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:	***2016.059a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Pepy6virus*, including two (2) new species in the family *Siphoviridae*.
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 10 are required)	**1** ☒	**2** ☒	**3** ☒	**4** ☐	**5** ☐
	6 ☐	**7** ☐	**8** ☐	**9** ☐	**10** ☒

Author(s):

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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 Bacterial and Archaeal Viruses Subcommittee

ICTV Study Group comments (if any) and response of the proposer:

Date first submitted to ICTV: June 2016
Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

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	***2016.059aB***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Pepy6virus* (new)**
Subfamily:	
Family:	***Siphoviridae***
Order:	***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.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Rhodococcus virus Pepy6 *Rhodococcus virus Poco6*	Rhodococcus phage ReqiPepy6 Rhodococcus phage ReqiPoco6	GU580941 GU580942

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**.
  - 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	***2016.059bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Siphoviridae***
Order:	***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 family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2016.059cB***	(assigned by ICTV officers)

To name the new genus: *Pepy6virus*

Assigning the type species and other species to a new genus

Code	***2016.059dB***	(assigned by ICTV officers)

To designate the following as the type species of the new genus

Rhodococcus virus Pepy6	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

Rhodococcus equi is an important equine pathogen causing severe pneumonia in foals. Rhodococcus phages ReqiPepy6 and ReqiPoco6 were enriched from soils around equine breeding farms [5]. Electron microscopy (Fig. 1) showed that ReqiPepy6 has an isometric head 80 nm in diameter and a long (285 nm) noncontractile tail. By comparison, ReqiPoco6's dimensions are – head: 82 nm; tail: 281 nm. Both phages have 3'-cohesive termini with the sequence CGCCGCCCT, which is remarkably similar to the sequence of the ends of members of the *Gordonvirus* and *Mudcatvirus* (CGCCGGCCT). The absence of integrases or recombinases in the genome sequence suggests that these are lytic phages. Unlike most phage proteomes which feature transmembrane domains (TMDs) in 8.5% of their proteins, 29% of the ReqiPepy6 and 24% of the ReqiPoco6 proteins possess TMDs [5].

NCBI BLASTN, CoreGenes (Table 1) [2], progressiveMauve [1] (Fig. 2), and phylogenetic analyses (Fig. 3) [3], all indicate that the proposed genus, *Pepy6virus*, is cohesive and distinct from other genera. On average, the genomes of this genus are 77.4 kb in length (53.3 mol% G+C), and encode 107 proteins and 1 tRNA.

Origin of the new genus name:

Based upon the name of *Rhodococcus* phage ReqiPepy6.

Reasons to justify the choice of type species:

Named after the 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. 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.

4. Agren J, Sundström A, Håfström T, Segerman B. Gegenees: fragmented alignment of multiple genomes for determining phylogenomic distances and genetic signatures unique for specified target groups. PLoS One. 2012;7(6):e39107.

5. Summer EJ, Liu M, Gill JJ, Grant M, Chan-Cortes TN, Ferguson L, Janes C, Lange K, Bertoli M, Moore C, Orchard RC, Cohen ND, Young R. Genomic and functional analyses of *Rhodococcus equi* phages ReqiPepy6, ReqiPoco6, ReqiPine5, and ReqiDocB7. Appl Environ Microbiol. 2011;77(2):669-83.

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 phages belonging to the genus *Pepy6virus*.

Rhodococcus phage	RefSeq No.	GenBank Accession No.	Genome length (kb)	Genome (mol% G+C)	No. CDS	No. tRNAs	% DNA Sequence identity *	% Homologous proteins **
ReqiPepy6	NC_023735	GU580941	76.8	53.4	107	1	100	100
ReqiPoco6	NC_023694	GU580942	78.1	53.3	107	1	74.0	87.8

* Determined using BLASTN; ** Determined using CoreGenes [2];

Fig. 1. Electron micrograph of negatively stained Rhodococcus phage ReqiPepy6 (LEFT) and Rhodococcus phage ReqiPoco6 (RIGHT) (Applied and Environmental Microbiology, ASM Press, with permission [5]).

