



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:	2013.029a-dB	(to be completed by ICTV officers)			
Short title: To create a new genus, the <i>Hk578likevirus</i> , within the family <i>Siphoviridae</i> (e.g. 6 new species in the genus <i>Zetavirus</i>)					
Modules attached (modules 1 and 9 are required)	1 <input checked="" type="checkbox"/> 6 <input type="checkbox"/>	2 <input checked="" type="checkbox"/> 7 <input type="checkbox"/>	3 <input checked="" type="checkbox"/> 8 <input type="checkbox"/>	4 <input type="checkbox"/> 9 <input checked="" type="checkbox"/>	5 <input type="checkbox"/>

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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-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	2013.029aB	(assigned by ICTV officers)
To create 5 new species within:		
Genus:	<i>Hk578likevirus</i> (new)	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.
Subfamily:		
Family:	<i>Siphoviridae</i>	
Order:	<i>Caudovirales</i>	
		GenBank sequence accession number(s) of reference isolate:
<i>Escherichia phage HK578</i> <i>Sodalis phage SO1</i> <i>Shigella phage EP23</i> <i>Enterobacteria phage JL1</i> <i>Enterobacteria phage SSL2009a</i>		JQ086375 GQ502199 JN984867 JX865427 FJ750948

<p>Reasons to justify the creation and assignment of the new species:</p> <ul style="list-style-type: none"> • Explain how the proposed species differ(s) from all existing species. <ul style="list-style-type: none"> ○ 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
<p>BLASTN analyses reveal that these five enterobacterial phages are related and distinct from any other phage. We have chosen 95% DNA sequence identity as the criterion for demarcation of species.</p>

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	2013.029bB	(assigned by ICTV officers)
To create a new genus within:		
Subfamily:		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
Family:	<i>Siphoviridae</i>	
Order:	<i>Caudovirales</i>	

naming a new genus

Code	2013.029cB	(assigned by ICTV officers)
To name the new genus: <i>Hk578likevirus</i>		

Assigning the type species and other species to a new genus

Code	2013.029dB	(assigned by ICTV officers)
To designate the following as the type species of the new genus		
<i>Escherichia phage HK578</i>		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 analyses reveal that these five enterobacterial phages are related and distinct from any other phage. EP23 infects <i>E.coli</i> strains and <i>Shigella</i> species. EP23 virions possess icosahedral heads 59nm in diameter and non-contractile flexible tails 142 nm in length (1). By comparison phage SSL-2009a has capsid 62nm in diameter and a tail 138nm long. (2). The tail genes of these phages are peripherally related to those of coliphage T1. Table 1 shows the general characteristics of this genus.
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Origin of the new genus name:

<i>Escherichia coli phage HK578</i>

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 as confirmed with the EMBOSS Stretcher algorithm.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

- 1: Chang HW, Kim KH. Comparative genomic analysis of bacteriophage EP23 infecting *Shigella sonnei* and *Escherichia coli*. J Microbiol. 2011 Dec;49(6):927-34.
- 2: Li S, Liu L, Zhu J, Zou L, Li M, Cong Y, Rao X, Hu X, Zhou Y, Chen Z, Hu F. Characterization and genome sequencing of a novel coliphage isolated from engineered *Escherichia coli*. Intervirology. 2010;53(4):211-20.
- 3: Darling AE, Mau B, Perna NT (2010) progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5: e11147
- 4: Rohwer F, Edwards RE (2002) The Phage Proteomic Tree: a genome-based taxonomy for phage. Journal of Bacteriology 184: 4529-4535

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.

1. Phage genomes belonging to the proposed genus

Phage	GenBank Accession No.	Genome size (bp)	Mol%G+C tRNA	Termini	% DNA sequence identity (a)	% Shared proteins (b)
<i>Escherichia</i> phage HK578	JQ086375	43,741	54.48	Presumed cohesive	100%	100%
<i>Sodalis</i> phage SO-1	GQ502199	45,169	54.58		81.6	86.7
<i>Shigella</i> phage EP23	JN984867	44,077	54.42		80.2	85.0
<i>Enterobacteria</i> phage JL1	JX865427	43,457	54.77		ND ^(c)	81.7
<i>Enterobacteria</i> phage SSL-2009a	FJ750948	39,792	54.72		ND	65.0

(a) Calculated using EMBOSS Stretcher (relative to HK578)

(b) Calculated using CoreGenes 2.0

(c) ND = not determined due to orientation of the genome (see Figure 2)

