



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:	2015.033a-dB	(to be completed by ICTV officers)			
Short title: Create one (1) new genus, <i>B4virus</i> , including five (5) new species within the family <i>Myoviridae</i> (e.g. 6 new species in the genus <i>Zetavirus</i>)					
Modules attached (modules 1 and 10 are required)	1 <input checked="" type="checkbox"/> 6 <input type="checkbox"/>	2 <input checked="" type="checkbox"/> 7 <input type="checkbox"/>	3 <input checked="" type="checkbox"/> 8 <input type="checkbox"/>	4 <input type="checkbox"/> 9 <input type="checkbox"/>	5 <input type="checkbox"/> 10 <input checked="" type="checkbox"/>

Author(s):

Andrew M. Kropinski – University of Guelph (Canada)
Jakub Barylski – University of Poznan (Poland)
Louise Temple – James Madison University (U.S.A.)
Sangryeol Ryu – Seoul National University (Korea)
Gabriel Kutty Everett – Texas A&M University (U.S.A.)
Evelien M. Adriaenssens – University of Pretoria (South Africa)

Corresponding author with e-mail address:

Andrew 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 and 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 *B4virus* rather than *B4likevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "like" from phage genus names.

Date first submitted to ICTV:

June 2015

Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

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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	2015.033aB	(assigned by ICTV officers)	
To create 5 new species within:			
Genus:	B4virus (new)	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.	
Subfamily:			
Family:	Myoviridae		
Order:	Caudovirales		
Name of new species:	Representative isolate: (only 1 per species please)	GenBank sequence accession number(s)	
<i>Bacillus virus B4</i>	Bacillus phage B4	JN790865	
<i>Bacillus virus Spock</i>	Bacillus phage Spock	KF669662	
<i>Bacillus virus Bigbertha</i>	Bacillus phage BigBertha	KF669647	
<i>Bacillus virus Troll</i>	Bacillus phage Troll	KF208639	
<i>Bacillus virus Riley</i>	Bacillus phage Riley	KJ489402	

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

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 3: **NEW GENUS**

creating a new genus

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

Code	2015.033bB	(assigned by ICTV officers)
To create a new genus within:		
Subfamily:		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
Family:	Myoviridae	
Order:	Caudovirales	

naming a new genus

Code	2015.033cB	(assigned by ICTV officers)
To name the new genus: <i>B4virus</i>		

Assigning the type species and other species to a new genus

Code	2015.033dB	(assigned by ICTV officers)
To designate the following as the type species of the new genus		
<i>Bacillus virus B4</i>		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:		
5		

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 B4 (Figure 1), which infects *Bacillus cereus*, has an isometric head (85 nm in diameter) with a nonflexible and contractile tail (213 nm in length), indicating that it belongs to the family *Myoviridae* (1). The properties of two *Bacillus thuringiensis* phages, BigBertha (2) and Spock (3) have been described in Genome Announcements (2,3).

The B4viruses, are all lytic *Bacillus* phages with the following average genome characteristics: size 164 kb (37.7 mol% G+C) encoding 286 proteins and no tRNA. BigBertha and Spock possess 2577 and 2800 bp direct terminal repeats. All phages share above 70% DNA sequence identity with the type phage (Table 1, Figure 2).

Motif analysis indicates that these phages all encode an S-adenosylmethionine-dependent methyltransferases (SAM or AdoMet-MTase) which is only rarely found in *Bacillus* phages.

A phylogenetic analysis (6) of the major capsid proteins (Fig.3), large subunit terminase (Fig. 4) and metallophosphatases (Fig 5) of all the current large *Bacillus* myoviruses reveal clustering which can be confirmed by total genome (BLASTN; progressiveMauve, 4) and proteomic

(CoreGenes, 5) analyses. Though higher level relationships could be proposed we have chosen, at this time, to concentrate on defining genera so that the SEA-PHAGES community (<http://seaphages.org/>) can classify its isolates in an ICTV-approved manner.

