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:	***2015.028a-dB***	(to be completed by ICTV officers)

Short title: Create one (1) new genus, *Kpp10virus*, including three (3) 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** ☒

Author(s):

Andrew M. Kropinski – University of Guelph (Canada)
Laurent Debarbieux – Institut Pasteur (France)
Rob Lavigne – KU Leuven (Belgium)
Evelien M. Adriaenssens – University of Pretoria (South Africa)

Corresponding author with e-mail address:

Andrew 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 and Archaeal Virus Subcommittee

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

Please note that we have chosen to refer to this new genus as *Kpp10virus* rather than *Kpp10likevirus* 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 *Pakpunavirus* genus.

Date first submitted to ICTV: June 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	***2015.028aB***	(assigned by ICTV officers)

To create 3 new species within:

Genus:	***Kpp10virus* (new)**
Subfamily:	
Family:	***Myoviridae***
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 KPP10 *Pseudomonas virus PAKP3* *Pseudomonas virus Ab03*	Pseudomonas phage KPP10 Pseudomonas phage PAK_P3 Pseudomonas phage vB_PaeM_PAO1_Ab03	AB472900.2 KC862299 LN610573

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**

creating a new genus

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

Code	***2015.028bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Myoviridae***
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	***2015.028cB***	(assigned by ICTV officers)

To name the new genus: *Kpp10virus*

Assigning the type species and other species to a new genus

Code	***2015.028dB***	(assigned by ICTV officers)

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

Pseudomonas virus KPP10

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

This group of phages has a global distribution having been isolated in Japan, France and Côte d'Ivoire and are lytic to their host, *Pseudomonas aeruginosa* (4,5,6). "Electron microscopy revealed that strain KPP10 had an isometrically hexagonal head (diameter, 72 nm) and a 116-nm-long contractile tail."(7). No dimensions are given for the other phages.

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. The average genome characteristics of the members of this genus are: genome size, 87.6 kb; mol%G+C, 54.8; encoding: 160 proteins and 3 tRNAs

Origin of the new genus name:

Pseudomonas phage KPP10

Reasons to justify the choice of type species:

The first fully sequenced member of this genus (1)

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.

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.
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. Uchiyama J, Rashel M, Takemura I, Kato S, Ujihara T, Muraoka A, Matsuzaki S, Daibata M. Genetic characterization of *Pseudomonas aeruginosa* bacteriophage KPP10. Arch Virol. 2012;157(4):733-8.
5.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.
6. 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.
7. Watanabe R, Matsumoto T, Sano G, Ishii Y, Tateda K, Sumiyama Y, Uchiyama J, Sakurai S, Matsuzaki S, Imai S, Yamaguchi K. Efficacy of bacteriophage therapy against gut-derived sepsis caused by *Pseudomonas aeruginosa* in mice. Antimicrob Agents Chemother. 2007;51(2):446-52. [KPP10]

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 PAK_P3 (provided by Laurent Debarbieux)

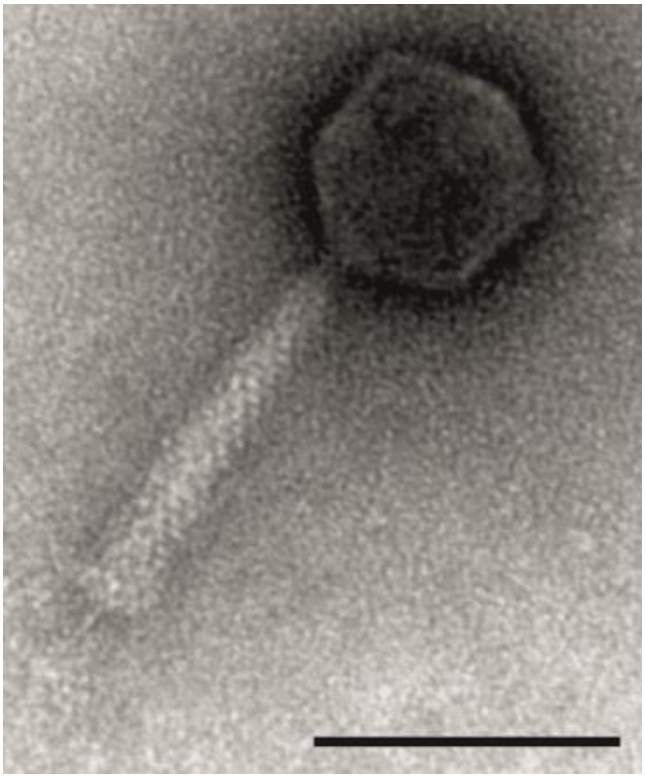

Table 1. Properties of the three phages belonging to the genus *Kpp10virus*

Phage	GenBank Accession No.	Genome size (kb)	Genome (mol%G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)*	% Homologous proteins **
KPP10	AB472900.2	88.32	54.8	158	3	100	100
PAKP3	KC862299	88.10	54.8	165	3	93	95.6
vB_PaeM_PAO1_Ab03	LN610573	86.25	54.7	156	3	85	89.2

