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:	***2015.037a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Sitaravirus*, including five (5) 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):

Eric Miller – North Carolina State University (U.S.A.)
Johannes Whittmann - Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Germany)
Andrew M. Kropinski – University of Guelph (Canada)
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 & 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 *Sitaravirus* rather than *Sitaralikevirus* 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	***2015.037aB***	(assigned by ICTV officers)

To create 5 new species within:

Genus:	***Sitaravirus (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)**
Paenibacillus virus Diva	Paenibacillus phage Diva	KP296791
Paenibacillus virus Shelly	Paenibacillus phage Shelly	KP296795
Paenibacillus virus Sitara	Paenibacillus phage Sitara	KP296796
Paenibacillus virus Rani	Paenibacillus phage Rani	KP296793
Paenibacillus virus Hb10c2	Paenibacillus phage HB10c2	KP202972

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

These phages all infect *Paenibacillus larvae*, the causative agent of American foulbrood in honey bees; and, exhibit elongated heads. All of the isolates were isolated in the USA, except phage HB10c2 which is a German isolate; and, are characterized by possessing a flexible tail and an elongated head (Figure 1).

While the phylogenetic analysis of the large subunit terminase protein (Fig. 3), genome length and mol%G+C (Table 1) would suggest that the Portuguese *Paenibacillus* phage phiIBB_Pl23 (4) should have precedence in the naming of this genus, its overall DNA sequence relatedness (Fig. 2) to this group of phages is low and it encodes a potential toxin not encoded by any of the other phages. The genome termini for phage phiIBB_Pl23 have not been determined.

BLASTN, CoreGenes (Table 1; 2), progressiveMauve alignment (Fig. 2; 1) and phylogenetic analyses (Fig. 3; 3) all indicate that the proposed genus, *Sitaravirus*, is cohesive and distinct from the other genera of viruses. The phages of this genus possess genome of approx. 40 kb (41.8 mol%G+C), and encode ca. 64 proteins and 0 tRNAs. The genomes possess 3'-protruding, single-stranded cohesive ends of 9 nucleotides (CGACTGCCC). They share >70% DNA sequence identity and >70% homologous proteins (Table 1).

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 *Sitaravirus* rather than *Sitaralikevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" and "*Phi*" from phage genus names.

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2015.037bB***	(assigned by ICTV officers)

To create a new genus within:

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 family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2015.037cB***	(assigned by ICTV officers)

To name the new genus: *Sitaravirus*

Assigning the type species and other species to a new genus

Code	***2015.037dB***	(assigned by ICTV officers)

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

Paenibacillus virus Diva

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:**

5

Reasons to justify the creation of a new genus:

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

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

Origin of the new genus name:

Named after the third phage of its type to be deposited in GenBank: *Paenibacillus* phage Sitara, since a plant virus genus, *Divavirus*, already exists; and, Ranivirus sounded too similar to *Ranavirus*.

Reasons to justify the choice of type species:

First phage of its type to be deposited in GenBank

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. 2013; 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.
4. Oliveira A, Melo LD, Kropinski AM, Azeredo J. Complete Genome Sequence of the Broad-Host-Range *Paenibacillus larvae* Phage phiIBB_Pl23. Genome Announc. 2013;1(5). pii: e00438-13.

Annex:

