This Word module should be used for all taxonomic proposals.

Please complete **Part 1** and:

either **Part 3** for proposals to create new taxa or change existing taxa

or **Part 2** for proposals of a general nature.

Submit the completed Word module, together with the accompanying Excel module named in Part 3, to the appropriate ICTV Subcommittee Chair.

For guidance, see the notes written in blue, below, and the help notes in file Taxonomic\_Proposals\_Help\_2018.

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

|  |  |  |
| --- | --- | --- |
| **Code assigned:** | ***2018.007D*** | (to be completed by ICTV officers) |
| **Short title: 8 new species in the family *Iridoviridae;* removal of 3 existing species** |
|  |
| **Author(s):** |
| VG Chinchar, P Hick, J Jancovich, K Subramaniam, T Waltzek, R Whittington, T Williams |
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| **List the ICTV study group(s) that have seen this proposal:** |
| A list of study groups and contacts is provided at <http://www.ictvonline.org/subcommittees.asp> . If in doubt, contact the appropriate subcommittee chair (there are six virus subcommittees: animal DNA and retroviruses, animal ssRNA-, animal ssRNA+, fungal and protist, plant, bacterial and archaeal) | **Iridoviridae SG** |
| **ICTV Study Group comments (if any) and response of the proposer:** |
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|  |
| Date first submitted to ICTV: | 6 June 2018 |
| Date of this revision (if different to above): | 18 June 2018 |

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| **ICTV-EC comments and response of the proposer:** |
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**Part 3:** **PROPOSED TAXONOMY**

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| **Name of accompanying Excel module: 2018.007D.N.v1.Iridoviridae\_8sp3sprem** |

The taxonomic changes you are proposing should be presented on an accompanying Excel module, 2017\_TP\_Template\_Excel\_module. Please enter the file name of the completed module in this box.

**Supporting material:**

| additional material in support of this proposal |
| --- |
| Please explain the reasons for the taxonomic changes you are proposing and provide evidence to support them. The following information should be provided, where relevant:* **Species demarcation criteria**: Explain how new species differ from others in the genus and demonstrate that these differences meet the criteria previously established for demarcating between species. If no criteriahave previously been established, and if there will now be more than one species in the genus, please state the demarcation criteria you are proposing.
* **Higher taxa**:
	+ There is no formal requirement to state demarcation criteria when proposing new genera or other higher taxa. However, a similar concept should apply in pursuit of a rational and consistent virus taxonomy.
	+ Please indicate the **origin of names** assigned to new taxa at genus level and above.
	+ For each new genus a **type species** must be designated to represent it. Please explain your choice.
* **Supporting evidence**: The use of Figures and Tables is strongly recommended (note that copying from publications will require permission from the copyright holder). For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.
 |

**Introduction:** Over the past several years, the genomic sequences of over 40 isolates within the family *Iridoviridae* have been determined and used to assess sequence identity and phylogenetic relatedness. Based on these and other criteria described below, we propose the establishment of several new virus species and the removal of three current species.

**Species demarcation criteria:** We propose, based on a set of concatenated genes, that viruses sharing 95% or greater amino acid sequence identity be tentatively considered as belonging to the same species. In addition, members of the same species will display the following features: (1) phylogenetic relatedness, (2) a co-linear arrangement of genes, (3) similar genomic size, and (4) similar G+C content. Viruses that fail to meet one or more of these criteria may be viewed as members of different species.

**Genus demarcation criteria:** Members of a given genus share less than 50% amino acid sequence identity with members of other genera. Furthermore, several additional criteria distinguish genera within the family. For example, phylogenetic analysis clearly separates members of one genus from that of others. In addition genera differ based on the principal host species infected, the presence of a DNA methytransferase, and characteristic pathology (e.g., wart-like growths following infection with lymphocystis disease virus, systemic disease characterized by internal and external hemorrhages and skin sloughing following infection with ranaviruses, and cell hypertrophy in the case of megalocytiviruses).

