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:	***2015.008a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Lit1virus*, including three (3) new species within the family *Podoviridae*.
(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):

Johannes Wittmann – Leibniz Institute DSMZ (Germany)
Andrew M. Kropinski – University of Guelph (Canada)
Hans-Wolfgang Ackermann – Université Laval (Canada)
Rob Lavigne – KU Leuven (Belgium)
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)	Bacterial & Archaeal Virus Subcommittee

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

Please note that we have chosen to refer to the new genera as *Lit1virus* rather than *Litunalikevirus*, since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" from phage genus names. Currently in GenBank there are over 30 fully sequenced N4-like phage genomes, which while displaying similar genome lengths; and, the presence of a high molecular weight virion-associated RNA polymerase; are poorly related at the phylogenetic (Fig. 1), genomic and proteomic levels. At this time we do not want to propose higher orders, until a firm molecular basis can be proposed to define these.

Date first submitted to ICTV: May 2015
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	***2015.008aB***	(assigned by ICTV officers)

To create 3 new species within:

Genus:	***Lit1virus (new)***
Subfamily:	
Family:	***Podoviridae***
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)**
Pseudomonas virus LIT1 *Pseudomonas virus PA26* *Pseudomonas virus Ab09*	Pseudomonas phage LIT1 Pseudomonas phage PA26 Pseudomonas phage vB_PaeP_C2-10_Ab09	FN422399 JX194238 HG962375

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. 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 *Lit1virus* rather than *Litunalikevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "like" from phage genus names.

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2015.008bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Podoviridae***
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	***2015.008cB***	(assigned by ICTV officers)

To name the new genus: *Lit1virus*

Assigning the type species and other species to a new genus

Code	***2015.008dB***	(assigned by ICTV officers)

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

Pseudomonas virus LIT1

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:**

3

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, CoreGenes and phylogenetic (Fig. 1) analyses all indicate that the proposed genus, *Lit1virus*, is cohesive and distinct from the other genera in the N4-superfamily of viruses. The next closest related phage is *Pseudomonas* phage LUZ7 (FN422398) which shares 41% DNA sequence identity; while *Escherichia* phage N4 which shares only <1% DNA sequence identity.

The phages of this genus possess genome of approx. 72.3 kb (54.9 mol%G+C), and encode 87 proteins and 0 tRNAs. The genome of LIT1 possesses 655 bp direct repeats. While the GenBank files on phages phi176, RWG and Pa2 (*Pseudomonas virus Ab09*) do not indicate direct repeats they are present: 663, 642 and 663 bp, respectively. The viruses in this genus share >91% DNA sequence identity and >92% homologous proteins (Table 1). Figure 2 derived from progressiveMauve analysis reveals the overall sequence similarity between the members of this genus.

These are *Pseudomonas*-specific phages. LIT1 possesses "a 70 nm icosahedral capsid attached to a short tail at the characteristic portal vertex. A narrow 30 nm long tail structure is attached to the LIT1 capsid but cannot be distinguished in LUZ7." (3). None of the other members has been characterized by electron microscopy.

Origin of the new genus name:

Pseudomonas phage LIT1

Reasons to justify the choice of type species:

The first virus of its type that was 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. Each of the proposed species differs from the others with more than 5% at the DNA level as confirmed with the BLASTN algorithm.

Therefore, *Pseudomonas* phages RWG (KM411958), phi176 (KM411960), Pa2 (KM411959) and YH6 (KM974184) are considered strains of *Pseudomonas virus Ab09*.

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.

3. Ceyssens PJ, Brabban A, Rogge L, Lewis MS, Pickard D, Goulding D, Dougan G, Noben JP, Kropinski A, Kutter E, Lavigne R. Molecular and physiological analysis of three *Pseudomonas aeruginosa* phages belonging to the "N4-like viruses". Virology. 2010;405(1):26-30.

4. Kim MS, Cha KE, Myung H. Complete genome of *Pseudomonas aeruginosa* phage PA26. J Virol. 2012;86(18):10244.

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. The virion RNA polymerases of the N4-like phages were aligned using ClustalW2 (http://www.ebi.ac.uk/Tools/phylogeny/clustalw2_phylogeny/) and the dnd file was rendered using FigTree (http://tree.bio.ed.ac.uk/software/figtree/). The branching position of N4 is illustrated in red. The RNA polymerase of *Shigella* phage pSb-1 is not included since it contains numerous frameshift errors.

