

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:	2016.060a-dB			(to be completed by ICTV officers)			
Short title: To create one (1) new genus, $Sep1virus$, including two (2) new species in the family $Myoviridae$. (e.g. 6 new species in the genus $Zetavirus$) Modules attached $1 \boxtimes 2 \boxtimes 3 \boxtimes 4 \subseteq 5 \subseteq 10$ (modules 1 and 10 are required) $1 \boxtimes 2 \boxtimes 3 \boxtimes 4 \subseteq 5 \subseteq 10$ (modules 1 and 10 are required)						5 🔲	
Author(s):							
Luis D.R. Melo—University of Minho (Portugal) Diana Gutiérrez—Instituto de Productos Lácteos de Asturias (Spain) Jens H. Kuhn—NIH/NIAID/IRF-Frederick, Maryland (USA) Andrew M. Kropinski—University of Guelph (Canada) Evelien M. Adriaenssens—University of Pretoria (South Africa) Pilar García—Instituto de Productos Lácteos de Asturias (Spain) Joana Azeredo—University of Minho (Portugal)							
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Andrew M. Kropinski Phage.Canada@gmail.com							
List the ICTV study group(s) that have seen this proposal:							
A list of study groups and contacts http://www.ictvonline.org/subcommin doubt, contact the appropriate schair (fungal, invertebrate, plant, pvertebrate viruses)	mittees.asp . If subcommittee	ICTV Subcom		and	Archaeal	Viruses	
ICTV Study Group comments (if any) and response of the proposer:							
Date first submitted to ICTV: Date of this revision (if different	Date first submitted to ICTV: June 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	Code $2016.060aB$ (assigned by IC			y ICTV officers)	CTV officers)			
To crea	To create 2 new species within:							
	Jennic.	Can Ivirus (n	04K)		Fill in all that apply. • If the higher taxon has yet to be			
Genus: Sep1virus (new) Subfamily:			cw)	created (i	n a later module, below) write			
Fa	amily:	Myoviridae		 If no genu 	"(new)" after its proposed name.If no genus is specified, enter			
(Order:	Caudovirales		"unassig	"unassigned" in the genus box.			
• •		Representative isola species please)	nte: (only 1 per	GenBank sequence accession number(s)				
Staphylococcus virus		Staphylococcus phag Staphylococcus phag phiIPLA-C1C		KF021268 KP027447				

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.
 - o 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

We have chosen 95% DNA sequence identity as the criterion for demarcation of species in this new genus. The members of each of the proposed species differ from those of other species by more than 5% at the DNA level as confirmed with the BLASTN algorithm.

MODULE 3: NEW GENUS

creating a new genus

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

Code	201	6.060bB	(assigned by I	ICTV officers)			
To create	a new	genus within:		Fill in all that apply.			
Subfai	mily:			If the higher taxon has yet to be created			
Fai	mily:	Myoviridae		(in a later module, below) write "(new)" after its proposed name.			
0	rder:	Caudovirales		 If no family is specified, enter "unassigned" in the family box 			

naming a new genus

Code	2016.060cB	(assigned by ICTV officers)				
To name the	he new genus: Sep1virus					

Assigning the type species and other species to a new genus

Code	2016.060dB	(assigned by ICTV officers)						
To designa	To designate the following as the type species of the new genus							
Staphyloco	Staphylococcus virus SEP1 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

Phage SEP1 was isolated from wastewater treatment plant raw sewage from ETAR de Frossos (Braga, Portugal)[4]. It displays a broad host range, but is particularly active against *Staphylococcus epidermidis*. It possesses an "isometric head of 90 nm diameter with capsomers and a contractile tail 207 nm long by 20 nm wide. Neck, conspicuous transverse tail striations, a baseplate and terminal bulbous spikes ~12 nm in length were also observable." [4]

Phage phiIPLA-C1C was enriched from a sewage treatment plant in Colunga, Asturias, Spain on *S. aureus* but grows better on *S. epidermidis* strains. phiIPLA-C1C has a capsid of 88 nm in diameter and a contractile tail that is 110 nm long. Upon contraction a double baseplate is seen which is typical of SPO1-related phages [5]. Its genome is flanked by LTRs. The authors of this paper noted its similarity to phage SEP1.

BLASTN, CoreGenes (Table 1), progressiveMauve (Fig. 2) and phylogenetic analyses (Fig. 2) [3] all indicate that the proposed genus, *Sep1virus*, is cohesive and distinct from other genera. On average, the genomes of members of this genus are 140.5 kb in length (27.9 mol% G+C), and encode 201 proteins and 0 tRNAs.

Origin of the new genus name:

Based upon the name of the first sequenced member of this genus.

Reasons to justify the choice of type species:

First sequenced member of this genus

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.

We have chosen 95% DNA sequence identity as the criterion for demarcation of species in this new genus. The members of each of the proposed species differ from those of other species by more than 5% at the DNA level as confirmed with the BLASTN algorithm.

