This form should be used for all taxonomic proposals. Please complete all those modules that are applicable.



For guidance, see the notes written in blue and the separate document “Help with completing a taxonomic proposal”

Please try to keep related proposals within a single document.

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

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Code assigned:** | ***2019.032*** | | | | (to be completed by ICTV officers) |
| **Short title:** Create one new genus (*Ohlsrhavirus*), including five new species, in the family *Rhabdoviridae*. | | | | | |
| **Modules attached**  (Modules 1, 4 and either 2 or 3 are required. | | **1**  **2  3  4** | | | |
| **Author(s):** | | | | | |
| ICTV *Rhabdoviridae* Study Group:  Peter J. Walker  Kim R. Blasdell  Ralf G. Dietzgen  Juliana Freitas-Astúa  Hideki Kondo  Gael Kurath  Ivan Kuzmin  David M. Stone  Robert B. Tesh  Nikos Vasilakis  Anna E. Whitfield | | | | | |
| **Corresponding author with e-mail address:** | | | | | |
| Peter J. Walker, [peter.walker@uq.edu.au](mailto:peter.walker@uq.edu.au) | | | | | |
| **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 *Rhabdoviridae* Study Group | | |
| **ICTV Study Group comments (if any) and response of the proposer:** | | | | | |
| Proposal supported by a majority of Study Group members (11 supporters and 2 non-responders). One Study Group member did not support the classification of NORCV as the complete coding sequence is not available. Two other Study Group members expressed concern about the classification of viruses without complete coding sequence. They felt that this should be considered on a case-by-case basis and that the available sequence for NORCV (lacking only M and a small part of the P gene) is sufficient for classification. All other respondents supported classification of NORCV to a new species. There was also some discussion about sequence divergence between NORCV and CRLV (9.2%) falling just below the species demarcation criterion requiring 10% divergence in L protein sequences. However, following discussion, it was agreed that species demarcation are an arbitrary guide and that separate species assignment requires that several criteria (but not necessarily all criteria) should be met. On this basis, it was agreed to support the classification of NORCV and CRLV to separate new species. | | | | | |
|  | | | | | |
| Date first submitted to ICTV: | | | |  | |
| Date of this revision (if different to above): | | | |  | |

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

**Part 2**: **PROPOSED TAXONOMY**

|  |
| --- |
| Present the proposed new taxonomy on accompanying spreadsheet |
| **Name of accompanying spreadsheet:** 2019.032M.A.v1.Ohlsrhavirus\_1gen5sp.xlxs |

**Part 4:** **APPENDIX**: supporting material

| additional material in support of this proposal |
| --- |
| **References:** |
| 1. **Shahhosseini N, Luhken R, Jost H, Jansen S, Borstler J, Rieger T, Kruger A, Yadouleton A, de Mendonca Campos R, Cirne-Santos CC, Ferreira DF, Garms R, Becker N, Tannich E, Cadar D, Schmidt-Chanasit J.** 2017. Detection and characterization of a novel rhabdovirus in *Aedes cantans* mosquitoes and evidence for a mosquito-associated new genus in the family *Rhabdoviridae*. Infection Genetics and Evolution **55:**260-268.  2. **Hang J, Klein TA, Kim HC, Yang Y, Jima DD, Richardson JH, Jarman RG.** 2016. Genome sequences of five arboviruses in field-captured mosquitoes in a unique rural environment of South Korea. Genome Announcements **4:**e01644-01615.  3. **Reuter G, Boros A, Pal J, Kapusinszky B, Delwart E, Pankovics P.** 2016. Detection and genome analysis of a novel (dima)rhabdovirus (Riverside virus) from *Ochlerotatus* sp. mosquitoes in Central Europe. Infection Genetics and Evolution **39:**336-341.  4. **Shi M, Neville P, Nicholson J, Eden JS, Imrie A, Holmes EC.** 2017. High-resolution metatranscriptomics reveals the ecological dynamics of mosquito-associated RNA viruses in Western Australia. Journal of Virology **91**.  5. **Coffey LL, Page BL, Greninger AL, Herring BL, Russell RC, Doggett SL, Haniotis J, Wang C, Deng X, Delwart EL.** 2014. Enhanced arbovirus surveillance with deep sequencing: Identification of novel rhabdoviruses and bunyaviruses in Australian mosquitoes. Virology **448:**146-158.  6. **de Lara Pinto AZ, de Carvalho MS, de Melo FL, Ribeiro ALM, Ribeiro BM, Slhessarenko RD.** 2017. Novel viruses in salivary glands of mosquitoes from sylvatic Cerrado, Midwestern Brazil. PLoS One **12:**e0187429.  7. **Kuwata R, Isawa H, Hoshino K, Tsuda Y, Yanase T, Sasaki T, Kobayashi M, Sawabe K.** 2011. RNA splicing in a new rhabdovirus from culex mosquitoes. Journal of Virology **85:**6185-6196.  8. **Charles J, Firth AE, Lorono-Pino MA, Garcia-Rejon JE, Farfan-Ale JA, Lipkin WI, Blitvich BJ, Briese T.** 2016. Merida virus, a putative novel rhabdovirus discovered in *Culex* and *Ochlerotatus* spp. mosquitoes in the Yucatan Peninsula of Mexico. Journal of General Virology **97:**977-987.  9. **Oncu C, Brinkmann A, Gunay F, Kar S, Oter K, Sarikaya Y, Nitsche A, Linton YM, Alten B, Ergunay K.** 2018. West Nile virus, Anopheles flavivirus, a novel flavivirus as well as Merida-like rhabdovirus Turkey in field-collected mosquitoes from Thrace and Anatolia. Infection Genetics and Evolution **57:**36-45.  10. **Cholleti H, Hayer J, Abilio AP, Mulandane FC, Verner-Carlsson J, Falk KI, Fafetine JM, Berg M, Blomstrom AL.** 2016. Discovery of novel viruses in mosquitoes from the Zambezi Valley of Mozambique. PLoS One **11:**e0162751.  11. **Temmam S, Monteil-Bouchard S, Robert C, Baudoin JP, Sambou M, Aubadie-Ladrix M, Labas N, Raoult D, Mediannikov O, Desnues C.** 2016. Characterization of viral communities of biting midges and identification of novel thogotovirus species and rhabdovirus genus. Viruses **8:**e77. |

