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:	***2015.005a-gB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Kayvirus*, including six (6) 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** ☒

Author(s):

Małgorzata Łobocka – Szkoła Główna Gospodarstwa Wiejskiego w Warszawie (Poland)
Hans-Wolfgang Ackermann – Université Laval (Canada)
Andrew M. Kropinski – University of Guelph (Canada)
Jens H. Kuhn - National Institute of Allergy and Infectious Diseases (U.S.A.)
Evelien M. Adriaenssens – University of Pretoria (South Africa)

Corresponding author with e-mail address:

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 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:

Date first submitted to ICTV: May 2015
Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

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

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	***2015.005aB***	(assigned by ICTV officers)

To create 6 new species within:

Genus:	***Kayvirus* (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 JD7	Staphylococcus phage JD007	JX878671
Staphylococcus virus SA12	Staphylococcus phage phiSA12	AB903967
Staphylococcus virus P108	Staphylococcus phage P108	KM216423
Staphylococcus virus G15	Staphylococcus phage G15	JQ686190
Staphylococcus virus MCE2014	Staphylococcus phage MCE-2014	KJ888149
Staphylococcus virus S253	Staphylococcus phage S25-3	AB853330

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

The *Kayvirus* genus is characterized by a terminally redundant genome averaging 142 kb (30.3 mol%G+C), encoding 215 proteins and 3-4 tRNAs. It is likely that the genome size is closer to 148 kb with the discrepancy due to incomplete sequencing of the terminal repeats of many of the members of this genus. Interestingly, none of the genomes of this genus possess GATC sites.

Phages of this genus have been proposed as therapeutics (7, 8, 10).

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 *Kayvirus* rather than *Klikevirus* 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	***2015.005bB***	(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	***2015.005cB***	(assigned by ICTV officers)

To name the new genus: *Kayvirus*

Assigning the type species and other species to a new genus

Code	***2015.005dB***	(assigned by ICTV officers)

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

Staphylococcus virus K

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: 7**

7

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, *Kayvirus*, is cohesive and distinct from the other genera of viruses.

Origin of the new genus name:

Named after the first phage of its type to be sequenced: *Staphylococcus* phage K

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 7: **REMOVE and MOVE**

Use this module whenever an existing taxon needs to be removed:

- *Either* to abolish a taxon entirely (when only part (a) needs to be completed)
- *Or* to move a taxon and re-assign it e.g. when a species is moved from one genus to another (when BOTH parts (a) and (b) should be completed)

Part (a) taxon/taxa to be removed or moved

Code	***2015.005eB***	(assigned by ICTV officers)

To remove the following taxon (or taxa) from their present position:

Staphylococcus phage G1 and *Staphylococcus phage K*

The present taxonomic position of these taxon/taxa:

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

Fill in all that apply.

If the taxon/taxa are to be abolished (i.e. not reassigned to another taxon) write "yes" in the box on the right

Reasons to justify the removal:
Explain why the taxon (or taxa) should be removed

Currently these phages are classified within the *Twortlikevirus* genus, but BLASTN, CoreGenes and phylogenetic analyses reveal that many of the large lytic *Staphylococcus* myoviruses are significantly different from *Staphylococcus* phage Twort. Phage K and Twort only share 31% DNA sequence identity (BLASTN) and 48.5 % homologous proteins as shown by CoreGenes analysis (2). Furthermore, phylogenetic analysis (Fig. 3) of the major capsid protein reveal that the proteins of phage Twort are significantly different from those of all other large lytic *Staphylococcus* myoviruses.

Part (b) re-assign to a higher taxon

Code	***2015.005fB***	(assigned by ICTV officers)

To re-assign the taxon (or taxa) listed in Part (a) as follows:

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

Fill in all that apply.

- If the higher taxon has yet to be created write "**(new)**" after its proposed name and complete relevant module to create it.

If no genus is specified, enter "**unassigned**" in the genus box.

