

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

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| **Code assigned:** | **2020.015M** |  |
| **Short title:**  Create one new genus (*Merhavirus*) including two new species (M*ononegavirales*: *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 (11 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 | 2 August 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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| 2020.001M\_014M\_015M\_016M.R.Rhabdoviridae.xlxs |

**Abstract**

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| The new genus *Merhavirus* is proposed to accommodate four currently unassigned rhabdoviruses that have been detected in culicine mosquitoes: Merida virus (MERDV), Merida-like virus Turkey (MLVT), Culex rhabdovirus (CXRV) and Culex tritaeniorhynchus rhabdovirus (CTRV). Based on high levels of amino acid sequence identity, MLVT and CXRV are considered to be variants of Merida virus. The viruses will be assigned to two new species (*Merida merhavirus* and *Tritaeniorhynchus merhavirus*) in the new genus. The new genus would be assigned to the proposed new subfamily *Alpharhabdovirinae*. |

**Text of proposal**

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| |  | | --- | | Merida virus (MERDV; strain Mex07) was detected in culicine mosquitoes (*Culex quinquefasciatus*) collected in Yucatan Peninsula, Mexico, in 2007 [1]. The complete genome sequence (11,798 nt) of the virus has been determined [1]. The virus has also been detected in culicine mosquitoes of other species (*Ochlerotatus taeniorhynchus* and *Ochlerotatus trivittatus*) [1]. We propose to assign Merida virus to the new species *Merida merhavirus*.  Merida-like virus Turkey (MLVT; strain 139-1-21) was detected in culicine mosquitoes (*Culex pipiens*) collected in Thrace, Turkey, in 2015 [3, 5]. The complete genome sequence (11,767 nt) has been determined [3]. Due to high levels of amino acid sequence identity (**Tables 1-3**) Merida-like virus Turkey is considered to be a variant of Merida virus.  Culex rhabdovirus (CXRV; strain Kern) was detected in a mosquito (*Culex* sp.) collected in California, USA, in 2016 [6]. The near-complete genome sequence (11,824 nt) has been determined, including complete coding sequences but incomplete 3' and 5' termini [6]. Due to high levels of amino acid sequence identity (**Tables 1-3**) Culex rhabdovirus is considered to be a variant of Merida virus.  Culex tritaeniorhynchus rhabdovirus (CTRV; strain TY) was isolated from culicine mosquitoes (*Culex* *tritaeniorhynchus*) collected in Chiba, Japan [4]. The complete genome sequence (11,308 nt) has been determined [4]. We propose to assign Culex tritaenioryhynchus rhabdovirus to the news species *Tritaeniorhynchus merhavirus*.  **Other related viruses**  Mopeia rhabdovirus (MOPRV; strain MZ2014-Mr) was detected in culicine mosquitoes (*Mansonia* sp.) collected in Mozambique in 2014 [2]. Only partial L gene sequence (2587 nt) is currently available and so it cannot be classified taxonomically at this time.  **Genome organization and expression**  Merhavirus genomes contain the five canonical rhabdovirus structural protein genes (N, P, M, G and L) (**Figure 1**). Uniquely in CTRV, the L gene contains a 76 nt intron, resulting in two consecutive open reading frames (L1 and L2) [4]. The full-length L protein is expressed by RNA editing [4].  A Clustal X alignment indicates that merhavirus G proteins are similar in structure and length, and share identifiable sequence identity, with each containing all 12 conserved cysteine residues that in vesicular stomatitis Indiana virus (VSIV) form six disulphide bonds in the folded protein (**Figure 2**).  Based on ML trees generated from complete L protein sequences, merhaviruses form a well-supported monophyletic clade that is distinct from all currently assigned genera and other currently unassigned rhabdoviruses (**Figure 3**). Amino acid sequence divergence between MERDV and CTRV in pair-wise alignments (p-distances) are 72.1% in the N proteins, 61.6% in the G proteins and 56.6% in the L protein (**Tables 1-3**).  **Species demarcation criteria**  Viruses assigned to different species within the genus *Merhavirus* 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.  The proposed members of the new genus meet demarcation criteria A, B, C and D.  **Derivation of the genus name**  *Merhavirus* is derived from Merida virus and rhabdovirus.  **Type species**  *Merida merhavirus* is designated as the type species of the genus as Merida virus was the first of proposed members of the genus to have been reported. | |

**Supporting evidence**

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**Figure 1.** Merhavirus genome organisations. Each genome contains long open reading frames (ORFs) in the N, P, M, G genes (open arrows). In MERDV (as well as the MLVT and CXRV variants of MERDV), the L gene also contains a single long ORF. In CTRV, the L gene contains two long ORFs separated by a 76 nt intron.

