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

Code assigned:	***2016.026a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Marthavirus*, including four (4) new species in the family *Myoviridae*.
(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)	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:

Code	*2016.026aB*		(assigned by ICTV officers)

To create 4 new species within:

Genus:	*Marthavirus* **(new)**	Fill in all that apply.
Subfamily:		• If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
Family:	*Myoviridae*	• If no genus is specified, enter "**unassigned**" in the genus box.
Order:	*Caudovirales*	

Name of new species:	Representative isolate: (only 1 per species please)	GenBank sequence accession number(s)
Arthrobacter virus Martha	Arthrobacter phage Martha	KU160656
Arthrobacter virus Sonny	Arthrobacter phage Sonny	KU160665
Arthrobacter virus Brent	Arthrobacter phage Brent	KT365401
Arthrobacter virus Jawnski	Arthrobacter phage Jawnski	KU160651

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.

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

Code	*2016.026bB*	(assigned by ICTV officers)

To create a new genus within:

		Fill in all that apply.
Subfamily:		• If the higher taxon has yet to be created (in a later module, below) write "**(new)**" after its proposed name.
Family:	*Myoviridae*	
Order:	*Caudovirales*	• If no family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	*2016.026cB*	(assigned by ICTV officers)

To name the new genus: *Marthavirus*

Assigning the type species and other species to a new genus

Code	*2016.026dB*	(assigned by ICTV officers)

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

Arthrobacter virus Martha	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:**

4

Reasons to justify the creation of a new genus:
Additional material in support of this proposal may be presented in the Appendix, Module 9

These lytic myoviruses were enriched from predominantly Pittsburgh, PA, USA, soil (exception is Arthrobacter phage Brent derived from Broomall, PA, USA on *Arthrobacter* sp. ATCC 21022. All phages possess circularly permuted genomes.
NCBI BLASTN, CoreGenes (Table 1) [2], progressiveMauve alignment (Fig. 2) [1], and phylogenetic analyses (Fig. 3) [3] all indicate that the proposed genus, *Marthavirus*, is cohesive and distinct from other genera. On average, the genomes of this genus are 50.3 kb in length (62.2 mol% G+C), and encode 75 proteins and 0 tRNAs.

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.

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 Arthrobacter phage TaeYoung (Left, http://phagesdb.org/phages/TaeYoung/); and Arthrobacter phage Jawnski (Right, http://phagesdb.org/phages/Jawnski/) - 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.
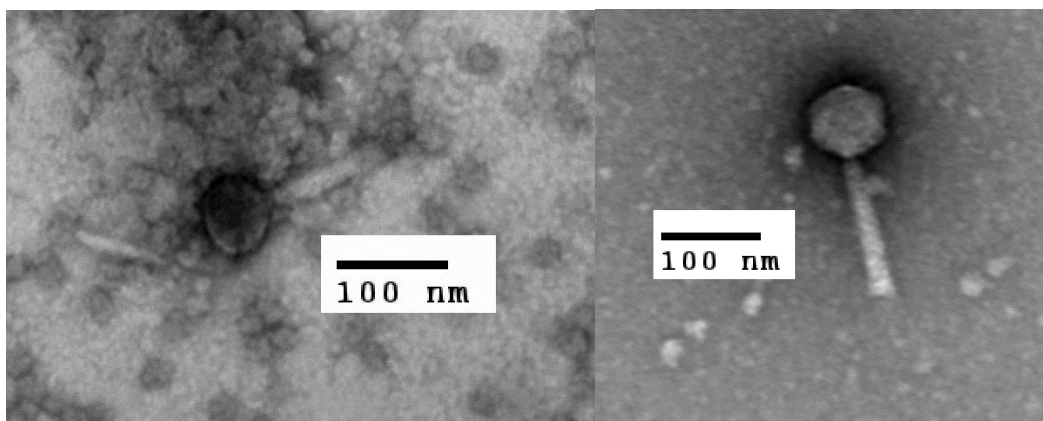
Table 1. Properties of the five phages belonging to the genus *Marthavirus*.

Arthrobacter phage	GenBank Accession No.	Genome length (kb)	Genome (mol% G+C)	No. CDS	DNA (% sequence identity)*	% Homologous proteins **
Martha	KU160656	51.03	61.0	77	100	100
Sonny	KU160665	50.91	61.1	77	91	93.5
Brent	KT365401	49.88	63.4	74	67	89.6
Jawnski	KU160651	49.42	63.4	73	66	88.3
TaeYoung***	KU160668	51.00	61.0	78	97	100

* Determined using BLASTN; ** Determined using CoreGenes [2]; *** should be considered a strain of Arthrobacter phage Martha.

