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:	***2015.042a-oB***	(to be completed by ICTV officers)

Short title: Create eight (8) new species and three (3) new genera to be assigned in a new family *Pleolipoviridae.*
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 10 are required)	**1** ☒	**2** ☒	**3** ☒	**4** ☐	**5** ☒
	6 ☐	**7** ☐	**8** ☐	**9** ☒	**10** ☒

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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)	

ICTV Study Group comments (if any) and response of the proposer:

Approved by Bacterial and Archaeal Virus Subcommittee Chair – Andrew M. Kropinski

Date first submitted to ICTV: June 3, 2015
Date of this revision: August 7, 2015

ICTV-EC comments and response of the proposer:

MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

Code	***2015.042aB***	(assigned by ICTV officers)

To create five new species within:

Genus:	*Alphapleolipovirus* (new)
Subfamily:	
Family:	***Pleolipoviridae* (new)**
Order:	

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Halorubrum virus HRPV-1	Halorubrum pleomorphic virus 1, HRPV-1	FJ685651
Halorubrum virus HRPV-2	Halorubrum pleomorphic virus 2, HRPV-2	JN882264
Halorubrum virus HRPV-6	Halorubrum pleomorphic virus 6, HRPV-6	JN882266
Haloarcula virus HHPV-1	Haloarcula hispanica pleomorphic virus 1, HHPV-1	GU321093
Haloarcula virus HHPV-2	Haloarcula hispanica pleomorphic virus 2, HHPV-2	KF056323

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 9

Although the genomes are collinear and all include the predicted replication initiation encoding gene, the sequence comparison of the viruses between species shows low identity over the whole genome nucleotide sequence.

MODULE 3: **NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	***2015.042bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Pleolipoviridae* (new)**
Order:	

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2015.042cB***	(assigned by ICTV officers)

To name the new genus:

Alphapleolipovirus

Assigning the type species and other species to a new genus

Code	***2015.042dB***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Halorubrum virus HRPV-1

Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain:**

5

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

Please see Annex.

Origin of the new genus name:

Pleolipoviridae (from the Greek pleo, for more or many; from the Greek lipos, for lipid)

Reasons to justify the choice of type species:

HRPV-1 is the most extensively characterized isolate (see Annex).

Species demarcation criteria in the new genus:

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

Species demarcation criteria in the genus *Alphapleolipovirus* are the following: Their genomes are collinear and all include the predicted rolling-circle replication initiation encoding gene. However, the sequence comparison of the viruses between species shows low identity over the whole genome nucleotide sequence.

MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

Code	***2015.042eB***	(assigned by ICTV officers)

To create two new species within:

Genus:	*Betapleolipovirus (new)*
Subfamily:	
Family:	***Pleolipoviridae* (new)**
Order:	

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Halorubrum virus HRPV-3	Halorubrum pleomorphic virus 3, HRPV-3	JN882265
Halogeometricum virus HGPV-1	Halogeometricum pleomorphic virus 1, HGPV-1	JN882267

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 9

Although the genomes of these two viruses are collinear and both include the gene encoding conserved haloarchaeal protein containing a winged-helix DNA binding domain showing 50% identity at the level of polypeptide sequence, the sequence comparison of the viruses between species shows low identity over the whole genome nucleotide sequence and different gene content in the variable regions of the genomes.

MODULE 3: **NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	***2015.042fB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Pleolipoviridae***
Order:	

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2015.042gB***	(assigned by ICTV officers)

To name the new genus:

Betapleolipovirus

Assigning the type species and other species to a new genus

Code	***2015.042hB***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Halorubrum virus HRPV-3

Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain:**

2

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

Please see Annex.

Origin of the new genus name:

Pleolipoviridae (from the Greek pleo, for more or many; from the Greek lipos, for lipid)

Reasons to justify the choice of type species:

HRPV-3 is the most extensively characterized isolate (see Annex).

