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:	***2016.024a-dB***	(to be completed by ICTV officers)

Short title: To create a new genus, *Luz7virus*, including 2 (two) 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** ☒

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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 & Archaeal Virus 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:

Currently in GenBank there are over 40 fully sequenced N4-like phage genomes, which while having 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 taxa, until a firm molecular basis can be proposed to define these.

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	***2016.024aB***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Luz7virus* (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 LUZ7 *Pseudomonas virus KPP21*	Pseudomonas phage LUZ7 Pseudomonas phage KPP21	FN422398 LC064302

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	***2016.024bB***	(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	***2016.024cB***	(assigned by ICTV officers)

To name the new genus: *Luz7virus*

Assigning the type species and other species to a new genus

Code	***2016.024dB***	(assigned by ICTV officers)

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

Pseudomonas virus LUZ7

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

These two phages are both specific for *Pseudomonas aeruginosa*. Pseudomonas phage LUZ7 [4,5] was isolated from hospital sewage samples taken at UH Leuven, Belgium; while Pseudomonas phage KPP21 [6] was isolated from an agricultural wastewater drain in Kochi City (Japan). "*Podoviridae* LUZ7 and LIT1 have slightly larger heads (diameter of 76 nm) incorporating a 70 kb genome. Tails (33 nm long) and a collar of 17 +/- 3 nm with straight fibres of 43 nm in length are also present" [4]. "A narrow 30 nm long tail structure is attached to the LIT1 capsid, but cannot be distinguished in LUZ7" [5]. The head diameter and tail length are 67.0 nm and 5.3 nm, respectively [6]. "The difference in gene number between LUZ7 and LIT1 is largely due to an extra cluster of 29 small genes, located directly upstream from the right terminal repeat of the LUZ7 genome" [5]. The authors of the manuscripts on these phages recognized that they were part of the N4-like phage group.

The phages of this genus, Pseudomonas phages LUZ7 and KPP21have genome lengths of 74,901 bp and 73,420 bp, respectively. While terminal repeats of 660 bp could be detected in Pseudomonas phage LUZ7, terminal repeats were probably used for cyclization of the Pseudomonas phage KPP21 genome during the genome assembly and therefore cannot be detected in the GenBank file. Both phages share about 82% sequence identity (Table 1)(Fig 2).

BLASTN, CoreGenes, and phylogenetic analyses (Fig. 1) all indicate that the proposed genus, *Luz7virus*, is distinct from other genera in the N4-superfamily of viruses. The next closest related phage is Pseudomonas phage LIT1 (FN422399); escherichia phage N4 shares only <1% DNA sequence identity with Pseudomonas phage LUZ7. Though Pseudomonas phages LIT1 and LUZ7 reveal a highly similar overall genome organization, a major difference could be detected in the early gene cluster revealing a much higher number of small ORFs in LUZ7.

Origin of the new genus name:

Pseudomonas phage LUZ7.

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. 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. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 2011 Oct;28(10):2731-9.

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. Sullivan MJ, Petty NK, Beatson SA (2011) Easyfig: a genome comparison visualizer. Bioinformatics 27:1009–1010

4. Ceyssens PJ, Noben JP, Ackermann HW, Verhaegen J, De Vos D, Pirnay JP, Merabishvili M, Vaneechoutte M, Chibeu A, Volckaert G, Lavigne R. Survey of *Pseudomonas aeruginosa* and its phages: *de novo* peptide sequencing as a novel tool to assess the diversity of worldwide collected viruses. Environ Microbiol. 2009;11(5):1303-13. [LUZ7]

5. 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. [LUZ7]

6. Shigehisa R, Uchiyama J, Kato S, Takemura-Uchiyama I, Yamaguchi K, Miyata R, Ujihara T, Sakaguchi Y, Okamoto N, Shimakura H, Daibata M, Sakaguchi M, Matsuzaki S. Characterization of Pseudomonas aeruginosa phage KPP21 belonging to family

Podoviridae genus N4-like viruses isolated in Japan. Microbiol Immunol. 2016;60(1):64-7.

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 several N4-like phages were aligned and the phylogenetic tree was constructed using MEGA5 (1). The members of the *Luz7virus* genus are boxed in red.

