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:	***2016.013a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Fri1virus*, including seven (7) new species in the subfamily *Autographivirinae*, 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** ☒

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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)	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	***2016.013aB***	(assigned by ICTV officers)

To create 7 new species within:

Genus:	***Fri1virus* (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)**
Acinetobacter virus Fri1	Acinetobacter phage Fri1	KR149290.1
Acinetobacter virus IME200	Acinetobacter phage IME-200	KT804908.1
Acinetobacter virus Abp1	Acinetobacter phage Abp1	JX658790.1
Acinetobacter virus phiAB1	Acinetobacter phage phiAB1	HQ186308.1
Acinetobacter virus PD6A3	Acinetobacter phage vB_AbaP_PD-6A3	KT388102.1
Acinetobacter virus PDAB9	Acinetobacter phage vB_AbaP_PD-AB9	KT388103.1
Acinetobacter virus AB3	Acinetobacter phage AB3	KC311669.1

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	***2016.013bB***	(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	***2016.013cB***	(assigned by ICTV officers)

To name the new genus: *Fri1virus*

Assigning the type species and other species to a new genus

Code	***2016.013dB***	(assigned by ICTV officers)

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

Acinetobacter virus Fri1	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:**

7

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

General introduction: As more and more phages are deposited in GenBank, we are noting that the original proposal to group viruses into genera based upon 40% homologous proteins [8] would lead to taxonomic lumping. This group of Acinetobacter phages is described as related to pseudomonas phage φKMV [4-7] but is sufficiently different based upon overall DNA and protein sequence identity and phylogeny to be considered a separate genus, distinct from members of *Phikmvvirus*.

BLASTN, CoreGenes (Table 1) [2], progressiveMauve alignment (Fig. 2) [1] and phylogenetic analyses (Fig. 3) [3] all indicate that the proposed genus, *Fri1virus*, is cohesive and distinct from other genera. On average the genomes of viruses of this genus are 41.54 kb in length (39.64 mol% G+C), encoding 50 proteins and no tRNAs. Bioinformatics analysis of these phage genomes demonstrates a high conservation of gene content. Each of these phages encodes genes solely on the Watson strand and exhibits a similar genomic organization consisting of modules of genes that are predicted to be expressed at the early middle and late stages of infection. Direct terminal repeats have been identified for Acinetobacter phages Fri1 and phiAB1. While the GenBank file for Acinetobacter phage Abp1 does not indicate direct repeats, a terminal repeat of 359 bp is present. Using OrthoMCL, the 337 proteins encoded by the 7 phages formed 58 groups consisting

of 2 or more proteins and 18 singleton proteins. A total of 24 groups are conserved across all members of this genus, a number that increases to 38 upon exclusion of the incomplete genome sequence of Acinetobacter phage AB3.
The presence of a virion-encoded single subunit RNA polymerase, encoded adjacent to the structural gene module, places these phages within the subfamily *Autographivirinae*.
Features that distinguish the proposed genus from *Phikmvvirus* and *Kp34virus* include the location and makeup of the lysis gene cassette and genes comprising the early gene module. In addition, the recognition and specificity loops of the RNA polymerase are conserved within members and differ substantially from those reported for members of *Phikmvvirus* and *Kp34virus*. The holin and endolysin genes are positioned between the tail fibre and DNA maturase A genes. The Fri1-related phages encode a class I holin with 3 transmembrane domains and an endolysin with a glycoside hydrolase family 19 domain (Pfam: PF00182). Acinetobacter phages Fri1, Abp1, and phiAB1 are distinguished from the other phages by insertion of a HNH homing endonuclease that has split the DNA polymerase. Interestingly, while the tail fibre encoded by each member shows a conserved N-terminal with a Phage_T7_tail domain, the C-terminal is divergent.

Origin of the new genus name:

Based upon the name of the first member of this genus for which the sequence and annotation is beyond reproach.

Reasons to justify the choice of type species:

The first sequenced member of this genus with a full and accurate annotation.

