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# Survey of high-resolution archaeal virus structures

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Archaeal viruses exhibit diverse morphologies whose structures are just beginning to be explored at high-resolution. In this review, we update recent findings on archaeal structural proteins and virion architectures and place them in the biological context in which these viruses replicate. We conclude that many of the unusual structural features and dynamics of archaeal viruses aid their replication and survival in the chemically harsh environments, in which they replicate. Furthermore, we should expect to find more novel features from examining the high-resolution structures of additional archaeal viruses.

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## Introduction

Advances in environmental sequencing have revealed a large number of previously unknown viruses that represent a deep pool of genetic diversity [1]. Classification of this collection of unknown viruses and characterization of their encoded genes is a challenging task, complicated by their limited homology to genes with known function [1–3]. Virion structure provides a compelling approach to organizing the diverse virosphere [4].

In contrast to ever increasing numbers of newly sequenced viral genomes, there is a countertrending decrease in discovery of new protein folds. At present, there are 1393 known

protein folds [5]. Of these, only 20 folds are known for the major capsid proteins (MCPs) of viruses [6,7]. Despite the genetic diversity of viruses, their structural proteins, and especially their MCPs, fit within a limited number of groups. This limited number of common structural themes unite viruses across all three domains of life (Eukarya, Bacteria, and Archaea) and evolutionary history [8]. Virion structure also allows for insights into viral assembly, release, attachment, and entry by comparing well-characterized members of a structural lineage with less characterized members [9–11].

Our understanding of viruses that infect Archaea (archaeal viruses), lags far behind those infecting Bacteria and Eukaryotes and we refer the reader to several excellent reviews on archaeal viruses [12–14]. Many archaeal viruses have unique morphologies [12,15–18]. Here, we focus on structural features unique to archaeal viruses, their role in virion architecture and dynamics, and we estimate the number of unknown archaeal structural proteins and virion architectures yet to be characterized.

## Survey of archaeal viruses

Out of the 125 known archaeal viruses representing 18 recognized or proposed taxonomic families, only 15 have high-resolution structural information available [13,19\*]. [Table 1](#) lists the archaeal viruses with high-resolution structural data, while [Figure 1](#) provides images of their MCPs and overall virion architecture. All the structurally characterized archaeal viruses replicate in either high temperature (>80°C) acidic (pH < 4) or high salt (>2.0 M) environments. Therefore, their virion morphologies and dynamics should be placed in the context of these unusual environments.

