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, etc.

MODULE 1: 111LE, AUTHURS, etc						
Code assigned:	ode assigned: 2013.021a-dB		(to be completed by ICTV officers)			
Short title: To create a new genus, Reylikevirus, within the family Siphoviridae						
(e.g. 6 new species in the ge Modules attached (modules 1 and 9 are require	,	1 ⊠ 6 □	2 ⊠ 7 □	3 ⊠ 8 □	4 ☐ 9 ⊠	5 🗌
Author(s) with e-mail address(es) of the proposer:						
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 conting://www.ictvonline.org/subin doubt, contact the approper 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 2013.021aB		(assigned by IC	TV offic	cers)	
To creat	To create 2 new species within:				
Subfar Far	enus: mily: mily: rder:	Reylikevirus (new) Siphoviridae Caudovirales	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 nan	ne the	e new species:			GenBank sequence accession number(s) of reference isolate:
Mycobact	terium	phage rey			JF937105
Mycobact	terium	phage bongo			JN699628

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.
 - 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. The proposed species show 62.0% identity at the DNA level as calculated 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	3.021bB	(assigned by I	CTV officers)
To create	a new	genus within:		Fill in all that apply.
Subfa	mily:			If the higher taxon has yet to be created
Fai	mily:	Siphoviridae		(in a later module, below) write "(new)" after its proposed name.
0	rder:	Caudovirales		 If no family is specified, enter "unassigned" in the family box

naming a new genus

Code	2013.021cB	(assigned by ICTV officers)
To name the new genus: Reylikevirus		

Assigning the type species and other species to a new genus

rissigning the type species and other species to a new genus					
Code	2013.021dB	(assigned by ICTV officers)			
To designate the following as the type species of the new genus					
Mycobacterium phage rey		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:					
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

This genus was originally recognized by the Mycobacteriophage group (www.phagesdb.org) as belonging to cluster M. Phages belonging to this genus share a comparable genome size (80 - 83 kb), a comparable GC content (~61.5%), the presence of a large number of tRNAs (19-21) and a genome with defined physical end. 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 rey*. We performed a CoreGenes 3.5 analysis [1–3] between Rey and Bongo (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 Rey

Reasons to justify the choice of type species:

The genus *Reylikevirus* is named after the first isolated and sequenced phage of this group, *Mycobacterium* phage Rey [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.

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. Pope WH, Ferreira CM, Jacobs-Sera D, Benjamin RC, Davis AJ, et al. (2011) Cluster K mycobacteriophages: insights into the evolutionary origins of mycobacteriophage TM4. PLoS One 6: e26750. doi:10.1371/journal.pone.0026750.
- 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.

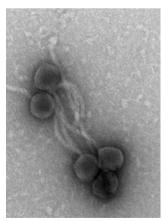


Figure 1: EM picture of isolates of phage Rey, the type species of the genus *Reylikevirus* (http://phagesdb.org/media/emPics/Rev.tif).

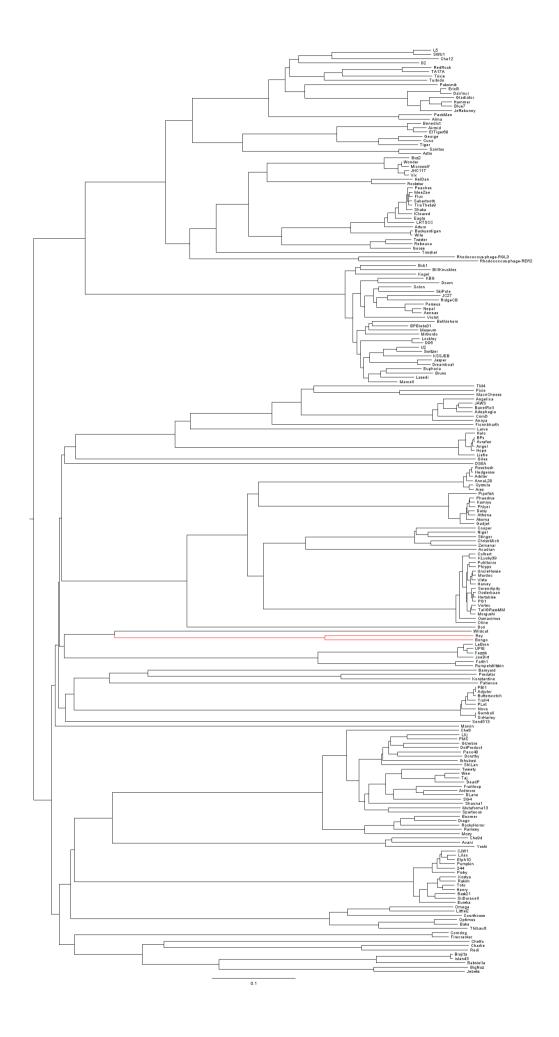


Figure 2: ClustalW phylogenetic tree (NJ) of complete genomes of all *Mycobacterium* siphoviruses in the NCBI database in November 2012. The proposed genus is colored in red. The scale bar represents 0.1 substitutions per site.



Figure 3: ClustalW phylogenetic tree of complete genomes of the isolates belonging to the genus *Reylikevirus*, excerpt of Figure 2. Phage Wildcat is an outlier, not belonging to this genus, sharing less than 40% proteins with phage Rey.

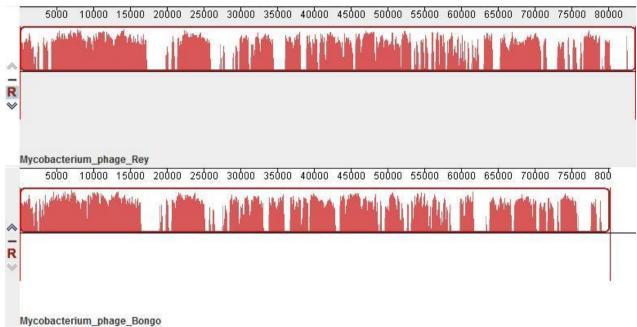


Figure 4: progressiveMauve alignment of the phage genomes belonging to the proposed genus [5]. Colored blocks indicate the regions of 1 to 1 best alignment with rearrangement breakpoints in a different random color. The degree of sequence similarity between regions is given by a similarity plot within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

Table 1: CoreGenes 3.5 and EMBOSS Stretcher analysis of selected phages with the type phage of the genus, *Mycobacterium phage rey*.

Phage Name	% proteins in common with Rey	% DNA similarity with Rey
Mycobacterium phage bongo	67.3	62.0