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:	***2015.013a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Biquartavirus*, including one (1) new species within the family *Myoviridae*

(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):

Andrew M. Kropinski – University of Guelph (Canada)
Hans-Wolfgang Ackermann – Laval University (Canada)
Elizabeth Kutter – The Evergreen State College (USA)
Evelien M. Adriaenssens – University of Pretoria (South Africa)

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 and 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 *Biquartavirus* rather than *Quarta4rrlikevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "like" from phage genus names. Until the committee has reexamined what constitutes higher level relationships we do not want to propose a subfamily containing the *Secunda5virus* and the *Biquartavirus*.

Date first submitted to ICTV: May 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	***2015.013aB***	(assigned by ICTV officers)

To create a new species within:

Genus:	***Biquartavirus (new)***
Subfamily:	
Family:	***Myoviridae***
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)**
Aeromonas virus 44RR2	Aeromonas phage 44RR2.8t	AY375531

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	***2015.013bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Myoviridae***
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	***2015.013cB***	(assigned by ICTV officers)

To name the new genus: *Biquartavirus*

Assigning the type species and other species to a new genus

Code	***2015.013dB***	(assigned by ICTV officers)

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

Aeromonas virus 44RR2	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

The current ICTV taxonomy lumps together three *Aeromonas* phages (25, 31 and 44RR2.8t) together with *Escherichia coli* phage T4 and its close relatives. Our analysis of the latter phages indicated that they are sufficiently distinct to warrant division into seven new genera. At the DNA level, phage 25 and T4 share <12% overall sequence identity; while at the protein level they share 47.9% homologous proteins. Therefore while they are related they are sufficiently different to warrant inclusion in a separate genus. *Aeromonas* phage 44RR2.8t was first described in 1968 (6) while phage 31 (AY962392) was described in 1970 (7). Since they share 98% sequence identity we consider them strains. It is morphologically related to coliphage T4. The genomes of this genus are characterized thusly; size: 173 kb (43.9 mol%G+C), encoding 250 proteins and 16 tRNAs.

BLASTN, CoreGenes (1) (Table 1), progressiveMauve alignment (2) (Fig. 2) and phylogenetic analyses (3) (Fig. 3) all indicate that the proposed genus, *Quarta4rrvirus*, is cohesive and distinct from the other genera of viruses.

Origin of the new genus name:

Aeromonas phage 44RR2.8t

Reasons to justify the choice of type species:

The first fully sequenced member of this genus

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.

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

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

1. 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. 2013; 6:140. doi: 10.1186/1756-0500-6-140.

2. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One. 2010; 5(6):e11147.

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. Fan H, Huang Y, Mi Z, Yin X, Wang L, Fan H, Zhang Z, An X, Chen J, Tong Y. Complete Genome Sequence of IME13, a *Stenotrophomonas maltophilia* bacteriophage with large burst size and unique plaque polymorphism. J Virol. 2012; 86(20):11392-3.

5. Kim JH, Son JS, Choi YJ, Choresca CH, Shin SP, Han JE, Jun JW, Park SC. Complete genomic sequence of a T4-like bacteriophage, phiAS4, infecting *Aeromonas salmonicida* subsp. *salmonicida*. Arch Virol. 2012;157(2):391-5.

6. Paterson, W. D. 1968. Some bacteriophages specific for *Aeromonas salmonicida*. M.Sc. Thesis, University of Guelph, Ont. Canada.

