MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	*2016.051a-dB*	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Tsarbombavirus*, including two (2) new species in 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)	ICTV Bacterial and Archaeal Viruses Subcommittee

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

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	*2016.051aB*	(assigned by ICTV officers)

To create 2 new species within:

		Fill in all that apply.
Genus:	*Tsarbombavirus* **(new)**	• If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
Subfamily:	*Spounavirinae*	
Family:	*Myoviridae*	• If no genus is specified, enter "**unassigned**" in the genus box.
Order:	*Caudovirales*	

Name of new species:	Representative isolate: (only 1 per species please)	GenBank sequence accession number(s)
Bacillus virus BCP78 *Bacillus virus TsarBomba*	Bacillus phage BCP78 Bacillus phage TsarBomba	JN797797.1 KT224359.1

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. 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.

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

Code	*2016.051bB*	(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	*2016.051cB*	(assigned by ICTV officers)

To name the new genus: *Tsarbombavirus*

Assigning the type species and other species to a new genus

Code	*2016.051dB*	(assigned by ICTV officers)

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

Bacillus virus TsarBomba	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

Bacillus phage TsarBomba, which infects *Bacillus thuringiensis* subsp. *Kurstaki*, was isolated in Russia [5]. It also infects *Bacillus thuringiensis* Bt DSM-350, Bt-18245 (PS52A1), Bt al Hakam, *Bacillus cereus* Gibson and FDA5, and *Bacillus anthracis* Sterne, but not *Bacillus subtilis* subtilis, *Bacillus simplex* RWR-2, *Bacillus megaterium* JJD118, *Bacillus pumilus* 113, or *Bacillus globegii*. The dimensions of Bacillus phage TsarBomba (Fig. 1) are the following:
 Capsid width (across the face) = 84.1 nm;
 Capsid length = 89.2 nm;
 Uncontracted tail length = 178.4 nm;
 Contracted tail length (neck to baseplate) = 96.3 nm.

 Bacillus phages BCP78 and BCU4 (Fig. 2) were isolated in Korea from *Bacillus cereus* [4]. Bacillus phages BCP79 and BCU4, have nearly identical genomes (>96% DNA sequence identity) [6], and could infect several strains of *B. cereus* (ATCC 27348, ATCC 1661, and NRRL B-569), *B. thuringienesis* (KCCM 11429, KCCM 11579), and *B. subtilis* (ATCC 23857). Furthermore, the endolysins of these phages have identical domain structures containing cell wall hydrolysis/autolysin (PF01520) and SH3-like domains (PF08460) [6].

BLASTN, CoreGenes (Table 1) [2], progressiveMauve alignment (Fig. 3) [1], and phylogenetic analyses (Fig. 4) [3] all indicate that the proposed genus, *Tsarbombdavirus*, is cohesive and distinct from other genera. On average, the genomes of members of this genus are 159.3 kb in length (40.0 mol% G+C), and encode 237 proteins and 19 tRNAs. A genomic map for phage BCP78 is shown in Fig. 5.

Origin of the new genus name:

Unfortunately, there already is an ICTV-accepted genus of myoviruses called *Bcep78virus* (former *Bcep78likevirus*). In our opinion, naming a new genus *Bcp78virus* would cause too much confusion and we therefore propose to name the new genus *Tsarbombavirus*.

Reasons to justify the choice of type species:

Member of this genus which will not cause confusion in genus naming when chosen as type species.

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. 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. Lee JH, Shin H, Son B, Ryu S. Complete genome sequence of *Bacillus cereus* bacteriophage BCP78. J Virol. 2012;86(1):637-8.

5. Erill I, Caruso SM. Complete Genome Sequence of *Bacillus cereus* Group Phage TsarBomba. Genome Announc. 2015 Oct 15;3(5). pii: e01178-15. doi: 10.1128/genomeA.01178-15. Erratum in: Genome Announc. 2015;3(6). pii: e01458-15.

6. Lee JH, Shin H, Ryu S. Characterization and comparative genomic analysis of bacteriophages infecting members of the *Bacillus cereus* group. Arch Virol. 2014; 159:871-884.

