

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

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| **Code assigned:** | **2021.004F** |  |
| **Short title:** Create one new family (*Hadakaviridae*) including one new genus (*Hadakavirus*) and one new species | | |
|  | | |

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

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**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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| **Is any taxon name used here derived from that of a living person (Y/N)** | N |

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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 | May 24, 2021 |
| Date of this revision (if different to above) | September 16, 2021 |

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

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| **EC Comments**: please address typos in the Word file and adjust legend of the Table 1.  **Response**: Done. |

**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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| 2021.004F.R.Hadakaviridae\_newfam.xlsx |

**Abstract**

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| This document proposes to create a new genus *Hadakavirus* and a new family *Hadakaviridae* in the phylum *Pisuviricota*. One new species termed *Hadakavirus nanga* is proposed to be assigned to the genus *Hadakavirus*. The members in the family *Hadakaviridae* are characterized by capsidless, multi-segmented (ten-eleven), positive-sense RNA genome [(+)RNA]. Known hadakavirids infect fungi. Hadakavirids are most closely related to the members in the family *Polymycoviridae*, which currently are classified as an unassigned family in the Realm *Riboviria*. Nevertheless, hadakavirids show several distinct features, e.g., phylogenetic distance, low RNA-dependent RNA polymerase (RdRP) amino acid sequence identity (<30.4%), and the absence of the polymycovirus hallmark protein (proline-alanine-serine-rich protein, PASrp). Hadakavirids, as well as polymycovirids, show phylogenetic affinity to animal (+)RNA viruses such as calicivirids and astrovirids in the phylum *Pisuviricota*. Thus, the family *Hadakaviridae* can be accommodated in this phylum in the kingdom *Orthornavirae,* the realm *Riboviria*. The term “hadaka” means “naked” in Japanese. |