Species demarcation criteria in the new genus:

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

Species demarcation criteria in the genus *Betapleolipovirus* are: The genomes are collinear. Sequence comparison of the genes between species shows low identity except for the gene encoding conserved haloarchaeal protein containing a winged-helix DNA binding domain, which is also the distinguishing feature for the genus. See Annex.

MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

Code	***2015.042iB***	(assigned by ICTV officers)

To create two new species within:

Genus:	*Gammapleolipovirus (new)*
Subfamily:	
Family:	***Pleolipoviridae* (new)**
Order:	

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no genus is specified, enter "**unassigned**" in the genus box.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Haloarcula virus His2	His2 virus	AF191797

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
  - If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
  - If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria.
- Further material in support of this proposal may be presented in the Appendix, Module 9

This virus is the only member of the genus.

MODULE 3: **NEW GENUS**

creating a new genus

Ideally, a genus should be placed within a higher taxon.

Code	***2015.042jB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Pleolipoviridae***
Order:	

Fill in all that apply.

- If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
- If no family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2015.042kB***	(assigned by ICTV officers)

To name the new genus:

Gammapleolipovirus (new)

Assigning the type species and other species to a new genus

Code	***2015.042lB***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Haloarcula virus His2

Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of species (including the type species) that the genus will contain:**

1

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

Please see Annex.

Origin of the new genus name:

Pleolipoviridae (from the Greek pleo, for more or many; from the Greek lipos, for lipid)

Reasons to justify the choice of type species:

His2 virus is the only representative of this genus (see Annex).

Species demarcation criteria in the new genus:

If there will be more than one species in the new genus, list the criteria being used for species demarcation and explain how the proposed members meet these criteria.

The genus *Gammapleolipovirus* currently contains only one proposed species, *Haloarcula virus His2*. The genome of this virus contains a gene encoding a putative protein-priming B-type DNA polymerase. See Annex.

MODULE 5: **NEW FAMILY**

creating and naming a new family

Code	***2015.042mB***	(assigned by ICTV officers)

To create a new family containing the subfamilies and/or genera listed below within the Order: *unassigned*

If there is no Order, write "**unassigned**" here.
If the Order has yet to be created (in Module 6) please write **"(new)"** after the proposed name.

Code	***2015.042nB***	(assigned by ICTV officers)

To name the new family: *Pleolipoviridae*

assigning subfamilies, genera and unassigned species to a new family

Code		(assigned by ICTV officers)

To assign the following subfamilies (if any) to the new family:
You may list several subfamilies here. For each subfamily, please state whether it is new or existing.

- If the subfamily is new, it must be created in Module 4
- If the subfamily already exists, please complete Module 7 to 'REMOVE' it from its existing family

Code	***2015.042oB***	(assigned by ICTV officers)

To assign the following genera to the new family:
You may list several genera here. For each genus, please state whether it is new or existing.

- If the genus is new, it must be created in Module 3
- If the genus already exists, please state whether it is currently unassigned or is to be removed from another family. If the latter, complete Module 7 to 'REMOVE' it from that family

***Alphapleolipovirus* – new**
***Betapleolipovirus* – new**
***Gammapleolipovirus* - new**

The new family will also contain any other new species created and assigned to it (Module 3) and any that are being moved from elsewhere (Module 7b). **Please enter here the TOTAL number of unassigned species that the family will contain (those NOT within any of the genera or subfamilies listed above):**

0

Reasons to justify the creation of the new family:
Additional material in support of this proposal may be presented in the Appendix, Module 9

Eight viruses have been isolated from globally distant locations, and they share a similar structural protein pattern, genome synteny, and sequence similarity (see Annex). These viruses do not show significant similarity to any other known archaeal virus and thus should be classified as a new family, *Pleolipoviridae*. One of these viruses, His2 virus, has previously been proposed to be a spindle-shaped virus distantly related to His1 virus, the type species of the *Salterprovirus* genus. However, a cryo-EM analysis (see Annex) shows that His2 virus is not spindle-shaped but rather spherical in shape resembling other pleolipoviruses. In addition, His2 virus has significant amino acid sequence similarity with the other viruses in the proposed family *Pleolipoviridae* but not with His1 virus.

