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.

The Word module explains and justifies your proposal. The Excel module is a critical document that will be used to implement the proposed taxonomic changes once they are approved and ratified. If proposals presented in the Word module are not presented accurately in the Excel module, the taxonomic changes cannot proceed.

For guidance, see the notes written in blue, below, and the Help Notes in file Taxonomic\_Proposals\_Help\_2019.

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

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| **Code assigned:** | ***2019.002F*** |  |
| **Short title:** Create one new family (*Polymycoviridae*) including one new genus (*Polymycovirus*) and ten new species |
|  |
| **Author(s) and email address(es):**  |
| List authors in a single line *Archives of Virology* citation format (e.g. Smith AB, Huang C-L, Santos, F) | Provide email address for each author in a single line separated by semi-colons |
| Kotta-Loizou I, Coutts RHA | i.kotta-loizou13@imperial.ac.uk; r.coutts@herts.ac.uk  |
| **Author(s) institutional address(es) (optional):**

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| --- |
| Provide institutional addresses, each on a single line followed by author(s) initials (e.g. University of Woolloomooloo [SAB, HCL]) |
| Imperial College London [IKL]University of Hertfordshire [RHAC] |

 |
| **Corresponding author** |
| Ioly Kotta-Loizou; i.kotta-loizou13@imperial.ac.uk |
| **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) |  |
| **ICTV Study Group comments (if any) and response of the proposer:** |
| The EC has recommended Ac, which means that the proposal will be accepted this year provided a number of modifications are made within the next 2 month period. Proposed changes are:1. As discussed, the phylogenetic tree should include a wider range of RNA viruses so the relationships of *Polymycoviridae* with other families is made more clear. Comparison groups might include representative sequences of different genera of caliciviruses along with members of other virus families that most closely polymycoviruses, *eg.* Partitiviruses.

A larger phylogenetic tree with a wider range of RNA viruses, including astroviruses, caliciviruses, partitiviruses, comoviruses, picornaviruses and potyviruses was constructed (Fig. 4). The analysis confirms that polymycoviruses are closely related to all these families but since there is no real bootstrap support for clustering above family level no further conclusions can be drawn.1. The species threshold was defined as 70% amino acid sequence similarity in their list. In this case the meaning of the sentence:

*All proposed species of the family Polymycoviridae are represented by fully sequenced viruses with publically available accession numbers and with an evolutionary distance larger than 0.3 from each other.*Is unclear.Fair point; the sentence has been modified as follows: *All proposed species of the family Polymycoviridae are represented by fully sequenced viruses with publically available accession numbers.*1. Can the coordinates of the region of RdRp analysed in the phylogenetic tree be provided.

Full length RdRp and methyltransferase sequences were analysed in the phylogenetic trees (Fig. 1 and 3). For the phylogenetic tree in Fig. 4, a region where there is some clear homology between different families was selected for the analysis.1. Sequence accession numbers should ideally be provided instead of protein accession numbers (since the former represent the source of the sequence).

Protein accession numbers have been replaced with nucleotide sequence accession numbers where applicable, e.g. on the phylogenetic tree.1. There is a link to external file embedded in the Word document – can this be resolved?

Apologies for this, the link has now been severed.The author should consider:1. The species threshold is rather high, and potentially the known viruses could (based on RdRp phylogeny at least) be potentially split into several (perhaps 3 or 4?) genera, if the authors believed this served a purpose to divide them into groups with identifiably different biological properties.

At this point in time, we would rather have one genus accommodating all known polymycoviruses. However, this is something will consider in the future, as the number of polymycovirus sequence and our knowledge of their biological characteristics increases.1. Do phylogeny relationships (topology) of the methyltransferase gene resemble RdRp?

Yes the topology of the methyltransferase sequences resembles that of the RdRp sequences, as shown in Figure 3.1. The root of the family name is the same as that of the genus. Although not obligatory, the authors could consider an alternative or modified root for the genus to avoid any confusion.

