



This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections).

For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	2011.008a-cB	(to be completed by ICTV officers)			
Short title: create the order <i>Ligamenvirales</i> containing the families <i>Rudiviridae</i> and <i>Lipothrixviridae</i> (e.g. 6 new species in the genus <i>Zetavirus</i>)					
Modules attached (modules 1 and 9 are required)	1 <input checked="" type="checkbox"/> 6 <input checked="" type="checkbox"/>	2 <input type="checkbox"/> 7 <input type="checkbox"/>	3 <input type="checkbox"/> 8 <input type="checkbox"/>	4 <input type="checkbox"/> 9 <input checked="" type="checkbox"/>	5 <input type="checkbox"/>

Author(s) with e-mail address(es) of the proposer:

Prangishvili, D. (david.prangishvili@pasteur.fr); Krupovic, M. (mart.krupovic@pasteur.fr)

List the ICTV study group(s) that have seen this proposal:

A list of study groups and contacts is provided at <http://www.ictvonline.org/subcommittees.asp> . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)

Prokaryote

ICTV-EC or Study Group comments and response of the proposer:

Date first submitted to ICTV:

22/06/11

Date of this revision (if different to above):

21/06/12

NEW ORDER *Ligamenvirales*

creating and naming a new order

Code	2011.008aB	(assigned by ICTV officers)
------	-------------------	-----------------------------

To create a new Order containing the families listed below

Rudiviridae and *Lipothrixviridae*

Code	2011.008bB	(assigned by ICTV officers)
------	-------------------	-----------------------------

To name the new Order:

Ligamenvirales

assigning families and genera to a new order

Code	2011.008cB	(assigned by ICTV officers)
------	-------------------	-----------------------------

To assign the following families to the new Order:

Rudiviridae (existing) and *Lipothrixviridae* (existing)

Code		(assigned by ICTV officers)
------	--	-----------------------------

To assign the following unassigned genera to the new Order (i.e. within the order but not assigned to any family):

none

Reasons to justify the creation of a new Order:

Originally, the two families of dsDNA viruses with linear genomes, the *Rudiviridae* and the *Lipothrixviridae*, were distinguished by differences in virion structure and this was later supported by comparative genomics. Nevertheless, recent advances in understanding the structural and genomic properties allow concluding close evolutionary relationship between the two families. The new order is proposed in order to acknowledge the common origin of the families *Rudiviridae* and *Lipothrixviridae*.

Origin of the new Order name:

'Ligamenvirales' (from the Latin *ligamen*, for “string, thread”).

additional material in support of this proposal

References:

Goulet, A., Blangy, S., Redder, P., Prangishvili, D., Felisberto-Rodriguesa, C., Forterre, P., Campanacci, V. and Cambillau, C. (2009). *Acidianus* filamentous virus 1 coat proteins display a helical fold spanning the filamentous archaeal viruses lineage. *Proc. Natl. Acad. Sci. USA*, **106**, 21155-21160.

Krupovic, M., Prangishvili, D. (2012) A new proposed taxon for double-stranded DNA viruses, the order “Ligamenvirales”, *Arch. Virol.* **157**, 791-795.

Krupovic, M; White, M., Forterre, P., Prangishvili, D. (2012) Postcards from the edge: structural genomics of archaeal viruses. *Adv. Virus Res.* **82**, 33-62.

References:

Pina M., Bize, A., Forterre, P., and Prangishvili, D. (2011) The Archeoviruses. *FEMS Microbiol. Rev.* **35**, 1035-1054.

Prangishvili, D., Forterre, P., and Garrett, R. A. (2006a) Viruses of the Archaea: a unifying view. *Nat. Rev. Microbiol.* **4**, 837-848.

Prangishvili, D., Garrett, R. A., Koonin, E. V. (2006b) Evolutionary genomics of archaeal viruses: Unique viral genomes in the third domain of life. *Virus Res.* **117**, 52-67

Steinmetz, N. F., Bize, A., Findlay, R. C., Lomonosoff, G. P., Manchester, M., Evans, D. J., and Prangishvili, D. (2008). Site-specific and spatially controlled addressability of a new viral nanobuilding block: *Sulfolobus islandicus* rod-shaped virus 2. *Adv. Funct. Mater* **18**, 3478-3486.

Szymczyna, B. R., Taurog, R. E., Young, M. J., Snyder, J. C., Johnson, J. E., and Williamson, J. R. (2009). Synergy of NMR, computation, and X-ray crystallography for structural biology. *Structure* **17**, 499-507.

