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:	***2015.038a-dB***	(to be completed by ICTV officers)

Short title: Create one (1) new genus, *Nit1virus*, including three (3) 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):

Gabriel Kuty Everett – Texas A&M University (U.S.A.)
Jakub Barylski – University of Poznan (Poland)
Andrew M. Kropinski – University of Guelph (Canada)
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 *Nit1virus* rather than *Nitunalikevirus* or *Nit1likevirus* 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: June 2015
Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

EC47 Decision: Uc. Remove final tree

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	***2015.038aB***	(assigned by ICTV officers)

To create 3 new species within:

Genus:	***Nit1virus* (new)**
Subfamily:	
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)**
Bacillus virus NIT1	Bacillus phage phiNIT1	AP013029
Bacillus virus Grass	Bacillus phage Grass	KF669652
Bacillus virus SPG24	Bacillus phage SPG24	AB930182

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	***2015.038bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
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	***2015.038cB***	(assigned by ICTV officers)

To name the new genus: *Nit1virus*

Assigning the type species and other species to a new genus

Code	***2015.038dB***	(assigned by ICTV officers)

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

Bacillus virus Nit1

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:**

3

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

"Some *Bacillus subtilis* strains, including natto (fermented soybeans) starter strains, produce a capsular polypeptide of glutamate with a γ-linkage, called poly-γ-glutamate (γ-PGA).(4)" Phages Grass, phiNIT1 and SPG24 all encode a 208-216 amino acid poly-gamma-glutamate hydrolase. Other diagnostic proteins produced by this group of phages include "an FtsK/SpoIIIE protein, an RtcB family protein, and a ParM protein. SpoIIIE is an ATPase common in sporulating bacteria that pumps DNA into the forespore. RtcB is an RNA ligase involved in tRNA splicing and repair. ParM is an actin homolog involved in plasmid segregation that is also found in the lytic *Bacillus* phage SPO1. How these proteins are involved in phage replication and assembly is unknown.(5)"

The genome of phiNIT1 contains 5103 bp LTRs (long direct terminal repeats) ; while the Grass genome contains 4208 bp terminal repeats.

A phylogenetic analysis (3) of the major capsid proteins (Fig. 3), large subunit terminase (Fig. 4) and metallophosphatases (Fig. 5) of all the current large *Bacillus* myoviruses reveals clustering which can be confirmed by total genome (BLASTN; progressiveMauve, 1) and proteomic (CoreGenes, 2) analyses.

Origin of the new genus name:

Bacillus phage phiNIT1

Reasons to justify the choice of type species:

The first fully sequenced member of this genus (1)

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. 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. Kimura K, Itoh Y. Characterization of poly-gamma-glutamate hydrolase encoded by a bacteriophage genome: possible role in phage infection of *Bacillus subtilis* encapsulated with poly-gamma-glutamate. Appl Environ Microbiol. 2003;69(5):2491-7. [phiNIT1]

5. Miller SY, Colquhoun JM, Perl AL, Chamakura KR, Kuty Everett GF. Complete Genome of *Bacillus subtilis* Myophage Grass. Genome Announc. 2013;1(6). pii: e00857-13.

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 three phages belonging to the genus *Nit1virus*

Phage	GenBank Accession No.	Genome size (kb)	Genome (mol%G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)*	% Homologous proteins **
phiNIT1	AP013029	155.63	42.1	219	4	100	100
Grass	KF669652	156.65	42.2	252	3	86	80.4
SPG24	AB930182	152.07	42.2	34***	4	90	***

* Determined using BLASTN; ** Determined using CoreGenes (2); *** severely underannotated.

Fig. 2. progressiveMauve alignment (3) of the annotated genomes of members of the *Nit1virus* genus – top (phiNIT1); middle (Grass) and bottom (SPG24). 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).

