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:	***2016.063aB***	(to be completed by ICTV officers)

Short title: One (1) new species in the genus *Alphasphaerolipovirus*, family *Sphaerolipoviridae*
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 11 are required)	**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)	Bacterial and Archaeal Viruses Subcommittee of ICTV

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

Date first submitted to ICTV: July 2, 2016
Date of this revision (if different to above):

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	***2016.063aB***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Alphasphaerolipovirus***
Subfamily:	
Family:	***Sphaerolipoviridae***
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 HCIV1	Haloarcula californiae icosahedral virus 1 (HCIV-1)	KT809302

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 11

HCIV-1 shares similar virion morphology (tailless icosahedral particles with an internal membrane), gene synteny, genome type (linear dsDNA), nucleic acid and protein sequences with the viruses belonging to the family *Sphaerolipoviridae*. The HCIV-1 sequences of the major capsid proteins and putative packaging ATPase are very similar with those of the viruses in *Alphasphaerolipovirus* genus.

MODULE 11: **APPENDIX**: supporting material

additional material in support of this proposal

References:

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

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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.

HCIV-1 as a member of *Alphasphaerolipovirus* genus of *Sphaerolipoviridae* family

Sphaerolipoviridae family

Currently the *Sphaerolipoviridae* family consists of icosahedral viruses with an internal membrane infecting either *Thermus* bacteria or members of *Halobacteriaceae* (Table 1; Pawlowski et al., 2014). HCIV-1 virion morphology is similar to those of the members of the *Sphaerolipoviridae* family. The virion structures have been solved for SH1, HHIV-2, and P23-77 and they obey $T = 28$ capsomer organization (Table 1). All sphaerolipoviruses have two major capsid protein species (MCPs) (or homologous genes). Genomes of sphaerolipoviruses are dsDNA molecules, which are either linear or circular.

Table 1. HCIV-1 and viruses classified to the family *Sphaerolipoviridae*.

Genus	Virus	Isolation source	Isolation host	Life cycle	Virion diameter	Capsid triangulation number (*T*)	Lipids demonstrated to be a structural component of the virion	Genome (GeneBank Acc No)	References
Alphasphaerolipovirus	HCIV-1	Solar saltern, Thailand	*Haloarcula californiae*	Lytic	~70 nm	-	Yes	Linear dsDNA, 31,314 bp (KT809302)	Atanasova et al., 2015; Demina et al., 2016
	SH1 (type virus)	Saline lake, Australia	*Haloarcula hispanica*	Lytic	~80 nm	*T* = 28, dextro	Yes	Linear dsDNA, 30,889 bp (AY950802)	Bamford et al., 2005; Jäälinoja et al., 2008; Kivelä et al., 2006; Porter et al., 2005
	HHIV-2	Solar saltern, Italy	*Haloarcula hispanica*	Lytic	~80 nm	*T* = 28, dextro	Yes	Linear dsDNA, 30,578 bp (JN968479)	Gil-Carton et al., 2015; Jaakkola et al., 2012
	PH1	Saline lake, Australia	*Haloarcula hispanica*	Lytic	~50 nm	-	-	Linear dsDNA, 28,072 bp (KC252997)	Porter et al., 2013
Betasphaerolipovirus	SNJ1 (type virus)	*Natrinema* sp. J7-1	*Natrinema* sp. J7-2	Lysogenic	~70-75 nm	-	Yes	Circular dsDNA, 16,341 bp (AY048850.1)	Liu et al., 2015; Zhang et al., 2012
Gammasphaerolipovirus	P23-77 (type virus)	Alkaline hot spring, New Zealand	*Thermus thermophilus*	Lytic or lysogenic	~78 nm	*T* = 28, dextro	Yes	Circular dsDNA, 17,036 bp (GQ403789)	Jaatinen et al., 2008; Jalasvuori et al., 2009; Rissanen et al., 2013
	IN93	*Thermus aquaticus* T Z2	*Thermus thermophilus*	Lysogenic		-	-	Circular dsDNA, 19,604 bp (AB063393)	Matsushita and Yanase, 2009

Alphasphaerolipovirus genus

All alphasphaeroliviruses have been isolated from halophilic environments and these virulent viruses infect *Haloarcula* sp. strains (Table 1). The HCIV-1 genome is colinear with the genomes of SH1, PH1 and HHIV-2 that are members of the *Alphasphaerolipovirus* genus (Figure 1). The genome type is the same for all the four viruses (linear dsDNA) and the lengths of the molecules are around 30 000 bp (Table 1). The overall levels of nucleotide identity of the HCIV-1 genome with the genomes of SH1, PH1, and HHIV-2 are ~63%, ~58%, and ~57%, respectively. There is a high level of gene synteny between the four viruses and the gene arrangement is highly conserved (Figure 1).

