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:	***2015.026a-rB***	(to be completed by ICTV officers)

Short title: To amend the description of the genus *Microvirus*; and, create three (3) new genera including 14 new species, within one (1) new subfamily, *Bullavirinae*.
(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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Corresponding author with e-mail address:

Andrew M. 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)	Bacterial & Archaeal Virus Subcommittee

ICTV Study Group comments (if any) and response of the proposer:

Please note that the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" and "*Phi*" from phage genus names, except where there was sufficient historical precedence for keeping this prefix.

Date first submitted to ICTV: June 2015
Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2015.026aB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	***Bullavirinae*** **(new)**
Family:	***Microviridae***
Order:	

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	***2015.026bB***	(assigned by ICTV officers)

To name the new genus: *Phix174microvirus*

Assigning the type species and other species to a new genus

Code	***2015.026cB***	(assigned by ICTV officers)

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

Escherichia phage phiX174 (proposed name ***Escherichia virus phiX174***)

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:**

1

Reasons to justify the creation of a new genus:

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

Whole genome BLASTN analysis, progressiveMauve alignment (1) (Fig. 4) and phylogenetic analyses (3) (Fig. 2) all indicate that the proposed genus, *Phix174microvirus*, is cohesive and distinct from the other genera of viruses.

Origin of the new genus name:

Named after *E.coli* phage phiX174

Reasons to justify the choice of type species:

First representative of this type of phage.

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 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	***2015.026dB***	(assigned by ICTV officers)

To create 7 new species within:

Genus:	***Alpha3microvirus* (new)**
Subfamily:	***Bullavirinae* (new)**
Family:	***Microviridae***
Order:	

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 WA45	Escherichia phage WA45	DQ079874
Escherichia virus NC35	Escherichia phage NC35	DQ079872
Escherichia virus ID21	Escherichia phage ID21	DQ079870
Escherichia virus NC28	Escherichia phage NC28	DQ079875
Escherichia virus NC29	Escherichia phage NC29	DQ079879
Escherichia virus ID32	Escherichia phage ID32	DQ079871
Escherichia virus ID62	Escherichia phage ID62	DQ079876

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

Please note that we have chosen to refer to this new genus as *Alpha3microvirus* rather than *Alpha3likevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" and "*Phi*" from phage genus names.

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	***2015.026eB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	***Bullavirinae* (new)**
Family:	***Microviridae***
Order:	

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	***2015.026fB***	(assigned by ICTV officers)

To name the new genus: *Alpha3microvirus*

Assigning the type species and other species to a new genus

Code	***2015.026gB***	(assigned by ICTV officers)

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

Escherichia phage alpha3 (proposed name ***Escherichia virus alpha3***)

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:**

3

Reasons to justify the creation of a new genus:

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

Whole genome BLASTN analysis, progressiveMauve alignment (2) (Fig. 5) and phylogenetic analyses (3) (Fig. 2) all indicate that the proposed genus, *Alpha3microvirus*, is cohesive and distinct from the other genera of viruses.

Origin of the new genus name:

Derived from first isolate *Escherichia* phage alpha3

Reasons to justify the choice of type species:

First representative of this type of phage.

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 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	***2015.026hB***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***G4microvirus*** **(new)**
Subfamily:	***Bullavirinae*** **(new)**
Family:	***Microviridae***
Order:	

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 ID52 *Escherichia virus Talmos*	Escherichia phage ID52 Escherichia phage ID2 Moscow/ID/2001	DQ079877 DQ079869

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

Please note that we have chosen to refer to this new genus as *G4microvirus* rather than *G4likevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" and "*Phi*" from phage genus names. In addition, the new species are named after their isolation host, not "Coliphage" or "Enterobacteria phage."

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	***2015.026iB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	***Bullavirinae* (new)**
Family:	***Microviridae***
Order:	

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	***2015.026jB***	(assigned by ICTV officers)

To name the new genus: *G4microvirus*

Assigning the type species and other species to a new genus

Code	***2015.026kB***	(assigned by ICTV officers)

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

Escherichia phage G4 (proposed name ***Escherichia virus G4***)	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:**

3

Reasons to justify the creation of a new genus:

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

Origin of the new genus name:

Named after *E.coli* phage G4

Reasons to justify the choice of type species:

First representative 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 4: **NEW SUBFAMILY**

creating a new subfamily

A subfamily can only be created within a family.

