

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

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| **Code assigned:** | ***2023.005M*** |  |
| **Short title:** Create one new genus and three new species in family *Bornaviridae* (*Mononegavirales*) | | |
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**List the ICTV Study Group(s) that have seen this proposal**

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| ICTV *Bornaviridae* Study Group |

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

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**ICTV Study Group votes on proposal**

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| --- | --- | --- | --- |
| **Study Group** | **Number of members** | | |
| **Votes support** | **Votes against** | **No vote** |
| ICTV *Bornaviridae* Study Group | 9 | 0 | 0 |

**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)** | |
|  | N |  | |

**Submission dates**

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| 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**

**Text of proposal**

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

**Name of accompanying Excel module**

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| 2023.005M.N.v1.Bornaviridae\_1ngen\_3nsp.xlsx |

**Abstract**

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| Using transcriptomic raw sequence read datasets (SRAs) from fish samples (superclass Osteichthyes and class Chondrichthyes), we assembled the likely complete genome sequences of three bornavirirds. Among them, two likely represent novel species within established genus *Cultervirus*, and one genome likely represents a novel species within a novel genus within family of *Bornaviridae*.  One of these novel genomes, identified in the dataset originating from the ampullae of Lorenzini tissue of a little skate (rajid *Leucoraja erinacea* [Mitchill, 1825]), has an unusual genomic architecture (N-Vp1-Vp2-X-P-G-M-L), i.e., the genome likely has two bornavirid-atypical open reading frames (tentatively named Vp1 and Vp2) that appear to represent ancient duplications of the viral glycoprotein open reading frame (G).  We propose to create two new species within the genus *Cultervirus*:  finepatterned puffer bornavirus (FPBV) → *Cultervirus inflati*  electric eel bornavirus (EEBV) → *Cultervirus electrophori*  Furthermore, we propose to create the new genus *Cartilovirus* within the family *Bornaviridae* including one new species:  little skate bornavirus (LSBV) → *Cartilovirus plani* |