7. Popoff, M. and J.-F. Vieu.1970. bactériophages et lysotypie d'*Aeromonas salmonicida*. Compt. Rend. 270:2219-2222.

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 phage 44RR2.8t negatively stained with uranyl acetate

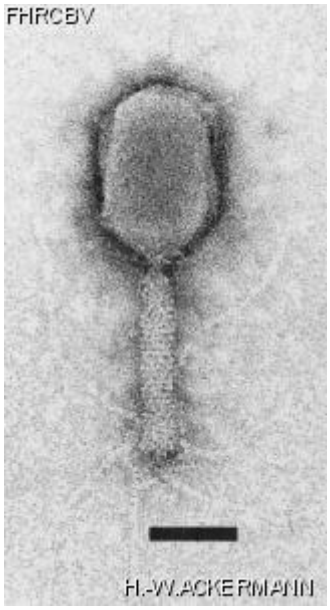

Table 1.Properties of the phages belonging to the *Biquartavirus* genus and by comparison, *Aeromonas* phage 25

Phage	GenBank Accession No.	Genome size (kb)	Genome (mol%G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)*	% Homologous proteins **
44RR2.8t	AY375531	173.59	43.9	252	17	100	100
31	AY962392	172.96	43.9	247	15	98	97.6
25	DQ529280	161.48	41.0	242	13	54	82.5

* Determined using BLASTN; ** Determined using CoreGenes (2); *** Suggests incomplete annotation; # not indicated in GenBank file

Fig. 2A. progressiveMauve alignment (1) of the annotated genomes of members of the *Biquartavirus* genus (44RR2.8t, top; and 31, bottom). 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).

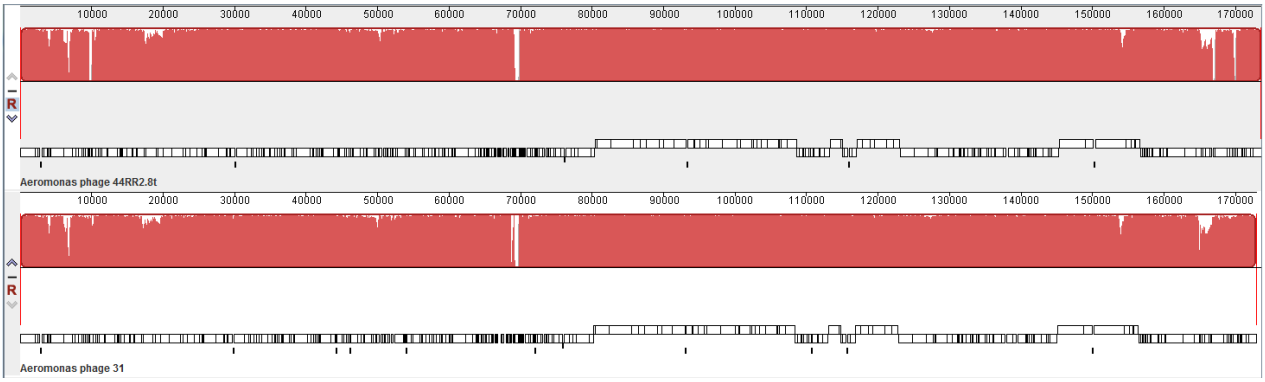

Fig. 2B. progressiveMauve alignment (1) of the annotated genomes of members of the *Aeromonas* phages 25 (top) and 44RR2.8t (bottom). Colored blocks indicate the regions of 1 to 1 best alignment with rearrangement breakpoints in a different random color. The degree of sequence similarity between regions 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).