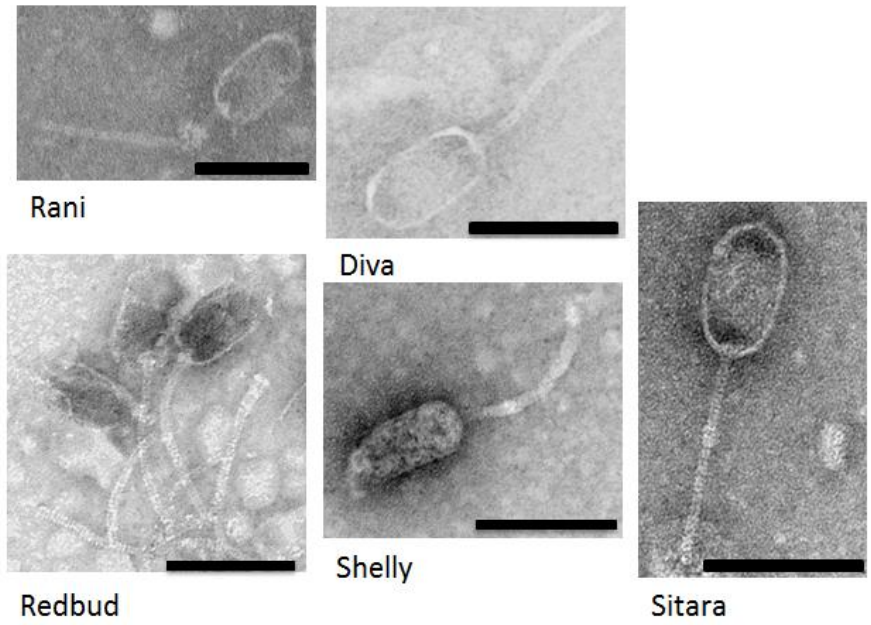
Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate 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.

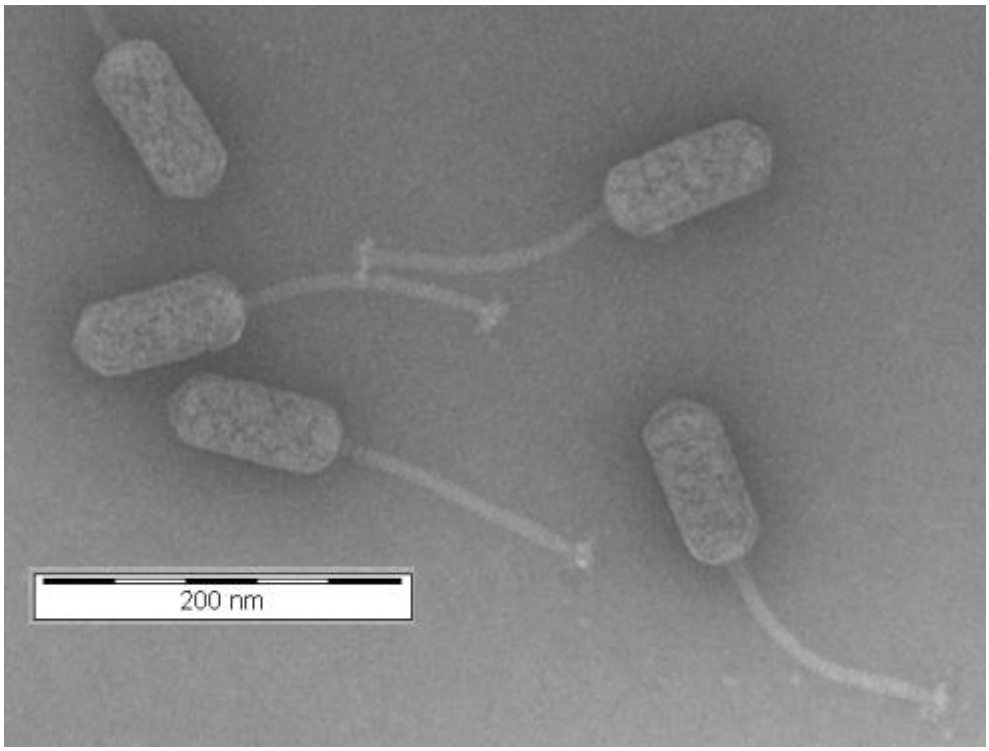
Table 1. Properties of the five phages belonging to the genus *Divavirus* plus one close outlier *Paenibacillus* phage phiIBB_Pl23.

Phage	GenBank accession No.	Genome length (kb)	Genome (mol%G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)*	Proteome (% homologous proteins)**
Diva	KP296791	37.25	42.1	60	0	100	100
Shelly	KP296795	41.15	41.5	68	0	94	95.0
Sitara	KP296796	43.74	41.6	74	0	92	95.0
Rani	KP296793	39.99	41.8	61	0	75	65.0
HB10c2	KP202972	35.64	41.8	56	0	71	70.0
phiIBB_Pl23	KF010834	41.29	40.9	68	0	69	68.3

* Determined using BLASTN; ** Determined using CoreGenes (2); *Paenibacillus* phage Redbud (KP296794) is a strain of *Paenibacillus* phage Rani.

