

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

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| **Code assigned:** | ***2023.021M*** |  |
| **Short title:** Create twenty-four new species, five new genera, and one new subfamily (*Deltarhabdovirinae*) (*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 to both the word document and spreadsheet. |

**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 | 10 | 0 | 4 |

**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)** |
| N/A | N/A | N/A |

**Submission dates**

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| Date first submitted to SC Chair | 6 July 2023 |
| Date of this revision (if different to above) | 26 July 2023 |

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

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| The question arose why genus *Platrhavirus* is not assigned to a new subfamily.  Response: A subfamily may be proposed in the future but the Study Group is not ready to do that as yet. Subfamily assignments of genera are not mandatory and there remain many unassigned invertebrate rhabdoviruses for which coding-complete genome sequences are available and their number continues to grow. The nodes in phylogenetic trees are often deep and branching is sometimes not sufficiently well supported to be sure of their evolutionary history. Trees begin to firm up and make more sense as new viruses are discovered. Once nodes become solid, subfamilies may be established. |

**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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| 2023.021M.A.v1.Rhabdoviridae\_24nsp\_5ngen.1nsf.xlsx |

**Abstract**

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| We propose the creation of two new species within the genus *Alphahymrhavirus,* one new species in the genus *Betahymrhavirus*,three new species within the genus *Betaricinrhavirus* and four new genera including 12 new species for viruses detected by metagenomic sequencing of bees and wasps (*Gammahymrhavirus*),hard ticks (*Gammaricinrhavirus*)and mosquitoes (S*tangrhavirus* and *Primrhavirus*). It is also proposed that these four new genera and seven existing genera (*Alphahymrhavirus*, *Betahymrhavirus*, *Betaricinrhavirus*, *Alphacrustrhavirus,* *Betapaprhavirus* and *Betanemrhavirus*) be assigned to a new subfamily (*Deltarhabdovirinae*) within the *Rhabdoviridae*. We also propose the creation of one new genus (*Platrhavirus*) including six new species for viruses detected by metagenomic sequencing of either flatworms or fox feces. This new genus will be assigned to the *Rhabdoviridae* but will not be assigned to a subfamily at this stage. |

