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:	***2016.037a-mB***	(to be completed by ICTV officers)

Short title: To create one (1) new subfamily, *Pclasvirinae*, including two new (2) genera and one existing genus in 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):

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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)	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	***2016.037aB***	(assigned by ICTV officers)

To create 4 new species within:

Genus:	***Fishburnevirus* (new)**
Subfamily:	***Pclasvirinae* (new)**
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)**
Mycobacterium virus Fishburne	Mycobacterium phage Fishburne	KC691256.1
Mycobacterium virus Brusacoram	Mycobacterium phage Brusacoram	KT347313.1
Mycobacterium virus Malithi	Mycobacterium phage Malithi	KP027200.1
Mycobacterium virus Donovan	Mycobacterium phage Donovan	KF841477.1

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	***2016.037bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	***Pclasvirinae* (new)**
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	***2016.037cB***	(assigned by ICTV officers)

To name the new genus: *Fishburnevirus*

Assigning the type species and other species to a new genus

Code	***2016.037dB***	(assigned by ICTV officers)

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

Mycobacterium virus Fishburne

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

The phages listed in Table 1 are all defined as members of Cluster P of Mycobacterium phages (http://phagesdb.org/clusters/P/) in the Actinobacteriophage Database. This is supported by phylogenetic analysis of the large subunit terminase (Fig. 1). However, two of the phages (BigNuz and Phayonce) are considerably different in DNA sequenced relatedness (Fig. 1), suggesting that they should be considered to belong to different, but related, genera. CoreGenes 3.5 [3] indicates that BigNuz unique proteins (>100 amino acids) include gp30, gp54, gp62, gp70, gp71; whereas gp21, gp23, gp29, gp32, gp82 are unique to Phayonce. One protein unique to the *Fishburnevirus* cluster is a HTH DNA-binding protein [Mycobacterium phage Brusacoram; AKY02585] though it is not annotated in Mycobacterium phage Jebeks.

Mycobacterium phage Fishburne was isolated by enrichment of *Mycobacterium smegmatis* mc²155 from soil from Charleston, SC (U.S.A,) in 2011.

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 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	***2016.037eB***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Phayoncevirus* (new)**
Subfamily:	***Pclasvirinae* (new)**
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)**
Mycobacterium virus Phayonce	Mycobacterium phage Phayonce	KR080195.1

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	***2016.037fB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	***Pclasvirinae* (new)**
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	***2016.037gB***	(assigned by ICTV officers)

To name the new genus: *Phayoncevirus*

Assigning the type species and other species to a new genus

Code	***2016.037hB***	(assigned by ICTV officers)

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

Mycobacterium virus Phayonce

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

The phages listed in Table 1 are all defined as members of Cluster P of Mycobacterium phages (http://phagesdb.org/clusters/P/) in the Actinobacteriophage Database. This is supported by phylogenetic analysis of the large subunit terminase (Fig. 1). However, two of the phages (BigNuz and Phayonce) are considerably different in DNA sequenced relatedness (Fig. 1), suggesting that they should be considered to belong to different, but related, genera. CoreGenes 3.5 [3] indicates that BigNuz unique proteins (>100 amino acids) include gp30, gp54, gp62, gp70, gp71; whereas gp21, gp23, gp29, gp32, gp82 are unique to Phayonce. One protein unique to the *Fishburnevirus* cluster is a HTH DNA-binding protein [Mycobacterium phage Brusacoram; AKY02585] though it is not annotated in Mycobacterium phage Jebeks.

Mycobacterium phage Fishburne was isolated by enrichment of *Mycobacterium smegmatis* mc²155 from soil from Charleston, SC (U.S.A,) in 2011.

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 4: **NEW SUBFAMILY**

creating a new subfamily

A subfamily can only be created within a family.

Code	***2016.037iB***	(assigned by ICTV officers)

To create a new subfamily within:

Family:	***Siphoviridae***
Order:	***Caudovirales***

If the family has yet to be created (in Module 5) please write "**(new)**" after the proposed name.

- If there is no Order, write "**unassigned**" here.

naming a new subfamily

Code	***2016.037jB***	(assigned by ICTV officers)

To name the new subfamily: *Pclasvirinae*

genera and species assigned to the new subfamily

Code	***2016.037kB***	(assigned by ICTV officers)

To assign the following genera to the new subfamily:

You may list several genera here. For each genus, please state whether it is new or existing.

