

<https://helda.helsinki.fi>

---

Pleolipoviridae, a newly proposed family comprising archaeal pleomorphic viruses with single-stranded or double-stranded DNA genomes

Pietilä, Maija K.

2016-01

---

Pietilä , M K , Roine , E , Sencilo , A , Bamford , D H & Oksanen , H M 2016 , ' Pleolipoviridae, a newly proposed family comprising archaeal pleomorphic viruses with single-stranded or double-stranded DNA genomes ' , Archives of Virology , vol. 161 , no. 1 , pp. 249-256 . <https://doi.org/10.1007/s00705-015-2613-x>

---

<http://hdl.handle.net/10138/173762>  
<https://doi.org/10.1007/s00705-015-2613-x>

---

other  
acceptedVersion

---

*Downloaded from Helda, University of Helsinki institutional repository.*

*This is an electronic reprint of the original article.*

*This reprint may differ from the original in pagination and typographic detail.*

*Please cite the original version.*

Archives of Virology – Virology Division News

1  
2  
3  
4  
5  
6 **“Pleolipoviridae”, a newly proposed family comprising archaeal**  
7  
8  
9 **pleomorphic viruses with single-stranded or double-stranded DNA**  
10  
11  
12 **genomes**  
13  
14  
15  
16  
17  
18  
19  
20

21 Maija K. Pietilä<sup>1</sup>, Elina Roine<sup>2</sup>, Ana Sencilo<sup>3</sup>, Dennis H. Bamford<sup>2</sup> and Hanna M. Oksanen<sup>2\*</sup>  
22  
23  
24  
25

26 <sup>1</sup>Department of Food and Environmental Sciences, P.O. Box 56, Viikinkaari 9, 00014 University of  
27  
28  
29 Helsinki, Finland  
30

31 <sup>2</sup>Institute of Biotechnology and Department of Biosciences, P.O. Box 56, Viikinkaari 9, 00014  
32  
33  
34 University of Helsinki, Finland  
35  
36

37 <sup>3</sup>Laboratory of Molecular Biology of Bacterial Pathogens, Institute of Microbiology of the ASCR,  
38  
39 v.v.i., Czech Academy of Sciences, 142 20, Prague 4, Czech Republic  
40  
41  
42  
43

44 \*Corresponding author. Mailing address: Institute of Biotechnology and Department of  
45  
46  
47 Biosciences, P.O. Box 56, Viikinkaari 9, 00014 University of Helsinki, Finland; Phone: +358-  
48  
49  
50 294159104; Fax: +35893867170; E-mail: hanna.oksanen@helsinki.fi  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Disclaimer

This article is related to an ongoing taxonomic proposal, submitted to the ICTV but not yet accepted at the time of submission. The taxonomy proposed here has not been endorsed by the ICTV Executive Committee, may differ from any new taxonomy that is ultimately approved by the ICTV, and is presented for discussion only but has no official standing.

## Abstract

1  
2  
3 Viruses infecting archaea show a variety of virion morphotypes, and they are currently classified  
4  
5 into more than ten viral families or corresponding groups. Pleomorphic virus morphotype is very  
6  
7 common among haloarchaeal viruses, and to date, several such viruses have been isolated. Here,  
8  
9 we propose classification of eight such viruses and formation of a new family, "Pleolipoviridae"  
10  
11 (from the Greek *pleo* for more or many and *lipos* for lipid), containing three genera, *Alpha-*, *Beta-*,  
12  
13 and *Gammapleolipovirus*. The proposal is currently under review by the International Committee  
14  
15 on Taxonomy of Viruses (ICTV). The members of the proposed family "Pleolipoviridae" infect  
16  
17 halophilic archaea and are nonlytic. They share structural and genomic features and differ from  
18  
19 any other classified virus. The virion of pleolipoviruses is composed of a pleomorphic membrane  
20  
21 vesicle enclosing the genome. All pleolipoviruses have two major structural protein species,  
22  
23 internal membrane and spike proteins. Although the genomes of the pleolipoviruses are single- or  
24  
25 double-stranded, linear or circular DNA molecules, they share genome organization and gene  
26  
27 synteny as well as show significant similarity at the amino acid level. The canonical features  
28  
29 common to all members of the proposed family "Pleolipoviridae" show that they are closely  
30  
31 related and thus form a new viral family.  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Introduction

1  
2  
3 Archaea and their viruses thrive in extreme environments and most of archaeal viruses  
4  
5 characterized so far infect extremophiles, either hyperhalophiles or hyperthermophiles [4-6, 29].  
6  
7 Viruses infecting archaea display diverse virion morphotypes, some of which are unique.  
8  
9 Consequently, archaeal viruses have been classified into over ten viral families and one floating  
10  
11 genus *Salterprovirus* by the International Committee on Taxonomy of Viruses (ICTV) [19, 29]. To  
12  
13 date, about 140 archaeal viruses have been isolated and most of these belong to the order  
14  
15 *Caudovirales* which is composed of three families of icosahedral tailed viruses [4-6, 19, 29]. In  
16  
17 addition to these, spherical and linear, spindle-, bottle- and droplet-shaped, and pleomorphic  
18  
19 viruses are known to infect archaea [4, 6, 29].  
20  
21  
22  
23  
24  
25

26 Archaeal viruses have revealed deep evolutionary relationships between viruses infecting  
27  
28 organisms from all three domains of life. Structural studies have shown that tailless icosahedral  
29  
30 viruses infecting archaea, bacteria, or eukaryotes share a common ancestor [1, 9, 10, 12, 13, 33].  
31  
32 Furthermore, icosahedral tailed viruses infecting archaea and bacteria have recently been shown  
33  
34 to have the same major capsid protein fold pointing to a common origin [28]. Pleomorphic viruses  
35  
36 infecting archaea and bacteria provide yet another example of viral relationships across domain  
37  
38 barriers as these viruses resemble each other at the virion level [16, 24-26]. The first isolate of  
39  
40 pleomorphic, membrane-containing viruses infecting archaea, *Halorubrum* pleomorphic virus 1  
41  
42 (HRPV-1), was discovered in 2009, and since then several other isolates have been characterized  
43  
44 [3, 21, 24, 34]. Comprehensive studies of the pleomorphic archaeal viruses have been performed  
45  
46 but yet they have remained unclassified [3, 18, 21, 24-26, 34, 36]. We have proposed to classify  
47  
48 these viruses into a new viral family designated as "Pleolipoviridae", and here, we summarize the  
49  
50 available information on their virion components, genomic data and relatedness.  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## “Pleolipoviridae”, a new family of eight archaeal pleomorphic viruses

