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:	***2016.023a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Kp32virus*, including six (6) new species in 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):

Zuzanna Drulis-Kawa—Uniwersytet Wrocławski (Poland)
Jens H. Kuhn—NIH/NIAID/IRF-Frederick, Maryland (USA)
Andrew M. Kropinski—University of Guelph (Canada)
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)	ICTV Bacterial and Archaeal Viruses 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:

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	***2016.023aB***	(assigned by ICTV officers)

To create 6 new species within:

Genus:	***Kp32virus* (new)**
Subfamily:	***Autographivirinae***
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)**
Klebsiella virus KP32	klebsiella phage KP32	GQ413937.1
Klebsiella virus K11	klebsiella phage K11	EU734173.1
Klebsiella virus KpV289	klebsiella phage vB_KpnP_KpV289	LN866626.1
Klebsiella virus Kp1	klebsiella phage vB_Kp1	KT367885.1
Klebsiella virus K5	klebsiella phage K5	KR149291.1
Escherichia virus K30	escherichia phage K30	HM480846.1

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	***2016.023bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	***Autographivirinae***
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	***2016.023cB***	(assigned by ICTV officers)

To name the new genus: *Kp32virus*

Assigning the type species and other species to a new genus

Code	***2016.023dB***	(assigned by ICTV officers)

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

Klebsiella virus KP32

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

Klebsiella pneumoniae strains producing plasmid-encoded beta-lactamases including ESBLs (Extended-Spectrum Beta-Lactamases), MBLs (Metallo-Beta-Lactamases, and KPCs (*Klebsiella pneumoniae* Carbapenemases) are among the prominent multidrug resistant (MDR) pathogens associated with nosocomial and community-acquired infections. *In vitro* studies have demonstrated high efficacy of *Klebsiella* bacteriophages in eradication of the multi-resistant strains as well as biofilm-forming bacteria. Most of the kp32viruses were propagated on multidrug-resistant *K. pneumoniae* strains [5].

BLASTN (Fig. 2), CoreGenes (Table 1) [2], progressiveMauve alignment (Fig. 3) [1], and phylogenetic analyses (Fig. 4) [3] all indicate that the proposed genus, *Kp32virus*, is cohesive and distinct from other genera. On average, the genomes of members of this genus are 41.0 kb in length (52.6 mol% G+C), and encode 48 proteins and 0 tRNAs. The genome termini possess direct repeats averaging 286 bp.

Escherichia phage K30 infects *Escherichia coli* E69 (O9a:K30:H12).

Origin of the new genus name:

Normally, genera are named after the first sequenced phage representing it but since there is another K11 phage, this would cause problems. We have therefore chosen the second sequenced member of this genus, klebsiella phage KP32, as the source of the genus name.

Reasons to justify the choice of type species:

The second sequenced member of this genus.

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. 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. doi: 10.1186/1756-0500-6-140.

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. Agren J, Sundström A, Håfström T, Segerman B. Gegenees: fragmented alignment of multiple genomes for determining phylogenomic distances and genetic signatures unique for specified target groups. PLoS One. 2012;7(6):e39107.

5. Kęsik-Szeloch A, Drulis-Kawa Z, Weber-Dąbrowska B, Kassner J, Majkowska-Skrobek G, Augustyniak D, Lusiak-Szelachowska M, Zaczek M, Górski A, Kropinski AM. Characterising the biology of novel lytic bacteriophages infecting multidrug resistant *Klebsiella pneumoniae*. Virol J. 2013;10:100.

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.

Table 1. Properties of the six phages belonging to the genus *Kp32virus*.

Phage	RefSeq No.	GenBank Accession No.	Genome length (kb)	Genome (mol% G+C)	bp in LTR	No. CDS	DNA (% sequence identity)*	% Homologous proteins **
Klebsiella phage KP32	NC_013647.1	GQ413937.1	41.12	52.4	nd	44	100	100
Klebsiella phage K11	NC_011043.1	EU734173.1	41.18	53.2	180	51	78	93.2
Klebsiella phage vB_KpnP_KpV289		LN866626.1	41.05	52.6	nd	51	79	86.4
Klebsiella phage vB_Kp1		KT367885.1	40.11	53.3	179	47	77	77.3
Klebsiella phage K5		KR149291.1	41.70	52.5	392	46	84	88.6
Escherichia phage K30	NC_015719.1	HM480846.1	40.94	51.4	393	49	77	86.4

* Determined using BLASTN; ** Determined using CoreGenes [2]; nd = not determined

Fig. 1. Electron micrograph of klebsiella phage KP15 negatively stained with 2% uranyl acetate and examined in the transmission electron microscope (TEM) JEM-100C (JEOL LTD, Tokyo, Japan) at 80 kV with magnification of 66 000× (provided by Zuzanna Drulis-Kawa - University of Wroclaw, Poland).

