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:** | ***2015.001a-kF*** | (to be completed by ICTV officers) |

**Short title: A new family and two new genera for classification of virophages**

two new species
(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:**

Date first submitted to ICTV: June 11, 2015
Date of this revision (if different to above):

**ICTV-EC comments and response of the proposer:**

Fungal and Protist Viruses Subcommittee Chair: Proposal approved for submission.

MODULE 2a: **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 | ***2015.001aF*** | (assigned by ICTV officers) |
|---|---|---|

**To create 2 new species within:**

| | |
|---|---|
| Genus: | ***Sputnikvirus*** **(new)** |
| Subfamily: | |
| Family: | ***Lavidaviridae*** **(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)** |
|---|---|---|
| *Mimivirus-dependent virus Sputnik* | Sputnik virus 1 | EU606015 |
| *Mimivirus-dependent virus Zamilon* | Zamilon virus | HG531932 |

**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

Both virus species represent satellite-like viruses of protists, also known as virophages, which depend for multiplication on members of the genus *Mimivirus* within the family *Mimiviridae*. Sputnik and Zamilon are able to replicate in *Acanthamoeba polyphaga* cells that are co-infected with Acanthamoeba polyphaga mimivirus (APMV) or Mont1 mimivirus, respectively [1, 2]. The latter virus is more closely related to "*Megavirus chilensis*" [3] than to APMV [4]. Both Sputnik and Zamilon have similarly-sized (17,276-18,342 base pairs), circular double-stranded DNA genomes that code for 20-21 proteins (Module 10; Figure 1) [1, 2, 5, 6]. The virions are icosahedral with a diameter of 60-75 nm, and are composed of at least two different proteins with jelly-roll folds, the minor and the major capsid protein (Module 10; Figure 2a) [7, 8]. The capsid proteins of viruses in the proposed genus are homologous and the major capsid protein can be used as a phylogenetic marker to demonstrate membership in the proposed genus (Module 10; Figure 3).

In addition to the two capsid protein genes, both species encode a FtsK-HerA family DNA-packaging ATPase, a cysteine protease, a primase-superfamily 3 helicase, a lambda-type integrase, a transposase, a Zinc-ribbon domain protein, a collagen-like protein, and six proteins of unknown function [1, 2].

MODULE 2b: **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 | ***2015.001bF*** | (assigned by ICTV officers) |
|---|---|---|

**To create 1 new species within:**

| | |
|---|---|
| Genus: | ***Mavirus* (new)** |
| Subfamily: | |
| Family: | ***Lavidaviridae* (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)** |
|---|---|---|
| *Cafeteriavirus-dependent mavirus* | Maverick-related virus (Mavirus), strain Spezl | HQ712116 |

**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

Mavirus replicates in the marine heterotrophic nanoflagellate *Cafeteria roenbergensis* in the presence of Cafeteria roenbergensis virus (CroV) [9, 10]. The circular, double-stranded DNA genome consists of 19,063 bp and encodes 20 proteins. The virions are icosahedral with a diameter of ~75 nm, and are composed of at least two different proteins with jelly-roll folds, the minor and the major capsid protein (Module 10; Figure 2b). The capsid proteins of viruses in the proposed genus are homologous and the major capsid protein can be used as a phylogenetic marker to demonstrate membership in the proposed genus (Module 10; Figure 3). In addition to the two capsid protein genes, Mavirus encodes a FtsK-HerA family DNA-packaging ATPase, a cysteine protease, a superfamily 3 helicase, a retroviral-type integrase, a lipase, and a FNIP repeat-containing protein [9]. Mavirus shares many features with the large, virus-like transposons of the Maverick/Polinton superfamily which are widespread in eukaryotes [11, 12]. Both types of elements encode 7 homologous proteins involved in virion morphogenesis (minor and major capsid proteins, FtsK-HerA-type genome packaging ATPase and cysteine protease homologous to adenoviral maturation proteases), genome replication (protein-primed family B DNA polymerase and superfamily 3 helicase) and integration (retrovirus-like integrase which belongs to a broad superfamily of DDE transposases) [9, 13]. Furthermore, the Mavirus genome contains long inverted repeats that resemble those found at the termini of Maverick/Polinton transposons.

