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:	***2016.039a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Prtbvirus*, including two (2) new species in the family *Podoviridae*.
(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** ☒

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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	***2016.039aB***	(assigned by ICTV officers)

To create 2 new species within:

Genus:	***Prtbvirus*** **(new)**
Subfamily:	
Family:	***Podoviridae***
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)**
Brucella virus Pr	Brucella phage Pr	JN939332.1
Brucella virus Tb	Brucella phage Tb	JN939331.1

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 9

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	***2016.039bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Podoviridae***
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	***2016.039cB***	(assigned by ICTV officers)

To name the new genus: *Prtbvirus*

Assigning the type species and other species to a new genus

Code	***2016.039dB***	(assigned by ICTV officers)

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

Brucella virus Pr

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

Introduction on virulent *Brucella* phages:

The history of Brucella phages and their use as a diagnostic tool for the identification of *Brucella* species began with the discovery of phage Tb (Tbilisi, Georgian SSR, USSR) in the 1960s [12-14] Some phages were isolated from *Brucella* cultures but lysogeny has not yet been demonstrated. On the basis of their host range, virulent Brucella phages are classified in seven groups (reference phages: Tb, Fi, Wb, Bk2, R/C, Iz, Np) routinely employed for *Brucella* typing [10, 11, 15]. Depending on the use of a specific phage plaques are turbid to clear with diameters of 0.5 to 2 mm on lawns of *Brucella* bacteria. With the exception of R/C, which is only active on rough Brucellae, other reference phages are highly lytic for a wide range of smooth Brucellae with distinct differences in some species or biovars of members. All virulent Brucella phages described so far have a podoviral morphology with icosahedral heads and short tails (Figure 1) [5-8]). Furthermore, the structural protein patterns of the reference phages are also closely related. Only within the protein patterns of phages Tb, Fi and R/C, minor differences were identified. Restriction analysis by the use of different endonucleases (*Ava*I, *Eco*RI, *Bgl*I and *Hin*dIII) indicate that the reference phages are only slightly different in genome length, ranging from 39 to 42 kb, but are closely related in regard to restriction patterns [9]. According to the obtained data, these phages were considered members of a single species comprising different host range

variants originating from a common ancestor [9,10, 13]. Later on, NGS phage genome sequencing confirmed these data. Genomic comparison of the diagnostic Brucella phages revealed a high sequence similarity but also distinct differences among the genomes. Some highly diverse genetic loci have been identified that may contribute to varying host specificity due to adaptive selection by phage/host interaction [5-8]). In general, Brucella phages have linear, double-stranded genomes with an average G+C content of ~48% with the majority of genes being transcribed in the same orientation.

Host range of Brucella phage F1:
Brucella phage F1 (propagated on *B. abortus* strain S19) formed clear plaques of 2 mm on lawns of *B. abortus* strain S19. Furthermore, this phage was highly lytic activity for a wide range of Brucellae, i.e. *B. abortus*, *B. suis* (bv1, bv5), *B. neotomae*, and *B. microti*.

Summary:
BLASTN, CoreGenes (Table 1) [2], progressiveMauve alignment (Fig. 2) [1], and phylogenetic analyses (Fig. 3) [3] all indicate that the proposed genus, *Prtbvirus*, is cohesive and distinct from other genera. On average, the genomes of members of this genus are 39.7 kb in length (48.2 mol% G+C), and encode ca 57 proteins and 0 tRNAs.

Origin of the new genus name:

Based upon the name of the two first sequenced members of this genus and two main species.

Reasons to justify the choice of type species:

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

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References:

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6. Farlow J, Filippov AA, Sergueev KV, Hang J, Kotorashvili A, Nikolich MP. Comparative whole genome analysis of six diagnostic Brucellaphages. Gene. 2014;541(2):115-22.

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10. Ackermann HW, Simon F, Verger JM. A survey of *Brucella* phages and morphology of new isolates. Intervirology. 1981;16(1):1-7.

11. Corbel MJ. Brucella phages: advances in the development of a reliable phage typing system for smooth and non-smooth *Brucella* isolates. Ann Inst Pasteur Microbiol. 1987;138(1):70-5.

12. Thomas EL, Corbel MJ. Isolation of a phage lytic for several *Brucella* species following propagation of Tbilisi phage in the presence of mitomycin C. Arch Virol. 1977;54(3):259-61.

13. Corbel MJ, Thomas EL. Properties of some new *Brucella* phage isolates; evidence for lysogeny within the genus. Dev Biol Stand. 1976;31:38-45.

