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:	***2013.015a-dB***	(to be completed by ICTV officers)

Short title: To create a new genus, *D3likevirus*, within the family *Siphoviridae*
(e.g. 6 new species in the genus *Zetavirus*)

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

Author(s) with e-mail address(es) of the proposer:

Evelien Adrieanssens Evelien.Adriaenssens@gmail.com
Andrew M. Kropinski kropinsk@queensu.ca
Rob Lavigne rob.lavigne@biw.kuleuven.be
Rob Edwards redwards@mail.sdsu.edu

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-EC or Study Group comments and response of the proposer:

Date first submitted to ICTV:	June 2013
Date of this revision (if different to above):	July 2014

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	***2013.015aB***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***D3likevirus (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.

	GenBank sequence accession number(s) of reference isolate:
Pseudomonas phage D3 *Pseudomonas phage PMG1*	AF165214 HQ711985

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

BLASTN analyses reveal that these two *Pseudomonas* phages are related and distinct from any other phage. We have chosen 95% DNA sequence identity as the criterion for demarcation of species.

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2013.015bB***	(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	***2013.015cB***	(assigned by ICTV officers)

To name the new genus: *D3likevirus*

Assigning the type species and other species to a new genus

Code	***2013.015dB***	(assigned by ICTV officers)

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

Pseudomonas phage D3

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

Pseudomonas aeruginosa temperate phage D3 has an isometric head 55 nm in diameter and a long flexible tail (113 nm x 7 nm) possessing six tail fibers with terminal knobs (2). It possesses three unusual features: (a) 3'-cohesive termini (4), (b) the major capsid subunit proteins are cross-linked creating oligomers (3), and (c) the ability to seroconvert lysogens (1). The second phage of this genus, the virulent *Pseudomonas* phage phiPMG1, does not share this ability, but was grouped in the genus because of a high DNA identity and shared protein content of 73.7% (5). General characteristics of this genus are listed in Table 1.
We propose 40% shared proteins with the type species for new phages to belong to this genus.

Origin of the new genus name:

Pseudomonas aeruginosa phage D3

Reasons to justify the choice of type species:

The original isolate of this group.

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 EMBOSS Stretcher algorithm.

MODULE 9: **APPENDIX**: supporting material

References:

1: Newton GJ, Daniels C, Burrows LL, Kropinski AM, Clarke AJ, Lam JS. Three-component-mediated serotype conversion in *Pseudomonas aeruginosa* by bacteriophage D3. Mol Microbiol. 2001; 39(5):1237-47. PubMed PMID: 11251840.

2: Kropinski AM. Sequence of the genome of the temperate, serotype-converting, *Pseudomonas aeruginosa* bacteriophage D3. J Bacteriol. 2000;182(21):6066-74.PubMed PMID: 11029426; PubMed Central PMCID: PMC94740.

3: Gilakjan ZA, Kropinski AM. Cloning and analysis of the capsid morphogenesis genes of *Pseudomonas aeruginosa* bacteriophage D3: another example of protein chain mail? J Bacteriol. 1999;181(23):7221-7. PubMed PMID: 10572124; PubMed Central PMCID: PMC103683.

4: Sharp R, Jansons IS, Gertman E, Kropinski AM. Genetic and sequence analysis of the cos region of the temperate *Pseudomonas aeruginosa* bacteriophage, D3. Gene. 1996;177(1-2):47-53. PubMed PMID: 8921844.

5: Krylov, S.V., Kropinski, A.M., Pleteneva, E.A., Shaburova, O.V., Burkal'tsevaa, M.V., Mirosnnikov, K.A., and Krylov, V.N. 2012. Properties of the new D3-like *Pseudomonas aeruginosa* bacteriophage phiPMG1: Genome structure and prospects for the use in phage therapy. Russian Journal of Genetics, 48 (9): 902–911.

6: Darling AE, Mau B, Perna NT (2010) progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5: e11147

7: Rohwer F, Edwards RE (2002) The Phage Proteomic Tree: a genome-based taxonomy for phage. Journal of Bacteriology 184: 4529-4535

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.

Figure 1. Electron micrograph of *Pseudomonas* phage D3 negatively stained with 1% (wt/vol) phosphotungstic acid (PTA) solution, pH 7.2 (3).

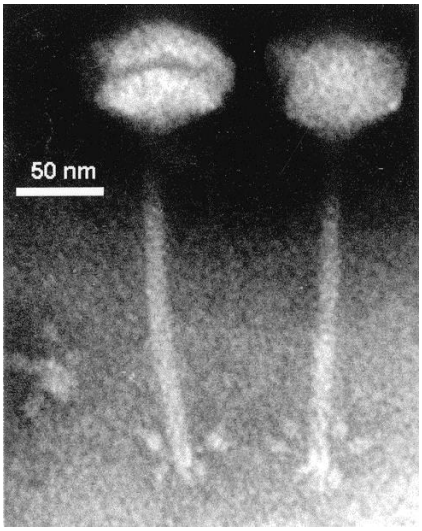

Table 1. Phage genomes belonging to this proposed genus

Phage	GenBank Accession No.	Genome size (bp)	Mol%G+C tRNA	Termini	tRNA	% DNA sequence identity (a)	% Shared proteins (b)
Pseudomonas phage D3	AF165214	56425	57.80	9-bp 3'-overhangs 5'-GCGCCCCCA-3'	4	100%	100%
Pseudomonas phage PMG1	HQ711985	54024	57.47	unknown	1	81.8%	73.7%

(a) Calculated using EMBOSS Stretcher (relative to D3)
(b) Calculated using CoreGenes 2.0