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| --- |
| **Annex:**  The new genus *Ohlsrhavirus* is proposed to accommodate five currently unassigned rhabdoviruses that have been detected in *Culex* and *Ochlerotatus* spp. mosquitoes from Europe, Asia and Australia. Each virus will be assigned to a new species within the new genus.  Ohlsdorf virus (OHSDV) was detected in mosquitoes (*Ochlerotatus cantans*) collected from Hamburg, Germany, in 2012 (1). The virus was also detected in mosquitoes of the same species collected from other parts of Germany from 2013-2015 (1). The near-complete genome sequence (11,624 nt) has been determined, including complete coding sequences but incomplete 3' and 5' termini. There is no virus isolate.  Tongilchon virus 1 (TCHV-1) was detected in mosquitoes (*Culex bitaeniorhynchus*) collected in the Republic of Korea, in 2012 (2). The partial genome sequence (13,343 nt) has been determined, including complete coding sequences. There is no virus isolate.  Riverside virus (RISV) was detected in three pools of mosquitoes (*Ochlerotatus* sp.) collected from near the Danube River and Drava River in Hungary, in 2014 (3). The near-complete genome sequence (11,713 nt) has been determined, including complete coding sequences but an incomplete 3' terminus. There is no virus isolate.  Culex rhabdo-like virus (CRLV) was detected in mosquitoes (*Culex quinquefasciatus*) collected near Perth in Western Australia, in 2015 (4). The virus was also detected in other culicine mosquitoes collected in 2015 at the same location and near Bunbury approximately 150 km south of Perth (*Culex globocoxitus* and *Culex australicus*, respectively). The near-complete genome sequence (11,503 nt) has been determined, including complete coding sequences but incomplete 3' and 5' termini. There is no virus isolate.  North Creek virus (NORCV) was detected in mosquitoes (*Culex sitiens*) collected at Ballina in New South Wales, Australia, in 1997 (5). Partial genome sequence has been reported, including complete N, G and L ORFs, and near-complete P ORF. There is no virus isolate. Although the coding sequence of NORCV is incomplete, there available sequence data may be considered sufficient for species assignment of the virus.  Ohlsrhavirus genomes range in length from approximately 11.2 kb to 13.4 kb, containing the five canonical rhabdovirus structural protein genes (*N*, *P*, *M*, *G* and *L*) (**Figure 1**). No alternative ORFs >180 nt occur in any of the genes of these viruses.  Based on ML trees generated from complete L protein sequences, ohlsrhaviruses form a well-supported monophyletic clade that is distinct from all currently assigned genera and other currently unassigned rhabdoviruses (**Figure 2**). Amino acid sequence divergence in pair-wise alignments (p-distances) are >10% in the N proteins, >30% in the G proteins and >10% in the L protein (except for 9.2% divergence between CRLV and NORCV L proteins) (**Tables 1-3**).  **Probable members of the genus:**  Lobeira virus (LOBV) was detected in culicine mosquitoes (*Stegomyia albopicta*) in the Chapada dos Guimaraes National Park, Mato Grosso, Brazil, in 2015 (6). Only partial genome sequence has been reported, including near-complete L ORF, and partial N, P, M and GORFs. The virus was isolated by passage in C6/36 mosquito cells.  **Other related viruses:**  Several other viruses that have been isolated from mosquitoes cluster phylogenetically in a sister clade to the ohlsrhaviruses in ML trees generated from complete L protein sequences. These include Culex tritaeniorhynchus rhabdovirus (CTRV) (7), Merida virus (MERDV) (8), Merida-like virus Turkey (MLVT) (9) and Beaumont virus (BEAUV) (5). However, the ohlsrhaviruses and this sister clade are relatively deeply rooted and Hubei dimarhabdovirus 3 (HbDRV3), which was isolated from dragonflies/damselflies (order Odonata), consistently falls between them (**Figure 3**). Therefore, we propose that these viruses should be excluded from the genus *Ohlsrhavirus* until the ecology is better defined. The other mosquito-associated could potentially form a distinct new genus.  Phylogenetic analysis using L gene fragments suggest that two other viruses are related to this larger virus complex but it is presently unclear where they fall. Mopeia rhabdovirus (MOPRV) was detected in mosquitoes (*Mansonia* spp.) collected in Cuacua, Zambezia Province, Mozambique, in 2014 (10). Several fragments of the *L* and *G* genes have been sequenced. Dielmo rhabdovirus (DIERV) was detected in biting midges (*Culicoides* spp.) collected in the Sine-Saloum region of Senegal, in November 2013 (11). Only fragments of the DIERV *L* gene have been reported.  **Species demarcation criteria**  Viruses assigned to different species within the genus *Ohlsrhavirus* 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 vertebrate hosts and or arthropod vectors.  All proposed members of the new genus meet demarcation criteria A, C, and F. They also meet criterion B, except for CRLV and NORCV which diverge by 9.2% (falling just below the arbitrary 10% cut-off). They all appear to share the same simple genome organisation (criterion D). As there are no virus isolates, cross-neutralisation tests have not been reported (criterion E).  **Derivation of the genus name.**  *Ohlsrhavirus* is derived from Ohlsdorf in Germany, where Ohlsdorf virus (assigned to the type species of the genus) was first detected in mosquitoes, and rhabdovirus.  **Type species.**  *Ohlsdorf ohlsrhavirus* is designated as the type species of the genus as Ohlsdorf virus was the first identified of the viruses assigned to the genus for which the complete coding sequence is available. |