Reasons to justify the re-assignment:

- If it is proposed to re-assign species to an existing genus, please 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.
- Provide accession numbers for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

Newly proposed genus

MODULE 8: **RENAME**

Use this module to change the name of one or more existing taxa (but note that stability of nomenclature is encouraged wherever possible). Insert extra lines in the table if needed.

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Renaming one or more taxa

Code	***2015.005gB***	(assigned by ICTV officers)

To rename the following taxon (or taxa):

Current name		**Proposed name**
Staphylococcus phage G1		*Staphylococcus virus G1*
Staphylococcus phage K		*Staphylococcus virus K*

Reasons to justify the renaming: Explain why the taxon (or taxa) should be renamed
To conform with the names of the newly created species in Module 2.

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. O'Flaherty S, Coffey A, Edwards R, Meaney W, Fitzgerald GF, Ross RP. Genome of staphylococcal phage K: a new lineage of *Myoviridae* infecting gram-positive bacteria with a low G+C content. J Bacteriol. 2004;186(9):2862-71.
5. Gill JJ. Revised Genome Sequence of *Staphylococcus aureus* Bacteriophage K. Genome Announc. 2014;2(1). pii: e01173-13.
6. Gu J, Liu X, Lu R, Li Y, Song J, Lei L, Sun C, Feng X, Du C, Yu H, Yang Y, Han W. Complete genome sequence of *Staphylococcus aureus* bacteriophage GH15. J Virol. 2012;86(16):8914-5.
7. Alves DR, Gaudion A, Bean JE, Perez Esteban P, Arnot TC, Harper DR, Kot W, Hansen LH, Enright MC, Jenkins AT. Combined use of bacteriophage K and a novel bacteriophage to reduce *Staphylococcus aureus* biofilm formation. Appl Environ Microbiol. 2014;80(21):6694-703. [MCE-2014]
8. Takemura-Uchiyama I, Uchiyama J, Kato S, Inoue T, Ujihara T, Ohara N, Daibata M, Matsuzaki S. Evaluating efficacy of bacteriophage therapy against *Staphylococcus aureus* infections using a silkworm larval infection model. FEMS Microbiol Lett. 2013;347(1):52-60. [S25-3]
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 six phages belonging to the *Kayvirus*.

Phage	GenBank accession No.	Genome length (kb)	Genome (mol%G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)*	Proteome (% homologous proteins)**
K	KF766114	148.32	30.4	233	4	100	100
JD007	JX878671	141.84	30.4	217	4***	92	83.7
phiSA12	AB903967	142.09	30.3	207	3***	90	81.6
P108	KM216423	140.81	30.2	226	3	89	80.7
G15	JQ686190	139.81	30.2	214	4***	88	80.3
MCE-2014	KJ888149	141.91	30.4	204	4***	83	76.4
S25-3	AB853330	139.74	30.2	206	3	89	77.7
Twort(#)	AY954970	130.71	30.3	195	1***	31	48.5

* 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 phages

Phage	GenBank Accession Number
676Z	JX080302.2
Staph1N	JX080300.2
A5W	EU418428.2
ISP	FR852584
G1	AY954969
MSA6	JX080304.2
Team1	KC012913
Fi200W	JX080303.2
P4W	JX080305.2
SA5	JX875065
A3R	JX080301.2
Sb-1	HQ163896
S25-4	AB853331