Origin of the new genus name:

Bacillus phage B4

Reasons to justify the choice of type species:

The first fully 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. 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. Lee JH, Shin H, Son B, Heu S, Ryu S. Characterization and complete genome sequence of a virulent bacteriophage B4 infecting food-borne pathogenic *Bacillus cereus*. Arch Virol. 2013;158(10):2101-8.
2. Ting JH, Smyth TB, Chamakura KR, Kutty Everett GF. Complete Genome of *Bacillus thuringiensis* Myophage BigBertha. Genome Announc. 2013;1(6). pii: e00853-13.
3. Maroun JW, Whitcher KJ, Chamakura KR, Kutty Everett GF. Complete Genome of *Bacillus thuringiensis* Myophage Spock. Genome Announc. 2013;1(6). pii:e00863-13.
4. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One. 2010; 5(6):e11147.
5. 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. doi: 10.1186/1756-0500-6-140.
6. 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. doi: 10.1093/nar/gkn180.

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 phage B4 (left) and B5S (right) negatively stained with 2 % aqueous uranyl acetate (1).

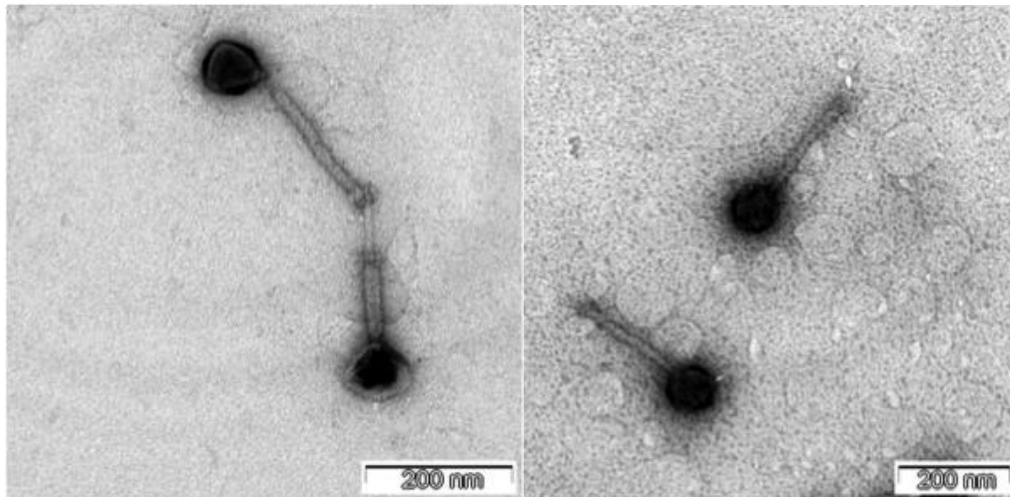


Table 1. Properties of the two phages belonging to the genus *B4virus*

Phage	GenBank Accession No.	Genome size (bp)	Genome (mol% G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)*	% Homologous proteins **
B4	JN790865	162,596	37.7	277	0	100	100
[B5S]***	JN797796	162,598	37.7	272	0	99	NA
Spock	KF669662	164,297	37.6	283	0	85	85.2
BigBertha	KF669647	165,238	37.8	291	0	71	85.6
Troll	KF208639	163,019	37.8	289	0	75	84.1
Riley	KJ489402	162,816	37.8	290	0	72	82.3

* Determined using BLASTN; ** Determined using CoreGenes *** DNA homology indicates strain of B4

Fig. 2. progressiveMauve alignment (4) of the annotated genomes of members of the *B4virus* genus (from top to bottom: B4, BigBertha, Riley, Spock and Troll). 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 major capsid proteins of b4viruses and variety of other *Bacillus* phage proteins constructed using “one click” at phylogeny.fr (6). N.B. The capsid gene of Hoody T contained a frameshift which was corrected prior to this analysis. "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."