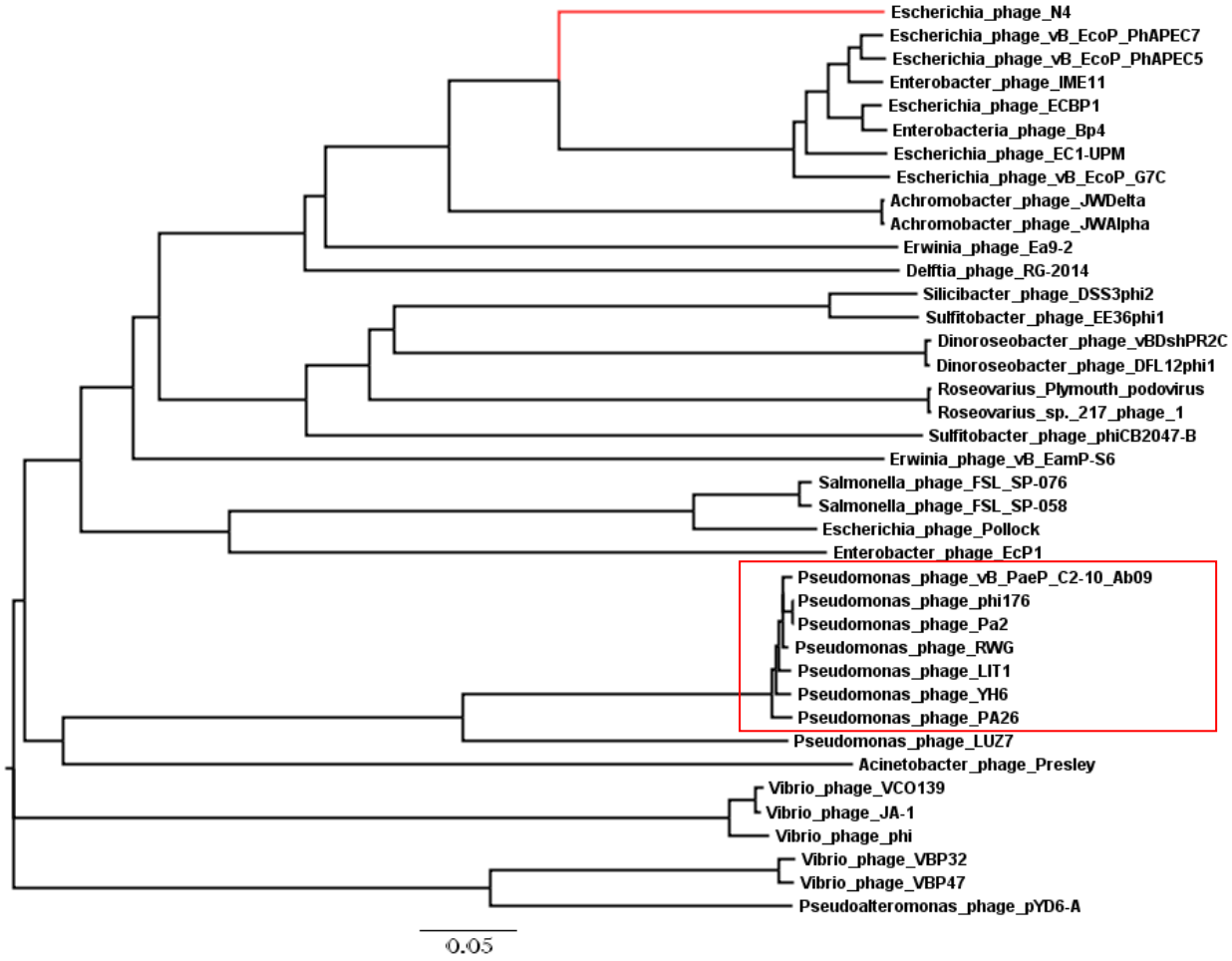

Fig. 2. Electron micrograph of negatively stained LIT1 (3).

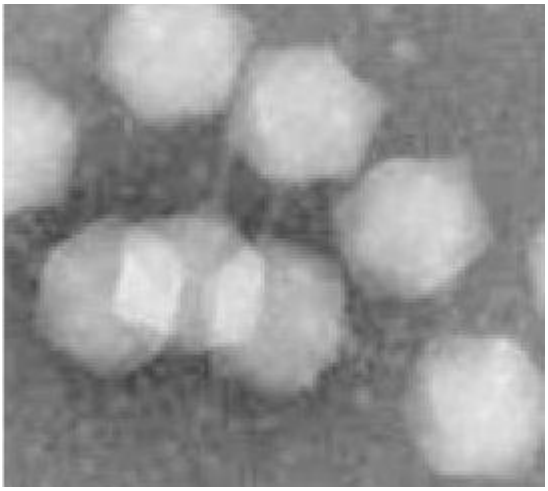

Table 1. Properties of the eight phages belonging to the genus *Lit1virus* and the genomic orphan N4.

Phage	GenBank accession No.	Genome length (kb)	Genome (mol%G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)*	Proteome (% homologous proteins)**
LIT1	FN422399	72.54	55.0	90	0	100	100
Ab09	HG962375	72.03	54.9	83	0	91	92.2
PA26	JX194238	72.32	54.8	88	0	94	94.4
N4	EF056009	70.15	41.3	72	0	<1	31.1

* Determined using BLASTN; ** Determined using CoreGenes (2)

Fig. 3. progressiveMauve alignment of the annotated genomes of members of the *Lit1virus* genus – top (LIT1), middle (PA26) and bottom (Ab09) (1). 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).