

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:	2016.008	8aB		(to be completed by ICTV officers)							
<b>Short title:</b> To add six (6) new species to the genus <i>Bxz1virus</i> within the family <i>Myovirida</i> (e.g. 6 new species in the genus <i>Zetavirus</i> )											
Modules attached (modules 1 and 10 are required)		$ \begin{array}{c} 1 \\ 6 \end{array} $	2 🔀 7 🗌	$3 \square $ $8 \boxtimes$	4 9	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 n doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)	ICTV Bacterial and Archaeal Viruses Subcommittee
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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 2016.008aB (assigned by ICTV officers) To create six (6) new species within: Fill in all that apply. • If the higher taxon has yet to be Genus: *Bxz1virus* created (in a later module, below) write Subfamily: "(new)" after its proposed name. Family: *Myoviridae*  If no genus is specified, enter Order: *Caudovirales* "unassigned" in the genus box. Name of new species: **Representative isolate:** (only 1 GenBank per species please) sequence accession number(s) Mycobacterium virus Bxz1 Mycobacterium phage Bxz1 AY129337.1 Mycobacterium phage Dandelion Mycobacterium virus Dandelion JN412588.1 Mycobacterium phage Nappy *Mycobacterium virus Nappy* JN699627.1 Mycobacterium virus Sebata Mycobacterium phage Sebata JN204348.1 Mycobacterium phage HyRo Mycobacterium virus HyRo KT281790.1 Mycobacterium virus Alice Mycobacterium phage Alice JF704092.1

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.

Phages in this genus possess genomes of ca. 155.4 kb in length (64.7 mol%G+C) and encode 230 proteins and 31 tRNAs on average. By comparison, their closest relative, phage Myrna, encodes approximately the same number of proteins but more tRNAs (41). Phage Myrna's genome is longer (164.6 kb) and possesses a higher GC-content (65.4%).

BLASTN analyses (NCBI, and Gegenees, Table 1, Fig. 1), coupled with progressiveMauve (Fig. 2), proteomic analyses using CoreGenes; and, phylogenetic analysis (Fig. 3) all indicate that the Bxz1viruses are a cohesive group of viruses.

## 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. Lavigne R, Darius P, Summer EJ, Seto D, Mahadevan P, Nilsson AS, Ackermann HW, Kropinski AM. Classification of Myoviridae bacteriophages using protein sequence similarity. BMC Microbiol. 2009;9:224.

#### 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 Mycobacterium phage Bxz1 (<u>http://phagesdb.org/phages/Bxz1/</u>) - 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. 2.** The BLASTN analysis of the phage genomes related to Mycobacterium phage Bxz1 determined using Gegenees [4]. The outlier phage Myrna is classified to the C2 subcluster of these viruses, and will become the member of the type species of another genus within a future subfamily once other similar phage genomes are deposited in GenBank.

