

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:	2015.004a-dB			(to be completed by ICTV officers)		
Short title: To create one (1) new genus, $Ff47virus$, including two (2) new species within the family $Siphoviridae$. (e.g. 6 new species in the genus $Zetavirus$) Modules attached (modules 1 and 10 are required) $1 \boxtimes 2 \boxtimes 3 \boxtimes 4 \square 5 \square$ $6 \square 7 \square 8 \square 9 \square 10 \boxtimes$						
Author(s):						
Andrew M. Kropinski – University of Guelph (Canada) Simone Basra – University of Guelph (Canada) Hany Anany – University of Guelph (Canada) Jens H. Kuhn – National Institute of Allergy and Infectious Diseases (U.S.A.) Evelien M. Adriaenssens – University of Pretoria (South Africa)						
Corresponding author with e	e-mail address:					
Andrew M. Kropinski Phage.	Canada@gmail.	<u>com</u>				
List the ICTV study group(s)	that have seer	this pro	posal:			
A list of study groups and contact http://www.ictvonline.org/subcommin in doubt, contact the appropriate schair (fungal, invertebrate, plant, pvertebrate viruses)	mmittees.asp . If e subcommittee Bacterial & Archaeal Virus Subcommittee					
ICTV Study Group comments (if any) and response of the proposer:						
Date first submitted to ICTV: Date of this revision (if different	rate first submitted to ICTV: May 2015 rate of this revision (if different to above):					
ICTV-EC comments and response of the proposer:						
Please note that we have chosen to refer to this new genus as <i>Ff47virus</i> rather than <i>Ff47likevirus</i> since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating " <i>like</i> " from phage genus names.						

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	<i>201</i>	5.004aB	(assigned by IC	(assigned by ICTV officers)			
To crea	To create 2 new species within:						
				Fill in all that apply.			
Genus: Ff47virus (new)				If the higher taxon has yet to be created (in a later module, below) wri "(new)" after its proposed name.			
Subfamily:							
Fa	Family: Siphoviridae			 If no genus is specified, enter 			
(Order: Caudovirales			"unassigned" in the genus box.			
		Representative isol (only 1 per species pl		GenBank sequence accession number(s)			
v		Mycobacterium virus FF47		JX901189			
		Mycobacterium viru Muddy	IS	KF024728			

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

Mycobacterium phages vB_MapS_FF47 (FF47) and Muddy were isolated in the same year in Guelph, Canada, and Durban, South Africa, respectively. These communities are approximately 13,890 kilometres apart, yet the two viruses show 94% identity at the nucleotide level. Muddy was isolated from the "underside of a partially decomposed aubergine" (2) while FF47 phage was isolated from fresh feces from a cow on a farm from Alberta that was suspected of having Johne's Disease (1). Their isolation hosts were Mycobacterium smegmatis and Mycobacterium avium subspecies paratuberculosis, respectively. Subsequent analysis reveals that FF47 can produce plaques on M. smegmatis. Electron micrographs of negatively stained FF47 (Fig. 1) show that it has an icosahedral head 55 nm in diameter and a long (168 nm) non-contractile tail ending in a small knob measuring 15 nm in length by 12 nm across, thus placing it in the family Siphoviridae.

The work of the Pittsburgh Bacteriophage Institute (PBI) further indicates that Muddy, and hence presumably FF47, has 11-bp 3'-cohesive termini with the following sequence CGTAGCGGCTT. Until the isolation of FF47 the PBI considered Muddy to be a singleton, i.e. it was unrelated to any of the more than 1400 phages that PBI have sequenced.

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.

The relatedness of these two phages was confirmed using CoreGenes (4) which the Bacterial and Archaeal Virus Subcommittee of ICTV has extensively used to compare the total proteomes (Table 1) of two viruses; progressiveMauve (Fig. 2); and, by phylogenetic analysis (5) of their major capsid proteins (Fig.3).

Please note that we have chosen to refer to this new genus as *Ff47virus* rather than *Ff47likevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" from phage genus names.

