

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

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| **Code assigned:** | **2020.005M** |  |
| **Short title:**  Create three new species in the genus *Ephemerovirus* (*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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| The proposal has been reviewed by the *Rhabdoviridae* Study Group and supported by a majority of members (11 supporters and 3 non-responders). |

**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 | 9 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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| 2020.005M.R.Ephemerovirus\_3nsp.xlsx |

**Abstract**

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| We propose to assign three viruses for which complete coding genome sequences are now available (Puchong virus, Hayes Yard virus and New Kent County virus) to three new species in the genus *Ephemerovirus*. The viruses cluster phylogenetically with the ephemeroviruses and have similar genome organisations to those of several other ephemeroviruses. The species demarcation criteria for ephemerovirus have been re-defined based on the observed genetic diversity of bovine ephemeral fever virus (BEFV; species *Bovine fever ephemerovirus*). The proposed new species meet the revised demarcation criteria. |

**Text of proposal**

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| |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | **Proposed new members of the genus**  Puchong virus (PUCV) was isolated from mosquitoes (*Mansonia uniformis*) collected in Selangor, Malaysia, in 1965 [6]. PUCV has been shown to cross-react strongly with bovine ephemeral fever virus (BEFV; species *Bovine fever ephemerovirus*), Berrimah virus (BRMV; species *Berrimah ephemerovirus*), Kimberley virus (KIMV; species *Kimberley ephemerovirus*) and several other ephemeroviruses in complement-fixation and/or indirect immunofluorescence tests [2, 6], and can be distinguished from other ephemeroviruses in serum neutralization tests [1]. The complete PUCV genome sequence (14,932 nt) has been determined [1]. We propose PUCV be assigned to the new species *Puchong ephemerovirus*.  Hayes Yard virus (HYV) was isolated in 2000 from a bull (*Bos indicus*) showing clinical signs of bovine ephemeral fever in the Northern Territory, Australia [1]. In thin sections of infected cells, HYV virions have the cone-shaped morphology that is typical of ephemeroviruses [1]. In serum neutralization tests, immune mouse ascitic fluid against PUCV has been shown to cross-react partially with HYV [1]. The complete HYV genome sequence (15,025 nt) has been determined [1]. We propose HYV be assigned to the new species *Hayes ephemerovirus*.  New Kent County virus (NKCV) was detected by NGS in hard ticks (*Ixodes scapularis*) collected in New Kent County, Virginia, in 2016 [9]. The near-complete NKCV genome sequence (14,814 nt) has been determined [9]. No isolate of NKCV is currently available. We propose NKCV be assigned to the new species *Kent ephemerovirus*.  **Other related viruses**  Mavingoni virus (MVGV) was detected by NGS in samples collected in 2017 from cattle with clinical signs similar to bovine ephemeral fever (‘cattle flu’) on Mayotte Island in the western Indian Ocean [3]. Only partial MVGV genome sequence (7,576 nt) has been determined [3]. Although the available sequence clearly suggests the virus should fall within the genus, the sequence lacks almost all of the L gene so it has been excluded at this time.  **Genome organizations**  The genomes of PUCV (14,932 nt) and HYV (15,025 nt) share the same genome organization as BEFV (**Figure 1**). Each contains the five canonical rhabdovirus structural protein genes (*N*, *P*, *M*, *G* and *L*) as well a long, complex region between the G gene and L gene containing ORFs encoding a non-structural glycoprotein (GNS), a viroporin (1) and three other proteins (2,  and ) of unknown function. Each has sequence homology with the cognate proteins of BEFV and other ephemeroviruses. The near complete NKCV genome sequence (14,814 nt) has the same genome features as above but includes an additional gene (**) located downstream of the ** gene, encoding a protein of unknown function. The  gene also occurs in two other ephemeroviruses, kotonkan virus (KOTV; species *Kotonkan ephemerovirus*) and Koolpinyah virus (KOOLV; species *Koolpinyah ephemerovirus*). The NKCV, KOTV and KOOLV  proteins share obvious amino acid sequences homology (**Figure** **2**).  **Phylogenetic analysis**  Based on ML trees generated from complete L protein sequences, PUCV, HYV and NKCV cluster with the ephemeroviruses in a distinct and well-supported monophyletic clade (**Figure 3**) (3). By this analysis, PUCV and HYV are most closely related to BEFV and BRMV. NKCV falls in a sub-clade that also includes KOOLV, KOTV and Yata virus (YATV; species *Yata ephemerovirus*).  **Amino acid sequence identities**  Pairwise sequence identities (p-distances) calculated in MEGA7 from ClustalW amino acid sequence alignments indicated that PUCV and HYV are closely related viruses, sharing 88.0% identity in L, 95.4% identity in N and 80.2% identity G (**Tables 1-3**) and confirmed that each is most closely related to BRMV and BEFV. Similarly, NKCV is most closely related to KOOLV and KOTV with amino acid sequence identities of 65.5-66.3% in L, 72.4% in N and 63.3-65.5% in G.  **Species demarcation criteria**  According to current criteria, viruses assigned to different species within the genus *Ephemerovirus* have several of the following characteristics: A) minimum amino acid sequence divergence of 15% in the L protein; B) minimum amino acid sequence divergence of 8% in the N protein; C) can be distinguished in serological tests; and D) significant differences in genome organization as evidenced by numbers and locations of ORFs.  The genetic distance criteria used here for L and N are based on the observed amino acid sequence divergence between BEFV and BRMV, the two most closely related ephemeroviruses that have been assigned to distinct species (see **Tables 2 and 3**). As PUCV and HYV are even more closely related than BEFV and BRMV, the genetic distance demarcation criteria were reviewed to determine if less stringent criteria should be used.  Sequences are available for more than BEFV 230 isolates from a vast geographic range extending from South Africa, Egypt, Israel, Turkey, Mayotte, India, Thailand, Australia, Taiwan, China and Japan. The time span covers some 61 years (1956-2017). Most sequences are of the G ORF and most of these are of complete G ectodomains. For more limited sets of viruses, sequences are available for complete L and N ORFs.  How do we define BEFV as a discrete virus? Based on G protein ectodomain sequences (and other more limited data sets for other genes/proteins), BEFV exists as a single clade with several sub-clades representative of the geographic distribution of isolates [8, 10]. BEFV isolates from different geographic locations and times of isolation strongly cross-neutralise. Vaccines from Australia, Japan and South Africa have been used successfully in distant locations [5, 7]. BEFV infects only bovines and causes a well characterised disease. BEFV has never been detected in apparently healthy bovines, despite intensive sampling of sentinel herds over many years that has yielded isolates of other ephemeroviruses from healthy cattle. As noted above, BRMV is the most closely related ephemerovirus to BEFV. BRMV was isolated from a healthy bovine and does not appear to cause disease [4]. Other ephemeroviruses cause disease rarely or not at all.  To determine the extent of diversity of BEFV isolates, pairwise identities (p-distances) were calculated in MEGA7 from ClustalW alignments of all available nucleotide and amino acid sequences of the complete G ORF (82 isolates) and the G ectodomain (233 isolates). Pairwise identities (p-distances) were also calculated for the smaller data sets of available sequences of the L ORF (11 isolates) and N ORF (12 isolates). Calculated p-distances are shown in **Appendix A**. For illustrative purposes, only representative sequences including those which were found to be most divergent are shown for the complete G and G ectodomain sequences.  The analysis indicated maximum divergence in the complete G coding sequence of 15.9% at the nucleotide level and 8.7% at the amino acid level. Similarly, maximum divergence for the larger data set of G ectodomain sequences was15.8% at the nucleotide level and 9.0% at the amino acid level. For the complete L coding sequences, maximum divergence was 16% at the nucleotide level and 6.1% at the amino acid level; for the complete N coding sequence, maximum diversity was 11.9% at the nucleotide level and 2.3% at the amino acid level. Although the L and N data sets were significantly more limited in size, they did include isolates from Australia and South Africa which were at or near the limits of divergence in the G and G ectodomain coding regions and so appear to be useful indicators.  On this basis, we propose to reset the sequence identity element of the species demarcation criteria for ephemeroviruses. Rather than reflecting the distance between viruses assigned to the two most closely related existing species (BEFV and BRMV), the new criteria will sit just beyond maximum diversity displayed by BEFV across its vast geographic range and over a time interval spanning more than 50 years.   |  |  |  | | --- | --- | --- | | Coding sequence | % divergence | | | nucleotide | amino acid | | G | 18% | 12% | | L | 18% | 8% | | N | 15% | 4% |   As some viruses assigned to different species are very divergent in nucleotide sequences, meaningful alignments are difficult to obtain, particularly for G gene sequences. Species demarcation will therefore use only % divergence (p-distance) estimations for amino acid sequences. Other species demarcation criteria pertaining to differences in genome organization and neutralization phenotype will remain unchanged. The revised demarcation criteria are as follows:  Viruses assigned to different species within the genus *Ephemerovirus* have several of the following characteristics: A) minimum amino acid sequence divergence of 12% in the G protein; B) minimum amino acid sequence divergence of 8% in the L protein; C) minimum amino acid sequence divergence of 4% in the N protein; D) can be distinguished in virus neutralization tests; and E) exhibit significant differences in genome organization as evidenced by numbers and locations of ORFs.  HYV, PUCV and NKCV meet sequence divergence criteria A, B and C. In virus neutralization tests, PUCV can be distinguished from other ephemeroviruses but PUCV immune ascites fluid does cross-react partially with HYV. As no isolate of NKCV is available, the neutralization phenotype is unknown. PUCV and HYV share the same genome organization as BEFV, BRMV and KIMV. The NKCV genome organization is similar to that of KOOLV and KOTV. | |