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

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

Pseudomonas phage CHA_P1	KC862295
Pseudomonas phage PAK_P5	KC862301
Pseudomonas phage P3_CHA	KC862296
Pseudomonas phage vB_PaeM_PAO1_Ab06	LN610582
Pseudomonas phage vB_PaeM_PAO1_Ab04	LN610581
Pseudomonas phage vB_PaeM_PAO1_Ab11	LN610583
Pseudomonas phage vB_PaeM_PAO1_Ab17	LN610576

Fig. 2. progressiveMauve alignment (1) of the annotated genomes of members of the *Kpp10virus* genus – top (KPP10); middle (PAK_P3) and bottom (Ab03). 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).

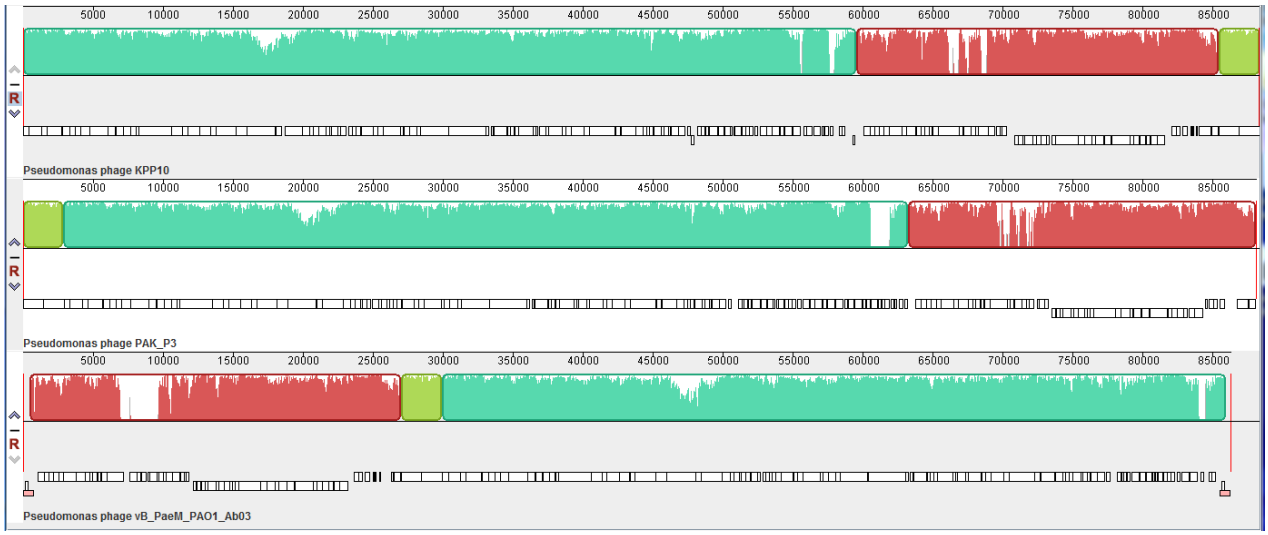

Fig. 3. Phylogenetic analysis of (**A**) the major capsid proteins, and, (**B**) terminase, large subunit proteins of KPP10-like viruses and variety of other phage proteins constructed using "one click" at phylogeny.fr (3). "The "One Click mode" targets users that do not wish to deal with program and 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