To date, eight pleomorphic membrane-containing viruses infecting halophilic archaea of the phylum *Euryarchaeota* have been discovered (Table 1). These viruses originate from globally distant locations, and five of them, *Halorubrum* pleomorphic viruses 1, 2, 3, and 6 (HRPV-1, HRPV-2, HRPV-3, and HRPV-6) as well as *Halogeometricum* pleomorphic virus 1 (HGPV-1), have been isolated using a host strain originating from the same sample (Fig. 1). *Haloarcula hispanica* pleomorphic viruses 1 and 2 (HHPV-1 and HHPV-2) and His2 have been isolated using a culture collection strain of *Haloarcula hispanica* [11, 21, 34]. These eight isolates share both genomic and structural features showing that they are related. The distinguishing characteristics of this virus group are virion morphology and structural components, genome organization and gene synteny as well as sequence similarity [7, 21, 24-26, 29, 34, 36]. When compared to the other known viruses, the only resemblance that haloarchaeal pleolipoviruses have, is to pleomorphic viruses infecting bacterial mycoplasmas [2, 7, 16, 24, 29]. However, only one of these phages, L2, has been classified (the family *Plasmaviridae*) [19]. This isolate shares no detectable sequence similarity with the pleomorphic archaeal viruses. Consequently, we propose formation of a new family, “Pleolipoviridae”, to classify the pleomorphic archaeal viruses. The name “Pleolipoviridae” originates from the Greek *pleo* for more or many and *lipos* for lipid as the members of the proposed family have pleomorphic virions which are composed of a proteinaceous lipid vesicle enclosing the genome (Fig. 2 and 3). The current members of the proposed family are divided into three genera (see below). The members are referred to as pleolipoviruses, and the model virus system HRPV-1 is the best characterized one.

His2 virus is one of the members of the proposed family “Pleolipoviridae”. It has previously been suggested to be distantly related to the spindle-shaped virus His1 infecting *Haloarcula hispanica* [11]. Currently, His1 is classified as the type species of a floating genus *Salterprovirus*

1 and His2 has been listed as a virus which may be a member of the genus *Salterprovirus* [19].  
2  
3 However, His1 and His2 share no significant amino acid sequence similarity except for their  
4  
5 putative DNA polymerases [11]. These protein-primed family B DNA polymerases of His1 and His2  
6  
7 have been independently acquired from archaeal transposon-like elements [20]. Cryo-electron  
8  
9 microscopy (cryo-EM) studies revealed that His2 is not spindle-shaped like His1, but rather  
10  
11 spherical in shape (Fig. 2) [17, 26]. In addition, His2 virion has a similar canonical structural protein  
12  
13 profile as the other isolates of the proposed family "Pleolipoviridae" (Fig. 3A), whereas His1 virion  
14  
15 protein pattern is unique [26, 27]. The genome synteny and amino acid sequence similarity also  
16  
17 suggest the relationship between His2 and the other pleolipoviruses [24, 34, 36]. Thus, we  
18  
19 propose that His2 should be classified in the proposed family "Pleolipoviridae".  
20  
21  
22  
23  
24  
25

26 In addition to the pleolipoviruses described above, three more haloarchaeal pleomorphic  
27  
28 viruses, *Halorubrum* pleomorphic viruses 7 and 8 (HRPV-7 and HRPV-8) and *Haloarcula*  
29  
30 pleomorphic virus 2 (HAPV-2) have recently been isolated [5]. These isolates display characteristics  
31  
32 of pleolipoviruses: pleomorphic virion morphotypes observed by the negative-staining  
33  
34 transmission EM, pleolipovirus-like simple structural protein patterns and hazy plaque  
35  
36 morphologies. Moreover, the infectivities are affected in the presence of chloroform (at least in  
37  
38 the case of HRPV-7 and HAPV-2) suggesting that there are a membrane in the virions [5]. However,  
39  
40 the genome sequences of these viruses are not available and this precludes their further  
41  
42 positioning within the pleolipovirus group. Thus, they are considered as related virus isolates  
43  
44 which may be members of the family "Pleolipoviridae".  
45  
46  
47  
48  
49  
50  
51  
52  
53

## 54 **Pleolipoviruses have nonlytic life cycles**

55 All studied pleolipoviruses infect hosts belonging to the family *Halobacteriaceae*. Furthermore,  
56  
57 they have a very narrow host range as in most cases they were able to infect only their isolation  
58  
59  
60  
61  
62  
63  
64  
65

1 strain when representatives from several haloarchaeal genera were tested [3, 5, 11, 24, 34]. All  
2 current members of the proposed family "Pleolipoviridae" are nonlytic, and they form hazy  
3 plaques on host lawn [21, 24, 26, 34]. In liquid cultures, progeny viruses are produced  
4 continuously resulting in host growth retardation [24, 26, 34]. Nonlytic nature of the life cycle as  
5 well as the enveloped pleomorphic appearance of the virion imply that pleolipoviruses use  
6 budding as an exit mechanism. Accordingly, the most likely entry mechanism is the fusion of the  
7 virion envelope with the host cell membrane.  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20

### 21 **Pleomorphic appearance of pleolipoviruses**

22  
23 Pleolipoviruses are sensitive to conditions of low salt concentration confirming their halophilic  
24 nature [24-26]. Negative-stain transmission EM of the highly purified virions suggested that the  
25 pleolipoviruses have flexible virion structure not defined by a rigid protein capsid [21, 24-26, 34].  
26 The pleomorphic appearance of the virions, which varied from spherical to elongated, did not  
27 resemble any of the previously described archaeal viruses [30, 32]. To avoid possible artifacts  
28 caused by negative staining, the virion morphology of the pleolipoviruses has also been studied  
29 using cryo-EM and cryo-electron tomography (cryo-ET). The cryo-electron micrographs show  
30 roughly spherical particles with decorating spikes on the virion surface (Fig. 2) [26]. It has been  
31 observed that the dimensions of the individual viruses vary. The smallest of the viruses is HRPV-1  
32 (41.1 ± 2.2 nm) and the largest is His2 (70.6 ± 3.6 nm) [26]. The pleomorphicity of the viruses is  
33 thus obvious in the range of sizes that each virus exhibits. In addition, cryo-ET of HRPV-1 has  
34 shown that there is an apparent lack of longitudinal order in the surface spikes emphasizing the  
35 pleomorphicity [26].  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59

