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

Code assigned:	*2015.029a-dB*	(to be completed by ICTV officers)

Short title: Create one (1) new genus, *Pakpunavirus,* including six (6) new 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** ☒

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List the ICTV study group(s) that have seen this proposal:

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

Please note that we have chosen to refer to this new genus as *Pakpaunavirus* rather than *Pakpaunalikevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" and "*Phi*" from phage genus names. At this time we do not propose to create a subfamily with the *Kpp10virus* genus.

Date first submitted to ICTV: June 2015
Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

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	*2015.029aB*	(assigned by ICTV officers)

To create 3 new species within:

Genus:	*Pakpunavirus* **(new)**	
Subfamily:		Fill in all that apply.
Family:	*Myoviridae*	• If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
Order:	*Caudovirales*	• 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 PAKP1	Pseudomonas phage PAK_P1	KC862297
Pseudomonas virus PAKP4	Pseudomonas phage PAK_P4	KC862300
Pseudomonas virus JG004	Pseudomonas phage JG004	GU988610.2
Pseudomonas virus CAb02	Pseudomonas phage vB_PaeM_C2-10_Ab02	LN610572
Pseudomonas virus PaP1	Pseudomonas phage PaP1	HQ832595
Pseudomonas virus CAb1	Pseudomonas phage vB_PaeM_C2-10_Ab1	HE983845

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.

MODULE 3: **NEW GENUS**

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

Code	*2015.029bB*	(assigned by ICTV officers)

To create a new genus within:

Fill in all that apply.

Subfamily:	
Family:	*Myoviridae*
Order:	*Caudovirales*

- 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	*2015.029cB*	(assigned by ICTV officers)

To name the new genus: *Pakpunavirus*

Assigning the type species and other species to a new genus

Code	*2015.029dB*	(assigned by ICTV officers)

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

Pseudomonas virus PAKP1

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:**

6

Reasons to justify the creation of a new genus:
Additional material in support of this proposal may be presented in the Appendix, Module 9

This group of phages has a global distribution having been isolated in Germany, France, Côte d'Ivoire and the People's Republic of China are lytic for their host, *Pseudomonas aeruginosa* (4,-7).
"Electron microscopy revealed that strain JG004 had an isometrically hexagonal head (diameter, 67 nm) and a 115-nm-long contractile tail."(6). The head is an icosahedron, as evidenced by the simultaneous presence of hexagonal and pentagonal capsids, and measures 68–70 nm between opposite apices. The head is separated from the tail sheath by an 8 nm-long neck. Uncontracted tails measure 138–140 x 17–20 nm." (7).

These phages possess terminally redundant genomes (vB_PaeM_C2-10_Ab2b [1165 bp], JG004 [1238 bp], PaP1 [1190 bp]. The average genome characteristics of the members of this genus are: genome size, 93.0 kb; mol%G+C, 49.4; encoding: 169 proteins and 12 tRNAs.

A phylogenetic analysis (3) of the major capsid proteins (Fig. 3A), large subunit terminase (Fig. 3B) and total genome (Fig. 3C) of all the related *Pseudomonas* myoviruses reveals clustering which was be confirmed by total genome (BLASTN; progressiveMauve, 1) and proteomic (CoreGenes, 2) analyses.

Origin of the new genus name:

Pseudomonas phage PAK_P1

Reasons to justify the choice of type species:

The first fully sequenced member of this genus (4,5)

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.

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.
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. Henry M, Lavigne R, Debarbieux L. Predicting in vivo efficacy of therapeutic bacteriophages used to treat pulmonary infections. Antimicrob Agents Chemother. 2013;57(12):5961-8.
5. Morello E, Saussereau E, Maura D, Huerre M, Touqui L, Debarbieux L. Pulmonary bacteriophage therapy on *Pseudomonas aeruginosa* cystic fibrosis strains: first steps towards treatment and prevention. PLoS One. 2011;6(2):e16963.
6. Garbe J, Bunk B, Rohde M, Schobert M. Sequencing and characterization of *Pseudomonas aeruginosa* phage JG004. BMC Microbiol. 2011;11:102.
7. Lu S, Le S, Tan Y, Zhu J, Li M, Rao X, Zou L, Li S, Wang J, Jin X, Huang G, Zhang L, Zhao X, Hu F. Genomic and proteomic analyses of the terminally redundant genome of the *Pseudomonas aeruginosa* phage PaP1: establishment of genus PaP1-like phages. PLoS One. 2013;8(5):e62933.