**Rationale for establishment of new species**

**Two new species in the genus *Lymphocystivirus*:** We propose establishing species for lymphocystis disease virus-China (LCDV-C) and lymphocystis disease virus-Sparus aurata (LCDV-Sa), named *Lymphocystis disease virus 2* and *Lymphocystis disease virus 3,* respectively, based on their separation from lymphocystis disease virus 1(LCDV-1, species *Lymphocystis disease virus 1*) following phylogenetic analysis (Fig. 1) and on the criteria described above. Specifically, the genomes of LCDV-Sa (208,501 bp) and LCDV-C (186,250 bp) are considerably larger than that of LCDV-1 (102,653 bp), the GC content of LCDV-Sa (33%) is higher than that of LCDV-1 (29%) and LCDV-C (27%), sequence identity at the nucleotide level among these three isolates is markedly low and ranges from 42-55%. LCDV-Sa showed marked genomic rearrangements compared to both LCDV-C and LCDV-1, and LCDV-1 shows no co-linearity with the genome of LCDV-C. (References 1-3)

**One new species within the genus *Megalocytivirus*:** Based on phylogenetic analysis, scale drop disease virus (SDDV) merits the establishment of a new species for it based on its marked divergence from the exemplar virus, infectious spleen and kidney necrosis virus (ISKNV), of the type (and single) species of the genus, *Infectious spleen and kidney necrosis virus*, and other viruses within the genus (Fig. 1). In addition, the genome of SDDV is larger (124 kbp) than that of ISKNV (111 kbp) and displays a lower (37% vs 55%) GC content, and the pathological outcome of infection is markedly different. (Reference 4)

**One new species within the genus *Iridovirus*:** Invertebrate iridescent virus 31 (IIV31) is proposed to be the member of a new species within the genus *Iridovirus* based on phylogenetic analysis (Fig. 1). Pairwise alignment using BLASTN of the IIV31 and IIV6 (member of the type species of the genus) genomes did not show marked levels of sequence identity at the nucleotide level. Likewise, clusters of co-linear genes were not detected between IIV31 and IIV6. IIV31 and IIV6 also differ in term of principal host species infercted.  IIV6 was originally isolated from the rice borer and is known to infect multiple species of insects, whereas IIV31 was isolated from a terrestial isopod.  (Reference 5)

**Four new species within the genus *Chloriridovirus*:** Based on phylogenetic analysis, IIV9, IIV22, IIV25, and Anopheles minimus iridovirus (AMIV) are proposed to join invertebrate iridescent virus 3(IIV3), member of the type species (*Invertebrate iridescent virus 3*), as members of separate new species within the genus *Chloriridovirus* (Fig.1). The members of these four new species display a lower (28-31%) GC% than IIV3 (48%), and the genome size of AMIV is markedly smaller (163 kbp) than that of the members of the other species within the genus (191-206 kbp). IIV3 and IIV9 show no evidence of genetic co-linearity; pairwise alignment of nucleotide sequences shows 74-83% identity between IIV22 and IIV9 over 60% of their length, whereas IIV25 and IIV9 display 85-92% identity over 88% of their length. (References 6-11)

**Table 1: Proposed species within the genus *Chloriridovirus***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Virus**  | **Acc. No.**  | **Genome size (bp)** | **No. ORFs** | **G+C%** |
| Invertebrate iridescent virus 3 | DQ643392 | 191,132 | 126 | 48 |
| Invertebrate iridescent virus 9 | GQ918152 | 206,791 | 191 | 31 |
| Invertebrate iridescent virus 22 | HF920633 | 197,693 | 167 | 29 |
| Invertebrate iridescent virus 25 | HF920635 | 204,815 | 177 | 30 |
| Anopheles minimus iridovirus | KF938901 | 163,023 | 148 | 39 |

**Removal of species *Invertebrate iridescent virus 1* within the genus *Iridovirus*:** IIV1 was the first iridescent virus discovered and a considerable body of information exists concerning its biology and replication. However, the complete genomic sequence of IIV1 has not yet been determined. Although sequence analysis of the IIV1 MCP gene clearly indicates IIV1 is a member of the family *Iridoviridae,* phylogenetic analysis places IIV1 closer to members of the genus *Chloriridovirus* than to members of the genus *Iridovirus.* Thus, in the absence of a completely sequenced IIV1 genome, we propose to abolish this species within the genus *Iridovirus*. Pending complete sequence analysis of its genome, IIV1 should be considered a tentative member of the genus *Chloriridovirus*.