PHAGE	ACCESSION NO.	AY129337.1	JN699013.1	JF704116.1	KF006817.1	KC748970.1	KR080196.1	JF704096.1	JN699625.1	EU826467.1	JN624850.1	KM881426.1	JQ911768.1	KT321476.1	KC748968.1	KF024734.1	EU826468.1	DQ398053.1	KJ595575.1	JN699626.1	EU826469.1	KC691257.1	EU826471.1	GQ303262.1	GQ303260.1	JN412588.1	JN699627.1	JN412592.1	JN204348.1	KT281790.1	JF704092.1	EU826466.1
Bxz1	AY129337.1	100.0	99.3	97.7	97.5	96.9	95.9	95.8	95.3	96.1	95.7	95.3	94.8	95.4	95.2	95.8	95.6	94.5	94.9	94.9	93.4	95.2	94.3	93.8	93.1	94.3	94.3	92.4	92.2	90.6	90.6	42.6
Pio	JN699013.1	99.2	100.0	97.3	97.4	96.7	95.8	95.7	95.3	96.1	95.7	95.4	94.8	95.3	95.3	95.9	95.5	94.2	94.9	95.0	93.3	95.5	94.5	93.9	93.1	94.3	94.5	92.5	92.1	90.7	90.7	44.9
Drazdys	JF704116.1	97.7	97.5	100.0	97.4	98.1	96.9	96.9	95.6	96.4	95.7	95.2	94.9	95.1	95.4	95.8	95.6	94.6	95.0	95.2	93.8	95.6	95.1	94.6	93.9	93.9	94.5	92.8	92.1	91.0	90.4	46.2
Breeniome	KF006817.1	97.4	97.3	97.2	100.0	97.3	96.3	96.1	95.7	96.7	95.8	95.5	95.2	95.2	95.4	95.9	94.7	95.0	95.6	94.4	93.7	95.0	94.9	94.6	93.6	93.8	94.8	92.5	92.1	91.0	90.5	43.4
ArcherS7	KC748970.1	96.5	96.4	97.9	97.3	100.0	97.6	97.8	95.8	96.5	95.6	95.5	95.1	95.2	95.3	96.0	94.9	95.2	95.4	94.5	93.7	95.6	95.3	94.9	94.3	93.6	94.6	92.5	91.7	91.5	91.1	42.9
Momo	KR080196.1	95.6	95.7	96.9	96.4	97.7	100.0	99.5	96.9	97.0	95.9	95.5	95.0	95.3	95.5	96.1	94.9	96.3	96.5	94.3	94.7	94.9	95.2	94.4	94.3	93.5	93.6	93.4	92.3	91.9	91.5	43.0
Ghost	JF704096.1	95.5	95.6	96.8	96.1	97.7	99.4	100.0	97.1	97.2	95.7	95.4	95.0	95.4	95.5	96.0	94.9	96.5	96.5	94.2	94.5	94.8	95.2	94.2	94.2	93.6	93.7	93.3	92.5	91.7	91.3	43.3
Wally	JN699625.1	95.2	95.3	95.8	95.8	96.0	97.1	97.3	100.0	98.4	96.7	96.8	96.3	96.7	96.3	96.7	96.1	97.7	97.8	95.0	95.5	95.0	95.1	94.7	94.2	94.0	94.1	94.2	93.1	92.1	91.7	45.0
Riza1	EU826467.1	96.0	96.2	96.4	96.9	96.6	96.9	97.1	98.3	100.0	97.5	97.6	97.0	97.2	97.0	96.6	96.6	97.3	97.7	95.7	95.0	95.5	95.5	95.2	94.2	94.5	94.5	93.6	92.5	91.4	91.2	42.5
Pleione	JN624850.1	95.6	95.6	95.6	95.8	95.7	95.8	95.7	96.5	97.5	100.0	98.3	97.6	97.6	98.1	97.1	96.0	95.6	97.4	95.8	95.0	95.4	95.1	94.9	94.1	94.2	94.2	93.7	92.9	91.7	91.4	43.5
ZygoTaiga	KM881426.1	95.0	95.1	95.0	95.2	95.2	95.2	95.2	96.4	97.2	98.2	100.0	98.8	97.9	97.9	96.9	96.2	95.6	98.3	95.1	95.3	95.3	95.0	94.6	94.1	94.0	94.1	93.3	92.5	91.5	91.5	44.8
Ava3	JQ911768.1	94.8	94.8	95.0	95.2	95.4	95.2	95.2	96.2	97.0	97.8	99.1	100.0	98.3	97.8	96.6	96.4	96.0	98.2	95.3	95.7	95.2	95.2	94.8	94.2	94.1	94.3	93.5	92.8	92.0	91.6	44.2
Zeenon	KT321476.1	95.3	95.3	95.1	95.1	95.2	95.3	95.3	96.7	97.2	97.7	98.1	98.2	100.0	97.8	96.1	96.2	96.0	97.1	95.0	95.2	95.1	94.6	94.3	94.1	94.4	94.1	93.9	93.0	92.2	91.9	43.6
Gizmo	KC748968.1	95.1	95.2	95.2	95.2	95.4	95.4	95.4	96.1	96.8	98.0	98.0	97.6	97.6	100.0	96.8	96.0	95.5	97.0	95.8	95.2	95.0	94.8	94.3	93.5	93.6	94.0	92.8	91.9	91.2	90.9	43.8