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

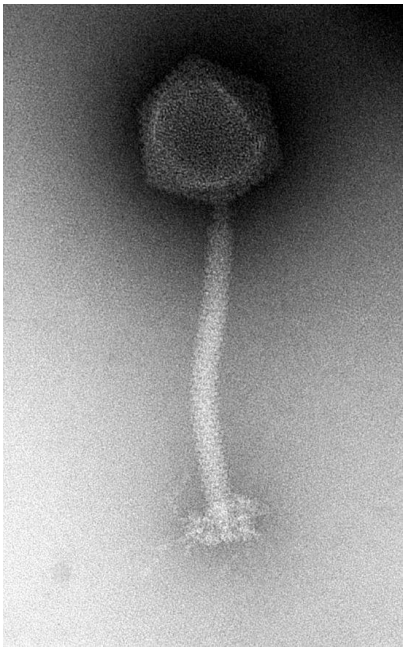

Fig. 2A. progressiveMauve alignment of the annotated genomes of members of the genus *Kayvirus* top to bottom: GH15, JD007, MCE-2014, P108, phiSA012, S25-3, and K (1). In the subsequent diagram Diva (top) is compared with phiIBB_Pl23 (bottom) showing that homologous blocks are only to be found at the 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. Some of the phage genomes are not collinear with K.

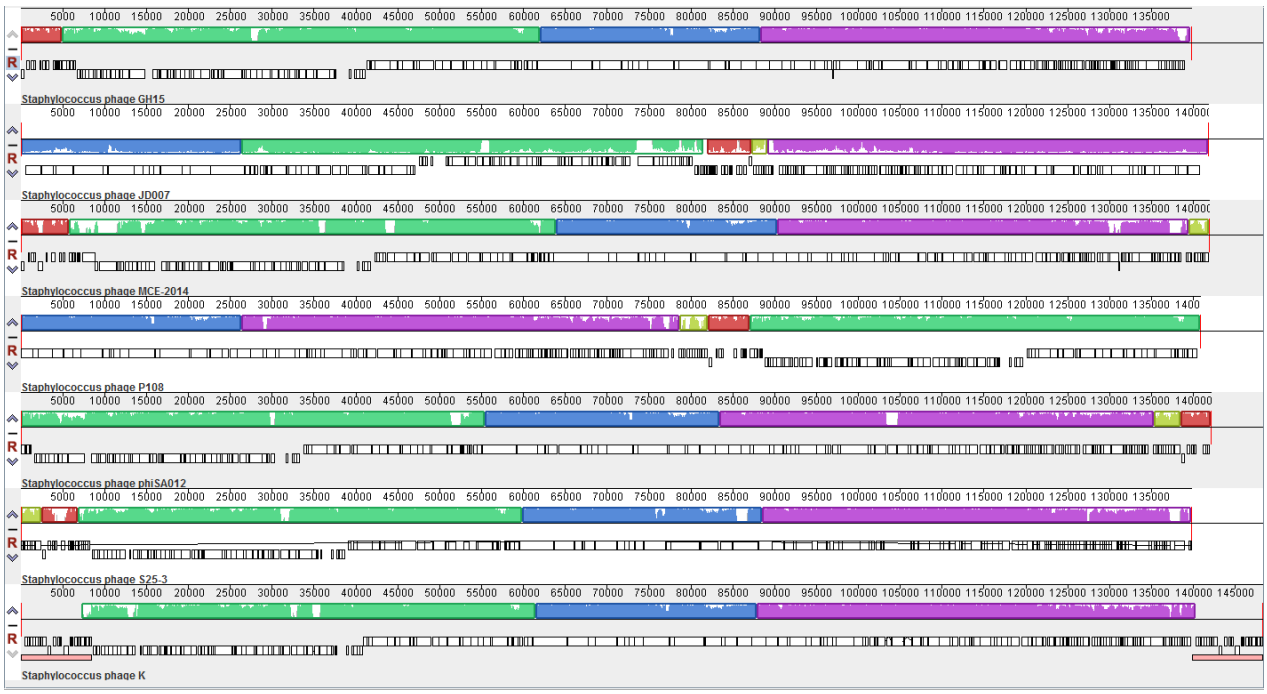

Fig. 2B. progressiveMauve alignment of K versus Twort illustrating the radical difference.