Origin of the new family name:
Pleolipoviridae (from the Greek pleo, for more or many; from the Greek lipos, for lipid)

MODULE 9: **NON-STANDARD**
Template for any proposal not covered by modules 2-8.

non-standard proposal

Code		(assigned by ICTV officers)

Title of proposal: List of other related virus isolates which may be members of the family *Pleolipoviridae*, but have not been approved as species.

Text of proposal:

Three haloarchaeal pleomorphic viruses, *Halorubrum* pleomorphic viruses 7 and 8 (HRPV-7 and HRPV-8) and *Haloarcula* pleomorphic virus 2 (HAPV-2) have been recently isolated and partially characterized (see Annex). These isolates display characteristic pleomorphic virion morphotype and structural protein pattern. They all infect halophilic archaea. The infectivity of HRPV-7 and HAPV-2 is affected by the presence of chloroform suggesting that there is a membrane in the virion. However, the genome sequences of these viruses are not available and this precludes their final taxonomic positioning. Thus, they are considered as tentative species of the family *Pleolipoviridae*.

Tentative members of the *Pleolipoviridae* family:
Halorubrum pleomorphic virus 7, HRPV-7
Halorubrum pleomorphic virus 8, HRPV-8
Haloarcula pleomorphic virus 2, HAPV-2

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Atanasova, N.S., Roine, E., Oren, A., Bamford, D.H., and Oksanen, H.M. (2012) Global network of specific virus-host interactions in hypersaline environments. *Environmental Microbiology* **14**: 426-440.

Atanasova, N.S., Demina, T.A., Buivydas, A., Bamford, D.H., and Oksanen HM. (2015) Archaeal viruses multiply: temporal screening in a solar saltern. *Viruses* **7**: 1902-1926.

Bath C., Cukalac T., Porter K., and Dyall-Smith M.L. (2006) His1 and His2 are distantly related, spindle-shaped haloviruses belonging to the novel virus group, *Salterprovirus*. *Virology* **350:** 228-239.

Dyall-Smith M.L., Pfeiffer F., Klee K., Palm P., Gross K., Schuster S.C., *et al.* (2011) *Haloquadratum walsbyi* : Limited diversity in a global pond. *PLoS One* **6:** e20968.

Kandiba L., Aitio O., Helin J., Guan Z., Permi P., Bamford D.H., Eichler J., Roine, E. (2012) Diversity in prokaryotic glycosylation: an archaeal-derived N-linked glycan contains legionaminic acid. *Molecular Microbiology* **84:** 578-593.

King A.M.Q., Adams M.J., Carstens E.B., Lefkowitz E.J. (2011) Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, London.

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 Research* **42**:2483-2492

Pietilä M.K., Atanasova N.S., Manole V., Liljeroos L., Butcher S.J., Oksanen H.M., Bamford D.H. (2012) Virion architecture unifies globally distributed pleolipoviruses infecting halophilic archaea. *Journal of Virology* **86**:5067-5079.

Pietilä M.K., Laurinavičius S., Sund J., Roine E., and Bamford D.H. (2010) The single-stranded DNA genome of novel archaeal virus *Halorubrum* pleomorphic virus 1 is enclosed in the envelope decorated with glycoprotein spikes. *Journal of Virology* **84:** 788-798.

Pietilä M.K., Roine E., Paulin L., Kalkkinen N., and Bamford D.H. (2009) An ssDNA virus infecting archaea: A new lineage of viruses with a membrane envelope. *Molecular Microbiology* **72:** 307-319.

Pina M., Bize A., Forterre P., and Prangishvili D. (2011) The archeoviruses. *FEMS Microbiology Reviews* **35:** 1035-1054.