- If the genus is new, it must be created in Module 3
- If the genus already exists, please state whether it is currently unassigned or is to be removed from another family. If the latter, complete Module 7 to 'REMOVE' it from that family

Bignuzvirus

Fishburnevirus **(new)**

Phayoncevirus **(new)**

The new subfamily 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 unassigned species that the subfamily will contain (those NOT within any of the genera listed above):**

0

Reasons to justify the creation of the new subfamily:

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

The phages listed in Table 1 are all defined as members of Cluster P of Mycobacterium phages (http://phagesdb.org/clusters/P/) in the Actinobacteriophage Database. This is supported by phylogenetic analysis of the large subunit terminase (Fig. 1). However, two of the phages (BigNuz and Phayonce) are considerably different in DNA sequenced relatedness (Fig. 1), suggesting that they should be considered to belong to different, but related, genera. CoreGenes 3.5 [3] indicates that BigNuz unique proteins (>100 amino acids) include gp30, gp54, gp62, gp70, gp71; whereas gp21, gp23, gp29, gp32, gp82 are unique to Phayonce. One protein unique to the *Fishburnevirus* cluster is a HTH DNA-binding protein [Mycobacterium phage Brusacoram; AKY02585] though it is not annotated in Mycobacterium phage Jebeks.

Origin of the new subfamily name:

Pclasvirinae includes the sigil "PCLAS" derived from **P Cl**uster of **A**ctinobacteriophage database

Siphovirus.

MODULE 7: **REMOVE and MOVE**

Use this module whenever an existing taxon needs to be removed:

- *Either* to abolish a taxon entirely (when only part (a) needs to be completed)
- *Or* to move a taxon and re-assign it e.g. when a species is moved from one genus to another (when BOTH parts (a) and (b) should be completed)

Part (a) taxon/taxa to be removed or moved

Code	***2016.037lB***	(assigned by ICTV officers)

To remove the following taxon (or taxa) from their present position:

Mycobacterium virus Jebeks

The present taxonomic position of these taxon/taxa:

Genus:	***Bignuzvirus***
Subfamily:	
Family:	***Siphoviridae***
Order:	***Caudovirales***

Fill in all that apply.

If the taxon/taxa are to be abolished (i.e. not reassigned to another taxon) write "yes" in the box on the right	

Reasons to justify the removal:
Explain why the taxon (or taxa) should be removed

The member of this species is viewed as sufficiently different from Mycobacterium phage BigNuz to warrant inclusion in its own genus.

Part (b) re-assign to a higher taxon

Code	***2016.037mB***	(assigned by ICTV officers)

To re-assign the taxon (or taxa) listed in Part (a) as follows:

Genus:	***Fishburnevirus* (new)**
Subfamily:	***Pclasvirinae* (new)**
Family:	***Siphoviridae***
Order:	***Caudovirales***

Fill in all that apply.

- If the higher taxon has yet to be created write "**(new)**" after its proposed name and complete relevant module to create it.

If no genus is specified, enter "**unassigned**" in the genus box.

Reasons to justify the re-assignment:

- If it is proposed to re-assign species to an existing genus, please 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 accession numbers for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

The member of this species has significantly more DNA sequence identity to Mycobacterium phage Fishburne compared to Mycobacterium phage BigNuz.

MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

1. 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.
2. 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.
3. Zafar N, Mazumder R, Seto D. CoreGenes: a computational tool for identifying and cataloging "core" genes in a set of small genomes. BMC Bioinformatics. 2002 ;3:12.

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 phages belonging to the *Pclasvirinae*.

Mycobacterium phage	**RefSeq**	**INSDC**	**Genome length (bp)**	**Mol% G+C**	**No. CDS**	**Termini**
BigNuz	NC_023692	JN412591.1	48984	66.7	82	12-bp 3' Sticky Overhang (CCTGCCCCCCCG)
Phayonce		KR080195.1	49203	66.7	77	12-bp 3' Sticky Overhang (CCTGCCCCCCGA)
Fishburne	NC_021302	KC691256.1	47109	67.3	77	12-bp 3' Sticky Overhang (CCCGCCCCCCGA)
Malithi	NC_026605	KP027200.1	46870	67.1	80	12-bp 3' Sticky Overhang (CCTGCCGCCCGA)
Donovan	NC_023552	KF841477.1	47162	67.2	78	12-bp 3' Sticky Overhang (CCTGCCCCCCGA)
Jebeks		JN572061.1	45580	67.3	70	12-bp 3' Sticky Overhang (CCCGCCCCCCGA)
Brusacoram		KT347313.1	47618	67.0	78	13-bp 3' Sticky Overhang

