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:	***2013.039a-dB***	(to be completed by ICTV officers)

Short title: To create a new genus, the *Chilikevirus*, within the family *Siphoviridae*, containing three new species
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 9 are required)	**1** ☒	**2** ☒	**3** ☒	**4** ☐	**5** ☐
	6 ☐	**7** ☐	**8** ☐	**9** ☒	

Author(s) with e-mail address(es) of the proposer:

Sangryeol Ryu sangryu@snu.ac.kr
Andrew M. Kropinski akropins@uoguelph.ca
Rob Lavigne rob.lavigne@biw.kuleuven.be
Roger Hendrix rhx@pitt.edu
Sherwood Casjens sherwood.casjens@path.utah.edu
Evelien Adriaenssens evelien.adriaenssens@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-EC or Study Group comments and response of the proposer:

Date first submitted to ICTV:	June 2013
Date of this revision (if different to above):	July 2014

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	***2013.039aB***	(assigned by ICTV officers)

To create 5 new species within:

Genus:	***Chilikevirus (new)***
Subfamily:	
Family:	***Siphoviridae***
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.

	GenBank sequence accession number(s) of reference isolate:
Salmonella phage Chi	JX094499
Salmonella phage SPN19	JN871591
Salmonella phage iEPS5	KC677662
Salmonella phage FSLSP088	KC139512
Salmonella phage FSLSP030	KC139519

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

BLASTN analyses reveal that these three enterobacterial phages are related and distinct from any other phage. We have chosen 95% DNA sequence identity as the criterion for demarcation of species.

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2013.039bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Siphoviridae***
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	***2013.039cB***	(assigned by ICTV officers)

To name the new genus: *Chilikevirus*

Assigning the type species and other species to a new genus

Code	***2013.039dB***	(assigned by ICTV officers)

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

Salmonella phage Chi

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

Phage Chi was isolated in 1935 by Sertic and Boulgakov and shown to only attack motile *Salmonella* strains. Further studies revealed its host range to include *Serratia marcescens* and *Escherichia coli* (2). "Bacteriophage chi attaches to the filament of a bacterial flagellum by means of a tail fiber, but the ultimate receptor site for the phage is located at the base of the bacterial flagellum.(1). Schade and Adler (2) observed circular DNA molecules by electron microscopy indicating cohesive termini. Unpublished work by Hendrix and Casjens reveals 5'-GCTCTGCGCACC cohesive termini.

Its buoyant density in CsCl is 1.48 g/cc.

The phage head is an icosahedron measuring 65.0 - 67.5 nm between the parallel sides. The tail is a flexible rod about 220 - 230 nm x 12.5 - 14 nm wide, displaying approximately 55 cross-striations. This is terminated by an extremely long tail fiber measuring 2.0 - 2.5 nm in width and approximately 200-220 nm in length (1).

Recently phage Chi and two relatives (4,5) have been isolated and sequenced. Phage iEPS5 features an isometric capsid (59 ± 2 nm) and non- contractile tail (216 ± 3 nm) (4). These three phages share a high degree of DNA identity and shared proteins (Table 1)

and are as such grouped in the same genus.

Origin of the new genus name:

Salmonella phage Chi

Reasons to justify the choice of type species:

The original isolate of this group.

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. Because many of these viruses are not collinear we have taken an alternative, but well accepted, approach to their grouping i.e. Pairwise Sequence Comparison (PASC) (7). The phage genomes were analyzed using BLASTN with the hits exported to Excel and the % coverage multiplied by the percent identity to give rise to a score showing the nucleotide relationship between the two phages.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

1: Schade SZ, Adler J, Ris H. How bacteriophage chi attacks motile bacteria. J Virol. 1967; 1(3):599-609. PubMed PMID: 4918241; PubMed Central PMCID: PMC375288.

2: Schade S, Adler J. Purification and chemistry of bacteriophage chi. J Virol. 1967; 1(3):591-8. PubMed PMID: 4918240; PubMed Central PMCID: PMC375286.

3: Meynell EW. A phage, phi chi, which attacks motile bacteria. J Gen Microbiol. 1961; 25: 253-90. PubMed PMID: 13770074.

4: Choi Y, Shin H, Lee JH, Ryu S. Identification and characterization of a novel flagellum-dependent *Salmonella*-infecting bacteriophage, iEPS5. Appl Environ Microbiol. 2013 Jun 7. [Epub ahead of print] PubMed PMID: 23747700.

5: Lee JH, Shin H, Choi Y, Ryu S. Complete genome sequence analysis of bacterial-flagellum-targeting bacteriophage chi. Arch Virol. 2013 Apr 19. [Epub ahead of print] PubMed PMID: 23605589.