### 60 **Virions of pleolipovirus are simple and resemble membrane vesicles**



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

In addition to the morphology, pleolipoviruses have a highly similar, simple structural protein profile (Fig. 3A). Although protein profile is not available for HHPV-2, the high similarity of all of its predicted genes to those of HHPV-1 suggests that the protein profiles of these two viruses are essentially the same. The virions of pleolipoviruses are composed of two major structural protein species [24-26, 34]. The smaller-sized protein contains predicted transmembrane domains and the larger-sized one has a C-terminal membrane anchor preceded by a predicted coiled-coil domain. Quantitative biochemical dissociation analyses have shown that the larger-sized proteins of pleolipoviruses are anchored to the membrane and the smaller ones are in the membrane facing the particle interior where the genome resides (Fig. 3B) [25, 26]. There are no nucleoproteins associated with the genome. Thus, the two major protein species have been designated as spike protein (VP4-like protein according to the HRPV-1 nomenclature; VP for virion protein) and internal membrane protein (VP3-like protein according to the HRPV-1 nomenclature). HHPV-1, HRPV-1, HRPV-2, HRPV-3, and HRPV-6 have one of each, His2 has two spike proteins and HGPV-1 has two internal membrane proteins (VP2 and VP3; Fig. 3A) [26]. The internal membrane protein VP27 of His2 sharing amino acid level sequence similarity only with the HGPV-1 protein VP3, is at the functional level VP3-like protein [26]. At amino acid level, VP3-like proteins are rather conserved in all pleolipoviruses, except in His2.

Cryo-electron tomography depicted that HRPV-1 spikes formed of protein VP4 are randomly distributed on the virion surface. Furthermore, HRPV-1 internal membrane protein, VP3, is mostly embedded in the envelope and does not form an ordered protein capsid or a thick matrix-like layer on the inner surface of the membrane [26]. In HRPV-1, one minor structural protein VP8, has been identified. HRPV-1 VP8 with its putative counterparts in other pleolipoviruses is predicted to be an ATPase [24, 36].

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

Some of the pleolipoviruses have modifications in their spike proteins. HRPV-1 VP4 is glycosylated [18, 25], and the major N-glycan is a pentasaccharide comprising glucose, glucuronic acid, mannose, sulphated glucuronic acid and a terminal 5-N-formyl-legionaminic acid residue [18]. This modification is involved in virus infectivity [18]. The spike proteins of His2 (VP28) and HGPV-1 (VP4) has been observed to be modified by unidentified lipid moiety(ies) [26].

Members of the proposed family "Pleolipoviridae" seem to acquire their lipid envelope from the host cell membrane, because the virions contain the same major polar lipids as their host cells (the lipid profile is not available for HHPV-2)[24-26, 34]. Furthermore it has been shown that the ratio of different lipids is the same in the viral and host membrane indicating that the pleolipoviruses acquire their lipids unselectively from the host lipid pool [25, 34]. Except for HGPV-1, the pleolipoviruses have three major phospholipids: phosphatidylglycerol (PG), phosphatidylglycerophosphate methyl ester (PGP-Me) and phosphatidylglycerosulfate (PGS) [24-26, 34]. The two major phospholipids of HGPV-1 and its host are PG and PGP-Me [26].

## **The pleolipoviral genomes are either single-stranded or double-stranded DNA molecules**

All archaeal viruses characterized so far have a DNA genome in contrast to known bacterial and eukaryotic viruses which have either an RNA or DNA genome [6, 19, 29]. Until 2009, the genomic landscape of the studied archaeal viruses was limited to double-stranded (ds) DNA genomes. HRPV-1 was the first archaeal virus to be described containing a single-stranded (ss) DNA genome [24]. Since the isolation of HRPV-1, four more ssDNA viruses infecting archaea have been described [21, 23, 36]. Three of them are members of the proposed "Pleolipoviridae".

The genomes of the eight pleolipoviruses discussed here have been sequenced (Table 1). The nucleotide sequence similarity of the genomes to other sequences in the databases is very

1 limited if the other pleolipoviruses are excluded. The genomes show collinear gene organization  
2 (Fig. 4), but the genomes of HRPV-1, HRPV-2, HRPV-6 and HHPV-2 are ssDNA molecules, whereas  
3  
4 HHPV-1 and His2 have dsDNA genomes [11, 21, 24, 34, 36]. HRPV-3 and HGPV-1 contain dsDNA  
5  
6 genomes, but with stretches of ssDNA [36]. His2 has a linear genome and the other virus isolates  
7  
8 have circular ones. The length of the circular genomes varies from 7,048 nt (HRPV-1) to 10,656 nt  
9  
10 (HRPV-2), and the linear His2 genome is 16,067 bp in size (Table 1). The GC content of the  
11  
12 genomes varies between 40% (His2) and 64% (HRPV-2). At the nucleotide sequence level the  
13  
14 genomes show similarity (60% or higher) only along very short stretches. Exceptions to this are the  
15  
16 HRPV-2 and HRPV-6 genomes as well as HHPV-1 and HHPV-2 genomes that show considerable  
17  
18 nucleotide sequence identity. The set of canonical core genes of the pleolipoviruses consists of the  
19  
20 internal membrane and spike protein coding genes and three conserved predicted downstream  
21  
22 genes of which one is predicted to encode an NTPase (Fig. 4) [11, 24, 34, 36].  
23  
24  
25  
26  
27  
28  
29  
30