14. Corbel MJ, Phillip JI. The relationship of *Brucella abortus* agglutinogenic antigens to the receptor sites for Tbilisi phage. Res Vet Sci. 1972;13(1):91-3.

15. Alton GG, Jones LM, Pietz DE. Laboratory techniques in brucellosis. Monogr Ser World Health Organ. 1975;(55):1-163.

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.

This TaxoProp is dedicated to the memory of Dr. Irina Antadze who worked on *Brucella* spp. and bacteriophages for many years at the G. Eliava Institute of Bacteriophages, Microbiology and Virology.

Fig. 1. Electron micrograph of negatively stained Brucella phage F1, grown on *B. abortus* strain S19 (provided by Dr. Jochen Reetz).

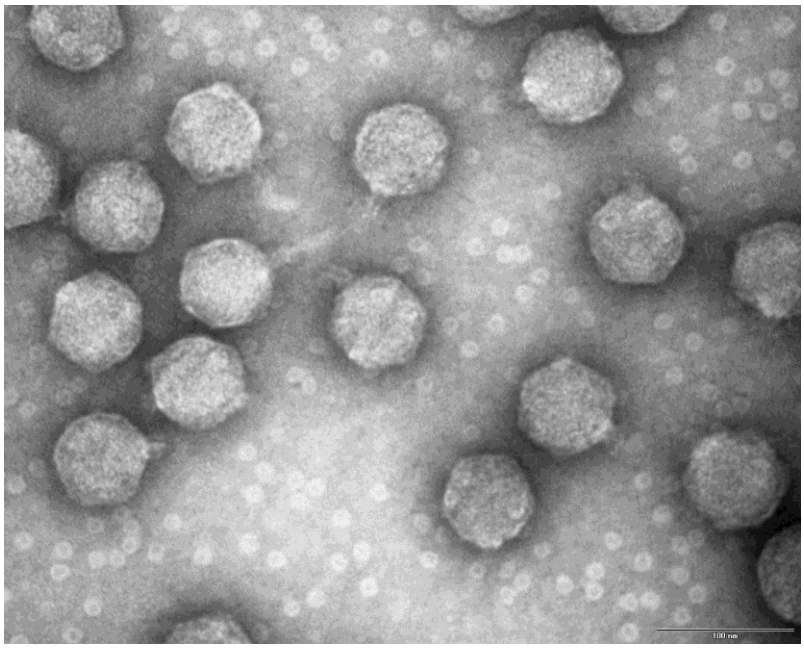


Fig. 2. Heat map of BLASTN relationships between all the Brucella phages determined using Gegenees [4].