Roine E., Kukkaro P., Paulin L., Laurinavičius S., Domanska A., Somerharju P., and Bamford D.H. (2010) New, closely related haloarchaeal viral elements with different nucleic acid types. *Journal of Virology* **84:** 3682-3689.

Roine, E. and Oksanen, H.M. (2011) Viruses from the hypersaline environment. In *Halophiles and Hypersaline Environments: Current Research and Future Trends.* Eds: Ventosa, A., Oren, A., and Ma, Y. pp. 153-172. Springer-Verlag Berlin Heidelberg.

Senčilo A., Paulin L., Kellner S., Helm M., Roine E. (2012) Related haloarchaeal pleomorphic viruses contain different genome types. *Nucleic Acids Research*. **40**: 5523-5534

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.

To date, eight pleomorphic viruses infecting halophilic archaea of the phylum *Euryarchaeota* have been isolated, HRPV-1, HRPV-2, HRPV-3, HRPV-6, HGPV-1, HHPV-1, HHPV-2 and His2 virus (Atanasova *et al*., 2012; Bath *et al*., 2006; Li et al., 2014; Pietilä *et al*., 2009 and 2012; Roine *et al*., 2010; Senčilo *et al.*, 2012). These viruses are composed of a membrane vesicle containing two major structural proteins (Fig. 1A). The biochemical analyses have shown that one major structural protein forms spikes (VP4-like protein according the nomenclature of HRPV-1) anchored to the membrane and the other one is associated with the membrane (VP3-like protein) facing the particle interior where the genome resides without associated nucleoproteins (Fig. 1B) (Pietilä *et al*., 2009, 2010 and 2012). HGPV-1 is an exception with three major structural proteins of which two are membrane proteins (Pietilä *et al*., 2012; Senčilo *et al.*, 2012).

Spindle-shaped His1 infecting *Haloarcula hispanica* is classified as the type species of the genus *Salterprovirus*. Currently, another virus infecting the same host, His2, has been proposed to be a member of this genus (King *et al*., 2011). His1 and His2 are proposed to be distantly related although they share no significant amino acid sequence similarity except for their putative DNA polymerases (Bath *et al*., 2006). Cryo-electron microscopy (cryo-EM) studies reveal that His2 is not spindle-shaped but spherical in shape and overall similar to the other pleomorphic viruses (Fig. 2) (Pietilä *et al*., 2012). In addition, His2 has a similar protein pattern as the other isolates, with the exception of two spike proteins (Fig. 1A) (Pietilä *et al*., 2012). The genome synteny and amino acid sequence similarity also suggest the relationship between His2 and the pleomorphic viruses (Pietilä *et al*., 2009; Roine *et al*., 2010; Senčilo *et al.*, 2012). Thus, we propose that His2 should be classified in the same group as the pleomorphic virus isolates.

Negative-stain transmission EM suggested that the pleomorphic viruses have flexible virion structure not defined by a rigid protein capsid (Pietilä *et al*., 2010 and 2012; Roine *et al*., 2010). To avoid possible artifacts caused by negative staining, the virion morphology was studied using cryo-electron microscopy and cryo-electron tomography (cryo-ET). The cryo-electron micrographs of the seven viruses show roughly spherical, intact particles, with decorating spikes on the virion surface (Fig. 2) (Pietilä *et al*., 2012). It was observed that the dimensions of the individual viruses varied. The smallest of the viruses is HRPV-1 (41.1 ± 2.2 nm) and the largest is His2 (70.6 ± 3.6 nm) (Pietilä *et al*., 2012). The pleomorphicity of the viruses is thus obvious in the wide range of sizes that each virus exhibits. In addition, cryo-ET of HRPV-1 showed that there is an apparent lack of longitudinal order in the surface spikes (Pietilä *et al*., 2012) emphasizing the pleomorphicity. HRPV-1 VP4 is glycosylated (Pietilä *et al.*, 2010), and the structure of the major N-glycan of VP4 has been determined to be a pentasaccharide comprising glucose, glucuronic acid, mannose, sulphated glucuronic acid and a terminal 5-N-formyl-legionaminic acid residue (Kandiba *et al.*, 2012).