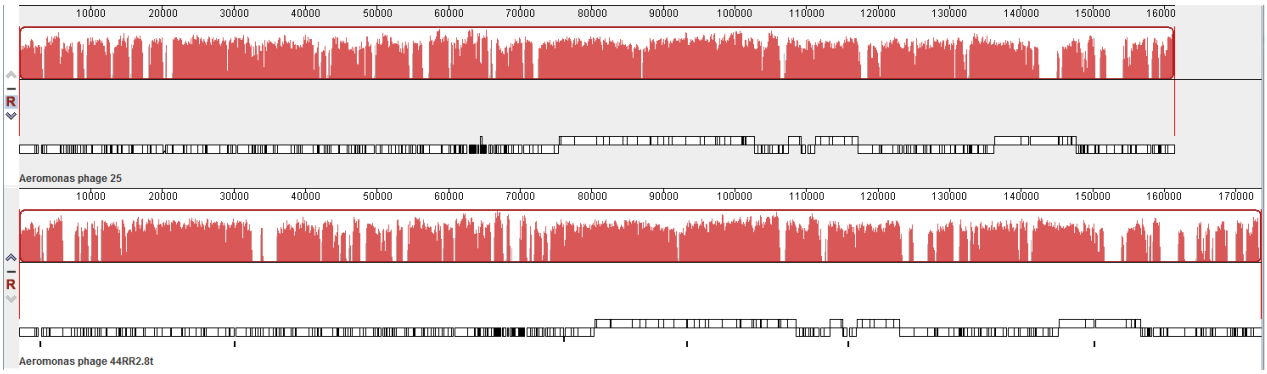

Fig. 3. Phylogenetic analysis of A. large subunit terminase protein, B. major capsid protein; and, C. tail sheath protein of quarta4rrviruses, and variety of other related phage proteins constructed using “one click” at phylogeny.fr (3). "The "One Click mode" targets users that do not wish to deal with 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. Large subunit terminase