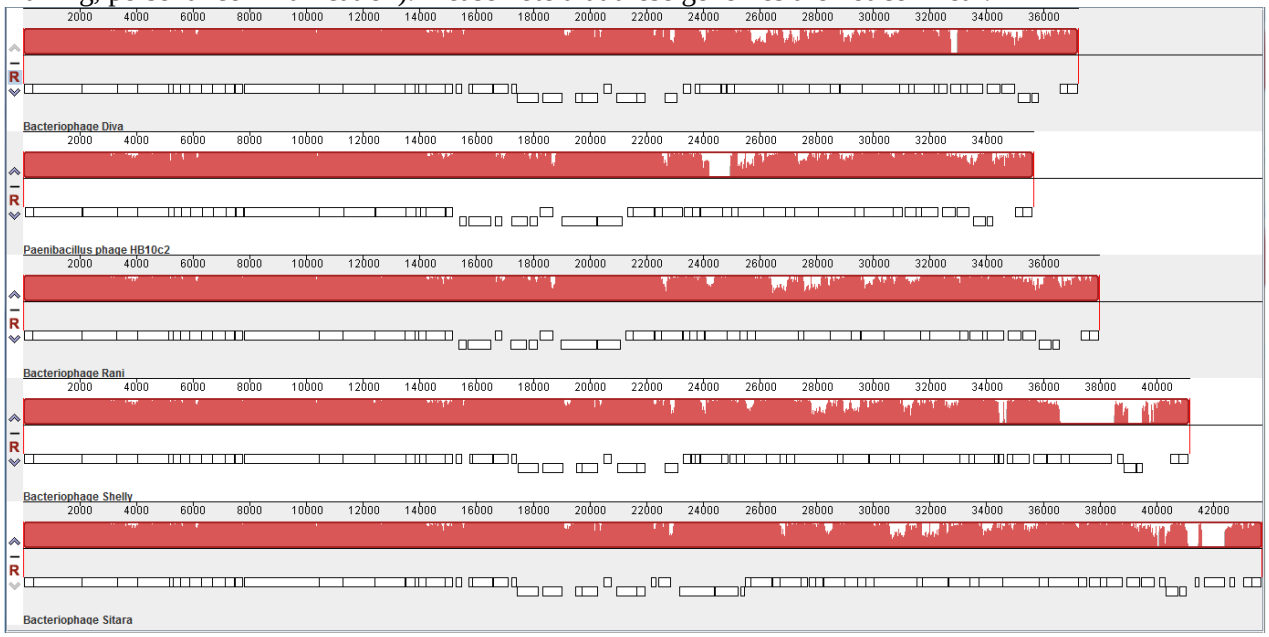
Fig. 1. Electron micrograph of *Paenibacillus* phage Diva and related phages stained with uranyl acetate.





Phage HB10c2

Fig. 2. progressiveMauve alignment of the annotated genomes of *Paenibacillus* phages top to bottom: Diva, HB10c2, Rani, Shelly and Sitara (1). In the subsequent diagram Diva (top) is compared with phiIBB_Pl23 (bottom) showing that homologous blocks are only to be found at the genome termini. 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). Please note that these genomes are not collinear.