At this point in time, we would rather keep the name of the genus as is and once we split we will adjust the names accordingly. |
| Date first submitted to ICTV: |       |
| Date of this revision (if different to above): |       |

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

**Part 3:** **PROPOSED TAXONOMY**

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| **Name of accompanying Excel module:** 2019.002F.A.v1.Polymycoviridae\_1newfam.xlxs |

The taxonomic changes you are proposing should be presented on an accompanying Excel module, 2019\_TP\_Template\_Excel\_module. Please enter the file name of the completed module in this box.

**Supporting material:**

The first member of the proposed family Polymycoviridae was reported by Kanhayuwa *et al*. in 2015 and since then at least fifteen related viruses have been found. These recent discoveries have led to the proposal for creation of novel taxa to accommodate these viruses (Table 1):

1. Creation of the family Polymycoviridae (from ‘poly’ = many + ‘myco’ = fungus in Greek).
2. Creation of the genus Polymycovirus within the proposed family Polymycoviridae.
3. Creation and assignment of ten new species to the proposed genus Polymycovirus.

Members of the family Polymycoviridae have 4-8 double stranded (ds) RNA genomic segments; the overall size of the genome ranges from 7.5 to 12.5 kbp. In all known polymycoviruses, dsRNA 1 encodes the RNA-dependent RNA polymerase (RdRP), dsRNA 2 encodes a putative scaffold protein, dsRNA 3 encodes a methyl transferase and dsRNA 4 encodes an intrinsically disordered proline-alanine-serine rich protein (PASrp) hypothesised to coat the non-conventionally encapsidated viral genome. When present, dsRNAs 5-8 encode proteins of unknown function that are non-homologous between different viruses. Only one polymycovirus, the octa-segmented Colletotrichum camelliae filamentous virus 1 (CcFV-1), is believed to have a capsid (Jia *et al*., 2017). It is feasible that the ability to form a capsid is dependent on the presence of the additional viral proteins, although the PASrp is the major capsid protein. To date, polymycoviruses have been found exclusively in fungi, both ascomycetes and, in the case of *Melampsora lini* (CAA45724), basidiomycetes.

As evident by the phylogenetic analysis (Fig. 1), putative members of the genus Polymycovirus, family Polymycoviridae group together. The proposed classification is also supported by the pairwise distance matrix (Fig. 2), illustrating that each virus has an evolutionary distance smaller than 0.65 compared to all putative members of the genus. Additionally, the phylogeny relationships of the methyltransferase are very similar to those of the RdRp sequences (Fig. 3). A more extensive phylogenetic analysis including members of the families *Astroviridae*, *Caliciviridae*, *Comoviridae*, *Partitiviridae*, *Picornaviridae* and *Potyviridae*, indicated that these are closely related taxa to polymycoviruses (Fig. 4), however the exact phylogenetic relationships among the families still remains to be elucidated. Members of the families *Chrysoviridae* and *Totiviridae* were used as outgroup.

The genomic organization of Aspergillus fumigatus tetramycovirus 1 (AfuTmV1), the proposed representative of the type species of the genus Polymycovirus, is depicted in Fig. 5. AfuTmV1 is the first and most well characterized polymycovirus and the first viral entity found to be infectious as double-stranded RNA (Kanhayuwa *et al*. 2015). Information on the exemplars representing the ten proposed species in the genus Polymycovirus can be found in Table 2.

The criteria to differentiate species within the family Polymycoviridae are:

* host of isolation
* amino acid sequence data (≤ 70% aa sequence identity in the RdRP)
* size and number of dsRNA segments
* presence of true capsid

All proposed species of the family Polymycoviridaeare represented by fully sequenced viruses with publically available accession numbers.

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**Figure 1:** Maximum likelihood phylogenetic tree created based on the RdRP sequences of polymycoviruses. The sequences were aligned with MUSCLE as implemented by MEGA 6 (Tamura *et al*., 2013), all positions with less than 30% site coverage were eliminated and the LG+G+F substitution model was used. The number in brackets after the virus name indicates the number of dsRNA genomic segments. At the end of the branches, blue and red indicate that the virus infects fungi and mammals, respectively; full circles indicate that the virus is a representative of a proposed species. The orange star indicates the representative of type species for the proposed genus Polymycovirus.

