

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

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| **Code assigned:** | **2020.007M** |  |
| **Short title:**  Create three new species in the genus *Ohlsrhavirus* (*Mononegavirales*: *Rhabdoviridae*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| *Rhabdoviridae* Study Group |

**ICTV study group comments and response of proposer**

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| Approved by all responding SG members (10 of 14) with minor revisions. |

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

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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 | 26 July 2020 |
| 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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| 2020.007M.R.Ohlsrhavirus\_3nsp |

**Abstract**

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| Three new species are proposed for classification in the genus *Ohlsrhavirus*. Like other members of the genus, Culex pseudovishnui rhabdo-like virus, Culex rhabdo-like virus Los Angeles and Lobeira virus were each detected in culicine mosquitoes. Based on L protein amino acid sequences, each virus clusters phylogenetically with members of the genus *Ohlsrhavirus* and meets demarcation criteria for assignment to a new species in the genus. |

**Text of proposal**

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| |  | | --- | | Culex pseudovishnui rhabdo-like virus (CpRLV; strain 17NGK-Cps2-874) was detected in the virome of mosquitoes (*Culex pseudovishnui*) collected in 2017 in Nagasaki Prefecture, Japan [1]. The near-complete genome sequence (11,583 nt) has been determined, including complete coding sequences but incomplete 3' and 5' termini [1]. We propose to assign Culex pseudovishnui rhabdo-like virus to a new species *Pseudovishnui ohlsrhavirus* in the genus *Ohlsrhavirus*.  Culex rhabdo-like virus Los Angeles (CRLVLA) was detected in the virome of mosquitoes (*Culex* sp.) collected in 2016 in California, USA [4]. The near-complete genome sequence (11,301 nt) has been determined, including complete coding sequences but incomplete 3' and 5' termini [4]. We propose to assign Culex rhabdo-like virus Los Angeles to a new species *Angeles ohlsrhavirus* in the genus *Ohlsrhavirus*.  Lobeira virus (LOBV; strain BR/MT-M05) was first detected in culicine mosquitoes (*Psorophora albigenu*) collected in 2014 in Mato Grosso, Brazil [2, 3]. The genome sequence includes complete L, G and M genes, and near complete N and P genes [2, 3]. We propose to assign Lobeira virus to a new species *Lobeira ohlsrhavirus* in the genus *Ohlsrhavirus*.  **Genome organizations**  The genome organisations of CpRLV, CRLVLA and LOBV are similar in organisation to those of other sunrhaviruses, containing only the five canonical rhabdovirus structural protein genes (N, P, M, G and L) (**Figure 1**). Although the LOBV N and P gene sequences are incomplete, we note that North Creek virus (NORCV; species *Northcreek ohlsrhavirus*) has already recently been assigned to the genus *Ohlsrhavirus* with only partial P and M gene sequences.  A Clustal X amino acid sequence alignment of ohlsrhavirus G proteins indicates that, like Ohlsdorf virus (OHLDV; species *Ohlsdorf ohlsrhavirus*), Tongilchon virus 1 (TCHV-1; species *Tongilchon ohlsrhavirus*), riverside virus (RISV; species *Riverside ohlsrhavirus*) and Culex rhabdo-like virus (CRLV; species *Culex ohlsrhavirus*), CpRLV and CRLVLA contain the 12 conserved cysteine residues that form 6 disulphide bonds in vesicular stomatitis Indiana virus (VSIV) (**Figure 2**). Like NORCV, LOBV lacks the CVII-CVIII disulphide bond and contains a single unpaired cysteine residue. (**Figure 2**).  Based on ML trees generated from complete L protein sequences CpRLV, CRLVLA and LOBV cluster phylogenetically with members of the genus *Ohlsrhavirus* (**Figure 3**).  Pairwise sequence identities (p-distances) calculated in MEGA7 from Clustal W alignments (**Tables 1, 2 and 3**) indicate that CpRLV is also most closely related to CRLV with in N (19.0 % divergence) and G (42.7 % divergence), and most closely related to TCHV-1 in L (11.7 % divergence). CRLVLA is most closely related to TCHV-1 in N (39.9 % divergence) and to RISV in G (56.1% divergence) and L (33.0 % divergence). LOBV is most closely related to CRLVLA in G (71.0 % divergence) and to OHLDV in L (40.7% divergence).  **Ecology**  All five currently assigned ohlsrhaviruses were isolated from or detected in culicine mosquitoes (*Culex* spp. or *Ochlerotatus* spp.) from Europe, Asia and Australia. CpRLV, CRLVLA and LOBV have also been detected in culicine mosquitoes (*Culex* sp. or *Psorophora* sp.) - from Asia or the Americas.    **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; B) minimum amino acid sequence divergence of 10% in L; C) minimum amino acid sequence divergence of 15% in G; D) significant differences in genome organization 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 arthropod vectors.  CpRLV meets species demarcation criteria A, B, C and F.  CRLVLA meets species demarcation criteria A, B and C. Although detected in a geographically distant location from other ohlsrhaviruses, species of mosquito in which CRLVLA was detected has not been clearly delineated (criterion F).  LOBV meets criteria B, C and F. Sequence divergence estimated for the incomplete N protein sequence indicate that it would also meet criterion A.  Neutralisation tests (criterion E) have not been conducted using DDRV as no virus isolates are currently available. CpRLV, CRLVLA and LOBV have similar genome organisations to other ohlsrhaviruses (criterion D). | |