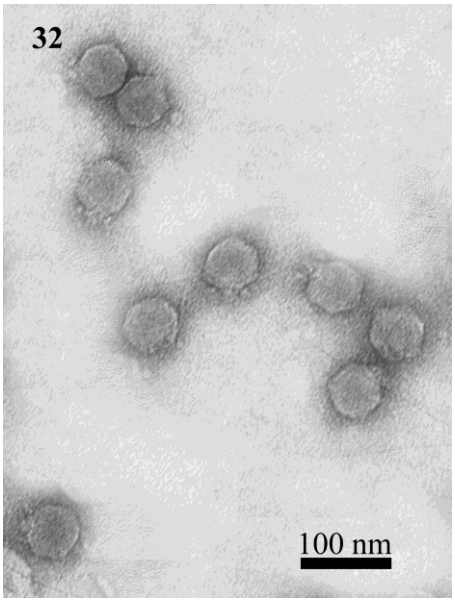

Fig. 2. Gegenees BLASTN analysis of the genomes of members of the subfamily *Autographivirinae* most closely related to klebsiella phage KP32 using the programs accurate settings: fragment size: 200 bp; step size: 100 bp. The results were exported to Excel and conditionally formatted. The data for the phages of interest is boxed.

PHAGE	ACCESSION NO.	AJ251805.1	KM253764.1	KP791807.1	EU547803.1	KP313531.1	KC960671.1	KM242061.1	GU071091.1	JX872508.1	KT321314.1	LN866626.1	KT367885.1	EU734173.1	KR149291.1	GQ413937.1	HM480846.1
phiYeO3-12	AJ251805.1	100.0	87.4	87.5	87.7	85.6	80.3	43.9	42.9	49.0	40.7	41.8	43.2	42.5	40.6	39.1	38.5
vB_YenP_AP5	KM253764.1	87.5	100.0	87.4	86.7	86.2	78.7	42.9	39.3	43.0	34.3	39.6	39.2	42.9	43.8	37.7	36.7
E-4	KP791807.1	87.4	87.8	100.0	87.6	88.3	80.0	47.2	42.0	43.9	38.0	36.3	40.4	41.3	44.9	38.6	34.4
phiSG-JL2	EU547803.1	87.3	86.9	88.3	100.0	86.8	82.3	46.7	42.8	45.6	36.3	44.0	42.9	43.5	42.7	41.9	43.0
phiCFP-1	KP313531.1	85.5	86.3	87.7	86.9	100.0	81.1	50.8	47.6	40.8	38.2	40.8	40.5	41.2	43.6	40.9	37.4
T3	KC960671.1	80.6	78.7	80.6	82.3	81.2	100.0	67.5	72.0	42.5	37.4	39.6	36.3	37.7	38.0	36.9	37.8
CICC80001	KM242061.1	46.0	46.3	47.4	45.8	51.2	67.9	100.0	76.6	48.5	38.3	36.5	34.4	35.8	36.4	35.7	36.5
T7	GU071091.1	42.7	40.6	41.7	42.9	46.4	72.2	76.3	100.0	45.4	37.8	31.6	32.6	31.7	30.6	34.2	33.8
IME15	JX872508.1	51.1	44.7	44.0	45.6	38.7	43.3	48.6	45.9	100.0	35.2	35.4	33.7	35.9	38.8	43.0	34.4
phiEap-1	KT321314.1	35.5	35.5	38.3	35.4	35.2	38.4	37.2	35.9	35.8	100.0	50.1	50.5	50.5	49.0	49.7	51.4
vB_KpnP_KpV289	LN866626.1	42.2	40.4	35.2	42.2	41.2	38.7	35.5	32.1	36.2	50.2	100.0	77.3	77.4	76.9	78.4	74.2
vB_Kp1	KT367885.1	42.1	41.1	40.3	42.2	43.3	36.8	33.7	32.7	35.1	50.8	76.8	100.0	82.2	77.9	77.0	74.9
K11	EU734173.1	44.4	44.7	41.4	42.9	41.5	37.6	34.4	30.3	36.2	51.7	77.5	82.9	100.0	78.6	78.1	77.6
K5	KR149291.1	39.5	43.4	42.4	39.6	44.0	37.1	36.9	29.9	38.3	48.7	76.5	78.3	78.4	100.0	80.3	75.6
KP32	GQ413937.1	39.1	39.3	38.5	39.7	42.9	37.6	34.8	35.9	42.1	50.3	78.7	77.8	77.6	81.0	100.0	76.4
KP30	HM480846.1	36.2	38.9	36.6	42.0	37.7	36.3	36.0	34.6	35.5	51.9	74.9	76.0	77.2	76.1	76.1	100.0

Fig. 3. progressiveMauve alignment [1] of the annotated genomes of members of the *Kp32virus* genus – from top to bottom: klebsiella phages K5 and K11, Escherichia phage K30, and klebsiella phages vB_Kp1, vB_KpnP_KpV289 and KP32. 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. 4. Phylogenetic analysis (A) DNA polymerase and (B) protein kinase of the kp32viruses and variety of homologous proteins from related phages 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." Members of the new genus are boxed in **red**.