31 Among the proposed pleolipoviruses, the highest identity at the amino acid level can be  
32  
33 found between the internal membrane VP3-like proteins (Fig. 4) [36]. One of the internal  
34  
35 membrane proteins of HGPV-1 (VP3) shows similarity to the corresponding protein of His2 (VP27)  
36  
37 and the other one (VP2) shows similarity to the internal membrane proteins of the other  
38  
39 pleolipoviruses. In addition to the core genes, HRPV-1, HHPV-1, HHPV-2, HRPV-2 and HRPV-6  
40  
41 share a predicted gene encoding a putative rolling-circle replication initiation protein. The  
42  
43 genomes of HRPV-3, HGPV-1 and His2 do not contain this putative gene, but encode a protein  
44  
45 homolog containing a C-terminal winged helix-turn helix (wHTH) domain (HRPV-3 and HGPV-1) or  
46  
47 a putative protein-primed family B-type DNA polymerase (His2) [11, 36]. Thus, HRPV-1, HRPV-2,  
48  
49 HRPV-6, HHPV-1 and HHPV-2 are proposed to use a rolling-circle replication mechanism [24, 34,  
50  
51 36]. The ends of the linear dsDNA genome of His2 contain inverted sequence repeats and terminal  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

proteins and most likely replicate using protein-priming, whereas the replication mechanisms of HRPV-3 and HGPV-1 remain unknown [11, 31, 36].

A total of fourteen putative pleolipovirus-like proviruses have been currently identified in the genomes of haloarchaeal strains from the genera *Haloarcula*, *Haloferax*, *Halomicrobium*, *Halopiger*, *Halorhabdus*, *Natrialba*, *Haloterrigena* and *Natronomonas* [15, 24, 34-36]. *Haloferax* plasmid pHK2 and *Halorubrum* plasmid pHRDV1 show gene synteny and significant amino acid sequence similarity to the pleolipovirus genomes. Thus, these plasmids are most likely proviruses related to the pleolipoviruses [14, 34]. Also, a metagenome from a hypersaline lake contained a sequence similar to the pleolipoviruses [37].

## Archaeal pleolipoviruses and bacterial mycoplasmaviruses

Pleolipovirus-like morphology has also been observed among bacterial mycoplasmaviruses. The pleomorphic, enveloped phages L2 and L172, which infect *Acholeplasma laidlawii* cells, have circular dsDNA and ssDNA genomes, respectively [16, 22]. However, there is no detectable DNA homology between these viruses [16]. Both L2 and L172 acquire their lipids unselectively from the host cell membrane as do pleolipoviruses [2]. Remarkably, the protein profile of L172 is highly similar to that of the pleolipoviruses as there are two major protein components and their estimated masses are close to those of the major structural proteins of the pleolipoviruses [16, 24, 26]. As there is no sequence data available for L172, its classification is currently unclear. The protein profile of L2 differs from that of L172 and the pleolipoviruses [16, 26], and L2 shows no sequence similarity to the pleolipoviruses. Thus, the proposal of a new family for archaeal pleolipoviruses is in line with the current classification of L2 into the family *Plasmaviridae*.

## Taxonomic structure of the family "Pleolipoviridae"

1 We propose that the genus and species demarcation criteria of the family “Pleolipoviridae” are the  
2 following (Table 2). (i) *Alphapleolipovirus*: The sequence comparison of the viruses between  
3 species shows low identity over the whole genome nucleotide sequence, but their genomes are  
4 collinear. All members encode a putative rolling-circle replication initiation protein. (ii)  
5  
6  
7  
8  
9  
10 *Betapleolipovirus*: The sequence comparison of the viruses between species shows very little  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

Among the canonical pleolipoviral gene products the VP3-like internal membrane protein shows the highest identity at the amino acid sequence level [36]. The relatedness of the VP3-like proteins can also be used to divide the current members of the “Pleolipoviridae” into the three genera in the same way, as with the above proposed criteria based on gene content. The relatedness of *Alphapleolipovirus* members, which have either an ssDNA or dsDNA genome, can be further verified on the basis of VP3-like protein relatedness. In this case, the genome type is not an adequate criterion.

In conclusion, our recent data show that the eight sequenced pleolipoviruses infecting halophilic archaea share conserved vesicle-like virion architecture. Based on this canonical virion architecture, pleolipoviruses differ from other known enveloped viruses as there is no nucleoprotein or matrix protein typical of such viruses. Despite the different genome types, the pleolipoviruses share genome synteny. Accordingly, we propose a new viral family for these viruses, “Pleolipoviridae”. Subdivision of the family into three genera, *Alphapleolipovirus*, *Betapleolipovirus*, and *Gammapleolipovirus*, is proposed. Traditionally, the genome type has been an important criterion in virus classification [8, 19]. Thus, the proposed family “Pleolipoviridae”

1 challenges this view by having both ssDNA and dsDNA viruses as well as both linear and circular  
2 genomes. This is most likely due to the replication strategies used resulting in different types of  
3 DNA molecules to be encapsidated into a virion. Further studies and comparisons will hopefully  
4 show whether the archaeal virus family "Pleolipoviridae" and the phage family *Plasmaviridae*  
5 could form an order for which we propose here a name of "Pleolipovirales".  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

## Acknowledgements

This work was supported by the Academy of Finland Postdoctoral Researcher funding grant (1274748; (M.K.P), and Academy Professor funding grants (283072 and 255342; D.H.B.). We thank Academy of Finland (grants 271413 and 272853) and University of Helsinki for the support to EU ESFRI Instruct Centre for Virus Production (ICVIR). We also acknowledge Czech Science foundation (grant P302/11/1940) and funding from RVO61388971 for the support.

We acknowledge all authors in the major original papers of pleolipoviruses (Table 1) for their valuable contribution in the alphabetical order: Aitio O, Atanasova NS, Bath C, Butcher SJ, Cukalac T, Domanska A, Dyll-Smith ML, Eichler J, Guan Z, Helin J, Helm M, Kalkkinen N, Kandiba L, Kellner S, Kukkaro P, Laurinavicius S, Li M, Liljeroos L, Manole V, Oren A, Paulin L, Permi P, Porter K, Somerharju P, Sund J, Wang R, Xiang H and Zhao D.

