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:	***2009.008a,bB***	(to be completed by ICTV officers)

Short title: create a new species named Microcystis aeruginosa phage Ma-LMM01 to be unassigned 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** ☒	

Author(s) with e-mail address(es) of the proposer:

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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	**N/A**

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

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	**2009.008aB**	(assigned by ICTV officers)

To create 1 new species with the name(s):

Microcystis aeruginosa phage Ma-LMM01

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	**2009.008bB**	(assigned by ICTV officers)

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

Genus:	**unassigned**
Subfamily:	**unassigned**
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

The phage Ma-LMM01 was shown to be infectious to the toxic cyanobacterium *Microcystis aeruginosa*. The morphology feature of Ma-LMM01 (having a contractile tail) suggests it is a member of the family *Myoviridae*.

The genome (162,109-bp long with 184 predicted protein-coding genes and two tRNA genes) showed no long-range colinearity with previously sequenced genomes of other myoviruses. The vast majority of the predicted genes did not show considerable similarity to other viruses; ex. except for ORF91 (tail sheath protein gene), no ORFs in the genome were similar to genes of the ever reported structural components (such as major capsid proteins) of other phages.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Yoshida, T., Nagasaki, K., Takashima, Y., Shirai, Y., Tomaru, Y., Takao, Y., Sakamoto, S., Hiroishi, S. and Ogata, H. Ma-LMM01 infecting toxic Microcystis aeruginosa illuminates diverse cyanophage genome strategies. J. Bacteriol. 190,1762-1772 (2008)

Yoshida, T., Takashima, Y., Tomaru, Y., Shirai, Y., Takao, Y., Hiroishi, S. and Nagasaki, K. Isolation and Characterization of a Cyanophage Infecting the Toxic Cyanobacterium Microcystis aeruginosa. Appl. Environ. Microbiol. 72, 1239-1247 (2006)

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.

Genome analysis of the *Myoviridae* shows that the proposed subfamilies and genera have large genomic diversity and myovirus genomes are more diverse than one may expect from their shared morphological features. From a proteomic perspective, phage Ma-LMM01 can be considered a 'unique' virus, lacking correlation to other known (sequenced bacteriophages) (Figure 1).

The head of the phage Ma-LMM01 is 86 nm in diameter and the tail complex consists of a central tube (9 nm in width); and has a contractile sheath (24 nm in width, 209 nm in length) that contracts to 90 nm in length. No fiber-like structure is observed (Fig. 2).

Genomic sequences of Ma-LMM01 were assembled into a circular 162,109 base-pair (bp) sequence with 184 putative protein coding genes and two tRNA genes (Fig. 3; accession number = AB231700). Ma-LMM01 phage particles contain a linear double-stranded DNA ca. 165 kb long, indicating that the genome has terminal redundancy of about 3 kb. The packaged genomic DNA is circularly permuted. The genomic G+C content was 46.0%. The Ma-LMM01 genome exhibited no long-range colinearity with previously sequenced genomes other myoviruses. BLAST searches (using an E-value threshold of 10^{-5}) revealed database homologs for only 44 (24%) of the 184 ORFs, leaving 140 ORFs with no detectable homologs in the databases. Only four Ma-LMM01 ORFs (ORF1, ORF41, ORF108, ORF109) showed their best BLAST scores against known viral sequences in the databases. Except for ORF91 (tail sheath protein gene), no ORFs in the genome showed considerable similarity to genes of known structural proteins of other phages.

Ma-LMM01 contains four major polypeptides of 84, 47, 38 and 26 kDa using SDS-PAGE (Fig. 4). The N-terminal amino acid sequences of the 47 kDa ("SDIPS") and 38 kDa ("SIHNV") polypeptides were found to respectively correspond to the deduced amino acid sequences of ORF86 and ORF87. These two ORFs showed no significant BLAST hits for any other proteins in the database, suggesting the long genetic distance between Ma-LMM01 and previously known viruses.

