

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

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| **Code assigned:** | **2021.035M** |  |
| **Short title:**  Create two new genera (*Amplylivirus*, *Replylivirus*) including two new species (*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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| Approved by all responding SG members (11 of 15) with minor revisions. |

**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 |
| **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 | May 28, 2021 |
| 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**

**Text of proposal**

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

**Name of accompanying Excel module**

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| 2021.035M.Ac.v1.Rhabdoviridae\_2ngen\_2nsp |

**Abstract**

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| We propose the establishment of two new genera in the *Rhabdoviridae* to accommodate two new species for viruses discovered in a frog and a lizard. These viruses are phylogenetically divergent from those assigned to the established and proposed genera. The new genera will be *Amplylivirus* (one species for an amphibian virus) and *Replylivirus* (one species for a reptilian virus). |

**Text of proposal**

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| |  | | --- | | We address two complete genome sequences, both deposited in GenBank (one described in a peer-reviewed publication, another not published), identified by metagenomics during recent years. These viruses have not been isolated. Both sequences demonstrate gene similarity and phylogenetic relatedness to lyssaviruses (family *Rhabdoviridae,* genus *Lyssavirus*) but can neither be included in this genus, nor placed together in one separate genus.  Viruses from genus *Lyssavirus* circulate exclusively in mammals, are transmitted in saliva via bite, and cause acute progressive encephalomyelitis with high case-fatality rate (rabies). Within the *Lyssavirus* genus, maximum divergence between nucleoprotein (N) sequences is 26% and between large polymerase protein (L) sequences it is 30%. The divergence is substantially greater between lyssavirus genes and genes of the proposed members of the genera *Amplylivirus* and *Replylivirus* (41-60% for N and 37-50% for L protein sequences). The divergence between genesof viruses in the genera *Amplylivirus* and *Replylivirus* is even greater (63% for N and 51.5% for L protein sequences) (**Tables 1-4**).  Phylogenetic trees based on each gene alignment placed both members of the genera *Amplylivirus* and *Replylivirus* ancestrally to cluster encompassing genus *Lyssavirus* with 100% bootstrap support but paraphyletic, as two separate lineages (**Figure 1**).  The single available member of the proposed genus *Amplylivirus* was identified in the brain of a frog with unknown clinical history whereas the single available member of the genus *Replylivirus* was identified in the brain and skin of a lizard without apparent clinical signs despite a long period of observation in captivity (Horie et al., 2021).  These genetic, phylogenetic, and biological properties suggest that both viruses are related to lyssaviruses and to each other but must be classified separately.   1. **Genus *Amplylivirus***   The new genus *Amplylivirus* includes the frog lyssa-like virus 1 (FLLV1; GenBank accession No. MK473367). We propose to assign this virus to a new species, *Amplylivirus cinereus*.  **Sequence origin**  FLLV1 was identified in the brain of the American green tree frog (*Hyla cinerea* or *Dryophytes cinereus* in different classifications). Geographic origin is not indicated in the GenBank record. But the host range (southern parts of North America and the Caribbean) and the fact that GenBank submission was made by the US group (Blavatnik Institute, Harvard Medical School, Boston, MA) suggests that it likely originated from the native host range. No details on clinical status of the animal and reason for sequencing are available.  **Genome organization and phylogenetic sequence relationships**  The nearly complete FLLV1 genome covering all coding sequences (12081 nt) contains the five canonical rhabdovirus structural protein genes (*N*, *P*, *M*, *G* and *L*) separated by short intergenic regions (**Figure 1**), with the absence of the long G-L intergenic region (400-700 nucleotides) typical to lyssaviruses. In the absence of complementarity of genome termini, we consider that either the leader, trailer, or both are truncated. Sequences of the most conserved viral genes N and L demonstrate maximum identity to several Eurasian, African and Australian bat lyssaviruses (N nucleotides, 51-53%; N amino acids, 40-41%, L nucleotides, 53-54%, L amino acids, 48-49%) (**Tables 1-4**).  Based on NJ and ML phylogenetic trees generated from each complete gene sequence, FFLV is ancestrally related to the members of *Lyssavirus* genus with 100% bootstrap support (**Figure 2**).  **Species demarcation criteria**  As only one member of the genus is proposed at this time, demarcation criteria are based on those in other rhabdovirus genera and are subject to refinement once more amplyliviruses are identified. At present time, we propose that viruses assigned to different species within the genus *Amplylivirus* have several of the following characteristics: A) minimum amino acid sequence divergence of 10% in N proteins; B) minimum sequence divergence of 10% in the L proteins; C) minimum amino acid sequence divergence of 15% in G proteins; D) can be distinguished in virus neutralisation tests; and E) occupy different ecological niches as evidenced by differences in vertebrate hosts and or arthropod vectors.  **Derivation of the genus name**  *Amplylivirus* is derived from the amphibian lyssa-like virus.  Derivation of the species name  *Amplylivirus cinereus* is derived from the host in which the virus was detected, the American green tree frog (*Hyla cinerea* or *Dryophytes cinereus*; because there is no agreement on the host genus name, we derived the virus species name from the host species name).   1. **Genus *Replylivirus***   The new genus *Replylivirus* includes the anole lyssa-like virus 1 (ALLV1; GenBank accession No. BR001666). We propose to assign this virus to a new species, *Replylivirus allogus*.  **Sequence origin**  ALLV1 was identified in the brain and skin of the Spanish flag anole (*Anolis allogus* or *Norops allogus* in different classifications). The lizard was a subject of physiological studies performed in Cuba during 2011 and did not show clinical signs of any disease.  **Genome organization and phylogenetic sequence relationships**  The nearly complete ALLV1 genome covering all coding sequences (11584 nt) contains the five canonical rhabdovirus structural protein genes (*N*, *P*, *M*, *G* and *L*) separated by short intergenic regions, with an elongated G-L intergenic region (180 nucleotides) resembling this genome region of lyssaviruses (**Figure 1**). In the absence of complementarity of genome termini, we consider that either the leader, trailer, or both are truncated. Sequences of the most conserved viral genes N and L demonstrate equal identities to various lyssaviruses (N nucleotides, 57-60%; N amino acids, 56-58%, L nucleotides, 61-62%, L amino acids, 62-63%) (**Tables 1-4**).  Based on NJ and ML phylogenetic trees generated from each complete gene sequence, AFLV is ancestrally related to the members of *Lyssavirus* genus with 100% bootstrap support (**Figure 2**).  **Species demarcation criteria**  As only one member of the genus is proposed at this time, demarcation criteria are based on those in other rhabdovirus genera and are subject to refinement once more amplyliviruses are identified. At present time, we propose that viruses assigned to different species within the genus *Replylivirus* have several of the following characteristics: A) minimum amino acid sequence divergence of 10% in N proteins; B) minimum sequence divergence of 10% in the L proteins; C) minimum amino acid sequence divergence of 15% in G proteins; D) can be distinguished in virus neutralisation tests; and E) occupy different ecological niches as evidenced by differences in vertebrate hosts and or arthropod vectors.  **Derivation of the genus name**  *Replylivirus* is derived from the reptilian lyssa-like virus.  Derivation of the species name  *Replylivirus allogus* is derived from the host in which the virus was detected, the Spanish flag anole (*Anolis allogus* or *Norops allogus* in different classifications; because there is no agreement on the host genus name, we derived the virus species name from the host species name). | |

