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:	***2015.051a-dB***	(to be completed by ICTV officers)

Short title: Create one (1) new genus, *Nonagvirus,* including four (4) new species within the family *Siphoviridae*
(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** ☒

Author(s):

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Andrey V. Letarov - Winogradsky Institute of Microbiology (Russia)

Corresponding author with e-mail address:

Andrew Kropinski Phage.Canada@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 Study Group comments (if any) and response of the proposer:

Please note that we have chosen to refer to this new genus as *Nonagvirus* rather than *Nonaglikevirus* 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: June 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	***2015.051aB***	(assigned by ICTV officers)

To create 4 new species within:

Genus:	***Nonagvirus* (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.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Escherichia virus 9g	Enterobacteria phage 9g	KJ419279
Escherichia virus JenK1	Enterobacteria phage JenK1	KP719134
Escherichia virus JenP2	Enterobacteria phage JenP2	KP719133
Escherichia virus JenP1	Enterobacteria phage JenP1	KP719132

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. Each of the proposed species differs from the others with 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	***2015.051bB***	(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:	***Siphoviridae***	
Order:	***Caudovirales***	

naming a new genus

Code	***2015.051cB***	(assigned by ICTV officers)

To name the new genus: *Nonagvirus*

Assigning the type species and other species to a new genus

Code	***2015.051dB***	(assigned by ICTV officers)

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

Escherichia virus 9g	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:**

4

Reasons to justify the creation of a new genus:

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

All four of these phages were isolated from animal fecal matter in Denmark or Russia (9g) against *Escherichia coli*. Only phage 9g has been the subject of a publication (4). "Phage 9 g has a slightly elongated capsid 62 × 76 nm in diameter and a non-contractile tail about 185 nm long." (Figure 1) (4). It has an unusual "carrier state" association with its host.

A unique feature of their gene complement is the presence of a complete queosine biosynthetic operon: queuosine tRNA-ribosyltransferase, GTP cyclohydrolase, 6-carboxy-5,6,7,8-tetrahydropterin synthase, 7-cyano-7-deazaguanine synthase; a feature that they share only with the *Seuratvirus*. This may be the reason why the DNA is resistant to most common restriction endonucleases.

Kullikov et al. "we propose to consider it as the representative of a novel genus of the Siphoviridae family." (4)

A phylogenetic analysis (3) of the large subunit terminase and major capsid protein (Fig. 3) together with whole genome BLASTN analysis reveal that these phages are related to the *Seuratvirus*. At present, due to ongoing discussions by the committee on what constitutes, in

molecular terms, a subfamily and higher taxons, we will not be proposing a taxonomic union of the *Seuratvirus* and *Nonagvirus*.

The average genome characteristics of the members of this genus are: genome size, 59.5 kb; mol%G+C, 43.6; encoding: 82 proteins and 0 tRNAs. Phage JenP1, JenP2 and JenK1 contain a module not found in 9g which affects the statistics on this genus.

Origin of the new genus name:

Escherichia phage 9g

Reasons to justify the choice of type species:

The first fully sequenced member of this genus (4)

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. 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.
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. Kulikov EE, Golomidova AK, Letarova MA, Kostryukova ES, Zelenin AS, Prokhorov NS, Letarov AV. Genomic sequencing and biological characteristics of a novel *Escherichia coli* bacteriophage 9g, a putative representative of a new *Siphoviridae* genus. Viruses. 2014;6(12):5077-92.

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. Electron micrograph of negatively stained phage 9g (A. Letarov)