**Signal domain CI**

MERDV\_G MLRMMLTILGICSFVEGLPVTHHGRLYIGPTGVVTPWKSISPESVDCKASVPRLGLELTP

CXRV\_G MLRMMLTILGICSFVEGLPVTHHGRLYIGPTGVVTPWKSISPESVDCKASVPRLGLELTP

MLVT\_G MLRIMLTIVGICSSVGGLPLTHHGRLYIGPTGVVTPWKSISPESVDCKASVPRLGLELTP

CTRV\_G MISLLIQLLALIATCSA-----DLQVYIGPERVTQPWRILSPGRIDCSAIGTGLGLESLE

VSIV\_G M-KCLLYLAFLFIGVNC------KFTIVFPHNQKGNWKNVPSNYHYCPSSSD-LNWHNDL

\* :: : : : \* \*: :.. \* : \*. .

**CII CIII CIV**

MERDV\_G EHKFVETKRWSPHVTAPTSGYTCSLIQKRTTCSRSFFGYDGIKKETKISLPSAQACREAF

CXRV\_G EHKFVETKRWSPHVTAPTSGYTCSLIQKRTTCSRSFFGYDGIKKETKISLPSAQASREAF

MLVT\_G EHKFVETKRWSPHVTAPTSGYTCSLIQKRTTCSRSFFGYDGIKKETKISLPSAQACREAF

CTRV\_G GAREFNVSRWTPHMVGSVTGYTCSKIIRRTRCSRSFFGYDSVTKESQISLPPASACADAY

VSIV\_G IGTALQVKMPKSHKAIQADGWMCHASKWVTTCDFRWYGPKYITHSIRSFTPSVEQCKESI

.:.. ..\* . . \*: \* \* \*. ::\* . :.:. : \*... . ::

**CV CVI**

MERDV\_G QRFRENHLEEIEHPYPTCHWLGDDTAEAKGLKISLVPVEYNPFSGKYTDHILAGGVCDSV

CXRV\_G QRFRENHLEEIEHPYPTCHWLGDDTAEAKGLKISSSAIEYSPFSGKYTDHILAGGVCDSV

MLVT\_G QKFRENHLEEIEHPYPTCHWLGDDTAEAKGLKISLVPVEYNPFSGKYTDHILAGGVCDSV

CTRV\_G QRYREHVNTDVEHPFPECRWLGDTVAESEAVEITISPVSFDPASGSYRDHLLAGDTCVKA

VSIV\_G EQTKQGTWLNPGFPPQSCGYATVTDAEAVIVQVTPHHVLVDEYTGEWVDSQFIDGKCSND

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**CVII CVIII**

MERDV\_G PCMVADRSGYWFNSSEPVGECFPDPA---IKIFFVD-RNDTLTPK-TEFVSLSLQARSFK

CXRV\_G PCMVADRSGYWFNSSEPVGECFPDPA---IKIFFVD-RNDTLTPK-TEFVSLSLQARSFK

MLVT\_G PCMVADRSGYWFNSSAPIGECFPDPS---IKIFFVD-RNDTLTTK-TEFVSLSLQARSFK

CTRV\_G PCLMANRKGYWVNTSDPESLCIEPEV---IGLFIKGSTNGSVLMD-KTFTSLSLAATSFR

VSIV\_G ICPTVHNSTTWHSDYKVKGLCDSNLISMDITFFSEDGELSSLGKEGTGFRSNYFAYETGD

\* .... \* . . \* \* :\* . .:: . . \* \* : :

**CIX CX CXI**

MERDV\_G GACKAKYCGLDGFLLNTNEWIENSRSFSNRYSSTSAIRDCYNMSKSYAYLTTQAIIGQVL

CXRV\_G GACKAKYCGLDGFLLNTNEWIENSRSFSNRYSSTSAIRDCYNMSKSXAYLTTQAIIGQVL

MLVT\_G GACKAKYCGLDGFLLNTNEWIENSRSFSNRFSSASAIRDCYNMSKSYAYLTTQAIIGQVL

CTRV\_G GGCLKSYCGKPGILLNTREWISGEPSLLAKLPGLAMLPSCSPGTAGYSSVSSGRVLSHVL

VSIV\_G KACKMQYCKHWGVRLPSGVWFEMAD---KDLFAAARFPECPEGSS-ISAPSQTSVDVSLI

.\* .\*\* \*. \* : \*:. . : : .\* : : : : ::