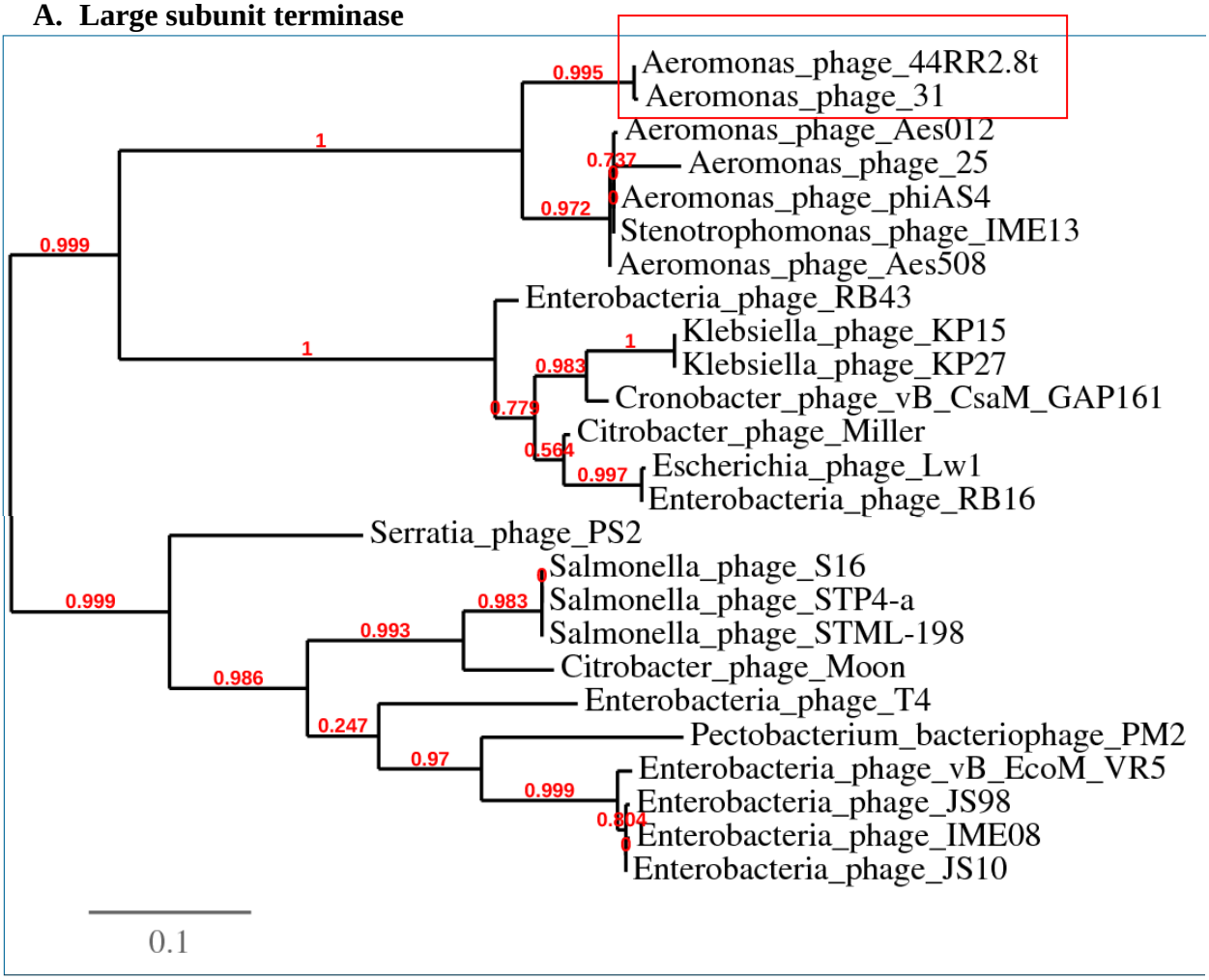

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

B. major capsid protein

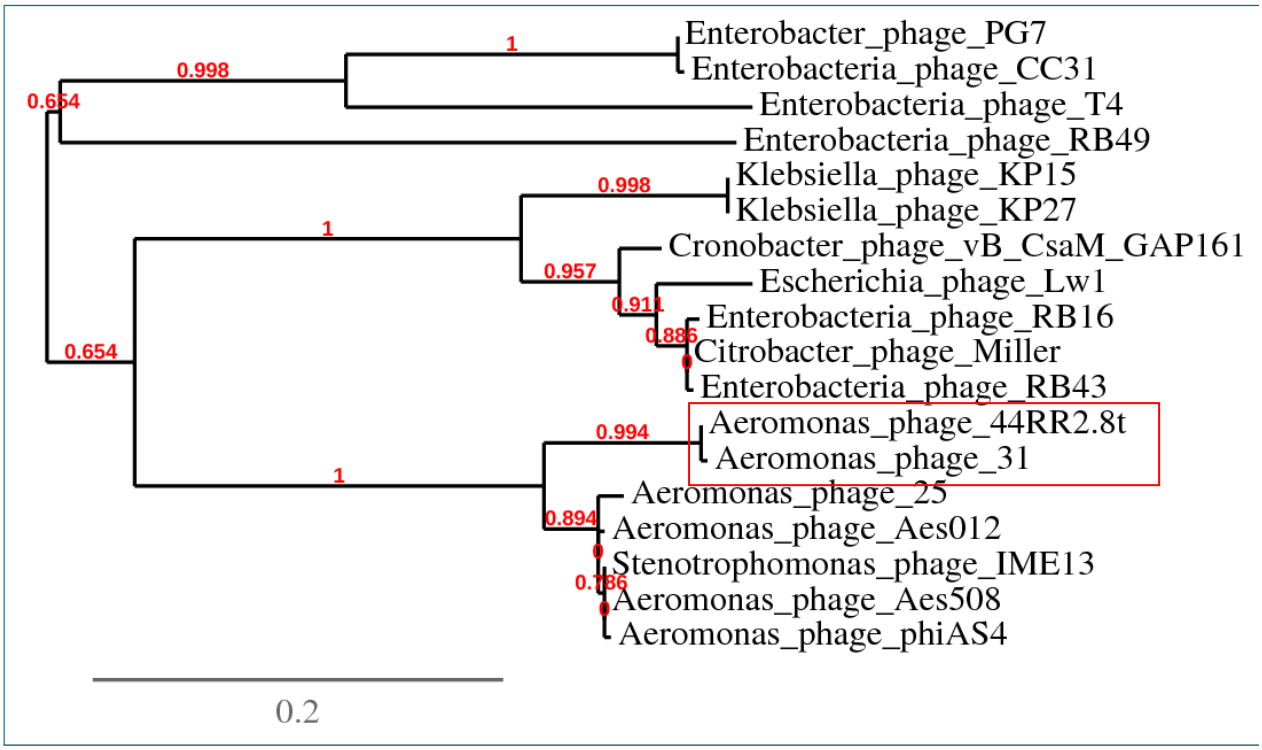