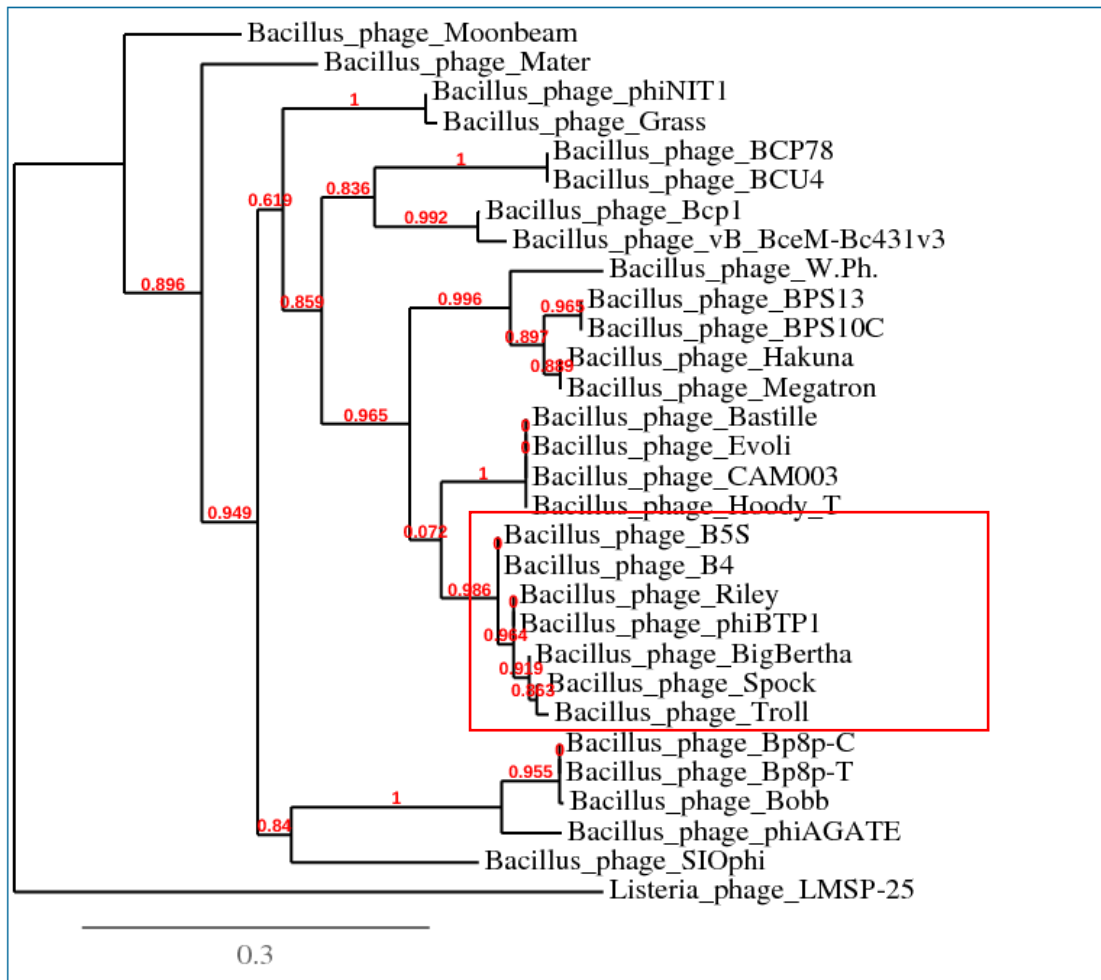


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).

Fig. 4. Phylogenetic analysis of large subunit terminase proteins of *B4virus* and variety of other *Bacillus* phage proteins constructed using “one click” at phylogeny.fr (6).

