

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

|  |  |  |
| --- | --- | --- |
| **Code assigned:** | **2022.002A** |  |
| **Short title:** Create one new order, ‘*Atroposvirales*’ and two new families, ‘*Verdandiviridae*’ and ‘*Skuldviridae*’ for classification of viruses of Asgardarchaeota | | |
|  | | |

**Author(s) and email address(es)**

|  |  |
| --- | --- |
| Medvedeva S, Sun J, Yutin N, Koonin EV,  Nunoura T, Rinke C, Krupovic M | [sof.medv@gmail.com](mailto:sof.medv@gmail.com); [jiarui.sun@uq.edu.au](mailto:jiarui.sun@uq.edu.au); [yutin@ncbi.nlm.nih.gov](mailto:yutin@ncbi.nlm.nih.gov); [koonin@ncbi.nlm.nih.gov](mailto:koonin@ncbi.nlm.nih.gov); [takuron@jamstec.go.jp](mailto:takuron@jamstec.go.jp); [c.rinke@uq.edu.au](mailto:c.rinke@uq.edu.au); [mart.krupovic@pasteur.fr](mailto:mart.krupovic@pasteur.fr) |

**Author(s) institutional address(es) (optional)**

|  |
| --- |
|  |

**Corresponding author**

|  |
| --- |
| Krupovic M |

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

|  |
| --- |
| Archaeal Viruses Subcommittee |

**ICTV Study Group comments and response of proposer**

|  |
| --- |
|  |

**ICTV Study Group votes on proposal**

|  |  |  |  |
| --- | --- | --- | --- |
| **Study Group** | **Number of members** | | |
| **Votes support** | **Votes against** | **No vote** |
|  |  |  |  |
|  |  |  |  |

**Authority to use the name of a living person**

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

|  |  |  |
| --- | --- | --- |
| **Taxon name** | **Person from whom the name is derived** | **Permission attached (Y/N)** |
|  |  |  |
|  |  |  |
|  |  |  |

**Submission dates**

|  |  |
| --- | --- |
| Date first submitted to SC Chair |  |
| Date of this revision (if different to above) |  |

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

|  |
| --- |
| Lokiarchaeia virus SkuldV3 was represented by the GenBank accession number of a metagenomics contig (rather than a dedicated accession for the virus genome). Now a GenBank accession number was obtained for the virus TPA (BK062752). |

**Part 3:** **TAXONOMIC PROPOSAL**

**Name of accompanying Excel module**

|  |
| --- |
| 2022.002A.N.v2.Verdandiviridae\_nf\_Atrospovirales\_no.xlsx |

**Abstract**

|  |
| --- |
| Asgardarchaeota are an expansive group of metabolically versatile archaea that thrive primarily in anoxic sediments around the globe. Here we propose the classification for two groups of viruses discovered by metagenomics and associated with asgardarchaeal hosts. The two groups, representing two new families, ‘*Verdandiviridae’* and ‘*Skuldviridae’*, are distantly related to bacterial and archaeal viruses of the classes *Caudoviricetes* and *Tectiliviricetes*, respectively. |