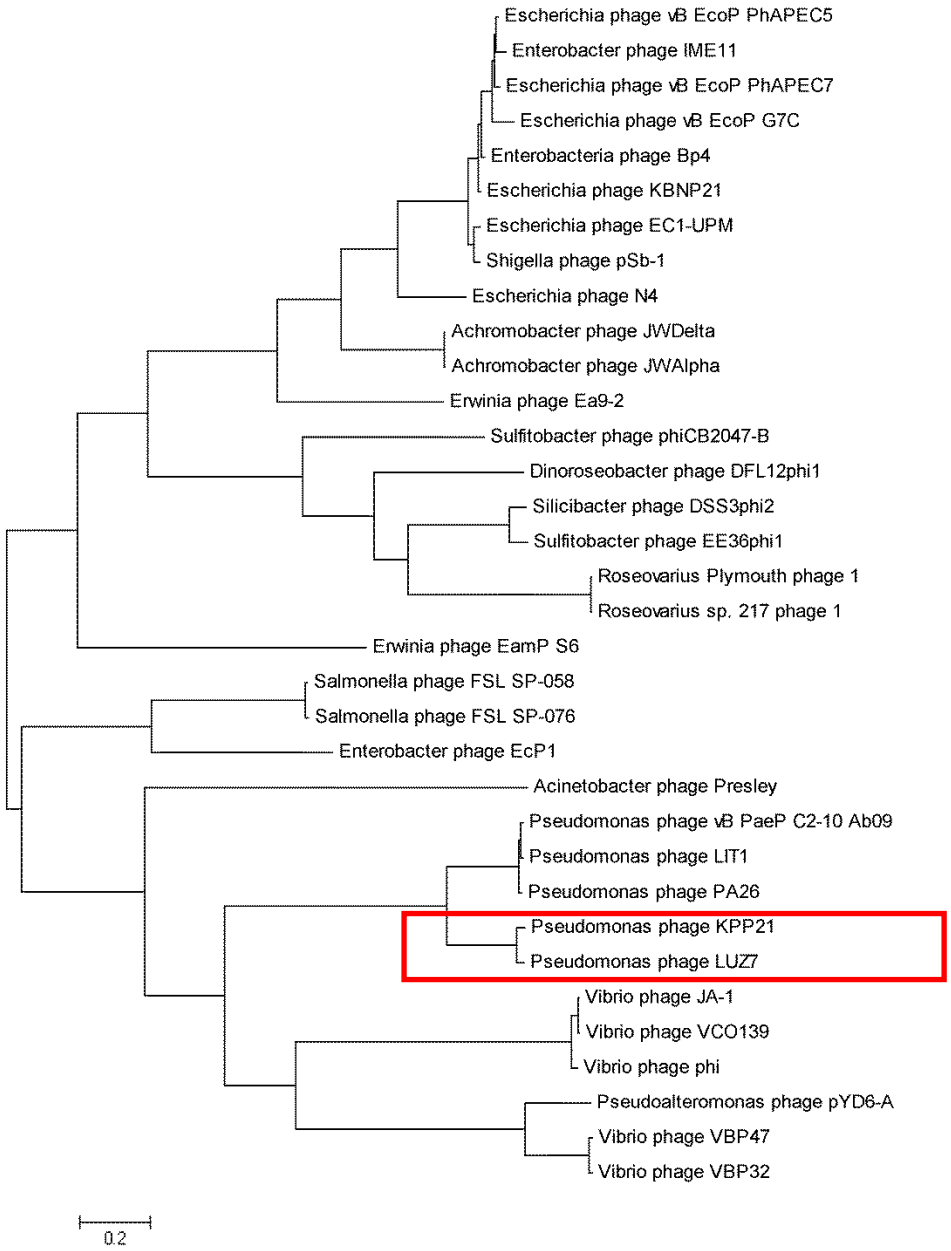

Fig 2. Synteny plot of luz7viruses (Pseudomonas phages LUZ7 and KPP21 in comparison with Pseudomonas phage LIT1 and escherichia phage N4 visualized with EasyFig [3]. The scale bar shows the level of nucleotide identity.