Annex:

Linear viruses of archaea belong to two distinct families, the *Rudiviridae* and the *Lipothrixviridae* (for reviews, see Prangishvili *et al.*, 2006a; Pina *et al.*, 2011). At least five proteins were found to constitute the rigid rod-shaped virions of rudiviruses. The highly basic major capsid protein (MCP; accounts for 99 % of virion proteins) associates with the genomic DNA to form a helical body of the virion, whereas the minor capsid proteins are implicated in the formation of terminal structures present at both ends of the linear virions (Steinmetz *et al.*, 2008). The structure of the the N-terminally truncated MCP from the rudivirus *Sulfolobus islandicus* rod-shaped virus (SIRV) revealed a novel four-helix bundle topology (Fig. 1) (Szymczyna *et al.*, 2009).

Unlike in rudiviruses, the filamentous virions of lipothrixviruses are flexible and possess a lipid envelope. Furthermore, lipothrixvirus virions are composed of two major capsid proteins (MCP1 and MCP2), not one as in the case of rudiviruses. Both MCPs were shown to interact with dsDNA and form virion-like filaments *in vitro*. The structures of the two MCPs from lipothrixvirus AFV1 have been recently determined by X-ray crystallography, revealing that they are structurally related to each other (Fig. 1A). However, the two proteins display a distinct hydrophobicity profile, which allowed the topological model of the two proteins in the AFV1 virion to be proposed. According to this model, the basic MCP1 protein forms a core around which the genomic dsDNA is wrapped, whereas MCP2 interacts with the genome with its basic N-terminal region and the hydrophilic C-terminal domain is embedded into the lipid envelope (Goulet *et al.*, 2009). Strikingly, MCP1 (and MCP2) of AFV1 is structurally remarkably similar to the MCP of rudiviruses (Fig. 1A). This fact is remarkable considering that there is only 17 % sequence identity between the MCP1 of AFV1 and MCP of SIRV, Fig. 1B). In the maximum-likelihood phylogenetic reconstruction, the MCPs of rudiviruses and lipothrixviruses are robustly segregated into separate clusters (Fig. 1 C), suggesting that

these MCP genes have evolved within their respective viral genomes for an appreciable period of time without being transferred between the members of the two families. Based on the structural relatedness of the lipothrixviral and rudiviral MCPs, it has been envisioned that lipothrixviruses have evolved from a “simpler” non-enveloped rudivirus-like ancestor (Goulet *et al.*, 2009).

Importantly, the four-helix bundle topology of the rudiviral and lipothrixviral MCPs has not been previously observed in the MCPs of other known dsDNA viruses (Krupovic and Bamford, 2011). Indeed, when rudiviral and lipothrixviral MCPs were compared against all currently available protein structures using the DALI server, the AFV1 MCP2 and SIRV MCP were reciprocally found to be the closest structural relatives with highly significant Z scores (13.3 and 13.4, respectively). No structural virion proteins from other viruses were identified as similar. Notably, the arrangement of the four helices in the MCPs of rudiviruses and lipothrixviruses is different from that found in the capsid protein of the ssRNA genome-containing Tobacco mosaic virus (Fig. 2; Goulet *et al.*, 2009), suggesting an independent origin for these archaeal and plant virus capsid proteins. Consequently, structural information not only illuminates the relationship between the two groups of linear archaeal viruses, but also highlights their distinctiveness from any other known virus group.

Comparative genomics of rudiviruses and lipothrixviruses has previously suggested that the two groups of viruses might be evolutionary related (Prangishvili *et al.*, 2006b; Krupovic *et al.*, 2012). Indeed, on the genome level, some lipothrixviruses are no more similar to other members of the *Lipothrixviridae* than they are to rudiviruses (Fig. 3). For example, lipothrixviruses AFV1 and SIFV share ten homologous genes. The same number of genes is also common to SIFV and rudivirus SIRV1. The two sets of genes (AFV1–SIFV and SIFV–SIRV) do overlap but are not identical (Fig. 3). The overlapping set of genes includes genes encoding glycosyl transferases, transcriptional factors and small genes of unknown functions. Such relatively close genetic relationship between viruses belonging to different families is unusual in the archaeal virosphere, where viruses from different families typically share only few (if any) homologous genes (Prangishvili *et al.*, 2006b). The Rudiviruses and lipothrixviruses do not encode recognizable integrases and are not known to lysogenize their hosts, a phenomenon which could potentially favour gene shuffling between unrelated viruses. Consequently, there is no reason to believe that horizontal gene exchange, that could explain the common gene content of rudiviruses and lipothrixviruses, is more vigorous between these linear viruses than between other hyperthermophilic archaeal viruses. We therefore conclude that the genomic relationship between the two groups of viruses most likely reflects their common ancestry, further reinforcing the hypothesis that rigid rod-shaped and flexible filamentous viruses have arisen from a common ancestor.