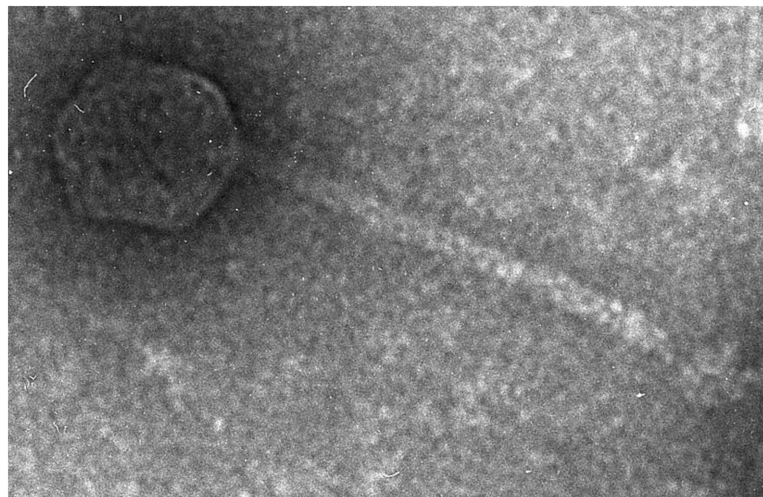

Table 1. Properties of the three phages belonging to the genus *Nonagvirus*; and, the type species of the *Seuratvirus* for comparison

Phage	GenBank Accession No.	Genome size (kb)	Genome (mol% G+C)	No. CDS	No. tRNAs	DNA (% sequence identity) *	% Homolog-ous proteins **
9g	KJ419279	56.70	43.9	71	0	100	100
JenP1	KP719132	60.75	43.2	87	0	79	91.6
JenP2	KP719133	59.80	43.2	87	0	82	95.8
JenK1	KP719134	60.75	43.9	86	0	93	94.4
Seurat	KM236243	56.78	44.6	88	0	18	62.0

* Determined using BLASTN; ** Determined using CoreGenes (2);

Fig. 2. progressiveMauve alignment (1) of the annotated genomes of members of the *Nonagvirus* genus – from top to bottom: 9g, JenK1, JenP1 and JenP2 . 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). N.B. The genomes are not collinear.

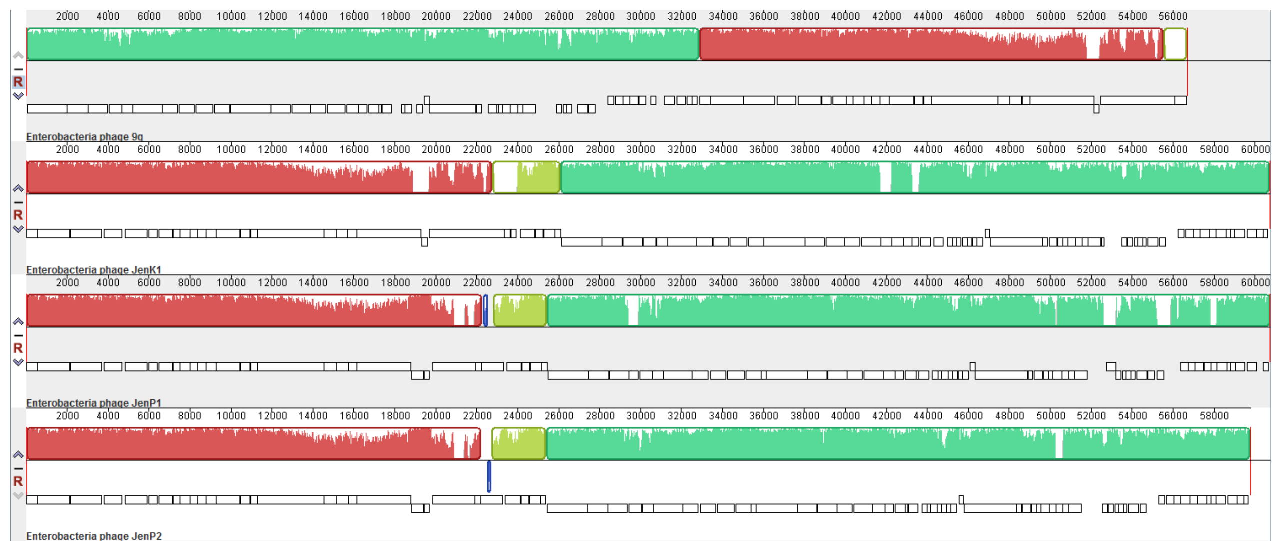

Fig. 3. Phylogenetic analysis of (A) the terminase, large subunit proteins add (B) the major capsid protein of *Nonagvirus* and variety of other phages constructed using “one click” at phylogeny.fr (3). "The "One Click mode" targets users that do not wish to deal with program and parameter selection. 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."

