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

Code assigned:	*2016.025a-dB*	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *M12virus*, including three (3) 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:

MODULE 2: **NEW SPECIES**

Code	*2016.025aB*		(assigned by ICTV officers)

To create 3 new species within:

Genus:	*M12virus* (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)
Sinorhizobium virus M12	Sinorhizobium phage phiM12	KF381361
Sinorhizobium virus M7	Sinorhizobium phage phiM7	KR052480
Sinorhizobium virus N3	Sinorhizobium phage phiN3	KR052482

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	*2016.025bB*	(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.
- If no family is specified, enter "**unassigned**" in the family box

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

naming a new genus

Code	*2016.025cB*	(assigned by ICTV officers)

To name the new genus: *M12virus*

Assigning the type species and other species to a new genus

Code	*2016.025dB*	(assigned by ICTV officers)

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

Sinorhizobium virus M12

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:**

3

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

Phage phiM12 was isolated from a commercial alfalfa inoculant manufactured in the USA and used for transduction in the *Sinorhizobium meliloti* laboratory strain Rm1021 [7]. The phage "has an icosahedral head (diameter, 83 nm ± 5), a rigid and contractile tail (length, 100 nm ± 10; width, 18 nm) constructed of a hollow rod surrounded by ca. 23 to 25 annuli that end a short distance from the head, and a bar or collar at the base of the head. Thin fibers may extend from the neck-collar region. The baseplate has projections that might be short flexible fibers and also has very thin, long, tail fibers (50 to 60 nm)". [7] The head possesses a novel T=19l triangulation number [5]. The cellular receptor for this virus is the essential porin RopA1 [6]. "No restriction was observed with *Bam*HI, *Bgl*Il, *Kpn*I, *Mst*II, *Pvu*I, *Pst*I, *Sal*l, *Sma*I, *Stu*I, *Xba*I, or *Xho*I" [7] suggesting modification of the DNA. The highlights of the major publication on the genome and proteome of this virus [4] are:

(a) This is the first complete genome of a rhizobium-infecting T4-superfamily phage;

(b) The structural genes of Sinorhizobium phage phiM12 are most similar to T4-like phages of cyanobacteria;

(c) It is the first report of a T4-superfamily phage lacking genes for class I RNR and

exonuclease dexA;

  (d) Sinorhizobium phage phiM12 is unique among T4-superfamily phages in possessing a class II B12-dependent RNR.

In this article the authors note that this phage is "the founder of a new group of T4-superfamily phages."

BLASTN, CoreGenes (Table 1) [2], progressiveMauve alignment (Fig. 2) [1], and phylogenetic analyses (Fig. 3) [3] all indicate that the proposed genus, *M12virus*, is cohesive and distinct from other genera. On average, the genomes of members of this genus are 196.6 kb in length (49.0 mol% G+C), and encode 380 proteins and 9 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.

4. Brewer TE, Stroupe ME, Jones KM. The genome, proteome and phylogenetic analysis of *Sinorhizobium meliloti* phage ΦM12, the founder of a new group of T4-superfamily phages. Virology. 2014 Feb;450-451:84-97.

5. Stroupe ME, Brewer TE, Sousa DR, Jones KM. The structure of *Sinorhizobium meliloti* phage ΦM12, which has a novel T=19l triangulation number and is the founder of a new

additional material in support of this proposal

References:

group of T4-superfamily phages. Virology. 2014 Feb;450-451:205-12.

6. Crook MB, Draper AL, Guillory RJ, Griffitts JS. The *Sinorhizobium meliloti* essential porin RopA1 is a target for numerous bacteriophages. J Bacteriol. 2013; 195(16):3663-71.

7. Finan TM, Hartweig E, LeMieux K, Bergman K, Walker GC, Signer ER. 1984. General transduction in *Rhizobium meliloti*. J. Bacteriol. 159:120–124.

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 (2% uranyl acetate) Sinorhizobium phage phiM12 (provided by D. R. Sousa and M.E. Stroupe).
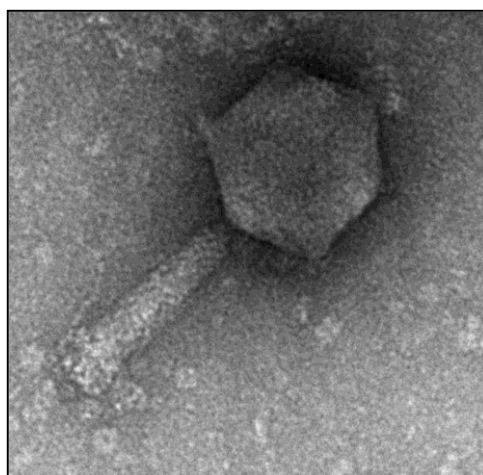

Table 1. Properties of the three phages belonging to the genus *M12virus*.

