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:	***2015.045a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Rheph4virus*, including one (1) species within the family *Myoviridae*.
(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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Evelien M. Adriaenssens – University of Pretoria (South Africa)

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:

Please note that we have chosen to refer to this new genus as *Rheph4virus* rather than *Rheph4likevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" and "*Phi*" from phage genus names.

Date first submitted to ICTV: June 2105
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	***2015.045aB***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Rheph4virus (new)***
Subfamily:	
Family:	***Myoviridae***
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)**
Rhizobium virus RHEph4	Rhizobium phage RHEph04	JX483876

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

Phages RHEph04, RHEph05, and RHEph06 are all lytic viruses which infect *Rhizobium etli*, and "were obtained from rhizosphere soil of bean plants from agricultural lands in Mexico"(2). "They had a polyhedral head of about 60 nm in diameter and a large retractile tail of about 92 nm." (2)

The phages of this genus possess genome of approx. 53 kb (56.4 mol%G+C), and encode 81 proteins and 0 tRNAs (Table 1).

BLASTN analysis reveals that they are very similar (i.e. share >94% nucleotide identity), with no close relatives. Phylogenetic analysis of the large subunit terminase protein (Fig. 3A) and major capsid protein (Fig. 3B) also indicate that these phages are not closely related to other viruses.

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.

Please note that we have chosen to refer to this new genus as *Rheph4virus rather than Rheph4likevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" and "*Phi*" from phage genus names.

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2015.045bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Myoviridae***
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	***2015.045cB***	(assigned by ICTV officers)

To name the new genus: *Rheph4virus*

Assigning the type species and other species to a new genus

Code	***2015.045dB***	(assigned by ICTV officers)

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

Rhizobium virus RHEph4

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:**

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

BLASTN and phylogenetic analyses (Fig. 3; 3) all indicate that the proposed genus, *Rheph4virus*, is cohesive and distinct from the other genera of viruses.

Origin of the new genus name:

Named after the first phage of its type to be deposited in GenBank: *Rhizobium* phage RHEph04

Reasons to justify the choice of type species:

First phage of its type to be deposited in GenBank

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. 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.
2. Santamaría RI, Bustos P, Sepúlveda-Robles O, Lozano L, Rodríguez C, Fernández JL, Juárez S, Kameyama L, Guarneros G, Dávila G, González V. Narrow-host-range bacteriophages that infect *Rhizobium etli* associate with distinct genomic types. Appl Environ Microbiol. 2014; 80(2):446-54.

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 sole phage belonging to the genus *Rheph4virus*.

Phage	GenBank accession No.	Genome length (kb)	Genome (mol%G+C)	No. CDS	No. tRNAs
RHEph04	JX483876	53.02	56.4	81	0

Table 2. Similar phages to RHEph4

Phage	**GenBank Accession No.**
RHEph05	JX483877
RHEph06	JX483878

Fig. 1. Electron micrograph of *Rhizobium* phage RHEph04 negatively stained with 2% uranyl acetate (2, with permission)

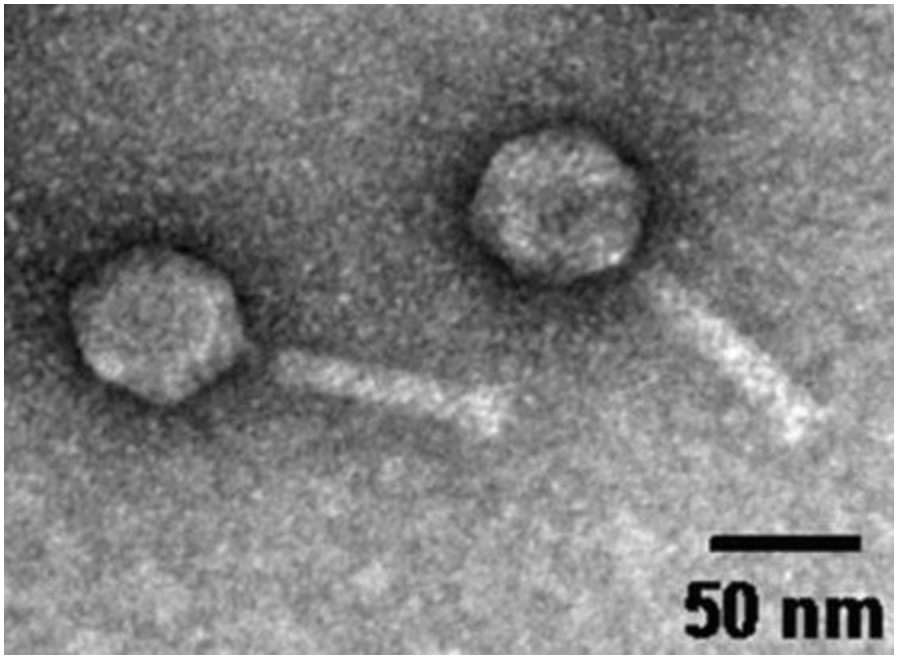

Fig. 2. Phylogenetic analysis of (A).the large subunit terminase; and, (B) major capsid protein of RHEph4-like viruses and some related phages constructed using "one click" at phylogeny.fr (1). "The "One Click mode" targets users that do not wish to deal with program and parameter selection. By default, 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