**Supporting evidence**

**Table 1.** Identity values between N amino acid sequences of ALLV1, FLLV1, and lyssaviruses from all species.



**Table 2.** Identity values between N gene nucleotide sequences of ALLV1, FLLV1, and lyssaviruses from all species.



**Table 3.** Identity values between L protein amino acid sequences of ALLV1, FLLV1, and lyssaviruses from all species**.**



**Table 4.** Identity values between L gene nucleotide sequences of ALLV1, FLLV1, and lyssaviruses from all species**.**





**Figure 1**. Genome organization of the anole lyssa-like virus 1 (ALLV1; proposed species *Replylivirus allogus*) and the frog lyssa-like virus 1 (FLLV1; proposed species *Amplylivirus cinereus*) compared to typical lyssavirus genome. Each genome contains five long open reading frames (ORFs) in the N, P, M, G and L genes (open arrows). In ALLV1, a long G-L intergenic region of 180 nt (ψ) is similar to those which occur in lyssaviruses.



***Lyssavirus***

**Figure 2.**  The evolutionary history was inferred from a Clustal W alignment of 170 complete L protein sequences of animal rhabdoviruses currently assigned or recently proposed for assignment to species in the subfamily *Alpharhabdovirinae*, family *Rhabdoviridae*. Phylogenetically informative sites were selected from the alignment using Gblocks resulting in 969 positions in the final dataset. The tree was inferred in MEGA7 by using the Maximum Likelihood method based on the Whelan and Goldman + Freq. model. The tree with the highest log likelihood (-130596.29) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using a JTT model, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Bootstrap values (100 iterations) are shown for each node.

**References**

1. Horie M, Akashi H, Kawata M, Tomonaga K. Identification of a reptile lyssavirus in *Anolis allogus* provided novel insights into lyssavirus evolution. Virus Genes. 2021; 57(1):40-49. doi: 10.1007/s11262-020-01803-y. PMID: 33159637