



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**

<b>Code assigned:</b>	<b>2016.002a-dB</b>	(to be completed by ICTV officers)
<b>Short title:</b> To create one (1) new genus, <i>Abouovirus</i> , including two (2) new species in the family <i>Myoviridae</i> . (e.g. 6 new species in the genus <i>Zetavirus</i> )		
<b>Modules attached</b> (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"/>

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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	<b>2016.002aB</b>		(assigned by ICTV officers)
<b>To create 2 new species within:</b>			
Genus:	<b><i>Abouovirus</i> (new)</b>		Fill in all that apply. • If the higher taxon has yet to be created (in a later module, below) write “ <b>(new)</b> ” after its proposed name. • If no genus is specified, enter “ <b>unassigned</b> ” in the genus box.
Subfamily:			
Family:	<b><i>Myoviridae</i></b>		
Order:	<b><i>Caudovirales</i></b>		
<b>Name of new species:</b>		<b>Representative isolate: (only 1 per species please)</b>	<b>GenBank sequence accession number(s)</b>
<i>Brevibacillus virus Abouo</i>		Brevibacillus phage Abouo	KC595517.1
<i>Brevibacillus virus Davies</i>		Brevibacillus phage Davies	KC595518.2

### **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. 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 3: **NEW GENUS**

creating a new genus

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

Code	<b>2016.002bB</b>	(assigned by ICTV officers)
<b>To create a new genus within:</b>		
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:	<i>Myoviridae</i>	
Order:	<i>Caudovirales</i>	

naming a new genus

Code	<b>2016.002cB</b>	(assigned by ICTV officers)
<b>To name the new genus: <i>Abouovirus</i></b>		

Assigning the type species and other species to a new genus

Code	<b>2016.002dB</b>	(assigned by ICTV officers)
<b>To designate the following as the type species of the new genus</b>		
<i>Brevibacillus virus Abouo</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 <b>TOTAL</b> 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

BLASTN (Fig. 2), CoreGenes (Table 1) [2], progressiveMauve alignment (Fig. 3) [1] and phylogenetic analyses (Fig. 4) [3] all indicate that the proposed genus, *Abouovirus*, is cohesive and distinct from other genera. This genus probably forms part of a subfamily that would include *Brevibacillus* phages Osiris and Jimmer 1. On average the genomes of members of this genus are 45.7 kb (39.1 mol% G+C), and encode 94 proteins and no tRNAs. These phages are likely to use headful packaging based on their large terminase homology with that of packaging phages P40. In addition to standard phage structural proteins and several genes that may be involved in breaking down the host cell wall, both *brevibacillus* phages Abouo and Davies encode an ABC transporter-like protein that was characterized as an extracellular protein produced by *Paenibacillus larvae*. They also each encode two XRE-family transcriptional regulators [7].

Both phages are specific for different environmental isolates of *Brevibacillus laterosporus*. There is evidence that *Brevibacillus* phage Davies can form stable lysogens in *B. laterosporus* [7], and thus both phages are likely to be temperate. Both are myoviruses based on electron microscopy.

#### Origin of the new genus name:

Based upon the name of the first sequenced member of this genus.

## Reasons to justify the choice of type species:

The first 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. 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. Agren J, Sundström A, Håfström T, Segerman B. Gegenees: fragmented alignment of multiple genomes for determining phylogenomic distances and genetic signatures unique for specified target groups. PLoS One. 2012;7(6):e39107.
5. Sheflo MA, Gardner AV, Merrill BD, Fisher JN, Lunt BL, Breakwell DP, Grose JH, Burnett SH. Complete Genome Sequences of Five *Paenibacillus larvae* Bacteriophages. Genome Announc. 2013;1(6). pii: e00668-13.
6. Sheflo MA, Gardner AV, Merrill BD, Fisher JN, Lunt BL, Breakwell DP, Grose JH, Burnett SH. Correction for Sheflo et al., Complete Genome Sequences of Five *Brevibacillus laterosporus* Bacteriophages. Genome Announc. 2015;3(5). pii: 01113-15.
7. Merrill BD, Grose JH, Breakwell DP, Burnett SH. Characterization of *Paenibacillus larvae* bacteriophages and their relationships to firmicute bacteriophages. BMC Genomics 2014; 15:745.