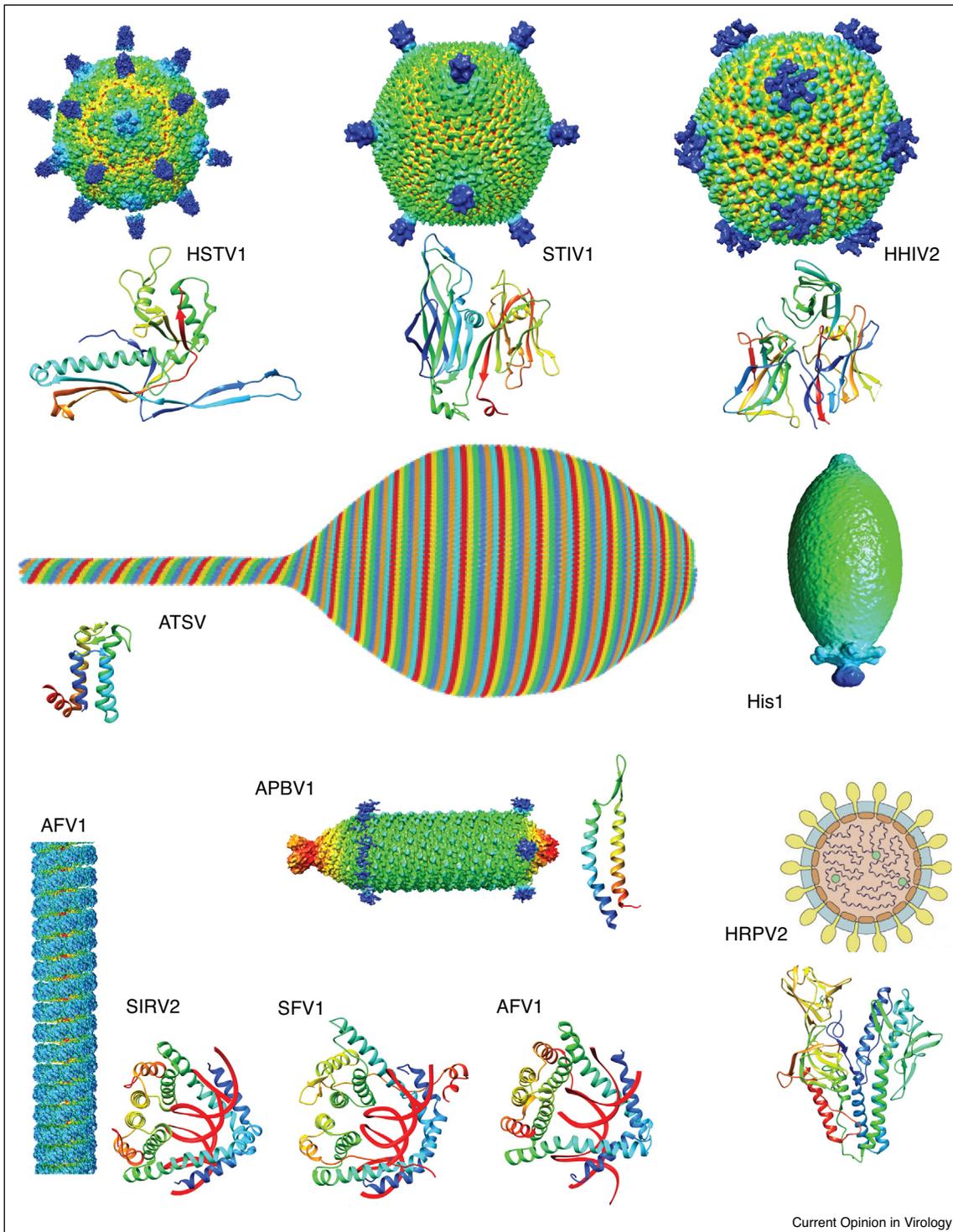
## Turriviridae and Sphaerolipoviridae

The ability of virion structural studies to identify distant evolutionary relationships is well reviewed [3,4,20,21]. Two archaeal viruses belonging to the PRD1 lineage are the Turriviridae and the Sphaerolipoviridae [22,23]. Viruses of the PRD1 lineage share MCPs exhibiting the double-jellyroll fold, with each jellyroll domain composed of two 4-stranded anti-parallel  $\beta$ -sheets packed against each other to give a  $\beta$ -sandwich or flattened  $\beta$ -barrel. The double-jellyroll fold is well suited for viral capsids with large facets and high triangulation numbers [21,24,25]. The double-jellyroll fold also displays extreme stability, being resistant to boiling temperatures, extremes in pH, and chemical denaturants [26–28].

