

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:	2016.052a	ı-dB		(to be completed by ICTV officers)					
Short title: To create one (1) r family <i>Siphoviridae</i> . (e.g. 6 new species in the genus A Modules attached (modules 1 and 10 are required)		s <i>virus</i> , in 1 ⊠ 6 □	cluding o 2 ⊠ 7 □	one (1) nev 3 ⊠ 8 □	w species i 4 9	n the 5 □ 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 <u>http://www.ictvonline.org/subcommittees.asp</u> . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)	ICTV Subcom	Bacterial nmittee	and	Archaeal	Viruses
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## ICTV Study Group comments (if any) and response of the proposer:

Date first submitted to ICTV: Date of this revision (if different to above): June 2016

## **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 201	6.052aB	(assigned by IC	TV office	rs)			
To create 1 n	ew species with	in:					
				all that apply.			
Genus:	Vegasvirus (ne	<ul> <li>If the higher taxon has yet to be</li> </ul>					
Subfamily:			<ul> <li>created (in a later module, below) write "(new)" after its proposed name.</li> <li>If no genus is specified, enter</li> </ul>				
Family:	Siphoviridae						
Order:	Caudovirales		"unassigned" in the genus box.				
Name of new	species:	Representative isolate 1 per species please)	e: (only	GenBank sequence accession number(s)			
Paenibacillus	virus Vegas	Paenibacillus phage Ve	egas	KT361654			

**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	201	6.052bB	(assigned by IC	CTV officers)
To create	a new	genus within:		Fill in all that apply.
Subfa	mily:			• If the higher taxon has yet to be created
Fa	mily:	Siphoviridae		(in a later module, below) write "(new)" after its proposed name.
С	Order:	Caudovirales		<ul> <li>If no family is specified, enter</li> <li>"unassigned" in the family box</li> </ul>

naming a new genus

Code	2016.052cB	(assigned by ICTV officers)
To name tl	ne new genus: <i>Vegasvirus</i>	

Assigning the type species and other species to a new genus

Code 2016.052dB	(assigned by ICTV officers)						
To designate the following as	the type species of the new genus						
Paenibacillus virus Vegas	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

Tsourkas *et al.* [5] characterized nine phages active against *Paenibacillus larvae* ERIC I, the causative agent of American Foulbrood Disease in honeybees. Paenibacillus phage Vegas capsids are prolate, 100 nm long by 50 nm wide, with tails about 150 nm long (Fig. 2 shows and electron micrograph of the related Paenibacillus phage Diane).

BLASTN analysis revealed that two main groups could be distinguished (Paenibacillus phages Vegas, Vadim, Hayley & Diane; and, Harrison & Paisley). Phylogenetic analysis, based upon the major capsid and TerL proteins revealed a single grouping (Fig. 4). The difference between the whole genome and single gene-based groupings was due to a modular genome nature in which the two marker genes were part of the shared module (Fig. 3).

These phages encode repressors and integrases.

Origin of the new genus name:

Based upon the name of the first sequenced member of this genus.

**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.

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. Tsourkas PK, Yost DG, Krohn A, LeBlanc L, Zhang A, Stamereilers C, Amy PS. Complete Genome Sequences of Nine Phages Capable of Infecting *Paenibacillus larvae*, the Causative Agent of American Foulbrood Disease in Honeybees. Genome Announc. 2015;3(5). pii: e01120-15.

#### 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.** Gegenees [4] BLASTN analysis of the genomes of Paenibacillus phage genomes run under accurate criteria (window: 200 bp; step size: 100 bp).

