

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

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| **Code assigned:** | ***2022.008M*** |  |
| **Short title:** Create two new species in the genus *Ephemerovirus* (*Mononegavirales*: *Rhabdoviridae*) | | |
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**Author(s) and email address(es)**

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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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| --- |
| Approved. |

**ICTV Study Group votes on proposal**

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| --- | --- | --- | --- |
| **Study Group** | **Number of members** | | |
| **Votes support** | **Votes against** | **No vote** |
| *Rhabdoviridae* SG | 14 | 0 | 1 |

**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 |

|  |  |  |
| --- | --- | --- |
| **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 | 5 May 2022 |
| 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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| --- |
| 2022.008M.N.v1.Ephemerovirus\_2nsp.xlsx |

**Abstract**

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| Two new species in the genus *Ephemerovirus* are proposed to accommodate two newly discovered viruses detected in pigs in China. The viruses cluster phylogenetically with the ephemeroviruses and have similar genome organisations to those of several other ephemeroviruses. The proposed new species meet the established demarcation criteria. |

**Text of proposal**

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| --- | --- |
| |  | | --- | | **Proposed new members of the genus**  Porcine ephemerovirus 1 (PoEV-1) and porcine ephemerovirus 2 (PoEV-2) were detected by high throughput sequencing of archived tissue samples (kidney, spleen and lymph  node) collected in China from pigs suffering classical swine fever [1]. PoEV-1 (strain HeN10) was detected in samples collected in Henan Province in 1998 and PoEV-2 was detected in samples collected in Guangdong Province in 2018. No isolates are yet available for these viruses. We propose PoEV-1 be assigned to the new species *Ephemerovirus* *henan* and PoEV-2 be assigned to the new species *Ephemerovirus guangdong*.  **Genome organizations**  The near-complete genome sequences of PoEV-1 (14,536 nt) and PoEV-2 (14,0390 nt) are available, lacking only extreme 3' and 5' termini. They each have a similar genome organization to bovine ephemeral fever virus (BEFV; species *Ephemerovirus febris*) (**Figure 1**) [1]. Each contains the five canonical rhabdovirus structural protein genes (*N*, *P*, *M*, *G* and *L*) as well as 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 ) that appear to share low levels of sequence identity with the corresponding proteins of other ephemeroviruses.  **Phylogenetic analysis**  Based on ML trees generated from complete L protein sequences, PoEV-1 and PoEV-2 cluster with the ephemeroviruses in a distinct and well-supported monophyletic clade (**Figure 2**) [1]. By this analysis, PoEV-1 and PoEV-2 fall within a sub-clade that also includes Yata virus (YATV; species *Ephemerovirus yata*), New Kent County virus (NKCV; species *Ephemerovirus kent*), kotonkan virus (KOTV; species *Ephemerovirus kotonkan*) and Koolpinyah virus (KOOLV; species *Ephemerovirus koolpinyah*).  **Amino acid sequence identities**  Pairwise sequence identities (p-distances) calculated in MEGA7 from ClustalW amino acid sequence alignments indicated that PoEV-1 and PoEV-2 are closely related viruses, sharing 70.7% identity in L, 78.1% identity in N and 59.9% identity in G (**Tables 1-3**) and confirmed that each is more closely related to YATV, NKCV, KOTV and KOOLV than to other ephemeroviruses.  **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 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.  PoEV-1 and PoEV-2 meet criteria A, B and C. No neutralization test data are yet available as there are currently no isolates of these viruses. The genome organisations of PoEV-1 and PoEV-2 appear to be similar to those of BEFV and several other ephemeroviruses. | |

**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) also occur in some genes but the significance of these is unknown.

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**Figure 2.** The evolutionary history was inferred from a MUSCLE alignment of complete L protein sequences of 189 rhabdoviruses that are currently assigned to species in the subfamily *Alpharhabdovirinae* as well as the proposed ephemeroviruses (porcine ephemerovirus 1 virus and porcine ephemerovirus 2). Phylogenetically informative sites were selected from the alignment using Gblocks resulting in 962 positions in the final dataset. The tree was inferred in MEGA11 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 (-134176.319) 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 | **PoEV-1** | **PoEV-2** | YATV | NKCV | KOTV | KOOLV |
| BEFV |  |  |  |  |  |  |  |  |  |  |  |  |  |
| BRMV | 75.3 |  |  |  |  |  |  |  |  |  |  |  |  |
| KIMV | 47.7 | 47.3 |  |  |  |  |  |  |  |  |  |  |  |
| HYV | 49.4 | 47.9 | 46.0 |  |  |  |  |  |  |  |  |  |  |
| PUCV | 48.6 | 47.9 | 44.3 | 76.0 |  |  |  |  |  |  |  |  |  |
| ARV | 28.0 | 26.8 | 25.3 | 26.0 | 26.2 |  |  |  |  |  |  |  |  |
| OBOV | 27.7 | 27.2 | 28.3 | 27.2 | 26.7 | 68.6 |  |  |  |  |  |  |  |
| **PoEV-1** | 26.3 | 26.6 | 28.9 | 24.2 | 25.2 | 28.9 | 28.5 |  |  |  |  |  |  |
| **PoEV-2** | 26.6 | 27.9 | 26.1 | 23.7 | 26.1 | 28.8 | 28.1 | 59.9 |  |  |  |  |  |
| YATV | 29.5 | 29.3 | 30.1 | 28.8 | 29.0 | 28.8 | 30.3 | 33.7 | 35.2 |  |  |  |  |
| NKCV | 27.6 | 27.6 | 29.4 | 27.2 | 27.3 | 28.1 | 29.2 | 34.6 | 35.7 | 40.2 |  |  |  |
| KOTV | 28.8 | 28.4 | 30.0 | 26.8 | 28.0 | 27.8 | 28.0 | 36.8 | 37.4 | 39.1 | 59.0 |  |  |
| KOOLV | 28.7 | 27.9 | 30.1 | 25.8 | 26.9 | 27.4 | 28.3 | 35.9 | 38.6 | 38.5 | 61.4 | 80.0 |  |