Code	***2015.026lB***	(assigned by ICTV officers)

To create a new subfamily within:

Family:	***Microviridae***
Order:	

If the family has yet to be created (in Module 5) please write "**(new)**" after the proposed name.

- If there is no Order, write "**unassigned**" here.

naming a new subfamily

Code	***2015.026mB***	(assigned by ICTV officers)

To name the new subfamily: *Bullavirinae*

genera and species assigned to the new subfamily

Code	***2015.026nB***	(assigned by ICTV officers)

To assign the following genera to the new subfamily:

You may list several genera here. For each genus, please state whether it is new or existing.

- If the genus is new, it must be created in Module 3
- If the genus already exists, please state whether it is currently unassigned or is to be removed from another family. If the latter, complete Module 7 to 'REMOVE' it from that family

Phix174microvirus – new

Alpha3microvirus – new

G4microvirus – new

The new subfamily 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 unassigned species that the subfamily will contain (those NOT within any of the genera listed above):**

0

Reasons to justify the creation of the new subfamily:

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

Our analysis of the current phages grouped within the genus *Microvirus* reveal that there are sufficient differences to warrant creation of three related genera, necessitating the creation of a subfamily (Figure 6). This had been noted as early as 2006 by Rokyta et al. (3) who also employed phylogenies based upon while genome alignments to define three clades: phiX174, G4 and alpha3-like viruses.

Origin of the new subfamily name:

Bulla (Latin for boss/knob/stud) to indicate the presence of the major spike protein G on the surface of these viruses (see Figure 5).

MODULE 7: **REMOVE and MOVE**

Use this module whenever an existing taxon needs to be removed:
- *Either* to abolish a taxon entirely (when only part (a) needs to be completed)
- *Or* to move a taxon and re-assign it e.g. when a species is moved from one genus to another (when BOTH parts (a) and (b) should be completed)

Part (a) taxon/taxa to be removed or moved

Code	***2015.026oB***	(assigned by ICTV officers)

To remove the following taxon (or taxa) from their present position:

Enterobacteria phage alpha3, Enterobacteria phage G4, Enterobacteria phage phiK, Enterobacteria phage phiX174 and *Enterobacteria phage St-1*

The present taxonomic position of these taxon/taxa:

Genus:	***Microvirus***
Subfamily:	
Family:	***Microviridae***
Order:	

Fill in all that apply.

If the taxon/taxa are to be abolished (i.e. not reassigned to another taxon) write "yes" in the box on the right

Reasons to justify the removal:
Explain why the taxon (or taxa) should be removed

The latter genus *Microvirus* currently contains five species - Enterobacteria phages alpha3, G4, phiK, phiX174 and St-1. BLASTN analysis reveals that this assemblage of viruses is not coherent and while these viruses are undoubtedly related they differ considerably in their overall DNA sequence identity. This had been noted as early as 2006 by Rokyta et al. (3) who also employed phylogenies based upon while genome alignments to define three clades: phiX174, G4 and alpha3-like viruses.

Part (b) re-assign to a higher taxon

Code	***2015.026pB***	(assigned by ICTV officers)

To re-assign the taxon (or taxa) listed in Part (a) as follows:

Enterobacteria phage phiX174 (proposed name *Escherichia virus phiX174*)

Genus:	***Phix174microvirus*** **(new)**
Subfamily:	***Bullavirinae*** **(new)**
Family:	***Microviridae***
Order:	

Fill in all that apply.
- If the higher taxon has yet to be created write "**(new)**" after its proposed name and complete relevant module to create it.

If no genus is specified, enter "**unassigned**" in the genus box.

Part (b) re-assign to a higher taxon

Code	***2015.026qB***	(assigned by ICTV officers)

To re-assign the taxon (or taxa) listed in Part (a) as follows:

Escherichia phage G4 (proposed name *Escherichia virus G4*)

Genus:	***G4microvirus* (new)**
Subfamily:	***Bullavirinae* (new)**
Family:	***Microviridae***
Order:	

Fill in all that apply.
- If the higher taxon has yet to be created write "**(new)**" after its proposed name and complete relevant module to create it.