MODULE 3a: **NEW GENUS**

creating a new genus

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

| Code | ***2015.001cF*** | (assigned by ICTV officers) |
|---|---|---|

**To create a new genus within:**

| | |
|---|---|
| Subfamily: | |
| Family: | ***Lavidaviridae* (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 | ***2015.001dF*** | (assigned by ICTV officers) |
|---|---|---|

**To name the new genus: *Sputnikvirus***

Assigning the type species and other species to a new genus

| Code | ***2015.001eF*** | (assigned by ICTV officers) |
|---|---|---|

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

| |
|---|
| ***Mimivirus-dependent virus Sputnik*** |

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

Viruses in this genus depend on members of the genus *Mimivirus* within the family *Mimiviridae* for multiplication and are able to replicate in *Acanthamoeba polyphaga* cells that are co-infected with their respective associated giant virus. All viruses in this genus contain similarly-sized (17,276-18,342 base pairs), circular double-stranded DNA genomes that code for 20-21 proteins. (Module 10; Figure 1) [1, 2, 5, 6]. The virions are icosahedral with a diameter of 60-75 nm, and are composed of at least two different proteins with jelly-roll folds, the minor and the major capsid protein (Module 10; Figure 2a) [7, 8].
In addition to the two capsid protein genes, both species encode a FtsK-HerA family DNA-packaging ATPase, a cysteine protease, a primase-superfamily 3 helicase, a lambda-type integrase, a transposase, a Zinc-ribbon domain protein, a collagen-like protein, and six proteins of unknown function [1, 2]. Based on the large number of homologous genes, it can be assumed that Sputnik and Zamilon are derived from a common ancestor and are best assigned within the same genus.

**Origin of the new genus name:**

Sputnik was the first virophage to be isolated and represents the type species of this genus.

**Reasons to justify the choice of type species:**

This representative has been well studied and its genome sequence as well as virion structure are available [1, 6, 8, 14, 15].

**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.

Membership in the genus is based on the dependence on a giant DNA virus of the genus *Mimivirus* within the family *Mimiviridae*, and on the presence of a set of about 15 homologous genes. These include a major and a minor capsid protein gene, a FtsK-HerA family DNA-packaging ATPase, a cysteine protease, a primase-superfamily 3 helicase, and a lambda-type integrase [2]. The capsid proteins of viruses in the proposed genus are homologous and the major capsid protein can be used as a phylogenetic marker to demonstrate membership in the proposed genus (Module 10; Figure 3).

MODULE 3b: **NEW GENUS**

creating a new genus

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

| Code | ***2015.001fF*** | (assigned by ICTV officers) |
|---|---|---|

**To create a new genus within:**

| | |
|---|---|
| Subfamily: | |
| Family: | ***Lavidaviridae* (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 | ***2015.001gF*** | (assigned by ICTV officers) |
|---|---|---|

**To name the new genus: *Mavirus***

Assigning the type species and other species to a new genus

| Code | ***2015.001hF*** | (assigned by ICTV officers) |
|---|---|---|

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

| | |
|---|---|
| *Cafeteriavirus-dependent mavirus* | 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

Mavirus depends for its multiplication on the giant virus CroV, a member of the genus *Cafeteriavirus* within the family *Mimiviridae*. Both Mavirus and CroV infect heterotrophic nanoflagellates related to the marine protist *Cafeteria roenbergensis*; however, only co-infection with CroV leads to productive Mavirus infection. Mavirus contains a circular double-stranded DNA genome of 19,063 kbp that codes for 20 proteins (Module 10; Figure 1) [9]. The virions are icosahedral with a diameter of ~75 nm, and are composed of at least two different proteins with jelly-roll folds, the minor and the major capsid protein (Module 10; Figure 2a). In addition to the two capsid protein genes, members of the proposed genus encode a FtsK-HerA family DNA-packaging ATPase, a cysteine protease, a superfamily 3 helicase, a retroviral DDE-type integrase, a protein-primed family B DNA polymerase, and a Zinc-ribbon domain protein, as well as further proteins of unknown function [9, 16]. A distinct feature of Mavirus and related viruses is their striking genetic similarity to eukaryotic DNA transposons of the Maverick/Polinton family, with whom they share 7 homologous proteins [9, 11, 12, 17, 18]. Both, host-range and genomic content, distinguish viruses of the proposed genus from other members of the proposed family *Lavidaviridae* and justify the creation of a new genus.