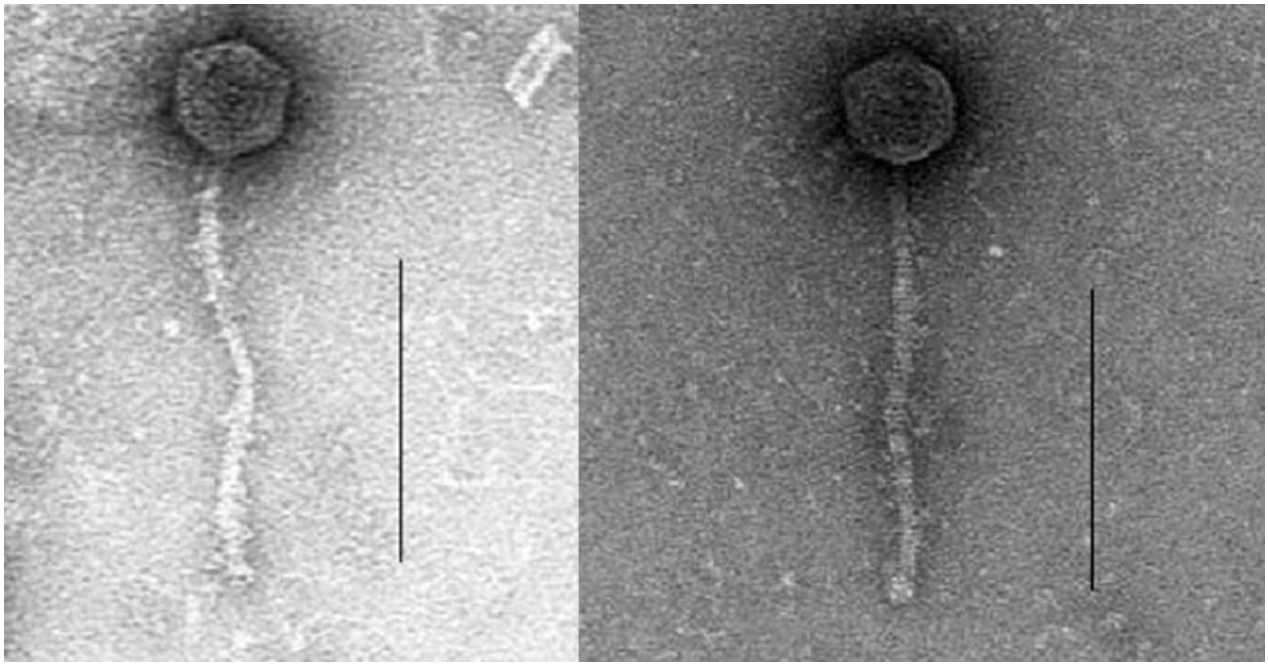

Fig. 2. progressiveMauve alignment [1] of the genomes of members of the *Pepy6virus* genus – from top to bottom: Rhodococcus phages ReqiPepy6 and ReqiPoco6. Colored blocks indicate the regions of 1 to 1 best alignment with rearrangement breakpoints in a different random color. The degree of sequence 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).

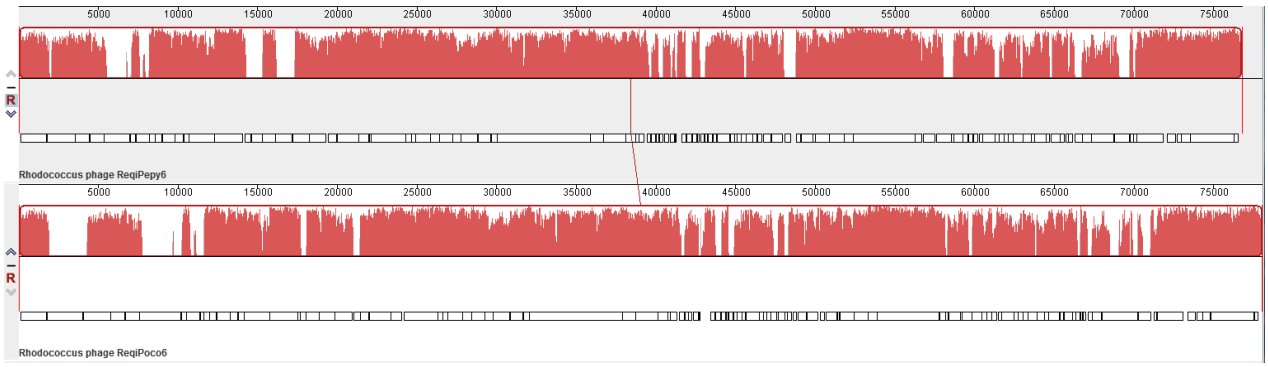

Fig. 3. Phylogenetic analysis of the (A) major capsid proteins and (B) tail tube proteins of Pepy6-like viruses and homologous proteins from a variety of other phages constructed using “one click” at phylogeny.fr [3]. "The "One Click mode" targets users that do not wish to deal with program and parameter 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. Major capsid protein