**Supporting evidence**

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**Figure 1.** Schematic representation of ephemerovirus genomes. N, P, M, G and L represent ORFs encoding the structural proteins. The GNS (aqua), α1 (yellow), α2 orange), β (blue), γ (green) and δ (purple) ORFs are highlighted. The GNS ORF encodes a non-structural class I transmembrane glycoprotein; the α1 ORF encodes a class 1a viroporin; other ORFs in the region between the G ORF and L ORF encode proteins of unknown function. Alternative ORFs (shaded grey) of significant length (>180 nucleotides) alsooccur in some genes but the significance of these is unknown.

KOTV\_delta MALIKIEGEVGTNEVRTELVITVIKEVNSIIMDILQCIGIPFQPDKMLTRRDIKMELDPE

KOOLV\_delta MALIMVEGEVGSSEASTGLVVTLVKEVNSLIMDILQCLDIPFEPGQMISRKDTTMEVDFE

NKCV\_delta MLSIQVEGEIGFMMWENHYLKWVIIQTNRAIADLFSLLDIGVNASNLVGPRDIQMMSDKE

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KOTV\_delta DHAVCFIRIEKSWETECDRVGKLSFLENLGGRGNFSYSGNMKLEISPKL

KOOLV\_delta DRSVCFIKIKKEWEVESIKVGTLSFIEELRGKGYFSYNGSMKLDILPKP

NKCV\_delta DHQVRFLSIRKQLYIETDQTIKYETTENLILPKEIRGSGSIKIIIK---

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

**Figure 2.** Clustal X alignment of the amino acid sequences of KOTV, KOOLV and NKCV delta () proteins. Grey shading indicates residues conserved between NKCV and either KOTV, KOOLV or both.

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**Figure 3.** The evolutionary history was inferred from a ClustalW alignment of complete L protein sequences of 160 animal rhabdoviruses currently assigned to species (or proposed to be assigned in other proposals) as well as the proposed ephemeroviruses (Hayes Yard virus, Puchong virus and New Kent County virus). Phylogenetically informative sites were selected from the alignment using Gblocks resulting in 974 positions in the final dataset. The tree was inferred in MEGA by using the Maximum Likelihood method based on the Whelan and Goldman + Freq. model. The tree with the highest log likelihood (-1118928.31) 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 identities (p-distance) of a CLUSTAL W alignment of ephemerovirus G protein sequences.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Viruses | BEFV | BRMV | KIMV | HYV | PUCV | ARV | OBOV | NKCV | KOTV | KOOLV | YATV |
| BEFV |  |  |  |  |  |  |  |  |  |  |  |
| BRMV | 78.5 |  |  |  |  |  |  |  |  |  |  |
| KIMV | 49.8 | 49.1 |  |  |  |  |  |  |  |  |  |
| HYV | 49.8 | 49.6 | 49.1 |  |  |  |  |  |  |  |  |
| PUCV | 50.5 | 50.2 | 47.7 | 80.2 |  |  |  |  |  |  |  |
| ARV | 31.0 | 31.1 | 28.6 | 29.5 | 29.9 |  |  |  |  |  |  |
| OBOV | 30.8 | 31.0 | 29.4 | 30.2 | 30.2 | 73.3 |  |  |  |  |  |
| NKCV | 28.8 | 29.0 | 31.3 | 29.7 | 29.0 | 28.6 | 29.7 |  |  |  |  |
| KOTV | 30.6 | 30.1 | 31.5 | 28.6 | 29.5 | 27.8 | 27.2 | 63.5 |  |  |  |
| KOOLV | 31.5 | 29.9 | 32.0 | 28.6 | 29.4 | 27.8 | 28.3 | 65.3 | 82.9 |  |  |
| YATV | 30.1 | 30.2 | 30.8 | 30.6 | 30.1 | 29.2 | 30.6 | 41.8 | 40.9 | 39.9 |  |