**Origin of the new genus name:**

*Ma-* for Maverick. The Maverick/Polinton DNA transposons of eukaryotes are genetically related to the Mavirus.

**Reasons to justify the choice of type species:**

Mavirus is the first and sole isolated representative of this proposed genus and its genome sequence is available.

**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.

Membership in the genus is based on the dependence on a giant DNA virus of the genus *Cafeteriavirus* within the family *Mimiviridae*, and on the presence of a set of at least 7 homologous genes. These include a major and a minor capsid protein gene, a FtsK-HerA family DNA-packaging ATPase, a cysteine protease, a protein-primed family B DNA polymerase, a superfamily 3 helicase, and a retrovirus-type integrase [9, 16]. The capsid proteins of viruses in the proposed genus are homologous and the major capsid protein can be used as a phylogenetic marker to demonstrate membership in the proposed genus (Module 10; Figure 3). Species in the proposed genus display a close genetic relationship to eukaryotic DNA transposons of the Maverick/Polinton family [9, 11, 12, 18, 17].

MODULE 5: **NEW FAMILY**

creating and naming a new family

| Code | ***2015.001iF*** | (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 | ***2015.001jF*** | (assigned by ICTV officers) |
|---|---|---|

**To name the new family: *Lavidaviridae***

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 | ***2015.001kF*** | (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

***Sputnikvirus* (new)**
***Mavirus* (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

All viruses in the proposed family encode a conserved set of six proteins or domains, which strongly suggests a monophyletic origin. This set consists of the morphogenetic module major capsid protein (hexon protein), minor capsid protein (penton protein), FtsK-HerA family DNA-packaging ATPase, and cysteine protease, as well as a primase-superfamily 3 helicase (S3H) and a Zinc-ribbon domain protein (Module 10, Figure 1) [17]. Although structurally homologous to jelly-roll capsid proteins from other viruses, the capsid protein genes of these viruses display no similarity to proteins outside this group with sequence-based homology detection methods such as BLASTp. Within the proposed family, however, the major capsid protein can be used as a phylogenetic marker to demonstrate membership with the family and to further subclassify members into genera (Module 10, Figure 3) [16, 19]. All viruses of the

proposed family, as well as further putative members from metagenomes without isolates, have circular or linear double-stranded DNA genomes that range in size from 17 kbp to 30 kbp and encode 16-34 ORFs [1, 9, 16, 20–22]. Cultured representatives have 40-80 nm icosahedral capsids with T=27 quasisymmetry (Module 10; Figure 1) [1, 2, 7, 8, 23]. All viruses in the proposed family depend on, or are found in association with, a large dsDNA virus of the family *Mimiviridae*. Co-infection with a suitable dsDNA virus is required for propagation of these viruses, as they replicate inside the cytoplasmic large virus factory and are presumed to use the transcription proteins encoded by the large dsDNA virus [1, 9, 24]. Viruses in the proposed family typically act as parasites of their associated large dsDNA viruses because they decrease the yield of the latter in co-infected cells, and have therefore been called "virophages" [1, 9, 14]. Based on these unifying characteristics which clearly set viruses of this group apart from other viruses, it seems appropriate to classify them within their own family.

**Origin of the new family name:**

*La-* for large, *vi-* for virus, *d-* for dependent, *a-* for associated (large virus dependent or associated)

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

**References:**


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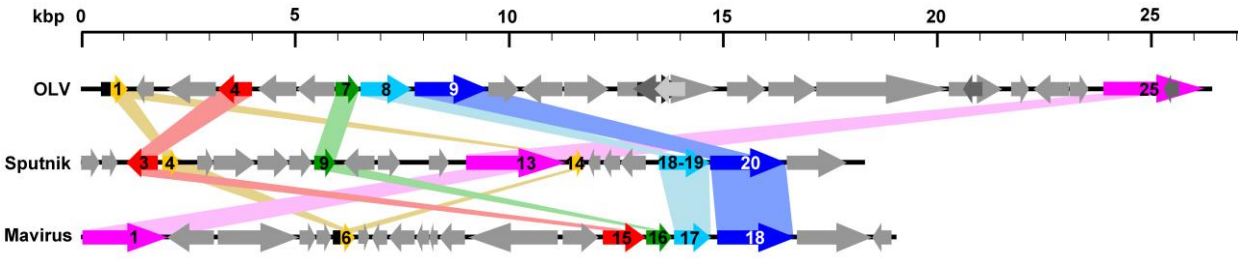


