

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

|  |  |  |
| --- | --- | --- |
| **Code assigned:** | **2020.186B** |  |
| **Short title:** Create one new realm (*Adnaviria*) for classification of filamentous archaeal viruses with linear dsDNA genomes | | |
|  | | |

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

|  |  |
| --- | --- |
| Krupovic M, Kuhn JH, Wang F, Baquero DP, Egelman EH, Koonin EV, Prangishvili D | [mart.krupovic@pasteur.fr](mailto:mart.krupovic@pasteur.fr); [kuhnjens@niaid.nih.gov](mailto:kuhnjens@niaid.nih.gov); [jerryfbwang@gmail.com](mailto:jerryfbwang@gmail.com); [diana-paola.baquero-uriza@pasteur.fr](mailto:diana-paola.baquero-uriza@pasteur.fr); [egelman@virginia.edu](mailto:egelman@virginia.edu); [koonin@ncbi.nlm.nih.gov](mailto:koonin@ncbi.nlm.nih.gov); [david.prangishvili@pasteur.fr](mailto:david.prangishvili@pasteur.fr) |

**Corresponding author**

|  |
| --- |
| Mart Krupovic |

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

|  |
| --- |
| Bacterial and Archaeal Viruses Subcommittee |

**ICTV study group comments and response of proposer**

|  |
| --- |
|  |

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

|  |  |  |
| --- | --- | --- |
| **Taxon name** | **Person from whom the name is derived** | **Permission attached (Y/N)** |
| *Zilligvirae* | Wolfram Zillig | N (deceased; 1925–2005) |

**Submission dates**

|  |  |
| --- | --- |
| Date first submitted to SC Chair | July 31, 2020 |
| Date of this revision (if different to above) |  |

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

|  |
| --- |
| EC request: Swap kingdom and realm names (realms are all after properties, not people). Answer: this has been done – realm name has been changed to *Adnaviria*. The Excel module has also been corrected. |

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

**Name of accompanying Excel module**

|  |
| --- |
| 2020.186B.R.Adnaviria.xlsx |

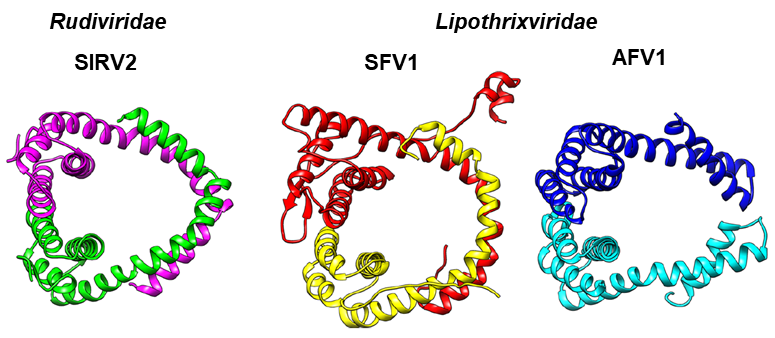
**Abstract**

|  |
| --- |
| Structural and comparative genomics studies have shown that filamentous archaeal viruses with linear double-stranded DNA (dsDNA) genomes belonging to families *Tristromaviridae*, *Rudiviridae*, and *Lipothrixviridae* are evolutionarily related to each other but do not share structural or genomic similarity with other characterized viruses of archaea, bacteria or eukaryotes. Thus, we propose creating a new taxon of the highest rank, the realm, *Adnaviria*, for the classification of these filamentous archaeal viruses. |