PHAGE	ACCESSION NO.	HG428758.1	JN939331.1	KC556894.1	KC556897.1	KJ133691.1	KJ133694.1	KJ133695.1	KJ133698.1	KJ133699.1	KJ133701.1	KJ133707.1	KJ133700.1	KJ133705.1	KJ133706.1	KJ133704.1	KJ133692.1	KJ133696.1	KJ133697.1	KJ133703.1	KJ133702.1	KJ133693.1	KJ133688.1	KJ133689.1	KJ133690.1	KC556896.1	KC556898.1	KC556893.1	KC556895.1	JN939332.1
F1	HG428758.1	100.0	99.2	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.4	99.3	99.3	99.3	99.3	99.3	92.4	92.4	92.4	92.4	92.4
Tb	JN939331.1	99.5	100.0	99.7	99.7	99.7	99.7	99.7	99.7	99.7	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	99.6	92.7	92.7	92.7	92.7	92.6
Fz	KC556894.1	99.8	99.7	100.0	100.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	92.9	92.9	92.9	92.9	92.8
Tb(2)	KC556897.1	99.8	99.7	100.0	100.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	92.9	92.9	92.9	92.9	92.8
11sa_141	KJ133691.1	99.7	99.7	99.9	99.9	100.0	100.0	100.0	99.9	100.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	92.9	92.9	92.9	92.9	92.8
141_)19	KJ133694.1	99.7	99.7	99.9	99.9	100.0	100.0	100.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	92.9	92.9	92.9	92.9	92.8
141_141	KJ133695.1	99.7	99.7	99.9	99.9	100.0	100.0	100.0	100.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	92.9	92.9	92.9	92.9	92.8
281_19	KJ133698.1	99.7	99.7	99.9	99.9	99.9	99.9	100.0	100.0	100.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	92.9	92.9	92.9	92.9	92.8
281_141	KJ133699.1	99.7	99.7	99.9	99.9	100.0	99.9	99.9	100.0	100.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	92.9	92.9	92.9	92.8	92.8
544_141	KJ133701.1	99.7	99.7	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	100.0	100.0	100.0	100.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	92.8	92.8	92.8	92.8	92.8
V_141	KJ133707.1	99.7	99.7	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	92.8	92.8	92.8	92.8	92.8
544_19	KJ133700.1	99.8	99.7	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	100.0	100.0	100.0	100.0	100.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	92.9	92.9	92.9	92.9	92.8
Tb_141	KJ133705.1	99.7	99.6	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.9	99.9	99.9	99.9	99.9	92.8	92.8	92.8	92.8	92.7
V_19	KJ133706.1	99.7	99.6	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	99.9	99.9	100.0	100.0	99.9	99.9	99.9	99.9	92.8	92.8	92.8	92.8	92.7
Tbilisi	KJ133704.1	99.7	99.6	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	100.0	100.0	100.0	100.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	92.8	92.8	92.8	92.8	92.7
110_19	KJ133692.1	99.7	99.6	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	99.9	100.0	100.0	99.9	100.0	100.0	100.0	100.0	100.0	99.9	100.0	100.0	100.0	92.8	92.8	92.8	92.8	92.7
177_19	KJ133696.1	99.7	99.6	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	99.9	99.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	92.8	92.8	92.8	92.8	92.7
177_141	KJ133697.1	99.7	99.6	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	99.9	99.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	92.8	92.8	92.8	92.8	92.7
1066_141	KJ133703.1	99.7	99.6	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	100.0	99.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	92.8	92.8	92.8	92.8	92.7
1066_19	KJ133702.1	99.7	99.6	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	99.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	92.8	92.8	92.8	92.8	92.7
110_141	KJ133693.1	99.7	99.6	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	92.8	92.8	92.8	92.8	92.7
02_19	KJ133688.1	99.7	99.6	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	92.8	92.8	92.8	92.8	92.7
02_141	KJ133689.1	99.7	99.6	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	92.8	92.8	92.8	92.8	92.7
11sa_19	KJ133690.1	99.7	99.6	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	92.8	92.8	92.8	92.8	92.7
S708	KC556896.1	99.0	98.9	99.2	99.2	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.0	99.1	99.1	99.1	99.1	99.1	99.0	99.0	99.0	100.0	100.0	100.0	99.9	99.9
Wb	KC556898.1	99.0	98.9	99.2	99.2	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.0	99.1	99.1	99.1	99.1	99.1	99.0	99.0	99.0	100.0	100.0	100.0	99.9	99.9
Bk	KC556893.1	99.0	98.9	99.2	99.2	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	100.0	100.0	100.0	100.0	99.9
R/C	KC556895.1	99.0	98.9	99.2	99.2	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.1	99.0	99.1	99.1	99.1	99.1	99.1	99.0	99.0	99.0	99.9	99.9	100.0	100.0	99.9
Pr	JN939332.1	98.9	98.9	99.1	99.1	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.0	99.9	99.9	99.9	99.9	100.0

Table 1. Properties of the phages belonging to the genus *Prtbvirus*.

Brucella phage	RefSeq No	GenBank Accession No.	Genome length (kb)	Genome (mol% G+C)	No. CDS	DNA (% sequence identity)*	% Homologous proteins **
Pr	NC_019447.1	JN939332.1	38.25	48.2	57	92.1	98.3
Tb	NC_019446.1	JN939331.1	41.15	48.2	58	100	100

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

Table 2. Brucella phages which are strains within this genus.

Brucella phage	Accession No.	Strain of Brucella phage
F1	HG428758.1	Tb
Fz	KC556894.1	Tb
Tb(2)	KC556897.1	Tb
11sa_141	KJ133691.1	Tb
141_)19	KJ133694.1	Tb
141_141	KJ133695.1	Tb
281_19	KJ133698.1	Tb
281_141	KJ133699.1	Tb
544_141	KJ133701.1	Tb
V_141	KJ133707.1	Tb
544_19	KJ133700.1	Tb
Tb_141	KJ133705.1	Tb
V_19	KJ133706.1	Tb
Tbilisi	KJ133704.1	Tb
110_19	KJ133692.1	Tb
177_19	KJ133696.1	Tb
177_141	KJ133697.1	Tb
1066_141	KJ133703.1	Tb
1066_19	KJ133702.1	Tb
110_141	KJ133693.1	Tb
02_19	KJ133688.1	Tb
02_141	KJ133689.1	Tb
11sa_19	KJ133690.1	Tb
S708	KC556896.1	Pr
Wb	KC556898.1	Pr
Bk	KC556893.1	Pr
R/C	KC556895.1	Pr

Fig. 2. progressiveMauve alignment [1] of the annotated genomes of members of the *Prtbvirus* genus – from top to bottom: Brucella phages Pr and Tb. 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).

