

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

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| **Code assigned:** | **2022.005F** |  |
| **Short title:** Create a new family ("*Mamonoviridae*"), a genus ("*Medusavirus*"), and two species ("*Medusavirus medusae*" and "*Medusavirus sthenus*") in the phylum *Nucleocytoviricota* |
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**List the ICTV Study Group(s) that have seen this proposal**

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| Fungal and Protist Viruses Subcommittee Chair |

**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** |
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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)** |  |

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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 | 23 May, 2022 |
| Date of this revision (if different to above) | 21 September,2022 |

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

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| EC Comments: 1. Please consider renaming genus and/or family names to avoid the same stem Medusavirus – Medusaviridae (optional).
2. Please indicate demarcation criteria for members of distinct species (indispensable).
3. Please consider changing ending of the species epithet to read "*Medusavirus medusae*" and "*Medusavirus sthenus*" to comply correct declension if intended to apply Latinized binomials (optional).

Authors response:1. We discussed proposed change and renamed family to "*Mamonoviridae*". We originally opted for "*Gorgonviridae*", but realized that word "Gorgon" has already been used for naming subfamily of bacterial viruses (*Gorgonvirinae*)
2. As requested, we reported species demarcation criteria in this version of the Taxonomic proposal
3. We accepted suggested changes in species names.
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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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| 2022.005F.N.v2.Mamonoviridae\_newfam.xlsx |

**Abstract**

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| Acanthamoeba castellanii medusavirus J1 is a “giant virus” isolated from a hot spring in Japan in 2019. Recently, a close relative of this virus was isolated in Japan named medusavirus stheno T3. Here, we propose to create two new species ("*Medusavirus medusae*" and "*Medusavirus sthenus*"), a new genus "*Medusavirus*" and a new family "*Mamonoviridae*" within the phylum *Nucleocytoviricota* to classify these two viruses. We describe their morphological and genomic features and gene content similarities along with phylogenetic analyses to support the proposal. |