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 Electron micrograph of negatively stained phage JG004 (left, taken from 6) and vB_PaeM_C2-10_Ab1 (right, Christine Pourcel).
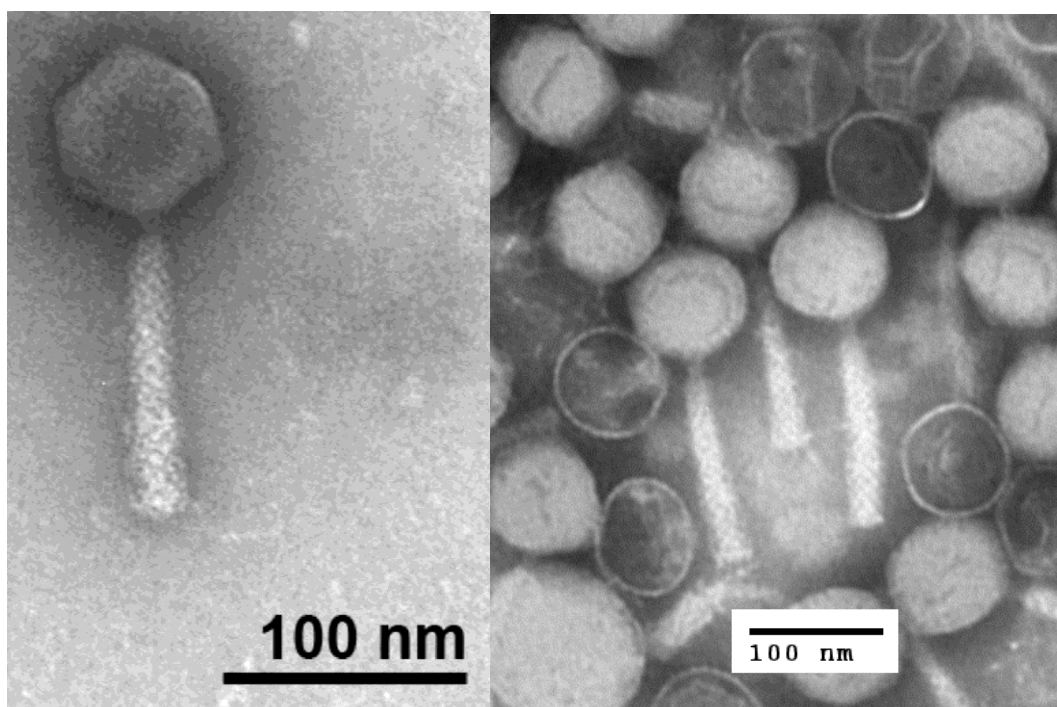
Table 1. Properties of the three phages belonging to the genus *Pakpunavirus*

Phage	GenBank Accession No.	Genome size (kb)	Genome (mol%G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)*	% Homologous proteins **
PAK_P1	KC862297	93.20	49.5	181	13***	100	100
PAK_P4	KC862300	93.15	49.3	174	14***	86	91.2
JG004	GU988610.2	93.02	49.3	161	12	86	82.3
Ab02	LN610572	93.85	49.4	175	12	86	92.8
PaP1	HQ832595	91.72	49.4	167	12	87	89.5
Ab1	HE983845	92.78	49.3	158	11	87	81.8

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

Table 2. Phage which are closely related to the species listed in Table 1.

Phage	GenBank Accession No.
Pseudomonas phage vB_PaeM_C2-10_Ab10	LN610586
Pseudomonas phage vB_PaeM_C2-10_Ab15	LN610587
Pseudomonas phage vB_PaeM_C2-10_Ab08	LN610575
Pseudomonas phage PAK_P2	KC862298

