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:	***2015.018a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Silviavirus*, including two (2) new species within the family *Myoviridae*.
(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)	Bacterial & Archaeal Virus Subcommittee

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

Please note that we have chosen to refer to this new genus as *Silviavirus* rather than *Silvialikevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" and "*Phi*" from phage genus names.

Date first submitted to ICTV: May 2015
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	***2015.018aB***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Silviavirus (new)***
Subfamily:	***Spounavirinae***
Family:	***Myoviridae***
Order:	***Caudovirales***

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)**
Staphylococcus virus Remus *Staphylococcus virus SA11*	Staphylococcus phage vB_SauM_Remus Staphylococcus phage SA11	JX846613 JX194239

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

Phages Romulus and Remus, are broad-host range lytic members of the family *Myoviridae*. "Both phages possess an isometric head with a diameter of 90 nm and a contractile tail with a length of 204 nm and a width of 17 nm." (5). The *Silviavirus* genus is characterized by a terminally redundant genome averaging 135 kb (30 mol%G+C), and encode 180 proteins and 0-1 tRNAs.

We have chosen 95% DNA sequence identity as the criterion for demarcation of species in this new genus. Each of the proposed species differs from the others with more than 5% at the DNA level as confirmed with the BLASTN algorithm.

Please note that we have chosen to refer to this new genus as *Silviavirus* rather than *Silvialikevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" and "*Phi*" from phage genus names.

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2015.018bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	***Spounavirinae***
Family:	***Myoviridae***
Order:	***Caudovirales***

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	***2015.018bB***	(assigned by ICTV officers)

To name the new genus: *Silviavirus*

Assigning the type species and other species to a new genus

Code	***2015.018dB***	(assigned by ICTV officers)

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

Staphylococcus virus Remus

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

BLASTN, CoreGenes (Table 1; 2), progressiveMauve alignment (Fig. 2; 1) and phylogenetic analyses (Fig. 3; 3) all indicate that the proposed genus, *Silviavirus*, is cohesive and distinct from the other genera of viruses. As Vandersteegen et al. remarked "Despite their relatedness to *Staphylococcus* phages K, G1, ISP, and Twort and Listeria phages A511 and P100, Romulus and Remus can be proposed as isolates of a new species within the *Twortlikevirus* genus." Furthermore the authors on the paper on *Staphylococcus* phage SA11 state "The analysis of the complete genomic sequence revealed that less than 21% of the genome shows any significant homology to those of previously reported *Staphylococcus* phages, including A5W, K, ISP, Sb-1, and G1.(4)"

Origin of the new genus name:

Named after Rhea Silvia, the mother of Romulus and Remus

Reasons to justify the choice of type species:

First phage of its type to be sequenced

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. Each of the proposed species differs from the others with 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. CoreGenes3.5: a webserver for the determination of core genes from sets of viral and small bacterial genomes. BMC Res Notes. 2013; 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. Kim MS, Myung H. Complete genome of *Staphylococcus aureus* phage SA11. J Virol. 2012; 86(18):10232.
5. Vandersteegen K, Kropinski AM, Nash JH, Noben JP, Hermans K, Lavigne R. Romulus and Remus, two phage isolates representing a distinct clade within the *Twortlikevirus* genus, display suitable properties for phage therapy applications. J Virol. 2013; 87(6):3237-47.
9. Kwan T, Liu J, DuBow M, Gros P, Pelletier J. The complete genomes and proteomes of 27 *Staphylococcus aureus* bacteriophages. Proc Natl Acad Sci U S A. 2005;102(14):5174-9. [Twort]
10. Łobocka M, Hejnowicz MS, Dąbrowski K, Gozdek A, Kosakowski J, Witkowska M, Ulatowska MI, Weber-Dąbrowska B, Kwiatek M, Parasion S, Gawor J, Kosowska H, Głowacka A. Genomics of staphylococcal Twort-like phages--potential therapeutics of the post-antibiotic era. Adv Virus Res. 2012;83:143-216.

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.

Table 1. Properties of the two phages belonging to the *Silviavirus*.

