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:	***2016.042a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Rsl2virus*, including two (2) new species, in the family *Myoviridae*.
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 11 are required)	**1** ☒	**2** ☒	**3** ☒	**4** ☐	**5** ☐
	6 ☐	**7** ☐	**8** ☐	**9** ☐	**10** ☒

Author(s):

Evelien M. Adriaenssens—University of Pretoria (South Africa)
Jens H. Kuhn—NIH/NIAID/IRF-Frederick, Maryland (USA)
Andrew M. Kropinski—University of Guelph (Canada)
Takashi Yamada—Hiroshima University (Japan)

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)	ICTV Bacterial and Archaeal Viruses Subcommittee

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

Date first submitted to ICTV: June 2016
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	***2016.042aB***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Rsl2virus***
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)**
Ralstonia virus RSL2	**Ralstonia phage RSL2**	**AP014693.1**
Ralstonia virus RSF1	**Ralstonia phage RSF1**	**AP014927.1**

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 11

We have chosen 95% DNA sequence identity as the criterion for demarcation of species in this new genus. The members of each of the proposed species differ from those of other species by 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	***2016.042bB***	(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	***2016.042cB***	(assigned by ICTV officers)

To name the new genus: *Rsl2virus*

Assigning the type species and other species to a new genus

Code	***2016.042dB***	(assigned by ICTV officers)

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

Ralstonia virus RSL2

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 11

"Jumbo phages infecting *Ralstonia solanacearum* were isolated in Thailand (ϕRSL2) and Japan (ϕRSF1)" [5]. The morphology indicates that Ralstonia phage RSF1 is a member of the *Myoviridae* (Fig. 1) possessing an icosahedral head (diameter: approximately 115 nm), and a long contractile tail (length:180 nm; width: 25 nm). The authors noted an evolutionary relationship to Pseudomonas phage PhiKZ [5].

Gegenees BLASTN and TBLASTX analyses (Fig. 2, Table 1), phylogenetic analyses (Fig. 3) [3], and progressiveMauve (Fig. 4) all indicate that the proposed genus, *Rsl2virus*, is cohesive and distinct from other genera. On average, the genomes of members of this genus are 223.4 kb in length (52.2 mol% G+C), and encode 227 proteins and 0 tRNAs.

Origin of the new genus name:

Ralstonia virus RSL2

Reasons to justify the choice of type species:

Under usual circumstances, this genus would be called *Rsfunavirus* after phage RSF1, which was the first sequenced member of this taxon, but it was thought that this name is too similar to

Rslunavirus (which is a related genus) and would lead to confusion.

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. The members of each of the proposed species differ from those of other species by more than 5% at the DNA level as confirmed with the BLASTN algorithm.

MODULE 11: **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. 2013; 6:140. doi: 10.1186/1756-0500-6-140.

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. Agren J, Sundström A, Håfström T, Segerman B. Gegenees: fragmented alignment of multiple genomes for determining phylogenomic distances and genetic signatures unique for specified target groups. PLoS One. 2012;7(6):e39107.

5. Bhunchoth A, Blanc-Mathieu R, Mihara T, Nishimura Y, Askora A, Phironrit N, Leksomboon C, Chatchawankanphanich O, Kawasaki T, Nakano M, Fujie M, Yamada T. Two asian jumbo phages, phiRSL2 and phiRSF1, infect *Ralstonia solanacearum* and show common features of phiKZ-related phages. Virology. 2016;494:56-66.

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 (2% ammonium molybdate pH 7.5) Ralstonia phage RSL2 (provided by: Dr. Takashi Yamada, Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima, 739-8530, Japan).

