

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:	2016.041a-fB			(to be completed by ICTV officers)				
Short title: To create one (1) in Siphoviridae. (e.g. 6 new species in the genus 2 Modules attached (modules 1 and 10 are required)	<i>C</i> ,	·	cluding one 2 7	` ,	1	the family 5 □ 10 ⊠		
Author(s):								
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Andrew M. Kropinski Phage.C	anada@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 Bacterial and Archaeal Viruse Subcommittee								
ICTV Study Group comments (if any) and response of the proposer:								
Date first submitted to ICTV: Date of this revision (if different								
ICTV-EC comments and response of the proposer:								

MODULE 3: NEW GENUS

creating a new genus

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

Code	<i>201</i>	6.041aB	(assigned by ICTV officers)				
To create a	a new	genus within:		Fill in all that apply.			
Subfar	nily:			• If the higher taxon has yet to be created			
Far	nily:	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	2016.041bB	(assigned by ICTV officers)
To name the	he new genus: Rer2virus	

Assigning the type species and other species to a new genus

Assigning the type species and other spec	tes to a new genus					
Code 2016.041cB	(assigned by ICTV officers)					
To designate the following as the type s	pecies of the new genus					
Rhodococcus virus RER2	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						
· · · · · · · · · · · · · · · · · · ·	. Please enter here the TOTAL number of species					
(including the type species) that the genus will contain:						
1						

Reasons to justify the creation of a new genus:

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

Rhodococcus phage RER2 was isolated from a wastewater treatment plant in Australia, but recently three closely related phages were isolated in the US. BLASTN (Table 1) [2], and phylogenetic analyses (Fig. 2) [3] all indicate that the proposed genus, *Rer2virus*, is cohesive and distinct from other genera. Morphologically, the member phage's capsid diameter is ca. 54 nm (when measured on opposite sides) and the tail is 170 nm long [4]. Rhodococcus phage RER2 lyses *Rhodococcus erythropolis* and *R. globerulus* and also two nocardiae, *Nocardia otididiscaviarum* and *N. carnea*. The US strains were isolated on *Rhodococcus erythropolis*. On average, the genomes of the members of this genus are 46.6 kb in length (58.6 mol% G+C), and encode 66 proteins and 3 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 7: MOVE

Use this module whenever an existing taxon needs to be moved and re-assigned (e.g. when a species is moved from one genus to another).

moving an existing taxon

Code	de 2016.041dB			(assigned by ICTV officers)			
To move	the following taxo	ı (or taxa) fro	om their p	oresent	t position:		
Rhodococ	ccus virus RER2, R	hodococcus v	irus RGL3	3			
The prese	ent taxonomic posi	tion of these t	taxon/taxa	a:			
	Genus:	L5virus					
	Subfamily:				Fill in all that apply.		
	Family:	Siphoviridae	2				
	Order:	Caudovirale	S				
Code	2016.041	eB		(assigr	ned by ICTV officers)		
To re-ass	ign the taxon (or ta	axa) listed in	Part (a) a	s follo	ws:		
Rhodoc	occus virus RER2				Fill in all that apply.		
	Genus:	Rer2virus (new)			If the higher taxon has yet to be and the higher taxon has yet to be and the higher taxon has yet to be and the higher taxon has yet to be		
	Subfamily:				created write "(new)" after itsproposed name and complete		
	Family:	Siphoviridae			relevant module to create it.		
	Order:	Caudovirales			If no genus is specified, enter "unassigned" in the genus box		
Code	2016.041fE	3	(assigned	by ICT	V officers)		
To re-as	sign the taxon (or	taxa) listed in	Part (a)	as follo	ows:		
Rhodoc	occus virus RGL3				Fill in all that apply.		
	Genus:	unassigned			 If the higher taxon has yet to be created write "(new)" after its 		
Subfamily:					proposed name and complete		
Family:		Siphoviridae			relevant module to create it.		
	Order:	Caudovirale	S		If no genus is specified, enter "unassigned" 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.
 - 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.
- Provide accession numbers for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 11

Rhodococcus phage RER2 was originally included in the L5virus genus on the basis of share proteins. The Actinobacteriophage Database considers them as separate, being members of the CA and A2 subclusters, respectively. At a molecular level Mycobacterium phage L5 has a 52.2 kb (62.3 mol%G+C) genome which encodes 85 proteins and 3 tRNAs. Rhodococcus phage RER2 has a 46.6 kb (58.6%G+C) genome encoding for 66 proteins and 3 tRNAs. Phlyogenetic analyses also reveal that these phages are significantly different, and should be in separate genera.

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. 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, 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.
- 4. Petrovski S, Seviour RJ, Tillett D. Characterization and whole genome sequences of the *Rhodococcus* bacteriophages RGL3 and RER2. Arch Virol. 2013; 158(3):601-9.

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 (2% uranyl acetate) Rhodococcus phage RER2 (provided by S.Petrovski). Scale bar = 50 nm.

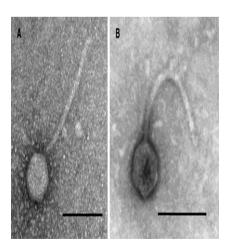


Table 1. Properties of the type phage belonging to the genus *Rer2virus*.

Rhdodococcus	RefSeq No.	GenBank	Genome	Genome	No.	No.	DNA (%	%
phage		Accession	length	(mol%	CDS	tRNAs	sequence	Homologous
		No.	(kb)	G+C)			identity)*	proteins **
RER2	NC_016653.1	JN116827.1	46.59	58.6	66	3	100	100
	-							

^{*} Determined using BLASTN; ** Determined using CoreGenes [2]; related phages which should be considered strains of Rhodococcus phage RER2 are: Rhodococcus phages TWAMP (KT959213), Rhodalysa (KT375356), and ComicsSans (KT372002.1)

Fig. 2. Phylogenetic analysis of (A) large subunit terminase proteins and major capsid proteins (B) of Rhodococcus phage RER2-like viruses 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."

A. Terminase, large subunit

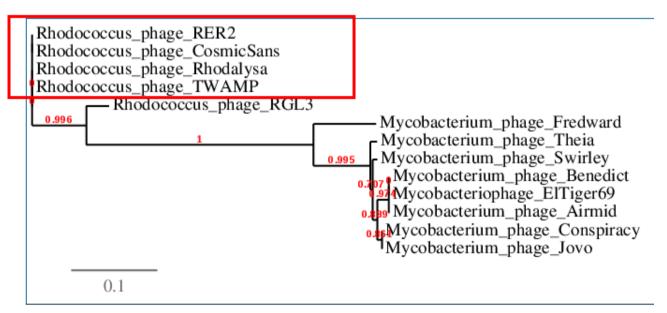


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).

B. Major capsid proteins

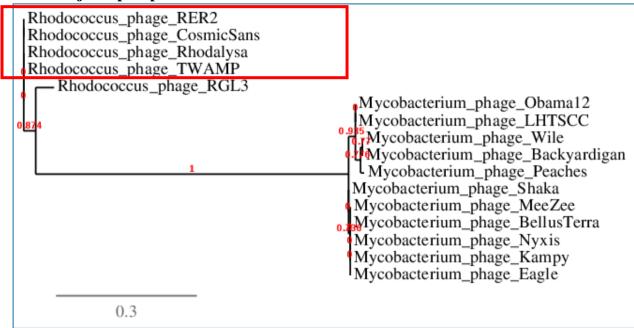


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).