Fig. 1. Electron micrograph of negatively stained (2% uranyl acetate) Bacillus phage TsarBomba (provided by Steven M. Caruso, Department of Biological Sciences, University of Maryland, Baltimore County Baltimore, MD, USA).
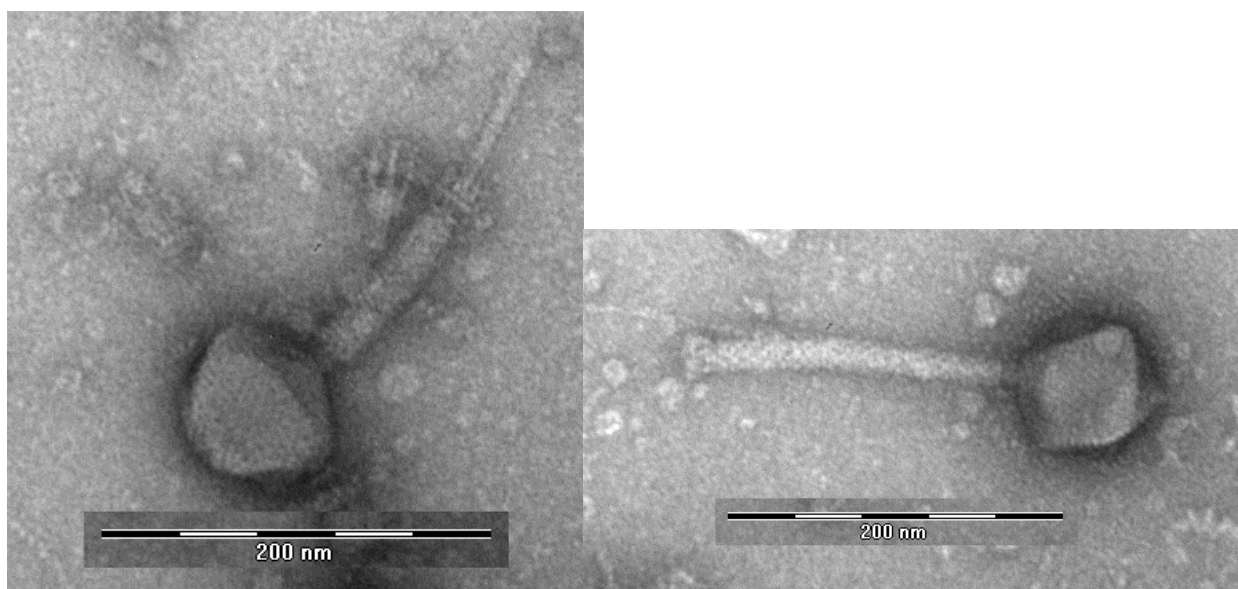

Fig. 2. Electron micrographs of negatively stained Bacillus phages BCP78 (left) and BCU4 (right; provided by: Sangryeol Ryu, Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea).
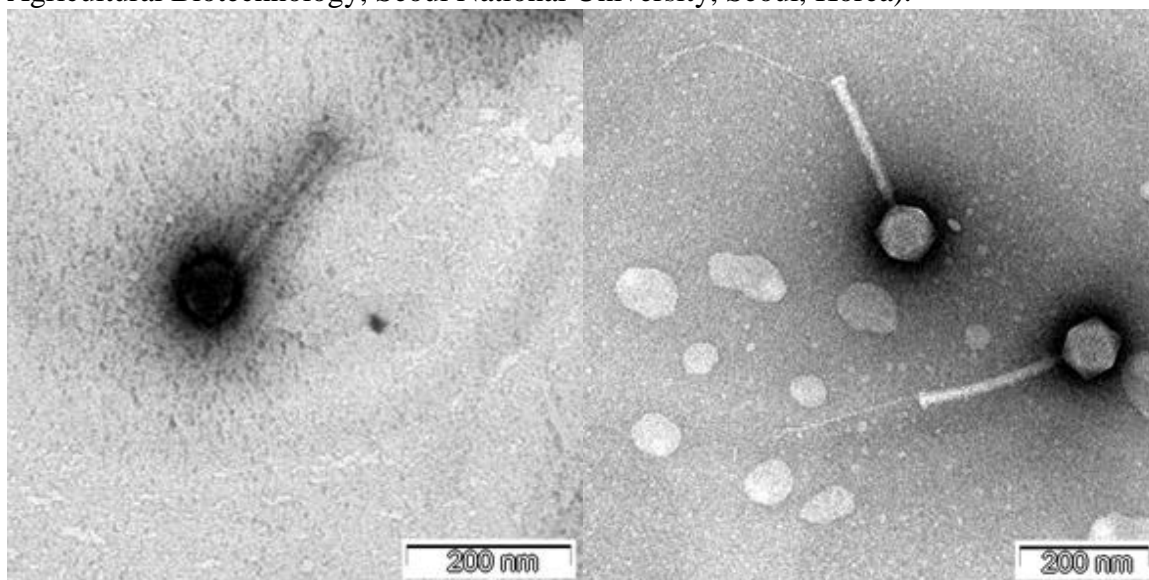
Table 1. Properties of the two phages belonging to the genus *Tsarbombavirus*.

