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:	***2016.022a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Kp15virus*, including five (5) new species in the subfamily *Tevenvirinae*
(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—University of Wroclaw (Poland)
Andrew M. Kropinski—University of Guelph (Canada)
Jens H. Kuhn—NIH/NIAID/IRF-Frederick, Maryland (USA)
Evelien M. Adriaenssens—University of Pretoria (South Africa)
Gabriel Kuty Everett—Texas A&M University (USA)

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	***2016.022aB***	(assigned by ICTV officers)

To create 5 new species within:

Genus:	***Kp15virus* (new)**
Subfamily:	***Tevenvirinae***
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)**
Klebsiella virus KP15	Klebsiella phage KP15	GU295964
Klebsiella virus KP27	Klebsiella phage KP27	HQ918180
Enterobacter virus Eap3	Enterobacter phage phiEap-3	KT321315
Klebsiella virus Matisse	Klebsiella phage Matisse	KT001918
Klebsiella virus Miro	Klebsiella phage Miro	KT001919

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	***2016.022bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	***Tevenvirinae***
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	***2016.022cB***	(assigned by ICTV officers)

To name the new genus: *Kp15virus*

Assigning the type species and other species to a new genus

Code	***2016.022dB***	(assigned by ICTV officers)

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

Klebsiella virus KP15

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:**

5

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 lytic myoviruses infect *Klebsiella* and *Enterobacter* strains and were isolated in Poland, U.S.A., and China [5-7]. On average the genomes of members of this genus are 175.4 kb (41.8 mol% G+C), and encode 274 proteins and 2 tRNAs.

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 kp15viruses were propagated on multidrug-resistant *K. pneumoniae* strains.

BLASTN (Fig. 4), CoreGenes (Table 1) [2], and phylogenetic analyses (Fig. 1) [3] all indicate that the proposed genus, *Kp15virus*, is cohesive and distinct from other genera.

Origin of the new genus name:

Named after the first phage of its type to be sequenced – Klebsiella phage KP15.

Reasons to justify the choice of type species:

The first phage of its type to be 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. 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 et al. (2012) Gegenees: fragmented alignment of multiple genomes for determining phylogenomic distances and genetic signatures unique for specified target groups. PLoS One.;7(6):e39107.

5. Provasek VE, Lessor LE, Cahill JL, Rasche ES, Kuty Everett GF. Complete Genome Sequence of Carbapenemase-Producing *Klebsiella pneumoniae* Myophage Matisse. Genome Announc. 2015; 3(5). pii: e01136-15.

6. Mijalis EM, Lessor LE, Cahill JL, Rasche ES, Kuty Everett GF. Complete Genome Sequence of Klebsiella pneumoniae Carbapenemase-Producing *K. pneumoniae* Myophage Miro. Genome Announc. 2015; 3(5). pii: e01137-15.

7. 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. [KP15 & KP27]

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 with 2% uranyl acetate Klebsiella phage KP15 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).

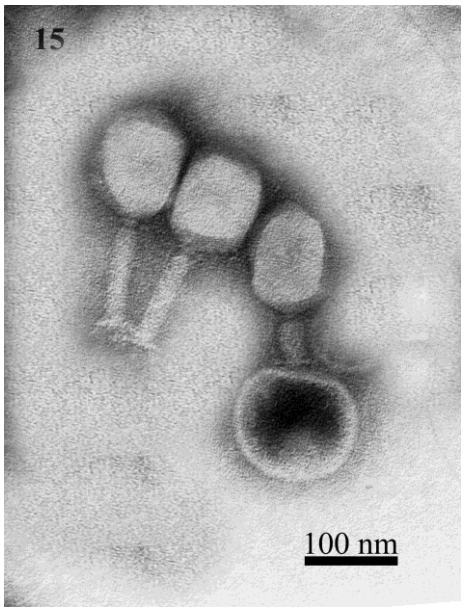

Table 1. Properties of the five phages belonging to the genus *Kp15virus*.

Phage	RefSeq No.	GenBank Accession No.	Genome length (kb)	Genome (mol% G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)*	% Homologous proteins **
Klebsiella phage KP15	NC_014036.1	GU295964.1	174.44	41.8	258	2	100	100
Klebsiella phage KP27	NC_020080.1	HQ918180.1	174.41	41.8	276	2	89	95.0
Klebsiella phage Matisse		KT001918.1	176.08	41.8	280	2	89	97.3
Klebsiella phage Miro		KT001919.1	176.06	41.8	277	2	88	96.1
Enterobacter phage phiEap-3		KT321315.1	175.81	42.0	278	2	88	95.7

* Determined using BLASTN; ** Determined using CoreGenes [2]

Fig. 2. progressiveMauve alignment [1] of the annotated genomes of members of the *Kp15virus* genus – from top to bottom: Klebsiella phages KP15, KP27, Matisse, Miro and Enterobacter phage phiEap-3. 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).