1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49

**Tables**

**Table 1.** Summary of the primary features of the "Pleolipoviridae" members.

| Virus species                                 | Abbreviation | Origin  | Host                               | Genome                    | Genome accession number | Virion diameter (nm) | Identified structural proteins | References  |
|---|--------------|---|------------------------------------|---------------------------|-------------------------|----------------------|--------------------------------|-------------|
| <i>Halorubrum virus</i><br><i>HRPV-1</i>      | HRPV-1       | Solar saltern, Trapani, Italy                                 | <i>Halorubrum</i> sp. PV6          | Circular ssDNA (7048 nt)  | FJ685651                | 41.1 ± 2.2           | VP3, VP4, VP8                  | [18, 24-26] |
| <i>Haloarcula virus</i><br><i>HHPV-2</i>      | HRPV-2       | Solar saltern, Samut Sakhon, Thailand                         | <i>Halorubrum</i> sp. SS5-4        | Circular ssDNA (10656 nt) | JN882264                | 54.0 ± 4.3           | VP4, VP5                       | [3, 26, 36] |
| <i>Halorubrum virus</i><br><i>HRPV-3</i>      | HRPV-3       | Experimental Dead Sea-Red Sea saltwater pond of Sedom, Israel | <i>Halorubrum</i> sp. SP3-3        | Circular dsDNA (8770 bp)  | JN882265                | 67.2 ± 5.2           | VP1, VP2                       | [3, 26, 36] |
| <i>Halorubrum virus</i><br><i>HRPV-6</i>      | HRPV-6       | Solar saltern, Samut Sakhon, Thailand                         | <i>Halorubrum</i> sp. SS7-4        | Circular ssDNA (8549 nt)  | JN882266                | 48.5 ± 2.7           | VP4, VP5                       | [26, 36]    |
| <i>Halogeometricum virus</i><br><i>HGPV-1</i> | HGPV-1       | Solar saltern, Cabo de Gata, Spain                            | <i>Halogeometricum</i> sp. CG-9    | Circular dsDNA (9694 bp)  | JN882267                | 55.5 ± 5.2           | VP2, VP3, VP4                  | [3, 26, 36] |
| <i>Haloarcula virus</i><br><i>HHPV-1</i>      | HHPV-1       | Solar saltern, Margherita di Savoia, Italy                    | <i>Haloarcula</i> <i>hispanica</i> | Circular dsDNA (8082 bp)  | GU321093                | 51.7 ± 4.0           | VP3, VP4                       | [26, 34]    |
| <i>Haloarcula virus</i><br><i>HHPV-2</i>      | HHPV-2       | Solar saltern, Hulu Island, Liaoning, China                   | <i>Haloarcula</i> <i>hispanica</i> | Circular ssDNA (8176 nt)  | KF056323                | ~50                  | NA                             | [21]        |
| <i>Haloarcula virus</i><br><i>His2</i>        | His2         | Hypersaline lake, Victoria, Australia                         | <i>Haloarcula</i> <i>hispanica</i> | Linear dsDNA (16067 bp)   | AF191797                | 70.6 ± 3.6           | VP27, VP28, VP29, VP32         | [11, 26]    |

NA – not analyzed



1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49

**Table 2.** Taxonomic structure of the proposed "Pleolipoviridae" family.

| <b>Genus</b>              | <b>Species</b>                                | <b>Representative isolate<sup>a</sup></b>                |
|---------------------------|---|--|
| <i>Alphapleolipovirus</i> | <i>Halorubrum virus HRPV-1</i> (type species) | <i>Halorubrum</i> pleomorphic virus 1 (HRPV-1)           |
|                           | <i>Halorubrum virus HRPV-2</i>                | <i>Halorubrum</i> pleomorphic virus 2 (HRPV-2)           |
|                           | <i>Halorubrum virus HRPV-6</i>                | <i>Halorubrum</i> pleomorphic virus 6 (HRPV-6)           |
|                           | <i>Haloarcula virus HHPV-1</i>                | <i>Haloarcula hispanica</i> pleomorphic virus 1 (HHPV-1) |
|                           | <i>Haloarcula virus HHPV-2</i>                | <i>Haloarcula hispanica</i> pleomorphic virus 1 (HHPV-2) |
| <i>Betapleolipovirus</i>  | <i>Halorubrum virus HRPV-3</i> (type species) | <i>Halorubrum</i> pleomorphic virus 3 (HRPV-3)           |
|                           | <i>Halogeometricum virus HGPV-1</i>           | <i>Halogeometricum</i> pleomorphic virus 1 (HGPV-1)      |
| <i>Gammapleolipovirus</i> | <i>Haloarcula virus His2</i> (type species)   | His2 virus (His2)  |

<sup>a</sup> Abbreviation of the virus is given in parentheses.

## Figure legends

**Figure 1.** Members of the family "Pleolipoviridae" are globally distributed. Dots indicate the origin of virus isolates. Orange indicates that the virus was isolated using a host strain isolated from the same location and green that the virus was isolated using a culture collection strain of *Haloarcula hispanica* [3, 11, 21, 24, 26, 34]. Source of the map: Wikimedia Commons.

**Figure 2.** Cryo-electron microscopy images of seven pleolipovirus isolates. Scale bar, 100 nm. Reproduced from [26] with permission.

**Figure 3.** Structural components of the seven pleolipoviruses. (A) Protein and lipid profile of the purified virions in a tricine-SDS-polyacrylamide gel stained with Coomassie blue and Sudan Black B for proteins and lipids, respectively. Numbers on the left indicate the molecular masses of the markers. Numbers on the gel indicate the gene encoding the protein. The theoretical position of VP2 protein of HGPV-1 is marked by a circle. Reproduced from [26] with permission. Protein and lipid profiles are not available for HHPV-2. (B) Schematic presentation of the HRPV-1 virion. HRPV-1 is the model virus of the proposed family "Pleolipoviridae". Genomes of the pleolipoviruses can be either ssDNA or dsDNA, linear or circular.

**Figure 4.** A linear representation of the pleolipovirus genomes. The identities (%) between the amino acid sequences of two predicted (or identified) gene products are indicated. Based on their genome organization and the relatedness of their VP3-like proteins, the pleolipoviruses can be divided into three genera, which are indicated on the left.

