

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:	2015.012	a-dB		(to be con officers)	npleted by I	CTV
<b>Short title:</b> To create one (1) new genus, <i>P100virus</i> , including one (1) new species within the family <i>Myoviridae</i> . (e.g. 6 new species in the genus <i>Zetavirus</i> )						
Modules attached (modules 1 and 10 are required)		1 🔀 6 🗌	2 🖂 7 🔀	3 8	4 9	5 🗌 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 <u>http://www.ictvonline.org/subcommittees.asp</u> . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)	Bacterial & Archaeal Virus Subcommittee
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## ICTV Study Group comments (if any) and response of the proposer:

Please note that we have chosen to refer to this new genus as *P100virus* rather than *P100likevirus* 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:May 2015Date 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 20	15.012aB	<b>B</b> (assigned by ICTV officers)		
To create 1 new species within:				
				all that apply.
Genus:	<i>P100virus</i> (n	new)		e higher taxon has yet to be
Subfamily:	Spounavirinae		<ul> <li>created (in a later module, below) write</li> <li>"(new)" after its proposed name.</li> <li>If no genus is specified, enter</li> </ul>	
Family:	Myoviridae			
Order:	Caudovirale	lovirales		assigned" in the genus box.
Name of new species: Representative isolate: per species please)		(only 1	GenBank sequence accession number(s)	
Listeria virus	P100	Listeria phage P100		DQ004855

#### **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

*Listeria* phage P100 and its relatives are all strictly lytic myoviruses. It and phage A511 possess extremely broad host ranges; the latter infecting "95% of the common *L. monocytogenes* strains of serovars 1/2 and 4 and 100% of *L. ivanovii* strains" (4). Phage A511 "features a head diameter of 87.4 nm, tail length of 199.4 nm, and tail diameter of 20.4 nm. Phage P100 is virtually identical in its dimensions to A511 (head diameter 90.0 nm; tail length 198.2 nm; tail diameter 19.0 nm. Long tail fibers with a six-fold symmetry are attached to the baseplate regions of A511 and P100." (4). The genome of A511 (~135-kb) possesses terminal repeats of 3.1 kb, which have not been described for other members of this group. Currently these phages are classified within the *Twortlikevirus* genus, but BLASTN, CoreGenes and phylogenetic analyses reveal that all the *Listeria* myoviruses of the proposed genus, *P100virus*, are cohesive and significantly different from *Staphylococcus* phage Twort. P100 and Twort only share 10% DNA sequence identity and 39.7 % homologous proteins as shown by CoreGenes analysis (2). Furthermore, phylogenetic analysis (Fig. 2) of the major capsid and large subunit terminase proteins reveal that the proteins of phage Twort are significantly different from the P100-like viruses, and indeed all of the *Staphylococcus* large myoviruses.

Based upon the complete genome of phage A511 the phages of this genus possess genomes of approx. 137 kb (36 mol%G+C), and encode 190 proteins and 16-18 tRNAs.

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.

Please note that we have chosen to refer to this new genus as *P100virus* rather than *P100likevirus* since

the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" and "*Phi*" from phage genus names.

## MODULE 3: NEW GENUS

creating a new genus

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

Code 20	15.012bB	(assigned by ICTV officers)	
To create a ne	w genus within:	Fill in all that apply.	
Subfamily	: Spounavirinae	If the higher taxon has yet to be created	
Family	: Myoviridae	(in a later module, below) write "(new)" after its proposed name.	
Order	: Caudovirales	<ul> <li>If no family is specified, enter</li> <li>"unassigned" in the family box</li> </ul>	

naming a new genus

Code	2015.012cB	(assigned by ICTV officers)
To name tl	ne new genus: <i>P100virus</i>	

Assigning the type species and other species to a new genus

(assigned by ICTV officers)	
e species of the new genus 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:

**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, CoreGenes (Table 1; 2), progressiveMauve alignment (Fig. 2; 1) and phylogenetic analyses (Fig. 3; 3) all indicate that the proposed genus, *P100virus*, is cohesive and distinct from the other genera of viruses.

## Origin of the new genus name:

Named after the first phage of its type to be sequenced: *Listeria* phage P100

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

First phage of its type to be sequenced

#### 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 7: **<u>REMOVE</u>** and <u>MOVE</u>

Use this module whenever an existing taxon needs to be removed:

- *Either* to abolish a taxon entirely (when only part (a) needs to be completed)
- Or to move a taxon and re-assign it e.g. when a species is moved from one genus to another (when BOTH parts (a) and (b) should be completed)

Part (a) taxon/taxa to be removed or moved

To remove the following taxon (or taxa) from their present position:				
Listeria phage P100 and Listeria phage A511				
The present taxonomic position of these taxon/taxa:				
Genus:	Twortlikevirus			
Subfamily:	Spounavirinae	Fill in all that apply		
Family:	Myoviridae	Fill in all that apply.		
Order:	Caudovirales			

#### **Reasons to justify the removal:**

Explain why the taxon (or taxa) should be removed

Currently these phages are classified within the *Twortlikevirus* genus, but BLASTN, CoreGenes and phylogenetic analyses reveal that all the large lytic *Listeria* myoviruses are essentially identical and significantly different from *Staphylococcus* phage Twort. P100 and Twort only share 10% DNA sequence identity (BLASTN) and 39.7 % homologous proteins as shown by CoreGenes analysis (2). Furthermore, phylogenetic analysis (Fig. 2) of the major capsid and large subunit terminase proteins reveal that the proteins of phage Twort are significantly different from those of the large lytic *Listeria* myoviruses.