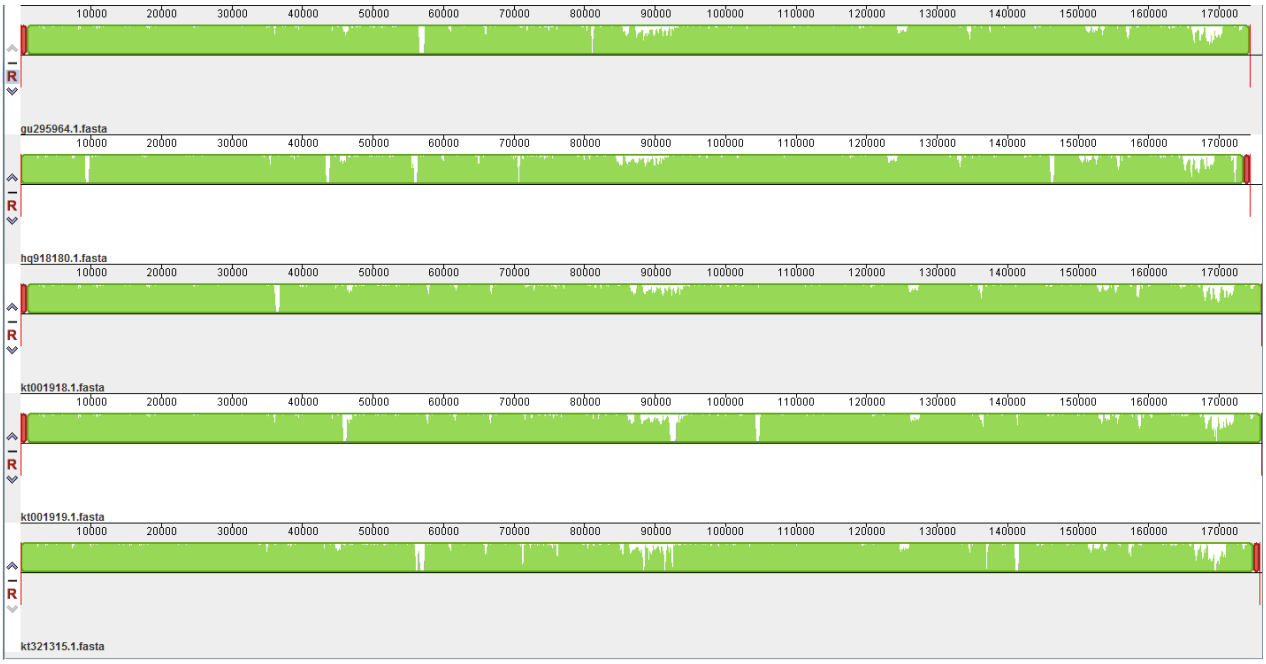

Fig. 3. Phylogenetic analysis of large subunit terminase proteins of kp15viruses and a variety of other similar phages constructed using “one click” at phylogeny.fr [3]. N.B. The capsid gene of Hoody T contained a frameshift which was corrected prior to this analysis. "The "One Click mode" targets users 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."

