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1 ***Adnaviria*: a new realm for archaeal filamentous viruses with linear A-form double-**  
2 **stranded DNA genomes**

3

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18

19 Keywords: A-form DNA, *Ligamenvirales*, *Tokiviricetes*, *Rudiviridae*, *Lipothrixviridae*,

20 *Tristromaviridae*, hyperthermophilic archaea, virus evolution, virus classification, virus

21 taxonomy, International Committee on Taxonomy of Viruses (ICTV), major capsid protein, virus

22 structure and assembly

23

24 **Abstract**

25 **The International Committee on Taxonomy of Viruses (ICTV) has recently adopted a**  
26 **comprehensive, hierarchical system of virus taxa. The highest rank in this hierarchy are**  
27 **realms, each of which is considered monophyletic but apparently originated independently**  
28 **of other realms. Here we announce the creation of a new realm, *Adnaviria*, which unifies**  
29 **archaeal filamentous viruses with linear A-form double-stranded DNA genomes and**  
30 **characteristic major capsid proteins unrelated to those encoded by other known viruses.**

31

32 **Text**

33 In 2018, the International Committee on Taxonomy of Viruses (ICTV) expanded the number of  
34 taxonomic ranks available for virus classification from 5 to 15 (1, 2). This development enabled  
35 formal recognition of the evolutionary kinships among distantly related viruses and led to  
36 creation of four realms—virus taxa that are roughly equivalent to the domain rank in cellular  
37 taxonomy (3): The realm *Riboviria* encompasses all RNA viruses and reverse-transcribing  
38 viruses that encode homologous RNA-directed RNA polymerases and reverse transcriptases,  
39 respectively; *Monodnaviria* comprises viruses with predominantly single-stranded (ss) DNA  
40 genomes and encoding rolling-circle replication initiation endonucleases of the HUH  
41 superfamily; *Duplodnaviria* unifies viruses with double-stranded DNA genomes that produce  
42 virions with icosahedral capsids formed by major capsid proteins (MCPs) with the HK97 fold;  
43 and *Varidnaviria* comprises dsDNA viruses with icosahedral capsids built from MCPs with  
44 double-jelly roll fold (4). However, many virus families remained unassigned to higher taxa due  
45 to the lack of demonstrable evolutionary relationships to viruses assigned to the four realms.

46 Here we announce the creation, and recent official acceptance by the ICTV  
47 (<https://talk.ictvonline.org/taxonomy/>), of a new realm, *Adnaviria*, which encompasses  
48 structurally related archaeal filamentous viruses with dsDNA genomes that adopt the A-form  
49 conformation within their virions.

50 Filamentous viruses infect hosts of all cellular domains but, despite overall similar morphology,  
51 virions of eukaryotic, bacterial, and archaeal filamentous viruses are built from capsid proteins  
52 with different structural folds (5). Furthermore, the types of the viral genomes that are protected  
53 by the corresponding capsid proteins are radically different among viruses infecting hosts from  
54 the different domains of life (6-11): All known eukaryotic filamentous viruses have linear ssRNA  
55 genomes and are classified in realm *Riboviria* (4); all known bacterial filamentous viruses have  
56 circular ssDNA genomes and belong to realm *Monodnaviria* (4, 12); and all known archaeal  
57 filamentous viruses have dsDNA genomes and, until recently, have remained unassigned to taxa  
58 ranked higher than order.

59 Archaeal viruses that form filamentous virions are currently classified into four families:  
60 *Clavaviridae*, *Lipothrixviridae*, *Rudiviridae*, and *Tristromaviridae* (13). Based on the shared  
61 gene content, structural similarity of their virions, and homology of the MCPs, families  
62 *Lipothrixviridae* and *Rudiviridae* were included in order *Ligamenvirales* (14). In virions of both  
63 families, the nucleoprotein helix is composed of asymmetric units containing two MCP  
64 molecules, a homodimer in the case of rudivirids and a heterodimer of paralogous MCPs in the  
65 case of lipothrixvirids (Figure 1) (11, 15). The MCPs of ligamenviral particles have a unique  $\alpha$ -  
66 helical fold first found in the MCP of rudivirid *Sulfolobus islandicus* rod-shaped virus 2 (SIRV2)  
67 (16). Lipothrixvirids and rudivirids share a characteristic feature in that the interaction between

68 the MCP dimer and the linear dsDNA genome maintains the DNA in the A form. Consequently,  
69 the entire genome adopts the A-form in virions (15-18).

