

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

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| **Code assigned:** | ***2023.010M*** |  |
| **Short title:** Create one new species (*Ephemerovirus huanggang*) 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* SG |

**ICTV Study Group comments and response of proposer**

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| Minor typos. Corrected. |

**ICTV Study Group votes on proposal**

|  |  |  |  |
| --- | --- | --- | --- |
| **Study Group** | **Number of members** | | |
| **Votes support** | **Votes against** | **No vote** |
| ICTV *Rhabdoviridae* Study Group | 13 | 0 | 1 |

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

|  |  |
| --- | --- |
| **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)** |
| N/A | N/A | N/A |

**Submission dates**

|  |  |
| --- | --- |
| Date first submitted to SC Chair | June 23, 2023 |
| 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**

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| N/A |

**Part 3:** **TAXONOMIC PROPOSAL**

**Name of accompanying Excel module**

|  |
| --- |
| 2023.010M.N.v1.Ephemerovirus\_1nsp.xlsx |

**Abstract**

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| We propose the creation of one new species in the genus *Ephemerovirus* for Huanggang rhabd tick virus 2 which was detected by metagenomic sequencing of ticks collected from cattle in China. The virus clusters phylogenetically with the ephemeroviruses and has a similar genome organisation to those of several other ephemeroviruses. The proposed new species meets the established demarcation criteria. |

**Text of proposal**

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| --- | --- |
| |  | | --- | | **Proposed new member of the genus**  Huanggang rhabd tick virus 2 (HgRTV2; sample TIGMIC 1) was detected by metagenomic sequencing of hard ticks (*Rhipicephalus microplus*) collected from cattle in Hubei Province, China, in 2019. We propose HgRTV2 be assigned to the new species *Ephemerovirus* *huanggang.*  **Genome organization**  The near-complete genome sequence of HrRTV2 (15,825 nt) is available, lacking only extreme 3' and 5' termini. The genome organization is similar to bovine ephemeral fever virus (BEFV; species *Ephemerovirus febris*) and Kimberley virus (KIMV; species *Ephemerovirus kimberley*). It 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 ) that appear to share low levels of sequence identity with the corresponding proteins of other ephemeroviruses (**Figure 1**). Intergenic sequences are relatively long compared to those of closely related ephemeroviruses.  **Phylogenetic analysis**  Based on ML trees generated from complete L protein sequences, HgRTV2 clusters with the ephemeroviruses in a distinct and well-supported monophyletic clade (**Figure 2**) [1]. By this analysis, it falls within a sub-clade that also includes BEFV, Berrimah virus (BRMV; species *Ephemerovirus berrimah*) and KIMV, and is most closely related to KIMV.  **Amino acid sequence identities**  Pairwise sequence identities (p-distances) calculated in MEGAX from ClustalW amino acid sequence alignments indicated that HgRTV2 is most closely related to KIMV, sharing 86.4% identity in L, 93.5% identity in N and 75.1% identity G (**Tables 1-3**).  **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.  HgRTV2 meets criteria A, B and C. No neutralization test data are yet available as there is currently no isolate of the virus. The genome organisation of HgRTV2 is similar to that of KIMV but differs in the intergenic regions which are relatively more extended. | |