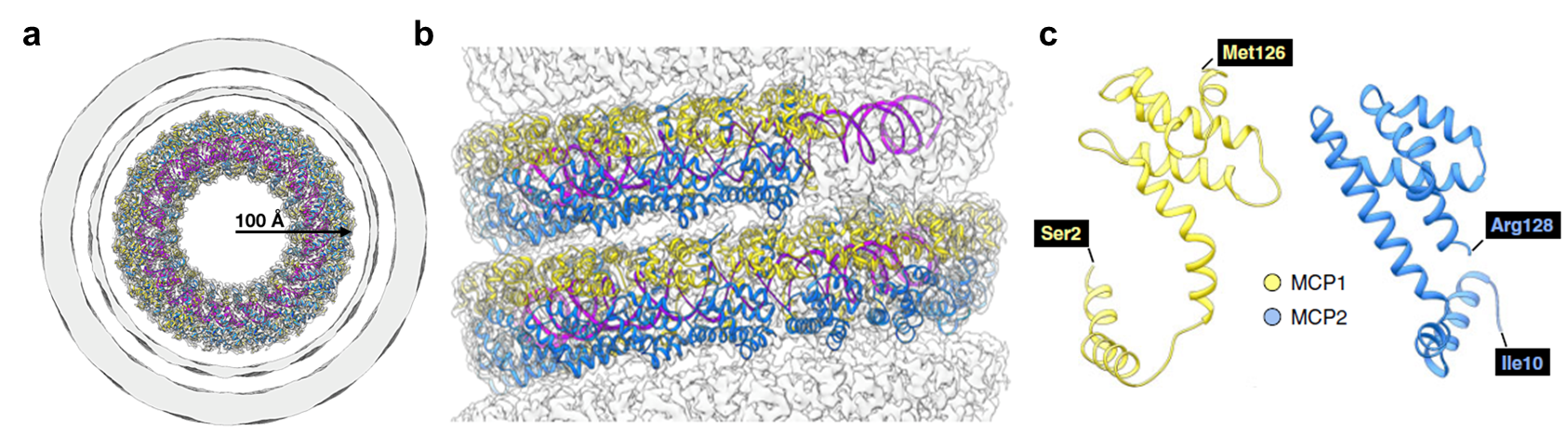
**Text of proposal**

|  |  |
| --- | --- |
| |  | | --- | | Filamentous viruses infect hosts in all three domains of life but, despite overall similar morphology, virions of these viruses are built from unrelated capsid proteins with different structural folds [1]. Furthermore, the ways the nucleic acids are protected by the corresponding capsid proteins are radically different in viruses from the different domains of life [2-7]. All known eukaryotic filamentous viruses have linear single-stranded RNA (ssRNA) genomes and are classified in realm *Riboviria* [8]; all known bacterial filamentous viruses have circular ssDNA genomes and belong to realm *Monodnaviria* [8]; all known archaeal filamentous viruses have dsDNA genomes and currently remain unassigned to taxa higher than family. Here, we propose establishing a separate realm for structurally related archaeal filamentous viruses.  Archaeal viruses that form filamentous virions are currently classified into four families: *Lipothrixviridae*, *Rudiviridae*, *Tristromaviridae,* and *Clavaviridae*. Based on the shared gene content and structural similarity of their virions, the families *Lipothrixviridae* and *Rudiviridae* are unified within the order *Ligamenvirales* [9]. In viruses from both families, the nucleoprotein helix is composed of asymmetric units containing two molecules of the major capsid protein (MCP): a homodimer in the case of rudivirids and a heterodimer of paralogous MCPs in the case of lipothrixvirids (Figure 1) [10]. The MCPs of ligamenvirals have a unique α-helical fold, first found in the MCP of rudivirid Sulfolobus islandicus rod‑shaped virus 2 (SIRV2) [2] and thus far, not observed in any other proteins. A characteristic feature of rudivirids and lipothrixvirids is that the interaction between the MCP dimer and the linear dsDNA genome results in transition of the B-form DNA to the A‑form DNA so that the entire genome exists in the virion in the A-form [2, 10-13].  Tristromavirids and clavavirids do not encode proteins recognizably similar at the sequence level to those of ligamenvirals. Hence, the two families were not included in the *Ligamenvirales*. Structural characterization of clavavirid Aeropyrum pernix bacilliform virus 1 (APBV1) particles by cryogenic electron microscopy (cryo-EM) confirmed that the fold of the MCP and the overall virion organization are unrelated to that of ligamenvirals or other characterized viruses [14, 15]. Recently, the structure of tristromavirid Pyrobaculum filamentous virus 2 (PFV2) has been characterized by cryo-EM (Figure 2). Unexpectedly, it turned out that the two nucleocapsid proteins of tristromavirids are structurally related to those of ligamenvirals and the virion organizations are remarkably similar, including the A‑form conformation of the genomic dsDNA [11] (Figure 3). Similar to lipothrixvirids, virions of tristromavirids are enveloped by a lipid membrane. Based on the structural similarity between members of the *Ligamenvirales* and *Tristromaviridae*, we infer that they share a common origin and propose the assignment of *Tristromaviridae* to a new order, *Primavirales,* and the unifying of the two orders into a new class, *Tokiviricetes*.  Members of the proposed class *Tokiviricetes* do not appear to be evolutionarily related to other known archaeal, bacterial, or eukaryotic viruses. This conclusion is supported by the lack of similarity in either virion structure or gene content. The only genes shared between members of the *Tokiviricetes* and other viruses include glycosyltransferases, ribbon‑helix‑helix transcription factors and anti-CRISPR proteins [16, 17], which are exchanged by horizontal gene transfer or independently acquired from the host by otherwise unrelated viruses. The mechanisms of genome replication used by members of the *Tokiviricetes* are also distinct from those described for other viruses. It has been proposed that rudivirid SIRV2 uses a combination of