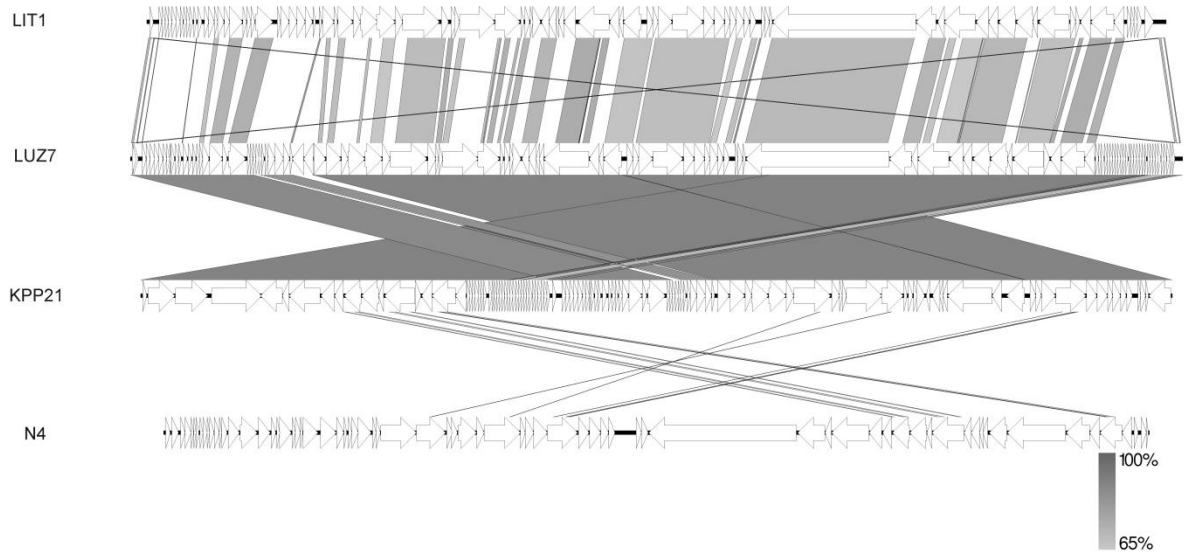

Fig.3 Electron micrographs of Pseudomonas phages KPP21 (A) and LUZ7 (B).

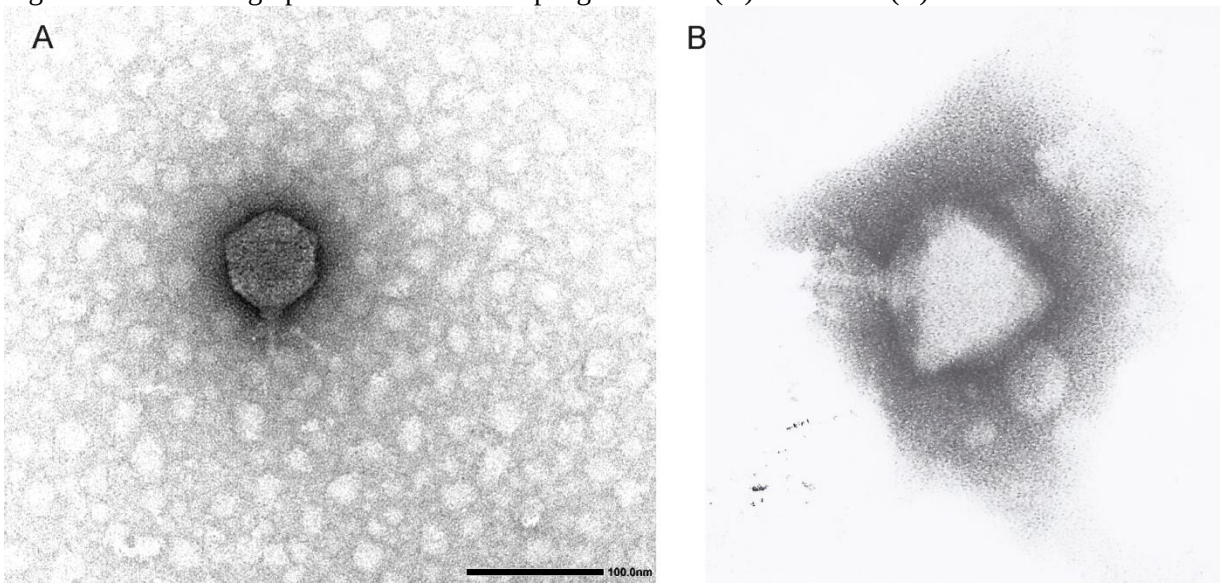

Table 1. Properties of the two phages belonging to the genus *Luz7virus* and the genomic orphan N4

Phage	GenBank acc. no.	Genome length (kb)	No. CDS	No. tRNAs	DNA (% sequence identity)*	Proteome (% homologous proteins)**
Pseudomonas phage LUZ7	FN422398	74.901	115	0	100	100
Pseudomonas phage KPP21	LC064302	73.420	113	0	82	90.4
N4	EF056009	70.153	72	0	<1	21.7

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