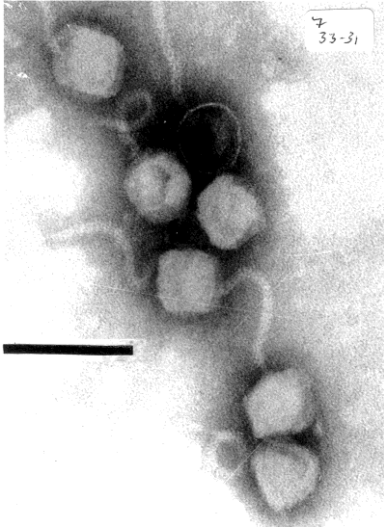


Figure 1. Electron micrograph of phage HK578 negatively stained with uranyl acetate. Bar = 100nm

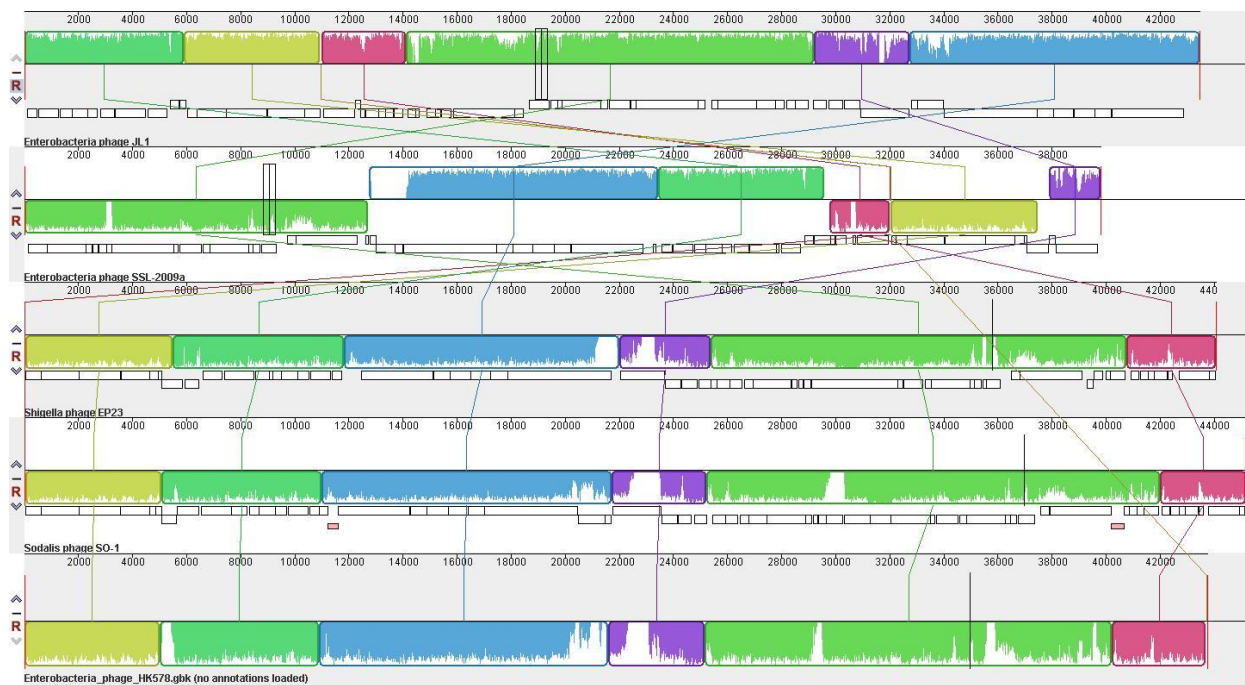


Figure 2. progressiveMauve alignment of the phage genomes belonging to the proposed genus (full genome represented by its annotated ORFs in white blocks) (3). Colored blocks indicate the regions of 1 to 1 best alignment with rearrangement breakpoints in a different random color. The degree of sequence 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. The comparison suggests considerable rearrangement in the case of JL1 and SSL-2009a.

