



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>2013.027a-dB</b>	(to be completed by ICTV officers)			
<b>Short title:</b> To create a new genus, the <i>Tp2unalikevirus</i> , within the family <i>Siphoviridae</i> (e.g. 6 new species in the genus <i>Zetavirus</i> )					
<b>Modules attached</b> (modules 1 and 9 are required)	1 <input checked="" type="checkbox"/>	2 <input type="checkbox"/>	3 <input type="checkbox"/>	4 <input type="checkbox"/>	5 <input type="checkbox"/>
	6 <input type="checkbox"/>	7 <input type="checkbox"/>	8 <input type="checkbox"/>	9 <input checked="" type="checkbox"/>	

**Author(s) with e-mail address(es) of the proposer:**

Evelien Adriaenssens [Evelien.Adriaenssens@gmail.com](mailto:Evelien.Adriaenssens@gmail.com)  
Andrew M. Kropinski [akropins@uoguelph.ca](mailto:akropins@uoguelph.ca)  
Rob Lavigne [rob.lavigne@biw.kuleuven.be](mailto:rob.lavigne@biw.kuleuven.be)  
Jochen Klumpp [jochen.klumpp@ilw.agrl.ethz.ch](mailto:jochen.klumpp@ilw.agrl.ethz.ch)

**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-EC or Study Group comments and response of the proposer:**

---

Date first submitted to ICTV: June 2013  
Date of this revision (if different to above): July 2014

---

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>2013.027aB</b>	(assigned by ICTV officers)
<b>To create 2 new species within:</b>		
Genus:	<b><i>Tp2unalikevirus</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>Siphoviridae</i></b>	
Order:	<b><i>Caudovirales</i></b>	
		<b>GenBank sequence accession number(s) of reference isolate:</b>
<i>Bacillus phage TP21</i> <i>Bacillus phage BMBtp2</i>		EU887664 JX887877

<p><b>Reasons to justify the creation and assignment of the new species:</b></p> <ul style="list-style-type: none"> <li>• Explain how the proposed species differ(s) from all existing species.             <ul style="list-style-type: none"> <li>○ If species demarcation criteria (see module 3) have previously been defined for the genus, <b>explain how the new species meet these criteria.</b></li> <li>○ 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.</li> </ul> </li> <li>• <a href="#">Further material in support of this proposal may be presented in the Appendix, Module 9</a></li> </ul> <p>BLASTN analyses reveal that these phages are related and distinct from other <i>Bacillus</i> phages. We have chosen 95% DNA sequence identity as the criterion for demarcation of species.</p>
---

### MODULE 3: NEW GENUS

creating a new genus

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

Code	<b>2013.027bB</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 “ <b>(new)</b> ” after its proposed name. • If no family is specified, enter “ <b>unassigned</b> ” in the family box
Family:	<i>Siphoviridae</i>	
Order:	<i>Caudovirales</i>	

naming a new genus

Code	<b>2013.027cB</b>	(assigned by ICTV officers)
<b>To name the new genus: <i>Tp2unalikevirus</i></b>		

Assigning the type species and other species to a new genus

Code	<b>2013.027dB</b>	(assigned by ICTV officers)
<b>To designate the following as the type species of the new genus</b>		
<i>Bacillus phage TP21</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). <b>Please enter here the TOTAL number of species (including the type species) that the genus will contain:</b>		
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

Unfortunately three different *Bacillus* phages are designated TP21 (1, 2). Phage TP21-L forms the basis of this new genus. Phage TP21-L is specific for *Bacillus cereus*; while BMBtp2 infects *Bacillus thuringiensis* subsp. *tenebrionis* (3). Both are temperate members of *Siphoviridae*. BLASTN analyses reveal that these phages are related and distinct from other *Bacillus* phages. They are related to prophage proCM3 (5). Phage TP21-L possesses an isometric head of 58.5 nm diameter and a long, non-contractile, flexible tail (144.8 nm x 11.0 nm) which is terminated by a single tail fiber (Figure 1). General characteristics of the genus members are listed in Table 1.

