels (unpublished data). To our knowledge freshwater mussels have not previously been described as hosts for rhabdoviruses, making this unusual host association a contributing factor to the justification of new genera for each of these viruses.**Summary**The justification for placement of each of the two viruses represented by the newly discovered coding-complete genome sequences from freshwater mussels within the family *Rhabdoviridae* is based on the similarity of their overall genome structure to known rhabdoviruses (containing the five canonical rhabdoviral genes in order *N*, *P*, *M*, *G* and *L*), high level of conservation of transcription regulatory sequences with those of known rhabdoviruses, and their strongly supported positions in the L protein phylogenetic tree.Justification for each virus representing a **new species in a new genus** within the *Rhabdoviridae* is as follows.**KILLV-1:** KILLV-1 contains overlapping duplicated *GNS* genes, two novel small accessory proteins, and is associated with mollusks (freshwater mussels in aquatic environments). KILLV-1 should not be classified as a member of the genus *Scophrhavirus,* to which it is most closely related,because these features are not shared with any of the scophrhaviruses. Furthermore, KILLV-1 is a phylogenetic outgroup to the scophrhaviruses based on L protein phylogeny (**Figure 1**). We also note that ICTV has specified “vertebrate hosts” in the species demarcation criteria for the scophraviruses, and KILLV-1 was identified in an invertebrate host. Thus, KILLV-1 merits classification within a new genus *Uniorhavirus.* Because KILLV-1 is the sole member of this genus, species demarcation criteria within the uniorhaviruses cannot yet be defined.**CHMFV-1:** CHMFV-1 has an unusually long coding-complete genome, markedly low GC content, two novel accessory proteins, a pseudogenized version of the *NV* gene, and is associated with mollusks (freshwater mussels in aquatic environments). CHMFV-1 should not be classified as a member of the genus *Novirhabdovirus,* to which it is most closely related,because these features are not shared with any of the novirhabdoviruses. Furthermore, CHMFV-1 is a clear phylogenetic outgroup to the novirhabdoviruses based on L protein phylogeny (**Figure 1**). Thus, CHMFV-1 merits classification within a new genus, *Margarhavirus.* Because CHMFV-1 is the sole member of this genus, species demarcation criteria within the margarhaviruses cannot yet be defined.Finally, we suggest that phylogenetic analyses including these sequences support the classification of CHMFV-1 as a member of the subfamily *Gammarhabdovirinae.* CHMFV-1 is the first virus closely related to the *Novirhabovirus* genus, such that its discovery and placement within the *Gammarhabdovirinae* shouldenhance our understanding of the taxonomic status of the *Novirhabdovirus* genus and should thereby help clarify the placement of the *Gammarhabdovirinae* with respect to the other rhabdoviral sub-families. |

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**Supporting evidence**

All supporting evidence is contained in the recent publication referenced below (and attached as 2023.002M.A.v1.Alpharhabdovirinae\_2ngen\_2nsp\_attachment). For convenience, we provide abbreviated legends as follows:

**Figure 1.** Maximum likelihood phylogenetic tree of members of the *Rhabdoviridae* showing the placement of KILLV-1 as a sister taxon to the genus *Scophrhavirus* within the *Alpharhabdovirinae* (bootstrap support 100%) and CHMFV-1 as a sister taxon to the genus *Novirhabdovirus* within the *Gammarhabdovirinae* (bootstrap support 100%).

**Figure 2.** Genome organization of KILLV-1 showing similarities and differences to other members of the *Alpharhabdovirinae*.

**Figure 3.** Transcriptional regulatory sequences of KILLV-1 showing similarities and differences to other members of the *Alpharhabdovirinae*.

**Figure 4.** Genome organization of CHMFV-1 showing similarities and differences to other members of the *Gammarhabdovirinae*.

**Figure 5.** Transcriptional regulatory sequences of CHMFV-1 showing similarities and differences to other members of the *Gammarhabdovirinae*.

**Table S2.** Nucleotide and amino acid identities of KILLV-1 and CHMFV-1 to their closest relatives for the *N*, G and *L* genes/proteins.

**Table S3.** Lengths and GC contents of KILLV-1 genes.

**Table S4.** Lengths and GC contents of CHMFV-1 genes.

**Reference**

1. Goldberg TL, Blevins E, Leis EM, Standish IF, Richard JC, Lueder MR, Cer RZ, Bishop-Lilly KA (2023) Plasticity, paralogy, and pseudogenization: rhabdoviruses of freshwater mussels elucidate mechanisms of viral genome diversification and the evolution of the finfish-infecting rhabdoviral genera. J Virol:e0019623. PMCID: PMC10231222 (available on 2023-11-08) DOI: 10.1128/jvi.00196-23.