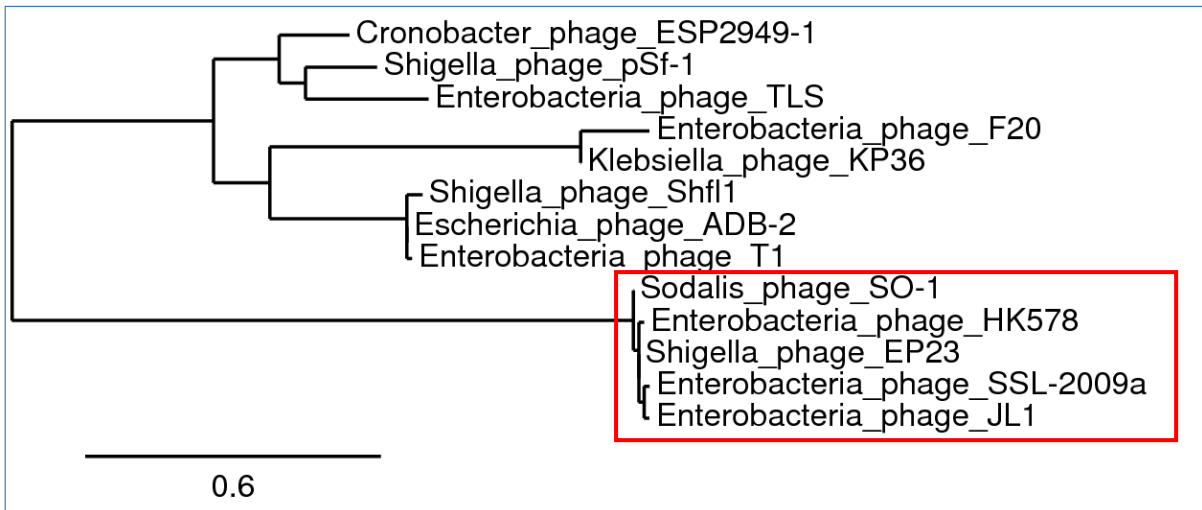


Figure 3. Phylogenetic analysis, using phylogeny.fr of the minor tail protein of members of this genus shows a peripheral relationship to the genus *Tunalikevirus*. The new genus is indicated with the red box.