**Text of proposal**

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| --- | --- |
| |  | | --- | | As of June 2023, the family *Bornaviridae* includes three genera (*Carbovirus*, *Cultervirus*, and *Orthobornavirus*). The genus *Cultervirus* currently includes a single species for a virus that has been discovered in fish (Wǔhàn sharpbelly bornavirus [WhSBV], species *Cultervirus hemicultri*) [1]. Recently, partial genomic sequences of an additional cultervirus, Murray-Darling carp bornavirus (MDCBV), were published but classification of this virus is pending [2].  Criteria for bornavirid genus affiliation and species demarcation are based on genomic characteristics, including genome organization, PAirwise Sequence Comparison (PASC) [3, 4], in combination with biological characteristics, such as antigenic relationships and natural host range [5]. In agreement with these criteria, the PASC species demarcation criterion was defined as 72–75% for coding-complete genome sequences [6, 7]. The genus-demarcation cut-off was tentatively set at approximately 45% genome nucleotide identity [6, 7].  During data mining of 116,082 raw transcriptomic SRA datasets from fish (jawless vertebrates, cartilaginous fishes, ray-finned fishes, and lungfishes), three potentially complete bornaviral genomes were assembled. In detail, electric eel bornavirus (EEBV; BK063519) was assembled from transcriptomic datasets of an electric eel (gymnotid *Electrophorus electricus* [Linnaeus, 1766]) kidney tissue. Finepatterned puffer bornavirus (FPBV; BK063517) was assembled from a transcriptomic dataset of finepatterned puffer (tetraodontid *Takifugu poecilonotus* [Temminck & Schlegel, 1850]) tissue pool of adult males. Little skate bornavirus (LSBV; BK063518) was assembled from a transcriptomic dataset of little skate (rajid *Leucoraja erinacea* [Mitchill, 1825]) ampullae of Lorenzini tissue of an adult female (compare **Table 1**). Finepatterned puffers and electric eels are ray-finned fishes (class Actinopterygii), whereas little skates are cartilaginous fish (class Chondrichthyes). In addition, we also found the first complete genome of MDCBV (BK063521) in a transcriptomic dataset of goldfish (cyprinid *Carassius auratus* [Linnaeus, 1758]) brain cells (compare **Table 1**).  **Sequence similarity and phylogeny**  In a phylogenetic analysis of the concatenated viral proteins N, G, and L, the novel bornavirids EEBV, FPBV and LSBV form separate phylogenetic entities that cluster alongside WhSBV and MDCBV, members of the genus *Cultervirus* (**Figure 1**). However, LSBV seems to be more distant to the other sequences.  PASC analysis confirmed that LSBV was most distant from other fish bornavirds (38.4-39.6%, **Table 2**, **Figure 2**). MDCBV and WhSBV were the most closely related viruses possessing 78.1% PASC identity. The PASC distances of EEBV, FPBV to MDCBV and WhSBV ranged from 43.7 to 51.5%.  **Genomic architecture**  The genomic architectures of MDCBV, EEBV, and FPBV, are similar in structure to those of culterviruses and carboviruses, following the pattern 3'-N-X/P-G-M-L-5' (**Figure 3**). The lengths of the predicted viral genomes are 9,148 and 9,397 nt, respectively. Notably, the LSBV genome structure differs from the other genomes, as it is significantly longer (11,090 nt) and contains two additional ORFs, tentatively referred to as viral protein 1 and 2 (Vp1 and 2) ORFs, resulting in the following genomic structure: 3'-N-Vp1-Vp2-X/P-G-M-L-5'. A phylogenetic analysis revealed that Vp1 and Vp2 may be the result of two ancient duplication events, as they share similarity with the G gene of LSBV. Interestingly, the deduced Vp1 and Vp2 expression products lack the transmembrane domain of G but contain conserved proteolytic cleavage sites.  **Proposal**  Based on the phylogenetic analysis and genomic architecture, we suggest creating two new species included in the genus *Cultervirus*:  finepatterned puffer bornavirus (FPBV) → *Cultervirus inflati*  electric eel bornavirus (EEBV) → *Cultervirus electrophori*  In previous TaxoProps for the classification of the genera *Carbovirus* and *Cultervirus*, the genus demarcation cut-off had been tentatively set to 45% PASC distance [8]. However, the data basis for this decision was rather weak, since the PASC histogram displayed a large gap between values at 38 and 55% (Figure 4A).  The PASC distances of EEBV to the two previously described culterviruses (WhSBV and MDCBV) is 49.7 to 51.5% and thus above the tentative cut-off (Table 2; Figure 4B). In contrast, the PASC distances of FPBV to WhSBV, MDCBV and EEBV are slightly below the tentative cut-off (43.7 to 44.6%; Table 2; Figure 4B), suggesting its classification in a new genus. However, due to the weak basis of the tentative cut-off, the lack of a clear gap at 45% (which may cause problems with the classification of further FPBV-like sequences potentially discovered in the future) and the identical genome structure, we suggest to rather adapt the tentative genus demarcation cut-off to 42% (Figure 4B) and classify FPBV in the genus *Cultervirus*.  Furthermore, we propose to create the new genus *Cartilovirus* within the family *Bornaviridae.* This is supported by the basal phylogenetic position, PASC distance (<39.6% to any known bornavirid) and especially the unique genomic architecture of LSBV. LSBV will be member of the sole species within this new genus:  little skate bornavirus (LSBV) → *Cartilovirus plani*  Furthermore, the first complete genome of MDCBV allowed for best practice PASC analysis and showed that MDCBV, in addition to WhSBV, is a member of the species *Cultervirus hemicultri* (**Table 2**; 78.1% PASC similarity; species demarcation cut-off: 72%).  **Etymology**  *Cultervirus electrophori*: “electrophori” from *Electrophorus electricus* (Linnaeus, 1766), one of three species for electric eel.  *Cultervirus inflati*: “inflati” (Latin): an act of inflation. Inspired by the inflation of pufferfish (originating sample was from finepatterned puffer).  *Cartilovirus:* “cartilo” (Latin): cartel or cartilage. Inspired by the cartilaginous fishes that have skeletons primarily composed of cartilage. Little skates are cartilaginous fishes.  *Cartilovirus plani*: “plani” (Latin): Inspired by the flat shape of rays, such as little skates. | |

