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

Code assigned:	*2015.036a-dB*	(to be completed by ICTV officers)

Short title: Create one (1) new genus, *Cp51virus,* 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 ☒

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

Please note that we have chosen to refer to this new genus as *Cp51virus* rather than *Cp51likevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" and "*Phi*" from phage genus names. Though the *Cp51virus* genus is clearly related to the *Spounalikevirus* genus of *Bacillus* phages, until the Bacterial and Archaeal Virus Subcommittee agrees on the molecular criteria for defining higher orders, we will, in the main, only define genera.

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

ICTV-EC comments and response of the proposer:

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	*2015.036aB*	(assigned by ICTV officers)

To create 2 new species within:

		Fill in all that apply.
Genus:	*Cp51virus* (new)	• If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
Subfamily:		
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 CP51 *Bacillus virus Shanette* *Bacillus virus JL*	Bacillus phage CP-51 Bacillus phage Shanette Bacillus phage JL	KF554508.2 KC595513.2 KC595512.2

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.

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

Code	*2015.036bB*	(assigned by ICTV officers)

To create a new genus within:

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

naming a new genus

Code	*2015.036c*	(assigned by ICTV officers)

To name the new genus: *CP51virus*

Assigning the type species and other species to a new genus

Code	*2015.036dB*	(assigned by ICTV officers)

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

Bacillus virus CP51	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

Bacteriophage CP-51 is infectious for *Bacillus cereus, B.anthracis* and *B. thuringiensis* strains (4). While lytic it is an efficient transducer. One unusual property of this virus is that it is unusually cold sensitive loosing up to 1 log viability in 24h at 4°C (5). Morphologically it resembles *Bacillus* phage SPO1 with an isometric head 80-90nm in diameter and a contractile tail 200 nm long (5). By comparison, phage Shanette and JL possess the same host range among this group of related bacteria, with heads 96 nm in diameter and tails approximately 153 nm in length (6, 7). The genomes of these phages possess the following average characteristics: size, 138.5 kb; mol%G+C, 40.8; encoding, 220 proteins and two tRNAs. The genomes of Shanette and JL possess long terminal direct repeats of 16480 and 16292, respectively. The data on phage CP-51 shows no evidence for direct repeats. The authors of the manuscript on these phages all pointed out the relationship between them and *Bacillus* phage SPO1. While recognizing that these viruses are indeed related; until the Bacterial and Archaeal Virus Subcommittee agrees on the molecular criteria for defining higher orders we will not be proposing a higher order to contain these viruses.

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 CP-51

Reasons to justify the choice of type species:

The first fully sequenced member of this genus (5)

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.

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. Thorne CB. Transduction in *Bacillus cereus* and *Bacillus anthracis*. Bacteriol.Rev. 1968.32,358–361.

5. Klumpp J, Schmuki M, Sozhamannan S, Beyer W, Fouts DE, Bernbach V, Calendar R, Loessner MJ. The odd one out: *Bacillus* ACT bacteriophage CP-51 exhibits unusual properties compared to related *Spounavirinae* W.Ph. and Bastille. Virology. 2014;462-463:299-308.

6. Grose JH, Belnap DM, Jensen JD, Mathis AD, Prince JT, Merrill BD, Burnett SH, Breakwell DP. The genomes, proteomes, and structures of three novel phages that infect the *Bacillus cereus* group and carry putative virulence factors. J Virol. 2014;88(20):11846-60.

7. Grose JH, Jensen JD, Merrill BD, Fisher JN, Burnett SH, Breakwell DP. Genome Sequences of Three Novel *Bacillus cereus* Bacteriophages. Genome Announc. 2014;2(1). pii: e01118-13.

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.