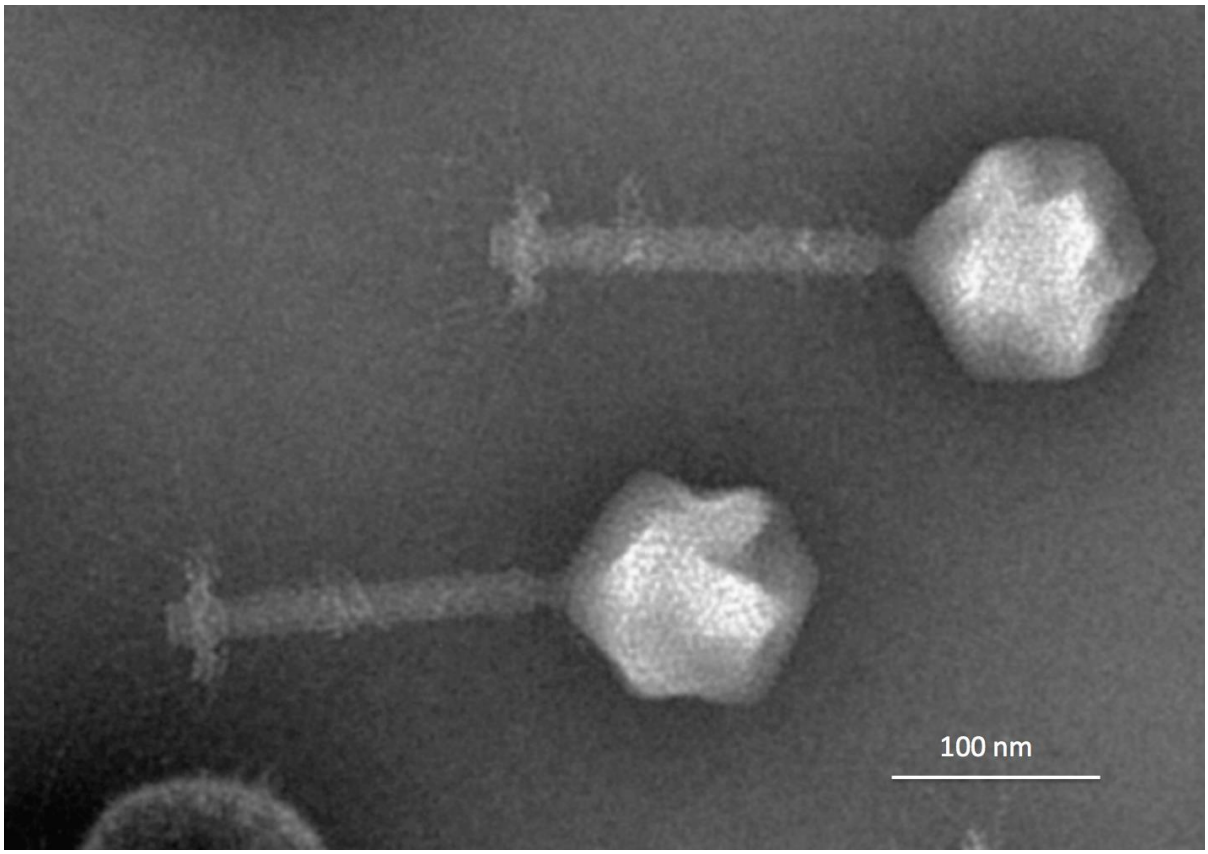


Table 1. Properties of the two phages belonging to the genus *Rsl1virus*.

Ralstonia phage	GenBank Accession No.	RefSeq No.	Genome length (kb)	Genome (mol% G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)*	Protein (% sequence identity) **
RSL2	AP014693.1	NC_028950.1	223.93	52.1	224	0	100	100
RSF1	AP014927.1	NC_028899.1	222.89	52.3	230	0	72	93.3

* Determined using BLASTN; ** Determined using CoreGenes [2];

Fig. 2. Gegenees BLASTN (Top) and TBLASTX (Bottom) analysis in accurate mode (fragment size: 200 bp; step size: 100 bp) of a subset of bacteriophages which possess genomes > 200kb. The phages boxed in black on the left diagram should be considered as strains.