The genome sequences of eight haloarchaeal viruses discussed here are available. The nucleotide sequence similarity of the genomes to other sequences in the databases is very limited. The genomes showed collinear gene organization, but the genomes of HRPV-1, HRPV-2, HRPV-6 and HHPV-2 are ssDNA molecules, whereas HHPV-1, HRPV-3, HGPV-1 and His2 have dsDNA genomes (Bath *et al.* 2006; Pietilä *et al*., 2009; Roine *et al*., 2010; Senčilo *et al.*, 2012, Li et al., 2014). His2 has a linear genome and the other virus isolates have circular ones. The size of the circular genomes varies from 7,048 nt (HRPV-1) to 10,656 nt (HRPV-2), and the linear His2 genome is 16,067 bp in size. The GC contents of the genomes vary between 40% (His2) and 64% (HRPV-2). At the nucleotide sequence level the genomes show similarity (60% or higher) only along very short stretches. Exceptions to this are the HRPV-2 and HRPV-6 genomes (80% similar) as well as the HHPV-1 and HHPV-2 genomes (approximately 80% identical). The core genes of the genomes consist of the small and large major structural protein genes and three conserved predicted downstream genes encoding putative NTPase and two assembly factors (Fig. 3). Within the pleolipoviruses, the highest identity at the amino acid level can be found between the small major structural proteins (VP3-like). HRPV-1, HHPV-1, HHPV-2, HRPV-2 and HRPV-6 additionally share a predicted gene encoding a putative rolling-circle replication initiation protein. The genomes of HRPV-3, HGPV-1 and His2 do not contain this putative gene, but encode a putative conserved protein containing a winged-helix DNA binding domain (HRPV-3 and HGPV-1) or a putative protein priming B-type DNA polymerase (His2) (Senčilo *et al.*, 2012; Bath *et al.* 2006).

Phylogenetic analysis of the pleolipoviral canonical gene products within each genus has been carried out previously (Senčilo *et al.*, 2012), except for the spike proteins (VP4-like), for which the amino acid sequence identities are too low for reliable analysis. This is expected as VP4-like proteins are responsible for host recognition and are considered to be under high selective pressure. For internal membrane protein (VP3-like), putative NTPase (VP8-like) and ORF7-like gene product, comparatively high conservation allowed reconstruction of the phylogenetic histories (Senčilo *et al.*, 2012). In these cases the predicted phylogenetic histories were similar.

The pleolipoviruses infecting haloarchaea have been isolated from six globally distant locations emphasizing the worldwide distribution of these viruses (Atanasova *et al*., 2012; Bath *et al*., 2006). Recently, three new pleomorphic viruses *Halorubrum* pleomorphic viruses 7 and 8 (HRPV-7 and HRPV-8) and *Haloarcula* pleomorphic virus 2 (HAPV-2) have been isolated from a solar saltern of Samut Sakhon, Thailand (Atanasova *et al.*, 2015). We propose that these three virus isolates are members of *Pleolipoviridae*. These isolates infect halophilic archaea and they display characteristics of pleolipoviruses: pleomorphic membrane vesicle appearance by negative-staining transmission electron microscopy, pleolipovirus-like simple protein pattern of purified virions, and hazy plaque morphology. In addition, possible proviruses related to the pleolipoviruses have been identified in the genomes of several haloarchaea (Dyall-Smith *et al*., 2011; Pietilä *et al*., 2009; Roine *et al*., 2010; Roine and Oksanen, 2011; Senčilo *et al.*, 2012).