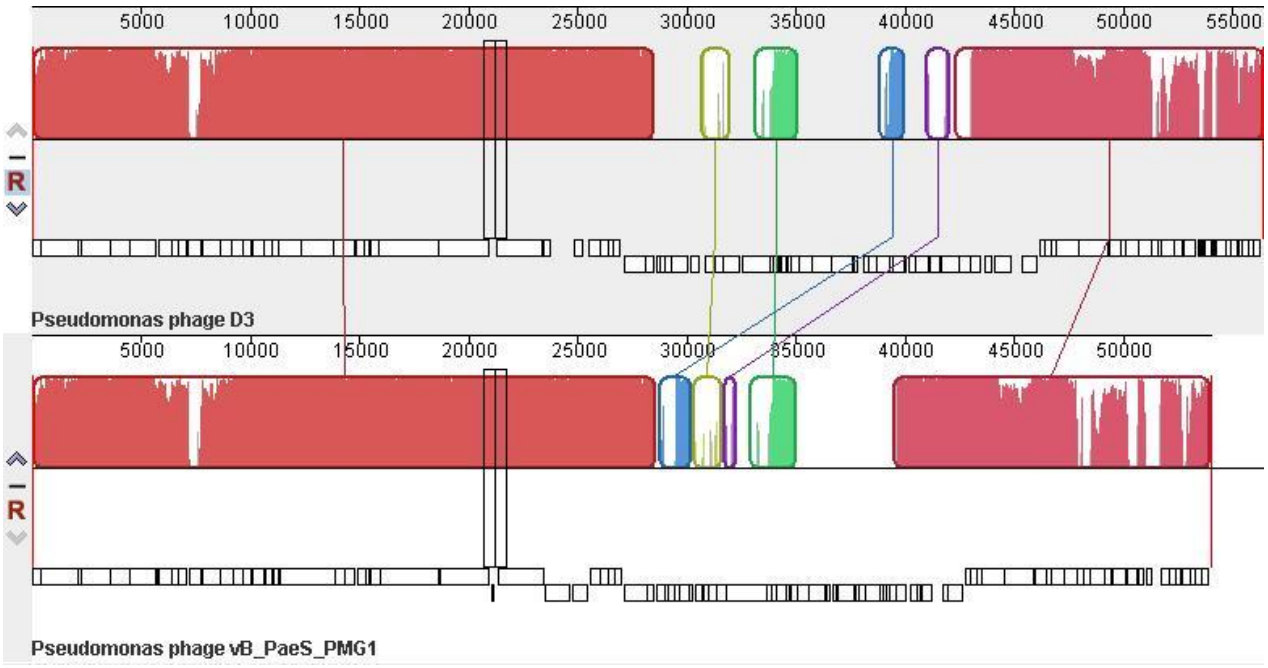

Figure 2. progressiveMauve alignment of the phage genomes (full genome represented by its annotated ORFs in white blocks) (6). Colored blocks indicate the regions of 1 to 1 best alignment with rearrangement breakpoints in a different random color. The degree of sequence similarity between regions is given by a similarity plot within the colored blocks with the height of the plot proportional to the average nucleotide identity.

Figure 3: Phage Proteomic Tree (Rohwer & Edwards, 2002) of all the *Siphoviridae* phages in the NCBI database November 2012. Briefly, all predicted proteins sequences are compared with all others and a length-corrected protein distance matrix was

calculated based on CLUSTALW alignment of sequences with a BLASTP e value < 0.001, with missing protein penalties of 10 and gap extension penalties of 0.20 (7). The tree was generated using FITCH. The proposed genus is colored in red. The scale bar represents protein distances of 2.0.

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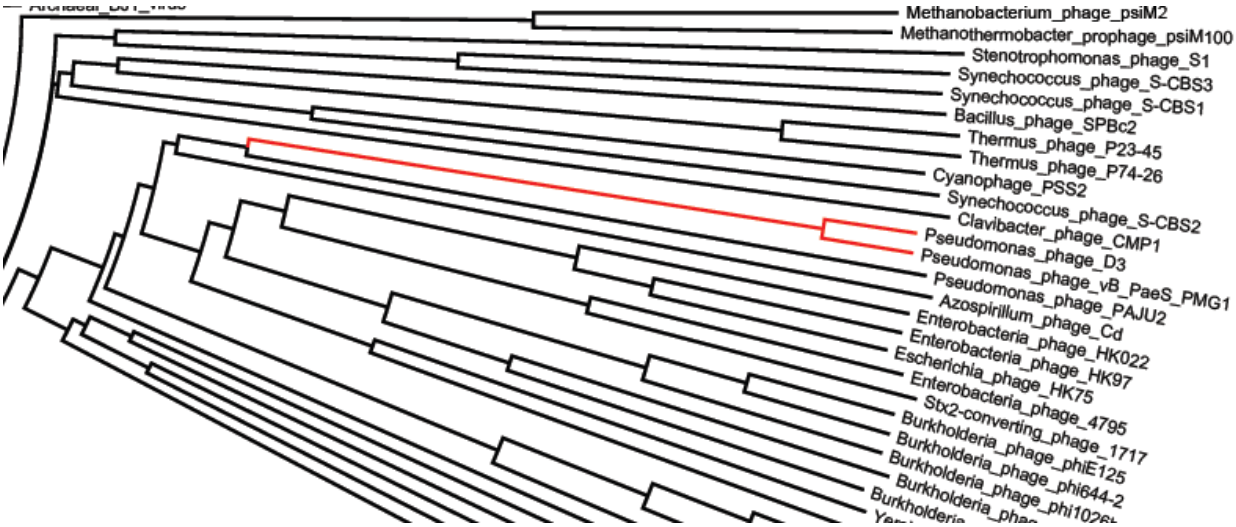

Figure 4: Fragment of the phylogenetic tree of Figure 3, zoomed in on the proposed genus.