

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

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| **Code assigned:** | **2020.005S** |  |
| **Short title:** Create one new species (*Limnipivirus D*) in the genus *Limnipivirus* (*Picornavirales*: *Picornaviridae*) |
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**List the ICTV Study Group(s) that have seen this proposal**

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| *Picornaviridae* Study Group |

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

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**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 | 02/06/2020 |
| 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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| --- |
| 2020.005S.A.v1.Limnipivirus\_1nsp.xlsx |

**Abstract**

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| The *Limnipivirus* genus presently comprises three species, *Limnipivirus A, B* and *C*. Another limnipivirus-like virus has been detected in gut/liver/gill organ pools of the spotted longbarbel catfish (*Hemibagrus guttatus*) in China. The virus has a typical picornavirus genome layout (5'-UTR[1AB-1C-1D-2A1npgp/2A2npgp/2A3npgp/2A4H-box/NC-2B-2Chel/3A-3BVPg-3Cpro-3Dpol]3'UTR) and significant sequence similarities to limnipiviruses (48.1-50.1% for P1, 62.5-71.5% for 2Chel, 71.7-75.1% for 3Cpro and 51.8-57.6% for 3Dpol) which justifies assignment to the genus *Limnipivirus*, but to a new species, *Limnipivirus D*. Divergences to sequences of other picornavirus genera are >72% for P1, >70% for 2Chel, >77% for 3Cpro and 61% for 3Dpol. |

**Text of proposal**

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| **Create one new species, *Limnipivirus D*, in the genus *Limnipivirus***The *Limnipivirus* genus presently comprises three species, *Limnipivirus A*, *B* and *C*. The known limnipiviruses infect freshwater fish and have been detected in tissue samples of various organs. Viable bluegill picornavirus and carp picornavirus has been isolated. Novel limnipivirus-like viruses were detected in organ pools (gut, liver, gill) of the bighead beaked sandfish (*Gonorynchus abbreviatus*) and the spotted longbarbel catfish (*Hemibagrus guttatus*) (Shi et al. 2018). Whereas the Wenling bighead beaked sandfish picornavirus comprises a second type of *Limnipivirus B*, the Guangdong spotted longbarbel catfish picornavirus exhibits differences which justify assignment to a new *Limnipivirus* species.**Relation to other picornaviruses:**1. The Guangdong spotted longbarbel catfish picornavirus has a typical picornavirus genome layout: 5'-UTR[1AB-1C-1D/2A1npgp-2A2npgp-2A3npgp-2A4H-box/NC-2B-2Chel/3A-3BVPg-3Cpro-3Dpol]3'UTR (compare Fig. 1 of supporting material)2. The Guangdong spotted longbarbel catfish picornavirus possesses typical hallmarks of picornaviruses:  **capsid proteins:** 1AB, 1C, 1D have **rhv** domains with drug-binding site,  **2Chel:** **G**xx**G**x**GKS** motif of helicases, **3BVPg:** **Y-3** residue, **3Cpro:** **C**x**CG**x14**G**x**H** motif, **3Dpol:** **KDE**, **PSG**, **YGDD**, **FLKR** motifs3. **Phylogenetic analyses** indicate clustering with the sequences of the picornavirus supergroup 4 (*Aalivirus, Aquamavirus, Avihepatovirus, Avisivirus, Crohivirus, Grusopivirus, Kunsagivirus, Limnipivirus, Orivirus, Parechovirus, Pasivirus, Potamipivirus, Shanbavirus*) in the P1 and 3D trees (compare Figs. 2 & 3 of supporting material). Closest relatives are limnipiviruses.**Distinguishing features of limnipivirus D1 (Guangdong spotted longbarbel catfish picornavirus) compared to other limnipiviruses:** 1. The Guangdong spotted longbarbel catfish picornavirus has **three 2A proteins** with a **NPGP sequence motifs**, whereas the other limnipiviruses have only two such proteins.2. Protein **2A4** of the Guangdong spotted longbarbel catfish picornavirus has a **H-box/NC** sequence motif whereas the other limnipiviruses lack such a protein (compare Fig. 1).3. **Within-genus divergence** (uncorrected p-distances) in comparisons with other limnipiviruses are >60% for the polyprotein, 48.1-50.1% for P1, 62.5-71.5% for 2Chel, 71.7-75.1% for 3Cpro and 51.8-57.6% for 3Dpol (compare Tables 1, 2). The **divergence data** suggest a new limnipivirus species, *Limnipivirus D*, with 1 type, limnipivirus D1 (GenBank acc. nos. MG600094).4. **Sequence divergence** (uncorrected p-distances) of orthologous proteins is high in pairwise comparisons with 40 acknowledged and proposed species of picornavirus supergroup 4. The amino acid divergences range from 72.8–81.9% for P1, 70.3–80.6% for 2Chel, 77.4–89.0% for 3Cpro and 61.2–71.8% for 3Dpol (compare Table 3). **Species demarcation criteria**Members of a species of the genus *Limnipivirus*  - share a common genome organization, - are less than 45% divergent in the polyprotein sequence, - are less than 25% divergent in the P1 aa sequence - are less than 45% divergent in the 2C+3CD aa sequence.**Exemplar virus of species:** ***Limnipivirus D***, limnipivirus D1 (Guangdong spotted longbarbel catfish picornavirus, GenBank acc. no. MG600094. |