Phage	GenBank accession No.	Genome length (kb)	Genome (mol%G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)*	Proteome (% homologous proteins)**
Remus	JX846612	134.64	30.0	174	1	100	100
SA11	JX194239	136.33	30.0	186	0	91	91.9
Twort(#)	AY954970	130.71	30.3	195	1***	32	64.4

* Determined using BLASTN; ** Determined using CoreGenes (2); *** None described in the GenBank records; #, the genome of this phage was resequenced in 2015.

Table 2. Related phage

Phage	GenBank Accession Number
Romulus	JX846613

Fig. 1. Electron micrographs of negatively stained *Staphylococcus* phage Remus

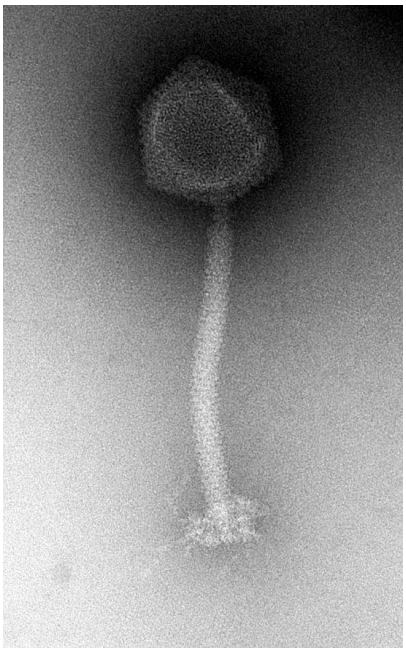

Fig. 2A. progressiveMauve alignment of the annotated genomes of *Staphylococcus* phage SA11 (top) and phage Remus (bottom) (1). In the subsequent diagram SA11 (top) is compared with Remus (bottom) showing that homologous blocks are only to be found at the genome termini. Colored blocks indicate the regions 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). N.B. SA11 is not collinear with Remus.

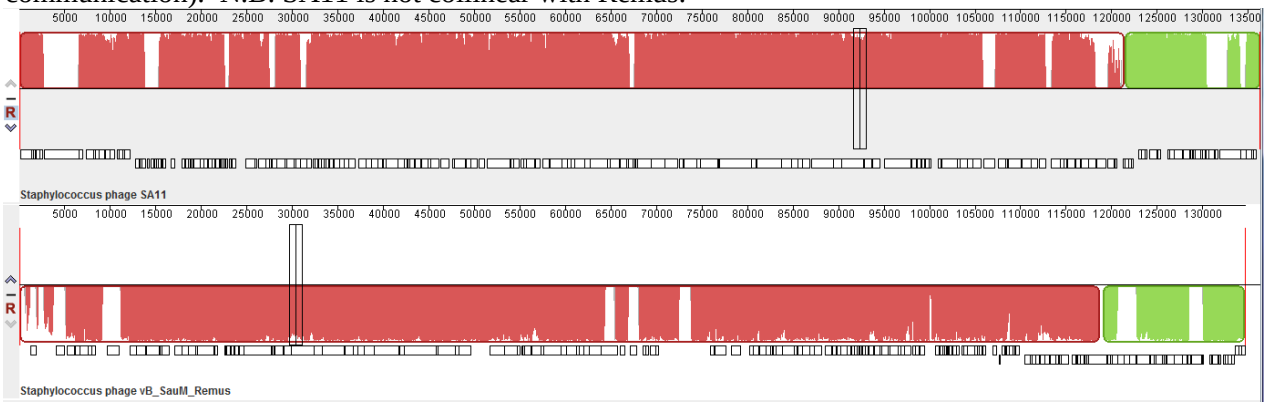

Fig. 2B. progressiveMauve alignment of Remus (top) versus Twort (bottom) illustrating the radical difference.