strand-displacement, rolling‑circle, and strand-coupled genome replication mechanisms, which generate multimeric, highly branched “brush-like” intermediates reaching a length of >1,200 kb (∼34 genome units) [18]. The latter are then processed into unit‑length, linear genomes with covalently linked hairpin ends. The genome replication of the lipothixvirus Acidianus filamentous virus 1 (AFV1) has been suggested to start by the formation of a D‑loop and progress by the strand displacement replication mechanism, whereas termination relies on recombination events through the formation of terminal‑loop‑like structures, though the genes involved in this unique mechanism of replication remain unknown [19]. Similar to those of rudivirids, genomes of tristromavirids contain terminal inverted repeats, but the mechanism of genome replication has not been investigated.  High-resolution structures are now available for six members of the *Tokiviricetes* [10]. As is the case with structurally related viruses in the two other realms of dsDNA viruses, *Varidnaviria* and *Duplodnaviria*, similarity between the capsid proteins of viruses from different families of the *Tokiviricetes* is undetectable at the sequence level, suggesting vast undescribed diversity of viruses in this part of the virosphere. Indeed, some of the already‑sequenced but currently unclassified archaeal viruses (e.g., hyperthermophilic archaeal virus 1) might be included in this assemblage following structural characterization of their virions. Regardless, the available data unequivocally shows that filamentous archaeal viruses do not fall into any of the four existing realms. Thus, given the lack of detectable relationship with other viruses, and to officially acknowledge the uniqueness of filamentous archaeal viruses, we propose creating a new taxon of the highest rank, the realm *Adnaviria*, for their classification.  Rule 3.22 of the International Code of Virus Classification and Nomenclature (ICVCN) permits establishing ranks that, for the moment, remain undefined; and Rule 3.24 indicates no restriction of ranks to be established. Thus, to bridge the gap between the class and realm taxa, we also propose establishing the intermediate kingdom and phylum taxa, with the names *Zilligvirae* and *Taleaviricota*, respectively.  **Taxon demarcation criteria**  We suggest the following provisional taxon demarcation criteria while being aware that these may have to be revisited whenever new members of the realm are being proposed:   1. *Adnaviria*: A virus is a member of this realm if it has a filamentous virion and dsDNA genome encoding a dimeric major capsid protein with the SIRV2-like fold 2. *Primavirales*: A realm member is a member of the order *Primavirales* if it infects archaea of the order Thermoproteales and shares gene content with members of the family *Tristromaviridae*. 3. *Ligamenvirales*: A realm member is a member of the order *Ligamenvirales* if it infects archaea of the order Sulfolobales and shares gene content with members of the families *Rudiviridae* and *Lipothrixviridae*.   If a principal rank taxon includes only a single lower-ranked taxon, then the definition of the lower-ranked taxon is, for now, identical to the definition of the higher-ranked taxon.  **Etymology of proposed taxa:**   * *Adnaviria*; from A-form DNA characteristic of viruses in this realm; the suffix ‑*viria* for realm taxa * *Zilligvirae*; after Wolfram Zillig (1925–2005), a pioneer of research on hyperthermophilic archaeal viruses; the suffix -*virae* for kingdom taxa * *Taleaviricota*; from Latin *talea*, meaning “rod” (referring to the virion morphology); the suffix -*viricota* for phylum taxa * *Tokiviricetes*; from Georgian *toki* (თოკი), meaning “thread”; the suffix ‑*viricetes* for class taxa * *Primavirales*; from Latin *prima*, meaning “first”, referring to the fact that Thermoproteus tenax virus 1, classified in this order, was the first hyperthermophilic archaeal virus to be isolated in 1983 [20] | |