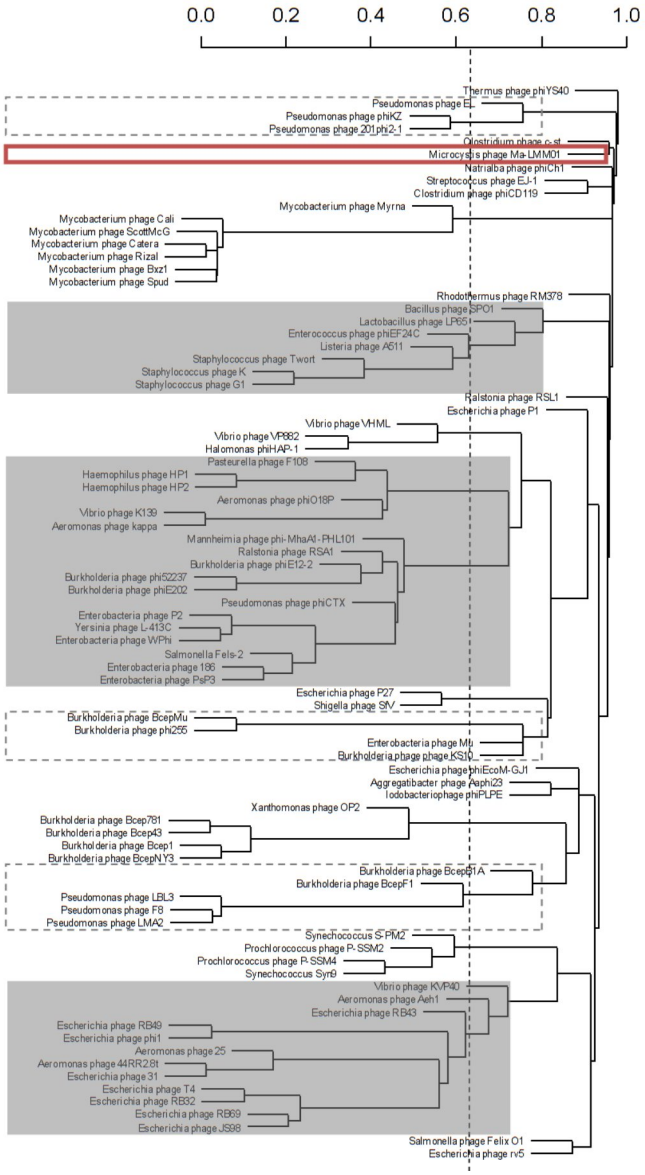

Fig. 1: Hierarchical cluster dendrogram of the *Myoviridae*
The relative dissimilarity between the phage proteomes (between 0.0 and 1.0) forms the basis for the proposed groupings. The dotted lines reflects the cut-off value used for the establishement of genera, used consistently for all *Myoviridae* and the previously defined *Podoviridae* (Lavigne et al., 2008). Subfamily and tentative subfamily groupings are indicated in the grey and dotted boxes, respectively. Phage Ma-LMM01 (red box) shows a large dissimilarity to other *Myoviridae* and can therefore be considered as a unassigned species within the *Myoviridae* family (pending the discovery of more closely related species which would warrant the establishment of a genus).