**Table 1****Archaeal viruses with structural data for their capsids and coat proteins**

Virus Family	Virus name	PDB/EMDB accession #	Method of acquisition	Resolution	Major discoveries	Reference
Bicaudaviridae	ATSV	5EQW	X-ray diffraction	1.679 Å	New capsid architectural principle consisting of a multi-start superhelix	ATSV: [44**]
Caudovirales	HSTV1	EMD2279	Single particle	8.9 Å	Linking of these halophilic archaeal viruses to the larger Caudovirales family of head-tail bacteriophages	HSTV1: [41] HVTV 1&2:
	HVTV1	EMD2234	Single particle	10.5 Å		
	HSTV2	EMD2235	Single particle	9.8 Å		
Claviviridae	APBV1	EMD3857 50XE	Helical reconstruction atomic model	3.7 Å	Unique capsid architecture	APBV1: [40*]
				3.7 Å		
Fuselloviridae	SSV1	Not avail.	Single particle	32 Å	Possible fullerene cone-based architecture	SSV1: [78]
	His1	EMD6222	Subtomogram averaging	20 Å	Dynamic capsid transformation from spindle to tube during DNA ejection	His1: [49]
Rudiviridae	SIRV-YNP	3F2E	X-ray diffraction	1.85 Å	First instance of a virus encapsidating A-form DNA likely to protect the genome from acid catalyzed degradation	SIRV: [30**,31]
	SIRV2	EMD6310 3J9X	Helical reconstruction atomic model	3.8 Å 3.8 Å		
Lipothrixviridae	AFV1	3FBZ and 3FBL EMD8780 5W7G	X-ray diffraction	2.3 Å and 1.95 Å	Rudiviridae and Lipothrixviridae share common MCP fold and nucleoprotein architecture	AFV1: [32,33**]
			Helical reconstruction atomic model	4.5 Å		
			Helical reconstruction atomic model	4.5 Å		
	SFV1	EMD7797 6D5F	Helical reconstruction atomic model	3.7 Å	Novel biological membrane with monolayer of lipids folded into a horseshoe configuration	SFV1: [34*]
				3.7 Å		
Sphaerolipoviridae	HHIV2	EMD3109 EMD1353	Single particle	13 Å	Insight into evolution of vertical β-barrel (Double Jelly Roll) fold	HHIV2: [79] P23-77: [80]
	P23-77 (phage)	3ZN6	Single particle	9.6 Å		
Turriviridae	STIV1	4IND 2BBB and 6BO3 EMD5584 3J31 EMD1679	X-ray diffraction	1.53 Å	The PRD1 viral lineage extends to archaeal viruses	STIV1: [23]
			X-ray diffraction	1.8 Å		
			X-ray diffraction	2.04 Å and 1.83 Å		
			Single particle	4.5 Å		
Pleolipoviridae	HRPV2	6QGI 6QGL EMD9779	Atomic model	4.5 Å	Jelly Roll folds are present in multiple capsid proteins indicating gene duplication and divergence from a single ancestral protein.	HRPV 2&6: [77**]
			Single particle	20 Å		
			Single particle	2.46 Å		
	HRPV6		X-ray diffraction	2.69 Å	The VP5 spike protein likely constitutes a new class of membrane fusion proteins	
			X-ray diffraction	2.69 Å		
			Subtomogram averaging	16 Å	The V-shaped fold has not been classified by SCOP but may represent a new protein fold	

Figure 1



Coat protein folds and capsid structures are shown for representative members of the Bicaudaviridae, Caudovirales, Claviviridae, Ligamenvirales, Fuselloviridae, Sphaerolipoviridae, Pleolipoviridae, and Turriviridae. All capsids are at the same relative scale, except SFV1 and APBV1 which are at 2× scale and have been truncated in length. Images were generated with UCSF Chimera (<https://www.cgl.ucsf.edu/chimera/>) except for the Pleolipoviridae capsid whose image is from ViralZone (reproduced with permission).

### Rudiviridae and Lipothrixviridae

The rod-shaped Rudiviridae (SIRV1, SIRV2) and Lipothrixviridae (AFV1, SFV1) have an abundance of orthologous genes [29]. The structural core of the MCPs from each family revealed nearly identical folds, a left handed 4-helix bundle, confirming their relatedness [30<sup>••</sup>,31]. The 4-helix MCP core is highly compact with short loops connecting each helix and extensive hydrophobic contacts making it well-adapted for a high temperature environment. Furthermore, in both families helix 1 shows a long, kinked, N-terminal extension, giving a curved appearance [30<sup>••</sup>,31,32,33<sup>••</sup>,34<sup>•</sup>].