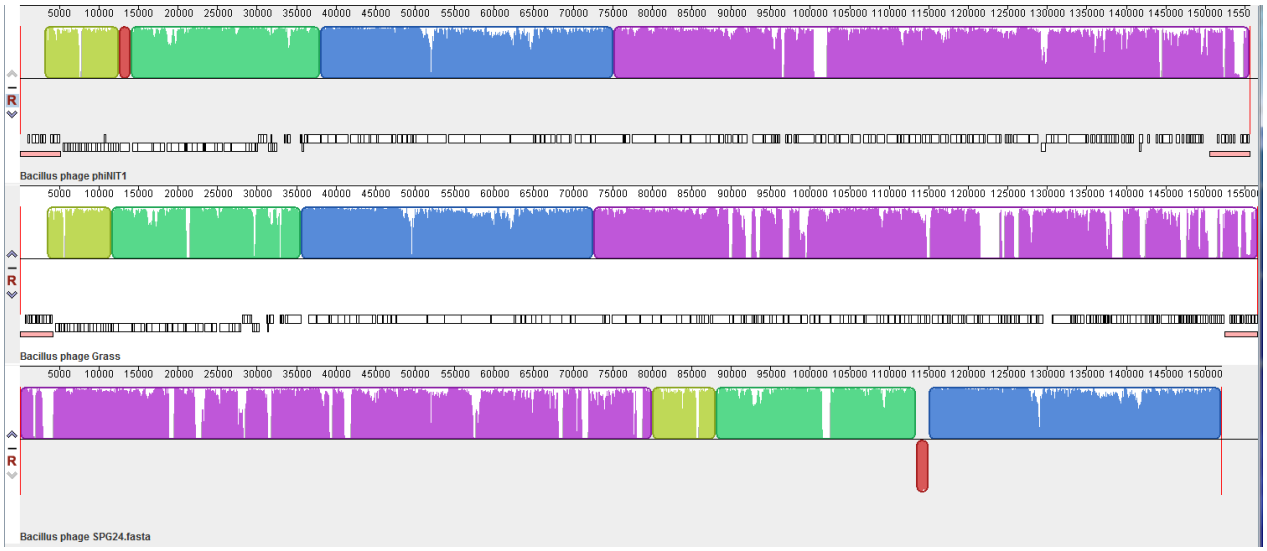

Fig. 3. Phylogenetic analysis of major capsid proteins of NIT1-like viruses and variety of other *Bacillus* phage proteins constructed using “one click” at phylogeny.fr (3). "The "One Click mode" targets users that do not wish to deal with program and parameter selection. By default, the pipeline is 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."