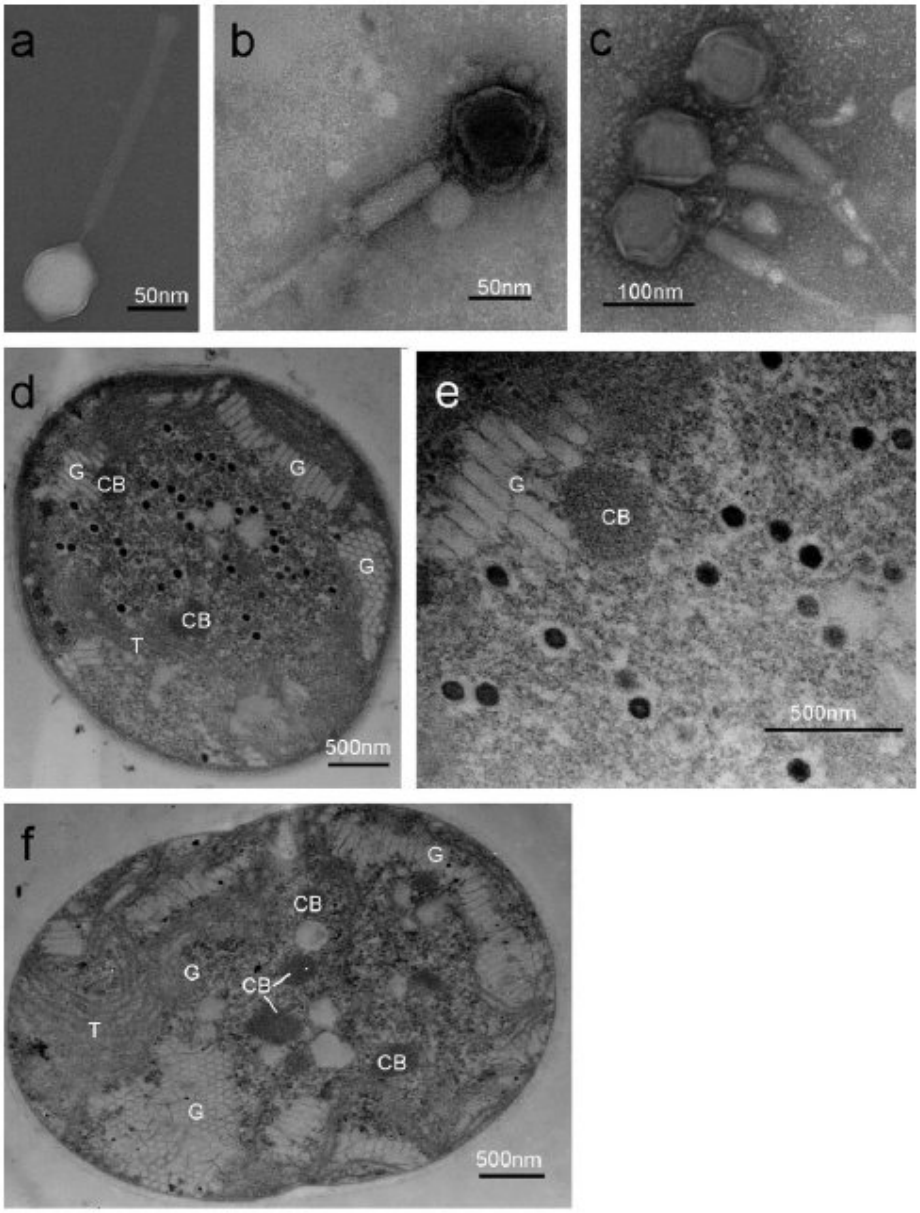

Fig. 2. Transmission electron micrographs of the cyanophage Ma-LMM01 and its host *Microcystis aeruginosa* NIES298: (a) A negatively stained virion of Ma-LMM01 with extended tail; (b) a negatively stained virion of Ma-LMM01 with a contracted tail; (c) negatively stained virions of Ma-LMM01 with a contracted tail when purified using CsCl step gradient ultracentrifugation; (d) thin section of a *M. aeruginosa* cell 52h after inoculation with Ma-LMM01; (e) higher magnification of Ma-LMM01 particles (d); (f) thin section of a healthy cells of *M. aeruginosa*. CB, carboxysome; G, gas vesicle; T, thylakoid.

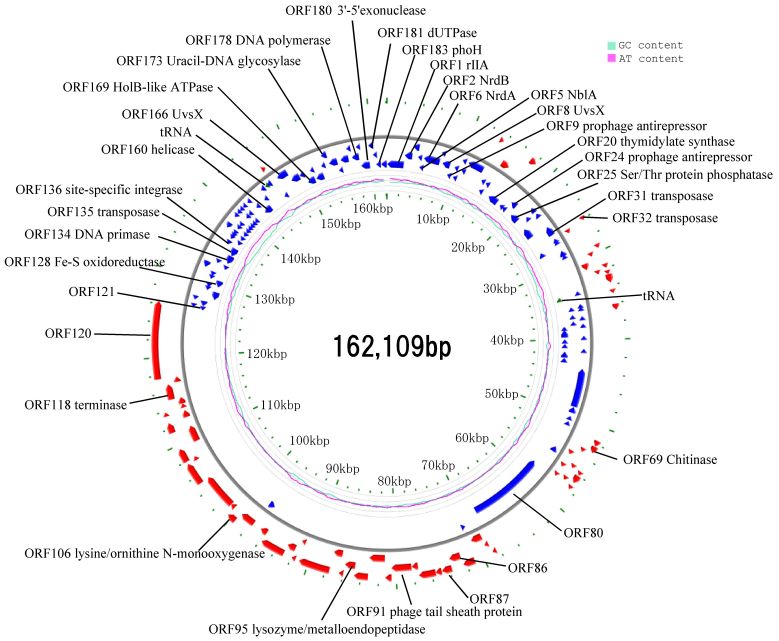

Fig. 3. Organization of the genome of Ma-LMM01. Red and blue arrows indicate putative open reading frames (ORFs). Pale blue and pink lines inside the circle show G+C and A+T content, respectively.

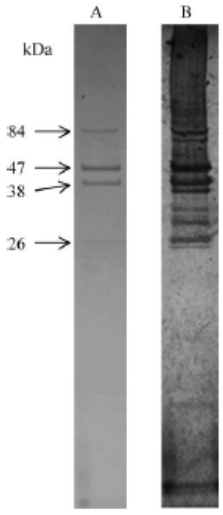

Fig.4. Major structural proteins of Ma-LMM01 stained by Coomassie Brilliant Blue G (left lane) or using the silver-staining method (right lane).