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:	***2015.007a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Kp34virus*, including five (5) species within the subfamily *Autographivirinae*.
(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 new genus as *Kp34virus* rather than *Kp34likevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" and "*Phi*" 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**

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	***2015.007aB***	(assigned by ICTV officers)

To create five new species within:

Genus:	***Kp34virus (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 KP34	Klebsiella phage KP34	GQ413938.2
Klebsiella virus SU503	Klebsiella phage vB_KpnP_SU503	KP708985
Klebsiella virus SU552A	Klebsiella phage vB_KpnP_SU552A	KP708986
Klebsiella virus F19	Klebsiella phage F19	KF765493.2
Klebsiella virus K244	Klebsiella phage NTUH-K2044-K1-1	AB716666

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

Klebsiella phages KP34, SU503, SU552A, F19 and NTUH-K2044-K1-1 are all strictly lytic podoviruses, which all share virtually identical morphological dimensions such as a head size of 63 nm and tail length of 15 nm (Fig.1; 1). KP34 and its relatives share an identical lysis cassette setup, with a unimolecular u-spanin, holin and endolysin that is distinct from its closest relative φKMV (Fig.2; 1). These phages share a similar G+C content of approximately 54 %, and similar genome size of almost 49 kb (Table 1). By comparison the genome of φKMV is 42.5 kb and has a mol%G+C content of 62.3. The RNA polymerase recognition loops are also identical among phages KP34, SU503, SU552A, F19 and NTUH-K2044-K1-1, however, there is a three amino acid difference in the recognition loops, which creates two subgroups, with KP34 andNTUH-K2044-K1-1 in one, and phages SU503, SU552A and F19 in the other (Table 2;1). CoreGenes (2) analysis revealed 29 conserved proteins among the phages, and a common gene arrangement. Phylogenetic analysis of the conserved RNA polymerase, head-tail connector protein and DNA maturase B genes between KP34 and its relative phages assemble into a monophyletic clade (Fig. 3; 1). 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.

Please note that we have chosen to refer to this new genus as *Kp34virus* rather than *Kp34likevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" and "*Phi*" from phage genus names.

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2015.007bB***	(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	***2015.007cB***	(assigned by ICTV officers)

To name the new genus: *Kp34virus*

Assigning the type species and other species to a new genus

Code	***2015.007dB***	(assigned by ICTV officers)

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

Klebsiella virus KP34

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

BLASTN (Table 1), *in silico* analysis of lysis cassette (Fig. 2) and phylogenetic analyses (Fig. 3), and RNA polymerase loop comparison (Fig. 4) all indicate that the proposed genus, *Kp34virus*, is cohesive and distinct from the other genera of viruses (1).

Origin of the new genus name:

Named after the first phage of its type to be sequenced: *Klebsiella* phage KP34

Reasons to justify the choice of type species:

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. 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. Eriksson, H.; Maciejewska, B.; Latka, A.; Majkowska-Skrobek, G.; Hellstrand, M.; Melefors, Ö.; Wang, J.-T.; Kropinski, A.M.; Drulis-Kawa, Z.; Nilsson, A.S. A Suggested New Bacteriophage Genus, "Kp34likevirus", within the *Autographivirinae* Subfamily of *Podoviridae*. Viruses 2015, 7, 1804-1822.
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. Whelan, S.; Goldman, N. A general empirical model of protein evolution derived from multiple protein families using a maximum-likelihood approach. Mol. Biol. Evol. 2001, 18, 691–699.
4. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 2013, 30, 2725–2729.

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 five phages belonging to the genus *Kp34virus*.

Phage	GenBank accession No.	Genome length (kb)	Genome (mol% G+C)	No. CDS	DNA (% sequence identity)*
Klebsiella phage KP34	GQ413938	43.8	54.1	57	100
Klebsiella phage vB_KpnP_SU503	KP708985	43.8	53.7	55	78.6
Klebsiella phage vB_KpnP_SU552A	KP708986	43.6	54.2	56	79.3
Klebsiella phage F19	KF765493	43.8	53.8	54	77.5
Klebsiella phage NTUH_NTUH-K2044-K1-1	AB716666	43.9	54.2	52	77.5