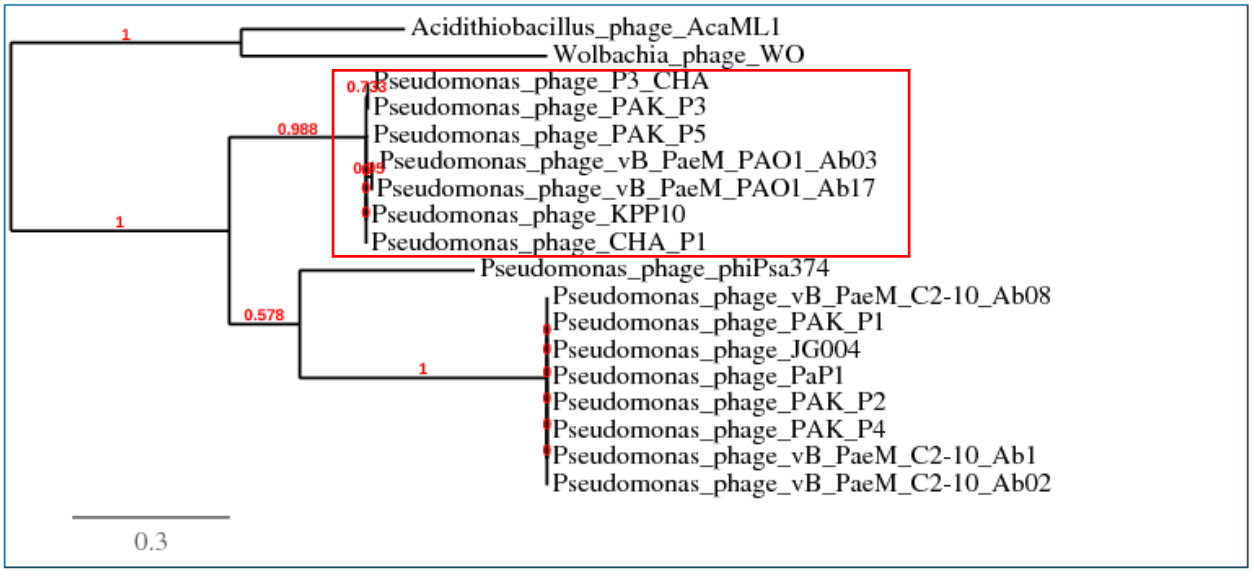

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

B. Terminase, large subunit

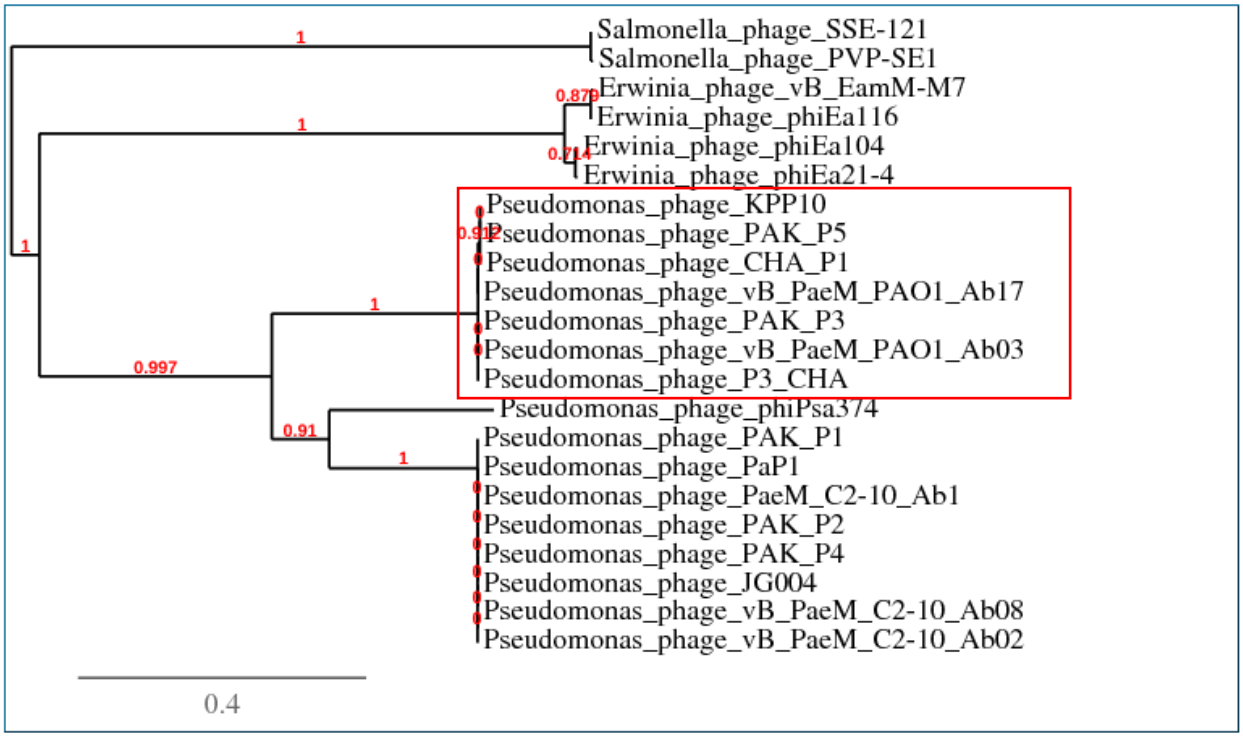

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

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 *Pakpunavirus* genus.