**Removal of species *Bohle iridovirus* and *European catfish virus* from the genus *Ranavirus***

**Bohle iridovirus:** BIV shares more than 95% amino acid sequence identity following comparison of a concatenated set of 26 conserved iridovirus genes, a similar genome size (104 kbp in BIV vs 105 kbp in frog virus 3 *(*FV3), exemplar virus of type species of the genus), similar GC content (55%), and displays genetic colinearity with FV3.Therefore,Bohle iridovirus is best viewed as an isolate belonging to species *Frog virus 3* rather than member of a distinct virus species. (Reference 12)

**European catfish virus (ECV):** Likewise ECV should be considered, along with European sheatfish virus (ESV), as a strain in species *Epizootic haematopoietic necrosis virus* rather than member of a distinct virus species. All three of these fish ranaviruses display high sequence identity, a larger genome size (126-128 kbp) than other members of the genus *Ranavirus* (104-106 kbp), and marked genetic co-linearity. (References 13-14)

**Criteria defining species within the genus *Ranavirus:*** With these and earlier changes, it is proposed that six virus species be recognized within the genus based on the criteria shown in Table 2. All viruses within a species must show >95% amino acid identity among a concatenated set of 26 core genes. In addition, among those viruses that show 95% identity, species differentiation will be made upon their phylogenetic position, extent of genetic co-linearity, genome size, and G+C content. The Study Group is considering whether a concatenated set of 26 core genes is the best way to classify novel viruses or whether a smaller number of key genes will provide sufficient information to generate a robust phylogeny.

**Table 2: Criteria defining species within the genus *Ranavirus***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Species****(exemplar virus)** | **% pairwise amino acid identity to FV3** | **Distinct phylogenetic position1**  | **Genetic Co-linearity2** | **Genome size (kbp)** | **G+C%** |
| *Frog virus 3* (FV3) | >95% | FV3, ATV, EHNV, CMTV | FV3-like | 103-106 | 55-56% |
| *Ambystoma tigrinum virus* (ATV) | >95% | FV3, ATV, EHNV, CMTV | ATV/ EHNV-like | 106 | 54% |
| *Epizootic haematopoietic necrosis virus* (EHNV) | >95% | FV3, ATV, EHNV, CMTV | ATV/ EHNV-like | 127 | 54-56% |
| *Common midwife toad virus* (CMTV) | >95% | FV3, ATV, EHNV, CMTV | CMTV-like | 106-108 | 55-56% |
| *Santee Cooper ranavirus* (SCRV) | 80% | SCRV | ND3 | ND | ND |
| *Singapore grouper iridovirus* (SGIV) | 70% | SGIV | SGIV-like | 140  | 49% |

1 Phylogenetic analysis (Fig. 1) shows that FV3, Ambystoma tigrinum virus (ATV), EHNV, and common midwife toad virus (CMTV) are closely linked, but that Santee-Cooper ranavirus (SCRV) isolates largemouth bass virus (LMBV), doctor fish virus (DFV), guppy virus 6 (GV6) and Singapore grouper iridovirus (SGIV) isolates SGIV and grouper iridovirus (GIV) occupy distinct branches of the tree.

2 Dot-Plot analysis shows little gene co-linearity between SGIV and the other viruses within the genus. In contrast, FV3, ATV, EHNV, and CMTV share marked conservation of gene order, but distinctive inversions are present allowing these viruses to be ordered into FV3-like, ATV/EHNV-like, and CMTV-like viruses.

