MODULE 1: **TITLE, AUTHORS, etc**

| Code assigned: | *2015.039a-dB* | (to be completed by ICTV officers) |
|---|---|---|

**Short title:** To create one (1) new genus, *Pagevirus*, including five (5) new species within 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) | 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 *Pagevirus* rather than *Pagelikevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" 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 | *2015.039aB* | | (assigned by ICTV officers) |
|---|---|---|---|

**To create 5new species within:**

Fill in all that apply.

| | |
|---|---|
| Genus: | *Pagevirus (new)* |
| Subfamily: | |
| Family: | *Podoviridae* |
| Order: | *Caudovirales* |

- 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) |
|---|---|---|
| *Bacillus virus Page* | Bacillus phage Page | KF669655 |
| *Bacillus virus Pony* | Bacillus phage Pony | KF669660 |
| *Bacillus virus Pookie* | Bacillus phage Pookie | KM236248 |
| *Bacillus virus Palmer* | Bacillus phage Palmer | KP411017 |
| *Bacillus virus Pascal* | Bacillus phage Pascal | KM236247 |

**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**

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

| Code | *2015.039bB* | (assigned by ICTV officers) |
|---|---|---|

**To create a new genus within:**

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. |
|---|---|---|
| Subfamily: | | |
| Family: | *Podoviridae* | |
| Order: | *Caudovirales* | • If no family is specified, enter "**unassigned**" in the family box |

naming a new genus

| Code | *2015.039cB* | (assigned by ICTV officers) |
|---|---|---|

**To name the new genus:** *Pagevirus*

Assigning the type species and other species to a new genus

| Code | *2015.039dB* | (assigned by ICTV officers) |
|---|---|---|

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

| *Bacillus virus Page* | 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 (1) (Table 1), progressiveMauve alignment (2) (Fig. 2) and phylogenetic analyses (3) of the terminase proteins (Fig. 3) all indicate that the proposed genus, *Pagevirus*, is cohesive and distinct from the other genera of viruses.

*Bacillus* phage Page, which infects *Bacillus megaterium* has an isometric head (61 nm in diameter) with a short non-contractile tail, indicating that it belongs to the family *Podoviridae* (Fig. 1). The properties of the 5 phages that currently compose this newly proposed genus (Page, Pony, Pookie, Pascal, and Palmer) have been described in Genome Announcements (4-8).

The *Pagevirus* members have the following average genome characteristics: size 39.9 kb (40.5 %G+C) encoding 50 proteins and 0 tRNA. *Pagevirus* phages use a pac-type DNA packaging strategy as determined by TerL sequence homology to the TerL of phages that are known to use pac-type DNA packaging.

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

**Origin of the new genus name:**

*Bacillus* phage Page

**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.

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.

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. Lopez MS, Hodde MK, Chamakura KR, Kuty Everett GF. Complete Genome of *Bacillus megaterium* Podophage Page. Genome Announc. 201; 2(2). pii: e00332-14.

5. Khatemi, BE, Chung On, CC, Chamakura KR, Kuty Everett GF. Complete Genome of *Bacillus megaterium* Podophage Pony. Genome Announc. 2013; 1(6). pii: e00860-13.

6. Ladzekpo TN, DeCrescenzo AJ, Hernandez AC, Kuty Everett GF. Complete Genome of *Bacillus megaterium* Podophage Pookie. Genome Announc. 2015; 3(1). pii: e01432-14.

7. Snowden JD, Vega Gonzalez AE, Maroun JW, Hernandez AC, Kuty Everett GF. Complete Genome of *Bacillus megaterium* Podophage Pascal. Genome Announc. 2015; 3(1). pii: e01429-14.

8. Hargrove EC, Lopez MS, Hernandez AC, Kuty Everett GF. Complete Genome of *Bacillus megaterium* Podophage Palmer. Genome Announc. Genome Announc. 2015;3(3). pii: e00358-15.

## 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.

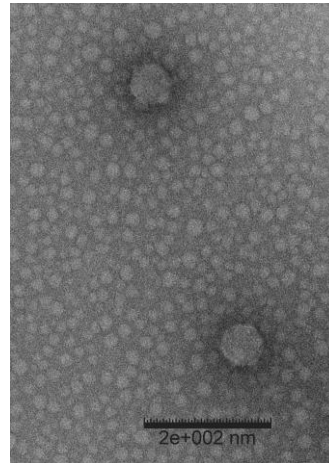**Fig. 1.** Electron micrograph of negatively stained phage Page



**Table 1**. Properties of the five phages belonging to the genus *Pagevirus*.

| Phage | GenBank Accession No. | Genome length (kb) | Genome (mol%G+C) | No. CDS | No. tRNAs | DNA (% sequence identity)* | % Homologous proteins ** |
|---|---|---|---|---|---|---|---|
| Page | KF669657 | 39.87 | 40.7 | 50 | 0 | 100 | 100 |
| Pony | KF669660 | 39.84 | 40.9 | 48 | 0 | 91 | 96.0 |
| Pookie | KM236248 | 40.21 | 40.57 | 52 | 0 | 89 | 98.0 |
| Palmer | KP411017 | 40.00 | 40.67 | 50 | 0 | 85 | 98.0 |
| Pascal | KM236247 | 39.64 | 39.9 | 50 | 0 | 59 | 90 |

* Determined using BLASTN; ** Determined using CoreGenes (2).