In summary, recent structural and genomic studies led to accumulation of compelling evidence that points to a common ancestry of rudiviruses and lipothrixviruses. Accordingly, in order to acknowledge the evolutionary relationship between linear viruses of the two families a new taxonomic order, the ‘Ligamenvirales’ (from the Latin *ligamen*, for string, thread) is being proposed (Prangishvili, Krupovic, 2012).

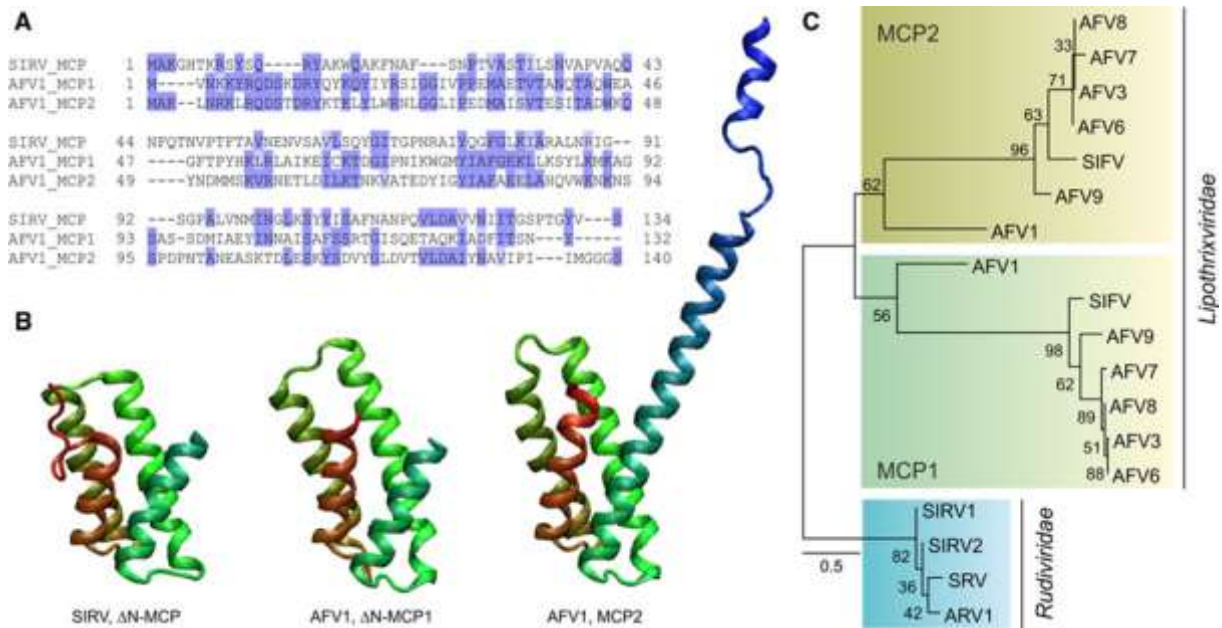


Figure 1. Major capsid proteins (MCP) of linear dsDNA viruses infecting *Archaea*. **A.** Sequence alignment of the MCP of the rudivirus SIRV (ORF134) with the two MCPs of the lipothrixvirus AFV1. The alignment is coloured according to sequence conservation (BLOSUM62 matrix). **B.** Comparison of the N-terminally truncated MCP of SIRV with the two MCPs of AFV1. The structures are colored using a rainbow colour gradient from the N terminus (blue) to the C terminus (red). **C.** Maximum likelihood phylogeny of the MCPs of rudiviruses and lipothrixviruses. For phylogenetic analysis homologous MCP sequences were collected using PSI-BLAST and aligned using PROMALS3D. MCP sequences of lipothrixviruses AFV2 and TTV1 could not be retrieved using sequence similarity-based approaches and are therefore not included in this analysis. Maximum likelihood phylogeny was constructed in MEGA5 using JTT matrix model (+I, +G [4 categories]). All positions containing gaps were eliminated and the final dataset consisted of 120 positions. Numbers at the branch-points represent bootstrap values (100 replicates). The scale bar represents the number of substitutions per site. The tree was rooted on the branch between rudiviral and lipothrixviral MCPs. GenBank accession numbers: *Rudiviridae* MCPs (SIRV1 ORF134, NP_666607; SIRV2 ORF134, NP_666560; SRV ORF134, CAQ58456; ARV gp24, YP_001542641), *Lipothrixviridae* MCP1 (AFV1 ORF132, YP_003749; AFV3 gp34, YP_001604376; AFV6 gp35, YP_001604193; AFV7 gp28, YP_001604252; AFV8 gp30, YP_001604311; AFV9 gp32, YP_001798550; SIFV ORF35, NP_445700) and MCP2 (AFV1 ORF140, YP_003750; AFV3 gp35, YP_001604377; AFV6 gp36, YP_001604194; AFV7 gp29, YP_001604253; AFV8 gp31, YP_001604312; AFV9 gp33, YP_001798551; SIFV ORF36, NP_445701).

