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

MODULE 1: TITLE AUTHORS Atc

MODULE 1: 111LE, AUTHORS, etc						
Code assigned:	2013.022	a-dB		(to be co	mpleted by	ICTV
Short title: To create a new genus, <i>Tm4likevirus</i> , within the family <i>Siphoviridae</i>						
(e.g. 6 new species in the go Modules attached (modules 1 and 9 are require	·	1 ⊠ 6 □	2 × 7 □	3 ⊠ 8 □	4 ☐ 9 ⊠	5 🗌
Author(s) with e-mail ad	dress(es) of the pro	oposer:				
Evelien M Adriaenssens, <u>evelien.adriaenssens@gmail.com</u> Andrew M Kropinski, <u>akropins@uoguelph.ca</u> Rob Lavigne, <u>rob.lavigne@biw.kuleuven.be</u> John Nash, <u>john.nash@phac-aspc.gc.ca</u>						
List the ICTV study group(s) that have seen this proposal:						
A list of study groups and conttp://www.ictvonline.org/subin doubt, contact the appropic chair (fungal, invertebrate, powertebrate viruses)	ocommittees.asp . If riate subcommittee					
ICTV-EC or Study Group comments and response of the proposer:						
Date first submitted to IC	ΓV:		June	2013		
Date of this revision (if di	fferent 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	201	3.022aB	(assigned by ICTV officers)		
To crea	To create 9 new species within:				
Genus: Tm4likevirus (new) Subfamily: Family: Siphoviridae Order: Caudovirales		• If c: "(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 genus is specified, enter "unassigned" in the genus box. 		
And na	me the	e new species:		GenBank sequence accession number(s) of reference isolate:	
Mycoba	cterium	phage TM4		AF068845	
Mycobacterium phage angelica			HM152764		
Mycobacterium phage jaws			JN185608		
Mycobacterium phage crimd		HM152767			
Mycobacterium phage anaya		JF704106			
Mycoba	cterium	phage pixie		JF937104	
Mycoba	cterium	phage macncheese		JX042579	
Mycoba	cterium	phage larva		JN243855	
Mycobacterium phage fionn			JN831653		

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
 - o If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
 - o 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 EMBOSS Stretcher algorithm.

MODULE 3: NEW GENUS

creating a new genus

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

Code	201	3.022bB	(assigned by ICTV officers)	
To create a	new	genus within:		Fill in all that apply.
Subfan	nily:			If the higher taxon has yet to be created
Fan	nily:	Siphoviridae		(in a later module, below) write "(new)" after its proposed name.
Or	rder:	Caudovirales		 If no family is specified, enter "unassigned" in the family box

naming a new genus

Code	2013.022cB	(assigned by ICTV officers)
To name the new genus: Tm4likevirus		

Assigning the type species and other species to a new genus

Code	2013.022dB	(assigned by ICTV officers)		
To designate the following as the type species of the new genus				
Mycobacterium phage TM4		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:				
9				

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

This genus was originally recognized by the Mycobacteriophage group ($\underline{www.phagesdb.org}$) as belonging to cluster K. Phages belonging to this genus share a comparable genome size (52-62 kb), a comparable GC content (65-68 %), and a genome with defined physical end and a comparable 3' overhang. Members of this genus also have a comparable morphology, with an isometric head and a long, non-contractile tail (Figure 1).

A ClustalW analysis of the complete genomes of this genus with all other *Mycobacterium* phages belonging to the *Siphoviridae* reveals that this genus is a clearly separate group (Figures 2 and 3). We propose a shared protein content of at least 40% with the type phage, *Mycobacterium phage TM4*. We performed a CoreGenes 3.5 analysis [1–3] with the four phages of this genus (Table 1). The CoreGenes analysis was also performed against the type species of other proposed genera of siphoviruses infecting *Mycobacterium* and the shared protein content was consistently below 40% (data not shown).

Origin of the new genus name:

Mycobacterium phage TM4

Reasons to justify the choice of type species:

The genus *Tm4likevirus* is named after the first isolated and sequenced phage of this group, *Mycobacterium* phage Tm4 [4].

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 EMBOSS Stretcher algorithm. The isolates BarrelRoll and Adephagia share 95.8% and 95.3% DNA identity with *Mycobacterium phage Jaws*, respectively, and thus belong to the same species.

MODULE 9: APPENDIX: supporting material

additional material in support of this proposal

References:

- 1. Mahadevan P, King JF, Seto (2009) Data mining pathogen genomes using GeneOrder and CoreGenes and CGUG: gene order, synteny and in silico proteomes. Int J Comput Biol Drug Des 2: 100–114.
- 2. Mahadevan P, King JF, Seto D (2009) CGUG: in silico proteome and genome parsing tool for the determination of "core" and unique genes in the analysis of genomes up to ca. 1.9 Mb. BMC Res Notes 2: 168. doi:10.1186/1756-0500-2-168.
- 3. Zafar N, Mazumder R, Seto D (2002) CoreGenes: A computational tool for identifying and cataloging "core" genes in a set of small genomes. BMC Bioinformatics 3: 12. doi:10.1186/1471-2105-3-12.
- 4. Ford ME, Stenstrom C, Hendrix RW, Hatfull GF (1998) Mycobacteriophage TM4: genome structure and gene expression. Tuber lung Dis 79: 63–73. doi:10.1054/tuld.1998.0007.
- 5. Darling AE, Mau B, Perna NT (2010) progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5: e11147. doi:10.1371/journal.pone.0011147.

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.