**Text of proposal**

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| |  | | --- | | 1. **Proposed new species in the genus *Alphahymrhavirus***   Lariophagus distinguendus negative strand RNA virus 1 (LdNSRV1) was detected by metagenomic sequencing of endoparasitoid wasps (*Lariophagus distinguendus*) of rice weevil (*Sitophilus oryzae*) larvae and pupae collected in China, in 2017 [9]. We propose LdNSRV1 be assigned to the new species *Alphahymrhavirus* *distinguendus.*  Xiangshan rhabdo-like virus 3 (XsRLV3; sample Novel\_25) was detected by metagenomic sequencing of a mixed sample of insects (Hymenoptera; Diptera; Lepidoptera)  collected in Beijing, China, in 2020 (PRJNA728541). We propose XsRLV3 be assigned to the new species *Alphahymrhavirus* *xiangshan.*  **Genome organizations**  The near-complete genome sequences of LdNSRV1 (11,633 nt) and XsRLV3 (11,774 nt) are available, lacking only extreme 3' and 5' termini [9]. Each genome contains only the five canonical rhabdovirus structural protein genes (*N*, *P*, *M*, *G* and *L*) (**Figure 1**).  **Phylogenetic analysis**  Based on ML trees generated from complete L protein sequences LdNSRV1 and XsRLV3 cluster with other alphahymrhaviruses in a moderately well-supported monophyletic subclade (BSP = 78) and are most closely related to hymenopteran rhabdo-related virus 46 (HRRV46; species *Alphahymrhavirus radians*) and hymenopteran rhabdo-related virus 109 (HRRV109; species *Alphahymrhavirus hirtum*) with which they form a strongly supported monophyletic subclade (BSP = 100) (**Figure 2**).  **Amino acid sequence identities**  Pairwise sequence identities (p-distances) calculated in MEGA7 from ClustalW amino acid sequence alignments indicated that LdNSRV1 is most closely related to HRRV109 in N (38.3% identity) and L (48.4% identity) and most closely related to HRRV109 in G (24.9% identity). Similarly, XsRLV3 is most closely related to HRRV109 in L (50.1% identity) and to HRRV46 in N (44.4% identity) and G (25.2% identity). (**Tables 1-3**)  **Species demarcation criteria**  Viruses assigned to different species within the genus *Alphahymrhavirus* 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) significant differences in genome organisation as evidenced by numbers and locations of ORFs; E) can be distinguished in virus neutralisation tests; and F) occupy different ecological niches as evidenced by differences in hosts and/or vectors.  The proposed members of the genus meet demarcation criteria A, B, and C. The genome organisations are similar to those of other alphahymrhaviruses (criterion D). As no virus isolates are currently available neutralisation tests have not been conducted (criterion E). LdNSRV1 appears to occupy a unique ecological niche amongst alphahymrhaviruses; the ecology of XsRLV3 is uncertain (criterion F).   1. **Proposed new species in the genus *Betahymrhavirus***   Xiangshan rhabdo-like virus 4 (XsRLV4; sample Novel\_26) was detected by metagenomic sequencing of a mixed sample of insects (Hymenoptera; Diptera; Lepidoptera)  collected in Beijing, China, in 2020 (PRJNA728541). We propose XsRLV4 be assigned to the new species *Betahymrhavirus* *xiangshan.*  **Genome organization**  The near-complete genome sequence of XsRLV4 (12,645 nt) is available, lacking only extreme 3' and 5' termini. Like other betahymrhaviruses, the genome contains the five canonical rhabdovirus structural protein genes (*N*, *P*, *M*, *G* and *L*) and an additional gene (*U1*) following the *M* gene in which there are overlapping reading frames (**Figure 1**).  **Phylogenetic analysis**  Based on ML trees generated from complete L protein sequences XsRLV4 clusters with other betahymrhaviruses in a well-supported monophyletic subclade (BSP = 100) and is most closely related to hymenopteran rhabdo-related virus 24 (HRRV24; species *Betahymrhavirus heterodontonyx*) (**Figure 2**).  **Amino acid sequence identities**  Pairwise sequence identities (p-distances) calculated in MEGA7 from ClustalW amino acid sequence alignments indicated that XsRLV4 is most closely related to HRRV24 with which it shares 45.0% identity in N and 58.9% identity in L and 51.6% identity in G (**Tables 4-6**).  **Species demarcation criteria**  Viruses assigned to different species within the genus *Betahymrhavirus* 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) significant differences in genome organisation as evidenced by numbers and locations of ORFs; E) can be distinguished in virus neutralisation tests; and F) occupy different ecological niches as evidenced by differences in hosts and/or vectors.  The proposed member of the genus meets demarcation criteria A, B, and C. The genome organisation is similar to those of other betahymrhaviruses (criterion D). As no virus isolates are currently available neutralisation tests have not been conducted (criterion E). The ecology of XsRLV4 is uncertain (criterion F).   1. **Proposed new species in the genus *Betaricinrhavirus***   Mudanjiang rhabd tick virus 1 (MjRTV1; sample TIGMIC7) was detected by metagenomic sequencing of hard ticks (*Ixodes persulcatus*) collected from the field in Jilin Province, China, in 2019 (PRJNA841744). We propose MjRTV1 be assigned to the new species *Betaricinrhavirus mudanjiang.*  Tongren rhabd tick virus 1 (TrRTV1; sample TIGMIC1) was detected by metagenomic sequencing of hard ticks (*Haemaphysalis hystricis*) collected from swine in Guizhou Province, China, in 2019 (PRJNA841744). We propose TrRTV1 be assigned to the new species *Betaricinrhavirus tongren.*  Yanbian rhabd tick virus 3 (YbRTV3; sample TIGMIC1) was detected by metagenomic sequencing of hard ticks (*Haemaphysalis japonica*) collected from the field in Jilin Province, China, in 2019 (PRJNA841744). We propose TrRTV3 be assigned to the new species *Betaricinrhavirus yanbian.*  Yanbian rhabd tick virus 2 (YbRTV2; sample TIGMIC3) was detected by metagenomic sequencing of hard ticks (*Haemaphysalis concinna*) collected from the field in Jilin Province, China, in 2019 (PRJNA841744). Although likely to be a member of the genus, the genome coding sequence is incomplete, lacking the essential *N* gene, and so YbRTV2 is not proposed for classification at this stage.  **Genome organization**  The near-complete genome sequences of MjRTV1 (13,702 nt), TrRTV1 (11,167 nt) and YbRTV3 (11,160 nt) are available, lacking only extreme 3' and 5' termini. Like other betaricinrhaviruses, the MjRTV1 genome contains the five canonical rhabdovirus structural protein genes (*N*, *P*, *M*, *G* and *L*) with an alternative long ORF in the *P* gene. The genomes of TrRTV1 and TbRTV3 are similar, containing four of the five canonical rhabdovirus structural protein genes (*N*, *P*, *M*, and *L*) but lacking the *G* gene. There is no alternative long ORF in the *P* gene of TrRTV1 or TbRTV3 (**Figure 1**). The absence of a *G* gene has been observed previously in the tick rhabdovirus, Wuhan tick virus 1 (species *Alpharicinrhavirus wuhan*) [6].  **Phylogenetic analysis**  Based on ML trees generated from complete L protein sequences MjRTV1, TrRTV1 and YbRTV3 cluster with other betaricinrhaviruses in a well-supported monophyletic subclade (BSP = 100) (**Figure 2**).  **Amino acid sequence identities**  Pairwise sequence identities (p-distances) calculated in MEGA7 from ClustalW amino acid sequence alignments indicate that MjRTV1 is most closely related to Chimay rhabdovirus (CRV: species *Betaricinrhavirus chimay*) with 63.8% identity in N, 87,5% identity in L and 61.5% identity in G. TrRTV1 and YbRTV3 (which both lack the *G* gene) are most closely related to each other sharing 28.0% identity in N and 58.7% identity in L, and are very distant from other betaricinrhaviruses, sharing only 10.7-12.3% identity in N and 35.9-36.5% identity in L. This extent of evolutionary divergence of the N proteins is extreme with levels of identity that are virtually insignificant (**Tables 7-9**).  **Species demarcation criteria**  Viruses assigned to different species within the genus *Betaricinrhavirus* 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) significant differences in genome organisation as evidenced by numbers and locations of ORFs; E) can be distinguished in virus neutralisation tests; and F) occupy different ecological niches as evidenced by differences in hosts and/or vectors.  The proposed member of the genus meets demarcation criteria A, B, and C. The genome organisation of MjRTV1 is similar to those of other betaricinrhaviruses but both TrRTV1 and YbRTV3 lack the *G* gene (criterion D). As no virus isolates are currently available neutralisation tests have not been conducted (criterion E). The viruses have been detected in hard ticks of different species from China (criterion F).   1. **Proposed new genus *Gammahymrhavirus***   The new genus *Gammahymrhavirus* is proposed for a distinct monophyletic clade of three related viruses detected in bees or wasps (order Hymenoptera).  Apis rhabdovirus 4 (ApRV4; sample 23-Acc030-ZJ2019) was detected by metagenomic sequencing of Asian honey bees (*Apis mellifera*) collected from cattle in Zhejiang Province, China, in 2019 (PRJNA706851). We propose ApRV4 be assigned to the new species *Gammahymrhavirus* *mellifera.*  Apis rhabdovirus 5 (ApRV5; sample 24-Am025-HLJ2017) was detected by metagenomic sequencing of European honey bees (*Apis cerana*) collected from cattle in Heilongjiang Province, China, in 2017 (PRJNA706851). We propose ApRV5 be assigned to the new species *Gammahymrhavirus* *cerana.*  Diachasminorpha longicaudata rhabdovirus (DlonRV; sample UGA) was detected by metagenomic sequencing of endoparasitoid wasps (*Diachasminorpha longicaudata*) of tephritid fruit flies collected in Florida, USA, in 2014 (PRJNA270235). We propose DlonRV be assigned to the new species *Gammahymrhavirus* *longicaudata.*  **Genome organization**  The near-complete genome sequences of ApRV4 (13,489 nt), ApRV5 (13,395 nt) and DlonRV (13,434 nt) are available, lacking only extreme 3' and 5' termini. Each genome contains the five canonical rhabdovirus structural protein genes (*N*, *P*, *M*, *G* and *L*) as well as one or two genes between the *M* gene and *G* gene (**Figure 1**). In ApRV4 and ApRV5, there are two genes (*U1* and *U2*) containing ORFs encoding three homologous proteins (U1, U1x and U2). Structural modeling predicts the U1 proteins to be very small secreted globular proteins with an N-terminal signal domain. The U1x proteins are encoded from an alternative ORF in the same gene, encoding small proteins with a C-terminal transmembrane domain. The U2 proteins have no predicted signal or transmembrane domains. ApRV5 also included alternative long ORFs in the *P* gene and *G* gene but it is not known if these are expressed. In DlonRV, there is only one gene (*U1*) in the region between the *M* gene and *G* gene. The DlonRV U1 ORF encodes a larger secreted globular protein that is unrelated in sequence to the U1, U1x or U2 proteins of ApRV4 and ApRV5.  **Phylogenetic analysis**  Based on ML trees generated from complete L protein sequences, ApRV4, ApRV5 and DlonRV cluster together as a well-supported monophyletic subclade (BSP = 100) within a larger clade of viruses that are currently not assigned to a subfamily but are proposed here to be assigned to the subfamily *Deltarhabdovirinae* (see below) (**Figure 2**).  **Amino acid sequence identities**  Pairwise sequence identities (p-distances) calculated in MEGA7 from ClustalW amino acid sequence alignments indicated that ApRV4 and ApRV5 are most closely related, sharing 81.2% identity in L, 88.0% identity in N and 57.6% identity in G (**Tables 10-12**).  **Species demarcation criteria**  Viruses assigned to different species within the genus *Gammahymrhavirus* 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) significant differences in genome organisation as evidenced by numbers and locations of ORFs; E) can be distinguished in virus neutralisation tests; and F) occupy different ecological niches as evidenced by differences in hosts and/or vectors.  All three viruses proposed as species in the new genus meet criteria A, B and C. The genome organisations of ApRV4 and ApRV5 are similar with differences only in the presence in ApRV5 of alternative long ORFs in the *P* and *G* genes. The DlonRV genome organization differs from those of ApRV4 and ApRV5 (criterion D). No neutralization test data are yet available as there is currently no isolate of the virus (criterion E). The viruses have been detected in hymenopteran insects of different species (criterion F).   1. **Proposed new genus *Gammaricinrhavirus***   The new genus *Gammaricinrhavirus* is proposed for a distinct monophyletic clade of two related viruses detected in ticks (order Ixodida).  Fuyun tick rhabdovirus (FyTRV; sample FY-19-20) was detected by metagenomic sequencing of ticks (*Hyalomma* sp.) collected in Fuyun, Xinjiang Province, China, in 2019 (Bioproject PRJNA871396). We propose FyTRV be assigned to the new species *Gammaricinrhavirus* *fuyun.*  Tacheng tick virus 7 (TcTV7; sampleTCRP-3) was detected by metagenomic sequencing of ticks (*Argas miniatus*) collected in Tacheng, Xinjiang Province, China, in 2012 [6]. We propose TcTV7 be assigned to the new species *Gammaricinrhavirus* *tacheng.*  Lhasa rhabd tick virus 1 (LsRTV1; sample TIGMIC1) was detected by metagenomic sequencing of ticks (*Alveonasus lahorensis*) collected from sheep in Tibet, China, in the year recorded as 1905 (Bioproject PRJNA841744). LhRTV1 was considered for assignment to the new genus but was excluded at this stage due to the poor bootstrap support (BSP = 52) for the node linking this virus to the two proposed members in the phylogenetic analysis based on L protein sequences (**Figure 2**).  **Genome organization**  The near-complete genome sequences of FyTRV (13,658 nt) and TcTV7 (13,408 nt) are available, lacking only extreme 3' and 5' termini. Each genome contains the five canonical rhabdovirus structural protein genes (*N*, *P*, *M*, *G* and *L*) as well one additional gene (*U1*) between the *M* and *G* genes (**Figure 1**). The *U1* genes, which encode homologous proteins, are not present in the LsRTV1 genome (which is not proposed for classification at this stage).  **Phylogenetic analysis**  Based on ML trees generated from complete L protein sequences, FyTRV and TcTV7 cluster together as a well-supported monophyletic subclade (BSP = 100) within a larger clade of viruses that are currently not assigned to a subfamily but are proposed here to be assigned to the subfamily *Deltarhabdovirinae* (see below) (**Figure 2**).  **Amino acid sequence identities**  Pairwise sequence identities (p-distances) calculated in MEGA7 from ClustalW amino acid sequence alignments indicated that FyTRV and TcTV7 share 63.0% identity in L, 54.8% identity in N and 37.1% identity in G.  **Species demarcation criteria**  Viruses assigned to different species within the genus *Gammaricinrhavirus* 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) significant differences in genome organisation as evidenced by numbers and locations of ORFs; E) can be distinguished in virus neutralisation tests; and F) occupy different ecological niches as evidenced by differences in hosts and/or vectors.  FyTRV and TcTV7 meet criteria A, B and C. The genome organisations of ApRV4 and ApRV5 are similar (criterion D). No neutralization test data are yet available as there is currently no isolate of the virus (criterion E). The viruses were detected in ticks of different genera (criterion F).   1. **Proposed new genus *Stangrhavirus***   The new genus *Stangrhavirus* is proposed for a distinct monophyletic clade of four related viruses detected in culicine mosquitoes (family Culicidae).  Stang virus (STNGV; sample CMS002\_053a\_PLCR) was detected by metagenomic sequencing of mosquitoes (*Culex erythrothorax*) collected in the Placer Valley, California, in 2017 [1]. We propose STNGV be assigned to the new species *Stangrhavirus stang.*  Elisy virus (ELSYV; sample CMS001\_039\_ALCO) was detected by metagenomic sequencing of mosquitoes (*Culex tarsalis*) collected in Alameda County, California, USA, in 2017 [1]. We propose ELSYV be assigned to the new species *Stangrhavirus elisy.*  Wuhan mosquito virus 9 (WhMV9; sample XY91455 was detected by metagenomic sequencing of mosquitoes (*Culex tritaeniorhynchus*) collected in Yunnan, China, in 2018 (Bioproject PRJNA778885). It had been detected previously in mosquitoes of the same species collected in China in 2013 but the reported genome sequence is corrupted in the *L* gene [6]. We propose ELSYV be assigned to the new species *Stangrhavirus wuhan.*  Guadeloupe Culex rhabdovirus (GCRV; sample 2017-PB-CQM-1-3) was detected by metagenomic sequencing of mosquitoes (*Culex quinquefasciatus*) collected in Guadeloupe, in 2017 [8]. It had been detected previously by metagenomic sequencing of mosquitoes (*Deinocerites* sp.) collected in Grenada in 2015 and named Grenada mosquito rhabdovirus 1 [10]. It was also detected in mosquitoes (*Culex quinquefasciatus*; *Aedes aegypti*) collected in Brazil, in 2017, and named cururu virus [3]. The three viruses are highly similar in nucleotide sequence and can be considered isolates of the same virus. As GCRV is the best described and publicly documented of the three viruses, it will serve as the exemplar isolate. We propose GCRV be assigned to the new species *Stangrhavirus guadeloupe.*  **Genome organization**  The near-complete genome sequences of STNGV (13,637 nt), ELSYV (13,816 nt), WhMV9 (13668 nt) and GCRV (14405 nt) are available, lacking only extreme 3' and 5' termini. Each genome contains the five canonical rhabdovirus structural protein genes (*N*, *P*, *M*, *G* and *L*) as well one additional gene (*U1*) between the *G* and *L* genes (**Figure 1**). The *U1* genes encode small homologous proteins. STNGV also contains an alternative ORF in the *M* gene but it is not known if it is expressed.  **Phylogenetic analysis**  Based on ML trees generated from complete L protein sequences, STNGV, ELSYV, WhMV9 and GCRV cluster together as a well-supported monophyletic subclade (BSP = 100) within a larger clade of viruses that are currently not assigned to a subfamily but are proposed here to be assigned to the subfamily *Deltarhabdovirinae* (see below) (**Figure 2**).  **Amino acid sequence identities**  Pairwise sequence identities (p-distances) calculated in MEGA7 from ClustalW amino acid sequence alignments indicated that STNGV and ELSYV are most closely related amongst the viruses to be assigned to the new genus, sharing 65.5% identity in L, 51.8% identity in N and 47.7% identity in G (**Tables 13-15**).  **Species demarcation criteria**  Viruses assigned to different species within the genus *Stangrhavirus* 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) significant differences in genome organisation as evidenced by numbers and locations of ORFs; E) can be distinguished in virus neutralisation tests; and F) occupy different ecological niches as evidenced by differences in hosts and/or vectors.  All members of the proposed new genus meet criteria A, B and C. The genome organisations are similar (criterion D). No neutralization test data are yet available as there is currently no isolate of the virus (criterion E). The viruses were detected in mosquitoes of different species (criterion F).   1. **Proposed new genus *Primrhavirus***   The new genus *Primrhavirus* is proposed for a distinct monophyletic clade of three related viruses detected in culicine mosquitoes (family Culicidae).  Primus virus (PRIMV; sample Ferlo) was detected by metagenomic sequencing of mosquitoes (*Aedes vexans*) collected in Senegal, in 2014 [5]. We propose PRIMV be assigned to the new species *Primrhavirus primus.*  San Gabriel mononegavirus (SGMNV; sample San Gabriel Valley) was discovered in a high-throughput RNA-Seq library from a mosquito colony (*Aedes albopictus*), established in San Gabriel, California, USA, that is deposited in the Sequence Read Archive (SRA) database [7]. It was also detected in other RNA-Seq libraries in the SRA from other colonies, wild-caught mosquitoes and various cell lines from *Aedes* spp. mosquitoes from various parts of the world [7]. We propose SGMNV be assigned to the new species *Primrhavirus gabriel.*  Atrato rhabdo-like virus 3 (AtRLV3; sample Cx 1773-3) was discovered by metagenomic sequencing of mosquitoes (*Culex sp.*) collected in Colombia, in 2016. We propose AtTRL3 be assigned to the new species *Primrhavirus atrato.*  **Genome organization**  The near-complete genome sequences of PRIMV (12,194 nt), SGMNV (12,620 nt), and AtRLV3 (12,076 nt) are available, lacking only extreme 3' and 5' termini. Each genome contains only the five canonical rhabdovirus structural protein genes (*N*, *P*, *M*, *G* and *L*) (**Figure 1**). Various alternative ORFs occur in each of these genes in a virus-specific manner but none appear to be homologous and they may not be expressed.  **Phylogenetic analysis**  Based on ML trees generated from complete L protein sequences, PRIMV, SGMNV, and AtRLV3 cluster together as a well-supported monophyletic subclade (BSP = 100) within a larger clade of viruses that are currently not assigned to a subfamily but are proposed here to be assigned to the subfamily *Deltarhabdovirinae* (see below) (**Figure 2**).  **Amino acid sequence identities**  Pairwise sequence identities (p-distances) calculated in MEGA7 from ClustalW amino acid sequence alignments indicated that PRIMV and SGMNV are most closely related amongst the viruses to be assigned to the new genus, sharing 60.4% identity in L, 36.1% identity in N and 40.0% identity in G (**Tables 16-18**).  **Species demarcation criteria**  Viruses assigned to different species within the genus *Primrhavirus* 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) significant differences in genome organisation as evidenced by numbers and locations of ORFs; E) can be distinguished in virus neutralisation tests; and F) occupy different ecological niches as evidenced by differences in hosts and/or vectors.  All members of the proposed new genus meet criteria A, B and C. The genome organisations are similar and the significance of alternative ORFs is not known (criterion D). No neutralization test data are yet available as there is currently no isolate of the virus (criterion E). The viruses were detected in mosquitoes of different species in different geographic locations (criterion F).   1. **Proposed new genus *Platrhavirus***   The new genus *Platrhavirus* is proposed for a distinct monophyletic clade of six related viruses detected in parasitic flatworms (phylum Platyhelminthes) or in animal feces.  Tritaenorhabdovirus 1 (TriRV1; sample TN1) was discovered in a high-throughput RNA-Seq library from a cestode parasite (*Triaenophorus nodulosus*) that is deposited in the Sequence Read Archive (SRA) database [4]. We propose TnRV1 be assigned to the new species *Platrhavirus nodulosus.*  Microrhabdovirus 1 (MicRV1; sample MSp2) was discovered in a high-throughput RNA-Seq library from a trematode parasite (*Microphallus* sp.2 LB-2020) that is deposited in the Sequence Read Archive (SRA) database [4]. We propose MicRV1 be assigned to the new species *Platrhavirus microphallus.*  Sphaeridiorhabdovirus 1 (SphRV1) was discovered in a high-throughput RNA-Seq library from a trematode parasite (*Sphaeridiotrema pseudoglobulus*) that is deposited in the Sequence Read Archive (SRA) database [4]. We propose SphRV1 be assigned to the new species *Platrhavirus pseudoglobulus.*  Schistorhabdovirus 1 (SchRV1; sample ST1) was discovered in a high-throughput RNA-Seq library from a trematode parasite (*Schistosoma turkestanicum*) that is deposited in the Sequence Read Archive (SRA) database [4]. We propose SchRV1 be assigned to the new species *Platrhavirus turkestanicum.*  Metorhabdovirus 2 (MetRV2; sample MO1) was discovered in a high-throughput RNA-Seq library from a trematode parasite (*Metorchis orientalis*) that is deposited in the Sequence Read Archive (SRA) database [4]. We propose MetRV2 be assigned to the new species *Platrhavirus orientalis.*  Fox fecal rhabdovirus (FFRV; sample S40) was detected by metagenomic sequencing of feces from a fox (*Vulpes vulpes*) collected in Spain, in 2013 [2]. We propose FFRV be assigned to the new species *Platrhavirus vulpes.*  **Genome organization**  The near-complete genome sequences of TriRV1 (15,554 nt), MicRV1 (12,249 nt), SphRV1 (12,317 nt), SchRV1 (12,715 nt), MetRV2 (13,061 nt) and FFRV (15,541 nt) are available, lacking only extreme 3' and 5' termini. Each genome contains the five canonical rhabdovirus structural protein genes (*N*, *P*, *M*, *G* and *L*) and one or more genes between the *G* and *L* genes (**Figure 1**). In several of these viruses, one of the additional genes encodes a predicted class I viroporin. Various alternative ORFs occur in some of these additional genes or the *P* or *M* genes, but none appear to be homologous and they may not be expressed.  **Phylogenetic analysis**  Based on ML trees generated from complete L protein sequences, TriRV1, MicRV1, SphRV1, SchRV1, MetRV2 and FFRV cluster together as a well-supported monophyletic subclade (BSP = 97). This clade is separate from all existing subfamilies (*Alpharhabdovirinae*, *Betarhabdovirinae* and *Gammarhabdovirinae*) and the clade of viruses that are currently not assigned to a subfamily but are proposed here to be assigned to the subfamily *Deltarhabdovirinae* (see below) (**Figure 2**).  **Amino acid sequence identities**  Pairwise sequence identities (p-distances) calculated in MEGA7 from ClustalW amino acid sequence alignments indicated that SchRV1 and SphRV1 are most closely related amongst the viruses to be assigned to the new genus, sharing 45.6% identity in L, 29.3% identity in N and 25.4% identity in G (**Tables 19-21**).  **Species demarcation criteria**  Viruses assigned to different species within the genus *Platrhavirus* 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) significant differences in genome organisation as evidenced by numbers and locations of ORFs; E) can be distinguished in virus neutralisation tests; and F) occupy different ecological niches as evidenced by differences in hosts and/or vectors.  All members of the proposed new genus meet criteria A, B and C. The genome organisations, although similar in overall structure, display significant differences (criterion D). No neutralization test data are yet available as there is currently no isolate of the virus (criterion E). The viruses were detected in platyhelminths (trematodes and cestodes) of different genera in different geographic locations (criterion F).  **NOTE:** Although occupying a distinct monophyletic clade and sharing the unique ecology of association with platyhelminth hosts, these viruses are very diverse in genome sequence. As such, the possible discovery of additional related viruses in the future may necessitate the splitting of the genus.   1. **Proposed new subfamily *Deltarhabdovirinae***   Further structural development of the family *Rhabdoviridae* is proposed through the creation of the new subfamily *Deltarhabdovirinae* for theseven existing genera that are currently not assigned to a subfamily (*Alphahymrhavirus*, *Betahymrhavirus*, *Alphadrosrhavirus*, *Alphacrustrhavirus*, *Betapaprhavirus*, *Betaricinrhavirus* and *Betanemrhavirus*) as well as four of the five new genera proposed above (*Gammahymrhavirus*, *Gammaricinrhavirus*, *Stangrhavirus* and *Primrhavirus*). Based on ML trees generated from complete L protein sequences, these 11 genera form a well-supported monophyletic clade (BSP = 93) that is distinct from the three existing subfamilies and from the newly proposed genus *Platrhavirus* (**Figure 2**).  We do not propose to assign the genus *Platrhavirus* to a subfamily at this stage as we expect that the future availability of additional virus sequences will reveal further structure in the family.   1. **Origin of the names of proposed new taxa**   genus *Gammahymrhavirus* – the gamma (third) clade of rhabdoviruses derived from hymenopteran hosts.  genus *Gammaricinrhavirus* – the gamma (third) clade of tick rhabdoviruses derived from *ricinus* (Latin, tick).  genus *Stangrhavirus* – fromStang virus(to be assigned to a species in the genus) and rhabdovirus*.*  genus *Primrhavirus* – fromPrimus virus(to be assigned to a species in the genus) and rhabdovirus*.*  genus *Platrhavirus* – a clade of rhabdoviruses derived from platyhemlinth hosts.  subfamily *Deltarhabdovirinae* – the delta (fourth) clade of rhabdoviruses to be assigned to a subfamily. | |

