

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:	2015.010a-dB			(to be completed by ICTV officers)			
Short title: To create one (1) new genus, <i>Nonanavirus</i> , including two (2) new species within the Family <i>Siphoviridae</i> . (e.g. 6 new species in the genus <i>Zetavirus</i>) Modules attached $1 \times 2 \times 3 \times 4 \times 5 \times 6 \times 100$ (modules 1 and 10 are required) $1 \times 2 \times 3 \times 4 \times 5 \times 100$							
Author(s):							
Andrew M. Kropinski – University of Guelph (Canada) Evelien M. Adriaenssens – University of Pretoria (South Africa) Andrea I. Moreno Switt – Center of Veterinary Medicine at Universidad Andrés Bello (Chile) Jens Kuhn – National Institutes of Health (U.S.A.)							
Corresponding author with e	e-mail address:						
Andrew M. Kropinski Phage.	Canada@gmail.	com					
List the ICTV study group(s)	that have seen	n this pro	posal:				
A list of study groups and contact http://www.ictvonline.org/subcomm in doubt, contact the appropriate schair (fungal, invertebrate, plant, pvertebrate viruses)	mmittees.asp . If the subcommittee 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 <i>Nonanavirus</i> rather than <i>Nonanalikevirus</i> since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating " <i>like</i> " from phage genus names.							
Date first submitted to ICTV: Date of this revision (if different	· · · · · · · · · · · · · · · · · · ·						
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	201	5.010aB	(assigned by ICTV officers)			
To crea	ate 2 no	ew species within:				
Genus: <i>Nonanavirus</i> (new) Subfamily:			Fill in all that apply. • If the higher taxon has yet to be created (in a later module, below) write			
F	amily: Order:	Siphoviridae Caudovirales		 "(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)		
Salmonella virus 9NA Salmonella virus SP069		Salmonella phage 9 Salmonella phage S		KJ802832 KC139649		

Reasons to justify the creation and assignment of the new species:

- Explain how the proposed species differ(s) from all existing species.
 - o If species demarcation criteria (see module 3) have previously been defined for the genus, **explain how the new species meet these criteria**.
 - o 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

Salmonella Typhimurium phage 9NA was isolated by P. H. Mäkelä (Central Public Health Laboratory, Helsinki, Finland) and studied by electron microscopy by Wollin (1). It has a "symmetrical head, which was about 60 nm long and about 60 nm wide, was attached an approximately 150-nm-long thin, noncontractile tail. A 30-nm-wide baseplate-like structure was attached to the distal end of the tail," thus placing it in the family *Siphoviridae*. It wasn't sequenced until 2014 (2). Its physico-chemical characteristics are listed in Table 1.

In 2013, Moreno Switt et al. (3) isolated and partially sequenced two *Salmonella* Newport phages, FSP SP-062 and FSP SP-069, which she subsequently proposed as the first members of a new genus: *Sp062likevirus* (4), but because its genome is incomplete we have chosen 9NA as the type phage. Casjens et al. (2) remarked on the relatedness of these three phages.

The relatedness of these two phages was confirmed using BLASTN; progressiveMauve (Fig. 1); and, by phylogenetic analysis (5) of their major capsid proteins (Fig.2).

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 *Nonanavirus* rather than *Nonanalikevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" from phage genus names.

MODULE 3: NEW GENUS

creating a new genus

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

Code	201	5.010bB	(assigned by ICTV officers)		
To create a	a new	genus within:		Fill in all that apply.	
Subfar	mily:			If the higher taxon has yet to be created	
Far	mily:	Siphoviridae		(in a later module, below) write "(new)" after its proposed name.	
O	rder:	Caudovirales		 If no family is specified, enter "unassigned" in the family box 	

naming a new genus

Code	2015.010cB	(assigned by ICTV officers)
To name the new genus: Nonanavirus		

Assigning the type species and other species to a new genus

Assigning the type species and other species to a new genus						
Code	2015.010dB	(assigned by ICTV officers)				
To designa	To designate the following as the type species of the new genus					
Salmonella virus 9NA		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: 2						

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, CoreGenes (1) (Table 1), progressiveMauve alignment (2) (Fig. 2) and phylogenetic analyses (3) (Fig. 3) all indicate that the proposed genus, Nonanavirus, is cohesive and distinct from the other genera of viruses.

Origin of the new genus name:

Named after the first phage of its type to be sequenced: Salmonella phage 9NA (1)

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.** Wollin R, Eriksson U, Lindberg AA. 1981. *Salmonella* bacteriophage glycanases: endorhamnosidase activity of bacteriophages, P27, 9NA, and KB1. J. Virol. **38:**1025–1033.
- **2.** Casjens SR, Leavitt JC, Hatfull GF, Hendrix RW. Genome Sequence of *Salmonella* Phage 9NA. Genome Announc. 2014; 2(4). pii: e00531-14.
- **3.** Moreno Switt AI, Orsi RH, den Bakker HC, Vongkamjan K, Altier C, M. 2013. Genomic characterization provides new insight into *Salmonella* phage diversity. BMC Genomics **14:**481.
- 4. Switt AI, Sulakvelidze A, Wiedmann M, Kropinski AM, Wishart DS, Poppe C, Liang Y. *Salmonella* phages and prophages: genomics, taxonomy, and applied aspects. Methods Mol Biol. 2015; 1225:237-87.
- 5. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One. 2010; 5(6):e11147.
- 6. 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.

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 two phages belonging to the Ssp2virus

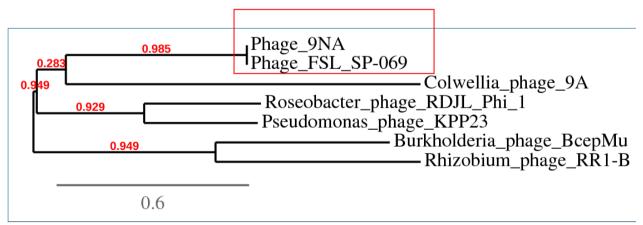
Phage	Genome	Genome	No. CDS	DNA (%
	length (bp)	(mol%G+C)		sequence
				identity)*
9NA	52,869	41.9	84	100
FSL SP-	>56,632	42.8	unknown	75
069 **				

^{*} Determined using BLASTN; ** This phage is >99% identical in sequence to *Salmonella* phage FSL SP-062 (KC139634), as shown using EMBOSS Stretcher

Fig. 1. progressiveMauve alignment of the annotated genomes of 9NA (top) and FSL SP-069 (bottom) (5). 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. 2. Phylogenetic analysis of major capsid proteins of nonanaviruses and variety of other siphovirus major capsid proteins constructed using "one click" at phylogeny.fr (6). "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."



 $\textbf{Figure 1:} \textit{Phylogenetic tree (the branch length is proportional to the number of substitutions per \textit{site})}.$