If no genus is specified, enter "**unassigned**" in the genus box.

Part (b) re-assign to a higher taxon

Code	***2015.026rB***	(assigned by ICTV officers)

To re-assign the taxon (or taxa) listed in section 2015.026oB as follows:

Enterobacteria phage alpha3 (proposed name *Escherichia virus alpha3*), *Enterobacteria phage phiK* (proposed name *Escherichia virus phiK*), *Enterobacteria phage St-1* (proposed name *Escherichia virus St1*)

Genus:	***Alpha3microvirus* (new)**
Subfamily:	***Bullavirinae* (new)**
Family:	***Microviridae***
Order:	

Fill in all that apply.
- If the higher taxon has yet to be created write "**(new)**" after its proposed name and complete relevant module to create it.

If no genus is specified, enter "**unassigned**" in the genus box.

Reasons to justify the re-assignment:

- If it is proposed to re-assign species to an existing genus, please 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.
- Provide accession numbers for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

BLASTN analysis reveals that existing virus genus, *Microvirus*, is not coherent and while these viruses are undoubtedly related both genetically and structurally (Figure 5) they differ considerably in their overall DNA sequence identity. We have proposed their incorporation in newly defined genera of Modules 3 above.

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. 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.
3. Rokyta DR, Burch CL, Caudle SB, Wichman HA. Horizontal gene transfer and the evolution of microvirid coliphage genomes. J Bacteriol. 2006;188(3):1134-42.

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. The phylogenetic relationship between 42 new isolates and the five laboratory strains (phiX174, S13, G4, alpha3, and phiK) was estimated using Bayesian inference on the whole-genome alignment Posterior probabilities are given above the relevant branches. The tree is mid-point rooted for visual clarity. Reprinted with permission from (3).