## References

1. Abrescia NG, Bamford DH, Grimes JM, Stuart DI (2012) Structure unifies the viral universe. *Annu Rev Biochem* 81:795-822
2. Al-Shammari AJN, Smith PF (1981) Lipid composition of two mycoplasmaviruses, MV-Lg-L172 and MVL2. *J Gen Virol* 54:455-458
3. Atanasova NS, Roine E, Oren A, Bamford DH, Oksanen HM (2012) Global network of specific virus-host interactions in hypersaline environments. *Environ Microbiol* 14:426-440
4. Atanasova NS, Bamford DH, Oksanen HM (2015) Haloarchaeal virus morphotypes. *Biochimie* In Press (doi:10.1016/j.biochi.2015.07.002)
5. Atanasova NS, Demina TA, Buivydas A, Bamford DH, Oksanen HM (2015) Archaeal viruses multiply: temporal screening in a solar saltern. *Viruses* 7:1902-1926
6. Atanasova NS, Oksanen HM, Bamford DH (2015) Haloviruses of archaea, bacteria and eukaryotes. *Curr Opin Microbiol* 25:40-48
7. Atanasova NS, Sencilo A, Pietilä MK, Roine E, Oksanen HM, Bamford DH (2015) Comparison of lipid-containing bacterial and archaeal viruses. *Adv Virus Res* 92:1-61
8. Baltimore D (1971) Expression of animal virus genomes. *Bacteriol Rev* 35:235-241
9. Bamford DH, Burnett RM, Stuart DI (2002) Evolution of viral structure. *Theor Popul Biol* 61:461-470
10. Bamford DH (2003) Do viruses form lineages across different domains of life? *Res Microbiol* 154:231-236
11. Bath C, Cukalac T, Porter K, Dyall-Smith ML (2006) His1 and His2 are distantly related, spindle-shaped haloviruses belonging to the novel virus group, *Salterprovirus*. *Virology* 350:228-239
12. Benson SD, Bamford JK, Bamford DH, Burnett RM (1999) Viral evolution revealed by bacteriophage PRD1 and human adenovirus coat protein structures. *Cell* 98:825-833
13. Benson SD, Bamford JK, Bamford DH, Burnett RM (2004) Does common architecture reveal a viral lineage spanning all three domains of life? *Mol Cell* 16:673-685
14. Chen S, Wang C, Xu JP, Yang ZL (2014) Molecular characterization of pHRDV1, a new virus-like mobile genetic element closely related to pleomorphic viruses in haloarchaea. *Extremophiles* 18:195-206
15. Dyall-Smith ML, Pfeiffer F, Klee K, Palm P, Gross K, Schuster SC, Rampp M, Oesterhelt D (2011) *Haloquadratum walsbyi*: limited diversity in a global pond. *PLoS One* 6:e20968
16. Dybvig K, Nowak JA, Sladek TL, Maniloff J (1985) Identification of an enveloped phage, mycoplasma virus L172, that contains a 14-kilobase single-stranded DNA genome. *J Virol* 53:384-390
17. Hong C, Pietilä MK, Fu CJ, Schmid MF, Bamford DH, Chiu W (2015) Lemon-shaped halo archaeal virus His1 with uniform tail but variable capsid structure. *Proc Natl Acad Sci U S A* 112:2449-2454
18. Kandiba L, Aitio O, Helin J, Guan Z, Permi P, Bamford DH, Eichler J, Roine E (2012) Diversity in prokaryotic glycosylation: an archaeal-derived N-linked glycan contains legionaminic acid. *Mol Microbiol* 84:578-593
19. King AMQ, Adams MJ, Carstens EB, Lefkowitz EJ (2012) *Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses*. Elsevier Academic Press, London
20. Krupovic M, Makarova KS, Forterre P, Prangishvili D, Koonin EV (2014) Casposons: a new superfamily of self-synthesizing DNA transposons at the origin of prokaryotic CRISPR-Cas immunity. *BMC Biol* 12:36
21. Li M, Wang R, Zhao D, Xiang H (2014) Adaptation of the *Haloarcula hispanica* CRISPR-Cas system to a purified virus strictly requires a priming process. *Nucleic Acids Res* 42:2483-2492
22. Maniloff J, Kampo GJ, Dascher CC (1994) Sequence analysis of a unique temperature phage: mycoplasma virus L2. *Gene* 141:1-8
23. Mochizuki T, Krupovic M, Pehau-Arnaudet G, Sako Y, Forterre P, Prangishvili D (2012) Archaeal virus with exceptional virion architecture and the largest single-stranded DNA genome. *Proc Natl Acad Sci U S A* 109:13386-13391