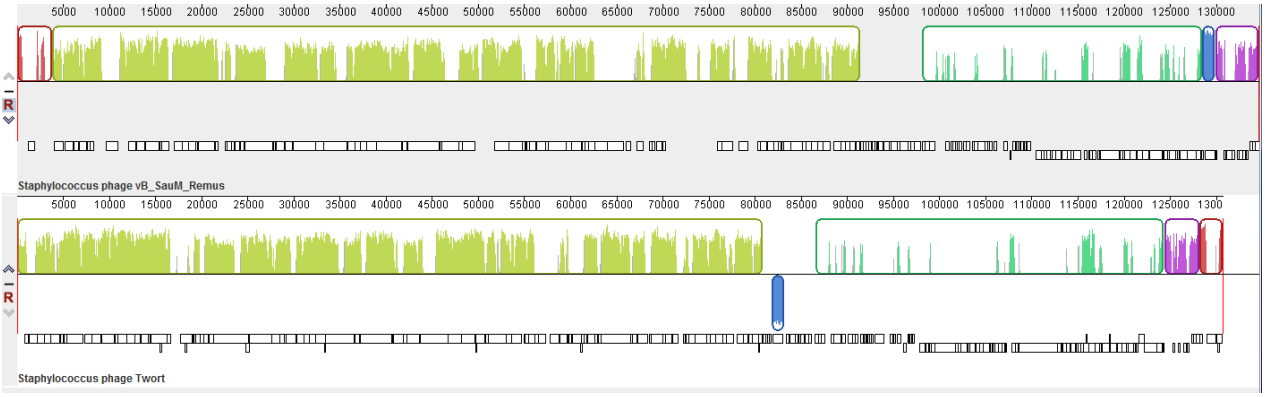

Fig. 3. Phylogenetic analysis of the major capsid protein (top) and tail sheath protein (bottom) of silviaviruses and some related *Staphylococcus* phages constructed using "one click" at phylogeny.fr (3). "The "One Click mode" targets users that do not wish to deal with program and parameter selection. 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." Due to errors in the sequence of *Staphylococcus* phage Twort, the sequence of its terminase had to be reconstructed from the genome sequence.