6: Moreno Switt AI, Orsi RH, den Bakker HC, Vongkamjan K, Altier C, Wiedmann M. Genomic characterization provides new insight into *Salmonella* phage diversity. BMC Genomics. 2013 Jul 17;14:481.

7: Bao Y., Kapustin Y. & Tatusova T. (2008). Virus Classification by Pairwise Sequence Comparison (PASC). Encyclopedia of Virology, 5 vols. (B.W.J. Mahy and M.H.V. Van Regenmortel, Editors). Oxford: Elsevier. Vol. 5, 342-348.

8: Darling AE, Mau B, Perna NT (2010) progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5: e11147

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.

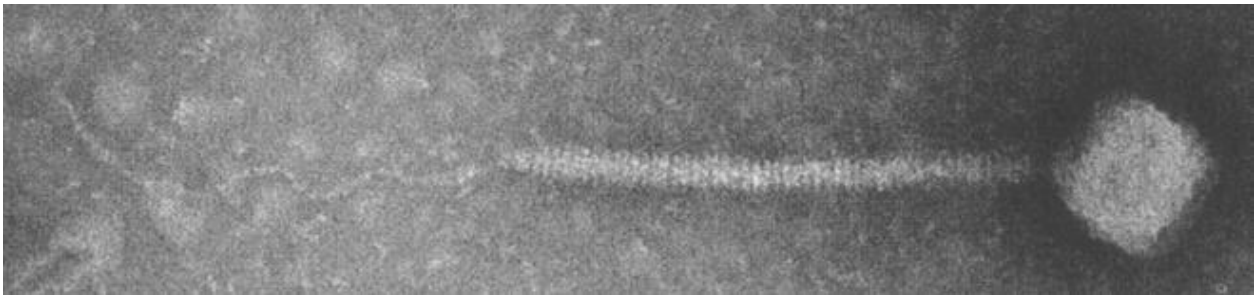

Figure 1. Electron micrograph of phage Chi negatively stained with 1% silicotungstic acid (1).

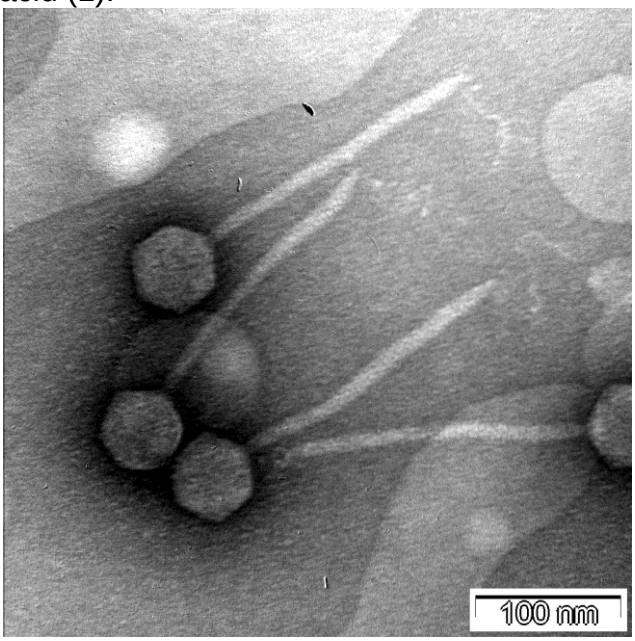

Figure 2. Electron micrograph of *Salmonella* phage iEPS5 negatively stained with 2% aqueous uranyl acetate

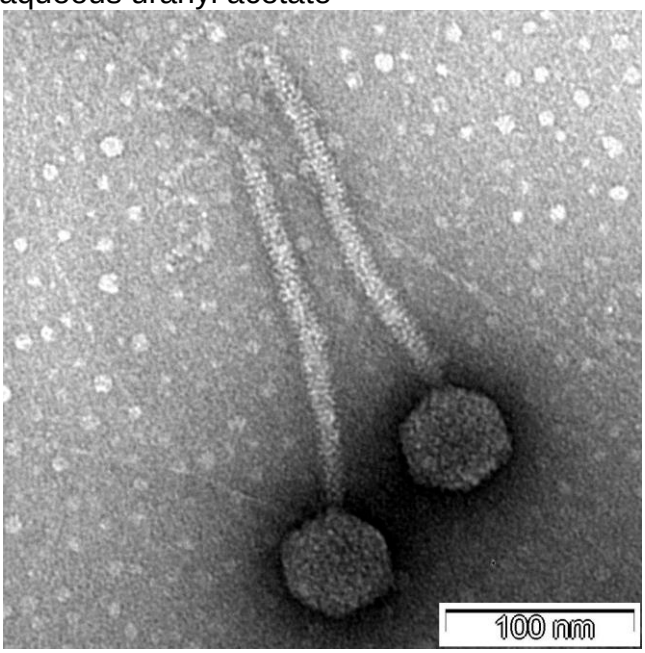

Figure 3. Electron micrograph of *Salmonella* phage SPN19 negatively stained with 2% aqueous uranyl acetate