**Text of proposal**

|  |  |
| --- | --- |
| |  | | --- | | Asgardarchaeota are an expansive group of metabolically versatile archaea that thrive primarily in anoxic sediments around the globe [4, 9, 14, 15, 17]. The vast majority of species in the Asgardarchaeota have been discovered through metagenomics, whereas only one species has been isolated and successfully grown in the laboratory [6]. Asgardarchaeota gained prominence due to their inferred key role in the origin of eukaryotes [10]. Indeed, Heimdallarchaeia form a sister group to eukaryotes in most phylogenetic analyses [9, 17]. No viruses infecting members of the Asgardarchaeota have been isolated, primarily due to the inherent difficulty to propagate asgardarchaeal hosts. However, three new virus groups (Fig. 1) have been recently discovered through metagenomics in deep-sea sediment samples and assigned to Asgardarchaeota hosts using various computational approached, including CRISPR matching and k-mer analysis [11]. Here we classify two of these groups for which complete genomes are available in GenBank.  The first of the two groups, which we refer to as verdandiviruses, represents viruses distantly related to tailed bacterial and archaeal viruses of the class *Caudoviricetes* and encode the HK97-like MCP, the hallmark protein of realm *Duplodnaviria*, large subunit of the terminase (genome packaging ATPase-nuclease), the portal protein as well as several other structural proteins, including tail components (Fig. 2a). Verdandivirus proteins generally show no significant sequence similarity (BLASTP, E<1e-5) to proteins encoded by other known viruses. However, functional annotation for some of the proteins was enabled by sensitive profile-profile comparisons, in which HK97-like MCP and TerL MCP were readily identified with highly significant scores. Structural modeling of the VerdaV1 MCP using RoseTTAFold [2], yielded a model with the canonical HK97-like fold (Fig. 2b). Given the extreme divergence of verdandiviruses from other known viruses, meaningful phylogenetic analysis can only be performed within the group.  We next used ViPTree to formally analyze the relationship between verdandiviruses and other archaeal members of the class *Caudoviricetes*. The proteome-based ViPTree analysis [12] has accurately recovered all established families of haloarchaeal and methanogenic *Caudoviricetes* viruses [8] and, based on the same genetic distances (branch length of 0.05) observed between the established archaeal virus families, placed verdnadiviruses into a separate family (Fig. 3). Thus, for their classification, we propose to create a new family, ‘*Verdandiviridae*’ (for Verdandi, one of the three Norns, the most powerful beings in Norse mythology that govern the lives of gods and mortals).  Genomes of 5 verdandiviruses were recovered as circular contigs, suggesting their completeness, with three of them available in GenBank. Gene-sharing network analysis using vConTACT v2.0 (Fig. 1), which has been specifically developed and calibrated to identify genus-level groupings of prokaryotic viruses [3], places the five viruses into two different genus-level clusters. Cluster 1 includes VerdaV2, whereas cluster 2 includes VerdaV1, VerdaV4, VerdaV5 and VerdaV6. The same grouping is also recovered in the ViPTree proteomic tree (Fig. 3). Viruses in the two clusters display appreciable sequence similarity only within the capsid formation and genome packaging modules, whereas the tail proteins are considerably more divergent (Fig. 3a). Furthermore, viruses from the two clusters were assigned to Asgardarchaeota lineages Thorarchaeia and Lokiarchaeia, respectively. Accordingly, for classification of the viruses from the two clusters, we propose creating two new genera, ‘*Tonitrusvirus*’ (from Latin *tonitrus* for thunder, referring to the predicted Thorarchaeia host of VerdaV2) and ‘*Dolusvirus*’ (from Latin *dolus* for trickery, referring to the predicted Lokiarchaeia host of viruses in this genus).  **Demarcation criteria**  We propose using 95% sequence identity as a species demarcation criterion, to be consistent with the classification of other bacterial and archaeal viruses in the class *Caudoviricetes*. Family demarcation was established based on the global proteomic tree calculated using ViPTree.  The second group of viruses associated with the Asgardarchaeota hosts, which we refer to as skuldviruses, is distantly related to members of the realm *Varidnaviria*. Similar to other varidnaviruses, skuldviruses encode double jelly-roll (DJR) major capsid proteins and putative genome packaging ATPases of the FtsK-HerA superfamily (which is not directly related to the terminases encoded by members of the *Duplodnaviria*).  Three skuldvirus genomes were discovered by metagenomics (Fig. 4a). SkuldV1 and SkuldV3 were assembled as circular contigs and are considered to represent complete genomes. The vast majority of skuldvirus proteins (97%; 66 of the 68 proteins from 3 skuldviruses) show no similarity to proteins encoded by other known viruses. Network analysis using CLANS [5] showed that the skuldvirus MCPs formed a cluster separate from the previously characterized [16] groups of DJR MCPs (Fig. 4b). Nevertheless, profile-profile comparisons showed that they are most closely related to the corresponding proteins of prokaryotic viruses of the families *Corticoviridae* (bacteriophage PM2: HHsearch probability of 98.3), *Turriviridae* (archaeal virus STIV: HHsearch probability of 97.8) and *Tectiviridae* (bacteriophage PRD1: HHsearch probability of 96.2), whereas eukaryotic viruses with DJR MCPs were recovered with considerably lower scores (*Phycodnaviridae*, Pyramimonas orientalis virus: HHsearch probability of 83.4). Structural comparison of the SkuldV1 MCP model obtained using RoseTTAFold [2] (Fig. 4b) further showed that it is most similar to the MCP of Pseudoalteromonas phage PM2 [1], a prototype of the *Corticoviridae* [13]. Nevertheless, skuldviruses do not share genes with other known viruses other than those encoding the MCP and the genome packaging ATPase. Corticoviruses, turriviruses and tectiviruses belong to the class *Tectiliviricetes* [7]. We propose that skuldviruses represent a separate virus family within the *Tectiliviricetes*, which we propose to name ‘*Skuldviridae*’ (for Skuld, another of the three Norns). To bridge the gap between the class and family ranks, as is the case of other families within *Tectiliviricetes*, we also propose creating a monotypic order, ‘*Atroposvirales*’ (for Atropos, one of the three Fates in Greek mythology, equivalent to Norns in Norse mythology).  SkuldV1 and SkuldV3 have collinear genomes (Fig. 4a), which display 87.12% nucleotide identity over 87% of their lengths. We propose using 95% sequence identity as a species demarcation criterion, to be consistent with the classification of most other bacterial and archaeal viruses. Accordingly, the two viruses will be classified into two separate species of the same genus, ‘*Delusorvirus*’ (from Latin *delusor* for deceiver, referring to the predicted Lokiarchaeia host of viruses in this genus).  The proposed taxonomy is summarized in Table 1. | |

