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:	***2009.001a-gB***	(to be completed by ICTV officers)

Short title: Create new genus named *Pb1likevirus* in the family *Myoviridae*
(e.g. 6 new species in the genus *Zetavirus*)

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

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Has this proposal has been seen and agreed by the relevant study group(s)? Please select answer in the box on the right	**Yes**

ICTV-EC or Study Group comments and response of the proposer:

[previous (EC41) decision: inconsistent with naming rules.]

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

MODULE 2: **NEW SPECIES**

Part (a) to create and name one or more new species.

If more than one, they should be a group of related species belonging to the same genus (see Part b)

Code	***2009.001aB***	(assigned by ICTV officers)

To create 7 new species with the name(s):

Pseudomonas phage PB1
Pseudomonas phage SN
Pseudomonas phage 14-1
Pseudomonas phage LMA2
Pseudomonas phage LBL3
Pseudomonas phage F8
Burkholderia phage BcepF1

Part (b) assigning new species to higher taxa

All new species must be assigned to a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family.

Code	***2009.001bB***	(assigned by ICTV officers)

To assign the species listed in section 2(a) as follows:

Genus:	***Pb1likevirus*** (new)
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.

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.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

P. aeruginosa phage PB1 was first described almost half a century ago (Holloway *et al*., 1960). Phage F8 was part of the Lindberg *P. aeruginosa* typing set (Lindberg and Latta, 1974) *Pseudomonas* phages SN, 14-1, LBL3 and LMA2 were isolated from water samples taken from very distinct natural environments, by different researchers, over a four-year period (Table 1). Burkholderia phage BcepF1 was isolated from agricultural soils in New York. All these phages belong to the A1 morphological group of the *Myoviridae*, carrying characteristic conspicuous capsomers that appear as 8 nm cup-like depressions on the phage heads. The non-flexible tail (ca. 140 nm) exhibits no transverse striations but presents a criss-cross pattern (Figure 1; Ackermann *et al*., 1988), a rare feature that has only been observed elsewhere with *Salmonella* phage FelixO1.

All genomes are fully sequenced and annotated: accession numbers can be found in Table 1. Whole-genome sequence analysis of the *Pseudomonas* phages showed little mutual variation in genome lengths (between 64.5 and 66.5 kb) and DNA similarity throughout the genomes (Table 1, Figure 2). For the more distantly related *Burkholderia* phage BcepF1, genome conservation is limited to the modules involved in virion formation and DNA replication (Figure 2). Since these modules (ORF17-74) account for more than half of the BCepF1 genome, this phage also belongs to the newly formed genus. Proteomic comparison between this genus and the other *Myoviridae* members visualizes the dissimilarity (molecular relationship) and allows demarcation of the "PB1-like viruses" (Figure 3).

Despite the conservation between the Pseudomonas phages, phages PB1, SN, 14-1, LMA2, LBL3 and F8 each carry at least one specific insertion/deletion which makes the phage unique. For example, ORF7.1 (YP_002154240) is unique for LMA; ORF 14.1 (YP_002154248) is only found in PB1 and LMA2; ORF2 (YP_002455932) is absent in LBL3 and SN; Based on these subtle differences in genome content, the creation of these six new phage species is justified.

MODULE 3: **NEW GENUS**

creating and naming a new genus

Code	***2009.001cB***	(assigned by ICTV officers)

To create a new genus to contain the species listed below

Code	***2009.001dB***	(assigned by ICTV officers)

To name the new genus: *Pb1likevirus*

assigning a new genus to higher taxa

Code	***2009.001eB***	(assigned by ICTV officers)

To assign the new genus as follows: Ideally, a genus should be placed within a higher taxon, but if not, write "**unassigned**" in the box below.

Subfamily:	
Family:	***Myoviridae***
Order:	***Caudovirales***

If any of these taxa has yet to be created (in module 4, 5 or 6) please write "**(new)**" after its proposed name.

assigning type species and other species to a new genus

Code	***2009.001fB***	(assigned by ICTV officers)

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

Pseudomonas phage PB1

Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered

Code	***2009.001gB***	(assigned by ICTV officers)

To assign the following as additional species of the new genus:

Pseudomonas phage SN (new)
Pseudomonas phage 14-1(new)
Pseudomonas phage LMA2(new)
Pseudomonas phage LBL3(new)
Pseudomonas phage F8(new)
Burkholderia phage BcepF1(new)

Reasons to justify the creation of a new genus:

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

All members described encapsulate linear, non-permuted genomes of approximately 65 kb within a solid, acid-resistant isometric capsid (diameter: 74 nm) and carry non-flexible, contractile tails of approximately 140 nm. Based on these observations, these phages can be classified into the A1 morphological group of the *Myoviridae*.(Table 1; Figure 1) Over the years, no less than 42 phages were reported to be PB1-related, mainly based on cross-DNA hybridization and morphological studies (Krylov *et al*., 1993; Ackermann and Dubow, 1987; Pleteneva *et al*., 2008).