**Origin of the new genus name:**

*Bacillus* phage TP21-L

**Reasons to justify the choice of type species:**

The original isolate of this group.

**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.

MODULE 9: **APPENDIX**: supporting material

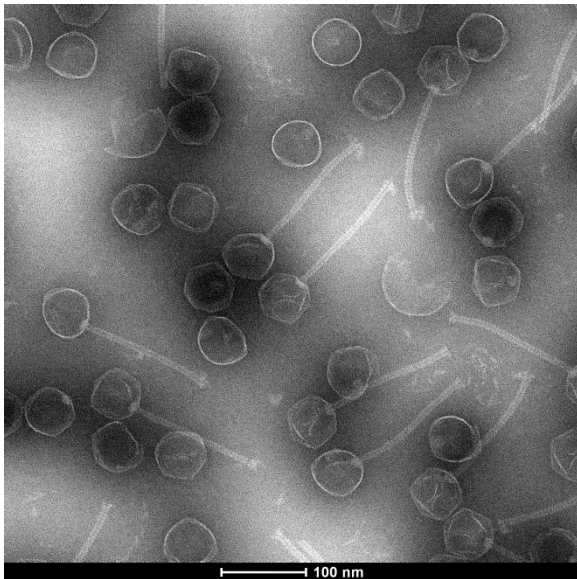
additional material in support of this proposal

**References:**

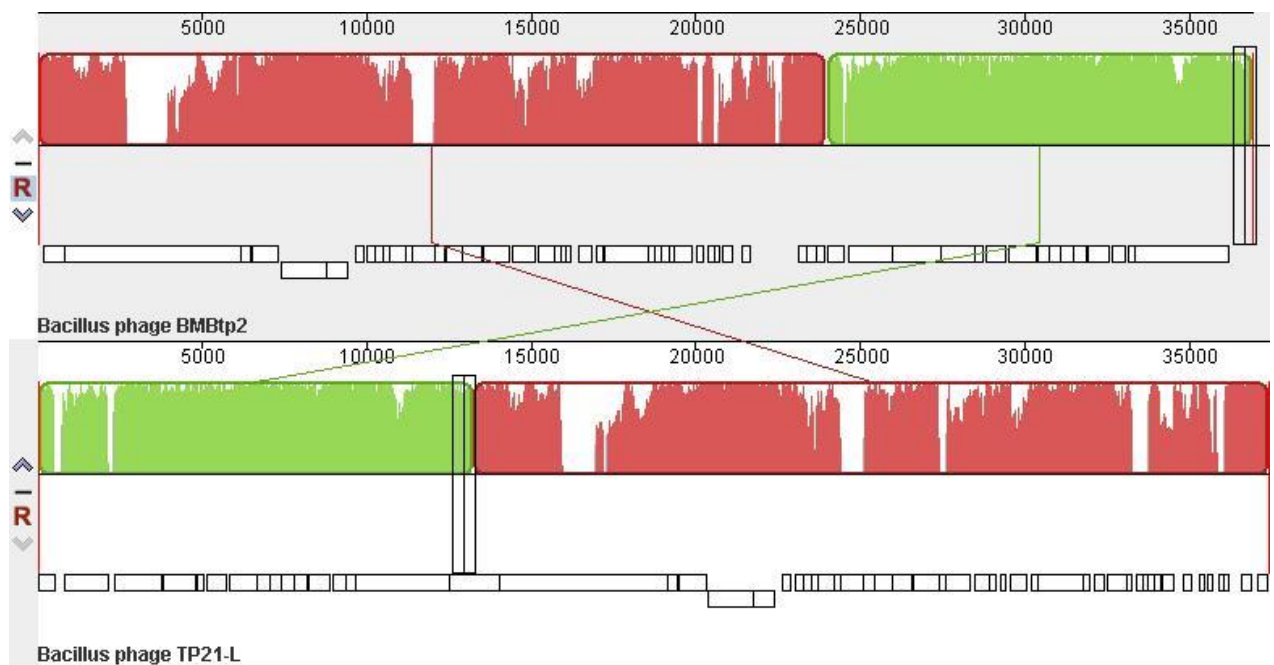
- 1: Klumpp J, Calendar R, Loessner MJ. Complete Nucleotide Sequence and Molecular Characterization of Bacillus Phage TP21 and its Relatedness to Other Phages with the Same Name. *Viruses*. 2010 Apr;2(4):961-71. Epub 2010 Apr 6. PubMed PMID: 21994663; PubMed Central PMCID: PMC3185655.
- 2: Loessner MJ, Maier SK, Daubek-Puza H, Wendlinger G, Scherer S. Three *Bacillus cereus* bacteriophage endolysins are unrelated but reveal high homology to cell wall hydrolases from different bacilli. *J Bacteriol*. 1997 May;179(9):2845-51. PubMed PMID: 9139898; PubMed Central PMCID: PMC179044.
- 3: Dong Z, Peng D, Wang Y, Zhu L, Ruan L, Sun M. Complete genome sequence of *Bacillus thuringiensis* bacteriophage BMBtp2. *Genome Announc*. 2013 1(1):e00011-12. doi:10.1128/genomeA.00011-12.
- 4: Darling AE, Mau B, Perna NT (2010) progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* 5: e11147
- 5: Yuan Y, Gao M, Peng Q, Wu D, Liu P, Wu Y. Genomic analysis of a phage and prophage from a *Bacillus thuringiensis* strain. *J Gen Virol*. 2014 Mar;95(Pt 3):751-61.