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. Huang G, Le S, Peng Y, Zhao Y, Yin S, Zhang L, Yao X, Tan Y, Li M, Hu F. Characterization and genome sequencing of phage Abp1, a new phiKMV-like virus

References:

infecting multidrug-resistant *Acinetobacter baumannii*. Curr Microbiol. 2013; 66(6):535-43. [Abp1]

5. Chang KC, Lin NT, Hu A, Lin YS, Chen LK, Lai MJ. Genomic analysis of bacteriophage ϕAB1, a ϕKMV-like virus infecting multidrug-resistant *Acinetobacter baumannii*. Genomics. 2011; 97(4):249-55. [phiAB1]

6. Zhang J, Liu X, Li XJ. Bioinformatic analysis of phage AB3, a phiKMV-like virus infecting *Acinetobacter baumannii*. Genet Mol Res. 2015;14(1):190-8. [AB3]

7. Kropinski AM. Accurate description of phages and their genomes - Genet. Mol. Res. 14 (1): 190-198 "Bioinformatic analysis of phage AB3, a phiKMV-like virus infecting *Acinetobacter baumannii*". Genet Mol Res. 2015;14(4):15902-3. [AB3]

8. Lavigne R, Seto D, Mahadevan P, Ackermann HW, Kropinski AM. Unifying classical and molecular taxonomic classification: analysis of the *Podoviridae* using BLASTP-based tools. Res Microbiol. 2008;159(5):406-14.

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. Cryo-EM picture of Acinetobacter phage Fri1 (provided by Dr. Petr Leiman, Laboratory of Structural Biology and Biophysics, École polytechnique fédérale de Lausanne, Switzerland; and Dr. Mikhail M. Shneider, Laboratory of Molecular Bioengineering, Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, Russia).

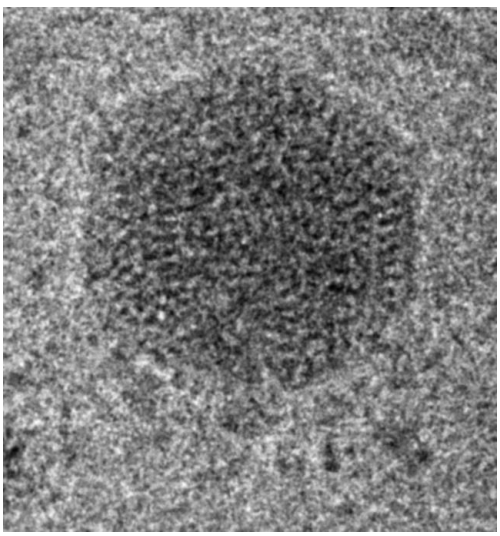


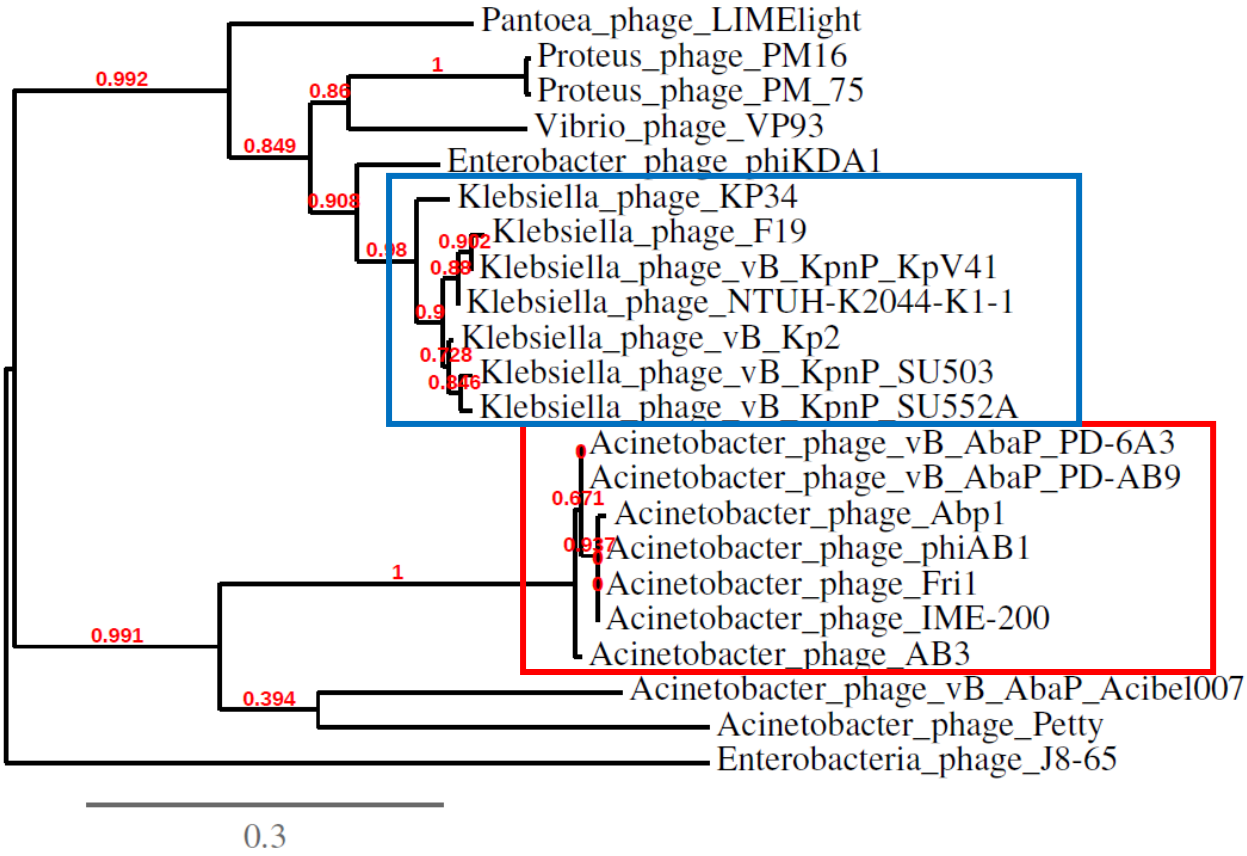
Table 1. Properties of the seven phages belonging to the genus *Fri1virus*.