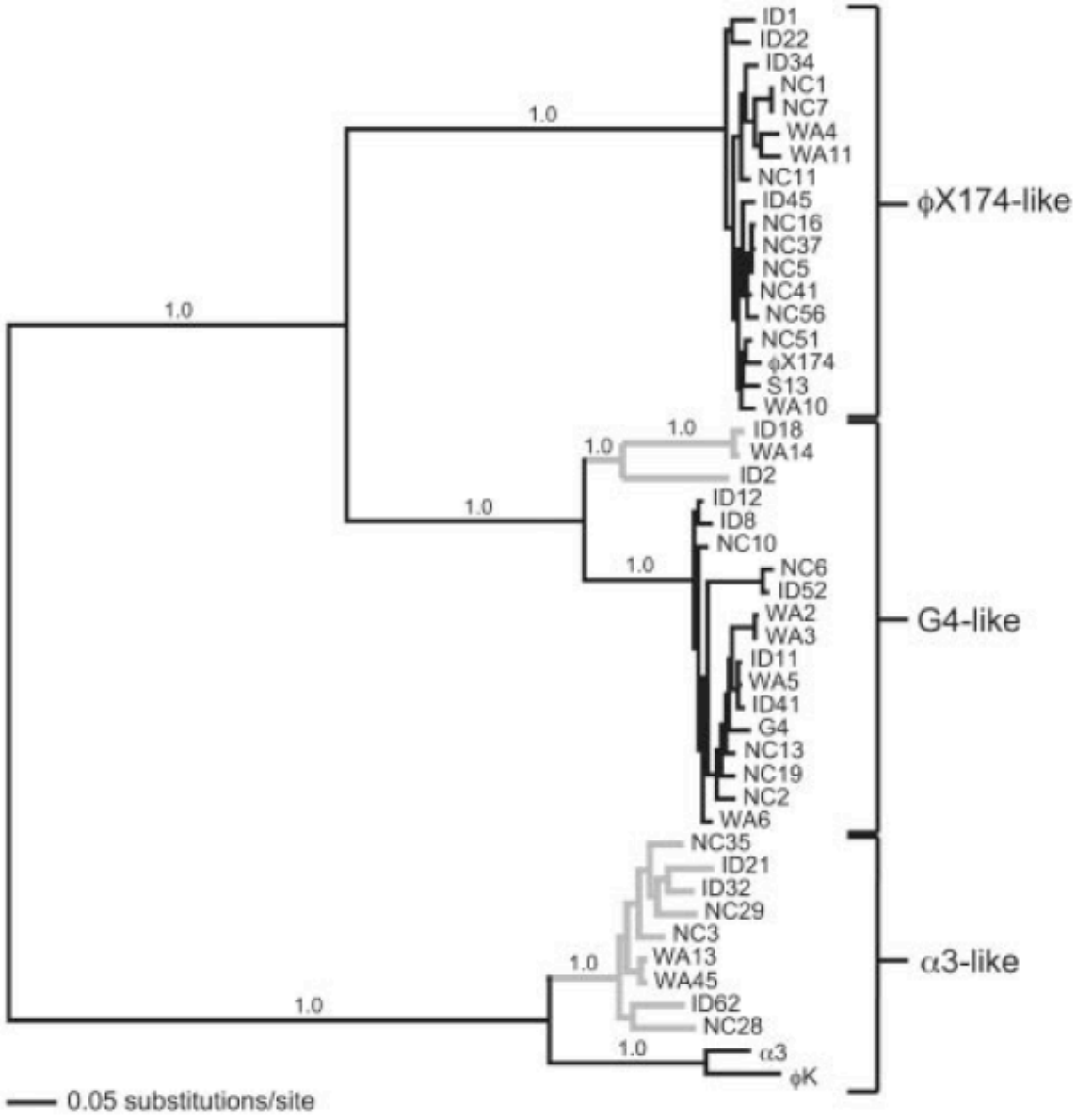


Fig. 2. Phylogenetic analysis of **A.** Major spike protein G and **B.** DNA replication protein A of members of the subfamily *Bullavirinae* constructed using "one click" at phylogeny.fr (2). "The "One Click mode" targets users that do not wish to deal with program and parameter selection. By default, 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. Major spike protein G

