

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

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| **Code assigned:** | **2020.002D** |  |
| **Short title:** Create two new species in the genus *Simplexvirus* (*Herpesvirales*: *Herpesviridae*) | | |
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**Author(s) and email address(es)**

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| Aaron W Kolb |

**List the ICTV Study Group(s) that have seen this proposal**

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| *Herpesvirales* Study Group |

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

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| The *Herpesvirales* Study Group felt that the proposal was justified; however the proposal should reflect current species demarcation criteria. We have changed the demarcation content of the proposal to reflect this by focusing on lethality phenotypes, an analysis of conserved concatenated proteins, and co-speciation of the viruses and hosts. As mentioned above, the study group suggested investigating at least one conserved gene. We performed a phylogenetic analysis of UL30/VP5/UL42 protein sequences. We have also moved the species delimiting method described in the original proposal to secondary species defining data and have changed the text to reflect that the delimiting values are “chosen” or selected, rather than determined. Also, on the Species spreadsheet the “Order” designation was incorrect and has been changed to *Herpesvirales*. |

**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)** |
| Not applicable |  |  |

**Submission dates**

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| Date first submitted to SC Chair | June 18, 2020 |
| Date of this revision (if different to above) | July 17, 2020 |

**ICTV-EC comments and response of the proposer**

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

**Name of accompanying Excel module**

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| 2020.002D.R.Herpesviridae\_2nsp |

**Abstract**

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| We propose creating two new species for current *Macacine alphaherpesvirus 1* (herpes B) strains derived from pig and lion-tailed macaques. The viruses would be assigned within the *Alphaherpesvirinae* subfamily, and the *Simplexvirus* genus. The reassignment is based mainly on mortality phenotypic data, phylogenetic analysis of a group of conserved protein sequences, and evidence of co-speciation with the hosts. |

**Text of proposal**

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| |  | | --- | | Herpes B-virus (*Macacine alphaherpesvirus 1*) is endemic to macaques, while PaHV-2 (*Papiine alphaherpesvirus 2*) and SA8 (*Cercopithecine alphaherpesvirus 2)* appear to be endemic to baboons. There are several genome sequenced herpes B strains which have been isolated from various macaque species including the crab-eating macaque or cynomolgus monkey (*M. fascicularis*), rhesus (*M. mulatta*), bonnet (*M. radiata*), Japanese (*M. fuscata*), lion-tailed (*M. silenus*) and pig-tailed (*M. nemestrina*) macaques. A study conducted by Eberle et al [1] examined the LD50 of the genome sequenced herpes B strains in mice. This study demonstrated different lethality phenotypes for the pig and lion-tailed macaque simplexviruses compared to the remaining herpes B strains [1]. The LD50 values for the pig and lion-tailed macaque simplexviruses were both >107 PFU, while the LD50 values for the remaining herpes B strains were approximately 104 PFU [1]. Additionally a phylogenetic network and maximum likelihood tree based on whole genome data (Figures 1A and 1B), as well as a phylogenetic network based on concatenated (UL30/VP5/UL42) protein sequences (Figures 2A and 2B) closely resemble a macaque phylogenetic tree (Figure 1C) based on work by Li et al [9]. This data suggests that the pig and lion-tailed macaque simplexviruses co-speciated with their hosts. Based on the different pathogenic characteristics, host identity, and sequence differences, we propose designating the pig and lion-tailed macaque simplexviruses as members of separate novel viral species.  While we acknowledge that the following analysis is not recognized as demarcation criteria by ICTV, we are presenting a quantitative delimiting analysis as secondary species defining data. A quantitative delimiting method was used to examine the 27 Old World monkey (OWM) herpes simplex isolates. First, the genomic sequences of the OWM simplexviruses were aligned, and then a phylogenetic network was generated (Figure 1A, 1B). The network shows that the virus and host phylogenies closely match (Figure 1; Figure 1C was modified from [9]). The phylogenetic network also shows the midpoint position of the pig and lion-tailed macaque derived viruses between the core herpes B strains and the baboon simplexviruses.  A quantitative delimiting method incorporating a kernel density graph superimposed over graphed maximum composite likelihood pairwise distances has been used to identify intraspecies viral clades [6, 8, 10]. This delimiting method was applied here to delimit viral species (Figure 3A). A species cutoff value (the selected trough between the two kernel density peaks) was 8.94%, where distance values above the cutoff were selected to be separate species. The genome-based nucleotide distances were then calculated, resulting in 10.1% distance between the lion and pig-tailed simplex viruses. The distances between the pig and lion-tailed simplex viruses vs herpes B core strains were each ~14% (Figure 3B). These distance values (both ~14%) were higher than the distance between established simplexvirus species PaHV-2 and SA8 (10.1%; Figure 3B). | |

**Supporting evidence**

Figure 1.

**A map with text

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Figure 1. Phylogenetic analysis of Old World monkey (OWM) simplexviruses. OWM viral genomic sequences (Table 1) were aligned with MAFFT ver. 7.394 and the optimal substitution model was calculated by IQ-Tree [3-5]. A) Phylogenetic network generated from the alignment using Splitstree ver. 4.14 and the HKY+G+I substitution model (gaps deleted; p-inv = 0.469; gamma = 1.138) [2]. B) A maximum likelihood tree generated using the OWM genomic alignment, in conjunction with the Hasegawa, Kishino, and Yano (HKY)+G+I nucleotide substitution model (1000 bootstrap replicates) with gaps by the Mega 7 software package. C) A macaque monkey phylogenetic tree based on data presented by Li et al [9].

Figure 2.

**A close up of a map

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Figure 2. Phylogenetic analysis of concatenated UL30/VP5/UL42 protein sequences of Old World monkey (OWM) simplexviruses. OWM viral genomic sequences were aligned with Mega 7 and the optimal substitution model was calculated by Mega 7 [7]. A) Phylogenetic network generated from the alignment using Splitstree ver. 4.14 and the JTT + G substitution model (gamma = 0.13) [2]. B) A maximum likelihood tree generated using the JTT+G substitution model with gaps (1000 bootstrap replicates) by the Mega 7 software package.

Figure 3.

**A close up of a map

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Figure 3. Establishing viral species cutoff value. Pairwise distances in the Old World monkey virus nucleotide alignment were calculated using Mega 7 [7], and the frequencies plotted using the R package. A kernel density plot was also generated and combined with the distance frequencies (A). A distance cutoff value was established by determining the trough of the kernel plot, which is depicted by a vertical dotted line (8.94%). Mega 7 was used to calculate between group distances which is shown in (B). The Maximum composite likelihood (MCL) pairwise distances between different OWM simplexvirus species or herpes B strains. The distances were calculated using the OWM monkey simplexvirus alignment by the Mega 7 package. The core herpes B grouping was defined above in Figure 1A.

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