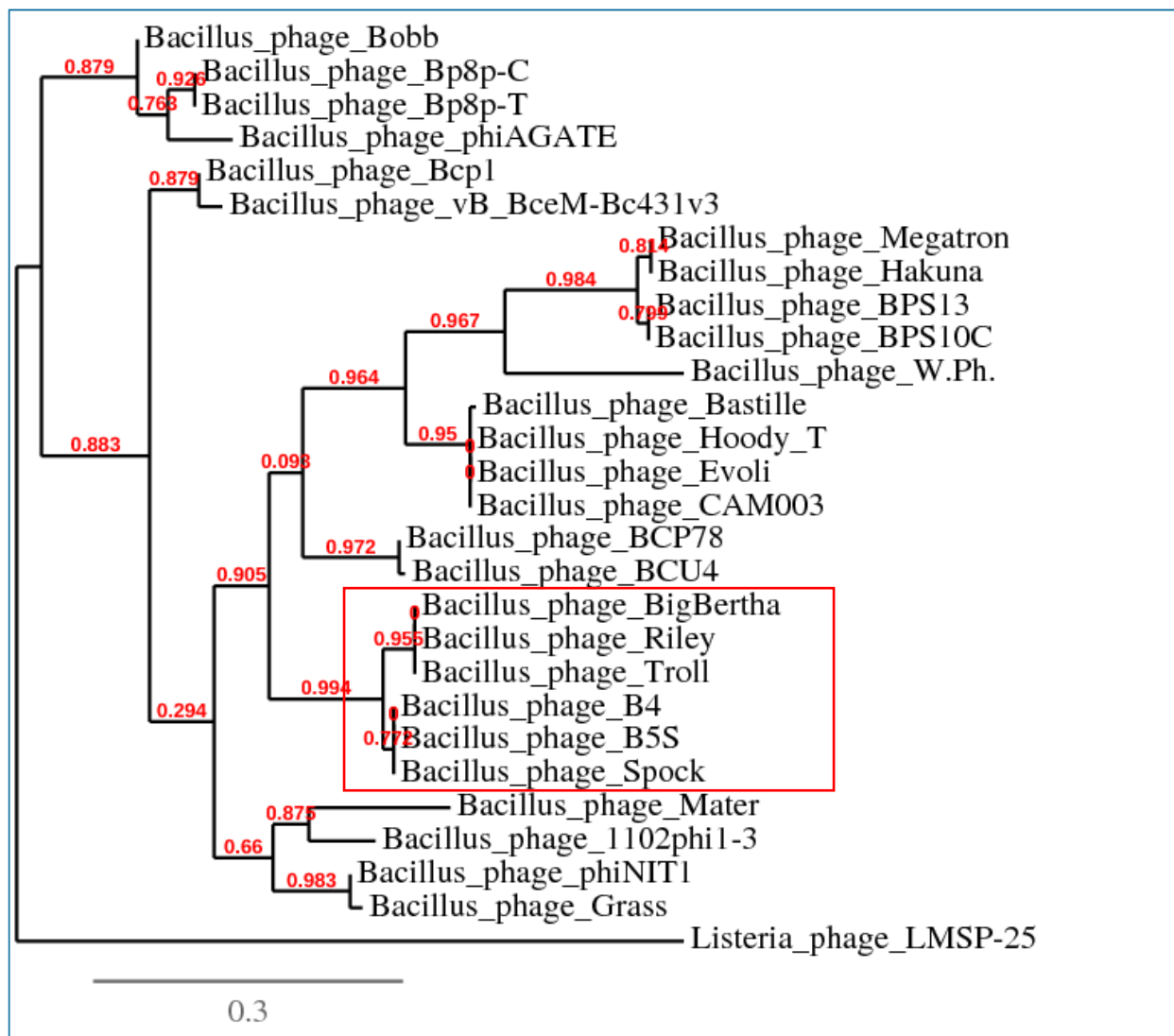


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).

Fig. 5. Phylogenetic analysis of the metallophosphatases of *B4virus* and variety of other *Bacillus* phage proteins constructed using “one click” at phylogeny.fr (6).