Restriction analysis after DNA treatment with Bal31 showed these phages carry a non-permuted, linear dsDNA genome (Ceyssens et al., submitted).

The genomes of all PB1-like phages (65-72 kb) are organized into at least seven transcriptional blocks alternating on both strands. These are separated by (in some cases bidirectional) factor-independent terminators with stem-loop structures which are conserved perfectly between the phages. A striking feature of the PB1-like genomes is the clustering of a large number of small genes encoding hypothetical proteins. Three clusters of 8 kb (ORF*1* through ORF*16*, except ORF*4*, Terminase), 4 kb (ORF*77* through ORF*91*) and 1 kb (ORF*60* through ORF*64*) are present, and encode small proteins of unknown functions. A similar single cluster of 20 kb, encoding 62 genes unrelated to PB1, is found in phage BCepF1.

Origin of the new genus name:

Named after type species PB1

Reasons to justify the choice of type species:

The strictly virulent and historically important *P. aeruginosa* phage PB1 was first described almost half a century ago (Holloway *et al*., 1960), and was already intensively studied in the 70s (Kropinski et al., 1977; Jarrell and Kropinski, 1977). Another historical important phage belonging to this genus, F8, was tested by Bergan (1972) for suitability as a typing phage. He suggested that F8 was isolated by Postic and Finland in 1961, but there is nothing in this paper to support this.

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.
- Provide Genbank accession numbers (not RefSeq accessions) for genomic sequences of new species

See module 2

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

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.

Table 1:

Phage	Isolation details Source & Location	Year	Genome size (%GC)	ORFs	Unique ORFs	DNA identity to F8 (%)	Accession No.
PB1	Sewage, Edinburgh, Scotland	1960	65,764 (55.5)	93	-	93.5	NC_011810
SN	Lake, Pokrov, Russia	2004	66,390 (55.6)	92	2	87.2	NC_011756
14-1	Sewage, Regensburg, Germany	2004	66,238 (55.6)	90	-	87.4	NC_011703
LMA2	River, Maastricht, Holland	2007	66,530 (55.6)	95	2	87.6	NC_011166
LBL3	Pond, Blanes, Spain	2006	64,427 (55.5)	88	2	88.6	NC_011165
F8	Unknown	1972	66,015 (55.6)	93	1	100	NC_007810
BcepF1	Soil, New York, USA	2003	72,415 (55.9)	127	-	-	NC_009015