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**Supporting evidence**

**Table 2: Diversities**

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| --- | --- | --- | --- | --- | --- |
|  | **LimV-A1****(BGPV)** | **LimV-B1****(CPV)** | **LimV-B2** | **LimV-C1****(FHMPV)** | **LimV-D1** |
| **Polyprotein** |
| **LimV-A1 (BGPV)** | - |  |  |  |  |
| **LimV-B1 (CPV)** | 0.571 | - |  |  |  |
| **LimV-B2** | 0.569 | 0.364 | - |  |  |
| **LimV-C1 (FHMPV)** | 0.576 | 0.477 | 0.490 | - |  |
| **LimV-D1** | 0.605 | 0.633 | 0.626 | 0.625 | - |
| **P1 protein** |
| **LimV-A1 (BGPV)** |  |  |  |  |  |
| **LimV-B1 (CPV)** | 0.413 | - |  |  |  |
| **LimV-B2** | 0.428 | 0.203 | - |  |  |
| **LimV-C1 (FHMPV)** | 0.426 | 0.293 | 0.323 | - |  |
| **LimV-D1** | 0.501 | 0.491 | 0.492 | 0.481 | - |
| **2C protein** |
| **LimV-A1 (BGPV)** | - |  |  |  |  |
| **LimV-B1 (CPV)** | 0.553 | - |  |  |  |
| **LimV-B2** | 0.528 | 0.385 | - |  |  |
| **LimV-C1 (FHMPV)** | 0.577 | 0.509 | 0.482 | - |  |
| **LimV-D1** | 0.625 | 0.715 | 0.686 | 0.678 | - |
| **3C protein** |
| **LimV-A1 (BGPV)** | - |  |  |  |  |
| **LimV-B1 (CPV)** | 0.677 | - |  |  |  |
| **LimV-B2** | 0.675 | 0.382 | - |  |  |
| **LimV-C1 (FHMPV)** | 0.665 | 0.630 | 0.648 | - |  |
| **LimV-D1** | 0.717 | 0.737 | 0.751 | 0.724 | - |
| **3D protein** |
| **LimV-A1(BGPV)** | - |  |  |  |  |
| **LimV-B1 (CPV)** | 0.506 | - |  |  |  |
| **LimV-B2** | 0.496 | 0.322 | - |  |  |
| **LimV-C1 (FHMPV)** | 0.509 | 0.416 | 0.429 | - |  |
| **LimV-D1** | 0.518 | 0.565 | 0.576 | 0.561 | - |