**Annex:**

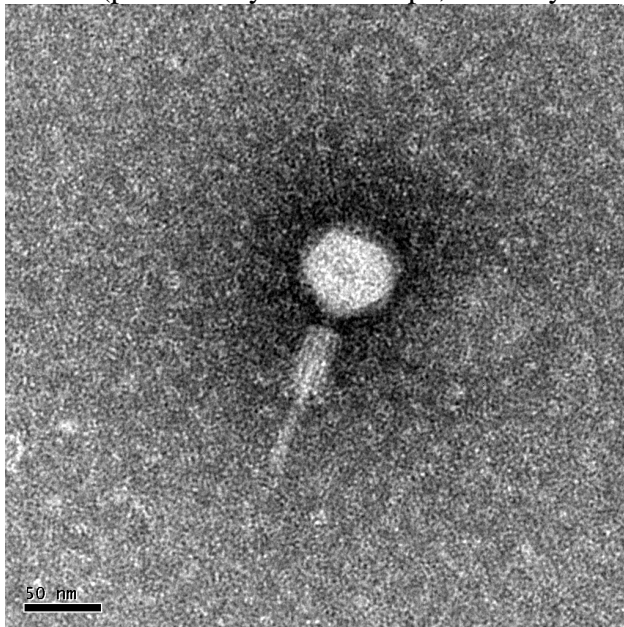
Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate 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 two phages belonging to the genus *Abouovirus*.

Phage	GenBank Accession No.	Genome length (kb)	Genome (mol% G+C)	No. CDS	% DNA sequence identity*	% Homologous proteins **
Brevibacillus phage Abouo	KC595517.1	45.6	39.1	94	100	100
Brevibacillus phage Davies	KC595518.2	45.8	39.1	94	83	90.4

\* Determined using BLASTN; \*\* Determined using CoreGenes [2]

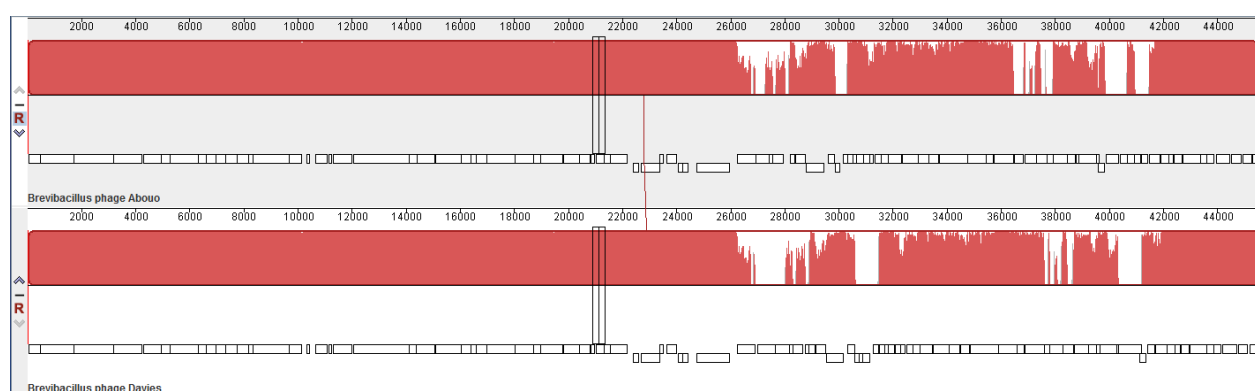
**Fig. 1.** Electron micrograph of negatively stained (phosphotungstic acid) brevivacillus phage Abouo (provided by Sandra Hope, taken by the BYU Microscopy Center).



**Fig.2.** BLASTN heat map generate using Gegenees [4] and accurate parameters – fragment size: 200 bp; step size: 100 bp.

PHAGE	ACCESSION NO.	KC595514.1	KC595515.1	KT151958.1	KT151956.1	KC595518.2	KC595517.1
Osiris	KC595514.1	100.0	99.5	89.2	86.8	38.3	35.2
Powder	KC595515.1	99.5	100.0	88.7	86.4	37.9	34.7
Jimmer2	KT151958.1	91.5	91.0	100.0	97.6	47.3	40.0
Jimmer1	KT151956.1	89.1	88.6	97.6	100.0	46.5	39.1
Davies	KC595518.2	45.4	44.9	54.9	54.0	100.0	83.2
Abouo	KC595517.1	41.8	41.2	46.4	45.5	83.9	100.0

**Fig. 3.** progressiveMauve alignment [1] of the annotated genomes of members of the *Abouovirus* genus – from top to bottom: brevibacillus phages Abouo and Davies. 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. 4.** Phylogenetic analysis of (A) large subunit terminase proteins and (B) major capsid proteins of *Brevibacillus* myoviruses 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. TerL proteins

