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:	***2015.024a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Vhmlvirus*, 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)	Bacterial & Archaeal Virus Subcommittee

ICTV Study Group comments (if any) and response of the proposer:

Please note that we have chosen to refer to this new genus as *Vhmlvirus* rather than *Vhmllikevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" from phage genus names.

Date first submitted to ICTV: May 2015
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	***2015.024aB***	(assigned by ICTV officers)

To create 3 new species within:

Genus:	***Vhmlvirus (new)***
Subfamily:	
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.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Vibrio virus VHML	Vibrio phage VHML	AY133112
Vibrio virus MAR	Vibrio phage vB_VpaM_MAR	JX556417
Vibrio virus VP585	Vibrio phage VP585	FN297812

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

Two of these phages, VHML and VP58.5 were isolated from lysogenic cultures of respectively *Vibrio harveyi* (1) and *V. parahaemolyticus* (serovar O3:K6; 2), respectively. Phage vB_VpaM_MAR (MAR) was isolated by enrichment from Mexican waters (3) using *V. parahaemolyticus* as a host. MAR caused a weak lytic response against *Vibrio alginolyticus* and *Photobacterium leiognathi*. BLASTN analysis revealed they are closely related. Two other myoviruses *Halomonas* phage ΦHAP-1 and *Vibrio* phage VP882 may be considered peripherally related but, while these share 39 and 47% protein homologs, they share ≤5% overall DNA sequence identity. At this time we do not want to consider higher level relationships.

MAR was characterized by electron microscopy (Fig. A) revealing an icosahedral head (74 nm long by 69 nm wide) and a contractile tail (234 nm long and 20 nm wide) with tail fibers. It is morphologically similar to *Vibrio vulnificus* phage P147 (7). Electron micrographs are presented in the paper on VP58.5 but no sizes were given.

The detailed worked on Vibrio phage VP58.5 by Zabala et al. (2) "show that it does not integrate into the host cell chromosome but replicates as a linear plasmid." It contains 5'-protruding ends. "VHML phage DNA has been reported to contain terminal inverted repeats. This repetitive sequence is similar to the telomere resolution site (telRL) of VP58.5."

We have chosen 95% DNA sequence identity as the criterion for demarcation of species in this new genus. Each of the proposed species differs from the others with more than 5% at the DNA level as confirmed with the BLASTN algorithm.

The relatedness of these two phages was confirmed using CoreGenes which the Bacterial and Archaeal Virus Subcommittee of ICTV has extensively used to compare the total proteomes (Table 1) of two viruses; progressiveMauve (Fig. 2); and, by phylogenetic analysis (5) of their protelomerases (Fig. 3). These proteins show a peripheral relatedness to the protelomerases from the *N15virus*. The latter viruses differ fundamentally from members of the *Vhmlvirus* being members of the *Siphoviridae*.

Please note that we have chosen to refer to this new genus as *Vhmlvirus* rather than *Vhmllikevirus* since the Bacterial and Archaeal Virus Subcommittee of ICTV has voted overwhelmingly in favour of eliminating "*like*" from phage genus names.

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2015.024bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
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 family is specified, enter "**unassigned**" in the family box

naming a new genus

Code	***2015.024cB***	(assigned by ICTV officers)

To name the new genus: *Vhmlvirus*

Assigning the type species and other species to a new genus

Code	***2015.024dB***	(assigned by ICTV officers)

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

Vibrio virus VHML

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

BLASTN, CoreGenes, progressiveMauve alignment and phylogenetic analyses all indicate that the proposed genus, *Vhmlvirus*, is cohesive and distinct from the other genera of viruses.