Fig. 2. progressiveMauve alignment [1] of the genomes of members of the *Marthavirus* genus – from top to bottom: Arthrobacter phages Martha, TaeYoung, Sonny, Jawnski, and Brent. 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).
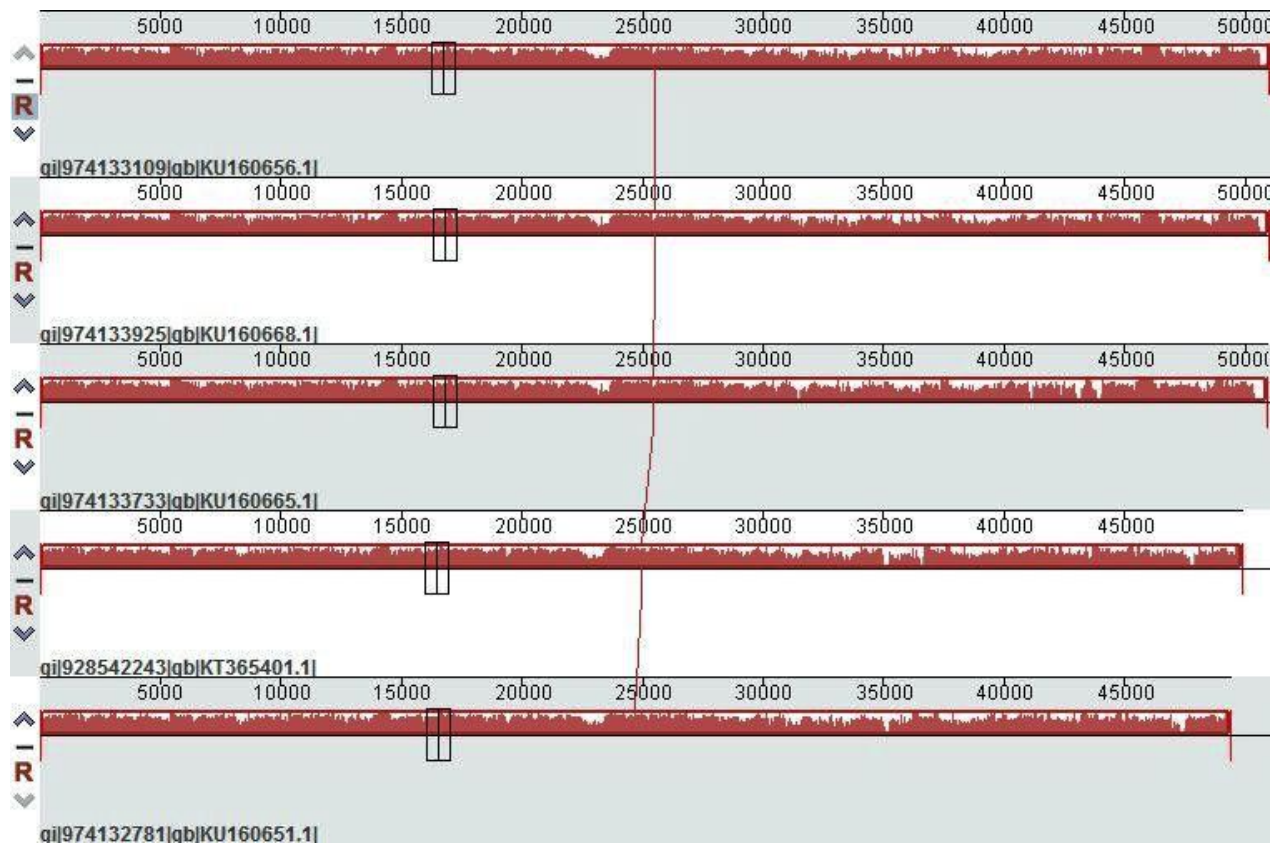

Fig. 3. Phylogenetic analysis of (A) large subunit terminase proteins, (B) major capsid proteins, and (C) tail sheath proteins of marthaviruses and homologous proteins from a variety of other phages constructed using "one click" at phylogeny.fr [3]. "The "One Click mode" targets users that do not wish 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."
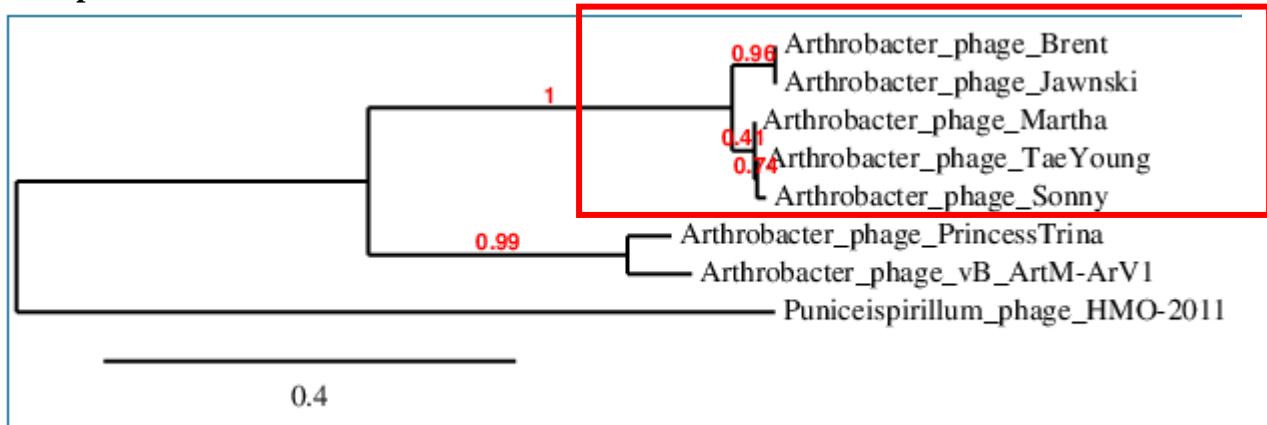
A. TerL proteins