Shrimp	KF024734.1	95.8	96.0	95.8	96.1	96.1	96.1	96.0	96.6	96.7	97.3	97.2	96.4	96.3	96.9	100.0	95.2	95.8	97.2	95.5	94.8	95.0	94.3	94.2	93.9	93.4	94.6	93.0	92.3	91.6	91.2	43.4
Spud	EU826468.1	95.6	95.9	95.5	94.9	95.2	95.0	95.0	96.3	96.7	96.4	96.7	96.5	96.4	96.3	95.4	100.0	96.5	96.2	95.8	97.0	95.2	94.9	94.8	93.6	94.0	93.8	93.4	92.5	91.9	91.8	47.1
Catera	DQ398053.1	94.6	94.7	95.0	95.2	95.5	96.7	96.9	97.9	97.6	95.9	96.2	96.2	96.2	96.0	96.1	96.7	100.0	97.3	95.2	96.8	94.5	94.8	94.3	93.9	93.8	93.6	93.6	92.6	92.7	92.4	46.7
Willis	KJ595575.1	94.6	94.8	95.0	95.5	95.4	96.5	96.5	97.7	97.6	97.5	98.6	98.1	97.2	97.1	97.1	95.9	97.0	100.0	95.3	95.7	94.8	95.0	94.5	94.3	94.1	94.2	94.3	93.4	92.3	92.1	45.5
MoMoMixon	JN699626.1	95.2	95.5	95.2	94.7	95.0	94.4	94.3	95.2	96.0	96.0	95.5	95.4	95.2	96.0	95.7	95.7	95.1	95.4	100.0	94.2	94.7	94.0	94.0	93.2	93.8	94.6	92.8	91.9	91.1	90.8	47.0
ScottMcG	EU826469.1	93.6	93.7	94.2	93.9	94.3	95.1	94.9	95.6	95.2	95.3	95.8	95.7	95.4	95.4	95.0	97.3	96.6	96.0	94.4	100.0	94.2	94.2	93.7	93.6	93.6	93.1	93.8	92.9	92.4	92.0	45.8
Astraea	KC691257.1	94.9	95.1	95.2	94.8	95.7	95.0	94.9	95.0	95.5	95.3	95.2	95.0	95.0	95.1	94.8	94.9	94.3	94.7	94.3	93.9	100.0	95.1	95.1	93.8	93.6	93.1	93.5	92.0	92.3	92.7	46.0
Cali	EU826471.1	94.2	94.4	94.8	94.5	95.1	94.9	94.9	94.9	95.1	94.9	95.0	94.8	94.4	94.6	94.0	94.5	94.2	94.9	93.8	93.7	94.8	100.0	96.9	95.5	93.6	93.6	93.6	92.8	91.9	91.2	44.6
LRRHood	GQ303262.1	93.7	93.9	94.6	94.4	94.9	94.5	94.4	94.6	95.0	94.7	94.8	94.5	94.3	94.2	94.2	94.4	93.9	94.5	93.7	93.3	94.9	97.2	100.0	95.5	93.7	93.7	94.0	93.2	92.9	92.1	44.3
ET08	GQ303260.1	93.0	93.3	94.0	93.5	94.4	94.3	94.3	94.1	94.3	94.2	94.4	94.0	94.1	93.7	94.0	93.5	93.5	94.2	92.9	93.2	93.9	96.1	95.5	100.0	92.6	93.1	93.8	93.0	92.5	93.6	46.0
Dandelion	JN412588.1	94.2	94.3	93.8	93.6	93.9	93.5	93.4	94.0	94.3	94.0	94.2	93.9	94.1	93.6	93.3	93.7	93.3	93.9	93.6	93.2	93.4	93.5	93.5	92.7	100.0	94.4	94.2	93.5	90.7	90.3	45.0
Nappy	JN699627.1	94.5	94.6	94.6	94.8	94.7	93.5	93.6	93.9	94.5	94.1	94.2	94.1	93.9	94.0	94.5	93.5	93.0	94.0	94.4	92.5	93.5	93.7	93.9	93.0	94.4	100.0	92.3	91.7	90.5	90.0	42.2
LinStu	JN412592.1	92.5	92.6	92.7	92.5	93.0	93.7	93.6	94.2	93.7	93.7	93.7	93.5	94.0	92.9	93.1	93.3	93.4	94.3	92.8	93.7	93.8	93.8	94.2	94.1	94.6	92.5	100.0	96.8	93.6	92.3	44.3
Sebata	JN204348.1	92.0	92.2	91.7	91.9	91.9	92.3	92.5	93.1	92.5	92.7	92.7	92.7	92.9	92.0	92.3	92.1	92.3	93.3	91.7	92.2	92.4	92.9	93.0	93.4	93.5	92.0	96.7	100.0	93.7	92.2	44.9
HyRo	KT281790 1	91.0	91.1	91.4	91.3	91.9	92.3	92.2	927	92.0	92.2	92.1	92.5	92.7	92.0	91.9	92.3	93.1	92.6	91.4	92.9	92.7	92.4	93.3	92.6	91.3	90.8	93.8	94.0	100.0	95.0	45.5
Alice	JF704092.1	90.3	90.4	90.6	90.4	91.0	91.5	91.4	91.9	91.2	91.5	91.8	91.6	91.8	91.2	91.2	91.6	92.0	92.0	90.5	91.6	92.8	91.8	92.1	93.8	90.8	90.2	92.3	92.2	94.5	100.0	44.0
Myrna	EU826466.1	43.0	44.4	44.4	43.7	44.4	43.2	43.2	43.5	42.7	43.8	44.0	45.0	45.0	45.5	43.3	45.6	45.0	43.9	45.4	45.8	44.7	44.4	45.1	45.5	44.9	43.4	44.9	45.3	45.3	43.6	100.0