* Determined using BLASTN

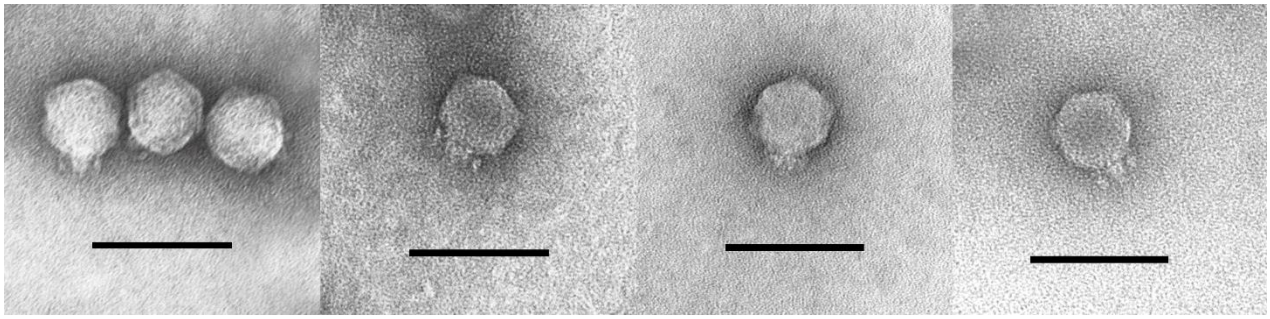

Fig. 1. Electron micrographs of negatively stained (2 % uranyl acetate) *Klebsiella* phages, from left to right, phages SU503, SU552A, KP34, NTUH-K2044-K1-1 (1).

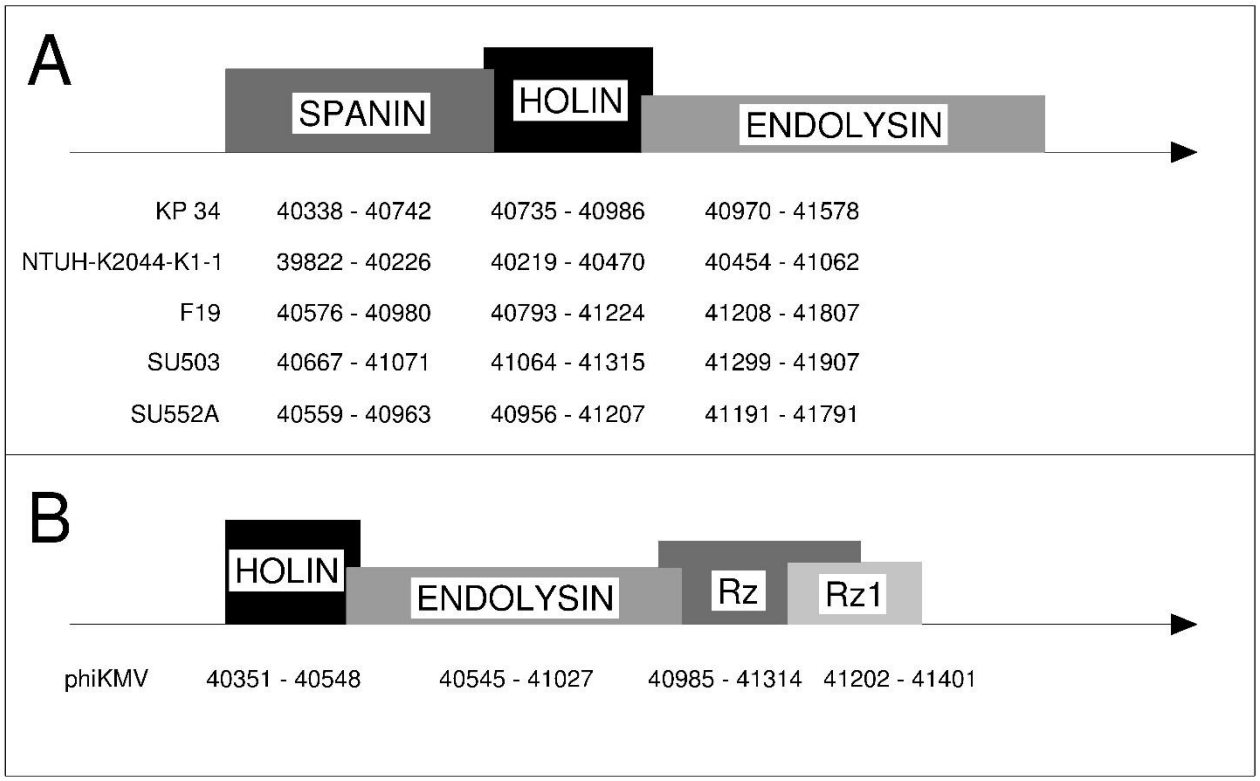

Fig. 2. *In silico* analysis of the lysis cassette of *Klebsiella* phages KP34, SU503, SU552A, F19 and NTUH-K2044-K1-1 together with their closest relative φKMV (1). KP34 and its relatives (A) all share a unimolecular u-spanin, holin and endolysin while the *Phikmvlikevirus* type phage φKMV (B) has a different order of its lysis cassette genes, and a i-spanin/o-spanin complex consisting of two proteins which are lambda Rz1 and Rz2 analogues (1).