**Text of proposal**

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| **To create a new family "*Mamonoviridae*"** Recently, two viruses were discovered by co-culturing with *Acanthamoeba castellanii*. The first one was isolated from a hot spring in Japan and was named Acanthamoeba castellanii medusavirus J1 (ACMV-J1), because the host amoebae tend to form cysts upon infection with this virus [1]. The second isolate was a close relative of the first virus and named medusavirus stheno T3 (MVS-T3), because Stheno is a sister of Medusa in Greek mythology [2]. Both viruses show substantial morphological and genomic similarities with the members of *Nucleocytoviricota*. However, these viruses were not phylogenetically close enough to any member of established families within *Nucleocytoviricota*. Thus, we propose to create two species, "*Medusavirus medusae*" and "*Medusavirus sthenus*", new genus "*Medusavirus*" and a new family "*Mamonoviridae*", in the class *Megaviricetes* of the phylum *Nucleocytoviricota*.The virions of ACMV-J1 (proposed to represent species "*Medusavirus medusae*") have an icosahedral shape with a diameter of approximately 260 nm, including surface spikes, as revealed by cryo-electron microscopy [3]. It includes a linear, double-stranded DNA (dsDNA) genome of 381,277 bp with a high G+C content (61.7%) [1]. A total of 461 open reading frames (ORFs) have been predicted in the genome. ACMV-J1 encodes five of the seven core genes of *Nucleocytoviricota* that are frequently used in phylogeny [4]. These are major capsid protein (MCP), superfamily II helicase (SFII), DNA polymerase family B (PolB), A32-like packaging ATPase (A32), and virus late transcription factor (VLTF3). However, ACMV-J1 is unique among other amoeba-infecting giant viruses in encoding a full set of histone proteins (i.e., linker histone H1, and core histones H2A, H2B, H3, and H4) and lacking two of the core genes, namely, RNA polymerase and DNA topoisomerase II (TopoII). Virus medusavirus stheno T3 (MVS-T3), representative of a proposed species "*Medusavirus sthenus*", was isolated in 2021 [2]. This virus has a G+C-rich (62.64%), 362,811 bp-long dsDNA genome. The average nucleotide identity (ANI) between ACMV-J1 and MVS-T3, proposed members of species "*Medusavirus medusae*" and "*Medusavirus sthenus*", is 79.5%. MVS-T3 has the same set of core genes as members of "*Medusavirus medusae*" and also encodes a full set of histones, but the genes for H3 and H4 are fused into a single gene. **Relationship with classified viruses**To clarify the relationship between medusaviruses and other members of the *Nucleocytoviricota*, we used the seven core genes from GVOGs, which have been reported to have the optimum performance for phylogenetic analysis of viruses belonging to the *Nucleocytoviricota* (i.e., PolB, SFII, A32, VLTF3, TopoII, TFIIB, RNAPL) [4]. The two medusaviruses formed an independent clade in the phylum *Nucleocytoviricota* with a high branch support (SH-aLRT = 100%, Ultrafast bootstrap = 100%) (Fig. 1), in consistent with a previous study that demonstrated that ACMV-J1 does not belong to any virus group identified so far [1]. In the tree, medusaviruses are close to Feldmannia species virus, Ectocarpus siliculosus virus 1, coccolithoviruses, pandoraviruses and molliviruses, which were previously suggested to form a putative order, "*Pandoravirales*" [4]. However, the branch support for this clade was weak (SH-aLRT = 97.9% and Ultrafast bootstrap = 58%). Thus, we propose to create a family "*Mamonoviridae*" and a genus "*Medusavirus*". **To create two new species within "*Mamonoviridae*"** We propose to create two new species "*Medusavirus medusae*" and "*Medusavirus sthenus*" within the proposed new family "*Mamonoviridae*". The criteria used for species demarcation are genome size of member viruses, number of predicted genes, number of shared ortholog groups (OGs), ANI, tetranucleotide similarity and tip distance from the tree.The two viruses proposed to be classified in "*Medusavirus medusae*" and "*Medusavirus sthenus*" showed similar genome size (381kbp and 362kbp), G+C contents (61.7% and 62.64%), number of predicted genes (461 and 429) and the core gene sets. Their genomes can be colinearly aligned (ANI = 79.5%, tetranucleotide similarity = 0.992) [2]. Among 429 predicted genes of MSV-T3 ("*Medusavirus sthenus*"), 81% had their best hit with ACMV-J1 genome ("*Medusavirus medusae*”).**Relationship between medusaviruses and clandestinovirus**Recently, another giant virus named clandestinovirus was isolated by co-culture with another host, *Vermamoeba vermiformis*, in France [5]. The clandestinovirus shows a larger genome, more genes, and a lower G+C content (581 kbp, 617 genes, 43.5%) than medusaviruses. In terms of core genes, clandestinovirus encodes all core genes that medusaviruses have and additionally encodes RNA polymerase and TopoII. The clandestinovirus also carries out a nucleo-cytoplasmic infection like medusaviruses that enter and turn the host nucleus into the viral factory. A previous study has shown that the closest relative of clandestinovirus is ACMV-J1 in terms of the core genes [5]. Here, we used a quantitative way to draw a family-level boundary to figure out the relationship between clandestinovirus and medusaviruses. We compared these three viruses in terms of the nucleotide level similarity, including ANI and tetra-nucleotide similarity (TETRA), phylogenomic distance by calculating the distance between tips on the phylogenomic tree, and number of shared OGs. We then compared these metrics between them to the inter- and intra-family metrics of other virus families. As a result, the relationship between medusaviruses and clandestinovirus lies in the middle of inter-family and intra-family levels.In terms of phylogenomic tree, the clandestinovirus branched together with medusaviruses with a high branch support (Ultrafast bootstrap = 100%, SH-aLRT = 98.8%) (Fig. 1). However, in terms of the distance between tips, the tip distances (3.92 to ACMV-J1 and 3.95 MVS-T3) lay between mean values for intra-family (2.46) and inter-family distances (7.30) (Fig. 2a).In terms of genome-level nucleotide similarity, ANI and TETRA were calculated by python package pyani [6]. The ANI between clandestinovirus and the two medusaviruses were both 0, whereas the averages of intra- and inter-family were 0.36 and 0.01, respectively. In addition, only kaumoebavirus had non-zero ANI (0.68) against clandestinovirus. The TETRA between medusaviruses and clandestinovirus were both 0.32, which was lower than the average of inter-family TETRA (0.38). In addition, TETRA between clandestinovirus and medusaviruses only ranked 134th and 139th among 220 comparisons between clandestinovirus and other viruses. (Fig. 2b&2c).We then used Orthofinder v.2.5.2 to identify OGs and calculated the gene-sharing level $S\_{ij}$ based on the number of shared OGs between viral genomes [7]. The number of shared OGs was normalized by the total number of OGs of each virus in comparison by the following formula:$$S\_{ij} = \frac{OG\_{ij}}{\sqrt{OG\_{i} × OG\_{j}}}$$Here, $OG\_{ij}$ is the number of shared OGs between virus $i$ and $j$, and $OG\_{i}$ is the total number of OGs in virus $i$. The gene-sharing level between clandestinovirus and medusaviruses (0.16 to ACMV-J1, ranked 30th among all comparisons between clandestinovirus and other viruses; 0.17 to MVS-T3, 25th) lay between the mean values for intra- and inter-family levels (0.47 and 0.07, respectively) (Fig. 2d).Among known viruses, clandestinovirus is the closest relative of medusaviruses. However, they show large divergence that places their phylogenetic relationships in the middle of intra- and inter-family levels. Thus, at this moment, we do not propose to include the clandestinovirus into the proposed new family "*Mamonoviridae*".**Proposed demarcation criteria**We propose the following criteria for species and genus demarcations within the family "*Mamonoviridae*":* If a virus exhibits >95% ANI, similar morphology, and comparable genome size to the members of a recognized species in the genus "*Medusavirus*" (e.g., "*Medusavirus medusae*" and "*Medusavirus sthenus*"), it should be considered a new isolate and be classified in the same species.
* If a new virus shares <95% ANI with recognized members and exhibits similar properties then such virus should represent a new species in the genus.
* We also propose a genus ANI cutoff of 70% ANI, along with similar morphology, and comparable compositions of core genes with members of the proposed genus "*Medusavirus*". This value (70% ANI) is comparable with intra-genus differences in the five families of the phylum *Nucleocytoviricota* (i.e., *Mimiviridae*, *Ascoviridae*, *Phycodnaviridae*, *Poxviridae* and *Iridoviridae*).