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

C. tail sheath protein

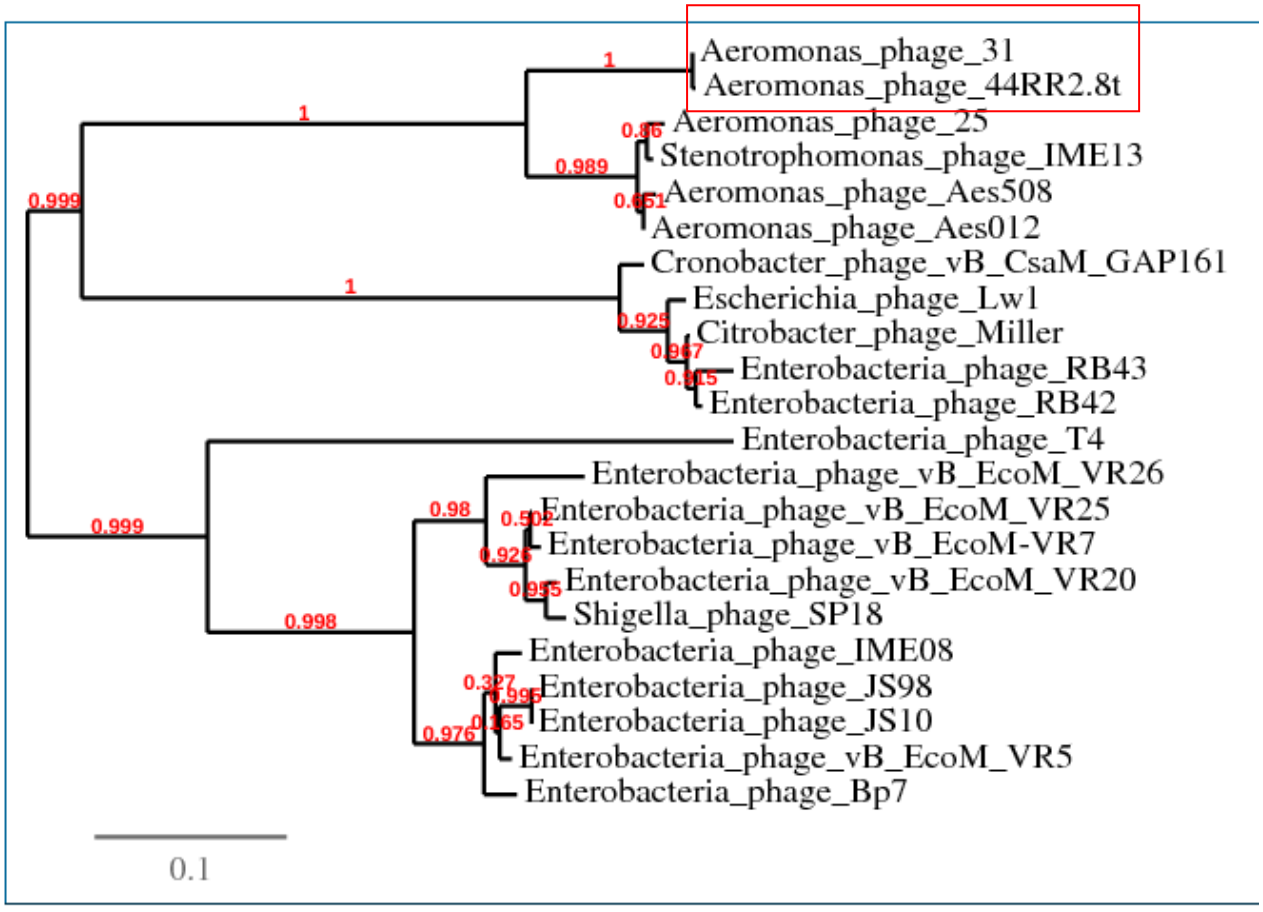

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*