PHAGE	ACCESSION NO.	AF399011.1	JX233784.1	NC_010821.1	NC_028999.1	KU726251.1	JN641803.1	JX316028.1	JX094500.1	NC_016571.1	NC_017975.1	NC_023557.1	KF623294.1	NC_028950.1	AP014927.1	KC131130.1	NC_007623.1
Pseudomonas phage phiKZ	AF399011.1	100.0	95.8	28.9	34.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas phage PA7	JX233784.1	96.0	100.0	33.6	31.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas phage 201phi2-1	NC_010821.1	31.2	31.0	100.0	31.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas phage PhiPA3	NC_028999.1	34.8	27.9	31.9	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Salmonella phage SEGD1	KU726251.1	0.0	0.0	0.0	0.0	100.0	97.2	44.1	30.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Salmonella phage SPN3US	JN641803.1	0.0	0.0	0.0	0.0	97.1	100.0	45.4	31.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Erwinia phage phiEaH2	JX316028.1	0.0	0.0	0.0	0.0	45.0	45.6	100.0	28.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cronobacter phage CR5	JX094500.1	0.0	0.0	0.0	0.0	30.9	32.2	27.6	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas phage OBP	NC_016571.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Halocynthia phage JM-2012	NC_017975.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
Erwinia phage Ea35-70	NC_023557.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0
Erwinia phage PhiEaH1	KF623294.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	14.8	0.0	0.0	0.0
Ralstonia phage RSL2	NC_028950.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.8	100.0	59.8	0.0	0.0
Ralstonia phage RSF1	AP014927.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	60.1	100.0	0.0	0.0
Vibrio phage VP4B	KC131130.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0	19.8
Pseudomonas phage EL	NC_007623.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	23.3	100.0

PHAGE	ACCESSION NO.	AF399011.1	JX233784.1	NC_028999.1	NC_010821.1	NC_028950.1	AP014927.1	KF623294.1	NC_023557.1	NC_017975.1	NC_016571.1	KC131130.1	NC_007623.1	JX094500.1	JX316028.1	JN641803.1	KU726251.1
Pseudomonas phage phiKZ	AF399011.1	100.0	92.3	34.3	29.8	17.3	17.4	16.7	17.2	15.4	15.9	15.7	15.8	16.2	16.0	16.0	16.1
Pseudomonas phage PA7	JX233784.1	96.2	100.0	34.8	30.3	17.5	17.6	16.8	17.3	15.5	15.9	15.7	15.8	16.3	16.1	16.1	16.2
Pseudomonas phage PhiPA3	NC_028999.1	32.0	31.6	100.0	32.6	17.6	17.5	17.0	17.0	14.8	15.7	15.6	16.0	16.3	16.3	16.2	16.2
Pseudomonas phage 201phi2-1	NC_010821.1	27.7	27.5	32.4	100.0	17.3	17.3	16.7	16.9	14.9	15.7	15.7	15.6	16.0	16.2	16.0	16.0
Ralstonia phage RSL2	NC_028950.1	17.9	17.8	18.7	18.3	100.0	67.0	17.7	17.7	15.1	15.9	15.8	16.0	16.8	16.8	16.7	16.7
Ralstonia phage RSF1	AP014927.1	18.1	17.9	18.6	18.4	67.2	100.0	17.9	17.7	15.0	15.8	15.8	16.0	16.7	16.7	16.6	16.6
Erwinia phage PhiEaH1	KF623294.1	17.3	17.2	18.0	17.5	17.9	17.8	100.0	18.2	15.0	15.8	15.6	16.1	17.0	17.0	16.7	16.7
Erwinia phage Ea35-70	NC_023557.1	17.0	16.8	17.3	17.2	17.2	17.1	17.3	100.0	14.8	16.9	16.5	16.7	16.4	16.4	16.2	16.2
Halocynthia phage JM-2012	NC_017975.1	16.7	16.7	16.6	16.4	16.2	16.2	16.2	16.4	100.0	15.8	16.0	15.4	15.6	15.7	15.9	15.9
Pseudomonas phage OBP	NC_016571.1	15.7	15.7	16.0	15.9	15.6	15.6	15.6	17.1	14.6	100.0	20.1	19.9	15.4	15.5	15.5	15.5
Vibrio phage VP4B	KC131130.1	15.9	15.9	16.4	16.3	16.0	15.9	15.7	17.2	15.0	21.4	100.0	22.5	15.6	15.6	15.6	15.6
Pseudomonas phage EL	NC_007623.1	16.0	15.9	16.7	16.2	15.9	16.0	16.2	17.4	14.5	21.5	23.1	100.0	15.6	15.8	15.6	15.6
Cronobacter phage CR5	JX094500.1	16.5	16.5	17.0	16.7	16.8	16.8	17.1	17.0	14.7	15.7	15.5	15.6	100.0	33.9	34.3	34.2
Erwinia phage phiEaH2	JX316028.1	16.0	15.9	16.7	16.5	16.7	16.5	16.9	16.6	14.5	15.5	15.3	15.6	32.4	100.0	51.3	50.9
Salmonella phage SPN3US	JN641803.1	16.1	16.1	16.8	16.6	16.6	16.5	16.7	16.5	14.7	15.6	15.4	15.5	33.0	52.1	100.0	96.7
Salmonella phage SEGD1	KU726251.1	16.1	16.1	16.8	16.6	16.6	16.4	16.7	16.6	14.7	15.6	15.4	15.5	32.9	51.9	97.0	100.0