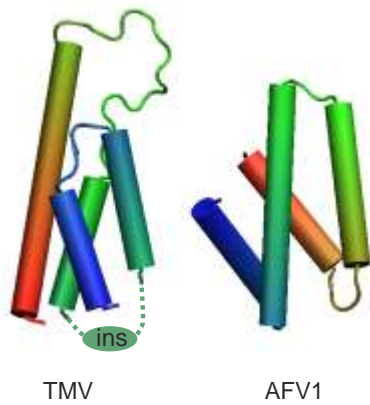


Figure 2. Different arrangement of α -helices in the four-helix bundle major capsid proteins of Tobacco mosaic virus (TMV; PDB ID:1EI7) and *Acidianus* filamentous virus 1 (AFV1; PDB ID:3FBL). The structures are colored using a rainbow color gradient from the N terminus (blue) to the C terminus (red). The insertion (ins) between the second and the third α -helices in the TMV protein was omitted for more convenient comparison.

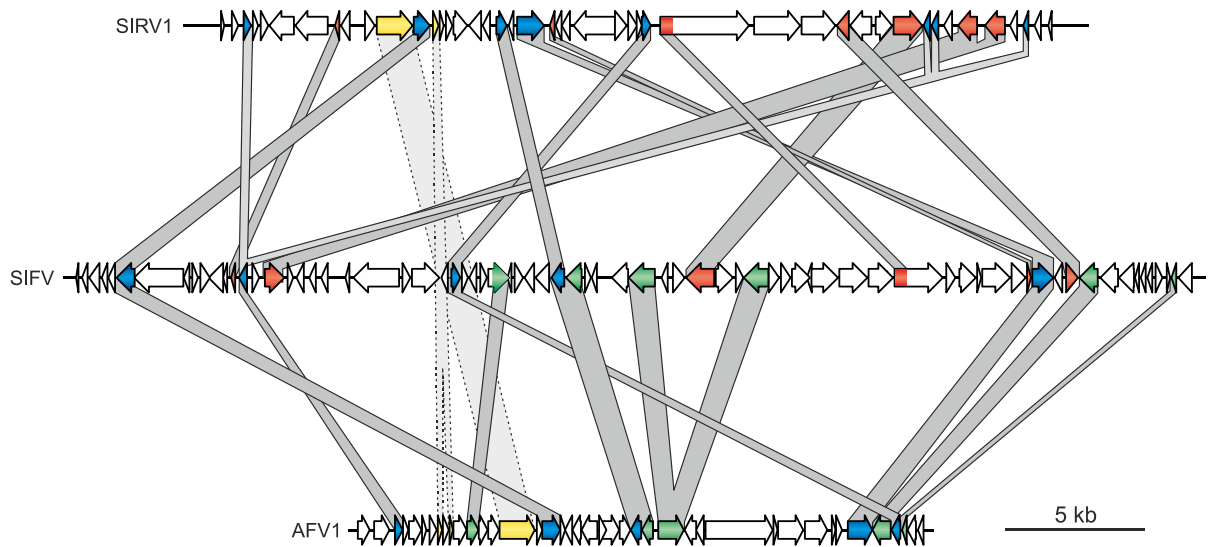


Figure 3. Genomic relationship between linear archaeal viruses of the *Rudiviridae* and *Lipothrixviridae* families. Genes shared by *Sulfolobus islandicus* rod-shaped virus 1 (SIRV1; *Rudiviridae*) and lipothrixviruses *Sulfolobus islandicus* filamentous virus (SIFV) and *Acidianus* filamentous virus 1 (AFV1) are shaded blue. Genes restricted to virus pairs SIRV1-SIFV, SIRV1-AFV1 and SIFV-AFV1 are shown in red, yellow and green, respectively.