**Supporting evidence**

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**Figure 1.** Schematic representation of (-) ssRNA ephemerovirus genomes shown in reverse polarity. 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 MAFFT alignment of complete L protein sequences of 207 rhabdoviruses that are currently assigned or are proposed to be assigned to species in the subfamily *Alpharhabdovirinae* as well as Huanggang rhabd tick virus 2. Phylogenetically informative sites were selected from the alignment using Trim Al resulting in 1649 positions in the final dataset. The tree was inferred in MEGAX 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 (-370622.07) 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.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | BEFV | BRMV | HYV | PUCV | KIMV | **HgRTV2** | ARV | OBOV | PoEV1 | PoEV2 | YATV | NKCV | KOTV | KOOLV |
| BEFV |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| BRMV | 75.3 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| HYV | 49.4 | 47.9 |  |  |  |  |  |  |  |  |  |  |  |  |
| PUCV | 48.6 | 47.9 | 76.0 |  |  |  |  |  |  |  |  |  |  |  |
| KIMV | 47.7 | 47.4 | 46.1 | 44.1 |  |  |  |  |  |  |  |  |  |  |
| **HgRTV2** | 47.2 | 48.2 | 45.6 | 44.9 | 75.1 |  |  |  |  |  |  |  |  |  |
| ARV | 28.1 | 27.1 | 26.2 | 26.3 | 25.2 | 26.1 |  |  |  |  |  |  |  |  |
| OBOV | 27.8 | 27.3 | 27.1 | 26.6 | 27.7 | 26.6 | 68.6 |  |  |  |  |  |  |  |
| PoEV1 | 26.1 | 26.5 | 24.6 | 25.5 | 28.4 | 26.8 | 28.9 | 28.5 |  |  |  |  |  |  |
| PoEV2 | 26.3 | 27.4 | 23.7 | 25.8 | 26.2 | 26.7 | 28.8 | 28.1 | 59.9 |  |  |  |  |  |
| YATV | 29.6 | 29.1 | 28.7 | 28.9 | 30.1 | 28.9 | 28.8 | 30.3 | 33.7 | 35.2 |  |  |  |  |
| NKCV | 27.6 | 27.5 | 26.9 | 26.9 | 29.6 | 29.6 | 28.1 | 29.2 | 34.6 | 35.7 | 40.2 |  |  |  |
| KOTV | 28.6 | 28.4 | 26.3 | 27.3 | 29.8 | 30.4 | 27.8 | 28.0 | 36.8 | 37.4 | 39.1 | 59.0 |  |  |
| KOOLV | 28.9 | 27.3 | 25.3 | 26.1 | 29.5 | 29.7 | 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.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | BEFV | BRMV | HYV | PUCV | KIMV | **HgRTV2** | ARV | OBOV | PoEV1 | PoEV2 | YATV | NKCV | KOTV | KOOLV |
| BEFV | 1000 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| BRMV | 84.0 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| HYV | 63.7 | 64.2 | 100.0 |  |  |  |  |  |  |  |  |  |  |  |
| PUCV | 63.4 | 63.7 | 86.8 |  |  |  |  |  |  |  |  |  |  |  |
| KIMV | 64.6 | 64.9 | 63.6 | 63.3 | 100. |  |  |  |  |  |  |  |  |  |
| **HgRTV2** | 65.1 | 65.4 | 63.2 | 62.8 | 86.4 |  |  |  |  |  |  |  |  |  |
| ARV | 49.2 | 48.8 | 47.4 | 47.2 | 48.4 | 48.0 |  |  |  |  |  |  |  |  |
| OBOV | 49.7 | 49.0 | 48.0 | 48.1 | 49.6 | 49.4 | 78.1 | 100.0 |  |  |  |  |  |  |
| PoEV1 | 49.5 | 49.3 | 48.9 | 49.0 | 49.9 | 49.8 | 47.2 | 47.3 |  |  |  |  |  |  |
| PoEV2 | 49.0 | 49.2 | 48.6 | 48.5 | 50.1 | 49.8 | 47.8 | 47.4 | 70.7 | 100.0 |  |  |  |  |
| YATV | 50.2 | 50.8 | 50.8 | 50.6 | 50.9 | 50.9 | 47.3 | 46.9 | 56.2 | 55.6 |  |  |  |  |
| NKCV | 51.1 | 51.4 | 50.6 | 50.0 | 51.3 | 51.1 | 46.4 | 47.0 | 56.3 | 56.5 | 59.7 | 100.0 |  |  |
| KOTV | 50.5 | 51.4 | 50.2 | 49.7 | 50.3 | 51.1 | 47.7 | 48.0 | 57.1 | 56.7 | 59.1 | 64.9 |  |  |
| KOOLV | 50.8 | 50.9 | 49.9 | 49.5 | 50.7 | 51.1 | 47.8 | 48.3 | 56.5 | 56.9 | 59.1 | 65.7 | 84.2 | 100.0 |

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

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | BEFV | BRMV | HYV | PUCV | KIMV | **HgRTV2** | ARV | OBOV | PoEV1 | PoEV2 | YATV | NKCV | KOTV | KOOLV |
| BEFV | 10.0 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| BRMV | 91.6 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| HYV | 75.9 | 76.8 | 10.0 |  |  |  |  |  |  |  |  |  |  |  |
| PUCV | 75.9 | 77.3 | 95.1 |  |  |  |  |  |  |  |  |  |  |  |
| KIMV | 76.1 | 77.0 | 74.8 | 74.1 |  |  |  |  |  |  |  |  |  |  |
| **HgRTV2** | 75.9 | 77.0 | 75.7 | 75.2 | 93.5 |  |  |  |  |  |  |  |  |  |
| ARV | 47.9 | 48.6 | 50.3 | 50.3 | 50.3 | 50.6 |  |  |  |  |  |  |  |  |
| OBOV | 49.5 | 49.3 | 51.7 | 51.5 | 51.0 | 50.6 | 86.9 |  |  |  |  |  |  |  |
| PoEV1 | 46.9 | 48.1 | 49.6 | 50.6 | 49.2 | 49.9 | 43.7 | 43.3 |  |  |  |  |  |  |
| PoEV2 | 49.5 | 48.8 | 48.9 | 48.7 | 51.5 | 51.1 | 47.5 | 47.0 | 78.1 | 100.0 |  |  |  |  |
| YATV | 45.4 | 45.9 | 45.8 | 45.5 | 47.2 | 47.9 | 41.0 | 38.4 | 49.3 | 47.9 |  |  |  |  |
| NKCV | 50.2 | 51.7 | 53.9 | 53.9 | 52.5 | 53.2 | 47.3 | 46.3 | 61.2 | 60.3 | 51.3 |  |  |  |
| KOTV | 51.4 | 52.1 | 53.2 | 53.2 | 52.7 | 53.6 | 47.8 | 46.6 | 59.9 | 58.7 | 51.3 | 71.9 |  |  |
| KOOLV | 52.1 | 52.4 | 52.0 | 52.5 | 52.2 | 52.7 | 46.8 | 46.6 | 59.2 | 58.7 | 50.8 | 71.9 | 92.7 | 100.0 |

**References**

The HgRTV2 sequence is publicly accessible in Genbank. There are no published papers describing the virus/sequence.