In summary, our recent data suggest that the eight pleolipoviruses infecting halophilic archaea share both morphological and structural features. Despite the different genome types, the viruses also share genome synteny and sequence similarity. Accordingly, we propose a new viral family

for these viruses, *Pleolipoviridae* (from the Greek pleo, for more or many; from the Greek lipos, for lipid). We propose that three genera, *Alphapleolipovirus*, *Betapleolipovirus*, and *Gammapleolipovirus*, are created within the family *Pleolipoviridae.* Species and genus demarcation criteria for the family in the three genera are based on the genome structure and gene content (Sencilo *et al.*, 2012). The subgrouping is also in line with the relatedness of the VP3-like proteins.

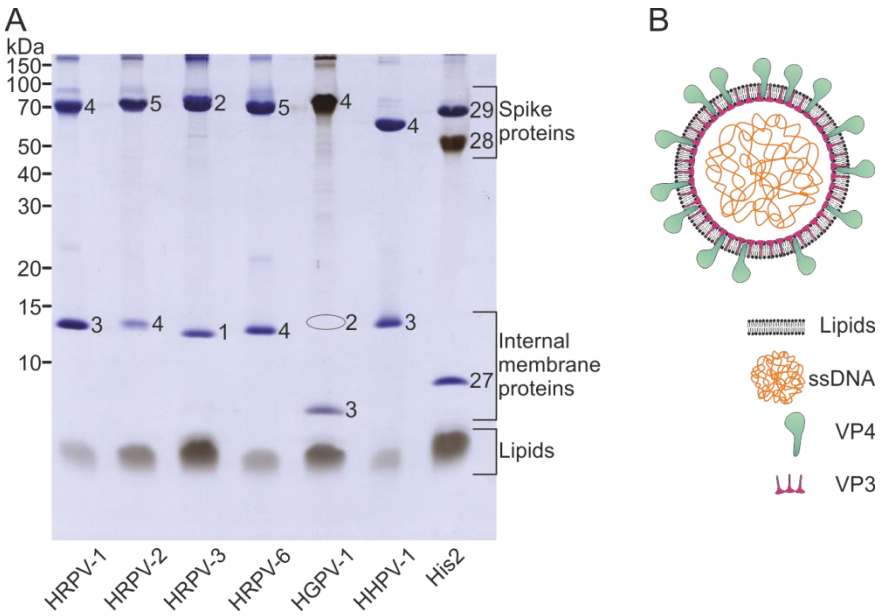

Figure 1. Structural components of the seven pleolipoviruses. (A) Protein and lipid profile of the purified virions in a tricine-SDS-polyacrylamide gel stained with Coomassie blue and Sudan Black B for proteins and lipids, respectively. Numbers on the left indicate the molecular masses of the markers. Numbers on the gel indicate the gene encoding the protein. The theoretical position of VP2 protein of HGPV-1 is marked by a circle. 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.

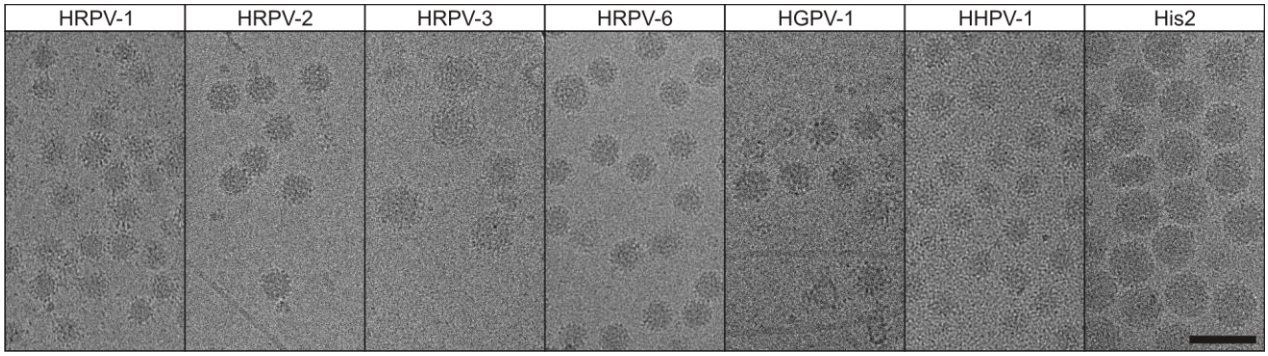

Figure 2. Cryo-EM images of seven pleolipovirus isolates. Scale bar, 100 nm. Reproduced from Pietilä *et al.*, 2012 with permission.