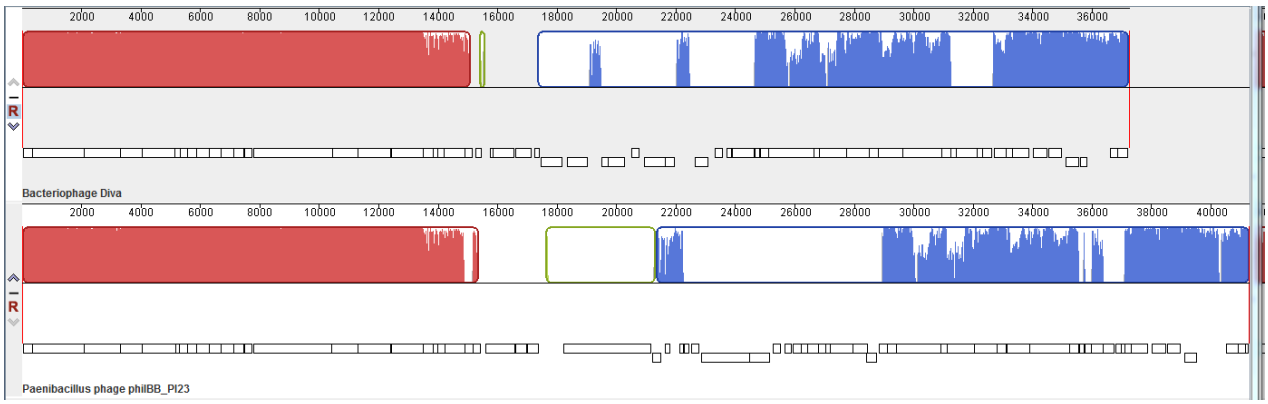


Fig. 3. Phylogenetic analysis of the large subunit terminase of sitaraviruses and some related phages constructed using “one click” at phylogeny.fr (3). "The "One Click mode" targets users that do not wish to deal with program and parameter selection. By default, the pipeline is already set up to run 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."

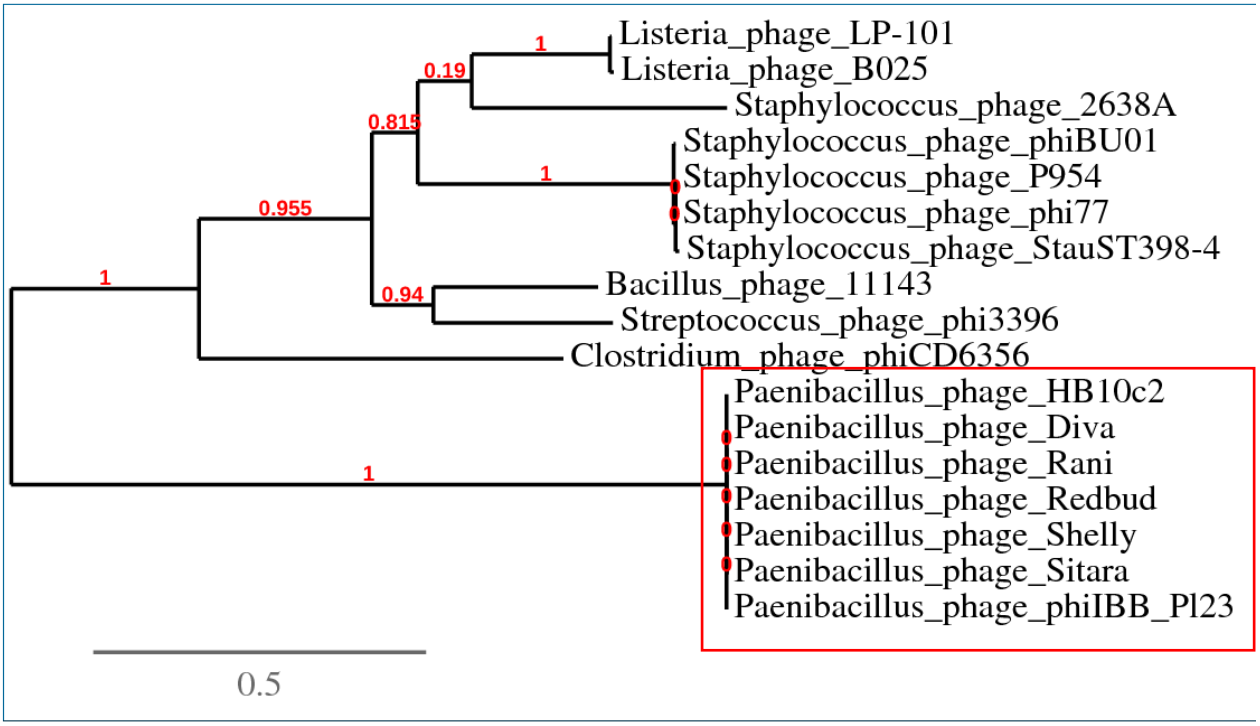


Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*