**Figure 1.** Comparative genomic maps of the virophages OLV (Organic Lake virophage), Sputnik, and Mavirus. ORFs are indicated with arrows. Conserved virophage genes are shown in colour: Superfamily 3 helicase, pink; Zinc-ribbon domain, yellow; FtsK-HerA family ATPase, red; Cys protease, green; minor capsid protein, light blue; major capsid protein, indigo. The scale bar shows distances in kilobase pairs.

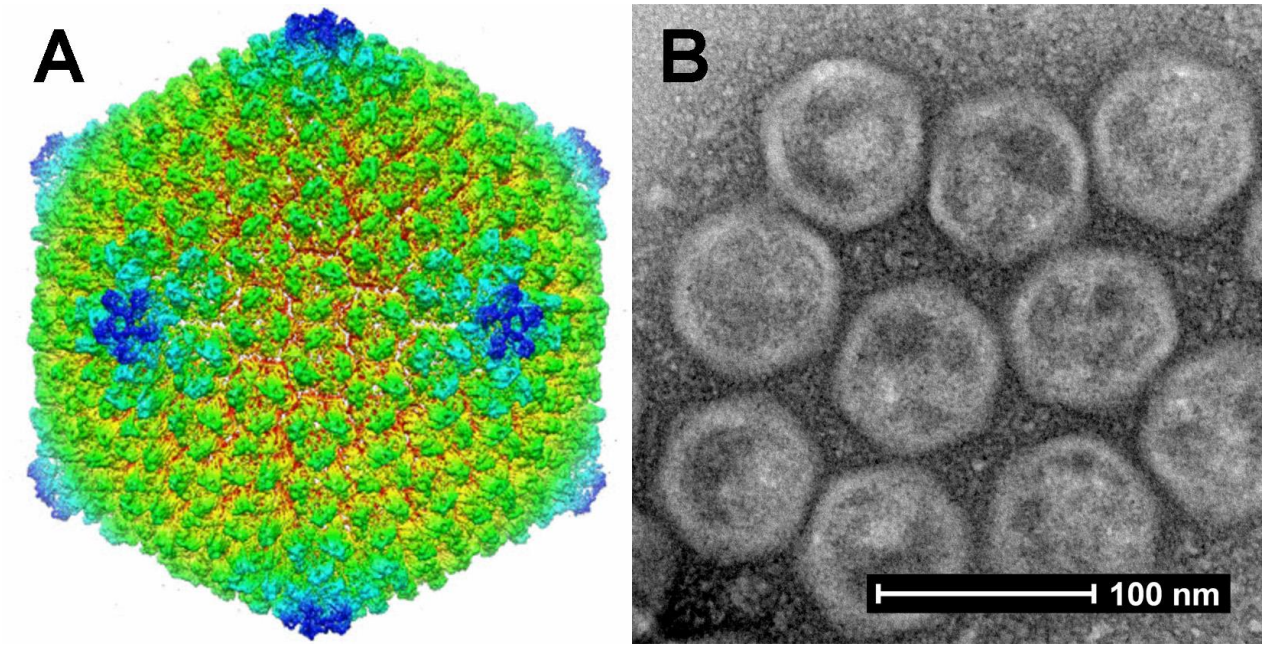


**Figure 2.** The virions of Mavirus and Sputnik. A. Cryo-EM reconstruction of the Sputnik virion (adapted from Ref. [8], Electron Microscopy Data Bank ID 5495). B. Negative stain electron micrograph of Mavirus particles (U. Mersdorf, Max Planck Institute for Medical Research).