Table 1. Phage genomes

Phage	GenBank Accession No.	Genome size (bp)	Mol%G+C	Termini	% DNA sequence identity (a)	% Shared proteins (b)
Salmonella phage Chi	JX094499	59,407	56.61	cohesive 12bp	100%	100%
Salmonella phage SNP19	JN871591	59,203	56.52		88.9	90.7
Salmonella phage iEPS5	KC677662	59,254	56.31		90.4	93.3
Salmonella phage FSL SP-088 **(c)**	KC139512	59,454	56.44			90.7
Salmonella phage FSL SP-30 **(d)**	KC139519	59746	56.56			92.0

(a) EMBOSS Stretcher (relative to Chi) – genomes colinearized
(b) CoreGenes 3.0
(c) High sequence identity to Salmonella phage FSL SP-124 (KC139515) – identical species
(d) High sequence identity to Salmonella phage FSL SP-39 (KC139514) – identical species

Figure 4. progressiveMauve alignment of the phage genomes belonging to the proposed genus (8). 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.

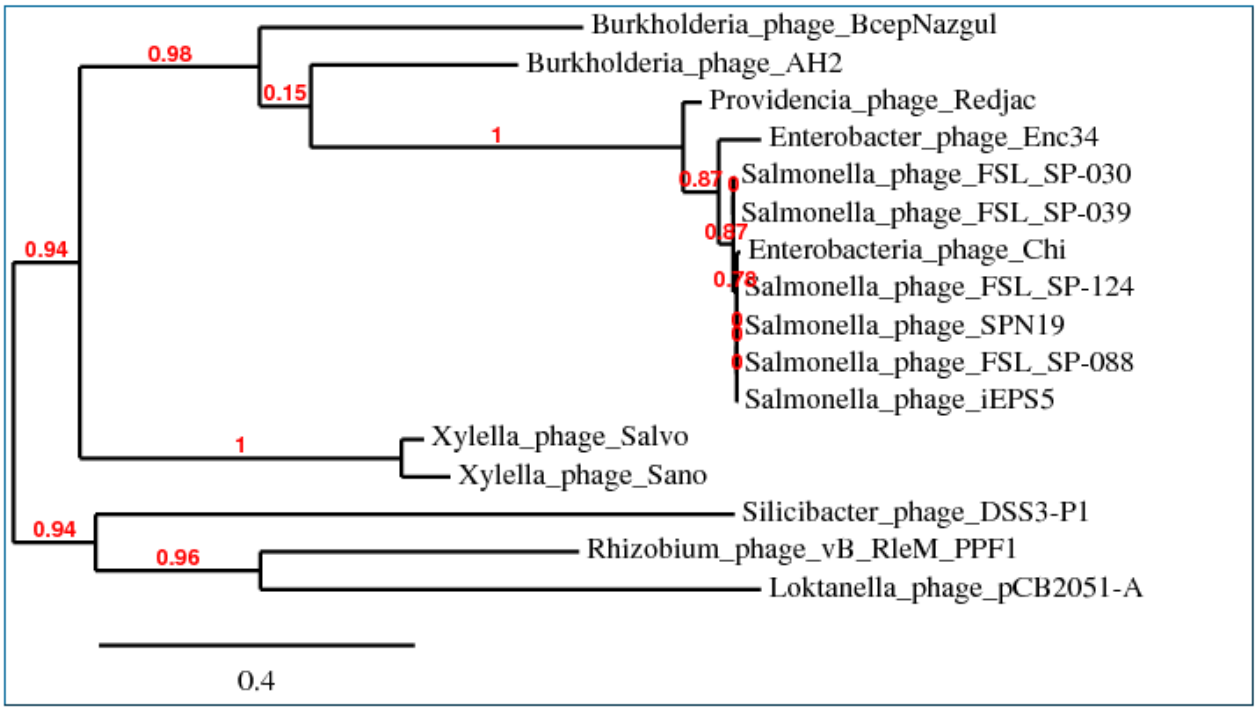

Figure 5. Phylogenetic tree constructed at phylogeny.fr using capsid proteins indicates a separate clade, with peripheral relationship to *Providencia* phage Redjac and *Enterobacter* phage Enc34.