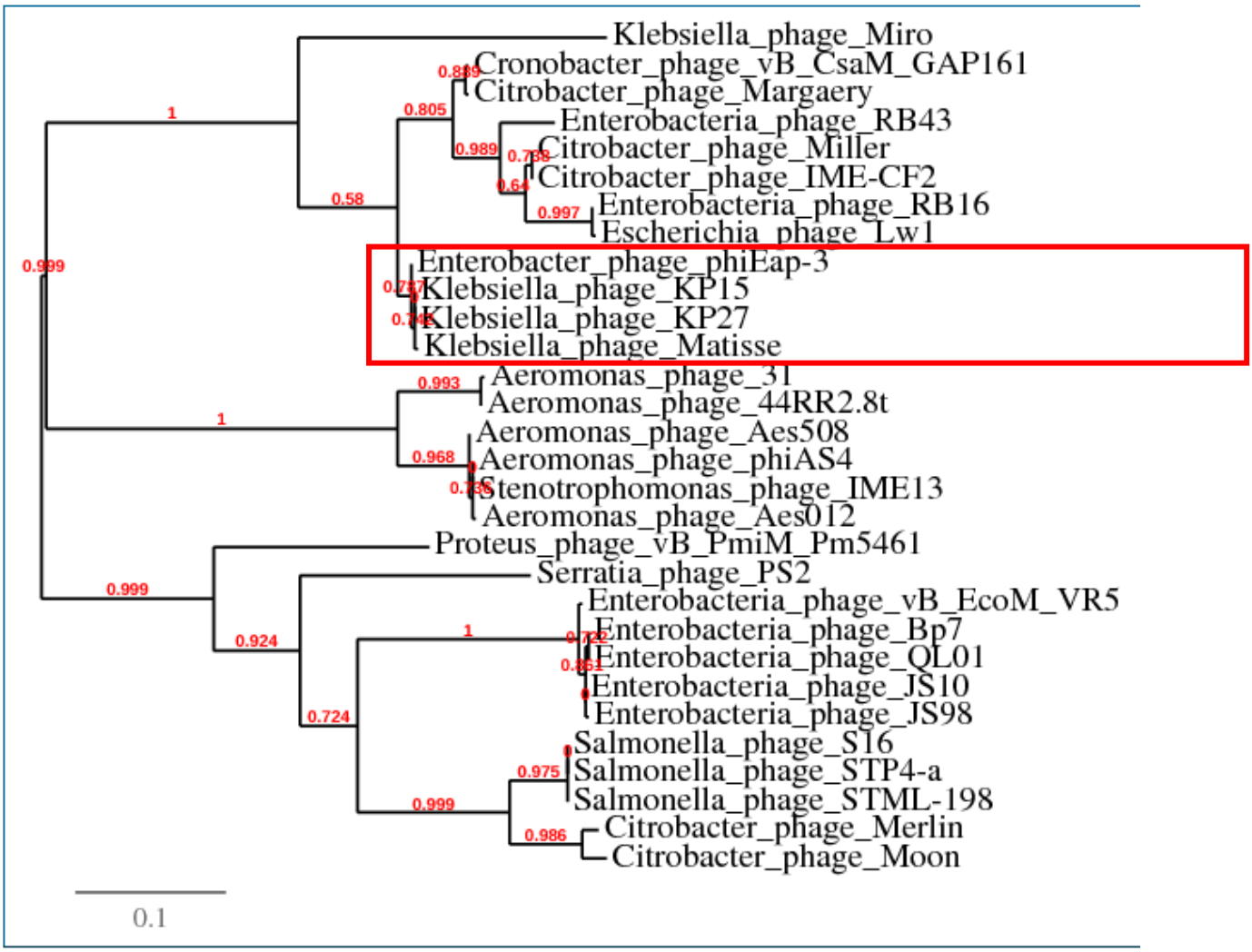

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

Fig. 4. Phylogenomic tree of kp15viruses and related species of bacteriophages. Similarity values were calculated using Gegenees 2.2.1 based on pairwise comparisons of the analyzed sequences (BLASTN method with "custom" settings of fragmenting algorithm - size: 100 bp, shift 50 bp) [4]. The results were exported to Excel.

PHAGE	ACCESSION NO.	ap014714.1	ap014715.1	kj101592.1	ay343333.1	ef437941.1	he978309.1	eu863408.1	kt381880.1	jn882287.1	hm134276.1	kc801932.2	ay967407.1	km236237.1	kr869820.1	kt321315.1	gu295964.1	kt001918.1	kt001919.1	hq918180.1
PEi20	ap014714.1	100.0	90.1	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
PEi26	ap014715.1	90.4	100.0	0.5	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PG7	kj101592.1	0.8	0.6	100.0	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.0	0.1	0.1	0.0	0.0	0.1
RB49	ay343333.1	0.0	0.0	0.1	100.0	87.5	87.3	81.6	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0
Phi1	ef437941.1	0.0	0.0	0.1	87.3	100.0	89.4	79.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
GEC-3S	he978309.1	0.0	0.0	0.1	87.2	90.0	100.0	80.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
JSE	eu863408.1	0.0	0.0	0.1	80.2	79.1	78.7	100.0	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Margaery	kt381880.1	0.0	0.0	0.1	0.0	0.0	0.1	0.0	100.0	71.3	30.8	23.4	20.6	22.6	22.1	5.5	5.5	5.9	6.2	5.9
GAP161	jn882287.1	0.0	0.0	0.1	0.0	0.0	0.1	0.0	71.6	100.0	36.1	24.0	20.2	22.3	23.0	4.4	5.6	5.8	6.1	6.2
RB16	hm134276.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	31.0	36.1	100.0	55.2	45.9	46.8	49.4	3.7	5.2	4.6	4.9	5.3
Lw1	kc801932.2	0.0	0.0	0.1	0.0	0.0	0.1	0.1	23.9	23.9	55.1	100.0	36.1	42.9	42.9	4.0	4.4	4.4	4.6	4.7
RB43	ay967407.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	20.4	19.7	45.0	35.1	100.0	65.9	65.4	3.8	4.2	4.0	3.9	4.1
Miller	km236237.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	22.7	22.3	46.4	42.3	67.0	100.0	84.2	4.0	4.2	4.2	4.6	4.6
IME-CF2	kr869820.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	22.4	23.1	49.4	42.2	66.3	84.4	100.0	4.0	5.0	4.3	4.5	4.7
phiEap-3	kt321315.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	5.3	4.4	3.9	4.1	4.0	3.9	3.9	100.0	87.3	86.4	84.3	84.2
Kp15	gu295964.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.5	5.7	5.3	4.7	4.4	4.3	5.0	87.8	100.0	89.8	88.4	88.5
Matisse	kt001918.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	5.6	5.8	4.5	4.4	4.2	4.3	4.3	86.1	88.9	100.0	90.8	88.9
Miro	kt001919.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	6.1	6.2	4.9	4.7	4.1	4.5	4.4	84.1	87.4	90.8	100.0	87.9
KP27	hq918180.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	5.9	6.4	5.4	4.8	4.3	4.6	4.7	85.0	88.5	89.7	88.7	100.0