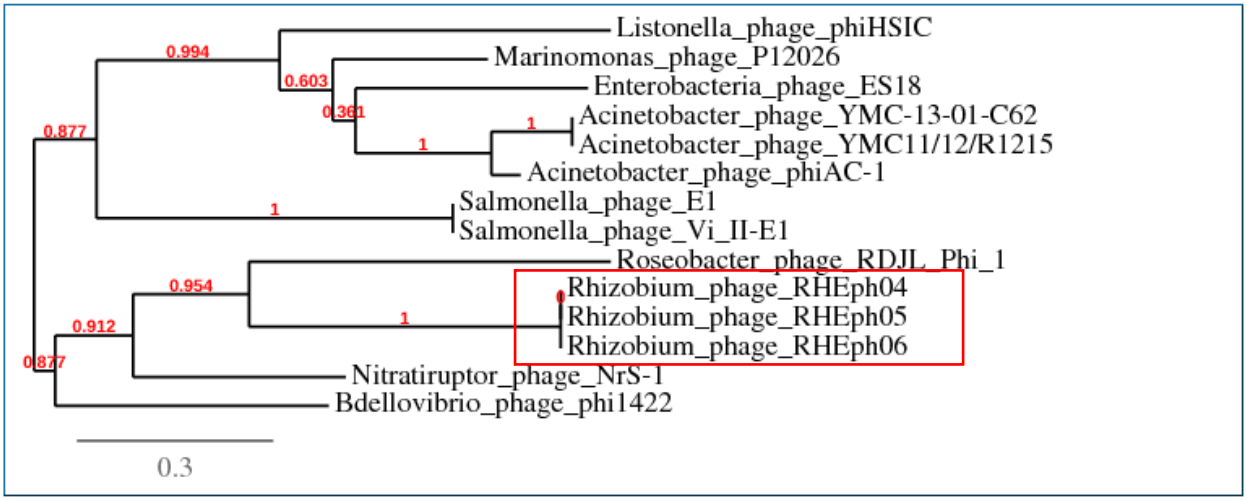

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

B. Major capsid protein

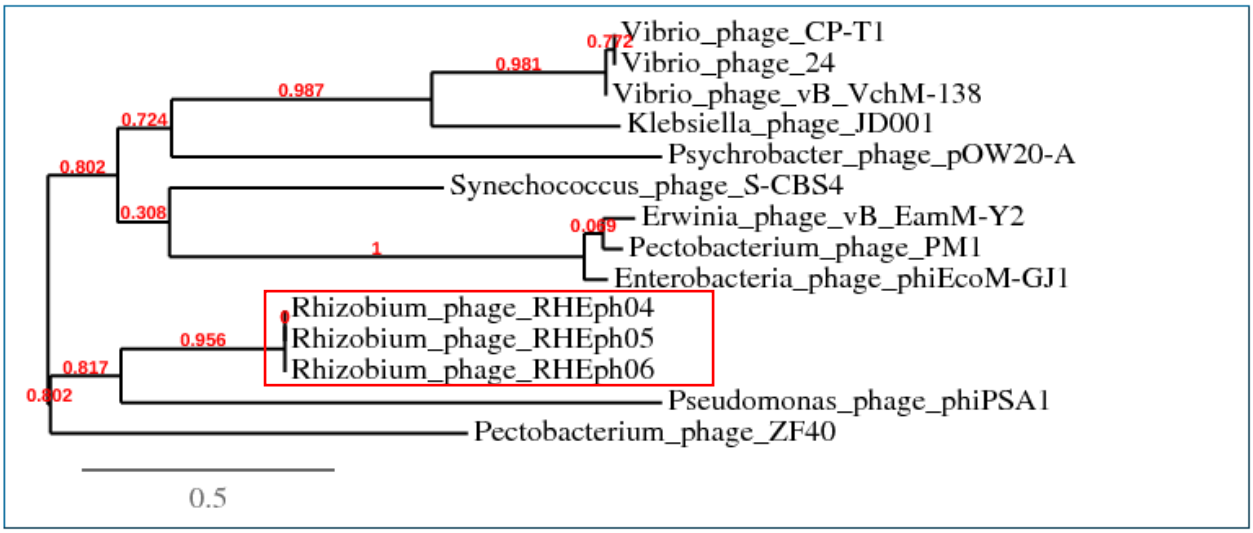

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*