PHAGE NAME	ACCESSION NO.	KF010834.1	KT361649.1	KT361650.1	KP202972.1	KP296794.1	KP296793.1	KP296796.1	KP296791.1	KP296795.1	KT361652.1	KP296792.1	KT361657.1	KT361656.1	КТ361654.1	КТ361655.1	КТ361651.1	KT361653.1
Paenibacillus phage philBB_Pl23	KF010834.1	100.0	64.7	64.7	53.2	59.4	60.4	59.6	53.6	52.1	50.6	12.2	13.1	13.1	13.2	12.7	34.1	34.1
Paenibacillus phage Fern	KT361649.1	70.4	100.0	100.0	60.5	66.1	67.4	65.5	61.8	58.7	57.0	12.6	15.0	15.0	15.2	14.5	18.5	18.5
Paenibacillus phage Willow	KT361650.1	70.4	100.0	100.0	60.5	66.1	67.4	65.5	61.8	58.7	57.0	12.8	15.0	15.0	15.2	14.6	18.5	18.5
Paenibacillus phage HB10c	KP202972.1	61.9	64.2	64.2	100.0	93.5	92.8	82.0	67.3	62.1	63.5	12.7	14.4	14.4	14.5	14.0	16.0	16.0
Paenibacillus phage Redbud	KP296794.1	64.4	66.2	66.2	87.9	100.0	98.4	88.0	67.2	66.0	64.9	14.2	13.1	13.1	13.2	12.8	14.1	14.1
Paenibacillus phage Rani	KP296793.1	65.8	67.4	67.4	87.1	98.4	100.0	89.2	67.9	67.2	65.5	14.4	13.8	13.8	13.9	13.3	14.9	14.9
Paenibacillus phage Sitara	KP296796.1	55.9	56.9	56.9	67.0	76.5	77.6	100.0	72.7	74.2	72.7	12.9	12.0	12.0	12.1	11.6	12.3	12.3
Paenibacillus phage Diva	KP296791.1	59.2	62.8	62.8	64.6	68.4	69.3	85.3	100.0	93.2	91.5	15.5	14.6	14.7	14.8	14.0	14.4	14.4
Paenibacillus phage Shelly	KP296795.1	52.0	54.3	54.3	53.9	61.0	62.3	78.8	84.6	100.0	98.3	21.8	13.4	13.4	13.6	12.9	14.0	14.0
Paenibacillus phage Zenia	KT361652.1	50.4	52.7	52.7	55.0	60.0	60.7	77.2	83.1	98.3	100.0	21.7	12.9	12.9	12.8	12.4	13.5	13.5
Paenibacillus phage Lily	KP296792.1	11.6	10.8	10.8	10.3	12.2	12.5	12.7	13.0	20.3	20.3	100.0	2.1	2.1	2.1	2.1	3.9	3.9
Paenibacillus phage Diane	KT361657.1	11.9	12.5	12.5	11.3	11.2	11.4	11.5	11.9	12.0	11.6	2.1	100.0	100.0	99.8	96.6	34.6	34.6
Paenibacillus phage Vadim	KT361656.1	11.9	12.5	12.5	11.3	11.2	11.4	11.5	11.9	12.0	11.6	2.1	100.0	100.0	99.9	96.6	34.6	34.6
Paenibacillus phage Vegas	KT361654.1	12.0	12.7	12.7	11.4	11.3	11.5	11.6	12.0	12.1	11.5	2.1	99.8	99.9	100.0	96.6	34.6	34.6
Paenibacillus phage Hailey	KT361655.1	11.8	12.4	12.4	11.3	11.2	11.2	11.3	11.8	11.8	11.4	2.1	99.5	99.5	99.5	100.0	36.0	36.0
Paenibacillus phage Harrison	KT361651.1	31.8	15.8	15.8	12.9	12.7	12.8	12.4	12.2	13.0	12.7	3.8	35.8	35.8	35.7	36.1	100.0	97.9
Paenibacillus phage Paisley	KT361653.1	31.8	15.8	15.8	12.9	12.6	12.8	12.4	12.2	13.2	12.9	3.8	35.8	35.9	35.8	36.2	98.0	100.0

**Fig. 2.** Electron micrograph of negatively stained Paenibacillus phage Diane (provided by Dr. Philippos Tsourkas, School of Life Sciences, University of Nevada, Las Vegas, USA).

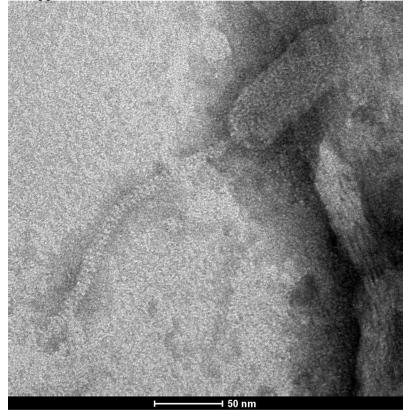


Table 1. Properties of Paenibacillus phage Vegas belonging to the genus Vegasvirus.

Paenibacillys	GenBank	Genome	Genome	No.	DNA (%	%
phage	Accession	length	(mol%	CDS	sequence	Homologous
	No.	(kb)	G+C)		identity)*	proteins **
Vegas***	KT361654	45.65	40.2	86	NA	NA

\* Determined using BLASTN; \*\* Determined using CoreGenes [2]; \*\*\* Paenibacillus phages Diane (KT361657), Vadim (KT361656), and Hayley (KT361655) should be considered strains Paenibacillus phage Vegas.

**Fig. 3.** progressiveMauve alignment [1] of the annotated genomes of Paenibacillus phages Harrison (top) and Vegas (bottom) indicating their differences. 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).

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**Fig. 4.** Phylogenetic analysis of (A) large subunit terminase proteins and (B) major capsid proteins of Paenibacillus phage Vegas-like viruses and homologous proteins from a variety of other 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."

# A. TerL proteins

	Paenibacillus_phage_Harrison Paenibacillus_phage_Paisley
0.999	Paenibacillus_phage_Vegas Paenibacillus_phage_Hayley Paenibacillus_phage_Vadim Paenibacillus_phage_Diane
0.999	Paenibacillus_phage_phiIBB_PI23 Paenibacillus_phage_HB10c2 Paenibacillus_phage_Diva Paenibacillus_phage_Rani Paenibacillus_phage_Redbud Paenibacillus_phage_Shelly Paenibacillus_phage_Sitara Paenibacillus_phage_Fern Paenibacillus_phage_Xenia Paenibacillus_phage_Willow

0.005

# B. Major capsid protein

Paenibacillus_phage_Harrison Paenibacillus_phage_Paisley	
Paenibacillus_phage_Vegas	
Paenibacillus_phage_Hayley	
Paenibacillus_phage_Vadim	
Paenibacillus phage Diane	
	Paenibacillus_phage_HB10c2
1	Paenibacillus_phage_Xenia
	Paenibacillus_phage_phiIBB_Pl23
	Paenibacillus_phage_Diva
	Paenibacillus_phage_Rani
	Paenibacillus_phage_Shelly
	Paenibacillus_phage_Sitara
	Paenibacillus_phage_Fern
	Paenibacillus_phage_Redbud
	Paenibacillus_phage_Willow
	acinoacinus_pnage_winow

0.07