Cryo-EM helical reconstructions of SIRV2, SFV1, and AFV1 confirm that their MCP homology extends to their capsid architecture [30<sup>••</sup>,33<sup>••</sup>,34<sup>•</sup>]. In each virion, the DNA is packaged with homodimers of capsid protein in which the extended N-terminal helices wrap around and completely encircle the DNA, resulting in a cylinder shaped, right-handed super helical nucleoprotein complex with a hollow interior [30<sup>••</sup>,33<sup>••</sup>,34<sup>•</sup>]. AFV1 and SFV1 both employ a heterodimer of paralogous proteins while SIRV2 uses a homodimer MCP [30<sup>••</sup>,33<sup>••</sup>,34<sup>•</sup>]. The SFV1 structure is further elaborated by a C-terminal extension, encompassing a 2-stranded antiparallel  $\beta$ -finger that packs against helices 3 and 4 on the outside of the nucleoprotein complex. This is followed by an extended random coil and two short  $\alpha$ -helices that are collectively involved in subunit interactions with an adjacent super-helical turn. Strikingly, all three viruses condense and desolvate their DNA into A-form, protecting it from hydrolysis in the acidic hot spring environment [30<sup>••</sup>,33<sup>••</sup>,34<sup>•</sup>].

Some members of Lipothrixviridae have higher genetic homology to Rudiviruses than to members of their own family, suggesting either high horizontal gene transfer or lack of monophylogeny [29,35]. Additionally, the heterodimers of SFV1 are structurally more similar to the SIRV2 homodimer than to the AFV1 heterodimer [34<sup>•</sup>]. The main feature distinguishing these two viral families is the presence or absence of an external lipid envelope.

Archaeal cyclic tetraether lipids ( $C_{40}$ ) such as GDGT-0 are known to span the entire membrane, contributing to the thermal stability of the host [36,37]. The high-resolution helical reconstruction of AFV1 revealed an additional surprise, the envelope is half the thickness of the host membrane [33<sup>••</sup>]. In the viral envelope, molecular dynamics simulations indicated that the individual tetraether GDGT-0 lipids are likely folded back on themselves into a horseshoe-shaped configuration [33<sup>••</sup>]. Critically, the tetraether GDGT-0 lipids lack cyclopentane rings present in GDGT-4, allowing the flexibility needed to adopt this unique configuration and explaining the preferential recruitment of GDGT-0 into the viral envelope. Similarly, SFV1 shows an external lipid envelope

thinner than expected. Interestingly, during budding the fusellovirus SSV1 also incorporates a GDGT-0 envelope with reduced thickness suggesting a related membrane structure, at least during this stage of the viral life-cycle [38,39].

### Claviviridae

Claviviridae family member APBV1 has a rod-shaped morphology reminiscent of the Rudiviridae. However, the 3 Å helical reconstruction by cryo-EM and *de novo* modeling of the MCP distinguish it as an unrelated virus family [40<sup>•</sup>]. The APBV1 glycosylated MCP uses a helix-turn-helix motif with a type-1  $\beta$  hairpin comprising the turn [40<sup>•</sup>]. Overall the virion is highly compact with extensive helix-helix packing and an abundance of hydrophobic contacts [40<sup>•</sup>]. The axis of each VP1 helix lies along the capsid surface with the helix curvature dictating the overall curvature of the virion. Although APBV1 shows gross morphological similarities to the Rudiviridae it uses hydrated  $\beta$ -form DNA packaged in the center of the virion [40<sup>•</sup>].

### Caudovirales

Head-tail virus morphologies, similar to Caudovirales family of bacteriophages, are abundant among euryarchaeal viruses commonly isolated from hypersaline environments [14]. Validation of their relatedness was established by cryo-EM reconstruction of HSTV1, rigid body fitting of the HK97 crystal structure, and modeling the HSTV1 MCP fold [41]. The HK97 fold is a complex mixed  $\alpha$ -helix  $\beta$ -sheet structure with two discontinuous domains and a large anti-parallel  $\beta$ -hairpin loop [42]. The signature HK97 covalent cross-links that weld the assembled capsid together do not appear conserved for HSTV1; compensatory mechanisms for achieving capsid stability have not been proposed but are likely present in this virus [41].