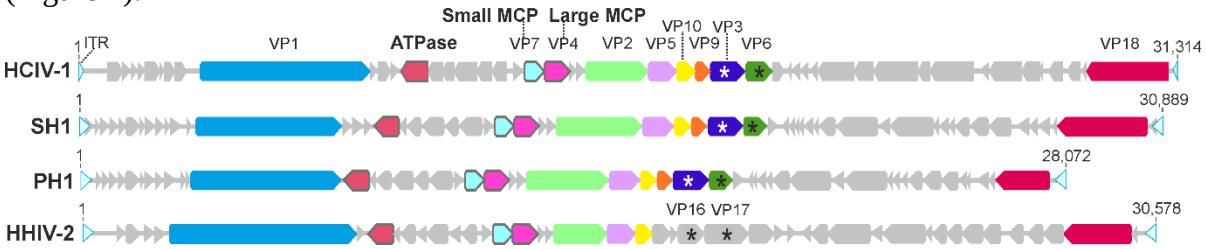

Figure 1. The genomes of HCIV-1 and alphasphaerolipoviruses SH1, PH1, and HHIV-2. ORFs/genes are shown as grey arrows, and homologous ones are colored (same colors in the four genomes). ORFs/genes products are shown on the top of the coding regions. The indicated protein names are the same for all four viruses, except two (putative) spike proteins (marked with asterisk): VP3 and VP6 for SH1, PH1, and HCIV-1, but VP16 and VP17 for HHIV-2. Inverted terminal repeats (ITRs) are shown as light blue arrows.

The HCIV-1 sequences of the MCPs and putative packaging ATPase are very similar with those of the viruses in *Alphasphaerolipovirus* genus (Tables 2 and 3). Alignment of the sequences of the MCPs and the putative packaging ATPases shows only few places where the amino acids are not similar (Figure 2). This high similarity suggests that the folds of the proteins could be the same. Phylogenetic analysis of the sequences of the MCPs and ATPases shows that HCIV-1 clusters with other members of the *Alphasphaerolipovirus* genus (Figure 3).

Table 2. Amino acid identities (%[a]) between sequences of small MCP (VP7), large MCP (VP4) and putative packaging ATPase of the alphasphaerolipoviruses.

	SH1	PH1	HHIV-2
HCIV-1	VP7 85.4 VP4 86.6 ATPase 88.8	VP7 85.9 VP4 87.5 ATPase 91.7	VP7 73.0 VP4 79.3 ATPase 85.0
SH1		VP7 98.4 VP4 94.0 ATPase 91.7	VP7 72.4 VP4 82.3 ATPase 79.2
PH1			VP7 71.9 VP4 81.5 ATPase 82.5

[a] Calculated with EMBOSS Needle (McWilliam et al., 2013).

Table 3. Amino acid similarities (%[a]) between sequences of small MCP (VP7), large MCP (VP4) and putative packaging ATPase of the alphasphaerolipoviruses.

	SH1	PH1	HHIV-2
HCIV-1	VP7 93.5 VP4 93.1 ATPase 96.2	VP7 93.5 VP4 92.2 ATPase 97.1	VP7 85.9 VP4 90.1 ATPase 92.9
SH1		VP7 98.9 VP4 96.6 ATPase 95.8	VP7 87.0 VP4 89.7 ATPase 90.8
PH1			VP7 87.6 VP4 89.7 ATPase 92.9

[a] Calculated with EMBOSS Needle (McWilliam et al., 2013).

Figure 2. Alignment of HCIV-1 small MCP (VP7), large MCP (VP4), and putative ATPase amino acid sequences with the homologs in alphasphaerolipoviruses SH1, PH1, and HHIV-2. Colour coding shows similarity. The sequences were aligned by MUSCLE (Edgar, 2004).