**Text of proposal**

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| |  |  |  |  |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | **Members in the proposed family *Hadakaviridae***  Members of the proposed family *Hadakaviridae* have multi-segmented positive-sense RNA [(+)RNA] genomes with a capsidless nature. This family is proposed to include a proposed new genus *Hadakavirus* containing a new species *Hadakavirus nanga.* This species includes two distinct isolates, namely hadaka virus 1 strain 7n (HadV1-7n) and hadaka virus 1 isolate 1NL (HadV1-1NL) (Table 1).  HadV1-7n, the exemplar isolate of *Hadakavirus nanga*, is the first hadakavirus which was discovered and characterized from an ascomycetous fungus *Fusarium oxysporum* (1). Hadaka virus name was coined to indicate capsidless nature of the virus, where ‘hadaka’ means ‘naked’ in Japanese (1). The second hadakavirus HadV1-1NL was reported from another ascomycetous fungus *Fusarium nygamai* (Khan et al. manuscript under submission). HadV1-7n has eleven genomic RNA segments (Fig. 1 left), while HadV1-1NL has ten (Fig. 1 right). HadV1-7n RNA1, RNA3 and RNA8 encode putative RNA-dependent RNA polymerase (RdRP), methyltransferase (MTR), and a protein containing zinc finger motif, respectively (Fig. 1). The putative functions of other proteins are unknown. HadV1-1NL RNA1 and RNA3 also encode RdRP or MTR, respectively, while lacks a segment homologous to HadV1-7n-RNA8.  **RdRP-based phylogeny of hadakavirids**  Based on a local sequence alignment (BLASTP), RdRPs of hadakavirids show sequence similarity only to the members of the family *Polymycoviridae* (1) (Khan et al. manuscript under submission), that show relationships with that of animal (+)RNA viruses in the genus *Vesivirus* in the family *Caliciviridae* (2). RdRP core motifs of hadakavirids show similarity to those of polymycovirids and viruses in the phylum *Pisuviricota* (“picornavirus supergroup", including calicivirids) (Fig. 2). However, like polymycovirids, the catalytic core residues in motif C of the RdRP of hadakavirids are GDNQ, a hallmark of many viruses in *Mononegavirales*, instead of GDD observed for most members of (+)ssRNA and dsRNA viruses (Fig. 2). RdRP-based phylogenetic analysis with polymycovirids and several viruses in the phylum *Pisuviricota* suggested that hadakavirids were most closely related to polymycovirids (Fig. 3). However, hadakaviruses belong to a lineage separated from polymycoviruses (Figs. 3 and 4).  Hadakavirids and polymycovirids clustered with several members in the family *Astroviridae*, though statistical support was not high (Fig. 3). Hadakavirids show closer phylogenetic affinity and evolutional distance to (+) RNA viruses such as astrovirids and calicivirids rather than dsRNA viruses in the family *Partitiviridae* (Figs. 3 and 4). Thus, hadakavirids should be classified as (+)RNA viruses based on the phylogenetic position, despite that relatively large amounts of their replicative form dsRNA accumulates as the case for of the established capsidless (+)ssRNA viruses of *Hypoviridae* and *Endornaviridae* (1). In conclusion, the family Hadakaviridae can be classified into the phylum *Pisuviricota*, the kingdom *Orthornavirae*, the realm *Riboviria*, while the class remained unassigned due to the lower branch probabilities. The family *Hadakaviridae* can be classified into a new order with the family *Polymycoviridae*, which is not determined here because the *Polymycoviridae* was not assigned to any order-kingdom.   |  | | --- | | Realm: *Riboviria* | | Kingdom: *Orthornavirae* | | Phylum: *Pisuviricota* | | Class: Unassigned | | Order: Unassigned | | Family: *Hadakaviridae* | | Genus: *Hadakavirus* |   **Difference between hadakavirids and polymycovirids**  Among the ten or eleven genomic segments of hadakavirids, three segments (RNA1, RNA2, and RNA3) encode hypothetical proteins [P1 (RdRP), P2 (a protein with unknown function), and P3 (MTR)] that show low-level of sequence identity to the counterparts of the polymycovirids (Figs. 5 and 6) (1) (Khan et al. manuscript in submission). Detected identity in P1, P2, and P3 sequences between hadakavirids and polymycovirids is less than 30.4%, 20.7%, and 23.3%, respectively (Fig. 5). However, hadakavirids lack the genomic segment encoding proline-alanine-serine-rich protein (PASrp) that is conserved among polymycovirids (1). PASrp is associated with double-stranded RNA (dsRNA) genome of polymycovirids (2-7).  The majority of polymycovirids lack true capsid and form an unstructured dsRNA-PASrp complex (semi-capsidless form), which is assumed to be the infectious (2-7). The dsRNA-PASrp complex of polymycovirids was precipitable by ultracentrifugation and tolerant to ribonuclease treatment in host tissue homogenates (1, 7). Unlike polymycovirids, genomic (+)ssRNA and replicative form of hadakavirids were not precipitable by ultracentrifugation and were susceptible to ribonuclease treatment in host tissue homogenates, due to the lack of PASrp and true capsid proteins (1). DsRNA of hadakavirids appears not to be infectious when experimentally introduced into protoplasts of the original host, *Fusarium oxysporum.* The infectious form of hadaka virus 1 remains unknown (1). In conclusion, hadakavirids have molecular properties distinct from polymycovirids. The family *Polymycoviridae* and *Hadakaviridae* can be distinguished by the phylogenetic distance, identity of the homologous proteins, the presence/ absence of PASrp, and the semi- or full-capsidless nature.  **Species criteria in the genus *Hadakavirus***  The proposed new genus *Hadakavirus* include only one species named *Hadakavirus nanga.* (Table 1) to classify two virus isolates (HadV1-7n and HadV1-1NL) that show 89.2% RdRP identity (Fig. 5A). As mentioned above, HadV1-7n and HadV1-1NL have similar but different genome segment number and organization (Fig. 1) and were isolated from different fungal species, *Fusarium oxysporum* and *F. nygami*. However, they should be classified into the same species according to the close phylogenetic distance and conserved amino acid sequence content of their RdRP proteins (Figs. 3-6). Species demarcation criteria will be set after more hadakavirids are reported.   |  |  |  | | --- | --- | --- | | New species | Exemplar isolate | GenBank sequence accession number | | *Hadakavirus nanga* | hadaka virus 1 isolate 7n | LC519840-LC519850 | | |