Bacillus phage	RefSeq No.	GenBank Accession No.	Genome length (kb)	Genome (mol% G+C)	No. CDS	No. tRNA	DNA (% sequence identity)*	% Homologous proteins **
TsarBomba	NC_028890.1	KT224359.1	162.49	40.1	247	20	100	100
BCP78	NC_018860.1	JN797797.1	156.18	39.9	227	18	83	89.4
***BCU4		JN797798.1	154.37	39.9	223	19	95	

* Determined using BLASTN; ** Determined using CoreGenes [2]; *** phage BCU4 (JN797798.1) should be considered a strain of Bacillus phage BCP78 in this genus, sequence identity listed relative to BCP78.

Fig. 3. progressiveMauve alignment [1] of the annotated genomes of members of the *Tsarbombavirus* genus – from top to bottom: Bacillus phages BCP78 and TsarBomba. 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. The genomes are not collinear. Since the genome of TsarBomba has 6364 bp direct terminal repeats it should be the model for further alignment. Both Bacillus phage BCP78 and BCU4 genomes contain regions which are homologous to the TsarBomba direct terminal repeats..
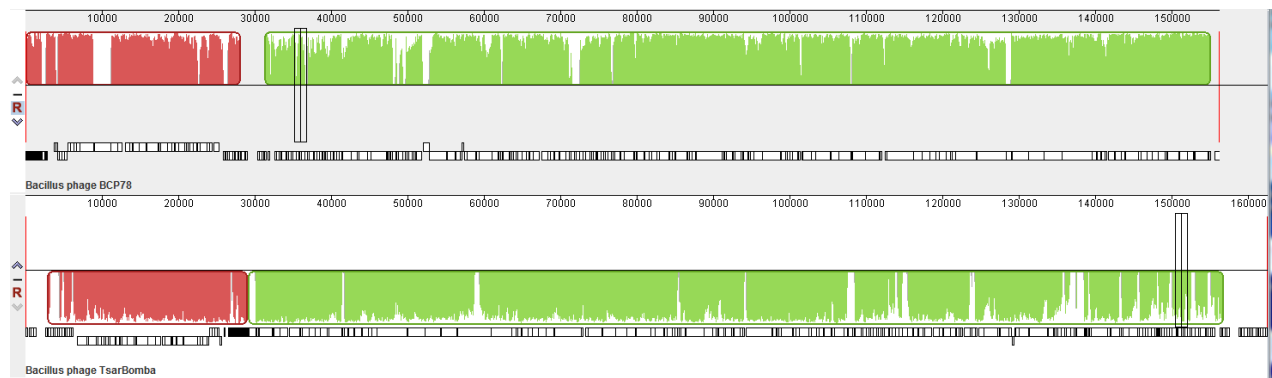

Fig. 4. Phylogenetic analysis of **A.** major capsid protein and **B.** large subunit terminase proteins of Bacillus phage TsarBomba-like viruses and homologous proteins from a variety of other phages constructed using "one click" at phylogeny.fr [3]. "The "One Click mode" targets users that do not wish 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."
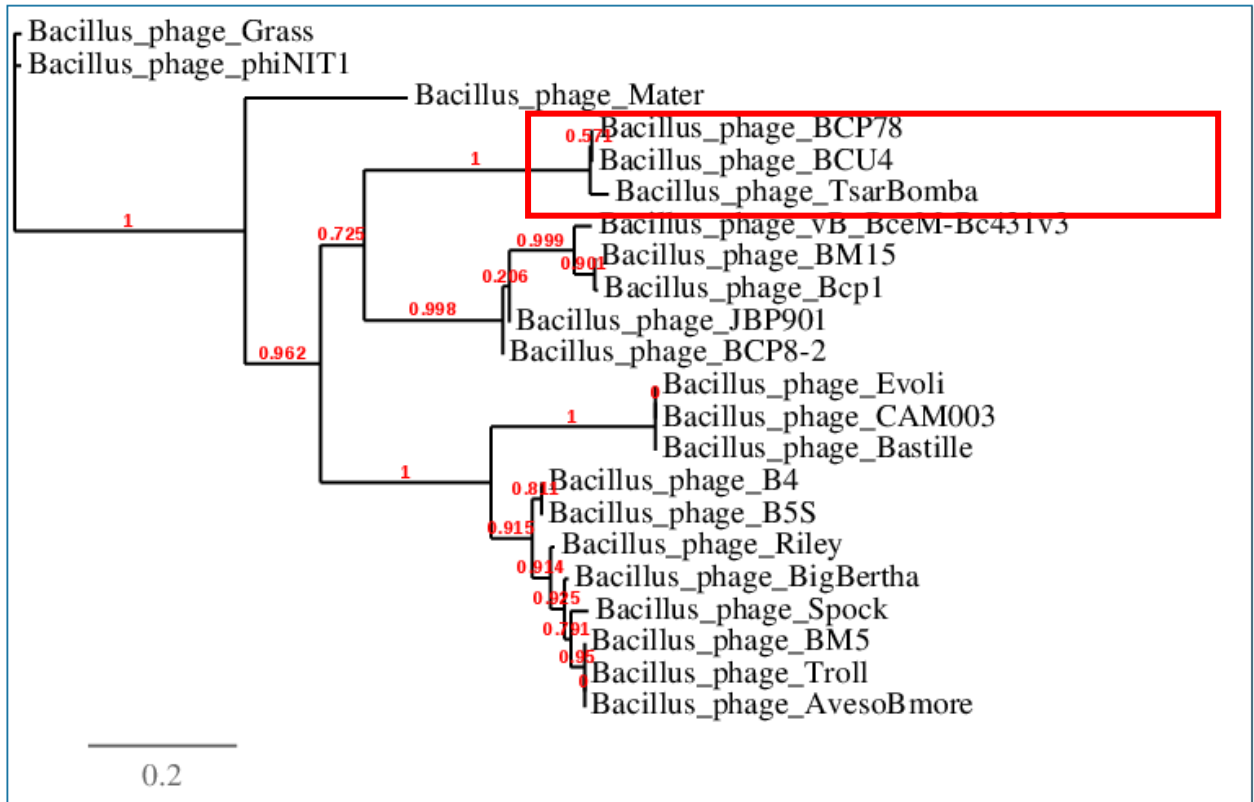
A. major capsid protein

