MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	*2015.009aB*	(to be completed by ICTV officers)

Short title: To create five (5) new species within the genus *Luz24virus* (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)	Bacterial & Archaeal Virus Subcommittee

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

Please note that we have chosen to refer to this genus as *Luz24virus* rather than *Luz24likevirus*, since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" from phage genus names.

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**

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	*2015.009aB*	(assigned by ICTV officers)

To create 5 new species within:

		Fill in all that apply.
Genus:	*Luz24likevirus* (proposed name *Luz24virus*)	• If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
Subfamily:		• If no genus is specified, enter "**unassigned**" in the genus box.
Family:	*Podoviridae*	
Order:	*Caudovirales*	

Name of new species:	Representative isolate: (only 1 per species please)	GenBank sequence accession number(s)
Pseudomonas virus CHU	Pseudomonas phage PhiCHU	KP233880
Pseudomonas virus Ab22	Pseudomonas phage vB_PaeP_C2-10_Ab22	LN610578
Pseudomonas virus PAA2	Pseudomonas phage phiIBB-PAA2	KF856712
Pseudomonas virus TL	Pseudomonas phage TL	HG518155
Pseudomonas virus PaP4	Pseudomonas phage PaP4	KC294142

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. Therefore, *Pseudomonas* phages MR299-2 (JN254801), vB_PaeP_p2-10_Or1 (HF543949) and vB_PaeP_C1-14_Or (HE983844) should be considered strains of *Pseudomonas virus PaP3*, which already belongs to this genus.

BLASTN, CoreGenes and phylogenetic (Fig. 1) analyses all indicate that the genus, *Luz24virus*, is cohesive and distinct from the other viral genera within the *Podoviridae*. The next closest related phage is *Pseudomonas* phage UFV-P2 (JX863101.2) which shares 34% DNA sequence identity. GenBank currently contains 10 phages which could be considered part of this genus. The phages of this genus possess genome of ca. 45.4 kb (52.3 mol%G+C),

and encode 70 proteins and 0-4 tRNAs; they share >71% DNA sequence identity (Table 1). A qualitative presentation of DNA-DNA relatedness is shown in Figure 2.

Please note that we have chosen to refer to this new genus as *Luz24virus* rather than *Luz24likevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" and "*Phi*" from phage genus names.

MODULE 10: **<u>APPENDIX</u>**: 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. 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. Essoh C, Blouin Y, Loukou G, Cablanmian A, Lathro S, Kutter E, Thien HV, Vergnaud G, Pourcel C. The susceptibility of *Pseudomonas aeruginosa* strains from cystic fibrosis patients to bacteriophages. PLoS One. 2013;8(4):e60575. [vB_PaeP_p2-10_Or1]

5. Alemayehu D, Casey PG, McAuliffe O, Guinane CM, Martin JG, Shanahan F, Coffey A, Ross RP, Hill C. Bacteriophages φMR299-2 and φNH-4 can eliminate *Pseudomonas aeruginosa* in the murine lung and on cystic fibrosis lung airway cells. MBio. 2012;3(2):e00029-12.

6. Pires DP, Kropinski AM, Azeredo J, Sillankorva S. Complete Genome Sequence of the *Pseudomonas aeruginosa* Bacteriophage phiIBB-PAA2. Genome Announc. 2014;2(1). pii: e01102-13.

Fig 1. Phylogenetic analysis of major capsid proteins of luz24viruses and their relatives 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."
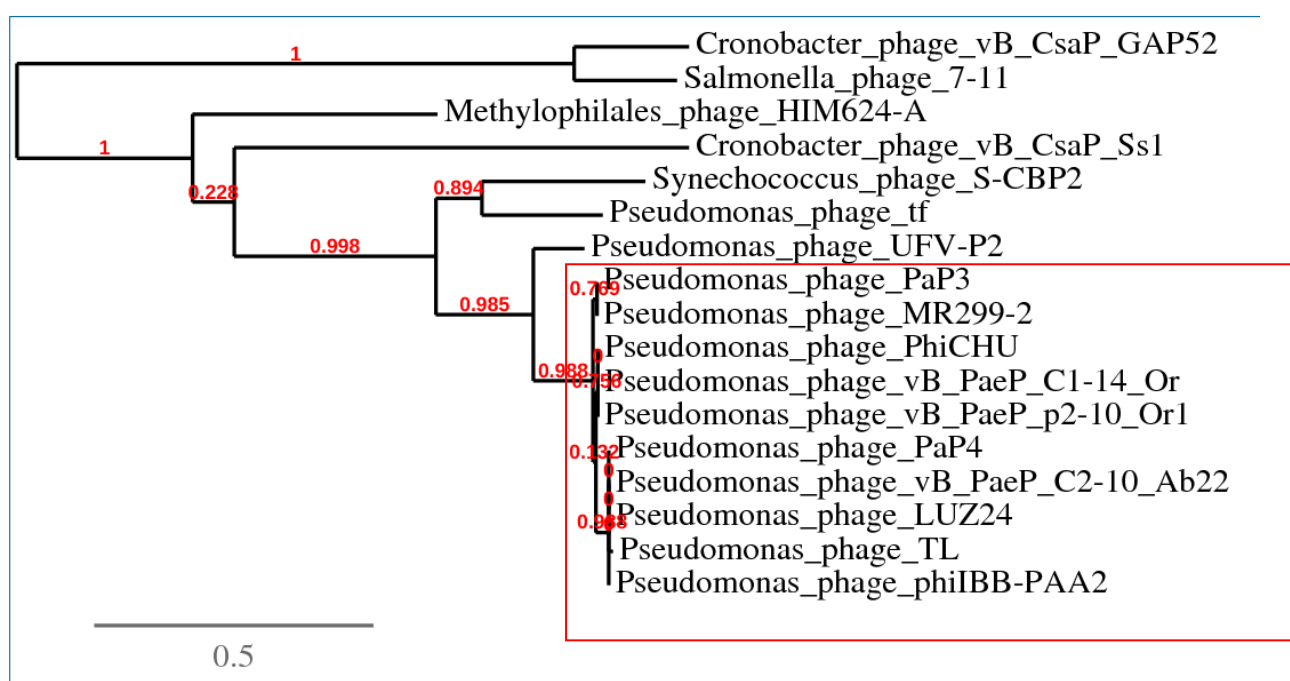

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

Table 1. Properties of the 7 phages belonging to the genus *Luz24virus*, plus its closest relative *Pseudomonas* phage UFV-P2.

Phage	GenBank accession No.	Genome length (kb)	Genome (mol%G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)*	Proteome (% homologous proteins)**
LUZ24	AM910650	45.63	52.2	68	2***	100	100
PaP3	AY078382.2	45.50	52.2	71	4	70.6	88.2
PhiCHU	KP233880	45.63	52.0	73	3	73.1	89.7
vB_PaeP_C2-10_Ab22	LN610578	45.81	52.4	71	3	91.2	89.7
phiIBB-PAA2	KF856712	45.34	52.3	69	3	88.3	86.8
TL	HG518155	45.70	52.4	65	2***	92.2	89.7
PaP4	KC294142	43.89	52.5	70	0	86.3	86.8
UFV-P2	JX863101.2	45.52	51.5	75	0	33.6	64.7

* Determined using BLASTN; ** Determined using CoreGenes (2); *** not indicated in GenBank RefSeq file

Fig. 2. progressiveMauve alignment of the annotated genomes of members of the *Luz24virus* genus − from top to bottom: LUZ24, PaP4, PhiCHU, PAA2, TL and Ab22 (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).