A. Terminase, large subunit

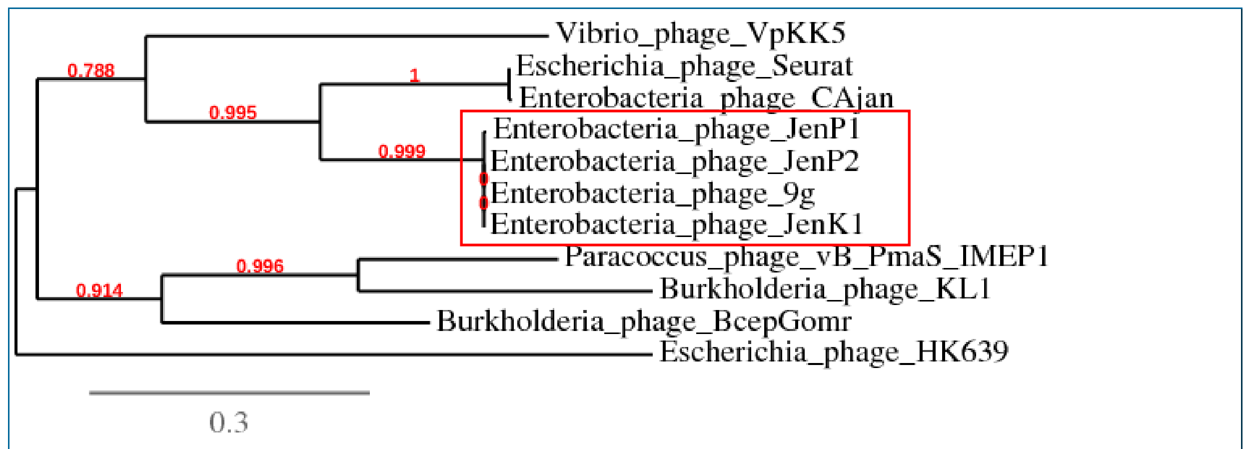

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

B. Major capsid protein

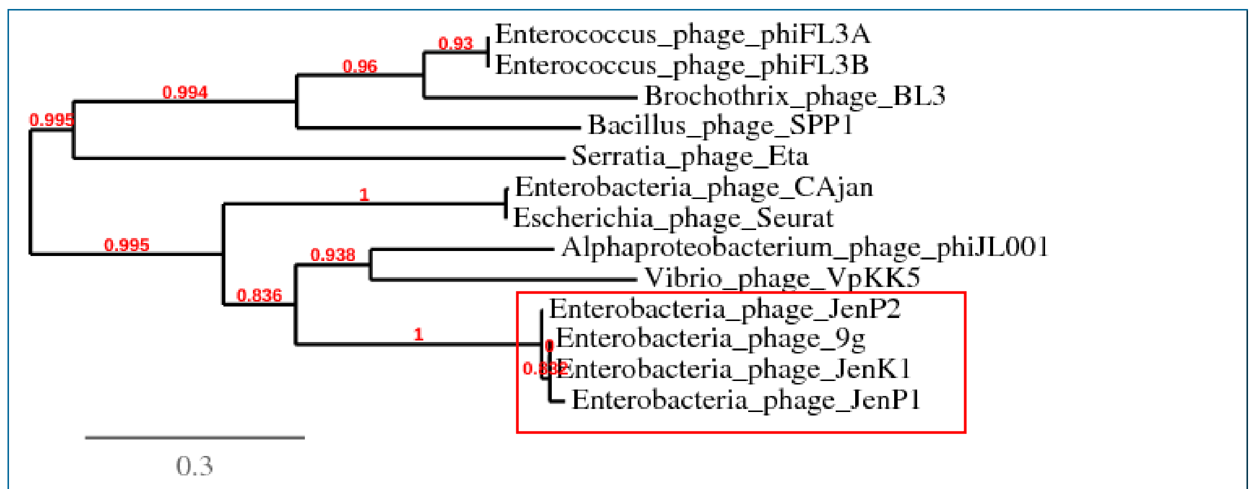

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*