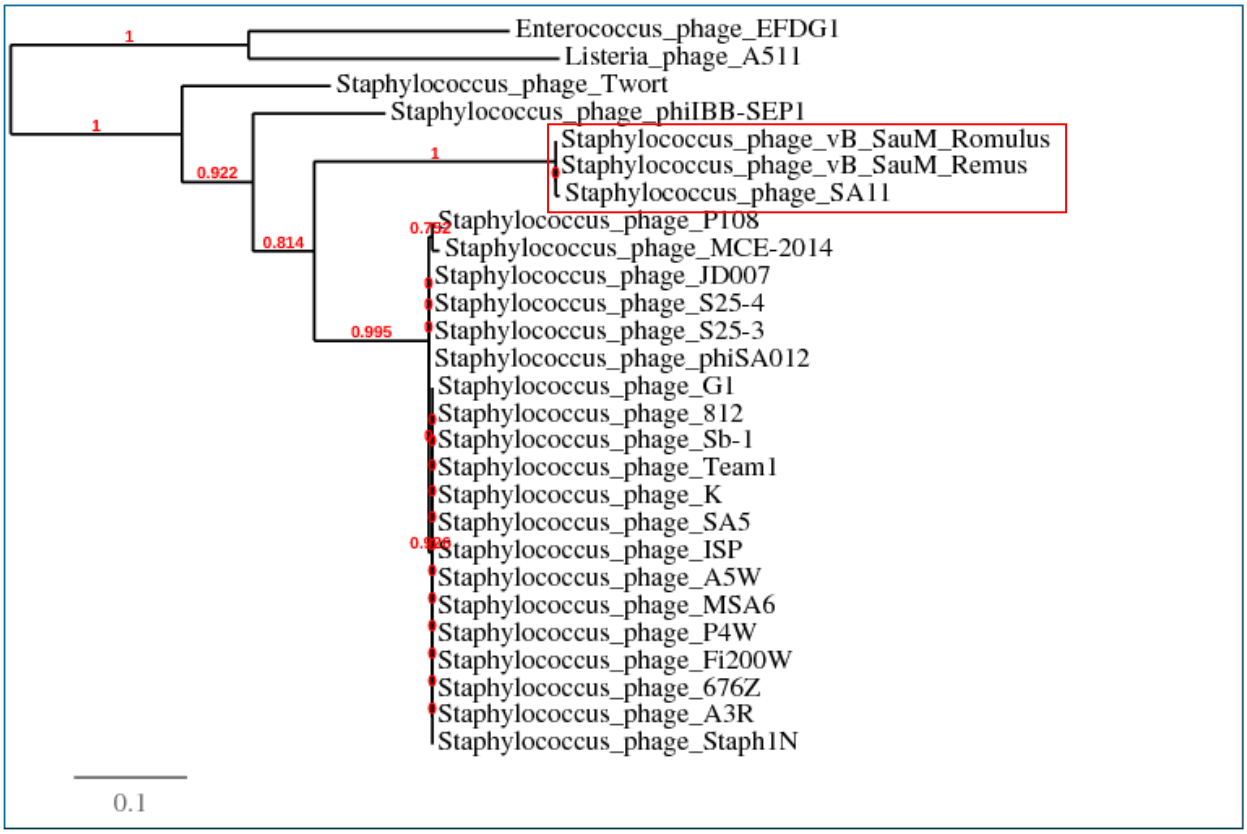

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

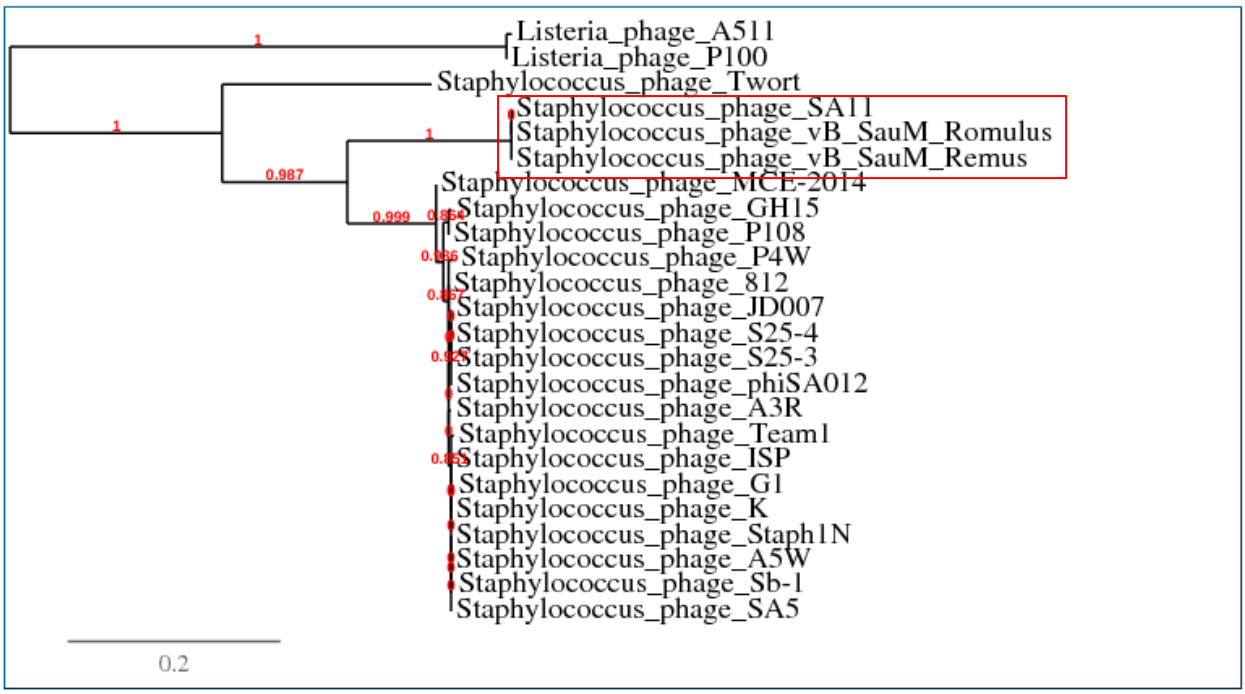

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*