**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 | **PoEV-1** | **PoEV-2** | YATV | NKCV | KOTV | KOOLV |
| BEFV |  |  |  |  |  |  |  |  |  |  |  |  |  |
| BRMV | 84.0 |  |  |  |  |  |  |  |  |  |  |  |  |
| KIMV | 64.8 | 65.0 |  |  |  |  |  |  |  |  |  |  |  |
| HYV | 63.6 | 64.2 | 63.6 |  |  |  |  |  |  |  |  |  |  |
| PUCV | 63.4 | 63.6 | 63.3 | 86.8 |  |  |  |  |  |  |  |  |  |
| ARV | 49.2 | 48.8 | 48.4 | 47.4 | 47.2 |  |  |  |  |  |  |  |  |
| OBOV | 49.7 | 49.0 | 49.5 | 48.0 | 48.1 | 78.1 |  |  |  |  |  |  |  |
| **PoEV-1** | 49.6 | 49.5 | 50.1 | 49.1 | 49.2 | 47.2 | 47.5 |  |  |  |  |  |  |
| **PoEV-2** | 49.2 | 49.4 | 50.3 | 48.6 | 48.6 | 47.9 | 47.4 | 70.7 |  |  |  |  |  |
| YATV | 50.4 | 51.0 | 51.1 | 50.9 | 50.7 | 47.4 | 46.9 | 56.2 | 55.6 |  |  |  |  |
| NKCV | 51.2 | 51.5 | 51.4 | 50.7 | 50.0 | 46.5 | 47.1 | 56.3 | 56.5 | 59.7 |  |  |  |
| KOTV | 50.5 | 51.7 | 50.3 | 50.2 | 49.8 | 47.5 | 47.9 | 57.1 | 56.7 | 59.1 | 64.9 |  |  |
| KOOLV | 51.0 | 51.1 | 50.7 | 50.0 | 49.6 | 47.8 | 48.4 | 56.5 | 56.9 | 59.1 | 65.7 | 84.2 |  |

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

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Viruses | BEFV | BRMV | KIMV | HYV | PUCV | ARV | OBOV | **PoEV-1** | **PoEV-2** | YATV | NKCV | KOTV | KOOLV |
| BEFV |  |  |  |  |  |  |  |  |  |  |  |  |  |
| BRMV | 91.6 |  |  |  |  |  |  |  |  |  |  |  |  |
| KIMV | 77.3 | 77.0 |  |  |  |  |  |  |  |  |  |  |  |
| HYV | 75.4 | 77.0 | 74.1 |  |  |  |  |  |  |  |  |  |  |
| PUCV | 75.6 | 77.5 | 73.6 | 95.1 |  |  |  |  |  |  |  |  |  |
| ARV | 48.7 | 49.2 | 50.1 | 50.1 | 49.9 |  |  |  |  |  |  |  |  |
| OBOV | 50.1 | 49.9 | 51.3 | 51.3 | 51.0 | 86.9 |  |  |  |  |  |  |  |
| **PoEV-1** | 46.6 | 47.5 | 49.4 | 49.4 | 50.8 | 44.0 | 43.3 |  |  |  |  |  |  |
| **PoEV-2** | 48.9 | 48.2 | 51.8 | 48.7 | 48.9 | 47.8 | 47.0 | 78.1 |  |  |  |  |  |
| YATV | 50.4 | 51.3 | 52.7 | 53.7 | 54.2 | 47.3 | 46.1 | 61.2 | 60.3 |  |  |  |  |
| NKCV | 45.8 | 46.2 | 48.1 | 46.7 | 46.0 | 41.8 | 39.5 | 50.5 | 49.1 | 51.4 |  |  |  |
| KOTV | 51.1 | 51.8 | 52.9 | 52.9 | 53.4 | 47.8 | 46.4 | 59.9 | 58.7 | 71.9 | 51.7 |  |  |
| KOOLV | 51.5 | 51.8 | 52.5 | 52.5 | 52.9 | 47.1 | 46.6 | 59.2 | 58.7 | 71.9 | 50.9 | 92.7 |  |

**References**

1. Wu Q, Yang Z, Lu Z, Mi S, Feng Y, He B, Zhu G, Gong W, Tu C (2022) Identification of two novel ephemeroviruses in pigs infected by classical swine fever virus. Infection Genetics and Evolution 100: e105273. doi.org/10.1016/j.meegid.2022.105273