**Supporting evidence**

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**Figure 1.** Schematic representation of deltarhabdovirus and platrhavirus (-) ssRNA genomes shown in reverse polarity. N, P, M, G and L represent ORFs encoding the structural proteins. Additional ORFs that appear to be homologous are shown in the same colour; those shown in grey are not homologous. The genomes are drawn to scale. The viruses representing proposed new species are listed in red text.

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**Figure 2.** The evolutionary history was inferred from a MAFFT alignment of complete L protein sequences of 360 rhabdoviruses that are currently assigned to species or proposed to be assigned in concurrent proposals plus 30 viruses that were considered for taxonomic assignment in this proposal. Viruses assigned to existing species and existing genera and subfamilies are shown in bold black type. Viruses proposed for taxonomic assignment to species as well as proposed new genera and the new subfamily are shown in red. Other viruses considered but not proposed for assignment are shown in blue. Phylogenetically informative sites were selected from the alignment using TrimAl resulting in 964 positions in the final dataset. The tree was inferred in MEGAX by using the Maximum Likelihood method based on the best-fit Le and Gascuel model with gamma distribution of evolutionary rates and invariable sites. The tree with the highest log likelihood (-369041.07) 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-Join 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. Several genera have been condensed together into single branches. Bootstrap values (100 iterations) are shown for each node.