Fig. 1. Electron micrograph of negatively stained phage CP-51 (left; magnification bar 100 nm; 4, with permission) and Shanette (right) showing contracted tail (provided by Julianne H. Grose).
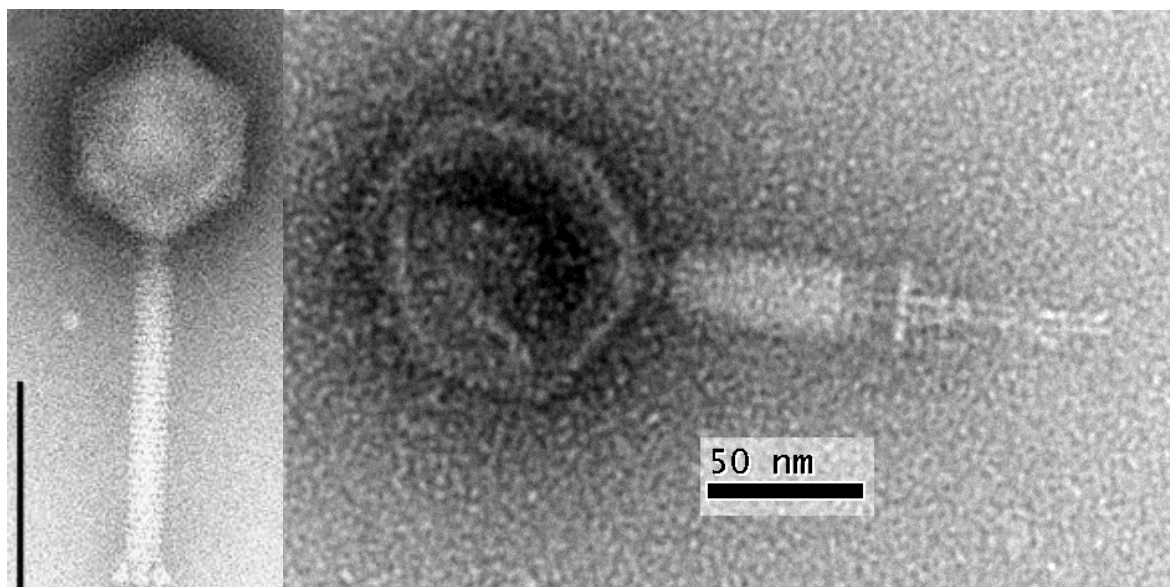
Table 1. Properties of the three phages belonging to the genus *Cp51virus* and their comparison with *Bacillus* phage SPO1.

Phage	GenBank Accession No.	Genome size (kb)	Genome (mol%G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)*	% Homologous proteins **
CP-51	KF554508.2	138.66	40.9	221	2***	100	100
Shanette	KC595513	138.88	40.8	220	2	84	84.6
JL	KC595512	137.92	40.8	218	2	83	84.6
SPO1	FJ230960	132.56	40.0	204	5	11	39.4

* Determined using BLASTN; ** Determined using CoreGenes (2); *** No tRNAs indicated in GenBank RefSeq record