3 The sequence of the 26 core genes has been determined for LMBV, GV6, and DFV, but due to difficulties in resolving repeat regions, the complete genomic sequence has not yet been determined.



**Fig. 1 Phylogenetic analysis of family *Iridoviridae****.*Accession numbers are shown for the viruses. Exemplars of accepted species are shown in boldface. The tree was constructed using maximum likelihood analysis in IQTREE and the concatenated amino acid (aa) sequences of 26 core genes (19,773 aa characters including gaps) from 45 completely sequenced genomes of members of the family. The tree was midpoint rooted and branch lengths are based on the number of inferred substitutions, as indicated by the scale bar. All branch points (i.e., nodes) separating genera are supported by bootstrap values greater than 99%. For other branch points all bootstrap values are >70% except for those displaying high levels of amino acid similarity, e.g., tiger frog virus vs. Bohle iridovirus/German gecko ranavirus, 66%; pike perch iridovirus vs. common midwife toad virus/2013/NL, 49%; turbot reddish body iridovirus vs. red seabream iridovirus, 57%; and lemon yellow croaker iridovirus vs. turbot reddish body iridovirus, 56%. (Figure provided by K Subramaniam and TB Waltzek.)

| **References:** |
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| 1. Tidona CA and Darai G. The complete DNA sequence of **lymphocystis disease virus**. Virology 230: 207 - 216, 1997. 2. Lopez-Bueno A et al., Concurrence of **iridovirus**, polyomavirus, and a unique member of a new group of fish papillomaviruses in lymphocystis disease-affected **gilthead sea bream**. J. Virol. 90: 8768 - 8779, 2016. 3. Zhang QY et al., Complete genome sequence of **lymphocystis disease virus** isolated from **China**. J. Virol. 78: 6982 - 6994, 2004. 4. De Groof A et al., A novel virus causes **scale drop disease** in *Lates calcarifer*. PLoS Pathogens 11: e1005074, 2015.5. Piegu B et al., Genome sequence of a crustacean iridovirus**, IIV31**, isolated from the pill bug, *Armadillidium vulgare*. J. Gen. Virol. 95: 1585 - 1590, 2014.6. Piegu B et al., Complete genome sequence of **invertebrate iridescent virus 22** isolated from a blackfly larva. J. Gen. Virology 94: 2112 - 2116, 2013. 7. Piegu B et al., Complete genome sequence of **invertebrate iridovirus IIV-25** isolated from blackfly larva. Arch. Virol. 159: 1181 - 1185, 2014. 8. Piegu B. et al., Complete genome sequence on invertebrate iridovirus **IIV30** isolated from corn earworm, *Helicoverpa zea*. J. Invert. Pathology 116: 43 - 47, 2014. 9. Wong CK et al., Genomic and proteomic analysis of **invertebrate iridovirus type 9**. J. Virology 85: 7900 - 7911, 2011. 10. Huang Y et al., Isolation and characterization of a novel invertebrate iridovirus from adult *Anopheles minimus* (**AMIV**) in China. J. Invert. Pathol. 127: 1 - 5, 2015. 11. Delhon G. et al., Genome of **invertebrate iridescent virus type 3** (Mosquito iridescent virus). J. Virol. 80: 8439 - 8449, 2006. 12. Hick P et al., Complete genome sequence of a **Bohle iridovirus** isolate from ornate burrowing frogs (*Limnodynastes ornatus*) in Australia. Genome Announcements 4: e00632-16, 2016.13. Fehér E et al., Whole genome sequencing and phylogenetic characterization of **brown bullhead (*Ameiurus nebulosus*)** origin ranavirus strains from independent outbreaks. Infect. Genet. Evol. 45: 402 - 407, 2016. 14. Mavian C et al., Genome Announcement: Complete genome sequence of the **European sheatfish virus**. J. Virology 86: 6365 - 6366, 2012. |