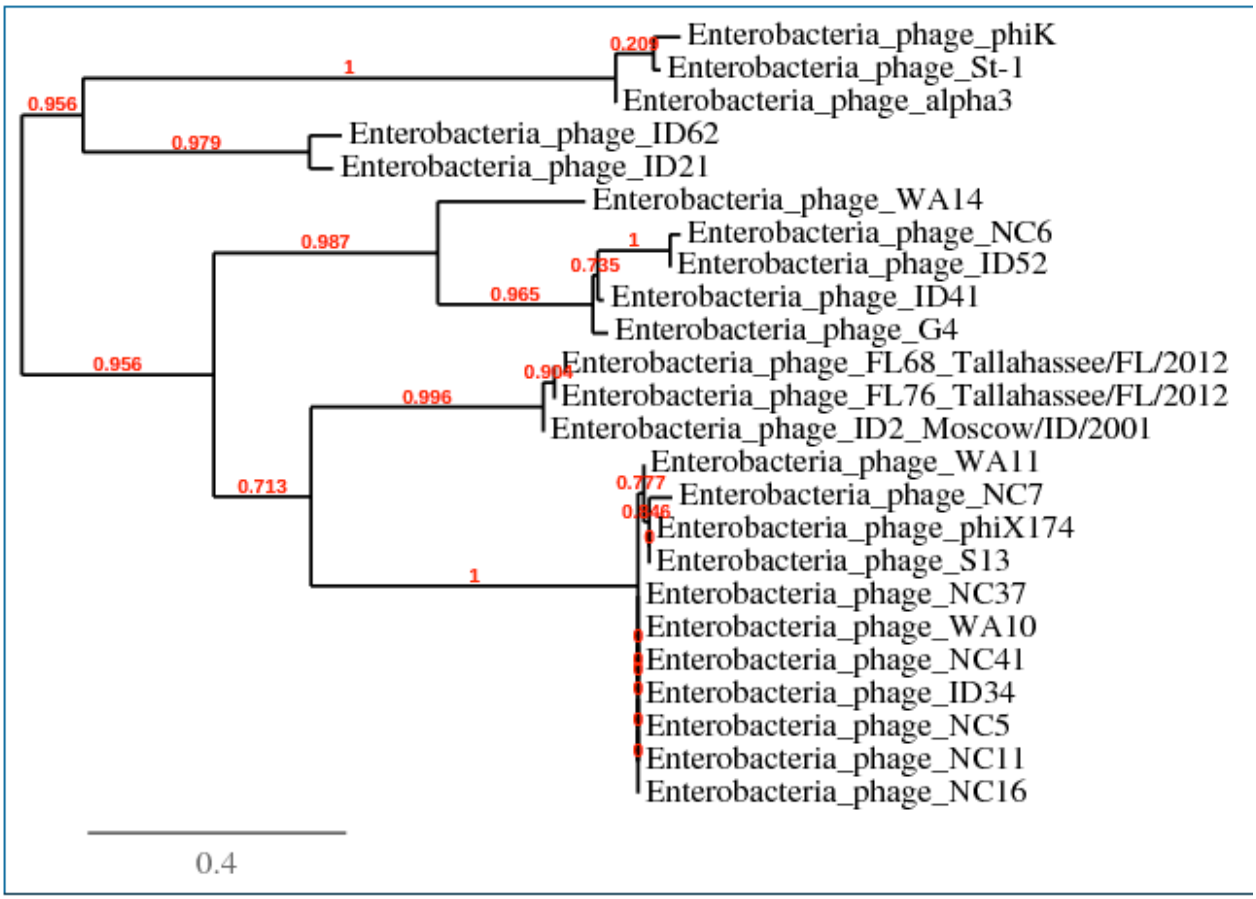


Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

B. DNA replication protein A

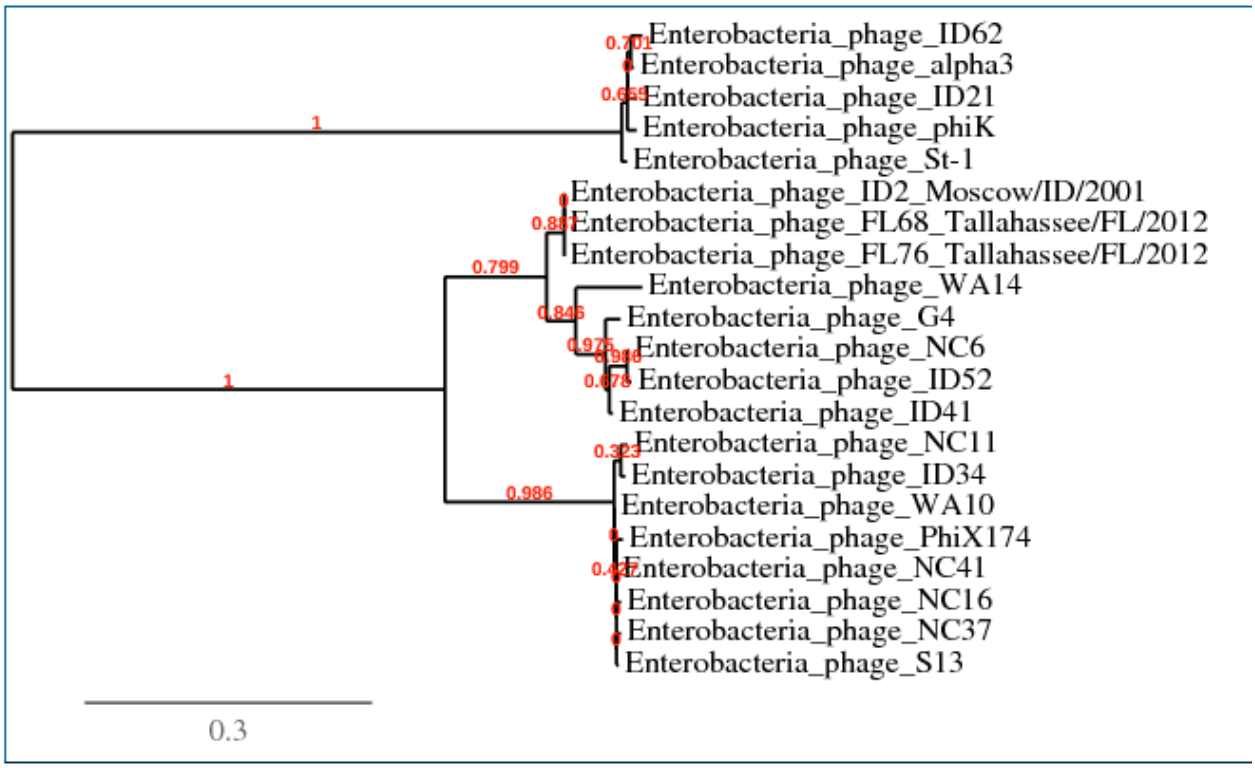


Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

Fig. 3. progressiveMauve alignment of the annotated genomes