Mycobacter ium Phage	RefSeq No.	GenBank Accession No.	Genome length (kb)	Genome (mol % G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)	% Homo- logous proteins **			
Bxz1	NC_004687	AY129337	156.1	64.8	225	28	100	100			
Dandelion	NC_023696	JN412588	157.6	64.7	239	35	94	96			
Nappy	NC_023725	JN699627	156.6	64.7	234	33	93	95.1			
Sebata		JN204348	155.3	64.8	232	35	92	94.7			
HyRo		KT281790	153.7	64.7	225	26	89	87.6			
Alice		JF704092	153.4	64.7	223	29	88	85.3			
Myrna	NC_011273	EU826466	164.6	65.4	229	41	26	44.9			

**Table 1**. Properties of the six phages belonging to the genus *Bxz1virus*.

\* Determined using BLASTN; \*\* Determined using CoreGenes [2]

**Table 2.** Phages which should be considered strains of the species *Mycobacterium virus Bxz1* within the *Bxz1virus* genus.

Phage	Accession No.
Mycobacterium phage Pio	JN699013.1
Mycobacterium phage Drazdys	JF704116.1
Mycobacterium phage Breeniome	KF006817.1
Mycobacterium phage ArcherS7	KC748970.1
Mycobacterium phage Momo	KR080196.1
Mycobacterium phage Ghost	JF704096.1
Mycobacterium phage Wally	JN699625.1
Mycobacterium phage Riza1	EU826467.1
Mycobacterium phage Pleione	JN624850.1
Mycobacterium phage ZygoTaiga	KM881426.1
Mycobacterium phage Ava3	JQ911768.1
Mycobacterium phage Zeenon	kt321476.1
Mycobacterium phage Gizmo	KC748968.1
Mycobacterium phage Shrimp	KF024734.1
Mycobacterium phage Spud	EU826468.1
Mycobacterium phage Catera	DQ398053.1
Mycobacterium phage Willis	KJ595575.1
Mycobacterium phage ScottMcG	EU826469.1
Mycobacterium phage Astraea	KC691257.1
Mycobacterium phage Cali	EU826471.1
Mycobacterium phage LRRHood	GQ303262.1
Mycobacterium phage ET08	GQ303260.1
Mycobacterium phage MoMoMixon	JN699626.1
Mycobacterium phage DTDevon	KT365398.1
Mycobacterium phage Dandelion	JN412588.1
Mycobacterium phage LinStu	JN412592.1

**Fig. 2.** progressiveMauve alignment [1] of the annotated genomes of members of the Bxz1virus genus – from top to bottom: Mycobacterium phages Bxz1, Dandelion, Nappy, Sebata, HyRo, and Alice. 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.** Maximum likelihood phylogenetic tree of phylogenetic tree of gp117 (AAN16773) from Mycobacterium phage Bxz1 and related viruses inferred using PhyML and GTR+I+G substitution model and aLRT branch support. The tree is rooted with Mycobacterium phage Myrna sequence.