**Table 3: Diversities of orthologous proteins \***

**Limnipivirus D1 (Guangdong spotted longbarbel catfish picornavirus) vs. P1 2Chel 3Cpro 3Dpol**

*Limnipivirus Limnipivirus A* 50.1% 62.5% 71.7% 51.8%

 *Limnipivirus B* 49.1% 71.5% 73.7% 56.5%

 *Limnipivirus C* 48.1% 67.8% 72.4% 56.1%

*Aalivirus Aalivirus A* 80.5% 75.3% 83.9% 61.4%

*Aquamavirus Aquamavirus A* 80.6% 77.9% 84.0% 71.8%

*Avihepatovirus Avihepatovirus A* 77.5% 75.6% 86.4% 64.0%

*Avisivirus Avisivirus A* 81.8% 73.3% 84.7% 67.6%

 *Avisivirus B* 79.5% 76.3% 83.3% 64.9%

 *Avisivirus C* 80.8% 73.8% 85.5% 66.8%

*Crohivirus Crohivirus A* 76.0% 76.0% 85.9% 63.8%

 *Crohivirus B* 76.0% 77.0% 85.3% 64.5%

*Grusopivirus Grusopivirus A* 80.0% 74.7% 82.7% 65.8%

 *Grusopivirus B* 78.7% 77.1% 85.5% 61.9%

*Kunsagivirus Kunsagivirus A* 83.1% 77.8% 84.4% 70.9%

 *Kunsagivirus B* 81.9% 77.1% 86.8% 71.3%

 *Kunsagivirus C* 79.6% 76.5% 84.4% 69.9%

*Orivirus Orivirus A* 79.4% 74.2% 85.4% 66.5%

*Parechovirus Parechovirus A* 78.6% 76.2% 85.8% 63.0%

 *Parechovirus B* 78.4% 75.2% 88.0% 62.0%

 *Parechovirus C* 79.7% 73.6% 89.0% 62.2%

 *Parechovirus D* 77.4% 75.9% 85.3% 61.4%

 *Parechovirus E* 78.6% 76.9% 86.9% 64.5%

 *Parechovirus F* 78.8% 75.5% 83.9% 61.5%

*Pasivirus Pasivirus A* 80.5% 77.2% 81.6% 69.3%

*Potamipivirus Potamipivirus A* 77.7% 74.4% 79.8% 61.2%

 *Potamipivirus B* 78.2% 74.8% 78.7% 62.7%

*Shanbavirus Shanbavirus A* 81.8% 80.6% 82.1% 68.2%

unassigned Beihai conger picornavirus 79.0% 74.1% 80.2% 64.9%

unassigned Wenling brown-lined puffer picornavirus 78.9% 78.0% 84.1% 64.6%

unassigned Wenling fish picornavirus 79.1% 77.4% 83.1% 61.2%

unassigned Wenling hoplichthys picornavirus 80.4% 76.4% 77.4% 62.6%

unassigned Wenling lepidotrigla picornavirus 77.4% 77.7% 77.4% 63.1%

unassigned Wenling rattails picornavirus 79.8% 70.3% 79.9% 64.3%

unassigned Wenling scaldfish picornavirus 1 77.1% 74.3% 79.0% 62.6%

unassigned Wenling scaldfish picornavirus 2 79.9% 72.9% 85.6% 63.9%

unassigned Wenling thamnaconus septentrionalis picornavirus 79.2% 78.2% 85.8% 67.2%

unassigned Western African lungfish picornavirus 72.8% 72.4% 81.3% 62.5%

unassigned Wuhan carp picornavirus 78.8% 74.7% 80.0% 64.2%

unassigned Wuhan sharpbelly picornavirus 1 79.4% 76.5% 83.1% 62.8%

unassigned Yancheng osbecks grenadier anchovy picornavirus 81.5% 75.9% 78.4% 62.7%

\* number of amino acid differences per site

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**Figure 1:** Genome organisation of limnipiviruses (schematic depiction). The genomes of limnipiviruses A1, B1, B2, C1 are is compared to the genome of the Guangdong spotted longbarbel catfish picornavirus, the exemplar virus of *Limnipivirus D*. The open reading frame is indicated by a box. Position of putative 3Cpro cleavage sites are indicated by a ▼ and the NPGP motif by a #. Non-orthologous 2A proteins are printed in colour. The names and lengths of the deduced proteins are presented. The 5’-UTRs may be incomplete.



**Figure 2:** Phylogenetic analysis of picornavirus P1 protein using Bayesian tree inference (MrBayes 3.2). Sixty-one picornavirus sequences of the *Aalivirus/Aquamavirus/Avihepatovirus/Avisivirus/ Crohivirus/Grusopivirus/Kunsagivirus/Limnipivirus/Orivirus/Parechovirus/Pasivirus/Potamipivirus/ Shanbavirus* supergroup were retrieved from GenBank; the cardiovirus sequence served as outgroup. [Note: the supergroup does not imply a taxonomic entity but reflects phylogenetic clustering of the respective genera observed in different tree inference methods (NJ, ML, Bayesian MCMC).] Presented are GenBank accession numbers, species names, type and—if available—common names in round brackets. Designations of isolates are given in square brackets. Genus names are presented at the right. Yet unassigned viruses are printed in blue. The proposed name is printed in red and indicated by a dot (●). Numbers at nodes indicate posterior probabilities obtained after 2,000,000 generations. The optimal substitution model (GTR+G+I) was determined with MEGA 5. The scale indicates substitutions/site.



**Figure 3:** Phylogenetic analysis of picornavirus 3D protein using Bayesian tree inference (MrBayes 3.2). Sixty picornavirus sequences of the *Aalivirus/Aquamavirus/Avihepatovirus/Avisivirus/ Crohivirus/Grusopivirus/Kunsagivirus/Limnipivirus/Orivirus/Parechovirus/Pasivirus/Potamipivirus/ Shanbavirus* supergroup were retrieved from GenBank; the cardiovirus sequence served as outgroup. [Note: the supergroup does not imply a taxonomic entity but reflects phylogenetic clustering of the respective genera observed in different tree inference methods (NJ, ML, Bayesian MCMC).] Presented are GenBank accession numbers, species names, type and—if available—common names in round brackets. Designations of isolates are given in square brackets. Genus names are presented at the right. Yet unassigned viruses are printed in blue. The proposed name is printed in red and indicated by a dot (●). Asterisks (\*) indicate incomplete genomes. Numbers at nodes indicate posterior probabilities obtained after 2,000,000 generations. The optimal substitution model (GTR+G+I) was determined with MEGA 5. The scale indicates substitutions/site.

**References**

1. Shi M, Lin XD, Chen X, Tian JH, Chen LJ, Li K, Wang W, Eden JS, Shen JJ, Liu L, Holmes EC, Zhang YZ. 2018. The evolutionary history of vertebrate RNA viruses. Nature 556:197-202. PMID: 29618816 DOI: 10.1038/s41586-018-0012-7