**Supporting evidence**



**Figure 1: Phylogenetic relationship within the family *Bornaviridae*.** The maximum-likelihood tree was based on the concatenated amino acid sequence alignments of the viral proteins N, G, and L of the potential fish bornavirids (bold) together with representative members of the genera *Cultervirus* (pink), *Carbovirus* (blue), and *Orthobornavirus* (grey). The proposed new genus *Cartilovirus* is highlighted in yellow. White lines indicate separated viral species within the same genus. Silhouettes depict the potential host organisms (black) or the sampling source (red) of the respective viruses. The tree was constructed using IQ-TREE (version 2.2.2.3), an optimal partition model and statistical support with each 1 million replicates for ultrafast bootstrap and SH-aLRT. The statistical support is shown for major branches using the format [ultrafast bootstrap/SH-aLRT]. Asterisks indicate statistical support ≥ 95% and ≥ 80% for ultrafast bootstrap and SH-aLRT, respectively.



**Figure 2**: **Nucleotide distances within the family *Bornaviridae* as calculated by PASC**. Colors correspond to demarcation cut-offs: ≤72% for the same species (green), >72% and ≤42% for the same genus (yellow), >42% different genera (salmon). The adapted genus demarcation cut-off of 42% is suggested in this proposal. Viruses discovered within transcriptomic SRA data from fish samples are highlighted in red.



**Figure 3: Genome architectures of current and potential novel bornavirids.** Representative overall genome organizations are shown for representative bornavirids along with the potential novel viruses (black stars). The (predicted) open reading frames (ORFs) are depicted as arrows along with their corresponding transcription start (S) and termination (T) sites. For each of the genomes, the potential hosts/sources are depicted for each virus. Note the different genomic arrangements: 3'-N-X-P-M-G-L-5' (genus *Orthobornavirus*) and 3'-N-X-P-G-M-L-5' (*Carbovirus* and *Cultervirus*). Little skate bornavirus shares the genomic structure of carboviruses and culterviruses but has two additional predicted ORFs: 3'-N-Vp1-Vp2-X-P-M-G-L-5'.



**Figure 4: Distribution of PASC distances within the family *Bornaviridae*. (A)** Current PASC distance distribution *excluding* the novel viruses from this proposal. Demarcation cut-offs: ≤72% for the same species (green), >72% and ≤45% for the same genus (yellow), >45% different genera (salmon). (B) PASC distance distribution *including* the novel viruses from this proposal. Demarcation cut-offs: ≤72% for the same species (green), >72% and ≤42% for the same genus (yellow), >42% different genera (salmon). Demarcation cut offs are shown as dashed lines. Note the changed genus demarcation cut offs in panel B (red arrow).

**Table 1**: Summary of SRA datasets selected for *de novo* assembly that resulted in bornaviral complete genomes

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| --- | --- | --- | --- | --- |
| SRA Accession | Sampled organism | Type of sample | Origin of sample | Reference |
| SRR1299086 | electric eel  *Electrophorus electricus* (Linnaeus, 1766) | kidney tissue | tropical fish retailer, Tri-County Tropicals (Richmond Hill, NY) | [9] |
| SRR13236436 | finepatterned puffer  *Takifugu poecilonotus* (Temminck & Schlegel, 1850) | tissue pool of adult males | Beibu Gulf of China | [10] |
| SRR9592747 | little skate  *Leucoraja erinacea*  (Mitchill, 1825) | ampullae of Lorenzini tissue of an adult female | unknown |  |
| SRR6207428 | goldfish  *Carassius auratus*  (Linnaeus, 1758) | radial glial cells from the brain of an adult female | commercial supplier  (Mt. Parnell Fisheries Inc.) | [11] |

**Table 2**: Selected PASC complete genome nucleotide identities (%)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | LSBV | EEBV | FPBV | WHSBV | MDCBV |
| little skate bornavirus (LSBV) | 100 | 39.6 | 39.2 | 38.4 | 38.2 |
| electric eel bornavirus (EEBV) | 39.6 | 100 | 44.6 | 49.7 | 51.5 |
| finepatterned puffer bornavirus (FPBV) | 39.2 | 44.6 | 100 | 44.1 | 43.7 |
| Wǔhàn sharpbelly bornavirus (WhSBV) | 38.4 | 49.7 | 44.1 | 100 | 78.1 |
| Murray-Darling carp bornavirus (MDCBV) | 38.2 | 51.5 | 43.7 | 78.1 | 100 |

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