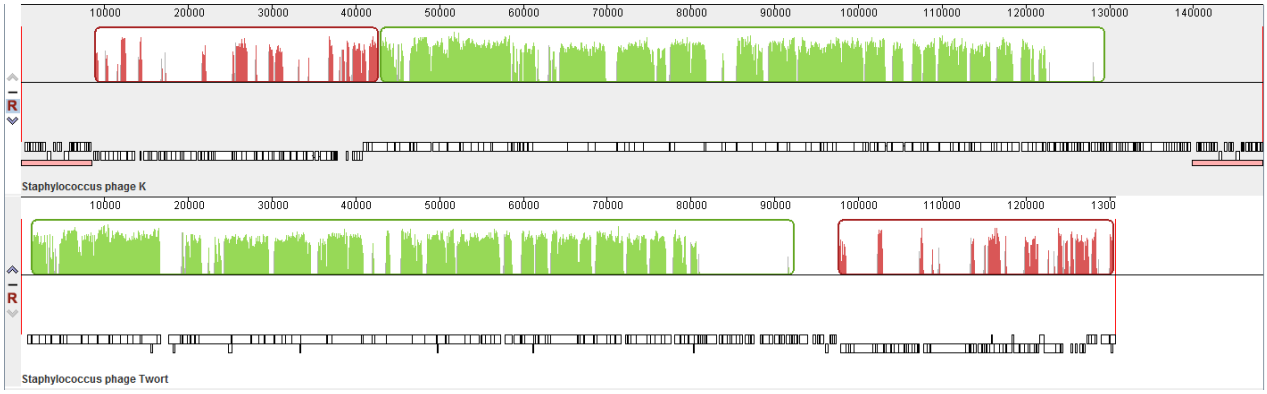

Fig. 3. Phylogenetic analysis of the major capsid protein (top) and tail sheat protein (bottom) of kayviruses and some related *Staphylococcus* phages constructed using “one click” at phylogeny.fr (3). "The "One Click mode" targets users that do not wish to deal with program and parameter selection. 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.