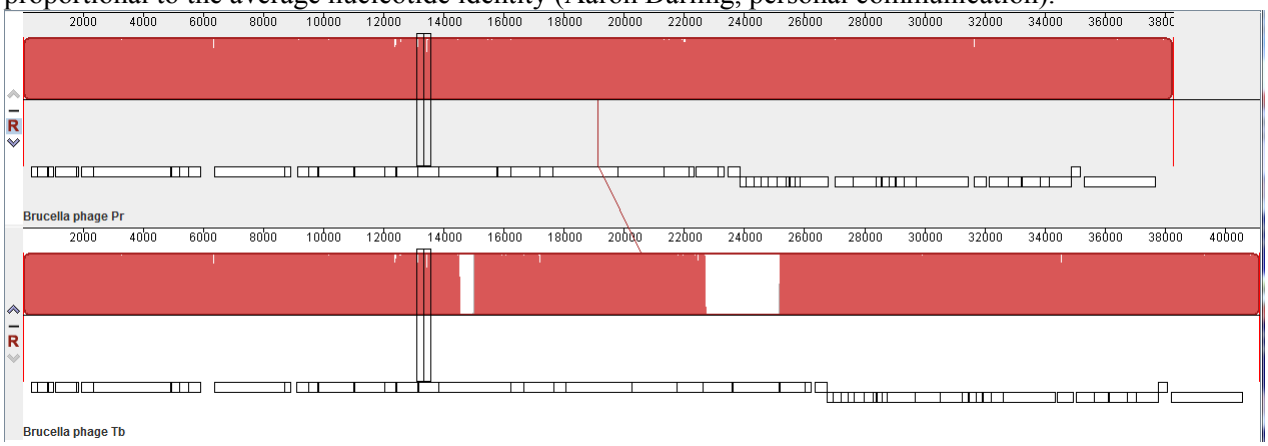
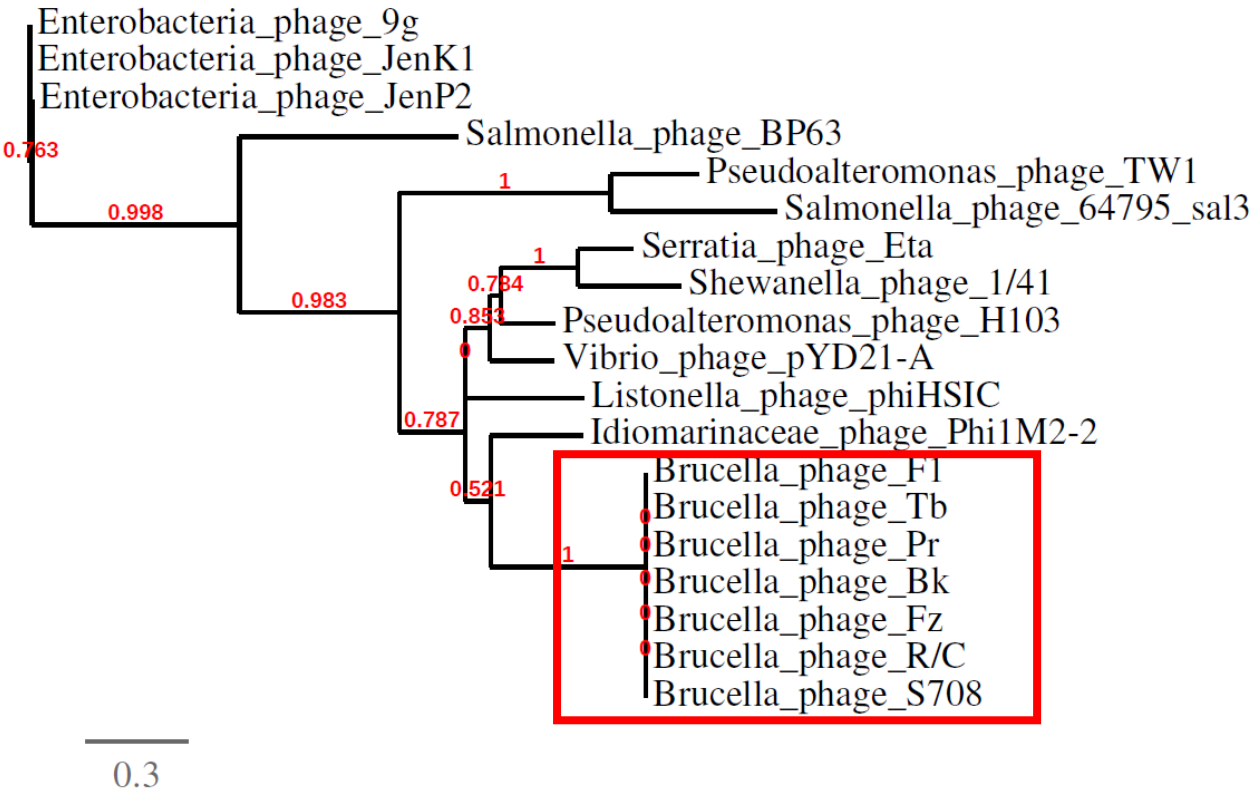
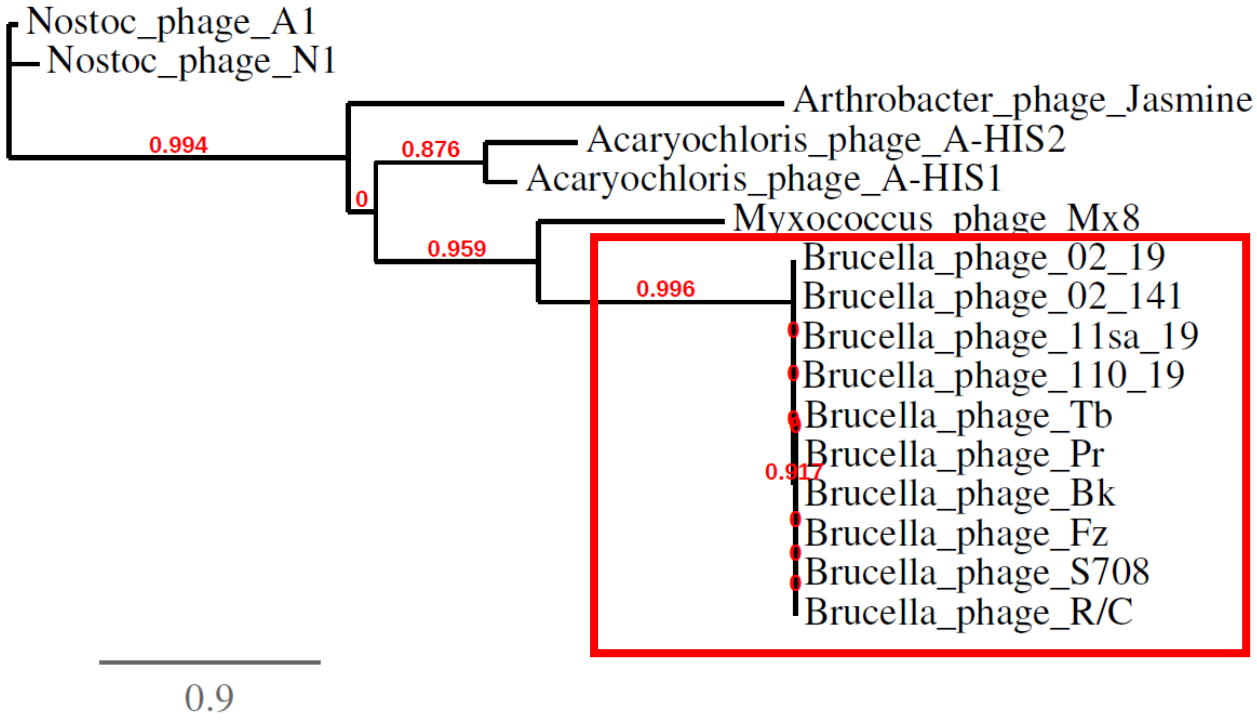


Fig. 3. Phylogenetic analysis of A. helicases of Brucella podoviruses and B. Major capsid proteins and the homologous protein constructed using “one click” at phylogeny.fr [3]. "The "One Click mode" targets users that do not wish to deal with program and parameter selection. By default, the pipeline 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. Helicases



B. Major capsid proteins


Nostoc_phage_A1
Nostoc_phage_N1
Arthrobacter_phage_Jasmine
Acaryochloris_phage_A-HIS2
Acaryochloris_phage_A-HIS1
Myxococcus_phage_Mx8
Brucella_phage_02_19
Brucella_phage_02_141
Brucella_phage_11sa_19
Brucella_phage_110_19
Brucella_phage_Tb
Brucella_phage_Pr
Brucella_phage_Bk
Brucella_phage_Fz
Brucella_phage_S708
Brucella_phage_R/C
0.994
0.876
0
0.959
0.996
0.917
0.9