### Spindle viruses: Bicaudaviridae and Fuselloviridae

Spindle-shaped viruses are only known to infect archaea. They are divided into two families, the Bicaudaviridae (large tailed spindles, 50–100 kb genome) and the Fuselloviridae (smaller tailless spindles, 15–20 kb). The coat protein structures have been determined for Bicaudaviridae members ATV and ATSV [43,44<sup>••</sup>]. The central fold consists of a classic right-handed antiparallel 4-helix bundle capped end on by a small  $\beta$ -sheet, and a short 5th helix at the C-terminus. Despite appearing evolutionarily unrelated, both Bicaudaviridae and Fuselloviridae are built upon a spindle-shaped capsid that transitions into an elongated cylindrical form, giving the appearance of tail growth. Two Bicaudaviridae members are known to undergo this morphologic change: ATV in response to temperature and SMV1 upon host cell contact [45,46]. For ATV this reduces the central spindle volume by 50% and could serve to prime it for genome ejection [45]. It is possible that other members like STSV1 and ATSV also

undergo this transition, given their heterogeneous lengths ranging from 50 nm spindles to 500 nm cylinders [47,48]. The Fusellovirus His1 can also be induced to undergo a related morphological transition, changing from a spindle-shaped body to a cylindrical tube when subjected to elevated temperatures, alkaline pH, or detergent [49]. In addition, the transition also results in partial genome ejection, where the internal capsid pressure of 10 atm is the driving force [50,51].

The capsid protein of ATSV crystalizes as a four-start or quadruple superhelical assembly, exhibiting structural similarity to the virus tail [44<sup>\*\*</sup>]. Combined with radially symmetric striations on the surface of the spindle-shaped capsid in cryo-EM micrographs; this led to a proposed architecture in which a multi-start helix of varying width forms both the capsid and tail [44<sup>\*\*</sup>]. Interactions in the crystal reveal extensive intrastrand contacts, but fewer interstrand contacts at the subunit interfaces [44<sup>\*\*</sup>]. This combination of strong intrastrand and weak interstrand forces could allow the capsid coils to slip past one another, smoothly altering the capsid diameter as it transitions to the lower energy cylindrical state [44<sup>\*\*</sup>]. It is likely that the dynamic nature of multi-start super helical assemblies will be a unifying feature of spindle-shaped virions, and that the transition from the metastable spindle-shape to the lower energy, tailed and cylinder-like structures are energetically responsible for genome delivery into the host cell. Additionally, we suspect that the ability to encapsidate a viral genome and then mature to a smaller pressurized particle might allow packaging genomes of varying length.

The spindle-shaped capsids are proposed to exist in a liquid crystalline, or smectic state [52<sup>\*</sup>] that can be pressurized. The equilibrium shape of liquid shells, enclosing a uniformly pressurized volume must be a surface of constant mean curvature [52<sup>\*</sup>]. Only a limited number of such surfaces exist (cylinder, sphere, unduloid, catenoid, nodoid) [53], and of these, only the unduloid approximates the shape of a lemon-shaped capsid [52<sup>\*</sup>]. While the unduloid differs from the sphere and cylinder in containing regions of both positive and negative curvature, as a surface of constant mean curvature, it suggests the physical basis for the occurrence of pressurized lemon-shaped virions in the archaea.

### Biophysical adaptation to extreme environments

Known archaeal viruses are predominantly either halophilic or thermophilic, and among the thermophiles, frequently acidophilic. All archaeal viruses characterized to date have enveloped capsids and/or glycosylated MCPs (Table 2; [19<sup>\*</sup>,33<sup>\*\*</sup>,34<sup>\*</sup>,39,40<sup>\*</sup>,54–57]). Additionally, viral encoded glycosyltransferases are a common feature [15,48,56,58–61]. Glycosylation often promotes increased protein stability by creating a network of glycan–glycan

and glycan–protein non-covalent interactions and by blocking proteolysis [62].