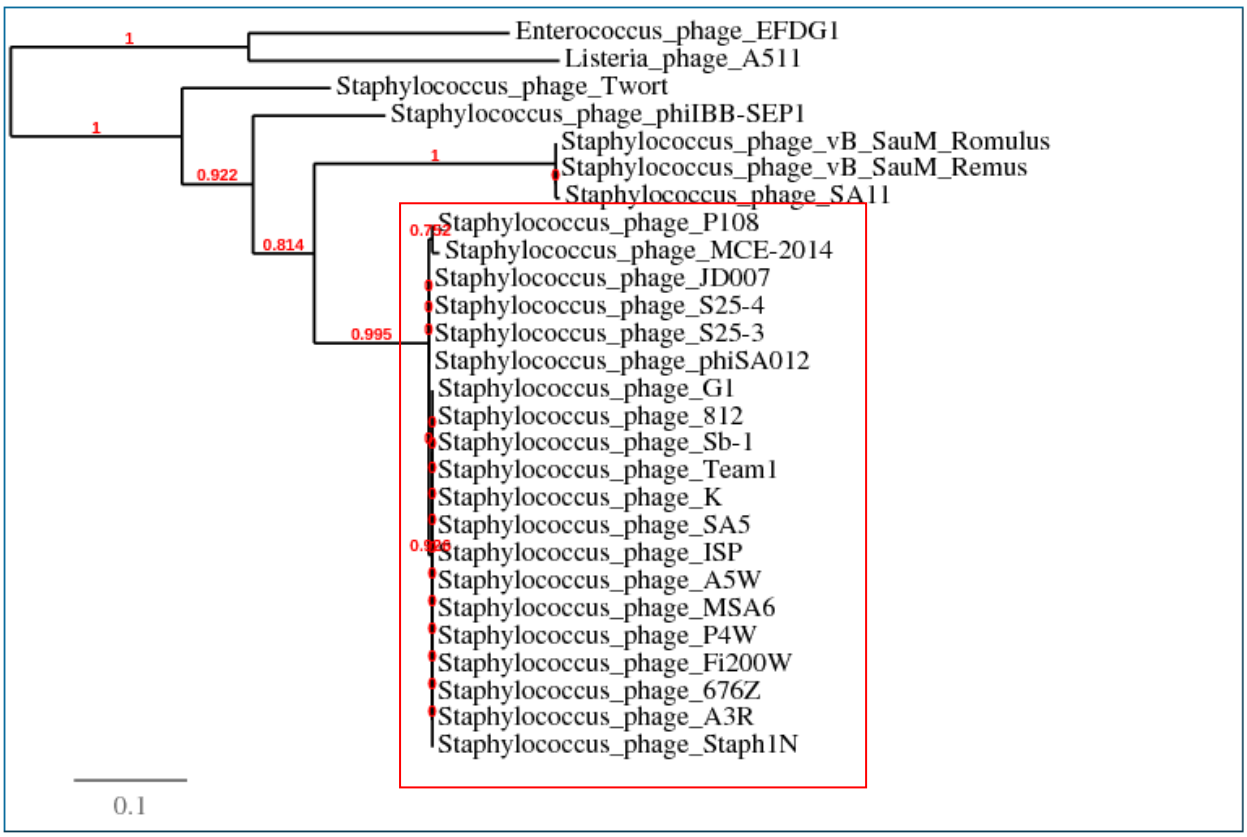

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

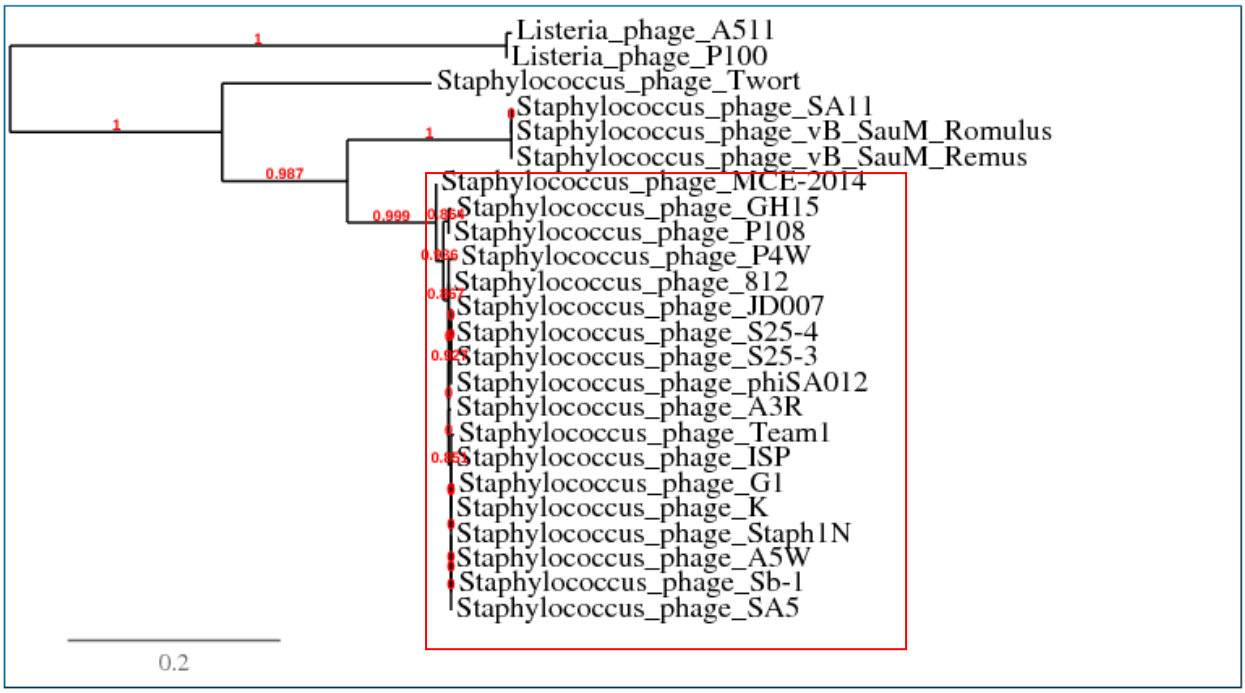

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*