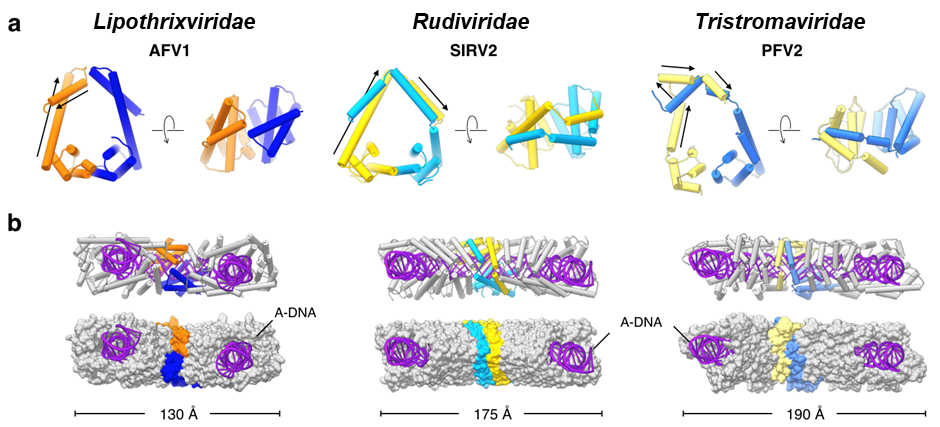
**Supporting evidence**

****

**Figure 1.** Structural conservation among the major capsid proteins of rudivirids and lipothrixvirids. Asymmetric unit in the rudivirid capsids is a homodimer (depicted in green and magenta for Sulfolobus islandicus rod shaped virus 2 [SIRV2]), whereas in lipothrixvirids, it is a heterodimer of two paralogous major capsid proteins (MCPs), shown in yellow and red for Sulfolobus filamentous virus 1 (SFV1) and in blue and cyan for Acidianus filamentous virus 1 (AFV1). The figure is modified from [12].

****

**Figure 2.** Reconstruction of tristromavirid Pyrobaculum filamentous virus 2 (PFV2). (a) A top view shows the nucleoprotein core and enveloping membrane. (b) In the side view, the membrane has been removed. Major capsid protein 1 (MCP1) and MCP2 subunits are shown in yellow and blue, respectively. (c) Three-dimensional alignment of MCP1 and MCP2 is shown. The figure is modified from [11].

****

**Figure 3.** Comparison of the filamentous lipothrixvirid Acidianus filamentous virus 1 (AFV1), rudivirid Sulfolobus islandicus rod shaped virus 2 (SIRV2), and tristromavirid Pyrobaculum filamentous virus 2 (PFV2). (a) Major capsid protein (MCP) dimer (asymmetric unit) comparison of AFV1, SIRV2, and PFV2. The MCP1 of AFV1, SIRV2, and PFV2 are shown in orange, gold, and yellow, respectively. The MCP2 of AFV1, SIRV2 and PFV2 are shown in blue, cyan, and light blue, respectively. The N-terminal helices of MCP1 in AFV1, SIRV2, and PFV2 are indicated with black arrows. (b) Wrapping of A-form DNA (A-DNA) in AFV1, SIRV2, and PFV2. Five MCP dimers are displayed: One MCP dimer is colored as in (a); the other four are shown in gray. Proteins are shown in ribbon representation (top) and as surfaces (bottom). The figure is reproduced from [11].