of the new members of the *Bullavirinae* subfamily – from top to bottom: alpha3, G4 and phiX174 (1). 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).

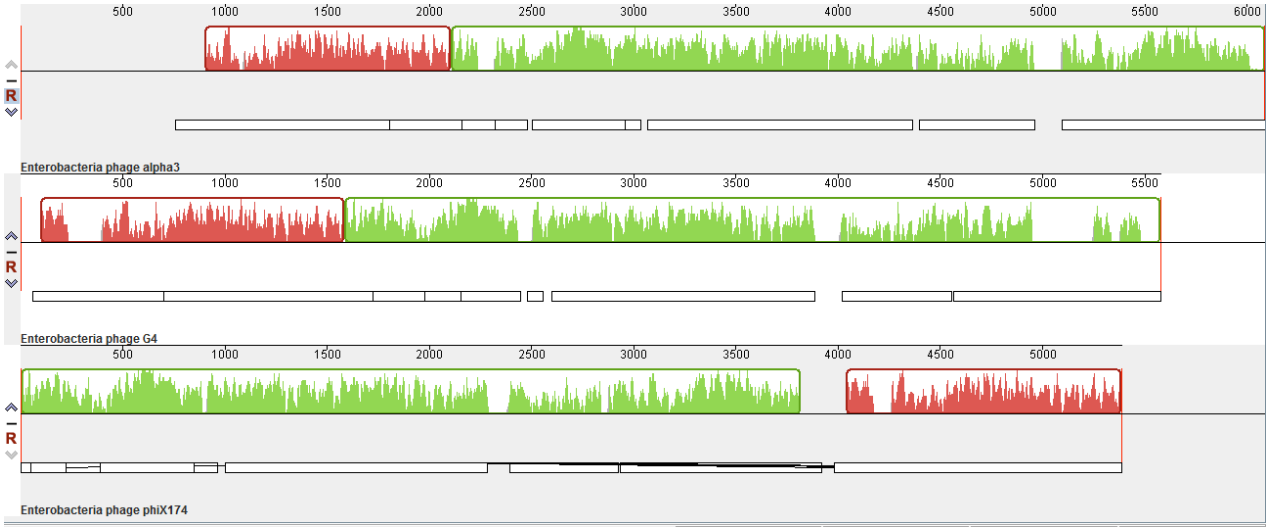
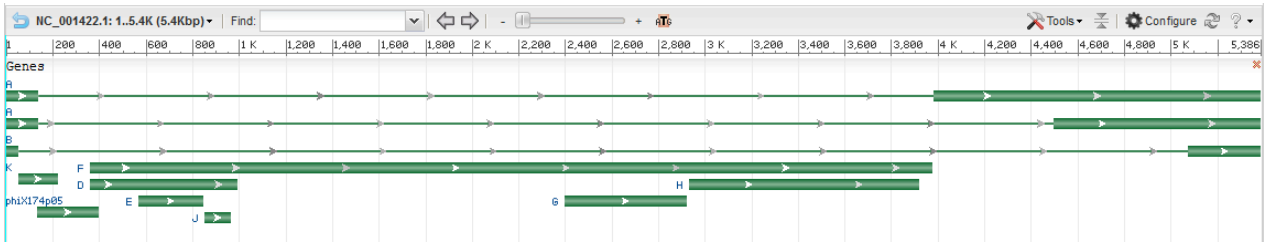
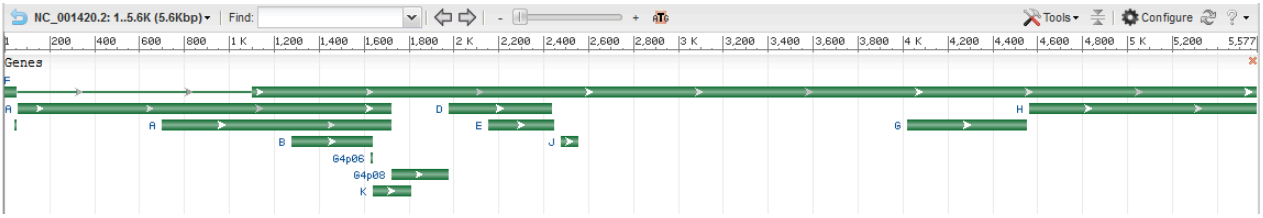