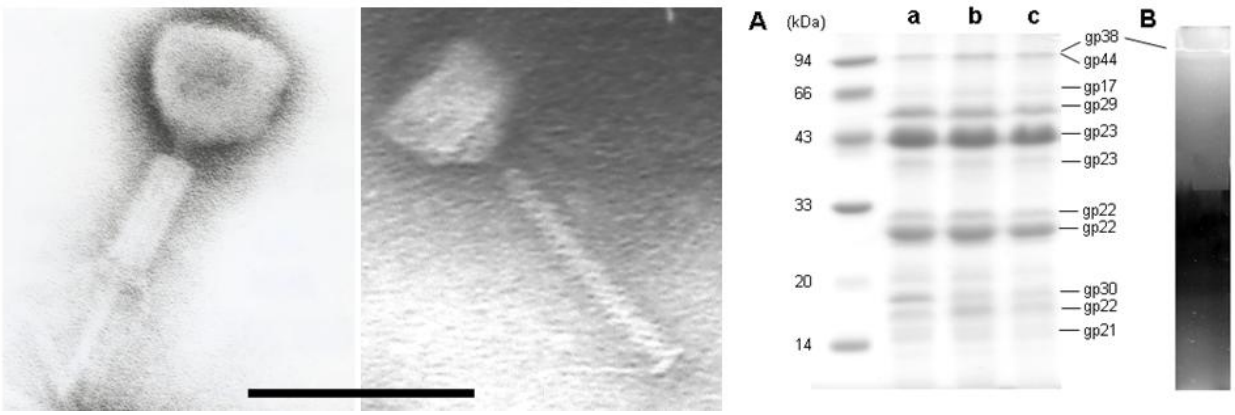
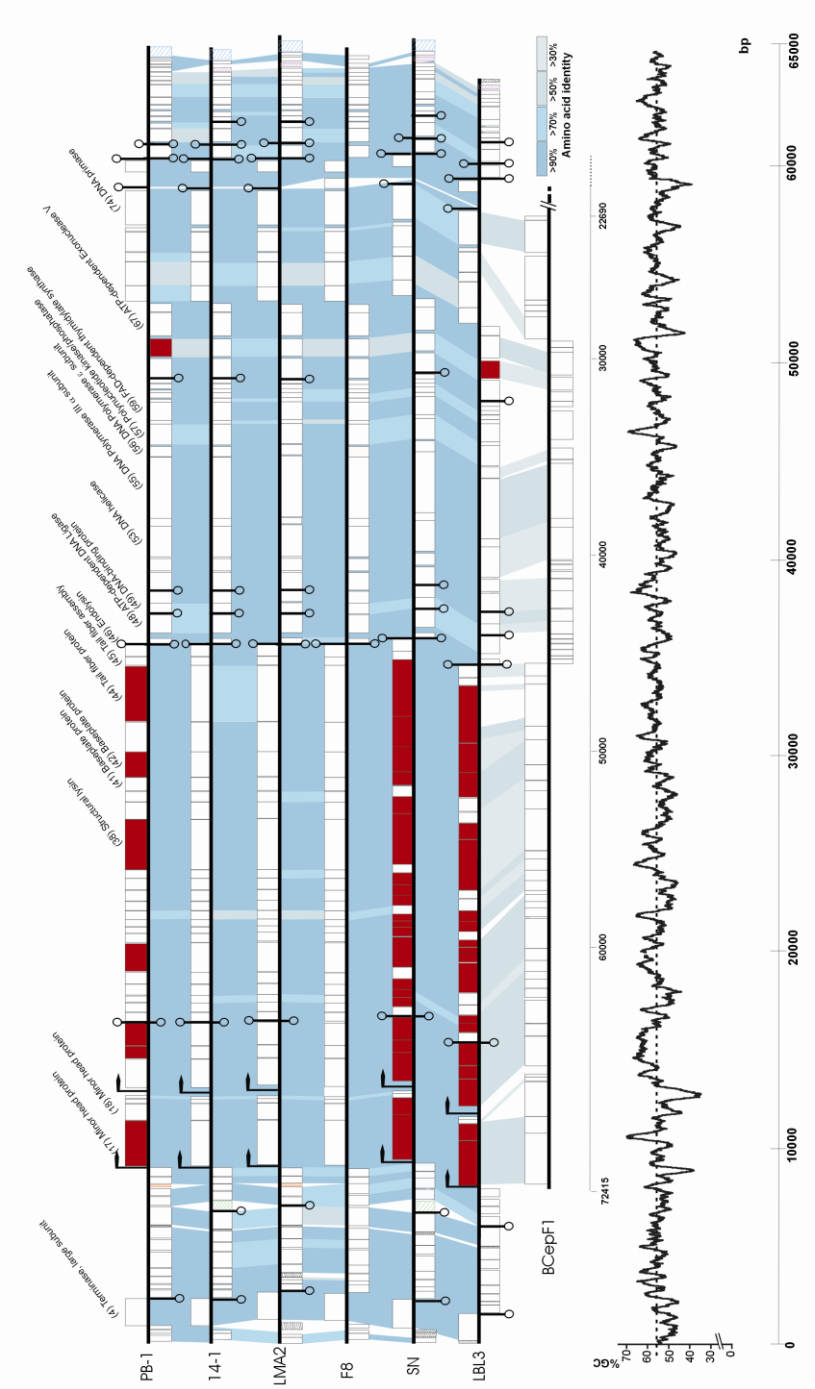