**Supporting evidence**

**Table 1.** Viruses in the family *Hadakaviridae*.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Species** | **Strain** | | | **Genome**  **info.** | **GenBank accession** | **Reference** |
| **Virus name** | **Isolate** | **Abbreviation** |
| *Hadakavirus nanga* | hadaka virus 1 | 7n | HadV1-7n | Complete | LC519840-LC519850 | [Sato et al. mBio2020](https://www.nature.com/articles/nmicrobiol20151) |
| 1NL | HadV1-1NL | Complete | LC592214-LC592223 | [Khan et al. 2021, manuscript under submission](https://link.springer.com/article/10.1007/s00705-013-1779-3) |

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**Figure 1: Genome organization of the two viral isolates of *Hadakavirus nanga*.** Accession numbers for the viruses are listed in Table 1. The left panel depicts eleven-genomic segments of hadaka virus 1 isolate 7n (HadV1-7n, the exemplar isolate of the species *Hadakavirus nanga*) genome. The right panel shows ten genomic segments of hadaka virus 1 isolate 1NL (HadV1-1NL) genome. The solid lines and black boxes represent genomic RNA segments and hypothetical open reading frames, respectively. Colored boxes indicate conserved domains [RNA-dependent RNA polymerase (RdRP) and methyltransferase (MTR) domains] or motif which were identified in proteins encoded by RNAs 1, 3 and 8 by BLASTP analyses. Arrows indicate homologous genomic segments conserved between HadV1-7n and HadV1-1NL.

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**Figure 2: Amino acid sequence alignment around the conserved motifs of RdRP.** The multiple sequence alignment of viral RdRPs listed in Table S1 was performed by online MAFFT version 7.475 with the L-INS-i method and default parameters (8). The alignment was visualized in MEGA X (9). Suffix for the viral order was omitted.

**ダイアグラム

自動的に生成された説明**

**Figure 3: RNA-dependent RNA polymerase (RdRP)-based phylogeny of hadakavirids.** A maximum-likelihood phylogenetic tree based on multiple amino acid sequence alignment of viral RdRPs is shown.Full names of viruses and accession numbers are listed in Table S1. This analysis involved the exemplar isolates of all the approved species in the family *Polymycoviridae* in addition to the several selected viruses from the diverse classes in the phylum *Pisuviricota*. The multiple sequence alignment was performed as described in the Fig. 2 legend above. The ambiguously aligned sites were deleted by online TrimAl version 1.3 with the Gappyout method (10). The phylogenetic tree was constructed with the best fit model LG+G+I (No of Discrete Gamma Categories = 5) in MEGA X version 10.2.3 (9). There was a total of 590 positions in the final dataset. The branch probability was tested by the bootstrap method with 500 iterations.