**Supporting evidence**

**Table 1. Proposed taxonomy of Asgard archaeal viruses.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Order** | **Family** | **Genus** | **Species** | **Virus name** | **Genome length (bp)** | **Accession number** |
|  | *Verdandiviridae* | *Tonitrusvirus* | *Tonitrusvirus shimokitaense* | Thorarchaeia virus VerdaV2 | 19541 | LC711078 |
|  |  | *Dolusvirus* | *Dolusvirus shimokitaense* | Lokiarchaeia virus VerdaV1 | 19940 | LC711077 |
|  |  | *Dolusvirus* | *Dolusvirus pacificense* | Lokiarchaeia virus VerdaV4 | 20488 | LC711080 |
| *Atroposvirales* | *Skuldviridae* | *Delusorvirus* | *Delusorvirus hikurangiense* | Lokiarchaeia virus SkuldV1 | 11290 | OK558607 |
|  |  | *Delusorvirus* | *Delusorvirus cascadiense* | Lokiarchaeia virus SkuldV3 | 11372 | BK062752 |

****

**Figure 1.** **Asgardarchaeal viruses and MGEs.** The network-based analysis of shared protein clusters (PCs) among asgardarchaeal viruses and the prokaryotic dsDNA viruses. The nodes represent viral genomes, and the edges represent the strength of connectivity between each genome based on shared PCs. Nodes representing genomes of the three groups of asgardarchaeal viruses are shown in red and the three groups are circled with yellow background. Nodes corresponding to other bacterial and archaeal viruses are shown in blue and orange, respectively.



**Figure 2. Diversity of verdandiviruses.** **a,** Genome maps of verdandiviruses. Homologous genes are shown using the same colors and the key is provided at the bottom of the panel. Also shown is the deduced schematic organization of the verdandivirus virion with colors marching those of the genes encoding the corresponding proteins. Genes encoding putative DNA-binding proteins with Zn-binding and helix-turn-helix domains are colored in black and grey, respectively. Colored circles indicate the positions of protospacers. Grey shading connects genes displaying sequence similarity at the protein level, with the percent of sequence identity depicted with different shades of grey (see scale at the bottom). Asterisks denote complete genomes assembled as circular contigs. Abbreviations: PAPSR, phosphoadenosine phosphosulfate reductase; TMP, tail tape measure protein; MTP, major tail protein; MCP, major capsid protein; TerL, large subunit of the terminase. **b,** Comparison of the structural model of the major capsid protein of verdnadivirus VerdaV1 with the corresponding structures of siphoviruses TW1 and HK97. The models are colored according to the secondary structure: α-helices, dark blue; β-strands, light blue.