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**Figure 1.** Ohlsrhavirus genome organisations. Each has a simple genome organisation containing only single long open reading frames (ORFs) in the *N*, *P*, *M*, *G* and *L* genes. Open arrows indicate the locations of each ORF which is located within a single transcriptional unit bounded by conserved transcription initiation and transcription termination/polyadenylation sequences. For NORCV, only full-length sequences of the N, G and L ORFs and near-complete sequence of the P ORF are available (as shown).



**Figure 2.** The evolutionary history was inferred from a Clustal W alignment of 130 complete L protein sequences of 119 animal rhabdoviruses currently assigned or recently proposed for assignment to species, five proposed members of the genus *Ohlsrhavirus* (OHLDV, RISV, TCHV-1, CRLV, NORCV), one probable members of the genus *Ohlsrhavirus* (LOBV), four other unclassified viruses detected in mosquitoes (MERDV, MLVT, CTRV, BEAUV) and an unclassified odonate-associated virus (HbDRV3). Phylogenetically informative sites were selected from the alignment using Gblocks resulting in 954 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 (-108494.0325) 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. Bootstrap values (100 iterations) are shown for each node.

**Table 1.** Percentage amino acid sequence identities (p-distance) of a CLUSTAL W alignment of ohlsrhavirus N proteins.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | OHLDV | RISV | TCHV-1 | CRLV | NORCV |
| OHLDV |  |  |  |  |  |
| RISV | 49.3 |  |  |  |  |
| TCHV-1 | 54.5 | 56.8 |  |  |  |
| CRLV | 52.3 | 53.1 | 79.6 |  |  |
| NORCV | 50.2 | 52.1 | 77.2 | 88.0 |  |

**Table 2.** Percentage amino acid sequence identities (p-distance) of a CLUSTAL W alignment of ohlsrhavirus G proteins.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | OHLDV | RISV | TCHV-1 | CRLV | NORCV |
| OHLDV |  |  |  |  |  |
| RISV | 28.0 |  |  |  |  |
| TCHV-1 | 26.4 | 37.5 |  |  |  |
| CRLV | 29.1 | 40.8 | 57.5 |  |  |
| NORCV | 26.2 | 37.7 | 57.9 | 68.5 |  |

**Table 3.** Percentage amino acid sequence identities (p-distance) of a CLUSTAL W alignment of ohlsrhavirus L proteins.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | OHLDV | RISV | TCHV-1 | CRLV | NORCV | LOBV |
| OHLDV |  |  |  |  |  |  |
| RISV | 68.0 |  |  |  |  |  |
| TCHV-1 | 64.3 | 67.3 |  |  |  |  |
| CRLV | 63.4 | 66.3 | 89.9 |  |  |  |
| NORCV | 62.5 | 65.9 | 87.8 | 90.8 |  |  |
| LOBV | 59.2 | 58.5 | 57.0 | 56.9 | 56.5 |  |