**Table 2.** Percentage amino acid identities (p-distance) of a CLUSTAL W alignment of ephemerovirus L protein sequences.

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Viruses | BEFV | BRMV | KIMV | HYV | PUCV | ARV | OBOV | NKCV | KOTV | KOOLV | YATV |
| BEFV |  |  |  |  |  |  |  |  |  |  |  |
| BRMV | 85.5 |  |  |  |  |  |  |  |  |  |  |
| KIMV | 66.0 | 66.4 |  |  |  |  |  |  |  |  |  |
| HYV | 64.9 | 65.4 | 64.8 |  |  |  |  |  |  |  |  |
| PUCV | 64.9 | 64.8 | 64.6 | 88.0 | ##### |  |  |  |  |  |  |
| ARV | 50.5 | 50.0 | 49.4 | 48.4 | 48.4 |  |  |  |  |  |  |
| OBOV | 50.8 | 50.1 | 50.6 | 49.1 | 49.4 | 80.0 | ##### |  |  |  |  |
| NKCV | 51.7 | 51.9 | 51.6 | 51.0 | 50.4 | 46.8 | 47.4 |  |  |  |  |
| KOTV | 51.8 | 52.8 | 51.4 | 51.0 | 50.9 | 48.6 | 48.8 | 65.5 |  |  |  |
| KOOLV | 52.0 | 52.0 | 51.7 | 50.8 | 50.7 | 48.7 | 49.2 | 66.3 | 85.5 | #VLUE! |  |
| YATV | 51.3 | 51.9 | 51.6 | 51.5 | 51.2 | 47.9 | 47.6 | 60.1 | 60.2 | 60.2 |  |

**Table 3.** Percentage amino acid identities (p-distance) of a CLUSTAL W alignment of ephemerovirus N protein sequences.

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Viruses | BEFV | BRMV | KIMV | HYV | PUCV | ARV | OBOV | NKCV | KOTV | KOOLV | YATV |
| BEFV |  |  |  |  |  |  |  |  |  |  |  |
| BRMV | 91.8 |  |  |  |  |  |  |  |  |  |  |
| KIMV | 78.7 | 78.4 |  |  |  |  |  |  |  |  |  |
| HYV | 76.7 | 78.2 | 75.1 |  |  |  |  |  |  |  |  |
| PUCV | 77.0 | 78.7 | 74.8 | 95.4 |  |  |  |  |  |  |  |
| ARV | 49.4 | 49.6 | 50.8 | 50.8 | 50.6 |  |  |  |  |  |  |
| OBOV | 50.6 | 50.1 | 52.0 | 51.8 | 51.6 | 87.1 |  |  |  |  |  |
| NKCV | 50.8 | 51.3 | 53.5 | 54.0 | 54.4 | 47.7 | 46.5 |  |  |  |  |
| KOTV | 51.3 | 51.6 | 53.0 | 52.3 | 53.0 | 48.4 | 46.8 | 72.4 |  |  |  |
| KOOLV | 51.8 | 51.6 | 52.5 | 51.8 | 52.5 | 47.7 | 47.0 | 72.4 | 92.6 |  |  |
| YATV | 46.0 | 46.8 | 48.2 | 46.5 | 45.8 | 42.0 | 39.6 | 51.3 | 52.0 | 51.3 |  |

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