**Part (b)** re-assign to a higher taxon

Code	(assigned by ICTV officers)		
To re-assign the taxon (or taxa) listed in Part (a) as follows:			
		Fill in all that apply.	
Genu	as: <i>P100virus</i> (new)	• If the higher taxon has yet to be	
Subfamil	y: Spounavirinae	created write "( <b>new</b> )" after its	
Famil	y: Myoviridae	proposed name and complete	
Orde	er: Caudovirales	relevant module to create it.	
		If no genus is specified, enter	
		" <b>unassigned</b> " in the genus box.	

#### **Reasons to justify the re-assignment:**

- If it is proposed to re-assign species to an existing genus, please 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.
- Provide accession numbers for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

Newly proposed genus (See Module 2)

## 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.

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. Klumpp J, Dorscht J, Lurz R, Bielmann R, Wieland M, Zimmer M, Calendar R, Loessner MJ. The terminally redundant, nonpermuted genome of *Listeria* bacteriophage A511: a model for the SPO1-like myoviruses of gram-positive bacteria. J Bacteriol. 2008;190(17):5753-65.

5. Denes T, Vongkamjan K, Ackermann HW, Moreno Switt AI, Wiedmann M, den Bakker

HC. Comparative genomic and morphological analyses of *Listeria* phages isolated from farm environments. Appl Environ Microbiol. 2014;80(15):4616-25.

6. Carlton RM, Noordman WH, Biswas B, de Meester ED, Loessner MJ. Bacteriophage P100 for control of *Listeria monocytogenes* in foods: genome sequence, bioinformatic analyses, oral toxicity study, and application. Regul ToxicolPharmacol. 2005;43(3):301-12.

#### 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 *P100virus* genus.

Phage	GenBank	Genome	Genome	No.	No.
	accession No.	length (kb)	(mol%G+C)	CDS	tRNAs
P100	DQ004855	131.4	36.0	174	18

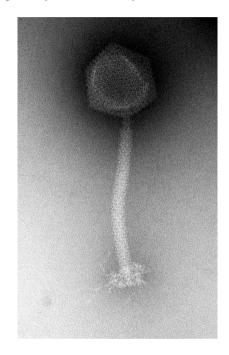
\* Determined using BLASTN; \*\* Determined using CoreGenes (2)

Table 2. Related phages

Phage	GenBank
	Accession Number
Listeria phage A511	DQ003638.2
Listeria phage vB_LmoM_AG20	JQ797329
Listeria phage LP-125	JX126918.2
Listeria phage LP-064	KJ094029
Listeria phage LP-083-2	KJ094030
Listeria phage LP-124	KJ094031
Listeria phage LP-048	KJ094033

Listeria phage List-36	KJ535721
Listeria phage LMSP-25	KJ535722
Listeria phage LMTA-94	KJ586795
Listeria phage LMTA-148	KJ591604
Listeria phage LMTA-57	KJ591605
Listeria phage WIL-1	KM373208

Fig. 1. Electron micrographs of negatively stained (uranyl acetate) Listeria phage P100



**Fig. 2.** Phylogenetic analysis of the large subunit terminase (top) and major capsid protein (bottom) of p100viruses and some related *Staphylococcus* 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." Due to errors in the sequence of *Staphylococcus* phage Twort, the sequence of its terminase had to be reconstructed from the genome sequence.

	0.999	Staphylococcus_phage_MSA6 Staphylococcus_phage_Team1 Staphylococcus_phage_phiSA0	
1		Staphylococcus_phage_GH15	
	Staphylococcus_phage_Strip		
	Enterococcus_phage_EFDG1		
└ <u></u>	0.005	Listeria_phage_LMTA-94	
	0.995	Listeria_phage_LMTA-57	
		Listeria_phage_LMSP-25	
		Listeria_phage_LMTA-34	
		Listeria_phage_A511	
		Listeria_phage_vB_LmoM_AG20	
		Listeria_phage_LP-125	
		Listeria_phage_List-36	
		Listeria_phage_LP-048	
		Listeria_phage_LP-083-2	
		Listeria_phage_WIL-1	
		Listeria_phage_P100	
		Listeria_phage_LP-064	
		Listeria_phage_LMTA-148	
		Listeria_phage_LP-124	
		Elsteria_priage_D1=12+	
0.2			

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

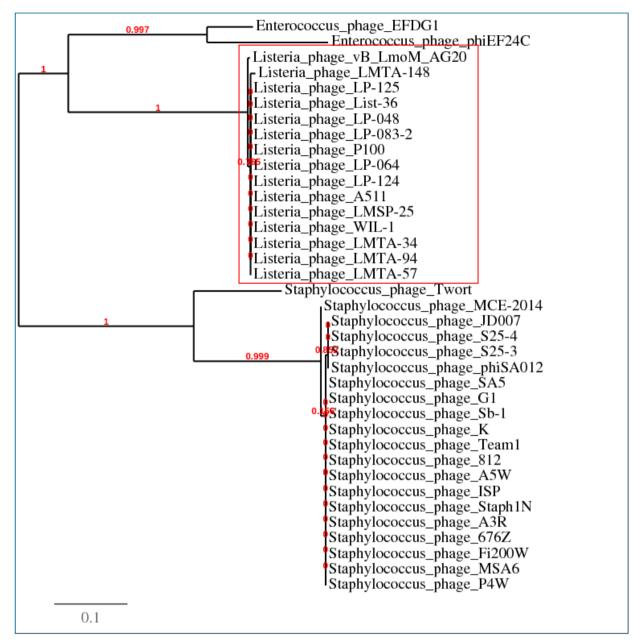


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).