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:	***2012.008a-dB***	(to be completed by ICTV officers)

Short title: Create new genus named *Yualikevirus* in family *Siphoviridae*, order *Caudovirales*

(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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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)	Prokaryote Virus Subcommittee

ICTV-EC comments:

use lower case within genus and species names. Provide Genbank accession numbers.

Date first submitted to ICTV:
Date of this revision (if different to above):

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	***2012.008aB***	(assigned by ICTV officers)

To create 3 new species within:

Genus:	***Yualikevirus*** (new)
Subfamily:	
Family:	***Siphoviridae***
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.

And name the new species:	**GenBank sequence accession number(s) of reference isolate:**
Pseudomonas phage Yua	AM749441
Pseudomonas phage M6	DQ163916
Phage Jl001	AY576273

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

The new species described here all fall within the genus criteria described below (Module 3). The entire YuA genome displays 91% DNA similarity to phage M6, which results in >80% amino acid identity with 92% of the predicted ORFs of M6. However, six genome regions contain unique YuA or M6 sequences, accounting for 15 differential gene products in total, 4 of which occur in YuA and 11 in M6, justifying demarcation of both species.

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2012.008bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	
Family:	***Siphoviridae***
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	***2012.008cB***	(assigned by ICTV officers)

To name the new genus: *Yualikevirus*

Assigning the type species and other species to a new genus

Code	***2012.008dB***	(assigned by ICTV officers)

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

Pseudomonas phage Yua

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**

Pseudomonas phage Yua (AM749441)
Pseudomonas phage M6 (DQ163916)
Phage Jl001 (AY576273)

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 9

Both YuA and M6 are significantly different from other siphoviruses deposited in the public databases, and display a characteristic morphology, i.e., an elongated head structure. These ~60 kb viruses share structural genes with other pili-specific ~40 kb *Pseudomonas* phages like PA73, B3, DMS3, and D3112, but are unrelated in the remainder of their genome. However, this only accounts for maximal 27.5% protein identity, in the case of phage PA73. The latter phages do not show the elongated head morphology typical of YuA.
Moreover, clear similarity –throughout the entire genome- exists between YuA proteins and proteins encoded by φJL001 (63,469 bp), a phage that infects an uncharacterized marine alphaproteobacterium, JL001. Phage φJL001 also displays the elongated head structure, and should be included the genus, despite the fact that the percentage of related proteins (37.5%) falls under the proposed threshold of 40%.

Origin of the new genus name:

Pseudomonas phage YuA

Reasons to justify the choice of type species:

Although *Pseudomonas phage M6* is known for a longer time, as it was part of the Lindberg typing set, *Pseudomonas phage Yua* was the first member of this genus which was profoundly characterized on a genome and proteome level.

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.

For species demarcation within the genus *Yualikevirus*, we propose a difference in DNA identity of more than 5% .

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Ceyssens PJ, Mesyanzhinov V, Sykilinda N, Briers Y, Roucourt B, Lavigne R, Robben J, Domashin A, Miroshnikov K, Volckaert G, Hertveldt K. The genome and structural proteome of YuA, a new Pseudomonas aeruginosa phage resembling M6. J Bacteriol. 2008 Feb;190(4):1429-35.

Ackermann, H. W., C. Cartier, S. Slopek, and J. F. Vieu. 1988. Morphology of *Pseudomonas aeruginosa* typing phages of the Lindberg set. Ann. Inst. Pasteur Virol. 139389-404.

Lindberg, R. B., and R. L. Latta. 1974. Phage typing of *Pseudomonas aeruginosa*: clinical and epidemiologic considerations. J. Infect. Dis. 130S33-S42

Lohr, J. E., F. Chen, and R. T. Hill. 2005. Genomic analysis of bacteriophage ÖJL001: insights into its interaction with a sponge-associated alpha-proteobacterium. Appl. Environ. Microbiol. 711598-1609.

Ehrlich, M., K. Ehrlich, and J. A. Mayo. 1975. Unusual properties of the DNA from Xanthomonas phage XP-12 in which 5-methylcytosine completely replaces cytosine. Biochim. Biophys. Acta 395109-119.