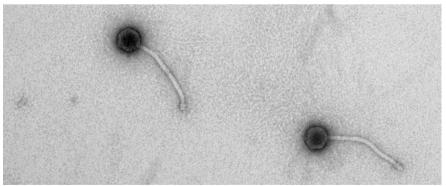


Figure 1: EM picture of isolates of phage Tm4, the type species of the genus *Tm4likevirus* (http://phagesdb.org/media/emPics/Tm4.tif).

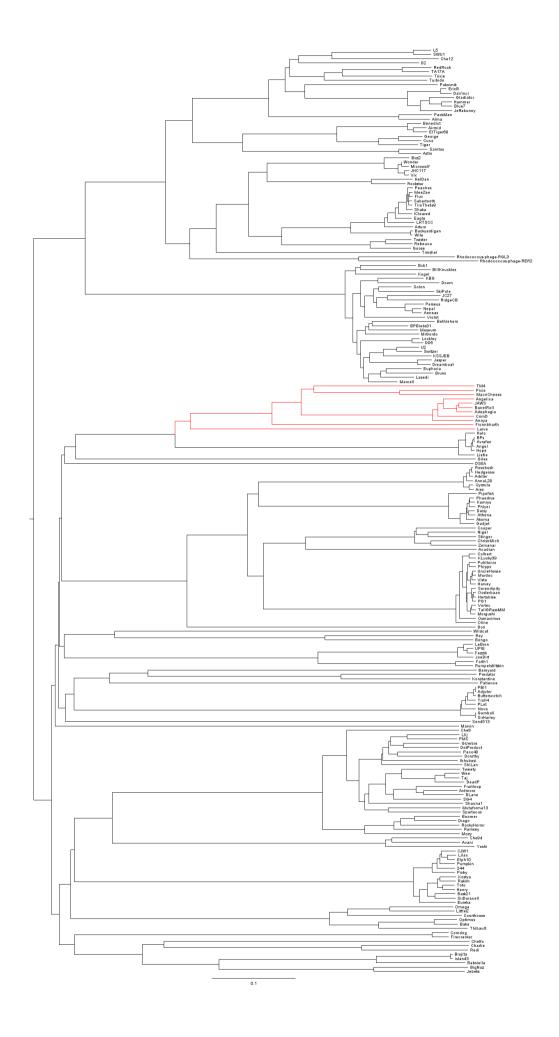


Figure 2: ClustalW phylogenetic tree (NJ) of complete genomes of all *Mycobacterium* siphoviruses in the NCBI database in November 2012. The proposed genus is colored in red. The scale bar represents 0.1 substitutions per site.

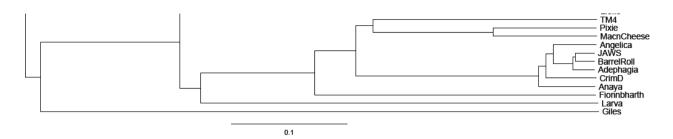


Figure 3: Clustal W phylogenetic tree of complete genomes of the isolates belonging to the genus Tm4likevirus, excerpt of Figure 2.

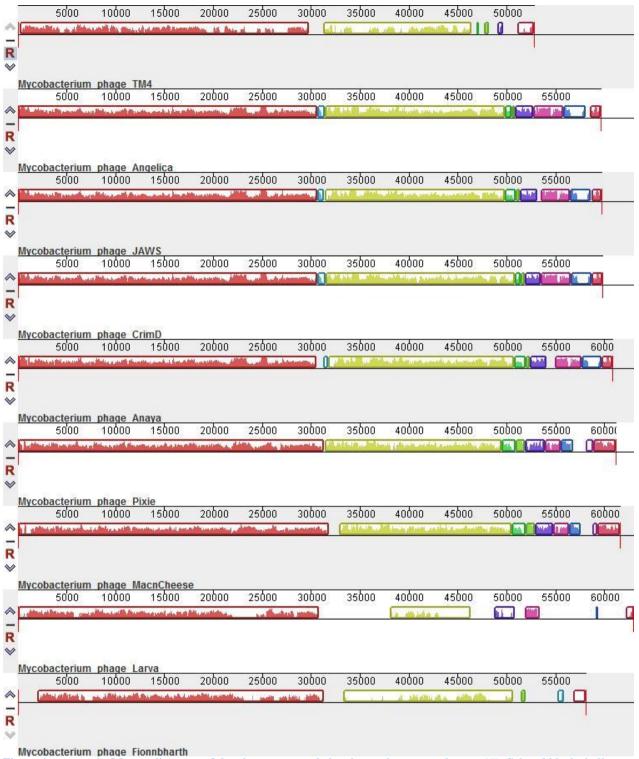


Figure 4: progressiveMauve alignment of the phage genomes belonging to the proposed genus [5]. Colored blocks indicate the regions of 1 to 1 best alignment with rearrangement breakpoints in a different random color. The degree of sequence similarity between regions is given by a similarity plot within the colored blocks with the height of the plot proportional to the average nucleotide identity.

Table 1: CoreGenes 3.5 (protein) and EMBOSS Stretcher (DNA) analysis of selected phages with the type phage of the genus, *Mycobacterium phage tm4*.

Phage Name	% proteins in common with TM4	% DNA similarity with TM4
Mycobacterium phage angelica	60.9	55.6

Mycobacterium phage jaws	64.1	56.1
Mycobacterium phage crimd	63.0	56.7
Mycobacterium phage anaya	62.0	55.9
Mycobacterium phage pixie	64.1	55.8
Mycobacterium phage	66.3	55.4
macncheese	00.5	
Mycobacterium phage larva	56.5	53.4
Mycobacterium phage fionn	46.7	55.7