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

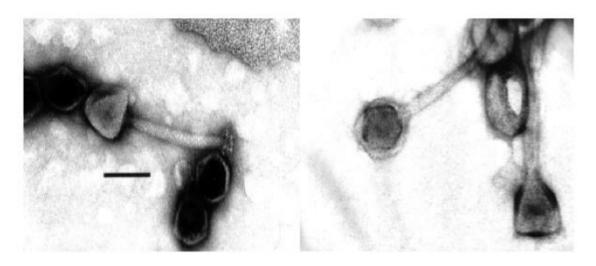
- 1. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One. 2010; 5(6):e11147.
- 2. Turner D, Reynolds D, Seto D, Mahadevan P. CoreGenes 3.5: a webserver for the determination of core genes from sets of viral and small bacterial genomes. BMC Res Notes. 2013; 6:140. doi: 10.1186/1756-0500-6-140.
- 3. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon S, Lefort V, Lescot M, Claverie JM, Gascuel O. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 2008; 36(Web Server issue):W465-9.
- 4. Melo LD, Sillankorva S, Ackermann HW, Kropinski AM, Azeredo J, Cerca N. Isolation and characterization of a new *Staphylococcus epidermidis* broad-spectrum bacteriophage. J Gen Virol. 2014 Feb;95(Pt 2):506-15.
- 5. Gutiérrez D, Vandenheuvel D, Martínez B, Rodríguez A, Lavigne R, García P. Two Phages, phiIPLA-RODI and phiIPLA-C1C, Lyse Mono- and Dual-Species Staphylococcal Biofilms. Appl Environ Microbiol. 2015 May 15;81(10):3336-48.

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.

Fig. 1. Electron micrograph of negatively stained *Staphylococcus* phage phiIBB-SEP1 (TOP; provided by Dr. Luís Daniel Rodrigues de Melo, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal); and phiIPLA-C1C (Bottom; provided by

Dr. Pilar García Suárez, Instituto de Productos Lácteos de Asturias (CSIC), Pso. Rio Linares s/n, 33300 – Villaviciosa – ASTURIAS – ESPAÑA)



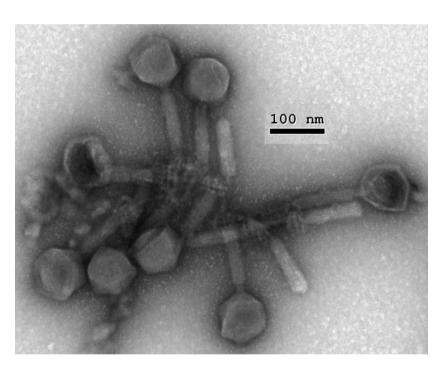


Table 1. Properties of the two phages belonging to the genus *Sep1virus*.

Staphylococcus	RefSeq No.	GenBank	Genome	Genome	No.	%	%
phage	_	Accession	length	(mol%	CDS	Overall	Homologous
		No.	(kb)	G+C)		DNA	proteins **
						Sequence	
						identity *	
phiIBB-SEP1		KF021268.1	140.00	27.9	200	100	100
phiIPLA-C1C	NC_028962.1	KP027447.1	140.96	28.0	203	86	88.5

^{*} Determined using BLASTN; ** determined using CoreGenes [2]

Fig. 2. progressiveMauve alignment [1] of the genomes of members of the *Sep1virus* genus – from top to bottom: phiIBB-SEP1 and phiIPLA-C1C. Colored blocks indicate the regions of 1 to 1 best alignment with rearrangement breakpoints in a different random color. The degree of sequence similarity between regions is given by a similarity plot within the colored blocks with the height of the plot proportional to the average nucleotide identity (Aaron Darling, personal communication).

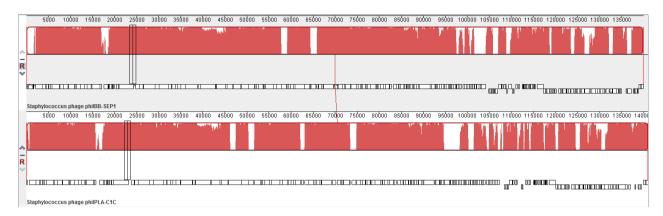


Fig. 3. Phylogenetic analysis of (A) large subunit terminase proteins, and (B) major capsid proteins of sep1viruses and homologous proteins from a variety of other phages constructed using "one click" at phylogeny.fr [3]. "The "One Click mode" targets users that do not wish to deal with program and parameter selection. By default, the pipeline is already set up to run and connect programs recognized for their accuracy and speed (MUSCLE for multiple alignment and PhyML for phylogeny) to reconstruct a robust phylogenetic tree from a set of sequences." It also includes the use of Gblocks to eliminate poorly aligned positions and divergent regions. "The usual bootstrapping procedure is replaced by a new confidence index that is much faster to compute. See: Anisimova M., Gascuel O. Approximate likelihood ratio test for branches: A fast, accurate and powerful alternative (Syst Biol. 2006;55(4):539-52.) for details." **Red** = *Sep1virus*.

A. TerL protein

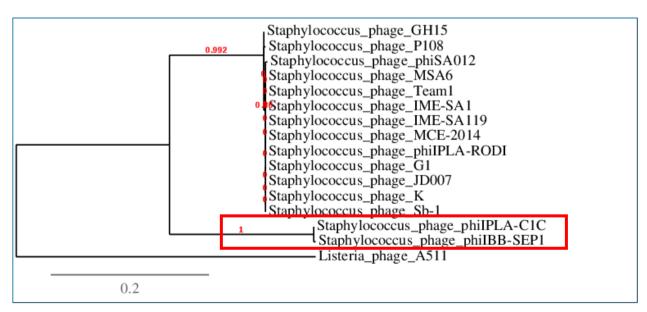


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).

B. Major capsid protein

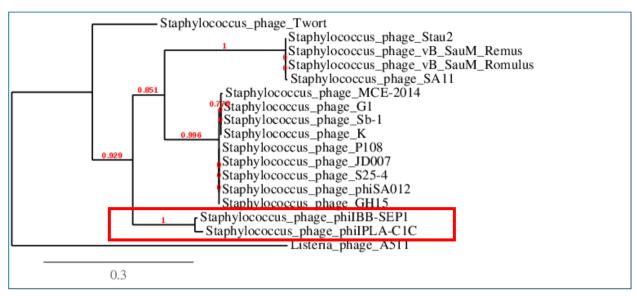


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).