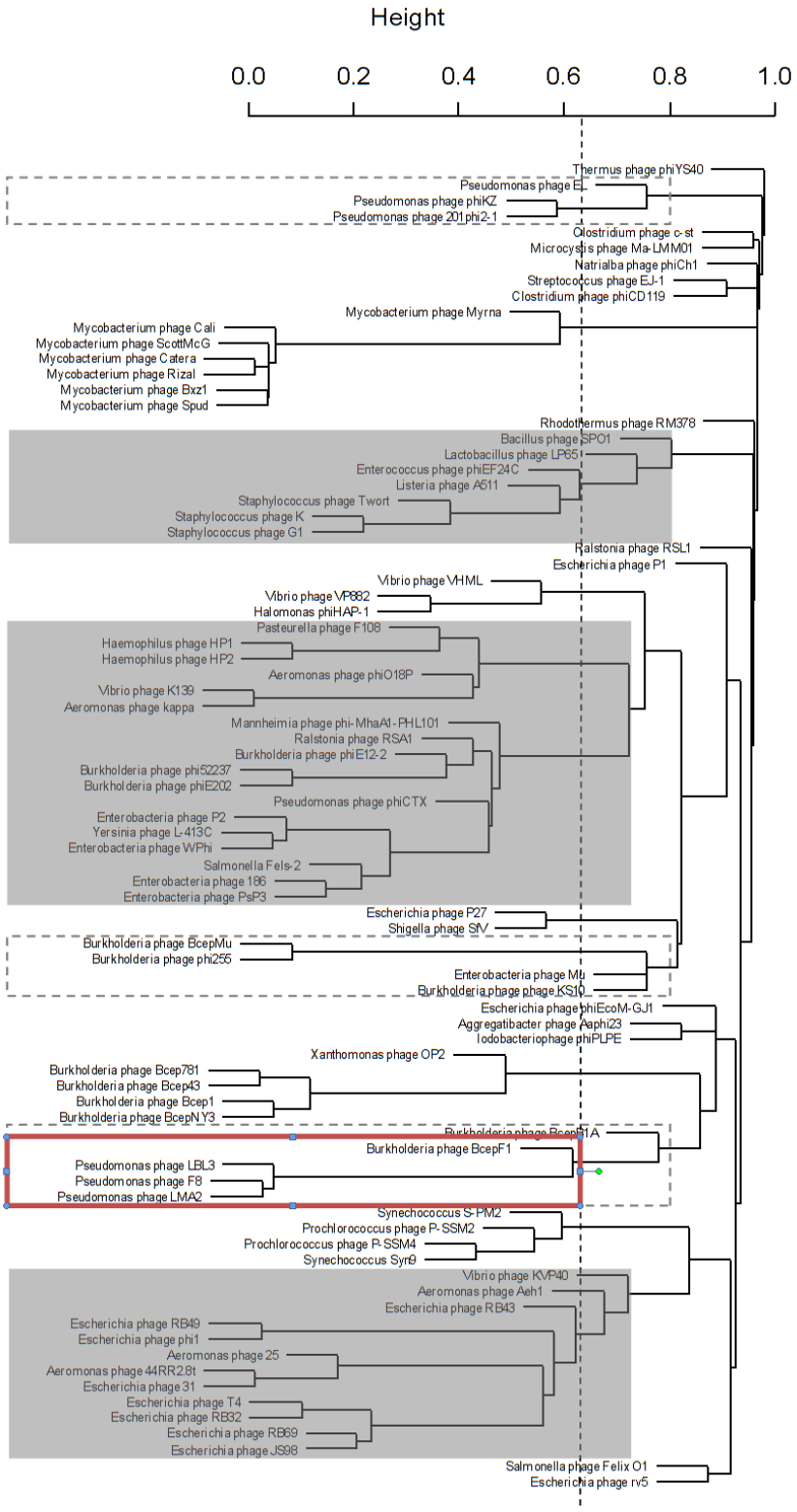
Figure 1:
Left: Electron microscopic images of negatively stained LBL3 (left) and SN (right) particles. Scale bar represents 100 nm
Right: A. Phage particle proteins separated on a 12% SDS-PAGE gel, in parallel to a LMW-size ladder (kDa) and stained with Simply BlueTM Safestain; a, 14-1; b, LBL3; c, LMA2. The positions of major structural proteins, as determined by ESI-MS/MS, are indicated. B. Zymogram analysis of phage 14-1. The clear zone associated with peptidoglycan degrading activity is indicated with an arrow.

Figure 2:



Bio-informatic analysis of the PB1 genus. Upper part. The predicted open reading frames of all PB1-like phages and their amino acid identity to the corresponding ORF in phage F8, indicated in different shades of blue. Predicted promoters and terminators are shown as open arrows and stem-loop structures, respectively. ORFs not present in F8 are hatched, and given unique colors when they appear in more than one phage. ORFs marked in red encode structural proteins which were identified as part of the phage particle using ESI-MS/MS. Lower part. Display of GC content throughout the PB1 genome. The plot was generated by the ISOCHORE tool on the EMBL-EBI website (http://www.ebi.ac.uk/Tools/emboss/cpgplot/index.html), with a window size of 200 bp.

Figure 3:



Hierarchical cluster dendrogram of the *Myoviridae*. The relative dissimilarity between the phage proteomes (between 0.0 and 1.0) forms the basis for the proposed groupings. The dotted lines reflects the cut-off value used for the establishement of genera, used consistently for all Myoviridae and the previously defined *Podoviridae* (Lavigne et al., 2008). Subfamily and tentative subfamily groupings are indicated in the grey and dotted boxes, respectively. The PB1-like viruses available in this analysis are mark within the red box.

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