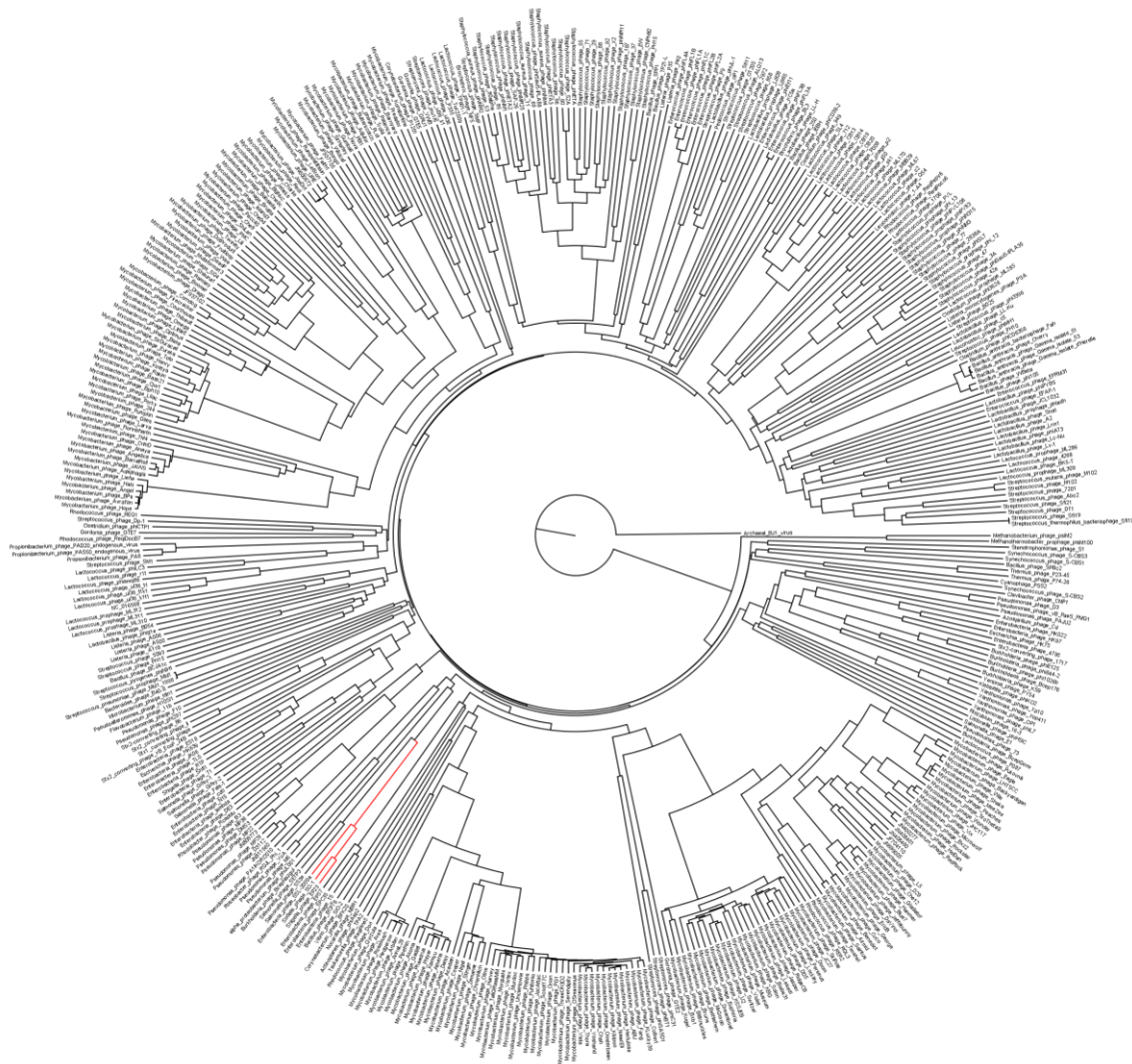


Figure 4: Phage Proteomic Tree (Rohwer & Edwards, 2002) of all the *Siphoviridae* phages in the NCBI database November 2012. Briefly, all predicted proteins sequences are compared with all others and a length-corrected protein distance matrix was calculated based on CLUSTALW alignment of sequences with a BLASTP e value < 0.001, with missing protein penalties of 10 and gap extension penalties of 0.20 (4). The tree was generated using FITCH. The proposed genus is in red. The scale bar represents protein distances of 2.0. Phage HK578 was not included in this tree, but will fall in this clade based on the high degree of DNA and protein similarity with SO1 and EP23 which are included.

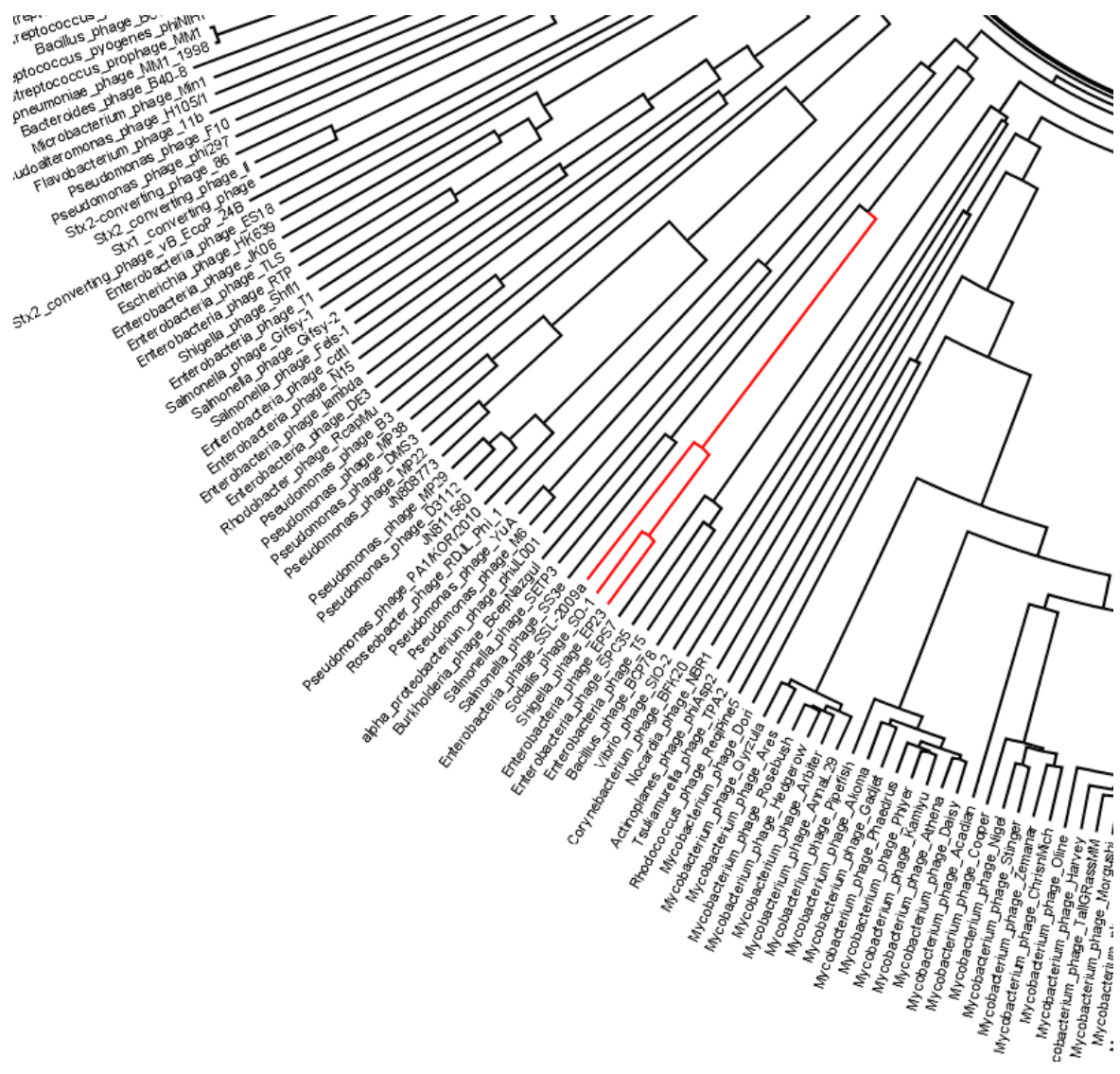


Figure 5: Fragment of the phylogenetic tree of Figure 4, zoomed in on the proposed genus.