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
24. Pietilä MK, Roine E, Paulin L, Kalkkinen N, Bamford DH (2009) An ssDNA virus infecting archaea: a new lineage of viruses with a membrane envelope. *Mol Microbiol* 72:307-319
  25. Pietilä MK, Laurinavičius S, Sund J, Roine E, Bamford DH (2010) The single-stranded DNA genome of novel archaeal virus *Halorubrum* pleomorphic virus 1 is enclosed in the envelope decorated with glycoprotein spikes. *J Virol* 84:788-798
  26. Pietilä MK, Atanasova NS, Manole V, Liljeroos L, Butcher SJ, Oksanen HM, Bamford DH (2012) Virion architecture unifies globally distributed pleolipoviruses infecting halophilic archaea. *J Virol* 86:5067-5079
  27. Pietilä MK, Atanasova NS, Oksanen HM, Bamford DH (2013) Modified coat protein forms the flexible spindle-shaped virion of haloarchaeal virus His1. *Environ Microbiol* 15:1674-1686
  28. Pietilä MK, Laurinmäki P, Russell DA, Ko CC, Jacobs-Sera D, Hendrix RW, Bamford DH, Butcher SJ (2013) Structure of the archaeal head-tailed virus HSTV-1 completes the HK97 fold story. *Proc Natl Acad Sci U S A* 110:10604-10609
  29. Pietilä MK, Demina TA, Atanasova NS, Oksanen HM, Bamford DH (2014) Archaeal viruses and bacteriophages: comparisons and contrasts. *Trends Microbiol* 22:334-344
  30. Pina M, Bize A, Forterre P, Prangishvili D (2011) The archeoviruses. *FEMS Microbiol Rev* 35:1035-1054
  31. Porter K, Dyall-Smith ML (2008) Transfection of haloarchaea by the DNAs of spindle and round haloviruses and the use of transposon mutagenesis to identify non-essential regions. *Mol Microbiol* 70:1236-1245
  32. Prangishvili D, Forterre P, Garrett RA (2006) Viruses of the Archaea: a unifying view. *Nat Rev Microbiol* 4:837-848
  33. Rice G, Tang L, Stedman K, Roberto F, Spuhler J, Gillitzer E, Johnson JE, Douglas T, Young M (2004) The structure of a thermophilic archaeal virus shows a double-stranded DNA viral capsid type that spans all domains of life. *Proc Natl Acad Sci U S A* 101:7716-7720
  34. Roine E, Kukkaro P, Paulin L, Laurinavičius S, Domanska A, Somerharju P, Bamford DH (2010) New, closely related haloarchaeal viral elements with different nucleic acid types. *J Virol* 84:3682-3689
  35. Roine E, Oksanen HM (2011) Viruses from the hypersaline environment. In: Ventosa A, Oren A, Ma Y (eds) *Halophiles and Hypersaline Environments: Current Research and Future Trends*. Springer-Verlag Berlin Heidelberg, pp 153-172
  36. Senčilo A, Paulin L, Kellner S, Helm M, Roine E (2012) Related haloarchaeal pleomorphic viruses contain different genome types. *Nucleic Acids Res* 40:5523-5534
  37. Sime-Ngando T, Lucas S, Robin A, Tucker KP, Colombet J, Bettarel Y, Desmond E, Gribaldo S, Forterre P, Breitbart M, Prangishvili D (2011) Diversity of virus-host systems in hypersaline Lake Retba, Senegal. *Environ Microbiol* 13:1956-1972

Figure 1  
[Click here to download Figure: Figure\\_1.tif](#)

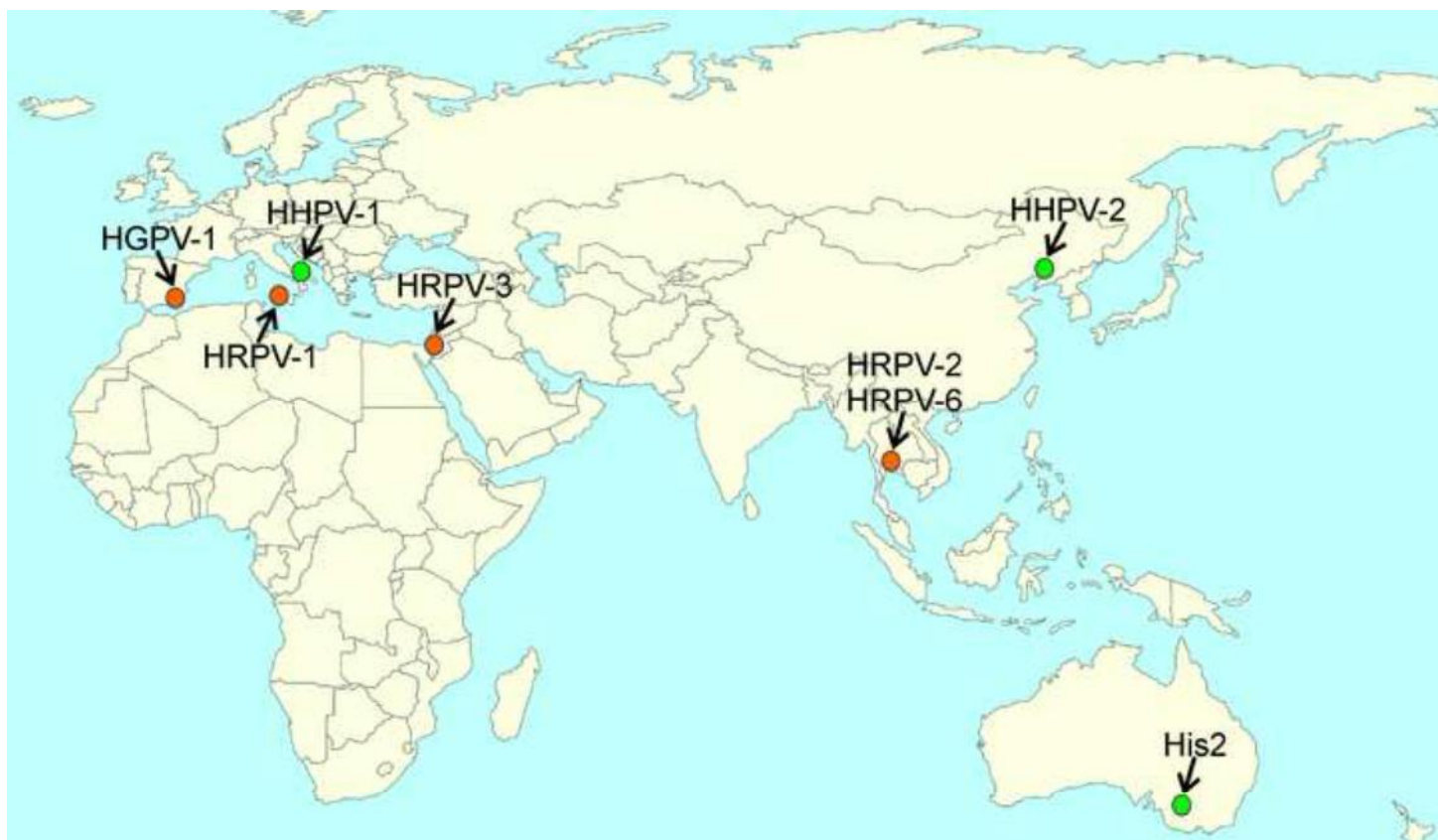


Figure 2  
[Click here to download Figure: Figure\\_2.tif](#)