Lipid envelopes are found in 11 of the 18 archaeal virus families [17,19<sup>\*</sup>,56,59,63]. Archaeal lipids composed of fused diethers or tetraether components show extremely low proton permeability indicating that one function in the virion could be the protection of the packaged genome [64,65]. Intriguingly, a number of these viruses selectively acquire a subset of host lipids [56,59,63]. The Fuselloviridae and Lipothrixviridae envelopes are composed primarily of GDGT-0 (SSV1 and AFV1) or archaeol (SFV1), which have much higher flexibility than the GDGT-4 (4 cyclopentane groups) that predominates in the membranes of their hosts [33<sup>\*\*</sup>,34<sup>\*</sup>,38,39]. These flexible lipids are likely required to facilitate the high membrane curvature of Fuselloviridae and Lipothrixviridae capsids [33<sup>\*\*</sup>,34<sup>\*</sup>]. Increased environmental stability is another rationale for selected lipid acquisition. SH1 enriches its membrane with PGP-Me which is known to increase membrane stability in the high salt environments inhabited by this virus [66,67].

Archaeal viruses isolated from acidic hot springs replicate in low cell density environments, with virus/cell ratios one to three orders of magnitude lower than those observed for marine environments [68,69]. Low host availability, combined with the harsh chemical conditions, indicate that these viruses are under strong selective pressure to rapidly find and infect a host. One strategy is to increase virion aspect ratio. High aspect particles undergo a tumbling motion which increases their effective surface area and thus also binding probability. ATV and SMV1 emerge as low aspect spindles that grow tails to mature into high aspect elongated cylinders [45,46].

Efficient virion attachment/entry processes represent another adaptation for hot spring environments. The thermo-acidophilic virus SIRV2 has an adsorption rate of  $2 \times 10^{-8}$  ml/min which is  $10\times$  faster than adsorption rates of bacteriophages, and  $1000$  to  $100\,000\times$  faster than halophilic archaeal virus adsorption rates [70–72]. The adsorption rate of SMV1, another thermo-acidophilic virus, is slightly slower at  $7 \times 10^{-9}$  ml/min but still double the common rates of bacteriophage attachment [46,72].

### New capsid morphologies and protein folds

The growing body of high-resolution structural data have tended to join viral families into a smaller number of lineages with shared capsid protein folds and architectures. Given this, can we expect to discover new archaeal viral morphologies or MCP folds? To address this, we analyzed an extensive archaeal viral metagenomic dataset, generated from thermal features across Yellowstone National Park. The assembled viral genomes grouped into 101 virus clusters, with 95 representing uncharacterized archaeal viruses [73] [Munson-Mcgee to be