Fig. 2. progressiveMauve alignment (1) of the annotated genomes of members of the *Pakpunavirus* genus from top to bottom: PAK_P1, JG004, PAK_P4, PaP1, Ab1 and Ab2. 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).
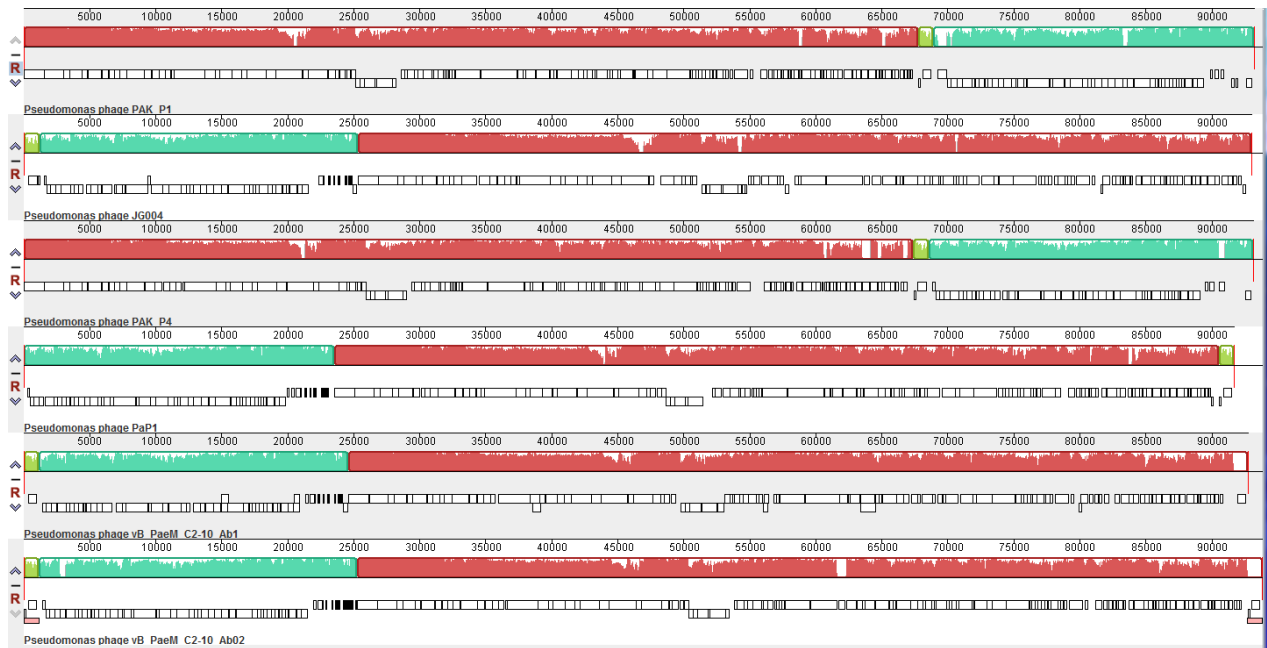

Fig. 3. Phylogenetic analysis of (**A**) the major capsid proteins, and, (**B**) terminase, large subunit proteins, and (**C**) DNA sequence of PAK_P1-like viruses. Phylogenetic tree of the proteins were constructed using "one click" at phylogeny.fr (3). "The "One Click mode" targets users that do not 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." N.B. The genome of *Pseudomonas* phage Ab11 is not annotated.
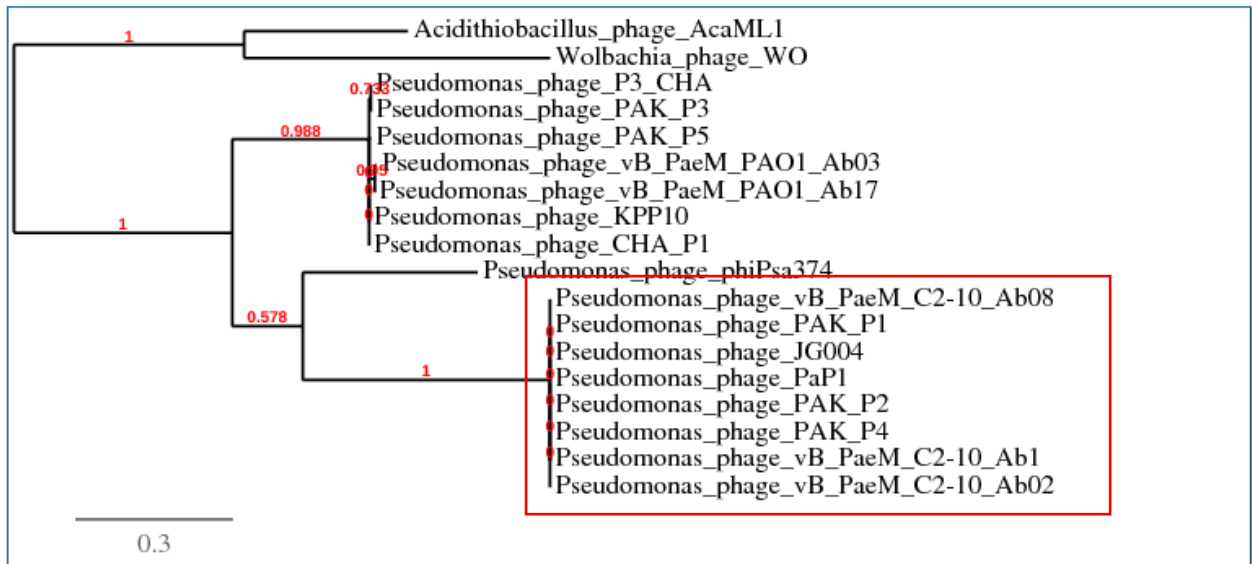
A. Major capsid protein