Fig. 4. Gene maps of phiX174 (**A**), G4 (**B**) and alpha3 (**C**) from NCBI genomic graphs.

A. **phiX174**



B. **G4**



C. **alpha3**

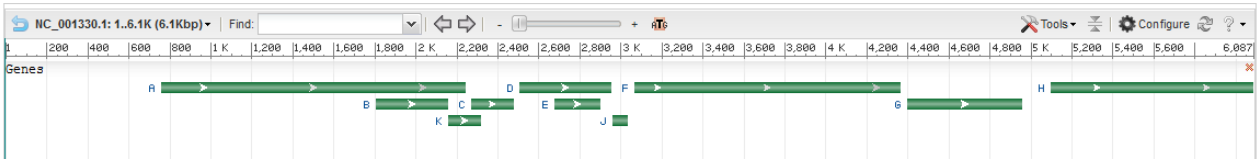


Figure 5. Structural similarity between the virions of Phix174, Alpha3 and G4. Top: Overall virions structures. Bottom: Ribbon representation of one asymmetric unit for each virion. Major capsid protein (F), magenta; major spike protein (G), pink; protein J, yellow. Note the structural differences between the major spike proteins of the three viruses. PDB accession numbers: Phix174, 2BPA; Alpha3, 1M06; G4, 1GFF. Images of the depicted virions were acquired from the VIPER database (viperdb.scripps.edu/).