Fig. 3. Phylogenetic analysis of the (A) major capsid protein, and (B) large subunit terminase proteins of a subset of myoviruses with genomes >200 kb 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. **Red** = *Rsl2virus.*

A. Major capsid protein

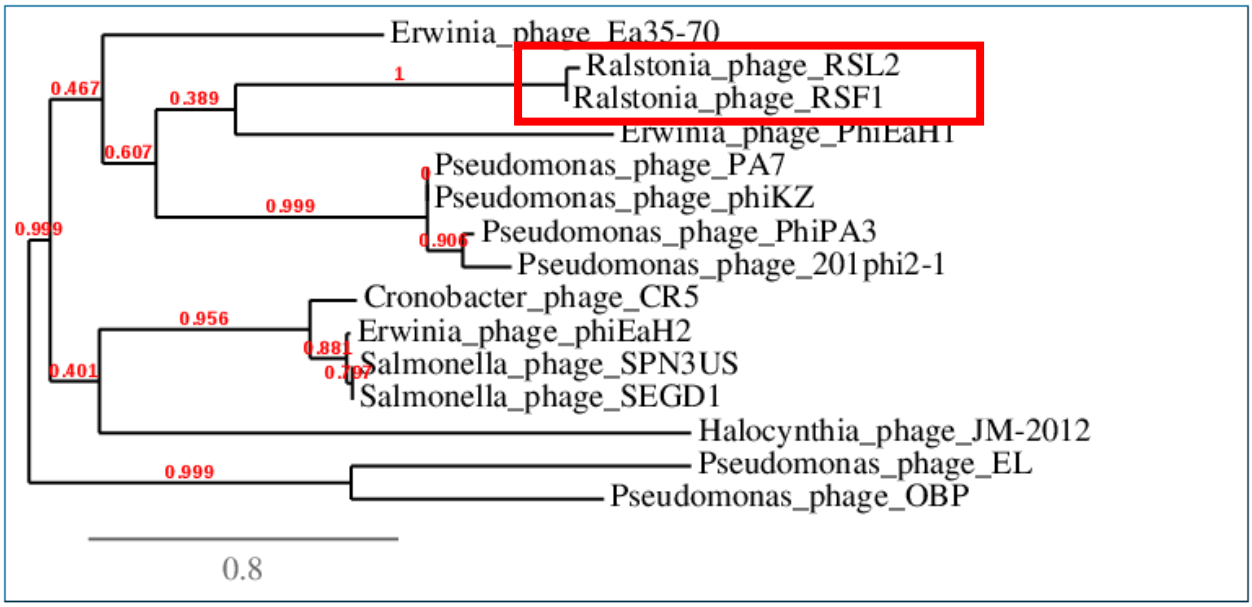


Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

B. TerL protein

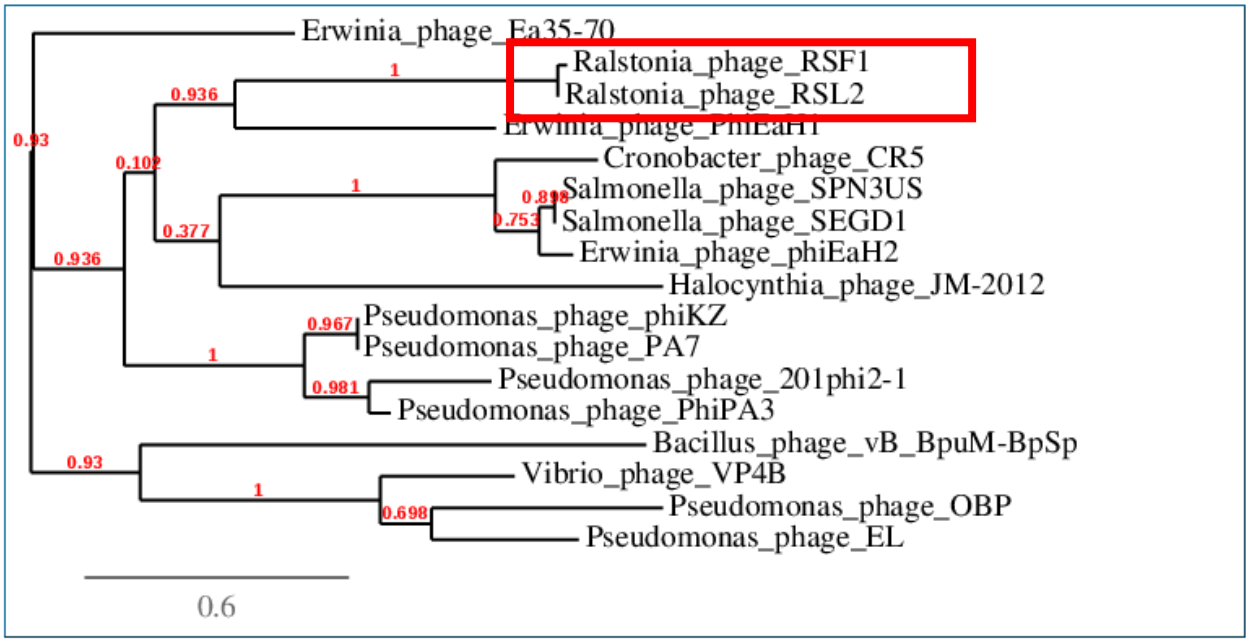
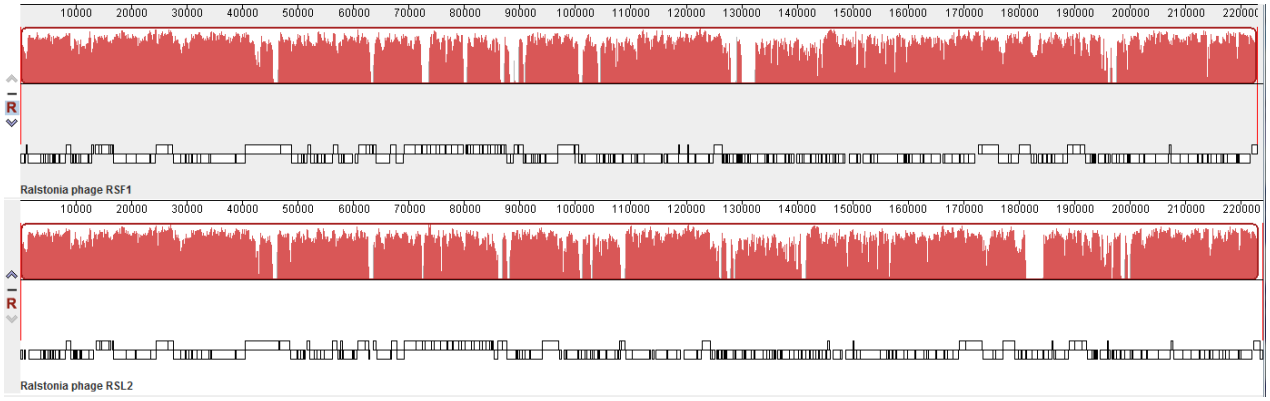


Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

Fig. 4. progressiveMauve alignment [1] of the annotated genomes of members of the *Rsl2virus* genus – from top to bottom: RSF1 and RSL2. 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).


10000 20000 30000 40000 50000 60000 70000 80000 90000 100000 110000 120000 130000 140000 150000 160000 170000 180000 190000 200000 210000 220000
R
Ralstonia phage RSF1
10000 20000 30000 40000 50000 60000 70000 80000 90000 100000 110000 120000 130000 140000 150000 160000 170000 180000 190000 200000 210000 220000
R
Ralstonia phage RSL2