Figure 1: *Phylogenetic tree.*

B. Major capsid proteins

Figure 1: *Phylogenetic tree.*

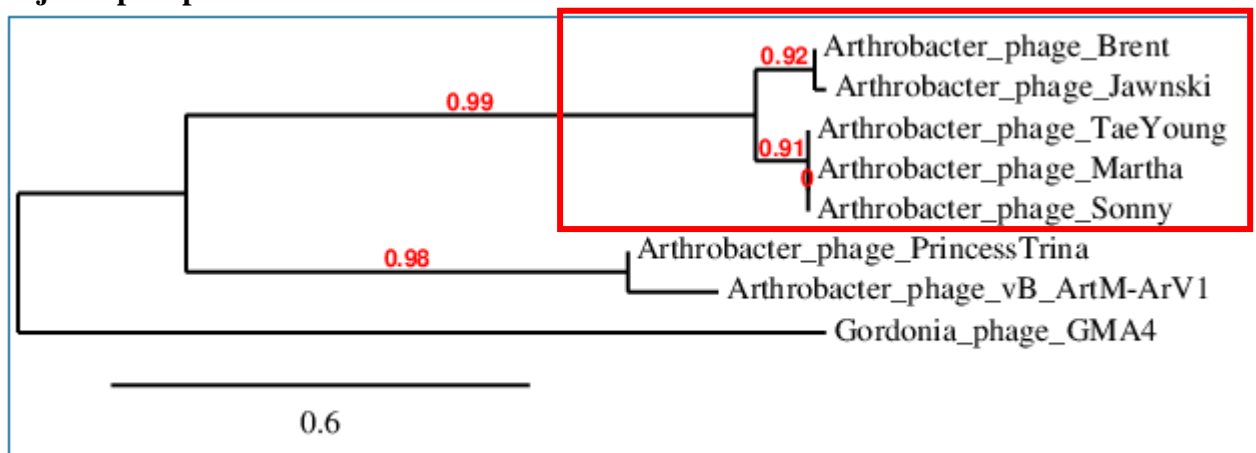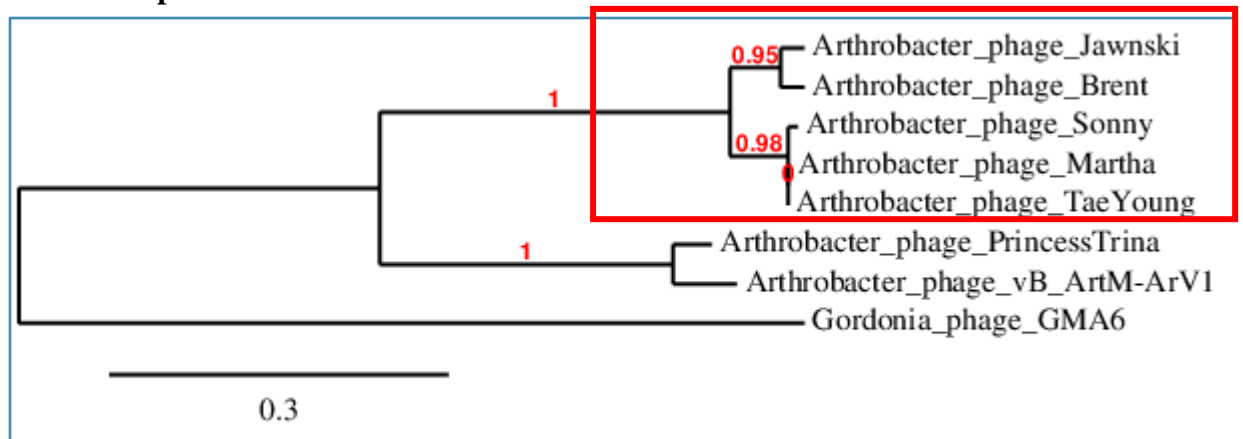### C. Tail sheath proteins

Figure 1: *Phylogenetic tree.*