						(CCTGCCGCCCCGA)

Fig. 1. Phylogenetic analysis of large subunit terminase proteins of a variety of *Mycobacterium* phages constructed using "one click" at phylogeny.fr [1]. "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." The TerL from Gaia contained a intein which was removed before the phylogenetic tree was constructed. The *Pclasvirinae* are boxed in red.

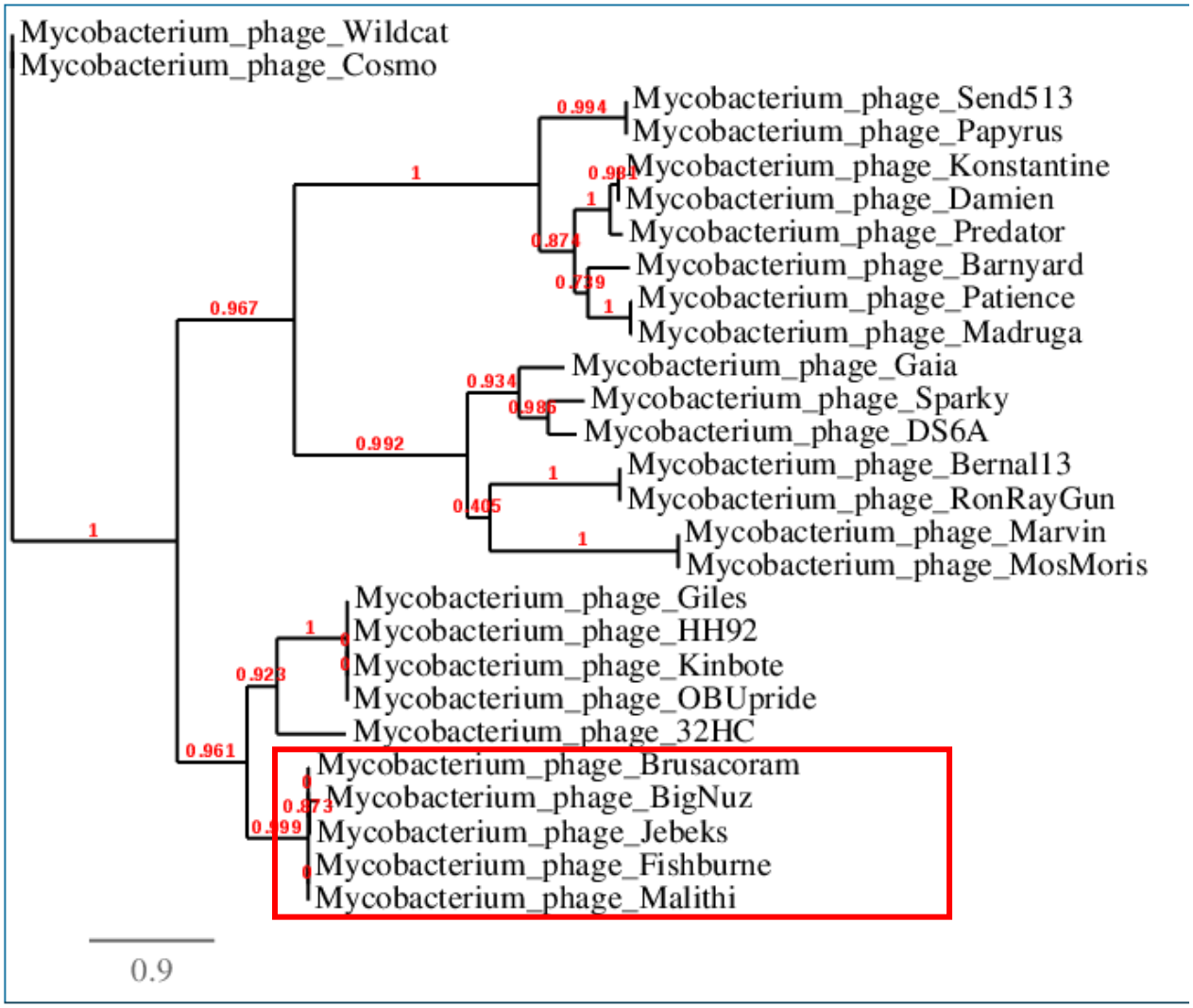


Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*

Fig. 2. BLASTN relationships between pcasviruses derived using Gegenees [2]. Two outliers, Mycobacterium phages Che9c and Sbash, are included for comparative purposes.