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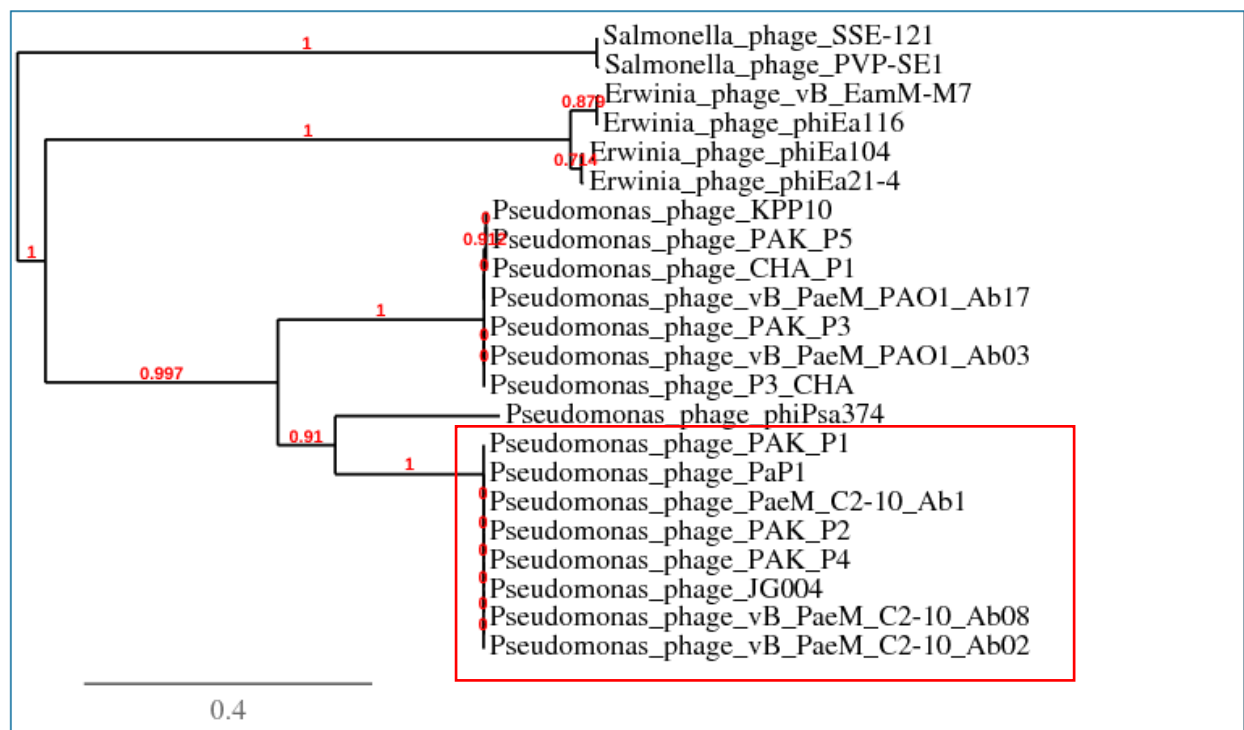
B. Terminase, large subunit

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

C. Whole genome DNA tree – a BLASTN search was conducted at NCBI, and the homologous sequences were selected for "Distance tree of results" analysis. The Neighbor Joining tree method was selected, and the results downloaded in "Newick Format." This file was edited with Notepad, and saved in dnd format. This was opened in FigTree (http://tree.bio.ed.ac.uk/software/figtree/) to produce the accompanying whole genome tree. At this time we do not propose to create a subfamily with the *Kppn10virus* genus.