

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

Short title: To create one (1) new genus, <i>Titanvirus</i> , including two (2) new species in the family <i>Siphoviridae</i> . (e.g. 6 new species in the genus <i>Zetavirus</i>) Modules attached (modules 1 and 10 are required) Author(s): Véronique A. Delesalle—Gettysburg College (USA) Jens H. Kuhn—NIH/NIAID/IRF-Frederick, Maryland (USA) Andrew M. Kropinski—University of Guelph (Canada) Evelien M. Adriaenssens—University of Pretoria (South Africa) David W. Bollivar—Illinois Wesleyan University (USA) Corresponding author with e-mail address: Andrew M. Kropinski Phage Canada@gmail.com 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 Study Group comments (if any) and response of the proposer: Date first submitted to ICTV: Date of this revision (if different to above): ICTV-EC comments and response of the proposer:	Code assigned:	2016.050a-dB			(to be completed by ICTV officers)			
Véronique A. Delesalle—Gettysburg College (USA) Jens H. Kuhn—NIH/NIAID/IRF-Frederick, Maryland (USA) Andrew M. Kropinski—University of Guelph (Canada) Evelien M. Adriaenssens—University of Pretoria (South Africa) David W. Bollivar—Illinois Wesleyan University (USA) Corresponding author with e-mail address: Andrew M. Kropinski Phage Canada@gmail.com 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 Study Group comments (if any) and response of the proposer: Date first submitted to ICTV: Date of this revision (if different to above):	family Siphoviridae. (e.g. 6 new species in the genus Zetavirus)							
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ICTV-EC comments and response of the proposer:								
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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 2016.050aB			(assigned by IC	(assigned by ICTV officers)			
To create 2 new species within:							
G	Senus:	Titanvirus (new	7)	Fill in all that apply. • If the higher taxon has yet to be			
Subfa	Subfamily:			created (in a later module, below) write "(new)" after its proposed name. • If no genus is specified, enter			
Fa	Family: Siphoviridae						
(Order: Caudovirales			"unassigned" in the genus box.			
_		Representative isola per species please)	ate: (only 1	GenBank sequence accession number(s)			
		Rhodobacter phage F Rhodobacter phage F		KR935213.1 KR935215.1			

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. The members of each of the proposed species differ from those of other species by more than 5% at the DNA level as confirmed with the BLASTN algorithm.

MODULE 3: NEW GENUS

creating a new genus

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

Code	201	6.050bB	(assigned by ICTV 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	2016.050cB	(assigned by ICTV officers)				
To name the	he new genus: Titanvirus					

Assigning the type species and other species to a new genus

Assigning the type species and other species to a new genus							
Code 2016.050dB	(assigned by ICTV officers)						
To designate the following as the type species of the new genus							
Rhodobacter virus RcTitan 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

The newly isolated bacteriophages for this proposed genus were isolated from water samples near a water treatment facility in Illinois, USA. The right end of their genomes includes a set of genes predicted to be involved in DNA replication, including a DNA polymerase, a replicative clamp, a DEAD box helicase, a replicative primase, and a virus replication repair endonuclease. These phages were isolated on *Rhodobacter capsulatus* as a host and are distinct from other bacteriophages that have been isolated using this host.

BLASTN, CoreGenes (Table 1) [2], progressiveMauve alignment (Fig. 2) [1], and phylogenetic analyses (Fig. 3) [3] all indicate that the proposed genus, *Titanvirus*, is cohesive and distinct from other genera. On average, the genomes of members of this genus are 44.3 kb in length (45.0 mol% G+C), and encode 61 proteins and 0 tRNAs.

Origin of the new genus name:

Based upon the name of the first sequenced member of this genus.

Reasons to justify the choice of type species:

The first sequenced member of this genus.

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. The members of each of the proposed species differ from those of other species by 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. 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. doi: 10.1186/1756-0500-6-140.
- 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.

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.

Fig. 1. Electron micrograph of negatively stained Rhodobacter phage RcTitan (provided by Dr. David W. Bollivar, Biology, Illinois Wesleyan University, Bloomington, IL, USA).

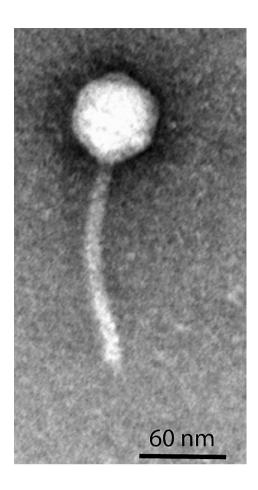


Table 1. Properties of the two phages belonging to the genus *Titanvirus*.

Rhodobacter	GenBank	Genome	Genome	No.	DNA (%	%
phage	Accession	length	(mol%	CDS	sequence	Homologous
	No.	(kb)	G+C)		identity)*	proteins **
RcTitan	KR935213	44.50	55.1	61	100	100
RcSpartan	KR935215	44.19	54.9	61	88	95.1

^{*} Determined using BLASTN; ** Determined using CoreGenes [2].

Fig. 2. progressiveMauve alignment [1] of the annotated genomes of members of the *Titanvirus* genus – from top to bottom: Rhodobacter phages RcTitan and RcSpartan. 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).

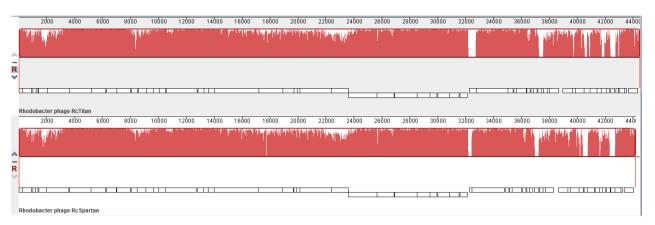


Fig. 3. Phylogenetic analysis of large subunit terminase proteins of titanviruses and homologous proteins from a variety of other phages constructed using "one click" at phylogeny.fr [3]. "The "One Click mode" targets users that do not wish to deal with program and parameter selection. By default, the 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."