**グラフ, ウォーターフォール図

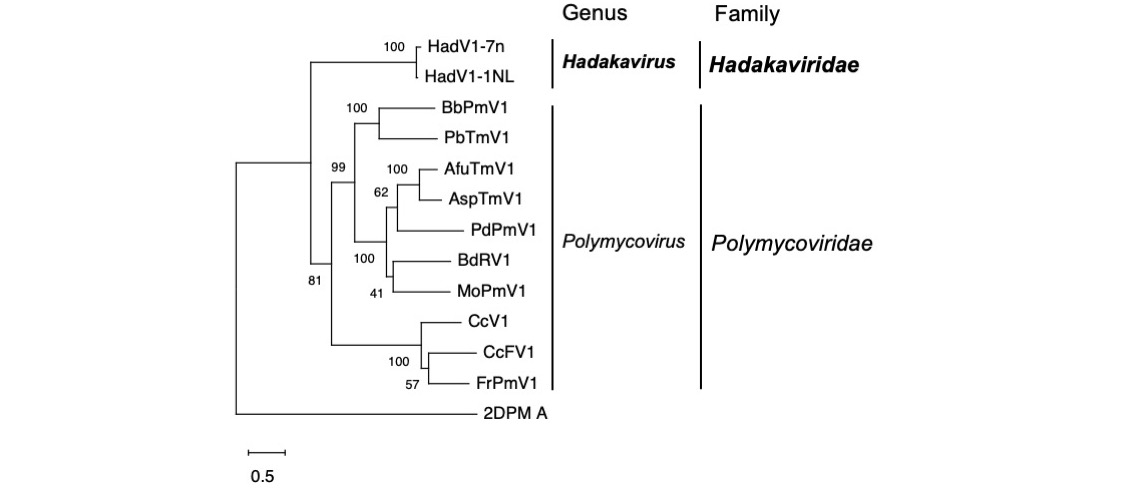
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**Figure 4: Pairwise evolutionary distance matrix based on the part of RdRP alignment.** The matrix illustrates the pairwise evolutionary distance calculated by the maximum likelihood method for the phylogenetic tree construction in Fig. 3. See Fig. 3 legend for the method. The evolutionary distance values were converted into the heatmap with R package “gplots” version 3.1.1. Suffix for the viral order was omitted.

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**Figure 5: Pairwise percent identity matrix based on a global amino acid sequence alignment.** Identity of hadakavirus-P1 (RNA-dependent RNA polymerase, RdRP) (**A**), -P2 (hypothetical protein with unknown function) (**B**), and -P3 (methyltransferase, MTR) (**C**) were compared with the homologous proteins of polymycovirids and the others. Full names of the viruses and accession numbers are listed in Table S1 and S2. “2DPM\_A” in (**C**) is a bacterial MTR whose UniProtKB/Swiss-Prot accession number is P04043.1. The global alignment was performed by Clustal Omega version 1.2.4 with default settings (11). The heatmap was drawn with R package “gplots” version 3.1.1.

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**Figure 6: Phylogenetic relationship of methyltransferase (MTR) of hadakavirids and polymycovirids.** A maximum-likelihood phylogenetic tree based on multiple amino acid sequence alignment of MTRs. Accession numbers for the MTR of polymycovirids are listed in Table S1.“2DPM\_A” is a bacterial MTR whose UniProtKB/Swiss-Prot accession number is P04043.1. The multiple sequence alignment was performed by online MAFFT version 7.475 with the L-INS-i method and default parameters (8). The ambiguously aligned sites were deleted by online TrimAl version 1.3 with the Gappyout method (10). The phylogenetic tree was constructed with the best fit model LG+G+I (No of Discrete Gamma Categories = 5) in MEGA X version 10.2.3 (9). There was a total of 569 positions in the final dataset. The branch probability was tested by the bootstrap method with 500 iterations.