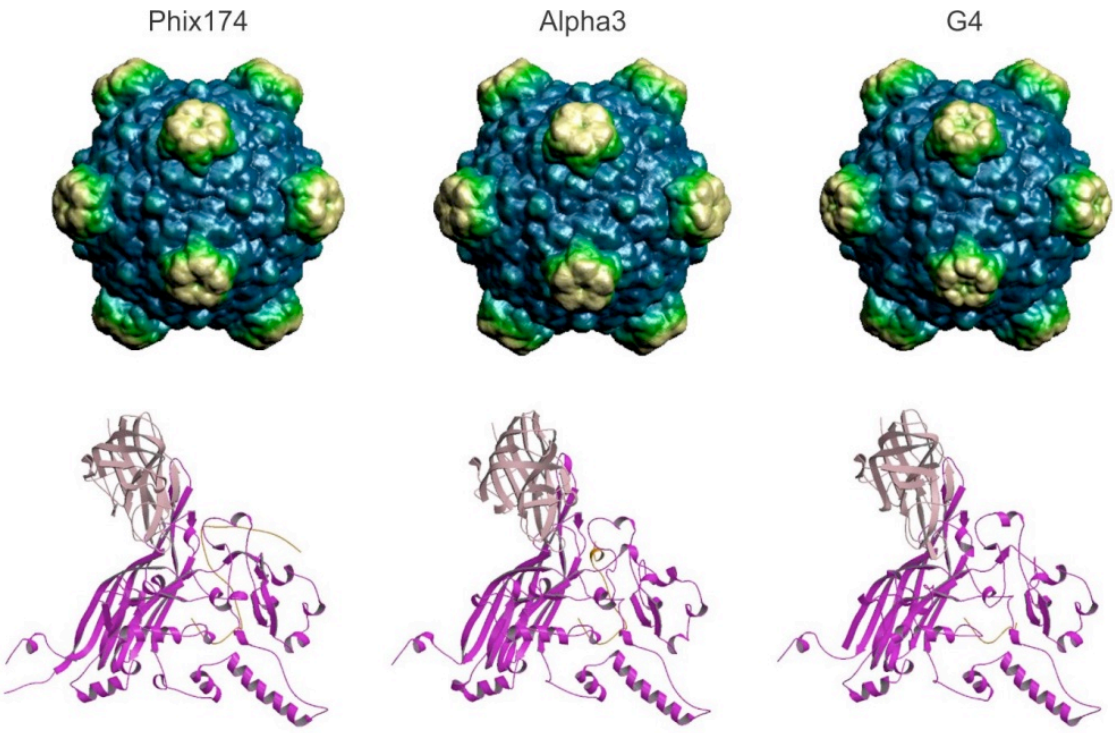


Fig 6: progressiveMauve alignment of the annotated genomes of all members of the *Bullavirinae* subfamily (1). Colored blocks indicate the regions of 1 to 1 best alignment with rearrangement breakpoints in a different random color. The degree of sequence similarity between regions is given by a similarity plot within the colored blocks with the height of the plot proportional to the average nucleotide identity (Aaron Darling, personal communication).


Enterobacteria phage phiX174
Enterobacteria phage alpha3
Enterobacteria phage St-1
Enterobacteria phage phiK
Enterobacteria phage WA45
Enterobacteria phage NC35
Enterobacteria phage ID21
Enterobacteria phage NC29
Enterobacteria phage ID32
Enterobacteria phage ID62
Enterobacteria phage G4
Enterobacteria phage ID2 Moscow/ID/2001
Enterobacteria phage ID52

Table 1. NCBI phage genomes closely related to phiX174

Phage*	Accession Number
Coliphage NC51	DQ079891.1
Bacteriophage S13	AF274751.1
Coliphage WA10	DQ079894.1
Coliphage ID45	DQ079883.1
Coliphage NC5	DQ079885.1
Coliphage NC41	DQ079890.1
Coliphage NC37	DQ079889.1
Coliphage NC16	DQ079888.1
Coliphage NC56	DQ079892.1
Coliphage NC11	DQ079887.1
Coliphage NC1	DQ079884.1
Coliphage NC7	DQ079886.1
Coliphage ID22	DQ079881.1
Coliphage ID34	DQ079882.1
Coliphage ID1	DQ079880.1
Coliphage WA4	DQ079893.1
Bacteriophage S13	M14428.1
Coliphage WA11	DQ079895.1

***Naming as per GenBank**

Table 2. NCBI phage genomes closely related to G4

Phage*	Accession Number
Enterobacteria phage G4 isolate Anc	AF454431.1
Enterobacteria phage G4 isolate G4_3_FR	JF719731.1
Genome of phage G4 (coliphage)	V00657.1
Enterobacteria phage G4 isolate G4_2_FR	JF719730.1
Enterobacteria phage G4 isolate G4_1_FR	JF719729.1
Coliphage NC13	DQ079901.1
Coliphage ID11	AY751298.1
Coliphage WA5	DQ079899.1
Coliphage ID41	DQ079903.1

Coliphage NC19	DQ079902.1
Coliphage NC2	DQ079900.1
Coliphage WA3	DQ079897.1
Coliphage WA2	DQ079896.1
Coliphage WA6	DQ079904.1
Coliphage NC10	DQ079906.1
Coliphage ID8	DQ079898.1
Coliphage ID12	DQ079905.1

***Naming as per GenBank**

Table 3. NCBI phage genomes closely related to alpha3

Phage*	Accession Number
Coliphage WA13	DQ079873
Coliphage NC3	DQ079878

***Naming as per GenBank**

Table 4. Properties of the three type viruses belonging to the subfamily *Bullavirinae*.

Phage	GenBank accession No.	Genome length (kb)	Genome (mol%G+C)	No. CDS	DNA (% sequence identity)*
phiX174	J02482	5.39	44.8	11	100
G4	V00657	5.58	45.7	11	52
alpha3	X60322	6.09	45.2	10	36

* Determined using BLASTN