**Table 2**

**Archaeal virus families and their adaptations to extreme environments**

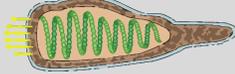
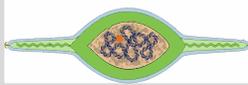
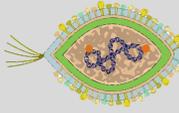
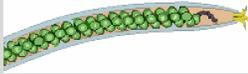
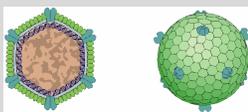
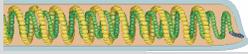
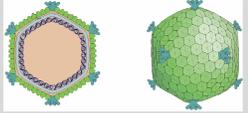
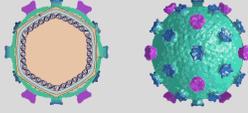
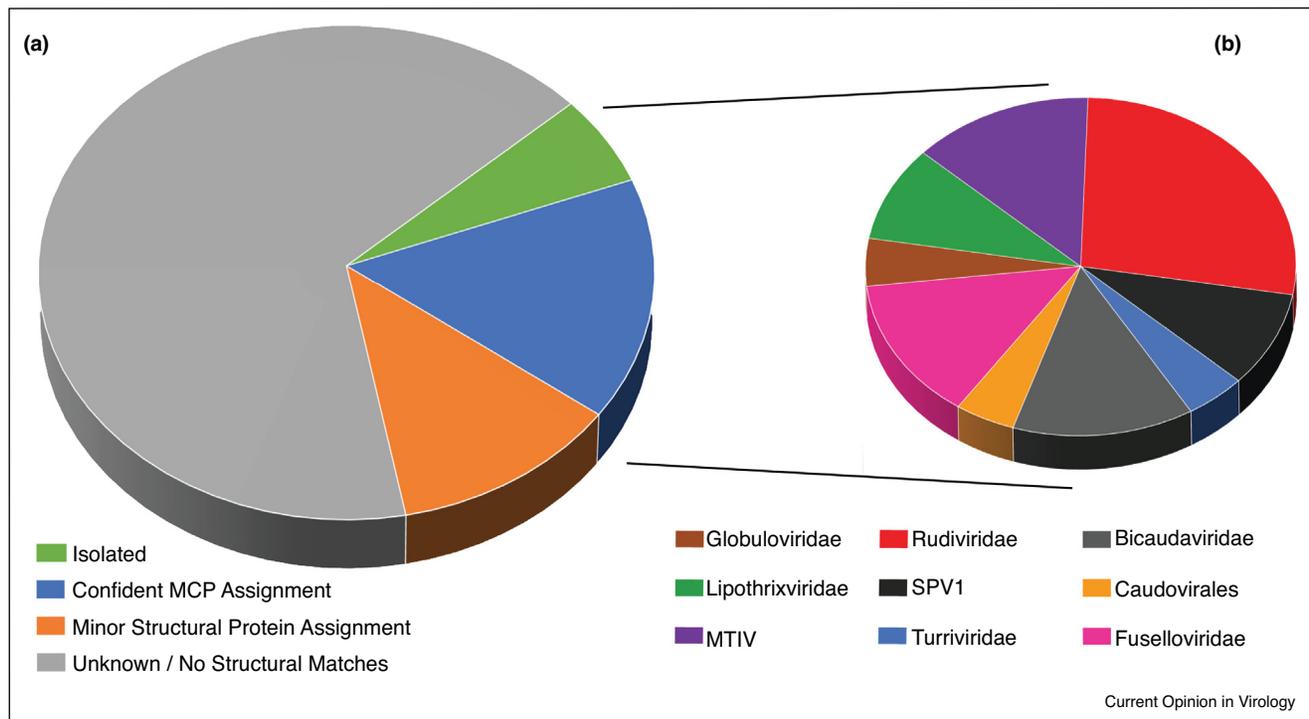
Virus family representatives (habitat)	MCP glycosylation	Enveloped	Morphology Source: ViralZone, SIB (reproduced with permission)	References
<u>Ampullaviridae</u> ABV (87–93° pH 1.5)	Putative glycosyltransferase	Likely enveloped		ABV: [18,60]
<u>Bicaudaviridae</u> ATV, ATSV, STSV1&2 (80–85° pH 2–3)	Putative glycosyltransferase	STSV1&2: ✓ ATV,ATSV: ?		STSV1: [81] STSV2: [47]
<u>Caudovirales</u> HSTV1 (hypersaline)	No data reported	⊘		HSTV1: [41]
<u>Claviviridae</u> APBV1 (90° pH 7)	✓	⊘		APBV1: [40*]
<u>Fuselloviridae</u> SSV1 (80° pH 3)	SSV1: ✓	SSV1: ✓		SSV1: [38,39] His1: [82]
<u>Salterproviridae</u> His1 (Hypersaline)	His1: ⊘	His1: lipid modified		PSV: [58]
<u>Globuloviridae</u> PSV (85° pH 6)	Putative glycosyltransferase	✓		
<u>Guttaviridae</u> APOV1 (90° pH 7) SNDV (80° pH 3)	No data reported	✓		SNDV: [17] APOV1: [17]
<u>Lipothrixviridae</u> AFV1, SFV1 (80–85° pH 2–3)	✓	✓		AFV1: [33**] SFV1: [34*]
<u>Pleolipoviridae</u> HRPV1 (hypersaline)	HRPV1: ✓ HRPV2&6: ⊘	✓		HRPV1: [55] HRPV2&6: [77**]
<u>Portogloboviridae</u> SPV1 (80° pH 3.7)	Putative glycosyltransferase	✓		SPV1: [59]
<u>Rudiviridae</u> SIRV2 (87–93° pH 1.5–2)	✓	⊘		SIRV2: [57]
<u>Sphaerolipoviridae</u> HHIV2, SH1, HCIV1 (hypersaline)	⊘	✓		SH1: [66] HHIV2: [79] HCIV1: [22]
<u>Spiraviridae</u> ACV (90° pH 7)	Putative glycosyltransferase	⊘		ACV: [15]
<u>Tristromaviridae</u> PFV1 (88° pH 6)	✓	✓		PFV1: [56]
<u>Turriviridae</u> STIV1 (80° pH 3)	✓	✓		STIV1: [23,54]
<u>Unclassified</u> MTIV (75–82° pH 2.1)	✓	✓		MTIV: [19*]