**Table 1.** Percentage amino acid identities (p-distance) of a ClustalW alignment of alphahymrhavirus N protein sequences.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | LnegV2 | HRRV38 | HRRV46 | HRRV109 | LdNSRV1 | XsRLV3 |
| LnegV2 |  |  |  |  |  |  |
| HRRV38 | 36.1 |  |  |  |  |  |
| HRRV46 | 26.9 | 23.1 |  |  |  |  |
| HRRV109 | 24.1 | 22.9 | 55.5 |  |  |  |
| LdNSRV1 | 25.6 | 24.1 | 37.6 | 38.3 |  |  |
| XsRLV3 | 21.2 | 19.9 | 44.4 | 40.7 | 37.1 |  |

**Table 2.** Percentage amino acid identities (p-distance) of a ClustalW alignment of alphahymrhavirus L protein sequences.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | LnegV2 | HRRV38 | HRRV46 | HRRV109 | LdNSRV1 | XsRLV3 |
| LnegV2 | 100.0 |  |  |  |  |  |
| HRRV38 | 49.4 |  |  |  |  |  |
| HRRV46 | 41.7 | 40.0 |  |  |  |  |
| HRRV109 | 41.3 | 40.2 | 64.6 | 100.0 |  |  |
| LdNSRV1 | 39.3 | 39.3 | 48.0 | 48.4 | 100.0 |  |
| XsRLV3 | 41.2 | 40.5 | 50.3 | 51.0 | 48.0 |  |

**Table 3.** Percentage amino acid identities (p-distance) of a ClustalW alignment of alphahymrhavirus G protein sequences.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | LnegV2 | HRRV38 | HRRV46 | HRRV109 | LdNSRV1 | XsRLV3 |
| LnegV2 | 100.0 |  |  |  |  |  |
| HRRV38 | 22.2 |  |  |  |  |  |
| HRRV46 | 18.6 | 19.1 |  |  |  |  |
| HRRV109 | 17.9 | 18.5 | 41.8 | 100.0 |  |  |
| LdNSRV1 | 16.6 | 17.0 | 27.1 | 24.9 |  |  |
| XsRLV3 | 18.0 | 17.2 | 25.2 | 22.8 | 23.8 | 100.0 |

**Table 4.** Percentage amino acid identities (p-distance) of a ClustalW alignment of betahymrhavirus N protein sequences.

|  |  |  |  |
| --- | --- | --- | --- |
|  | HRRV23 | HRRV24 | XsRLV4 |
| HRRV23 |  |  |  |
| HRRV24 | 47.7 |  |  |
| XsRLV4 | 44.8 | 45.0 |  |

**Table 5.** Percentage amino acid identities (p-distance) of a ClustalW alignment of betahymrhavirus L protein sequences.

|  |  |  |  |
| --- | --- | --- | --- |
|  | HRRV23 | HRRV24 | XsRLV4 |
| HRRV23 |  |  |  |
| HRRV24 | 57.7 |  |  |
| XsRLV4 | 57.7 | 58.9 |  |

**Table 6.** Percentage amino acid identities (p-distance) of a ClustalW alignment of betahymrhavirus G protein sequences.

|  |  |  |  |
| --- | --- | --- | --- |
|  | HRRV23 | HRRV24 | XsRLV4 |
| HRRV23 |  |  |  |
| HRRV24 | 52.1 |  |  |
| XsRLV4 | 48.8 | 51.6 |  |

**Table 7.** Percentage amino acid identities (p-distance) of a ClustalW alignment of betaricinrhavirus N protein sequences.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | CRV | BLTRV1 | MjRTV1 | TrRTV1 | YbRTV3 |
| CRV |  |  |  |  |  |
| BLTRV1 | 42.5 |  |  |  |  |
| MjRTV1 | 63.8 | 42.9 |  |  |  |
| TrRTV1 | 12.1 | 12.3 | 12.0 |  |  |
| YbRTV3 | 12.3 | 11.2 | 10.7 | 28.0 |  |

**Table 8.** Percentage amino acid identities (p-distance) of a ClustalW alignment of betaricinrhavirus L protein sequences.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | CRV | BLTRV1 | MjRTV1 | TrRTV1 | YbRTV3 |
| CRV | 10.0 |  |  |  |  |
| BLTRV1 | 75.6 |  |  |  |  |
| MjRTV1 | 87.5 | 75.3 | 100.0 |  |  |
| TrRTV1 | 36.2 | 36.2 | 36.3 |  |  |
| YbRTV3 | 35.9 | 36.5 | 36.5 | 58.7 | 100.0 |

**Table 9.** Percentage amino acid identities (p-distance) of a ClustalW alignment of betaricinrhavirus G protein sequences.

|  |  |  |  |
| --- | --- | --- | --- |
|  | CRV | BLTRV1 | MjRTV1 |
| CRV | 100.0 |  |  |
| BLTRV1 | 42.8 |  |  |
| MjRTV1 | 61.5 | 43.9 | 100. |

**Table 10.** Percentage amino acid identities (p-distance) of a ClustalW alignment of gammahymrhavirus N protein sequences.

|  |  |  |  |
| --- | --- | --- | --- |
|  | ApRV4 | ApRV5 | DlonRV |
| ApRV4 |  |  |  |
| ApRV5 | 88.0 |  |  |
| DlonRV | 18.6 | 19.6 |  |

**Table 11.** Percentage amino acid identities (p-distance) of a ClustalW alignment of gammahymrhavirus L protein sequences.

|  |  |  |  |
| --- | --- | --- | --- |
|  | ApRV4 | ApRV5 | DlonRV |
| ApRV4 |  |  |  |
| ApRV5 | 81.2 |  |  |
| DlonRV | 38.2 | 37.4 |  |

**Table 12.** Percentage amino acid identities (p-distance) of a ClustalW alignment of gammahymrhavirus G protein sequences.