**Supporting evidence**

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**Figure 1.** Ohlsrhavirus genome organisations.

**Figure 2.** Clustal X amino acid sequence alignment of the ohlsrhavirus G proteins. Conserved cysteine residues in the VSIV G protein are marked (CI-CXII).

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**Figure 3.** The evolutionary history was inferred from a Clustal W alignment of 169 complete L protein sequences of 166 animal rhabdoviruses currently assigned or recently proposed for assignment to species in other genera and three rhabdoviruses which we propose to be assigned to a new species in the genus *Ohlsrhavirus*. Phylogenetically informative sites were selected from the alignment using Gblocks resulting in 915 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 (-127314.573) 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.

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

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | NORCV | CRLV | TCHV | CpRLV | CRLVLA | RISV | OHLDV |
| NORCV |  |  |  |  |  |  |  |
| CRLV | 88.0 | ! |  |  |  |  |  |
| TCHV | 77.2 | 79.6 |  |  |  |  |  |
| CpRLV | 78.4 | 81.0 | 78.2 |  |  |  |  |
| CRLVLA | 57.0 | 58.5 | 60.1 | 58.2 |  |  |  |
| RISV | 51.9 | 53.1 | 56.6 | 50.0 | 52.1 | ###### |  |
| OHLDV | 50.2 | 52.3 | 54.5 | 52.8 | 53.8 | 49.8 |  |

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

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | NORCV | CRLV | TCHV | CpRLV | CRLVLA | RISV | OHLDV | LOBV |
| NORCV |  |  |  |  |  |  |  |  |
| CLRLV | 69.0 |  |  |  |  |  |  |  |
| TCHV | 58.6 | 58.1 |  |  |  |  |  |  |
| CpRLV | 55.1 | 57.3 | 55.3 |  |  |  |  |  |
| CRLVLA | 39.2 | 41.4 | 37.6 | 39.2 |  |  |  |  |
| RISV | 37.4 | 40.8 | 37.4 | 38.2 | 43.9 |  |  |  |
| OHLDV | 26.2 | 29.6 | 26.8 | 28.6 | 28.4 | 28.4 |  |  |
| LOBV | 25.6 | 27.0 | 27.4 | 28.0 | 29.0 | 27.6 | 26.8 |  |

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

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| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | NORCV | CRLV | TCHV | CpRLV | CRLVLA | RISV | OHLDV | LOBV |
| NORCV |  |  |  |  |  |  |  |  |
| CLRLV | 90.8 |  |  |  |  |  |  |  |
| TCHV | 87.8 | 90.0 |  |  |  |  |  |  |
| CpRLV | 85.5 | 87.3 | 88.3 |  |  |  |  |  |
| CRLVLA | 65.6 | 66.4 | 66.3 | 66.8 |  |  |  |  |
| RISV | 65.9 | 66.3 | 67.3 | 67.5 | 67.0 |  |  |  |
| OHLDV | 62.5 | 63.4 | 64.3 | 63.6 | 62.5 | 68.0 |  |  |
| LOBV | 56.6 | 57.0 | 57.0 | 57.4 | 57.8 | 58.5 | 59.3 |  |

**References**

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