For a virus distantly related to the members of this proposed family "*Mamonoviridae*", its inclusion in or exclusion from the family should be considered based on phylogenomic analyses like those we presented in this study. We acknowledge that these criteria are flexible and could be revisited in the future. **Etymology** Species and genus nomenclature derives from names of two Gorgon sisters from Greek mythology (Medusa and Stheno), while family name comes from Japanese word “mamono” (魔物) meaning “monster”.  |

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**Supporting evidence**



Fig. 1 Maximum-likelihood phylogenetic trees based on concatenated amino acid sequence of seven marker genes constructed using MAFFT (v.7.471), trimAl (v.1.4.1), and IQ-TREE 2 (v.2.1.3) [8–10]. The model was LG+F+R8 selected by the built-in Modelfinder of IQ-TREE 2 [11]. The branch supports were computed by 1000 ultrafast bootstrap and SH-aLRT [12]. The tree was visualized by iTOL, the round labels on branches represent high confidence supports with Ultrafast bootstrap ≥ 95%, SH-aLRT ≥ 80%.



Fig. 2 Density plot of (a) Tip distance; (b) ANI; (c) TETRA; (d) Normalized OGs sharing level. Blue area represents intra-family comparisons, while red area represents inter-family comparisons. The vertical black line represents the value between clandestinovirus and Acantoamoeba castellanii medusavirus J1 (ACMV-J1, an exemplar isolate of the proposed species "*Medusavirus medusae*"). The dashed line represents the average value of intra-family levels and the dotted line represents the average value of inter-family level.

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