Origin of the new genus name:

Named after the first phage of its type to be sequenced: *Vibrio* phage VHML (1)

Reasons to justify the choice of type species:

First phage of its type to be sequenced

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. Each of the proposed species differs from the others with 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. Oakey HJ, Owens L. A new bacteriophage, VHML, isolated from a toxin-producing strain of *Vibrio harveyi* in tropical Australia. J Appl Microbiol. 2000; 89(4):702-9.
2. Zabala B, Hammerl JA, Espejo RT, Hertwig S. The linear plasmid prophage Vp58.5 of *Vibrio parahaemolyticus* is closely related to the integrating phage VHML and constitutes a new incompatibility group of telomere phages. J Virol. 2009; 83(18):9313-20.
3. Alanis Villa A, Kropinski AM, Abbasifar R, Griffiths MW. Complete genome sequence of *Vibrio parahaemolyticus* bacteriophage vB_VpaM_MAR. J Virol. 2012; 86(23):13138-9.
4. Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One. 2010; 5(6):e11147.
5. 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.
6. 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.
7. Pelon W, Siebeling RJ, Simonson J, Luftig RB. Isolation of bacteriophage infectious for *Vibrio vulnificus*. Curr Microbiol. 1995;30(6):331-6.

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 the two phages belonging to the genus *Vhmlvirus*

Phage	Genome length (bp)	Genome (mol%G+C)	No. CDS	DNA (% sequence identity)*	Proteome (% homologous proteins)**
VHML	43,198	50.6	57	100	100
MAR	41,351	51.3	62	88	77
VP58.5	42,612	50.9	58	84	79

* Determined using BLASTN; ** Determined using CoreGenes (5)

Fig. 1. Electron micrograph of *Vibrio parahaemolyticus* phage MAR negatively stained with 2% uranyl acetate showing intact tail (left) and contracted tails and empty heads (right).

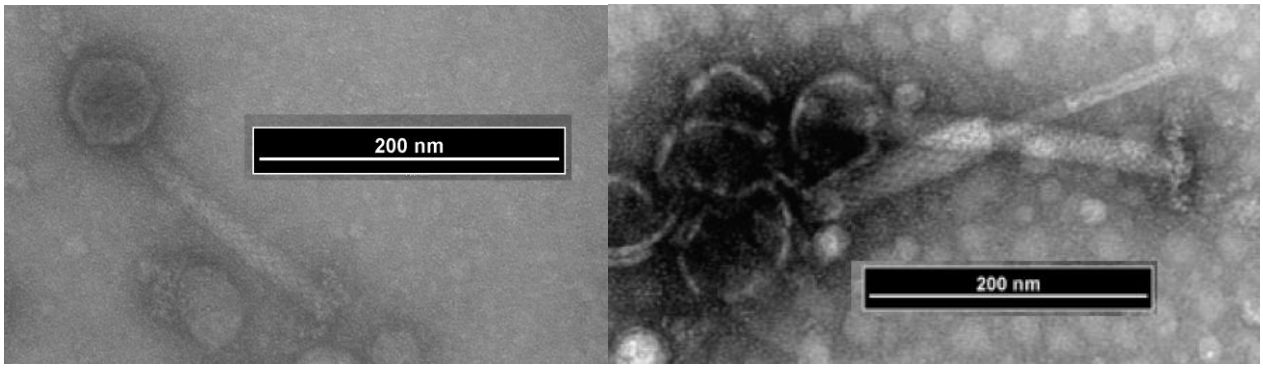

Fig. 2. progressiveMauve alignment (4) of the annotated genomes of VHML (top), MAR (middle) and VP58.5 (bottom). Colored blocks indicate the regions of 1 to 1 best alignment with rearrangement breakpoints in a different random color. The degree of sequence similarity between regions is given by a similarity plot within the colored blocks with the height of the plot proportional to the average nucleotide identity (Aaron Darling, personal communication).