|  |  |  |  |
| --- | --- | --- | --- |
|  | ApRV4 | ApRV5 | DlonRV |
| ApRV4 |  |  |  |
| ApRV5 | 57.6 |  |  |
| DlonRV | 22.9 | 22.8 |  |

**Table 13.** Percentage amino acid identities (p-distance) of a ClustalW alignment of stangrhavirus N protein sequences.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | STNGV | ELSYV | WhMV9 | GCRV | GMRV1 | CRRUV |
| STNGV | 100. |  |  |  |  |  |
| ELSYV | 51.8 |  |  |  |  |  |
| WhMV9 | 48.8 | 47.7 |  |  |  |  |
| GCRV | 27.1 | 25.3 | 28.2 |  |  |  |
| GMRV1 | 26.6 | 25.1 | 28.6 | 99.4 |  |  |
| CRRUV | 27.1 | 25.3 | 28.2 | 100.0 | 99.4 |  |

**Table 14.** Percentage amino acid identities (p-distance) of a ClustalW alignment of stangrhavirus L protein sequences.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | STNGV | ELSYV | WhMV9 | GCRV | GMRV1 | CRRUV |
| STNGV |  |  |  |  |  |  |
| ELSYV | 65.7 |  |  |  |  |  |
| WhMV9 | 65.0 | 63.9 |  |  |  |  |
| GCRV | 41.9 | 42.9 | 42.2 |  |  |  |
| GMRV1 | 41.9 | 42.9 | 42.2 | 99.5 |  |  |
| CRRUV | 41.9 | 42.9 | 42.2 | 99.3 | 99.3 |  |

**Table 15.** Percentage amino acid identities (p-distance) of a ClustalW alignment of stangrhavirus G protein sequences.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | STNGV | ELSYV | WhMV9 | GCRV | GMRV1 | CRRUV |
| STNGV |  |  |  |  |  |  |
| ELSYV | 47.7 |  |  |  |  |  |
| WhMV9 | 45.4 | 46.6 |  |  |  |  |
| GCRV | 27.8 | 27.4 | 30.3 |  |  |  |
| GMRV1 | 27.6 | 27.2 | 30.1 | 99.2 |  |  |
| CRRUV | 27.6 | 27.2 | 30.1 | 98.9 | 99.4 | 0 |

**Table 16.** Percentage amino acid identities (p-distance) of a ClustalW alignment of primrhavirus N protein sequences.

|  |  |  |  |
| --- | --- | --- | --- |
|  | PRIMV | SGMNV | AtRLV2 |
| PRIMV |  |  |  |
| SGMNV | 36.1 |  |  |
| AtRLV2 | 24.6 | 25.8 |  |

**Table 17.** Percentage amino acid identities (p-distance) of a ClustalW alignment of primrhavirus L protein sequences.

|  |  |  |  |
| --- | --- | --- | --- |
|  | PRIMV | SGMNV | AtRLV2 |
| PRIMV |  |  |  |
| SGMNV | 60.4 |  |  |
| AtRLV2 | 45.8 | 45.5 |  |

**Table 18.** Percentage amino acid identities (p-distance) of a ClustalW alignment of primrhavirus G protein sequences.

|  |  |  |  |
| --- | --- | --- | --- |
|  | PRIMV | SGMNV | AtRLV2 |
| PRIMV |  |  |  |
| SGMNV | 40.0 |  |  |
| AtRLV2 | 23.2 | 23.9 |  |

**Table 19.** Percentage amino acid identities (p-distance) of a ClustalW alignment of platrhavirus N protein sequences.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | FFRV | TriRV1 | MetRV2 | MicRV1 | SchRV1 | SphRV1 |
| FFRV |  |  |  |  |  |  |
| TriRV1 | 9.9 |  |  |  |  |  |
| MetRV2 | 15.0 | 10.6 |  |  |  |  |
| MicRV1 | 15.0 | 9.6 | 12.9 |  |  |  |
| SchRV1 | 15.0 | 11.7 | 13.4 | 12.0 |  |  |
| SphRV1 | 11.8 | 11.9 | 11.9 | 13.1 | 29.3 |  |

**Table 20.** Percentage amino acid identities (p-distance) of a ClustalW alignment of platrhavirus L protein sequences.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | FFRV | TriRV1 | MetRV2 | MicRV1 | SchRV1 | SphRV1 |
| FFRV |  |  |  |  |  |  |
| TriRV1 | 22.2 |  |  |  |  |  |
| MetRV2 | 23.1 | 22.7 |  |  |  |  |
| MicRV1 | 19.9 | 21.0 | 23.3 |  |  |  |
| SchRV1 | 22.3 | 20.6 | 25.2 | 22.8 |  |  |
| SphRV1 | 23.9 | 21.9 | 25.9 | 23.2 | 45.6 |  |

**Table 21.** Percentage amino acid identities (p-distance) of a ClustalW alignment of platrhavirus G protein sequences.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | FFRV | TriRV1 | MetRV2 | MicRV1 | SchRV1 | SphRV1 |
| FFRV |  |  |  |  |  |  |
| TriRV1 | 18.2 |  |  |  |  |  |
| MetRV2 | 15.4 | 20.0 |  |  |  |  |
| MicRV1 | 17.0 | 18.4 | 17.8 |  |  |  |
| SchRV1 | 9.0 | 11.8 | 7.8 | 7.9 |  |  |
| SphRV1 | 7.4 | 13.6 | 8.6 | 8.5 | 25.4 |  |

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