Seguritan, V., I.-W. Feng, F. Rohwer, M. Swift, and A. M. Segall. 2003. Genome sequences of two closely related *Vibrio parahaemolyticus* phages, VP16T and VP16C. J. Bacteriol. 1856434-6447.

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.

Morphological characteristics of the members of the genus *Yualikevirus*

Electron microscopic imaging reveal yualikeviruses as a typical member of the *Siphoviridae* family of double-stranded DNA bacteriophages (*Caudovirales*) having a flexible, noncontractile tail (Fig.1). In contrast to the well-known *Pseudomonas*-infecting *Siphoviridae* phages D3, B3, and D3112, which resemble phage λ, phage YuA has an elongated head (B2 morphotype). Their phage head size is ~72 by ~51 nm, and the tail length is ~145 nm. Besides an elongated head, both YuA and M6 have striated tails which are terminally and subterminally decorated with short fibers. Phage M6 is reported to be morphologically identical to *Xanthomonas oryzae* phage XP12 and has been shown to adsorb to nonretractile host pili.

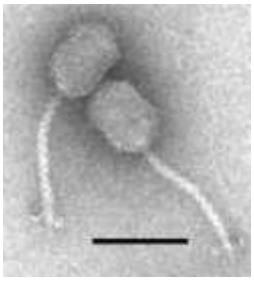

Figure 1. Electron microscopic image of phage YuA particles. Scale bar = 100 nm. Phage YuA has an elongated head and a flexible tail.

Resistance against restriction endonucleases.

YuA and M6 genomic DNA is insensitive to the activities many common restriction enzymes, including HindIII, BglII, and EcoRI and methylation-dependent DpnI (GA^{m}/TC). Given YuA's sensitivity to digestion with Sau3A (/GATC) and the methylation-sensitive SmaI (CsCCs/GGG), it can be concluded that YuA contains unmethylated adenine and cytosine residues. This is in contrast with the morphologically related phage XP12, which is known to contain a 5-methylcytosine instead of cytosine in its genome. In silico analysis revealed the absence of 9 out of 10 recognition sites, although 4 EcoRI sites are present despite YuA's insensitivity to that restriction enzyme. Furthermore, it became clear during genome sequencing that isolated YuA DNA is rather inaccessible to standard PCR amplification using various primers, annealing temperatures, and commercially available DNA polymerases. These observations suggest the presence of another base substitution or modification. Resistance to restriction during phage infection could also be provided by gene product 45 (gp45) of YuA, which displays high similarity to the ArdB antirestriction protein (Pfam E value of 10^{-30}). This plasmid-encoded protein inhibits efficient restriction by members of the three known families of type I restriction endonucleases.

Tentative lysogenic behavior

Thus far, no stable, lysogenic *P. aeruginosa* PAO1 strain carrying integrated YuA-like viruses has been isolated from turbid plaques. Similar results were reported for phage φJL001 and *Vibrio parahaemolyticus* phages VP16T and VP16C. Strikingly, the YuA integrase exhibits 32% amino acid identity with the integrases of vibriophages VP5 and VP2, which share similarity with the VP16T and VP16C integrases. These vibriophage-like integrase proteins are also unrelated to the well-studied tyrosine or serine recombinase families and exhibit distinct integrase behavior. The YuA integrase may require specific—but not yet determined—physiological conditions or a different host strain for stable lysogenic establishment.

Genetic relationships within and without the genus *Yualikevirus*

The circular permuted genome of YuA comprises 58,663 bp and has a G+C content of 64.3%, strongly resembling the G+C average (65%) of its host.For YuA, in total 78 ORFs (ORFs 1 to 77 and ORF 60.1) were predicted (Fig. 2), all oriented in the same direction and leaving only 4% of the YuA genome as noncoding. No tRNA genes are predicted. The genome of the YuA is neither Mu- nor λ-like and encodes gene products that cluster in three major regions involved in (i) DNA metabolism and replication, (ii) host interaction, and (iii) phage particle formation and host lysis. At the DNA level, YuA is 91% identical to the recently (July 2007) annotated phage M6 of the Lindberg typing set. Despite this level of DNA homology throughout the genome, both phages combined have 15 unique genes that do not occur in the other phage.