Sinorhizobium phage	RefSeq No.	GenBank Accession No.	Genome length (kb)	Genome (mol% G+C)	No. CDS	No. tRNAs	DNA (% sequence identity)*	% Homologous proteins **
phiM12	NC_027204	KF381361	194.70	49.0	377	10	100%	100%
phiM7		KR052480	188.43	49.0	361	10***	92%	85.7
phiN3	NC_028945	KR052482	206.71	49.1	402	6	90%	87.5

* Determined using BLASTN; ** Determined using CoreGenes [2]. Sinorhizobium phage phiM19 (KR052481) should be considered a strain of Sinorhizobium phage phiM12. *** Three should be considered pseudo tRNAs.

Fig. 2. progressiveMauve alignment [1] of the annotated genomes of members of the *M12virus* genus – from top to bottom: Sinorhizobium phages phiM7, phiM12, and phiN3. 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).
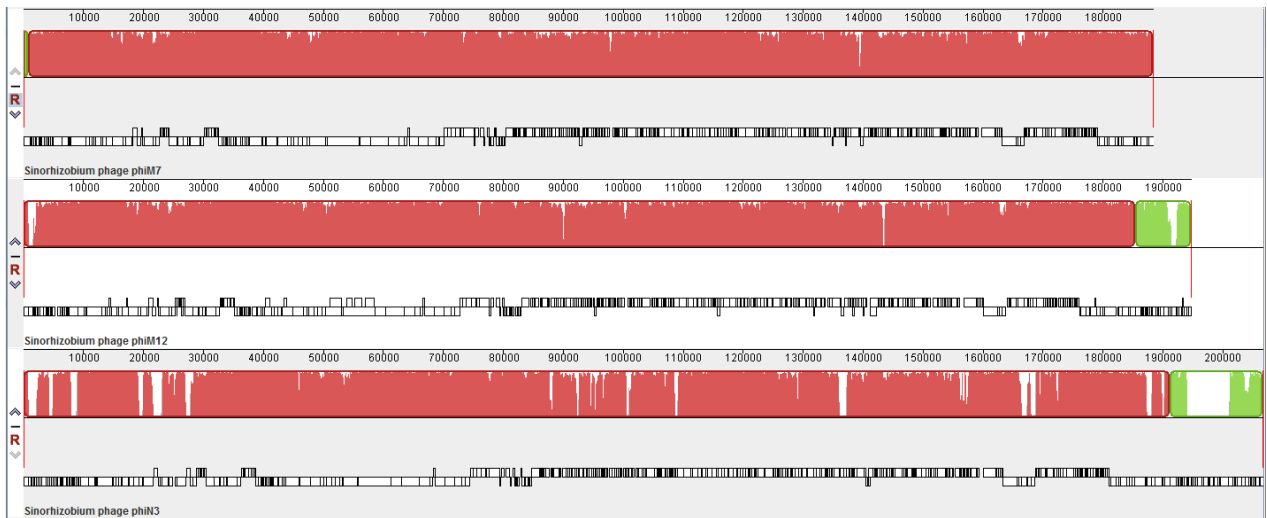

Fig. 3. Phylogenetic analysis of large subunit terminase proteins of Sinorhizobium phage phiM12-like viruses and homologous proteins from other phages constructed using "one click" at phylogeny.fr [3]. "The "One Click mode" targets users that do not wish to deal with program and parameter selection. 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."
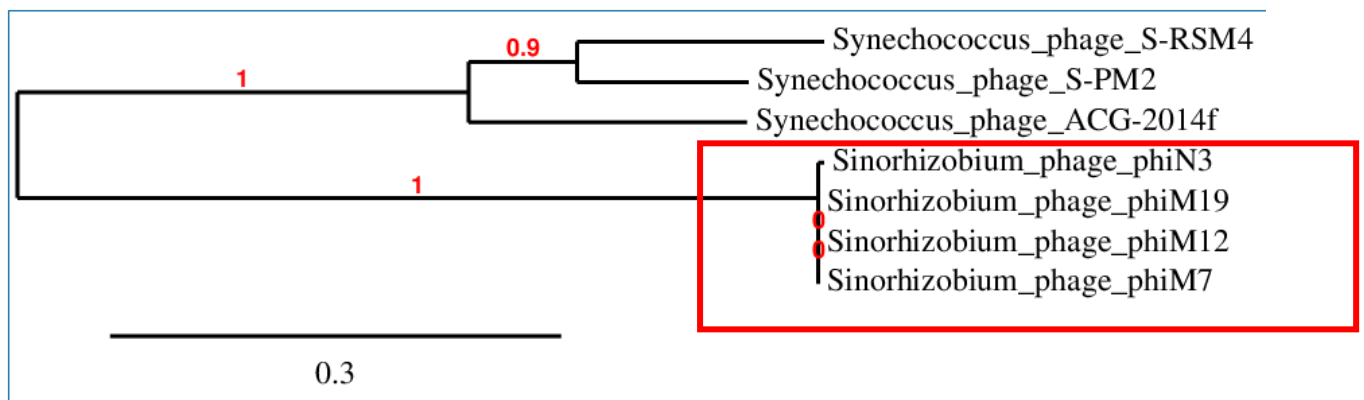

Figure 1: *Phylogenetic tree.*