**CXII**

MERDV\_G GSSQSDALLAECRKVKDKLMLGEPISRTDLQLFSPESEGRGPVYRFFNGSLQVATAKYES

CXRV\_G GSSQSDALLAECRKVKDKLMLGEPISRTDLQLFSPESEGRGPVYRFFNGSLQVATAKYES

MLVT\_G GSSQSDALLAECRKVKDKLMLGEPISRTDLQLFSPESEGRGPVYRFFNGSLQVATAKYES

CTRV\_G HAYAVSSELKECSRIRTKLMLNETVSRSDIYHLSPRVIGIGPVYRYNKDHWESSIAKYIP

VSIV\_G QDVERILDYSLCQETWSKIRAGLPISPVDLSYLAPKNPGTGPAFTIINGTLKYFETRYIR

\* . \*: . .:\* \*: ::\*. \* \*\*.: :. : ::\*

MERDV\_G LVFPDDTAASLHGYSLGATVNTSTPVLWP--HAIRINRDIVDGPNGMFWFRGRLIHPRTW

CXRV\_G LVFPDDTAASLHGYSLGATVNTSTPVLWP--HAIRINRDIVDGPNGMFWFRGRLIHPRTW

MLVT\_G LVFPDDTAASLHGYSLGATVNTSTPVLWP--HAIRINRDIVDGPNGMFWFRGRLIHPRTW

CTRV\_G IRLPKTTDG-HHGNSLGLQGNTSMHVVWG--HPVAFSQDVVDGPNGIFWYRGELVVPGLY

VSIV\_G VEIAAPILSRMVGMISGTTTERELWDDWAPYEDVEIGPNGVLRTSSGYKFPLYMIGHGML

: :. . \* \* : . \* . : :. : \* ... : : ::

MERDV\_G EGRIQEVSQHLVQLYSLKFKTPGVPDIDQGNIVEPLSNWEWTSPAVIR------PVAHLT

CXRV\_G EGRIQEVSQHLVQLYSLKFKTPGVPDIDQGNIVEPLSNWEWTSPAVIR------PVAHLT

MLVT\_G EGRIQEVSQHLVQLYSLKFRTPGVPDIDQGNIVEPLSHWEWTSPAVIR------PVAHLT

CTRV\_G GGDISEITESLTKALTRSSKTSGAMAHLPDVLSRLGEETSWTSPEVIR------PIAHLS

VSIV\_G DSDLRLSSKAQVFEHP-HIQDAASQLPDDETLFFGDTGLSKNPIELVEGWFSGWKSSIAS

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**Transmembrane domain**

MERDV\_G LLESLALLTGGVLILTLCVKITVWRR-TKRPRLSAGVQPSWR--

CXRV\_G LLESLALLTGGVLILTLCVKITVWRR-TKRPRLSAGVQPSWR--

MLVT\_G LLESLALLAGGVVILILCVKITAWRR-AKQPNRFSGVQPSWR--

CTRV\_G VFWSVVLIIIGILTTWLIVTWLKSRGGDKKPSTKGFPMYQW---

VSIV\_G FFFIIGLIIGLFLVLRVSIYLCIKLKHTKKRQIYTDIEMNRLGK

.: : \*: .: : : \*: .

**Figure 2**. A Clustal X alignment of the VSIV G protein with the merhavirus 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 165 animal rhabdoviruses currently assigned or recently proposed for assignment to species in other genera and four viruses proposed to be assigned to the genus *Merhavirus* (as two species). 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 merhavirus N proteins.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | MERDV | CXRV | MLVT | CTRV |
| MERDV |  |  |  |  |
| CXRV | 100 |  |  |  |
| MLVT | 92.5 | 92.5 |  |  |
| CTRV | 27.9 | 27.9 | 26.9 |  |

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | MERDV | CXRV | MLVT | CTRV |
| MERDV |  |  |  |  |
| CXRV | 98.8 |  |  |  |
| MLVT | 95.4 | 94.2 |  |  |
| CTRV | 38.4 | 38.0 | 37.6 |  |

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

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | MERDV | CXRV | MLVT | CTRV |
| MERDV |  |  |  |  |
| CXRV | 98.6 |  |  |  |
| MLVT | 95.7 | 95.3 |  |  |
| CTRV | 43.4 | 43.1 | 43.6 |  |

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