 

**Figure 2:** Pairwise distance matrix created based on the RdRP sequences of polymycoviruses and related viruses. The sequences were aligned with MUSCLE as implemented by MEGA 6 (Tamura *et al*., 2013), all positions with less than 30% site coverage were eliminated and the p-distance substitution model/method was used. Red background indicates high conservation while green background indicates low conservation.

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**Figure 3:** Maximum likelihood phylogenetic tree created based on the RdRP (top) and methyltransferase (bottom) sequences of polymycoviruses. The sequences were aligned with MUSCLE as implemented by MEGA 6 (Tamura *et al*., 2013) and all positions with less than 30% site coverage were eliminated. The LG+G+F and LG+G+I+F substitution models were used for the RdRP and methyltransferase sequences, respectively. The number in brackets after the virus name indicates the number of dsRNA genomic segments.

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**Figure 4:** Maximum likelihood phylogenetic tree created based on the RdRP sequences of polymycoviruses and other related viruses. The sequences were aligned with MAFTT using the G-INS-I algorithm; the phylogenetic tree was constructed by MEGA 6 (Tamura *et al*., 2013), all positions with less than 30% site coverage were eliminated and the JTT+G+F substitution model was used. The number in brackets after the virus name indicates the number of dsRNA genomic segments. At the end of the branches, blue, light blue, yellow, green, purple and red indicate that the virus infects filamentous fungi, yeast, protozoa, plants, birds and mammals, respectively.

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| **Figure 5:** Schematic representation of the genomic organization of Aspergillus fumigatus tetramycovirus 1, exemplar virus for the type species of genus Polymycovirus, family Polymycoviridae. The genome consists of four dsRNAs, each containing one ORF flanked by 5’- and 3’-UTRs. The light coloured boxes represent known and/or predicted motifs and domains. **Table 1:** Proposed organization of the family Polymycoviridae |
| **FAMILY: Polymycoviridae** |
|  **GENUS: Polymycovirus** |
|  **SPECIES:** Aspergillus fumigatus polymycovirus 1 |
|  Aspergillus spelaeus polymycovirus 1 |
|  Beauveria bassiana polymycovirus 1 |
|  Botryosphaeria dothidea polymycovirus 1 |
|  Cladosporium cladosporioides polymycovirus 1 |
|  Colletotrichum camelliae polymycovirus 1 |
|  Fusarium redolens polymycovirus 1 |
|  Magnaporthe oryzae polymycovirus 1  |
|  Penicillium brevicompactum polymycovirus 1 |
|  Penicillium digitatum polymycovirus 1  |