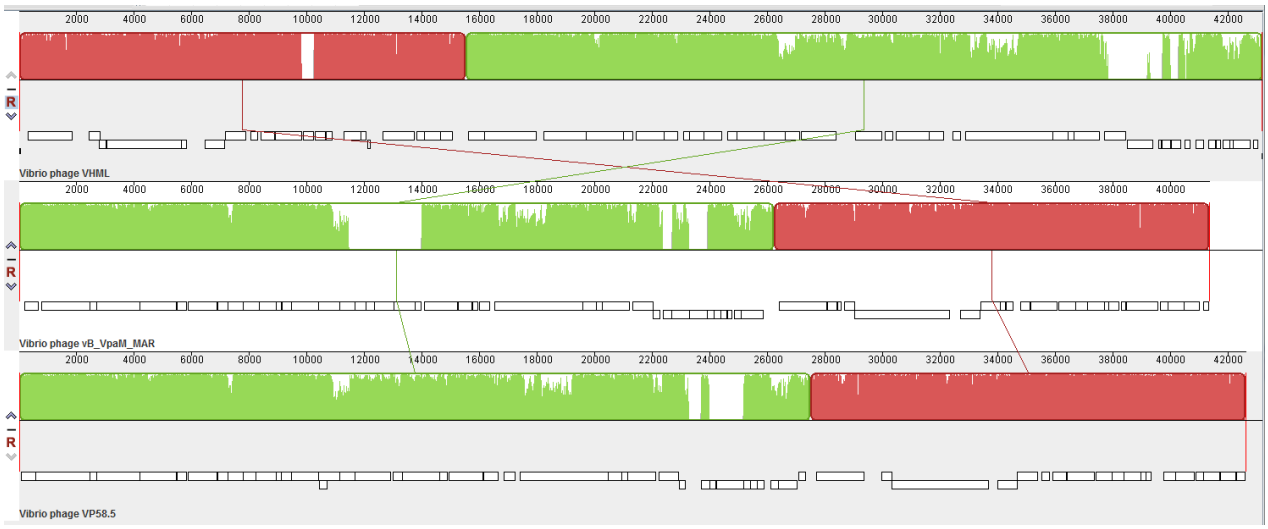

Fig. 3. Phylogenetic analysis of protelomerases of vhmlviruses and n15viruses (N15 and phiKO2) constructed using “one click” at phylogeny.fr (6). "The "One Click mode" targets users that do not wish to deal with program and parameter selection. By default, the pipeline is already set up to run and connect 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."

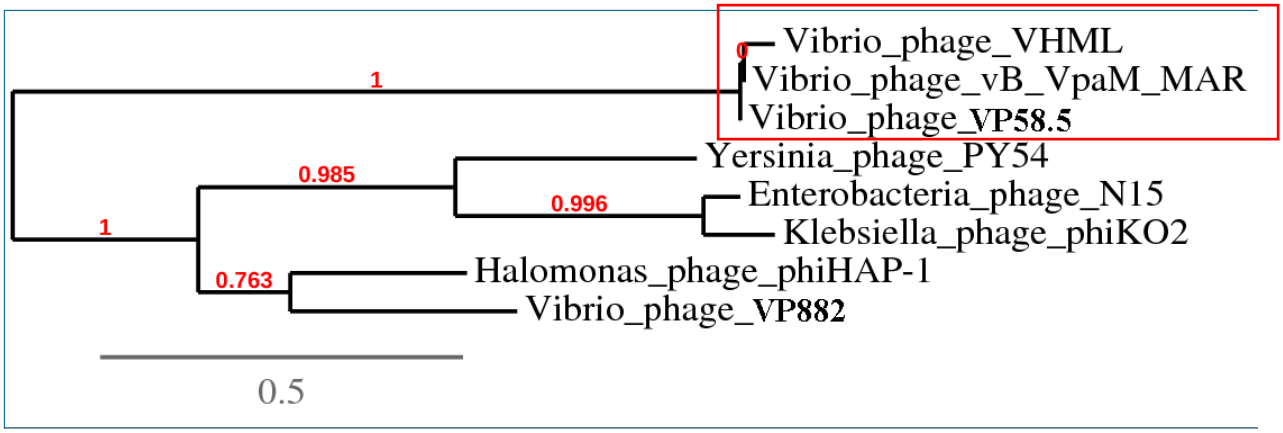

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*