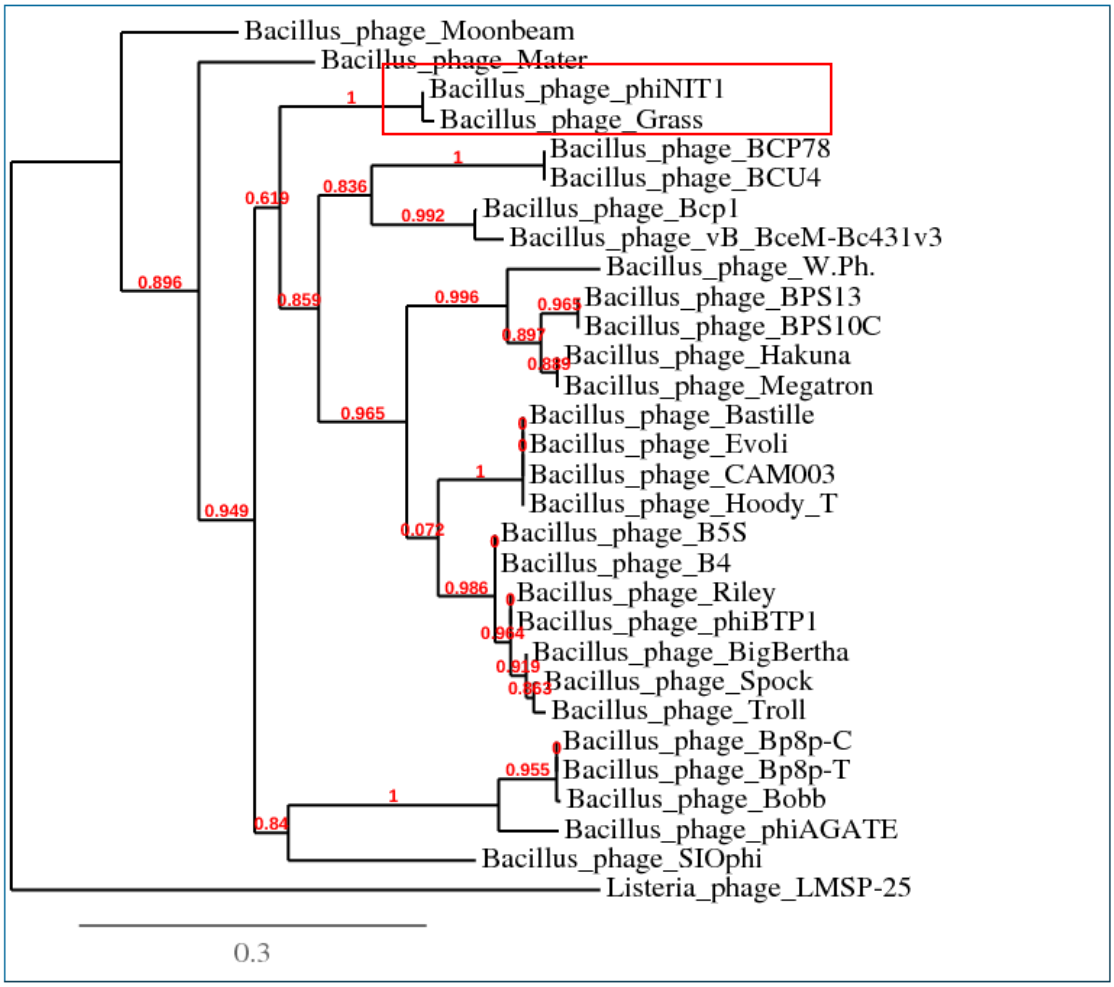

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

Fig. 4. Phylogenetic analysis of large subunit terminase proteins of NIT1-like viruses and variety of other *Bacillus* phage proteins constructed using "one click" at phylogeny.fr (3).

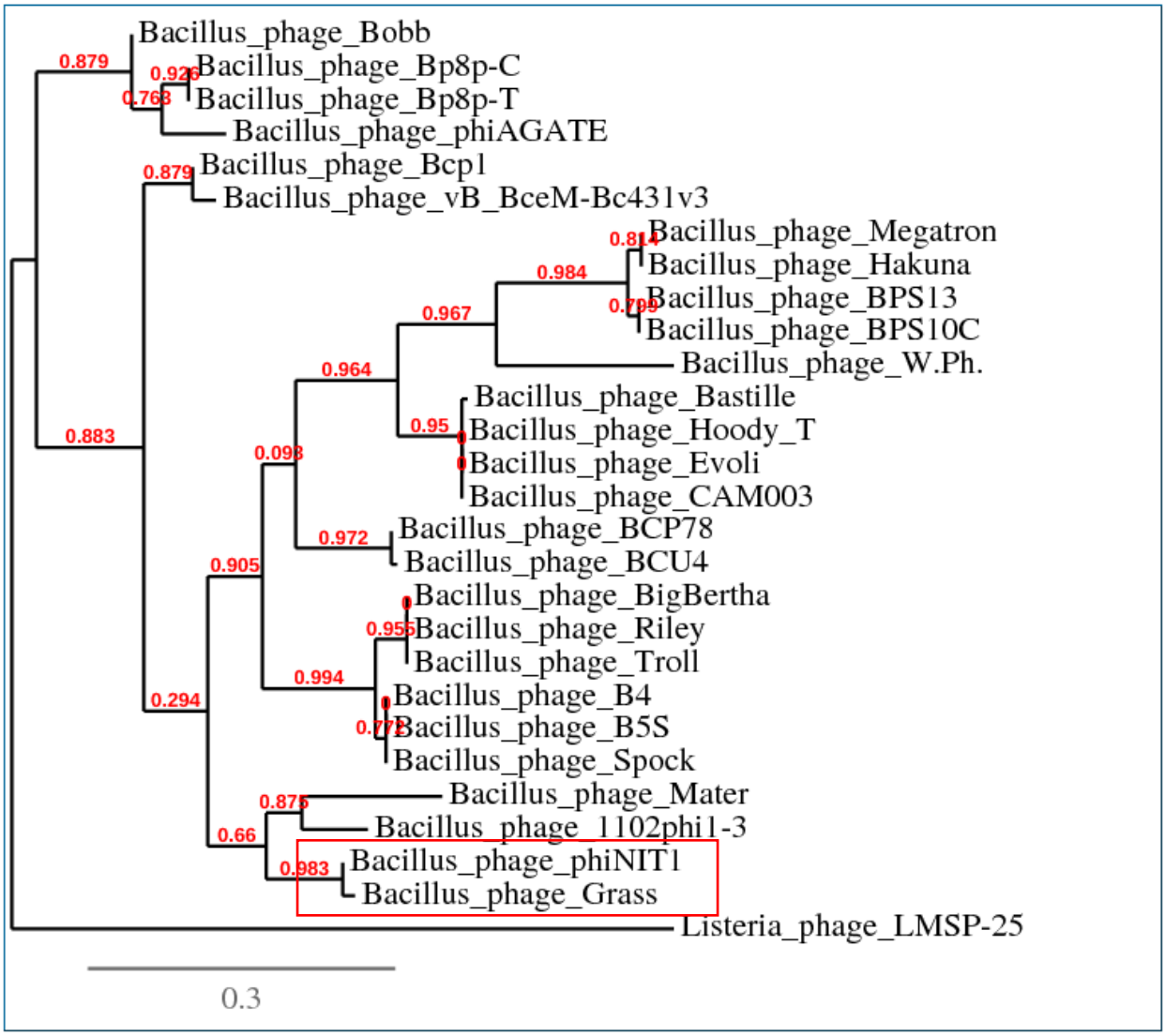

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

Fig. 5. Phylogenetic analysis of the metallophosphatases of NIT1-like viruses and variety of other *Bacillus* phage proteins constructed using "one click" at phylogeny.fr (3).