70 Clavavirids (19, 20) and tristromavirids (21) do not encode proteins with recognizable sequence  
71 similarity to proteins of ligamenvirals (22). Hence, the two families were not included in  
72 *Ligamenvirales*. Recently, the structure of particles produced by tristromavirid *Pyrobaculum*  
73 filamentous virus 2 (PFV2) has been characterized by cryogenic electron microscopy (cryo-EM)  
74 (23). Unexpectedly, the two nucleocapsid proteins of tristromavirids were found to be  
75 structurally related to those of ligamenvirals, and the virion organizations were discovered to be  
76 remarkably similar, including the A-form conformation of the genomic dsDNA (23) (Figure 1).  
77 Similar to lipothrixvirids, virions of tristromavirids are enveloped by a lipid membrane (24).  
78 Based on the structural similarity between members of *Ligamenvirales* and *Tristromaviridae*, we  
79 inferred that they share a common origin (23). To formalize this evolutionary relationship,  
80 *Tristromaviridae* was assigned to a new order, *Primavirales*, which, along with *Ligamenvirales*,  
81 was included in a new class, *Tokiviricetes*.

82 Members of the *Tokiviricetes* represent one of the most commonly detected virus groups in  
83 terrestrial hot springs and are globally distributed (25-27). Rudivirids have been extensively  
84 studied in particular, and many aspects of their life cycles are understood in considerable detail  
85 (28). Notably, the mechanisms of genome replication do not appear to be conserved among  
86 tokiviricetes. It has been proposed that rudivirus SIRV2 uses a combination of strand-  
87 displacement, rolling-circle, and strand-coupled genome replication mechanisms, which generate  
88 multimeric, highly branched, “brush-like” intermediates reaching lengths of >1,200 kb (~34  
89 genome units) (29). The latter are then processed into unit-length, linear genomes with  
90 covalently linked hairpin ends. The genome replication of the lipothrixvirid *Acidianus*

91 filamentous virus 1 has been suggested to start by the formation of a D loop and to progress by  
92 the strand displacement replication mechanism, whereas termination relies on recombination  
93 events through the formation of terminal loop-like structures, though the genes involved in this  
94 unique mechanism of replication remain unknown (30). Similar to rudivirid genomes,  
95 tristromavirid genomes have terminal inverted repeats (21), but the replication mechanism of  
96 these viruses has not been investigated.

97 High-resolution structures are now available for virions of six distinct tokiviricetes (11, 15). Like  
98 many structurally related viruses in the two other realms of dsDNA viruses (*Duplodnaviria* and  
99 *Varidnaviria*), there is no detectable sequence similarity among the capsid proteins of viruses  
100 from different tokiviricete families, suggesting a vast undescribed diversity of viruses in this part  
101 of the virosphere. Indeed, it has been suggested that tokiviricetes were present in the last archaeal  
102 common ancestor, and possibly even in the last universal cellular ancestor (LUCA) (31).  
103 Regardless, the available data unequivocally show that archaeal filamentous viruses do not fall  
104 into any of the four established realms. Thus, given the lack of detectable relationship with other  
105 viruses and officially acknowledging the uniqueness of the three families of archaeal filamentous  
106 viruses that contain A-form DNA in their virions, a new taxon of the highest rank, realm  
107 *Adnaviria*, was created for their classification. To bridge the gap between the class and realm  
108 taxa, intermediate kingdom and phylum taxa, named *Zilligvirae* and *Taleaviricota*, respectively,  
109 were established.

110 In contrast, structural characterization of clavavirid *Aeropyrum pernix* bacilliform virus 1  
111 (APBV1) particles by cryo-EM confirmed that the fold of its MCP and its overall virion  
112 organization are unrelated to that of ligamenviral, tristromavirid, or other characterized virus

113 particles (32). Therefore, notwithstanding their filamentous virions, clavavirids are not included  
114 in realm *Adnaviria* and likely represent another realm to be established in the future.