Acinetobacter phage	RefSeq No.	GenBank Accession No.	Genome length (kb)	Genome (mol% G+C)	No. CDS	DNA (% sequence identity)*	% Homologous proteins **
Fri1	-	KR149290.1	41.80	39.69	54	100	100
IME-200	-	KT804908.1	41.24	39.73	52	84	81.48
Abp1	NC_021316.1	JX658790.1	42.18	39.2	54	82	81.48
phiAB1	-	HQ186308.1	41.53	39.58	46(49)	83	75.93
vB_AbaP_PD-6A3	-	KT388102.1	41.56	39.92	48	83	79.63
vB_AbaP_PD-AB9	-	KT388103.1	40.94	39.73	48	83	79.63
AB3	-	KC311669.1	31.18***	39.2	(29)	61	48.15

* Determined using BLASTN; ** Determined using CoreGenes [2]; *** genome is incomplete [7]; Numbers in brackets denote revised numbers of CDS following re-annotation.

Fig. 2. progressiveMauve alignment [1] of the annotated genomes of members of the *Fri1virus* genus – from top to bottom: Acinetobacter phages Fri1, phiAB1, Abp1, AB3, vB_AbaP_PD-6A3, vB_AbaP_PD-AB9, and IME-200. 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 the major capsid proteins of Acinetobacter phage Fri1-like viruses and homologous proteins from a variety of other 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." The members of this genus are boxed in **red**, while the members of the recently approved genus *Kp34virus* are boxed in **blue**.


Pantoea_phage_LIMElight
Proteus_phage_PM16
Proteus_phage_PM_75
Vibrio_phage_VP93
Enterobacter_phage_phiKDA1
Klebsiella_phage_KP34
Klebsiella_phage_F19
Klebsiella_phage_vB_KpnP_KpV41
Klebsiella_phage_NTUH-K2044-K1-1
Klebsiella_phage_vB_Kp2
Klebsiella_phage_vB_KpnP_SU503
Klebsiella_phage_vB_KpnP_SU552A
Acinetobacter_phage_vB_AbaP_PD-6A3
Acinetobacter_phage_vB_AbaP_PD-AB9
Acinetobacter_phage_Abp1
Acinetobacter_phage_phiAB1
Acinetobacter_phage_Fri1
Acinetobacter_phage_IME-200
Acinetobacter_phage_AB3
Acinetobacter_phage_vB_AbaP_Acibel007
Acinetobacter_phage_Petty
Enterobacteria_phage_J8-65
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1
0.86
0.849
0.908
0.98
0.902
0.9
0.671
0.957
1
0.991
0.394
0.3