Table 2. *In silico* comparison of the RNA polymerase specificity and recognition loops of *Klebsiella* phages KP34, SU503, SU552A, F19 and NTUH-K2044-K1-1 together with their closest relative phage, φKMV (1). Phage KP34 and its relatives share an identical RNA polymerase recognition loop, and a highly similar specificity loop with three amino acid substitutions in phages KP34 and NTUH-K2044-K1-1.

Phage	Recognition loop	Specificity loop
φKMV	HQEAKAAKPAAKL	EEVRVRLRAEAVEYVTLYEAK-DEL
KP34	MRNVKAPGIGGKY	EEVRVRIDCMNLSAVLVHNRDFKTC
K2044	MRNVKAPGIGGKY	EEVRVRIDCMNLSAVLVHNRDFKTC
F19	MRNVKAPGIGGKY	EEVRVRIDCMNLTIMRVHNRDFKTC
SU503	MRNVKAPGIGGKY	EEVRVRIDCMNLTIMRVHNRDFKTC
SU552A	MRNVKAPGIGGKY	EEVRVRIDCMNLTIMRVHNRDFKTC

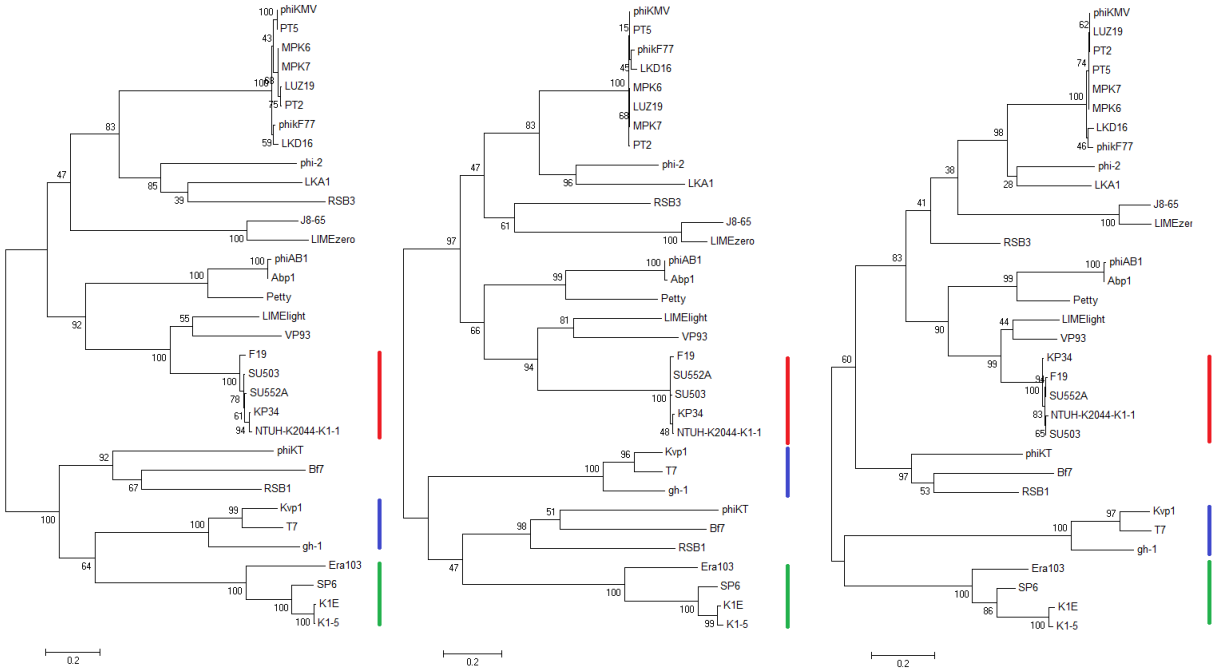

Fig. 3. Phylogenetic analysis of the RNA polymerase (left) and head-tail connector protein (middle) and DNA maturase B (right) of the conferred amino acid sequence of three conserved genes of 33 *Autographivirinae* phages (1). *Klebsiella* phages KP34, SU503, SU552A, F19 and NTUH-K2044-K1-1 form a distinct monophyletic clade (red line), apart from the established *Autographivirinae* genera *T7likevirus* (blue line), *SP6likevirus* (green line) and *Phikmvlikevirus* (scattered in the upper branches of the phylogram). Phylogenetic analysis was performed using the maximum likelihood model, utilizing the Whelan Gold position scoring matrix (3). Uniform substitution rates were assumed, positions containing gaps and missing data were excluded and Nearest-Neighbor-Interchange heuristic method utilized. 500 bootstrap replicas were performed, with bootstrap percentages next to each node in the phylograms. Initial tree was were generated using the maximum parsimony method. Phylogenetic analyses were performed in MEGA6 (4).