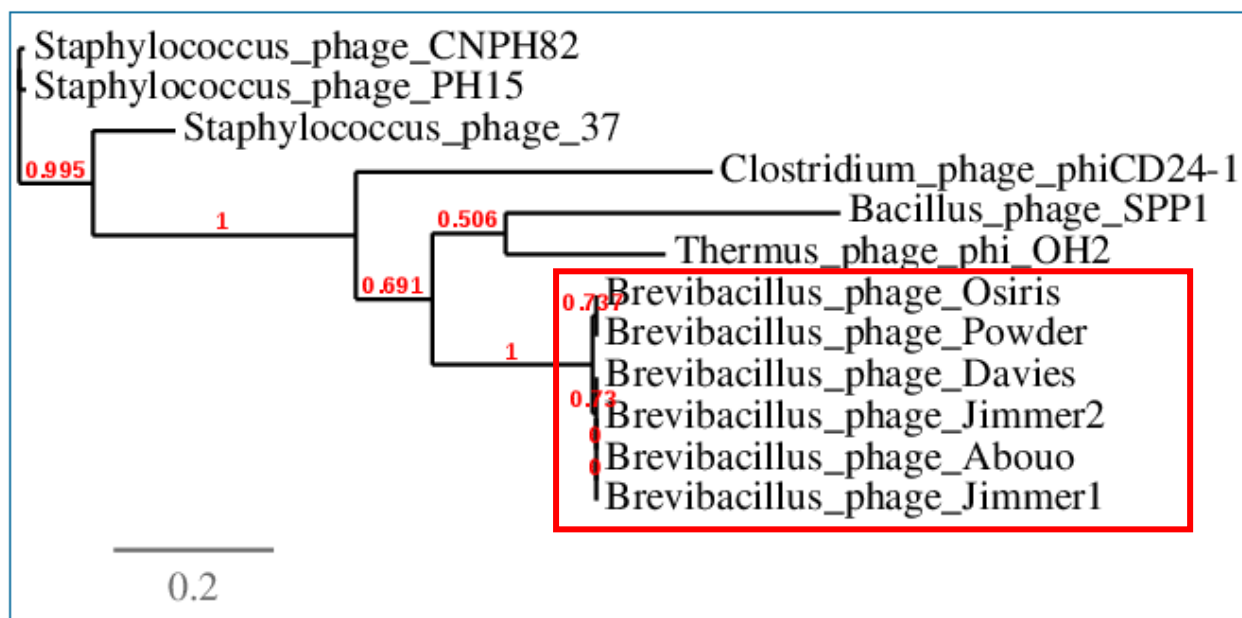


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).

## B. Major capsid proteins

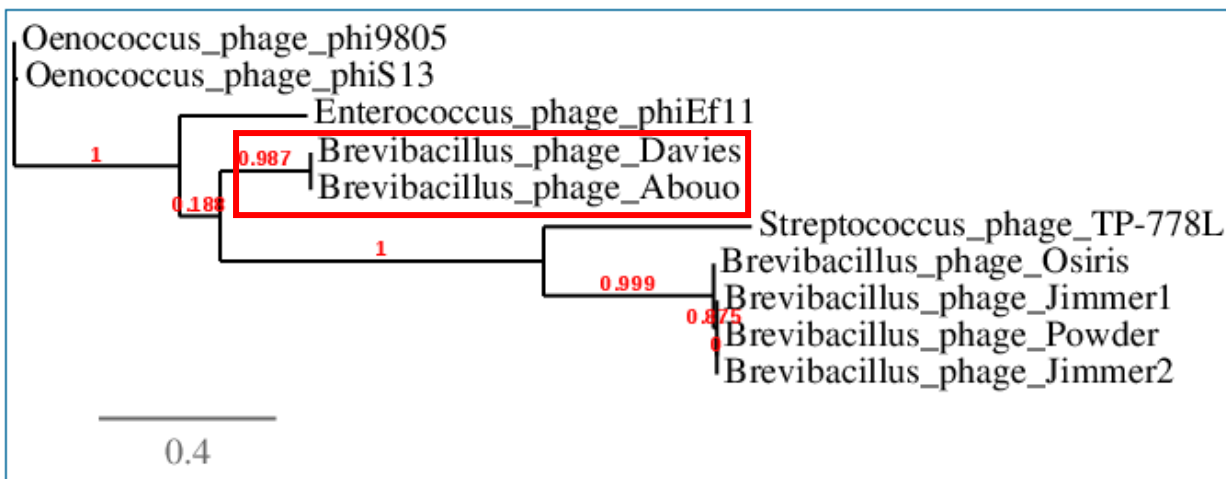


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).