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| **Table 2:** Exemplars of the proposed species in genus Polymycovirus, family Polymycoviridae |
| **virus name & abbreviation** | **accession number & size** | **Reference** |
| Aspergillus fumigatus tetramycovirus 1 | dsRNA 1: HG975302 (2403 bp) | Kanhayuwa *et al*., 2015 |
| (AfuPmV1) | dsRNA 2: HG975303 (2233 bp) |  |
|  | dsRNA 3: HG975304 (1970 bp) |  |
|  | dsRNA 4: HG975305 (1131 bp) |  |
| Aspergillus spelaeus tetramycovirus 1 | dsRNA 1: MG887754 (2384 bp)  | unpublished |
| (AspTmV1) | dsRNA 2: MG887755 (2219 bp) |  |
|  | dsRNA 3: MG887756 (1968 bp) |  |
|  | dsRNA 4: MG887757 (1194 bp) |  |
| Beauveria bassiana polymycovirus 1 | dsRNA 1: LN896307 (2425 bp) | Kotta-Loizou & Coutts, 2017 |
| (BbPmV1) | dsRNA 2: LN896308 (2260 bp) |
|  | dsRNA 3: LN896309 (1921 bp) |
|  | dsRNA 4: LN896310 (1373 bp) |
| Botryosphaeria dothidea RNA virus 1  | dsRNA 1: KP245734 (2379 bp) | Zhai *et al.*, 2016 |
| (BdRV1) | dsRNA 2: KP245735 (2184 bp) |  |
|  | dsRNA 3: KP245736 (1967 bp) |  |
|  | dsRNA 4: KP245737 (1131 bp) |  |
|  | dsRNA 5: KP245738 (1060 bp) |  |
| Cladosporium cladosporioides virus 1  | dsRNA 1: KJ787686 (2434 bp) | unpublished |
| (CcV1) | dsRNA 2: KJ787687 (2241 bp) |  |
|  | dsRNA 3: KJ787688 (2008 bp) |  |
|  | dsRNA 4: KJ787689 (1261 bp) |  |
|  | dsRNA 5: KJ787690 (942 bp) |  |
| Colletotrichum camelliae filamentous virus 1  | dsRNA 1: KX778766 (2444 bp) | Jia *et al*., 2017 |
| (CcFV1) | dsRNA 2: KX778767 (2253 bp) |  |
|  | dsRNA 3: KX778768 (2012 bp) |  |
|  | dsRNA 4: KX778769 (1299 bp) |  |
|  | dsRNA 5: KX778770 (1122 bp) |  |
|  | dsRNA 6: KX778771 (1085 bp) |  |
|  | dsRNA 7: KX778772 (1053 bp) |  |
|  | dsRNA 8: KX778773 (990 bp) |  |
| Fusarium redolens polymycovirus 1  | dsRNA 1: MK609920 (2350 bp) | Mahillon *et al*., 2019 |
| (FrPmV1) | dsRNA 2: MK609921 (2256 bp) |  |
|  | dsRNA 3: MK609922 (2040 bp) |  |
|  | dsRNA 4: MK609923 (1298 bp) |  |
|  | dsRNA 5: MK609924 (1224 bp) |  |
|  | dsRNA 6: MK609925 (1015 bp) |  |
|  | dsRNA 7: MK609926 (1003 bp) |  |
|  | dsRNA 8: MK609927 (890 bp) |  |
| Magnaporthe oryzae polymycovirus 1  | dsRNA 1: MH231406 (2401 bp) | unpublished |
| (MoPmV1) | dsRNA 2: MH231407 (2233 bp) |  |
|  | dsRNA 3: MH231408 (1963 bp) |  |
|  | dsRNA 4: MH231409 (1324 bp) |  |
| Penicillium brevicompactum tetramycovirus 1 | dsRNA 1: MG887750 (2400 bp) | unpublished |
| (PbcTmV1) | dsRNA 2: MG887751 (2259 bp) |  |
|  | dsRNA 3: MG887752 (1930 bp) |  |
|  | dsRNA 4: MG887753 (1206 bp) |  |
| Penicillium digitatum polymycoviruses 1 | dsRNA 1: MF317878 (2382 bp) | Niu *et al*., 2018 |
| (PdPmV1) | dsRNA 2: MF317879 (2336 bp) |  |
|  | dsRNA 3: MF317880 (2016 bp) |  |
|  | dsRNA 4: MF317881 (1292 bp) |  |

| **References:** |
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| Jia H, Dong K, Zhou L, Wang G, Hong N, Jiang D, Xu W (2017) A dsRNA virus with filamentous viral particles. *Nat Commun* 8:168.Kanhayuwa L, Kotta-Loizou I, Özkan S, Gunning AP, Coutts RHA (2015) A novel mycovirus from *Aspergillus fumigatus* contains four unique dsRNAs as its genome and is infectious as dsRNA. *Proc Natl Acad Sci U S A* 112:9100-5.Kotta-Loizou I, Coutts RHA (2017) Studies on the virome of the entomopathogenic fungus *Beauveria bassiana* reveal novel dsRNA elements and mild hypervirulence. *PLoS Pathog* 13:e1006183.Mahillon M, Decroës A, Liénard C, Bragard C, Legrève A (2019) Full genome sequence of a new polymycovirus infecting *Fusarium redolens*. *Arch Virol* doi: 10.1007/s00705-019-04301-1.Niu Y, Yuan Y, Mao J, Yang Z, Cao Q, Zhang T, Wang S, Liu D (2018) Characterization of two novel mycoviruses from *Penicillium digitatum* and the related fungicide resistance analysis. *Sci Rep* 8:5513.Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* 30:2725-2729.Zhai L, Xiang J, Zhang M, Fu M, Yang Z, Hong N, Wang G (2016) Characterization of a novel double-stranded RNA mycovirus conferring hypovirulence from the phytopathogenic fungus *Botryosphaeria dothidea*. *Virology* 493:75-85. |