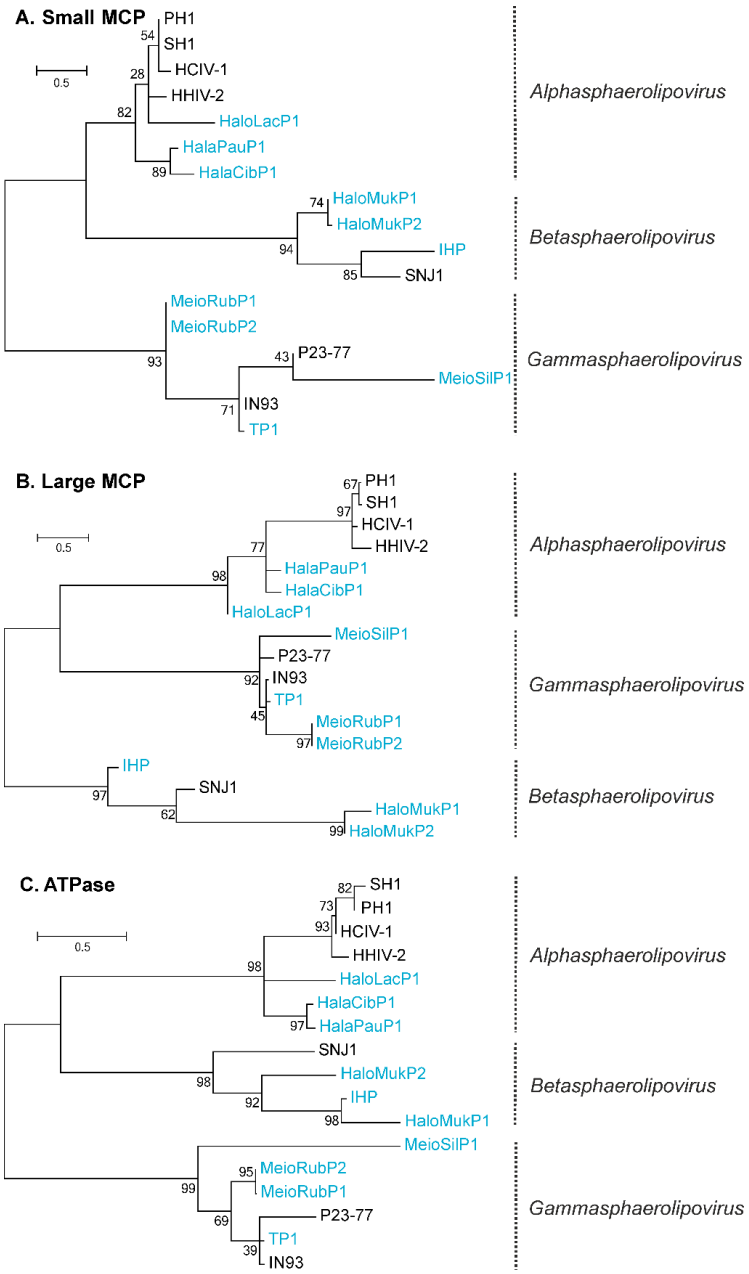

Figure 3. Maximum likelihood phylogenetic tree of the MCPs and ATPase amino acid sequences of HCIV-1 and sphaerolipoviruses, as well as related proviruses (blue). The sequences were aligned with MUSCLE (Edgar, 2004), and the phylogenetic analysis was done using JTT amino acid substitution model in MEGA 5.05 (Tamura et al., 2011). Bootstrap values are shown next to the nodes, and bar (0.5) indicates inferred number of substitutions per site.

The alphasphaerolipovirus structural protein patterns are highly similar (Figure 4A). For SH1, HHIV-2 and HCIV-1, it has been shown that the lipids are selectively acquired from the host cell membrane (Table 1). Cryo- electron microscopy based structures of SH1 and HHIV-2 have revealed that the internal membrane follows the icosahedral shape of the capsid (Jäälinoja et al., 2008; Gil-Carton et al., 2015). Based on the similarities between the structural protein species of alphasphaerolipoviruses, schematic model for HCIV-1 virion organization can be proposed (Figure 4B). HCIV-1 virions have a double-layered appearance when visualized by transmission electron microscopy (the layers of the protein capsid and lipid bilayer enclosing the genome; Figure 4C).

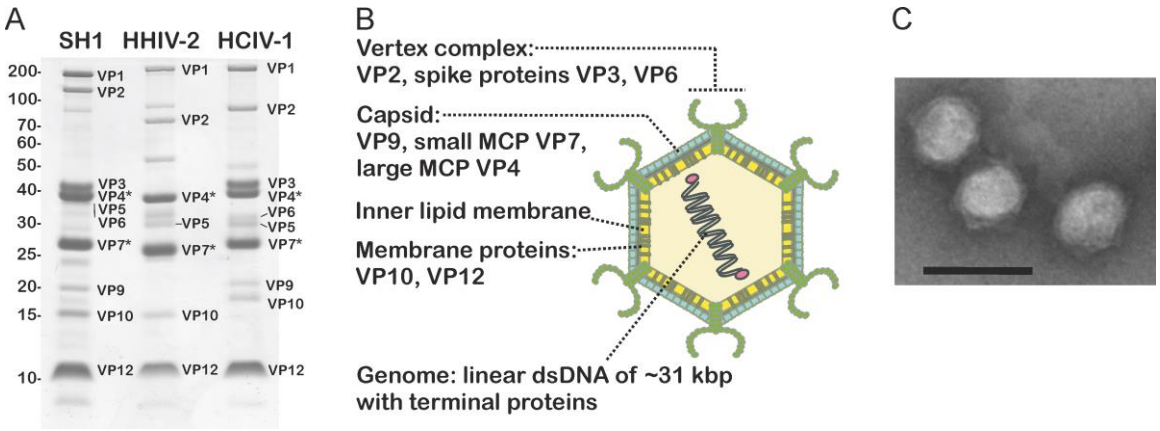

Figure 4. (A) Protein profiles of HCIV-1 and alphasphaerolipoviruses SH1 and HHIV-2. MCPs are marked with asterisk. (B) Schematic model of HCIV-1 virion. (C) Electron micrograph of HCIV-1 highly purified particles stained with 3% (w/v) uranyl acetate. Scale bar 100 nm.