MODULE 3: NEW GENUS

creating a new genus

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

Code	201	5.004bB	(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
Fa	mily:	Siphoviridae		(in a later module, below) write "(new)" after its proposed name.
O	rder:	Caudovirales		 If no family is specified, enter "unassigned" in the family box

naming a new genus

Code	2015.004cB	(assigned by ICTV officers)			
To name the new genus: Ff47virus					

Assigning the type species and other species to a new genus

Assigning the type species and other species to a new genus						
Code	2015.004dB	(assigned by ICTV officers)				
To designa	To designate the following as the type species of the new genus					
Mycobacte	rium virus Ff47	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

See Module 2

Origin of the new genus name:

Named after the first phage of its type to be sequenced: Mycobacterium phage FF47 (1)

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 10: APPENDIX: supporting material

additional material in support of this proposal

References:

- 1. Basra S, Anany H, Brovko L, Kropinski AM, Griffiths MW. Isolation and characterization of a novel bacteriophage against *Mycobacterium avium* subspecies *paratuberculosis*. Arch Virol. 2014:159(10):2659-74.
- 2. http://phagesdb.org/phages/Muddy/
- 3. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One. 2010; 5(6):e11147.
- 4. Turner D, Reynolds D, Seto D, Mahadevan P. CoreGenes 3.5: a webserver for the determination of core genes from sets of viral and small bacterial genomes. BMC Res Notes. 2013; 6:140.
- 5. 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.

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 Ff47virus

Phage	Genome	Genome	No. CDS	DNA (%	Proteome
	length (bp)	(mol%G+C)		sequence	(%
				identity)*	homologous
					proteins)**
FF47	47,724	58.6	73	100	100
Muddy	48,228	58.8	71	94	90

^{*} Determined using BLASTN; ** Determined using CoreGenes (4)

Fig. A. Electron micrograph of *Mycobacterium* phage FF47 negatively stained with uranyl acetate.

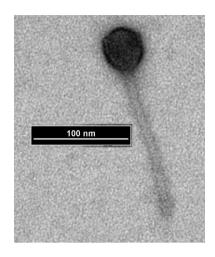


Fig. B. progressiveMauve alignment of the annotated genomes of Muddy (top) and FF47 (bottom) (3). Colored blocks indicate the regions of 1 to 1 best alignment with rearrangement breakpoints in a different random color. The degree of sequence similarity between regions is given by a similarity plot within the colored blocks with the height of the plot proportional to the average nucleotide identity (Aaron Darling, personal communication).

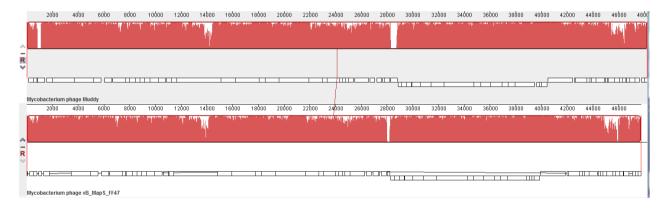


Fig. C. Phylogenetic analysis of major capsid proteins of ff47viruses and tm4viruses (TM4, Anaya, Angelica etc) constructed using "one click" at phylogeny.fr (5). "The "One Click mode" targets users that do not wish to deal with program and parameter selection. By default, the pipeline is already set 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."

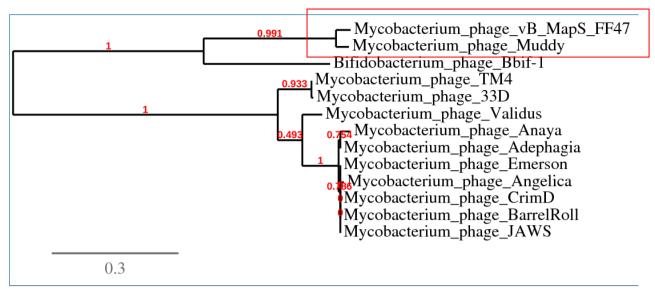


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).