A picture containing graphical user interface

Description automatically generated

**Figure 4.** Classification of archaeal *Caudoviricetes*. A. The Viral Proteomic Tree (ViPTree) of archaeal tailed viruses. The tree is constructed using BIONJ based on all-versus-all genomic similarity matrix, and mid-point rooted. Branch lengths are log-scaled. The branch length for family-level demarcation is around 0.05.



**Figure 4.** **Diversity of skuldviruses.** **a,** Genome maps of skuldviruses and PM2 virus. Homologous genes are shown using the same colors and the key is provided at the bottom of the panel. Also shown is the deduced schematic organization of the skuldvirus virion with colors marching those of the genes encoding the corresponding proteins. Colored circles indicate the positions of protospacers. Grey shading connects genes displaying sequence similarity at the protein level, with the percent of sequence identity depicted with different shades of grey (see scale on the right). Asterisks denote complete genomes assembled as circular contigs. Abbreviations: RCRE, rolling circle replication endonuclease; DJR-MCP, double jelly-roll major capsid protein; HTH, helix-turn-helix. Genome map of corticovirus PM2 is shown for comparison. **b,** Sequence similarity network of prokaryotic virus DJR MCPs. Protein sequences were clustered by the pairwise sequence similarity using CLANS. Lines connect sequences with CLANS P-value ≤ 1e−04. CLANS uses p-values of BLASTP comparisons calculated from Poisson distribution of high scoring segment pairs. Different previously defined groups [16] of DJR MCP are shown as clouds of differentially colored circles, with the key provided on the right. PRD1, Toil, and Bam35 subgroups are named after the corresponding members of the family *Tectiviridae*. Skuldviruses are highlighted within a yellow circle. When available, MCP structures of viruses representing each group are shown next to the corresponding cluster (PDB accession numbers are given in the parenthesis). The skuldvirus cluster is represented by a structural model of the SkuldV1 MCP. The models are colored according to the secondary structure: α-helices, dark blue; β-strands, light blue.

**References**

1. Abrescia NG, Grimes JM, Kivela HM, Assenberg R, Sutton GC, Butcher SJ, Bamford JK, Bamford DH, Stuart DI (2008) Insights into virus evolution and membrane biogenesis from the structure of the marine lipid-containing bacteriophage PM2. Mol Cell 31:749-761. Doi:10.1016/j.molcel.2008.06.026. PMID:18775333

2. Baek M, DiMaio F, Anishchenko I, Dauparas J, Ovchinnikov S, Rie Lee G, Wang J, Cong Q, Kinch LN, Schaeffer RD, Millán C, Park H, Adams C, Glassman CR, DeGiovanni A, Pereira JH, Rodrigues AV, van Dijk AA, Ebrecht AC, Opperman DJ, Sagmeister T, Buhlheller C, Pavkov-Keller T, Rathinaswamy MK, Dalwadi U, Yip CK, Burke JE, Garcia KC, Grishin NV, Adams PD, Read RJ, Baker D (2021) Accurate prediction of protein structures and interactions using a three-track neural network. Science 373:871-876. Doi:10.1126/science.abj8754.

3. Bin Jang H, Bolduc B, Zablocki O, Kuhn JH, Roux S, Adriaenssens EM, Brister JR, Kropinski AM, Krupovic M, Lavigne R, Turner D, Sullivan MB (2019) Taxonomic assignment of uncultivated prokaryotic virus genomes is enabled by gene-sharing networks. Nat Biotechnol 37:632-639. Doi:10.1038/s41587-019-0100-8. PMID:31061483

4. Dombrowski N, Teske AP, Baker BJ (2018) Expansive microbial metabolic versatility and biodiversity in dynamic Guaymas Basin hydrothermal sediments. Nat Commun 9:4999. Doi:10.1038/s41467-018-07418-0. PMID:30479325

5. Frickey T, Lupas A (2004) CLANS: a Java application for visualizing protein families based on pairwise similarity. Bioinformatics 20:3702-3704. Doi:10.1093/bioinformatics/bth444. PMID:15284097