Fig. 2. progressiveMauve alignment (3) of the annotated genomes of members of the *Cp51virus* genus – top (CP-51); middle (JL) and bottom (Shanette). 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).
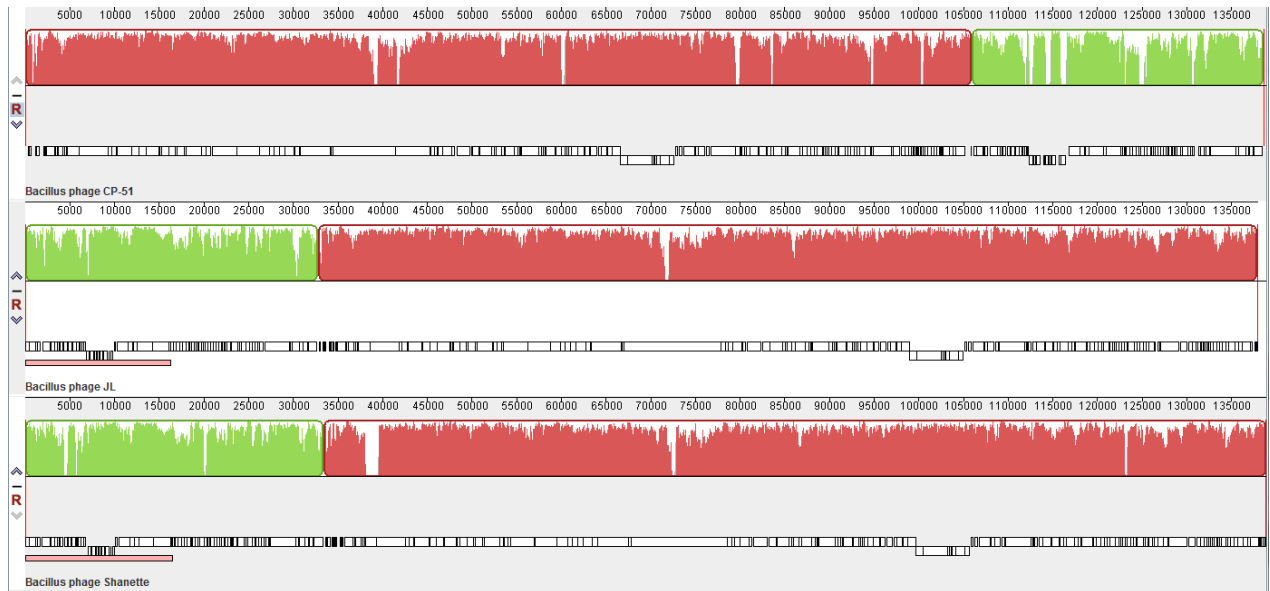
Fig. 3. Phylogenetic analysis of major capsid proteins of CP-51-like viruses and variety of other *Bacillus* phage proteins constructed using "one click" at phylogeny.fr (3). N.B. The capsid gene of Hoody T contained a frameshift which was corrected prior to this analysis. "The "One Click mode" targets 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."
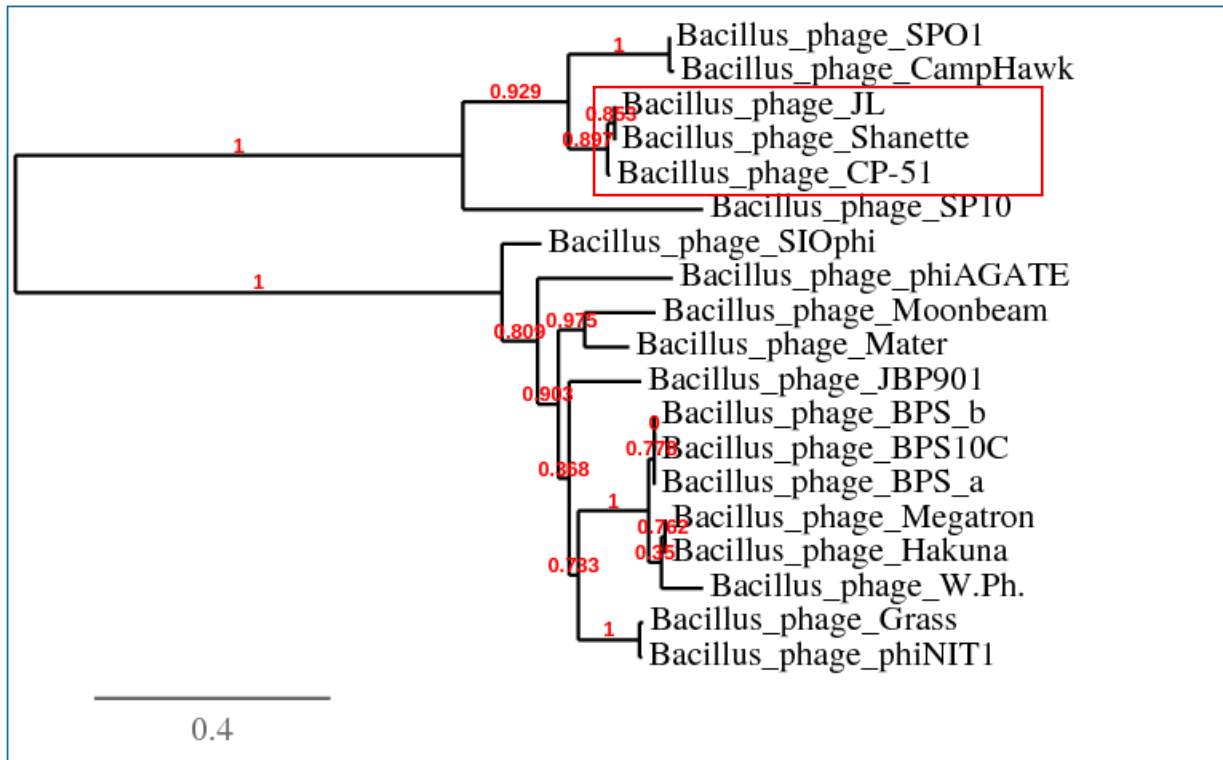