A. DNA polymerase

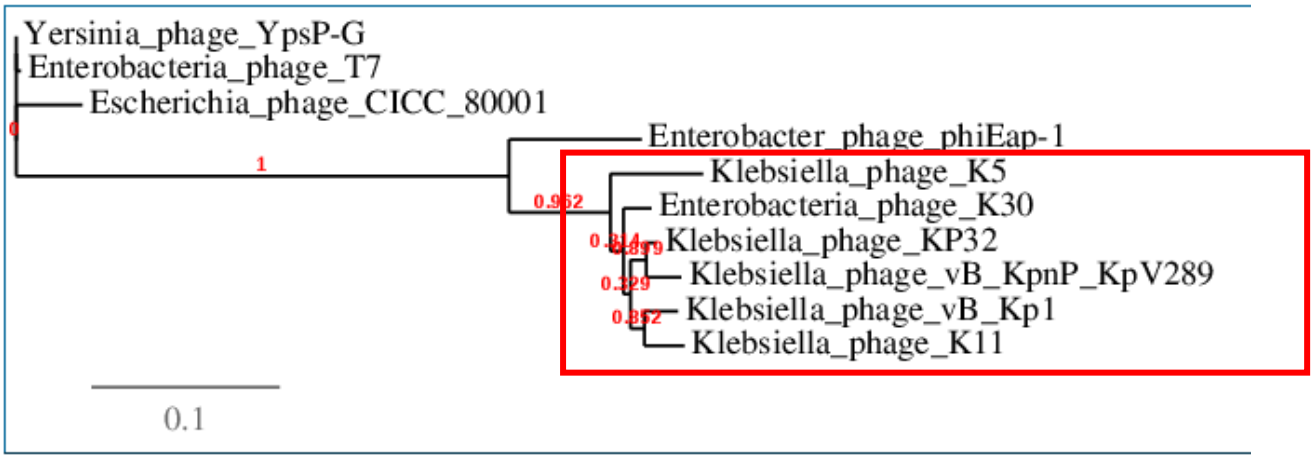

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

B. Protein kinase

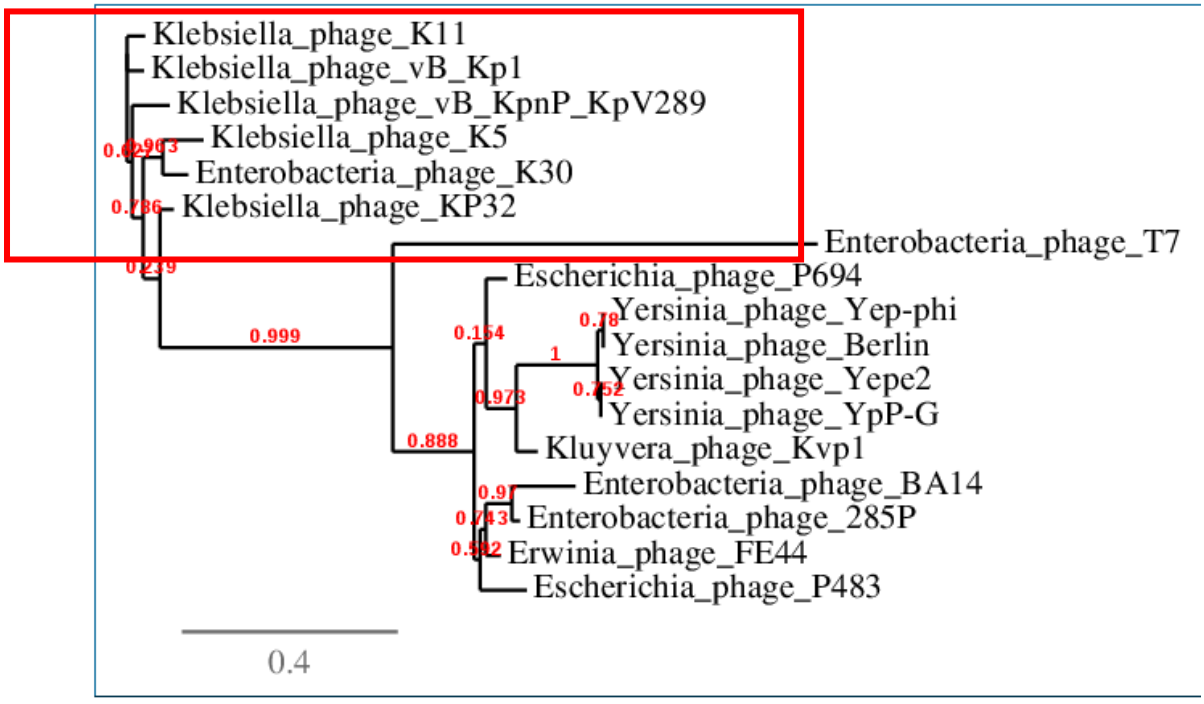

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*