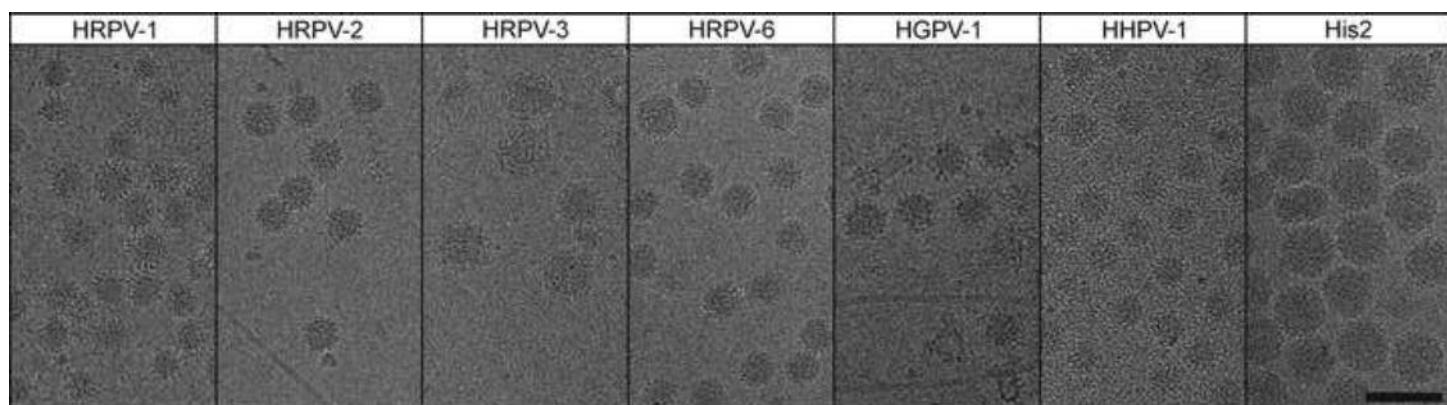


Figure 3  
[Click here to download Figure: Figure\\_3.tif](#)

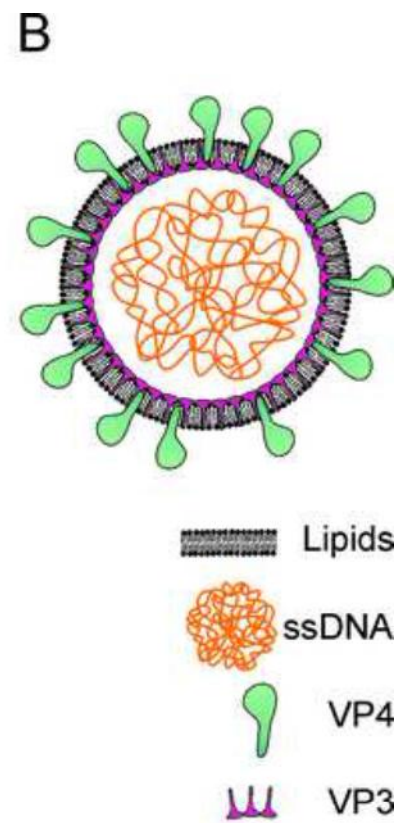
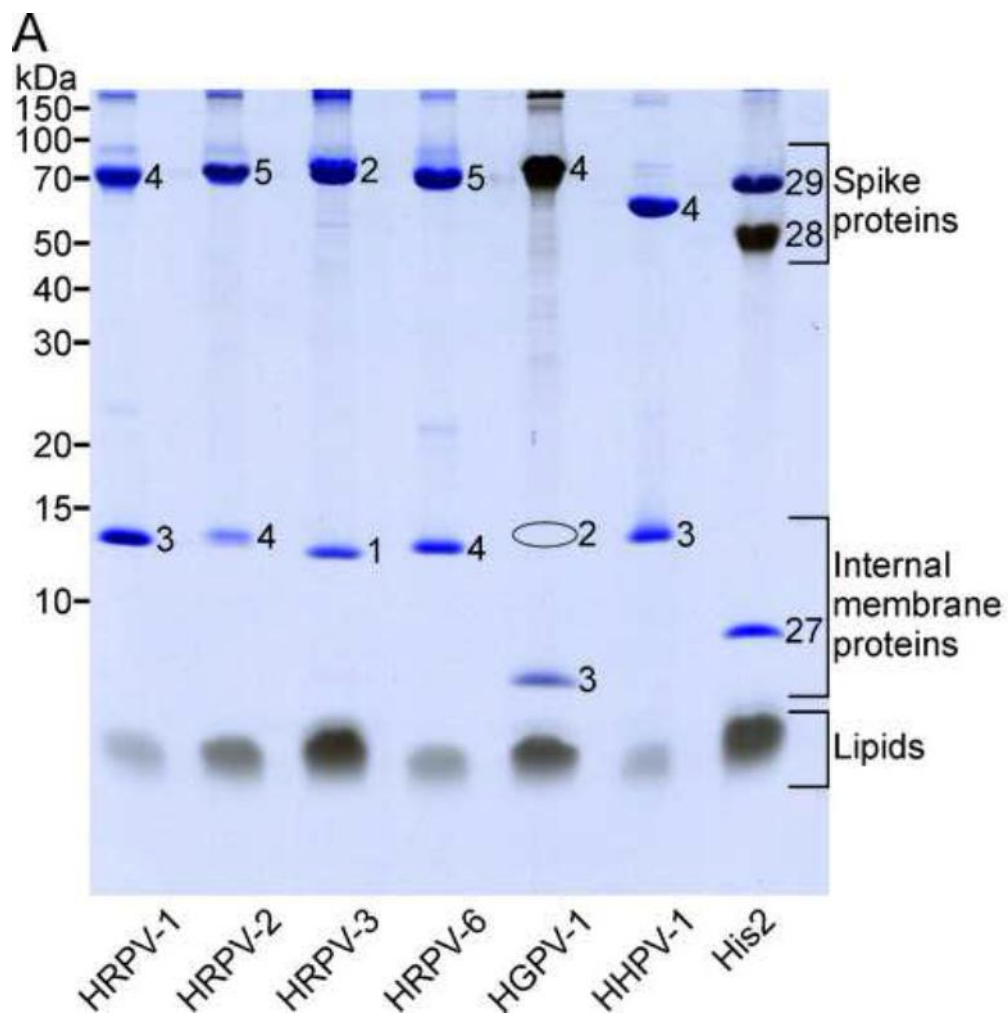


Figure 4  
[Click here to download Figure: Pleoviridae\\_Figure 4\\_revision.tif](#)