These ~60 kb viruses share structural genes with other pili-specific ~40 kb Pseudomonas phages like PA73, B3, DMS3, and D3112, but are unrelated in the remainder of their genome. However, this only accounts for maximal 27.5% protein identity, in the case of phage PA73 (Figure 2). The latter phages do not show the elongated head morphology typical of YuA.

Moreover, clear similarity exists between 29 predicted YuA proteins and proteins encoded by φ JL001 (63,469 bp), a phage that infects an uncharacterized marine alphaproteobacterium, JL001. Unlike any other phage sequenced so far, it displays homology to YuA thoughout its genome,

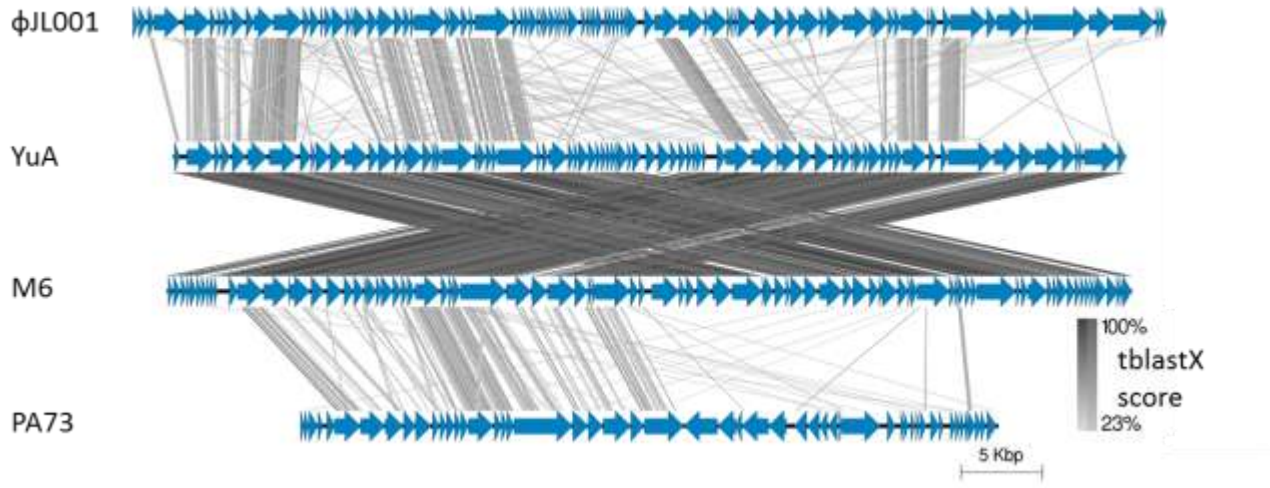

Figure 2. Genomes of YuAlikeviruses compared to the distantly related phage PA73. The choice of the YuA genome sequence zero point was based on genome comparisons with phages φJL001, D3112, DMS3, and B3; predicted gene functions; and promoter prediction/identification in phage YuA. The YuA zero point differs from the phage M6 zero point, which might be reconsidered for consistency among these related phages.

with the exception of the region encoding the tail proteins.. Phage φJL001 also displays the elongated head structure, and is included the genus.

Other genetic features of yualikeviruses

Many remarkable gene products are present in the genomes of this genus. Firstly, a dUMP hydroxymethylase (dUMP-HMase) function is predicted for gp17 of YuA. This enzyme, dUMP serves to generate the modified base hydroxymethyl-dUMP. The presence of this modified base is also predicted for φJL001 and M6.
Unique to YuA is the presence of a diguanylate cyclase or GGDEF domain (ORF 44), which is widespread in bacterial proteins, functioning as a global second messenger controlling motility and adhesion in bacterial cells.
The YuA lysis cassette is located within the genome region encoding phage particle proteins, presumably between the head and tail morphogenesis genes. Compared to the genes of the lysis cassette in lambdoid phages, the genes within this cassette in YuA are rearranged, since the putative endopeptidase *Rz* (gp60) gene and the embedded *Rz1* (gp60.1) (reading frame +1) gene precede the holin (gp61) gene and the endolysin (gp62) gene.