**Fig. 2.** progressiveMauve alignment (1) of the annotated genomes of members of the *Pagevirus* genus (from top to bottom: Page, Palmer, Pony, Pookie and Pascal). 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).
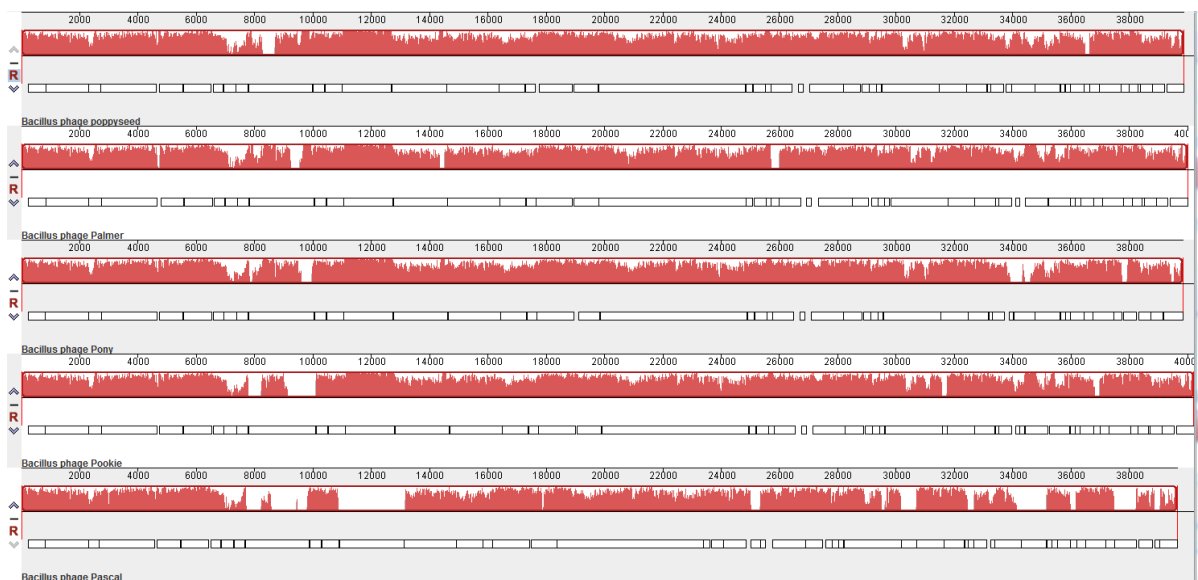
**Fig. 3.** Phylogenetic analysis of large subunit terminase proteins of the pageviruses and variety of other related phage proteins constructed using "one click" at phylogeny.fr (3). "The "One Click mode" targets users that do not wish to deal with program and parameter selection. By default, the pipeline 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."
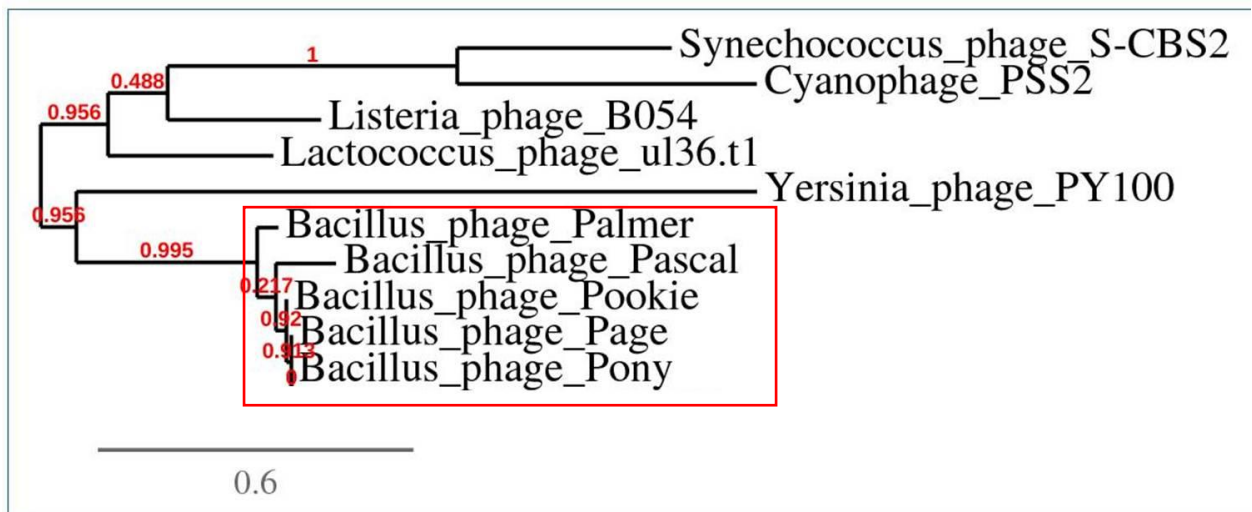


**Figure 1:** *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*