PHAGE	ACCESSION NO.	AY129333.1	KP027201.1	KR080195.1	JN412591.1	KT347313	KP027200.1	JN572061.1	KC691256.1	KF841477.1
Che9c	AY129333.1	100.0	42.6	7.0	4.0	1.9	1.8	2.0	2.7	2.8
Sbash	KP027201.1	43.4	100.0	7.5	4.7	2.4	2.1	1.4	2.3	2.3
Phayonce	KR080195.1	8.1	8.6	100.0	27.9	17.0	16.2	17.0	16.3	16.7
BigNuz	JN412591.1	4.8	5.6	28.3	100.0	26.9	26.4	26.7	27.7	27.5
Brusacoram	KT347313.1	2.1	2.6	17.2	28.3	100.0	82.8	78.4	77.5	73.3
Malithi	KP027200.1	2.0	2.4	16.8	27.6	84.1	100.0	85.8	80.3	77.4
Jebeks	JN572061.1	2.4	1.6	19.0	29.7	81.6	88.0	100.0	84.7	86.7
Fishburne	KC691256.1	3.1	2.8	17.0	30.6	78.3	79.9	82.1	100.0	89.7
Donovan	KF841477.1	3.1	2.7	17.5	29.1	73.6	77.2	83.8	89.5	100.0

Fig. 3. Electron micrograph of negatively stained Mycobacterium phage Fishburne (http://phagesdb.org/phages/Fishburne/) - Limited permission was granted by The Actinobacteriophages Database, funded by the Howard Hughes Medical Institute, to use this electron micrograph for this taxonomy proposal; it cannot be reused without permission of The Actinobacteriophages Database.

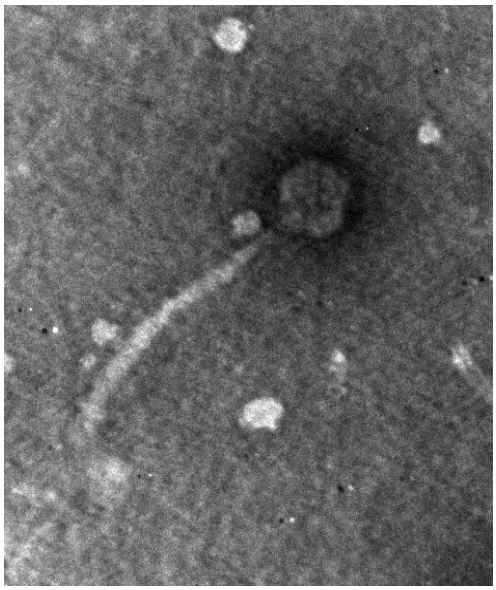
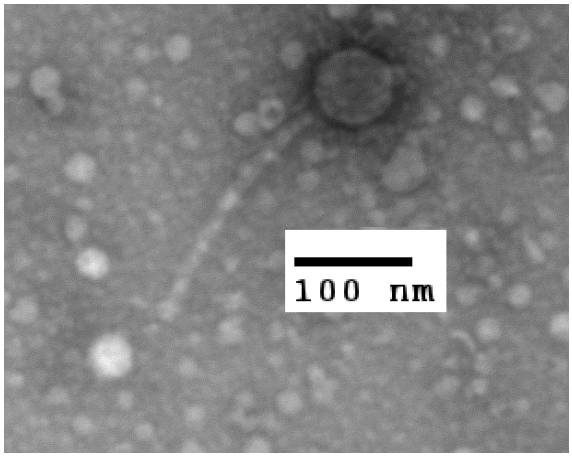


Fig. 4. Electron micrograph of negatively stained Mycobacterium phage Phayonce (http://phagesdb.org/phages/Phayonce/) - Limited permission was granted by The Actinobacteriophages Database, funded by the Howard Hughes Medical Institute, to use this electron micrograph for this taxonomy proposal; it cannot be reused without permission of The Actinobacteriophages Database.


100 nm