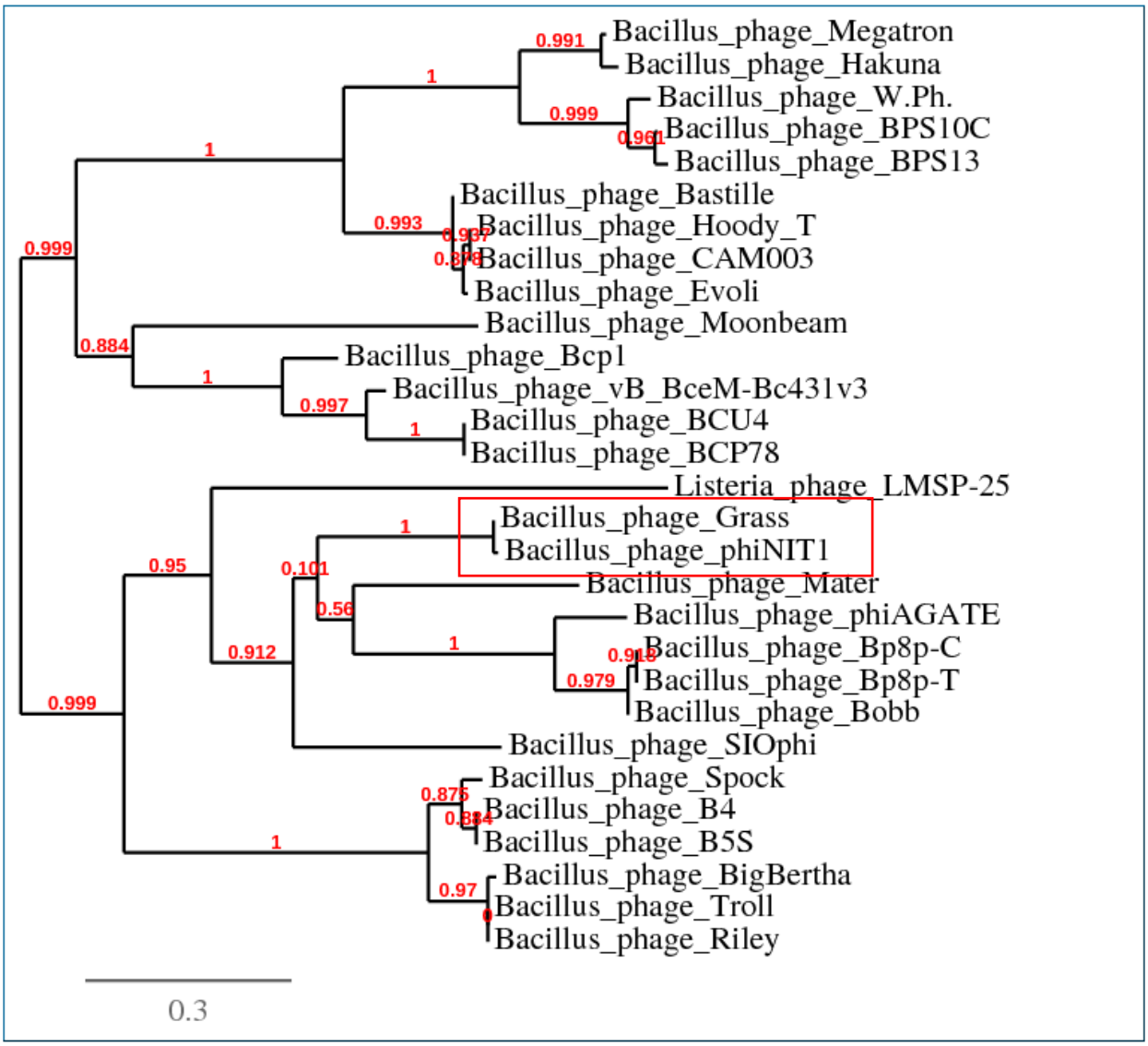

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*