Figure 2



Hot Spring metavirome partitions linked to known viruses and structural proteins. **(a)** Level of assignment for the 101 partitions. Isolated: Each partition is more similar to an isolated archaeal virus, than to any other partition in the network. Confident MCP Assignment: Linked by *hmmScan* and *jackhammer* to a known viral MCP. Minor Structural Protein Assignment: Linked by *hmmScan* and *jackhammer* to a minor structural protein. **(b)** Number of isolated and confident partitions matching to each viral family or in the case of MTIV and SPV1 to an unclassified virus.

published]. These 95 clusters were computationally investigated for virion structural proteins. First, alignments were generated for all archaeal virus structural proteins and then used to build Hidden Markov Model (HMM) profiles with *hmmBuild* (HMMER: <http://hmmer.org>) in combination with HMM profiles previously obtained from the pVOG database and alignments published in Yutin *et al.*, and Krupovic *et al.* [16,74,75]. Next, the metagenomic viral partitions were queried against the HMM profiles using *hmmScan*. However, some archaeal structural proteins lack homologues in the public databases, preventing alignment construction. The second approach remedies this by not requiring predetermined alignments using *jackhammer* to search all known archaeal structural proteins against the viral network (HMMER: <http://hmmer.org>). In both approaches, E-values below  $1E-5$  were considered valid.

We could confidently assign viral structural proteins in 34 of the 101 viral partitions (Figure 2a). As expected, six of the 34 clusters were linked to previously characterized viruses, while 28 clusters were predicted to have known structural proteins. The assigned partitions displayed strong homology to MCPs, and often also identified other minor structural proteins (Figure 2b). Sixty-seven viral clusters (66%) lacked sequences with significant

homology to known structural proteins. These unexplored clusters of archaeal virus diversity almost certainly contain divergent homologues of previously described folds. Still, they are also a prime hunting grounds for entirely new protein folds and viral architectures. As a comparison, the same analysis performed on bacteriophage sequences from the human gut resulted in the identification of MCPs in 71% of the viral clusters, 9% of clusters with tentative assignments, and 20% of viral clusters with no MCPs detected [76]. While this methodology limits identification only to virion structural proteins with recognizable sequence homology, it suggests that there are many new archaeal structural proteins and novel capsid architectures yet to be discovered.

## Conclusion

The last decade has seen a major expansion and understanding of archaeal virus high-resolution structures in their biological context. However, entire virus families and morphologies lack structural characterization. Bioinformatic analysis indicates that there may be more archaeal virus structural protein diversity to discover.

## Conflict of interest statement

Nothing declared.

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- of special interest
- of outstanding interest

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