115

116 **Etymology of new taxa recently accepted by the ICTV:**

- 117 • *Adnaviria*; from A-form DNA characteristic of viruses in this realm and suffix *-viria* for  
118 realm taxa.
- 119 • *Zilligvirae*; after Wolfram Zillig (1925–2005), a pioneer of research on  
120 hyperthermophilic archaeal viruses, and the suffix *-virae* for kingdom taxa.
- 121 • *Taleaviricota*; from Latin *talea*, meaning “rod” (referring to the virion morphology), and  
122 suffix *-viricota* for phylum taxa.
- 123 • *Tokiviricetes*; from Georgian თოკო (*toki*), meaning “thread”, and suffix *-viricetes* for  
124 class taxa.
- 125 • *Primavirales*; from Latin *prima*, meaning “first”, referring to the fact that Thermoproteus  
126 tenax virus 1, classified in this order, was the first hyperthermophilic archaeal virus to be  
127 isolated in 1983 (33).

128

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143 Department of Health and Human Services or of the institutions and companies affiliated with  
144 the authors.

145



146 **Figure legend**

147 **Figure 1. Taxonomic organization of realm *Adnaviria*.** **A.** Taxonomic ranks from family to  
148 realm are rendered with different colors. **B-D.** Cryogenic electron microscopy shows structures  
149 of the major capsid protein (MCP) homodimer (characteristic of rudivirids) and heterodimer  
150 (characteristic of lipothrixvirids and tristromavirids) (**B**), MCPs bound to viral double-stranded  
151 (ds) DNA genome in A-form conformation (**C**), and side views of the viral nucleocapsid are  
152 shown for each constituent family (**D**). *Rudiviridae*, *Lipothrixviridae*, and *Tristromaviridae* are  
153 represented by structures of *Sulfolobus islandicus* rod-shaped virus 2 (PDB entry: 3J9X),  
154 *Acidianus filamentous virus 1* (PDB entry: 5WTG), and *Pyrobaculum filamentous virus 2* (PDB  
155 entry: 6VTB), respectively. The figure is modified from ref ([11](#)).

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- 255

A

Realm:

*Adnaviria*

Kingdom:

*Zilligvirae*

Phylum:

*Taleaviricota*

Class:

*Tokiviricetes*

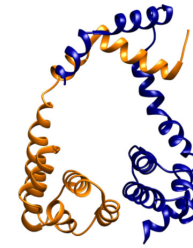
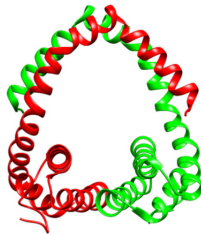
Order:

*Ligamenvirales**Primavirales*

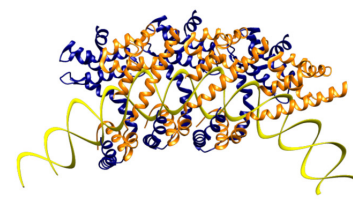
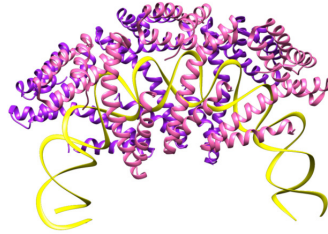
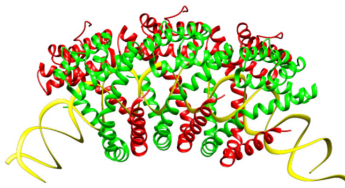
Family:

*Rudiviridae**Lipothrixviridae**Tristromaviridae*

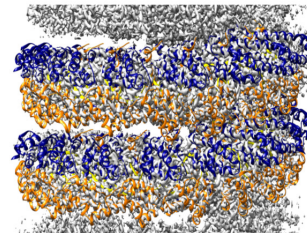
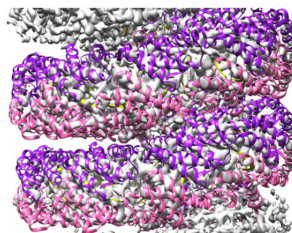
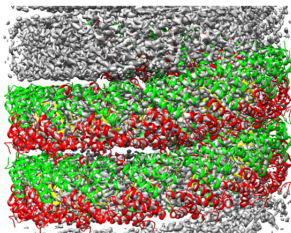
B

MCP  
dimer

C

3 MCP  
dimers  
bound to  
A-DNA

D

virion  
superhelix  
(side view)