**Table S1.** Taxonomy of the viruses and accession numbers of the viral RdRP used for the phylogenetic analysis.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Phylum** | **Class** | **Order** | **Family** | **Genus** | **Species** | **Virus abbrev.** | **RdRP accession** |
| Unassigned | Unassigned | Unassigned | *Polymycoviridae* | *Polymycovirus* | *Aspergillus fumigatus polymycovirus 1* | AfuTmV1 | CDP74618.1 |
| *Aspergillus spelaeus polymycovirus 1* | AspTmV1 | AYP71805.1 |
| *Beauveria bassiana polymycovirus 1* | BbPmV1 | YP\_009352879.1 |
| *Botryoshaeria dothidea polymycovirus 1* | BdRV1 | YP\_009342446.1 |
| *Cladosporium cladosporioides polymycovirus 1* | CcV1 | YP\_009052470.1 |
| *Colletotrichum camelliae polymycovirus 1* | CcFV1 | ASV63092.1 |
| *Fusarium redolens polymycovirus 1* | FrPmV1 | QDH44656.1 |
| *Magnaporthe oryzae polymycovirus 1* | MoPmV1 | QAU09249.1 |
| *Penicillum brevicompactum polymycovirus 1* | PbTmV1 | AYP71801.1 |
| *Penicillium digitatum polymycovirus 1* | PdPmV1 | YP\_009551548.1 |
| *Pisuviricota* | *Pisoniviricetes* | *Picornavirales* | *Caliciviridae* | *Lagovirus* | *Rabbit hemorrhagic disease virus* | RHDV | NP\_062875.1 |
| *Nebovirus* | *Newbury 1 virus* | N1V | YP\_529550.1 |
| *Norovirus* | *Norwalk virus* | NV | NP\_056820.1 |
| *Sapovirus* | *Sapporo virus* | SV | YP\_077278.1 |
| *Vesivirus* | *Vesicular exanthema of swine virus* | VESV | NP\_066255.1 |
| *Picornaviridae* | *Cardiovirus* | *Cardiovirus A* | EMCV-1 | NP\_056777.1 |
| *Enterovirus* | *Enterovirus C* | CVA2 | YP\_009505603.1 |
| *Hepatovirus* | *Hepatovirus A* | HAV | NP\_041007.1 |
| *Kobuvirus* | *Aichivirus A* | AiV-A1 | NP\_047200.1 |
| *Parechovirus* | *Parechovirus A* | HPeV-1 | YP\_009505617.1 |
| *Stelpaviricetes* | *Stellavirales* | *Astroviridae* | *Avastrovirus* | *Avastrovirus 1* | TAstV | NP\_853540.1 |
| *Mamastrovirus* | *Mamastrovirus 1* | HAstV | YP\_009275017.1 |
| *Duplopiviricetes* | *Durnavirales* | *Partitiviridae* | *Alphapartitivirus* | *White clover cryptic virus 1* | WCCV1 | YP\_086754.1 |
| *Betapartitivirus* | *Atkinsonella hypoxylon virus* | AhV | NP\_604475.1 |
| *Cryspovirus* | *Cryptosporidium parvum virus 1* | CSpV1 | YP\_009508065.1 |
| *Deltapartitivirus* | *Pepper cryptic virus 1* | PCV1 | YP\_009466859.1 |
| *Gammapartitivirus* | *Penicillium stoloniferum virus S* | PsV-S | YP\_052856.2 |
| Unassigned | Yadokarivirales | Yadokariviridae | Alphayadokarivirus | Alphayadokarivirus 1 | YkV1 | YP\_009551451.1 |
| Betayadokarivirus | Betayadokarivirus 3 | YkV3 | BBB86810.2 |

**Table S2.** Accession numbers of the polymycovirus-encoded P2 and P3 proteins.

|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Virus abbrev.** | **Accession** | |
| **P2** | **P3 (MTR)** |
| *Aspergillus fumigatus polymycovirus 1* | AfuTmV1 | CDP74619.1 | CDP74620.1 |
| *Aspergillus spelaeus polymycovirus 1* | AspTmV1 | AYP71808.1 | AYP71807.1 |
| *Beauveria bassiana polymycovirus 1* | BbPmV1 | YP\_009352876.1 | YP\_009352877.1 |
| *Botryoshaeria dothidea polymycovirus 1* | BdRV1 | YP\_009342447.1 | YP\_009342470.1 |
| *Cladosporium cladosporioides polymycovirus 1* | CcV1 | YP\_009052471.1 | YP\_009052472.1 |
| *Colletotrichum camelliae polymycovirus 1* | CcFV1 | ASV63093.1 | ASV63094.1 |
| *Fusarium redolens polymycovirus 1* | FrPmV1 | QDH44657.1 | QDH44658.1 |
| *Magnaporthe oryzae polymycovirus 1* | MoPmV1 | QAU09250.1 | QAU09251.1 |
| *Penicillum brevicompactum polymycovirus 1* | PbTmV1 | AYP71804.1 | AYP71803.1 |
| *Penicillium digitatum polymycovirus 1* | PdPmV1 | YP\_009551551.1 | YP\_009551549.1 |

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