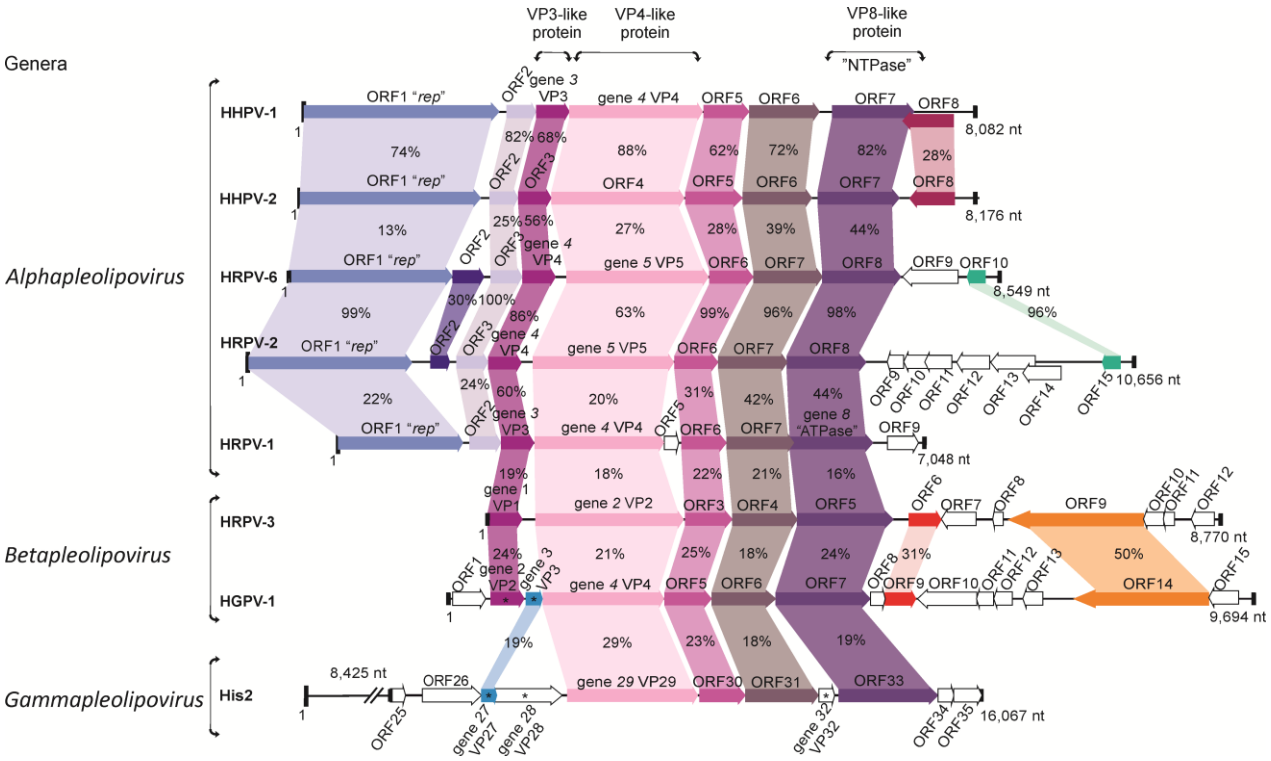

Figure 3. A linear representation of the pleolipovirus genomes. The identities (%) between the amino acid sequences of two predicted (or identified) gene products are indicated. More information of the pair-wise comparisons can be found from Senčilo *et al.*, 2012. Based on the genome organization, the pleolipoviruses can be divided into three genera which are indicated on the left.