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

Fig. 4. Phylogenetic analysis of large subunit terminase proteins of CP-51-like viruses and variety of other *Bacillus* phage proteins constructed using "one click" at phylogeny.fr (3).
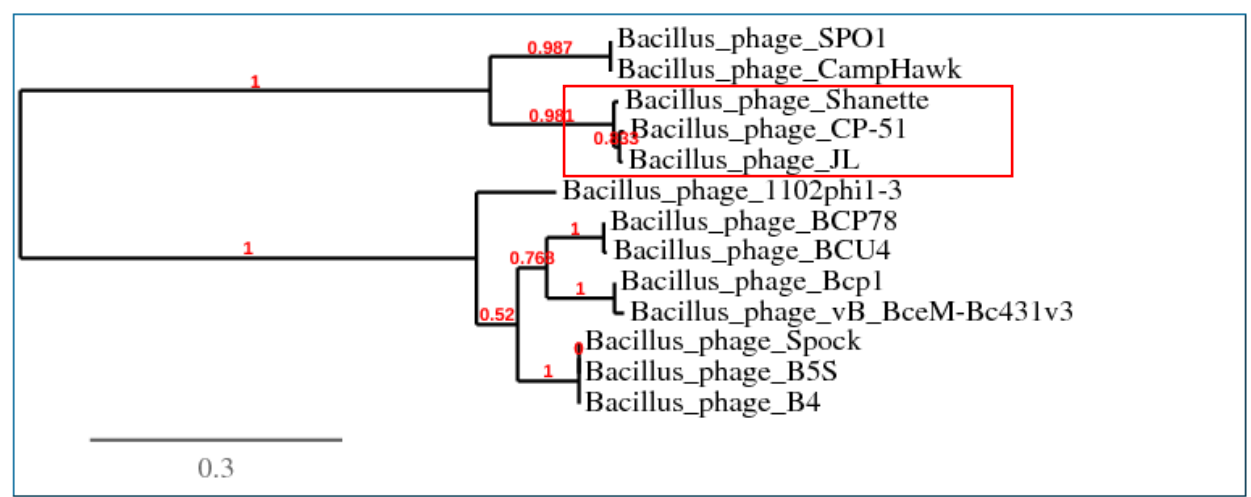

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

Fig. 5. Phylogenetic analysis of the DNA polymerases of CP-51-like viruses and variety of other *Bacillus* phage proteins constructed using "one click" at phylogeny.fr (3).
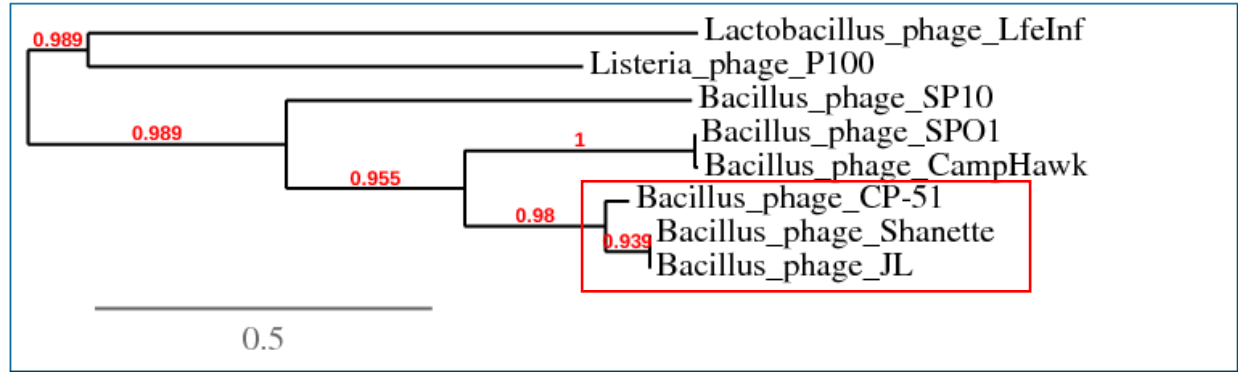

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*