6. Imachi H, Nobu MK, Nakahara N, Morono Y, Ogawara M, Takaki Y, Takano Y, Uematsu K, Ikuta T, Ito M, Matsui Y, Miyazaki M, Murata K, Saito Y, Sakai S, Song C, Tasumi E, Yamanaka Y, Yamaguchi T, Kamagata Y, Tamaki H, Takai K (2020) Isolation of an archaeon at the prokaryote-eukaryote interface. Nature 577:519-525. Doi:10.1038/s41586-019-1916-6. PMID:31942073

7. Koonin EV, Dolja VV, Krupovic M, Varsani A, Wolf YI, Yutin N, Zerbini FM, Kuhn JH (2020) Global Organization and Proposed Megataxonomy of the Virus World. Microbiol Mol Biol Rev 84Doi:10.1128/MMBR.00061-19. PMID:32132243

8. Liu Y, Demina TA, Roux S, Aiewsakun P, Kazlauskas D, Simmonds P, Prangishvili D, Oksanen HM, Krupovic M (2021) Diversity, taxonomy, and evolution of archaeal viruses of the class *Caudoviricetes*. PLoS Biol 19:e3001442. Doi:10.1371/journal.pbio.3001442. PMID:34752450

9. Liu Y, Makarova KS, Huang WC, Wolf YI, Nikolskaya AN, Zhang X, Cai M, Zhang CJ, Xu W, Luo Z, Cheng L, Koonin EV, Li M (2021) Expanded diversity of Asgard archaea and their relationships with eukaryotes. Nature 593:553-557. Doi:10.1038/s41586-021-03494-3. PMID:33911286

10. Lopez-Garcia P, Moreira D (2020) The Syntrophy hypothesis for the origin of eukaryotes revisited. Nat Microbiol 5:655-667. Doi:10.1038/s41564-020-0710-4. PMID:32341569

11. Medvedeva S, Sun J, Yutin N, Koonin EV, Nunoura T, Rinke C, Krupovic M (2022) Three families of Asgard archaeal viruses identified in metagenome-assembled genomes. Nat Microbiol In press.

12. Nishimura Y, Yoshida T, Kuronishi M, Uehara H, Ogata H, Goto S (2017) ViPTree: the viral proteomic tree server. Bioinformatics 33:2379-2380. Doi:10.1093/bioinformatics/btx157. PMID:28379287

13. Oksanen HM, ICTV Report Consortium (2017) ICTV Virus Taxonomy Profile: *Corticoviridae*. J Gen Virol 98:888-889. Doi:10.1099/jgv.0.000795. PMID:28581380

14. Spang A, Saw JH, Jorgensen SL, Zaremba-Niedzwiedzka K, Martijn J, Lind AE, van Eijk R, Schleper C, Guy L, Ettema TJG (2015) Complex archaea that bridge the gap between prokaryotes and eukaryotes. Nature 521:173-179. Doi:10.1038/nature14447. PMID:25945739

15. Sun J, Evans PN, Gagen EJ, Woodcroft BJ, Hedlund BP, Woyke T, Hugenholtz P, Rinke C (2021) Recoding of stop codons expands the metabolic potential of two novel Asgardarchaeota lineages. ISME Commun 1:30.

16. Yutin N, Bäckström D, Ettema TJG, Krupovic M, Koonin EV (2018) Vast diversity of prokaryotic virus genomes encoding double jelly-roll major capsid proteins uncovered by genomic and metagenomic sequence analysis. Virol J 15:67. Doi:10.1186/s12985-018-0974-y. PMID:29636073

17. Zaremba-Niedzwiedzka K, Caceres EF, Saw JH, Backstrom D, Juzokaite L, Vancaester E, Seitz KW, Anantharaman K, Starnawski P, Kjeldsen KU, Stott MB, Nunoura T, Banfield JF, Schramm A, Baker BJ, Spang A, Ettema TJ (2017) Asgard archaea illuminate the origin of eukaryotic cellular complexity. Nature 541:353-358. Doi:10.1038/nature21031. PMID:28077874