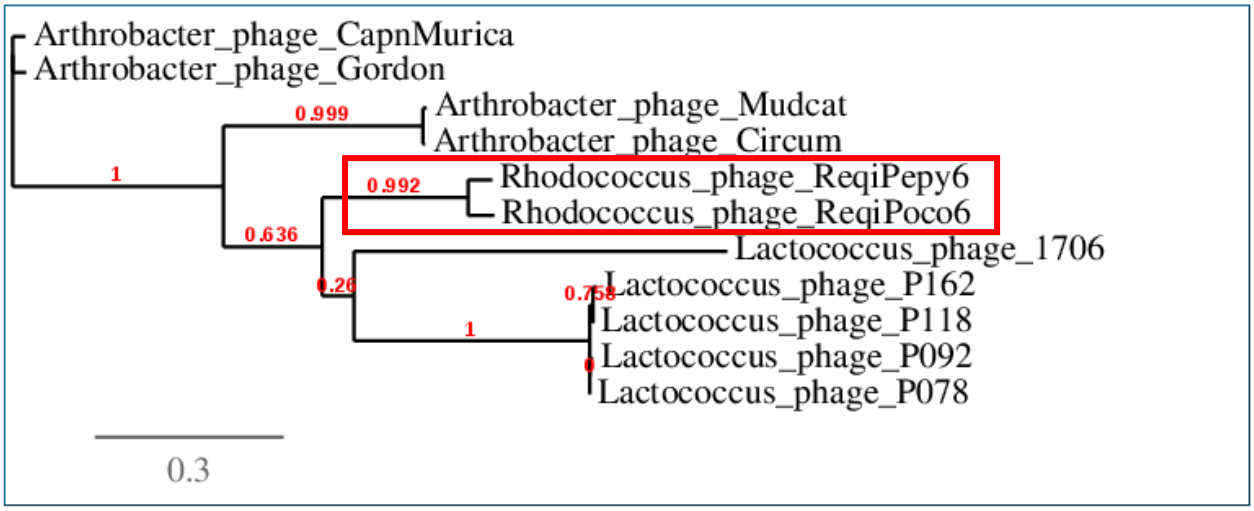

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

B. Tail tube protein

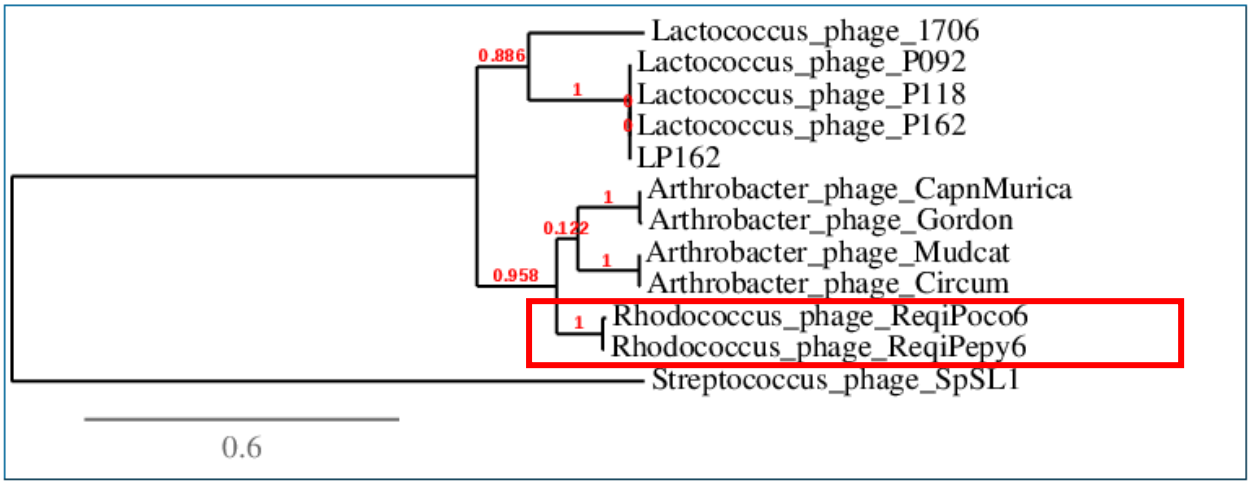

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*