**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.



**Figure 1.** Electron micrograph of *Bacillus* phage TP21-L (kindly provided by J. Klumpp; Institute of Food, Nutrition and Health, ETH Zurich, 8092 Zurich, Switzerland)



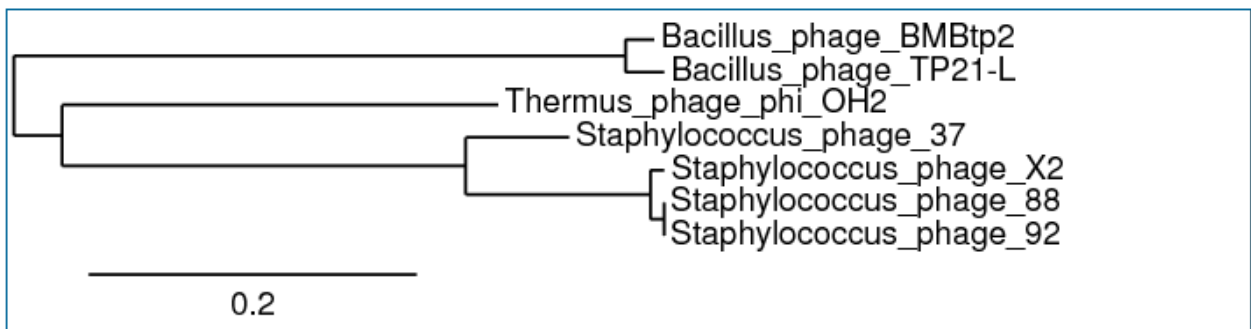
**Figure 2.** progressiveMauve alignment of the phage genomes belonging to the proposed genus (full genome represented by its annotated ORFs in white blocks) (4). Colored blocks indicate the regions of 1 to 1 best alignment with rearrangement breakpoints in a different random color. The degree of sequence 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.

**Table 1. Phage genomes belonging to the proposed genus Tp2unalikevirus**

Phage	GenBank Accession No.	Genome size (bp)	Mol%G+C	% DNA sequence identity (a)	% Shared proteins (b)
TP21-L	EU887664	37456	37.80	100%	100%
BMBtp2	JX887877	36932	37.79	86.9%	82.1%

(a) Calculated using EMBOSS Stretcher – reoriented genome to compensate for different origins

(b) Calculated using CoreGenes 2.0



**Figure 3.** Phylogenetic tree constructed using “one click” phylogeny.fr and phage large subunit terminase proteins.