**References**

1. Krupovic M, Koonin EV. Multiple origins of viral capsid proteins from cellular ancestors. Proc Natl Acad Sci U S A. 2017; 114(12):E2401-E2410. doi: 10.1073/pnas.1621061114. PMID: 28265094
2. DiMaio F, Yu X, Rensen E, Krupovic M, Prangishvili D, Egelman EH. Virology. A virus that infects a hyperthermophile encapsidates A-form DNA. Science. 2015; 348(6237):914-7. doi: 10.1126/science.aaa4181. PMID: 25999507
3. DiMaio F, Chen CC, Yu X, Frenz B, Hsu YH, Lin NS, Egelman EH. The molecular basis for flexibility in the flexible filamentous plant viruses. Nat Struct Mol Biol. 2015; 22(8):642-4. doi: 10.1038/nsmb.3054. PMID: 26167882
4. Stubbs G, Kendall A. Helical viruses. Adv Exp Med Biol. 2012; 726:631-58. doi: 10.1007/978-1-4614-0980-9\_28. PMID: 22297534
5. Klug A. The tobacco mosaic virus particle: structure and assembly. Philos Trans R Soc Lond B Biol Sci. 1999; 354(1383):531-5. doi: 10.1098/rstb.1999.0404. PMID: 10212932
6. Tarafder AK, von Kügelgen A, Mellul AJ, Schulze U, Aarts DGAL, Bharat TAM. Phage liquid crystalline droplets form occlusive sheaths that encapsulate and protect infectious rod-shaped bacteria. Proc Natl Acad Sci U S A. 2020; 117(9):4724-4731. doi: 10.1073/pnas.1917726117. PMID: 32071243
7. Zamora M, Méndez-López E, Agirrezabala X, Cuesta R, Lavín JL, Sánchez-Pina MA, Aranda MA, Valle M. Potyvirus virion structure shows conserved protein fold and RNA binding site in ssRNA viruses. Sci Adv. 2017; 3(9):eaao2182. doi: 10.1126/sciadv.aao2182. PMID: 28948231
8. Koonin EV, Dolja VV, Krupovic M, Varsani A, Wolf YI, Yutin N, Zerbini FM, Kuhn JH. Global Organization and Proposed Megataxonomy of the Virus World. Microbiol Mol Biol Rev. 2020; 84(2):e00061-19. doi: 10.1128/MMBR.00061-19. PMID: 32132243
9. Prangishvili D, Krupovic M. A new proposed taxon for double-stranded DNA viruses, the order "*Ligamenvirales*". Arch Virol. 2012; 157(4):791-5. doi: 10.1007/s00705-012-1229-7. PMID: 22270758
10. Wang F, Baquero DP, Beltran LC, Su Z, Osinski T, Zheng W, Prangishvili D, Krupovic M, Egelman EH. Structures of filamentous viruses infecting hyperthermophilic archaea explain DNA stabilization in extreme environments. Proc Natl Acad Sci U S A. 2020; 117(33):19643-19652. doi: 10.1073/pnas.2011125117. PMID: 32759221
11. Wang F, Baquero DP, Su Z, Osinski T, Prangishvili D, Egelman EH, Krupovic M. Structure of a filamentous virus uncovers familial ties within the archaeal virosphere. Virus Evol. 2020; 6(1):veaa023. doi: 10.1093/ve/veaa023. PMID: 32368353
12. Liu Y, Osinski T, Wang F, Krupovic M, Schouten S, Kasson P, Prangishvili D, Egelman EH. Structural conservation in a membrane-enveloped filamentous virus infecting a hyperthermophilic acidophile. Nat Commun. 2018; 9(1):3360. doi: 10.1038/s41467-018-05684-6. PMID: 30135568
13. Kasson P, DiMaio F, Yu X, Lucas-Staat S, Krupovic M, Schouten S, Prangishvili D, Egelman EH. Model for a novel membrane envelope in a filamentous hyperthermophilic virus. Elife. 2017; 6:e26268. doi: 10.7554/eLife.26268. PMID: 28639939
14. Ptchelkine D, Gillum A, Mochizuki T, Lucas-Staat S, Liu Y, Krupovic M, Phillips SEV, Prangishvili D, Huiskonen JT. Unique architecture of thermophilic archaeal virus APBV1 and its genome packaging. Nat Commun. 2017; 8(1):1436. doi: 10.1038/s41467-017-01668-0. PMID: 29127347
15. Prangishvili D, Mochizuki T, Liu Y, Krupovic M, ICTV Report Consortium. ICTV Virus Taxonomy Profile: *Clavaviridae*. J Gen Virol. 2019; 100(9):1267-1268. doi: 10.1099/jgv.0.001295. PMID: 31271351
16. Iranzo J, Koonin EV, Prangishvili D, Krupovic M. Bipartite Network Analysis of the Archaeal Virosphere: Evolutionary Connections between Viruses and Capsidless Mobile Elements. J Virol. 2016; 90(24):11043-11055. doi: 10.1128/JVI.01622-16. PMID: 27681128
17. Iranzo J, Krupovic M, Koonin EV. The Double-Stranded DNA Virosphere as a Modular Hierarchical Network of Gene Sharing. mBio. 2016; 7(4):e00978-16. doi: 10.1128/mBio.00978-16. PMID: 27486193
18. Martínez-Alvarez L, Bell SD, Peng X. Multiple consecutive initiation of replication producing novel brush-like intermediates at the termini of linear viral dsDNA genomes with hairpin ends. Nucleic Acids Res. 2016; 44(18):8799-8809. doi: 10.1093/nar/gkw636. PMID: 27407114
19. Pina M, Basta T, Quax TE, Joubert A, Baconnais S, Cortez D, Lambert S, Le Cam E, Bell SD, Forterre P, Prangishvili D. Unique genome replication mechanism of the archaeal virus AFV1. Mol Microbiol. 2014;92(6):1313-25. doi: 10.1111/mmi.12630. PMID: 24779456
20. Janekovic D, Wunderl S, Holz I, Zillig W, Gierl A, Neumann H. TTV1, TTV2 and TTV3, a family of viruses of the extremely thermophilic, anaerobic, sulfur reducing archaebacterium *Thermoproteus tenax*. Mol Gen Genet. 1983; 192:39-45. doi: 10.1007/BF00327644.