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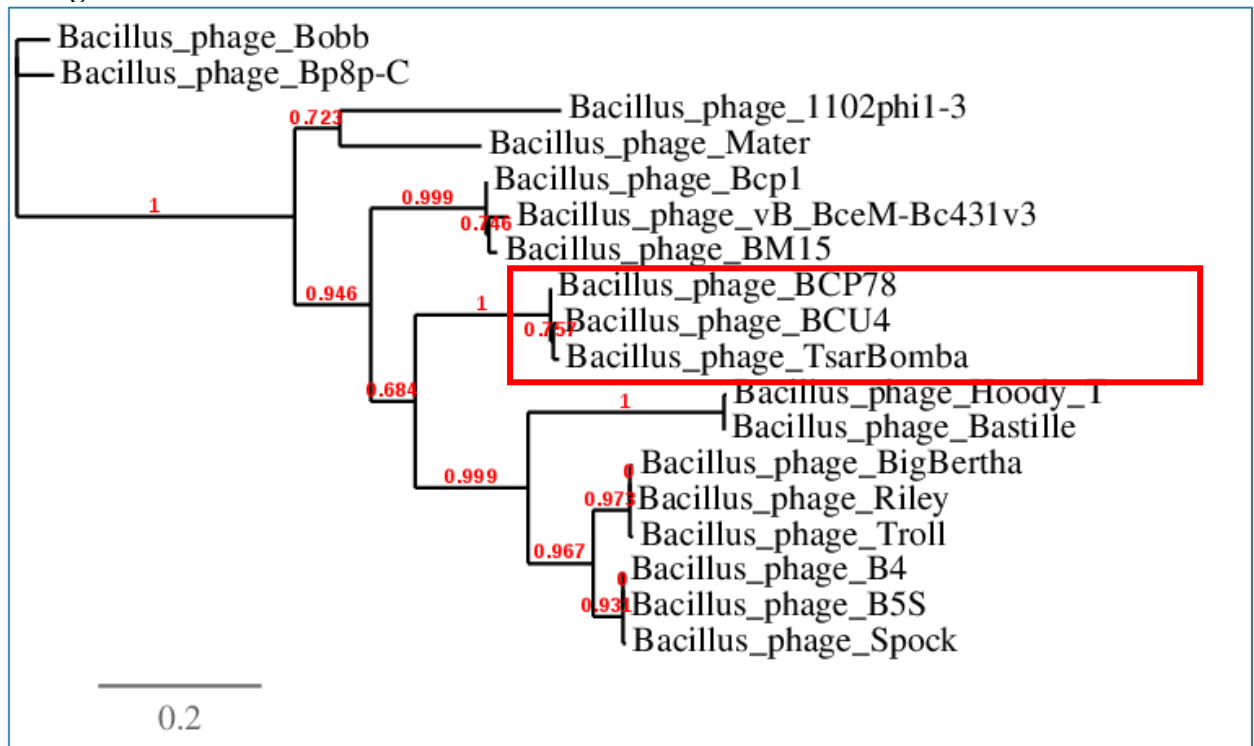
B. large subunit terminase

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

Fig. 5. Genomic map of the phage BCP78