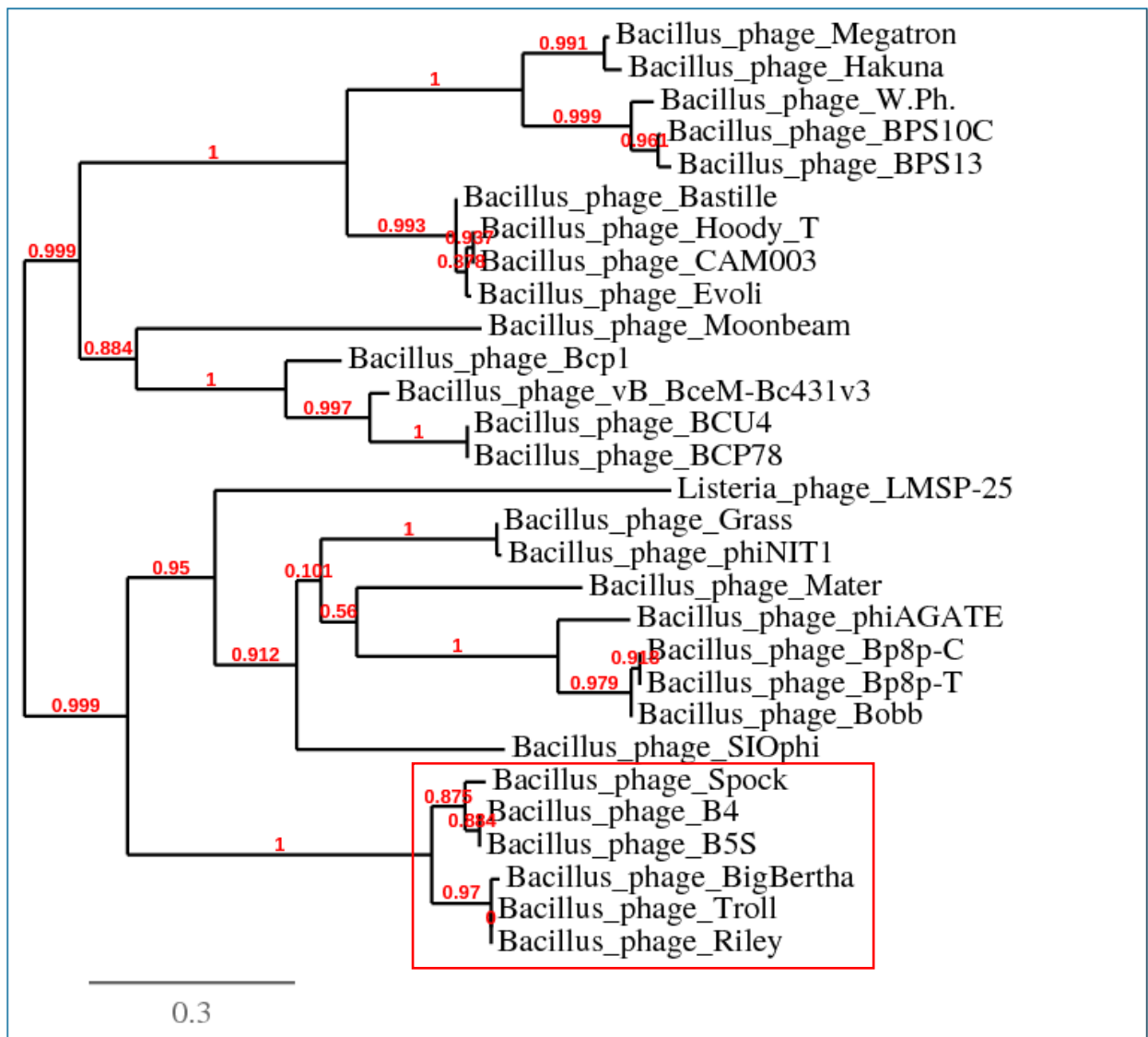


Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*