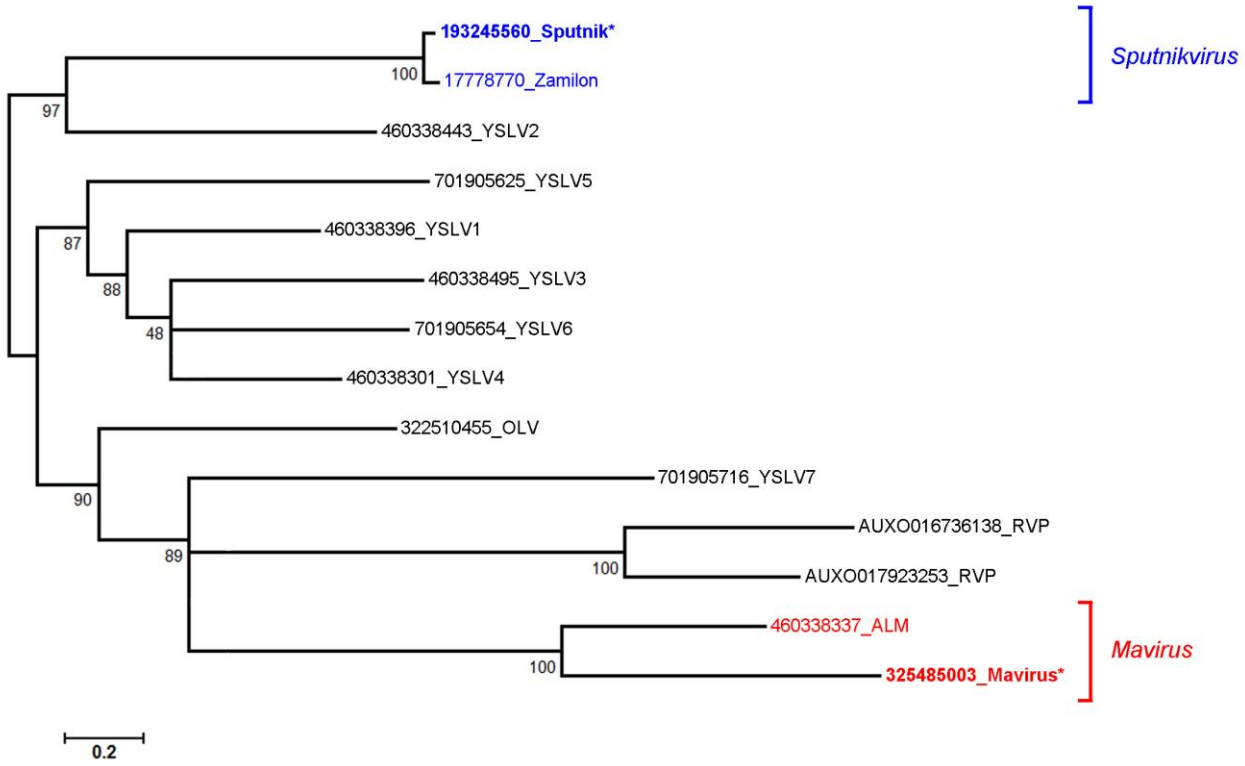


Figure 3. Phylogenetic analysis of the major capsid proteins of virophages. Branches are coloured according to the proposed genera of virophages: "*Sputnikvirus*", blue; "*Mavirus*", red. The proposed type species of the two tentative genera are designated with asterisks. ALM represents a metagenomic virophage, whose genome sequence makes it a member of the proposed genus *Mavirus*. However, since no isolates for ALM are available, it cannot be classified at this point. The multiple sequence alignment for phylogenetic analysis was constructed using PROMALS3D

[9] with the Sputnik Cryo-EM structure (protein data bank ID 3j26) as 3D structure template. Columns containing gaps were removed from the alignment. Maximum-likelihood phylogenetic analysis was carried out using PhyML 3.1 [10], with the Whelan and Goldman (WAG) model of amino acid substitutions, including a gamma law with four substitution rate categories. Numbers at the branch points represent SH (Shimodaira–Hasegawa)-like local support values. The scale bar represents the number of substitutions per site. All taxa are indicated with the corresponding GenBank identifiers, or in the case of rumen virophages with the Shotgun Assembly Sequence identifier. Abbreviations: ALM, Ace Lake mavirus; OLV, Organic Lake virophage; RVP, rumen virophage; YSLV, Yellowstone Lake virophage.