

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:	2016.081a-dB			(to be completed by ICTV officers)			
Short title: To create one (1) r family <i>Podoviridae</i> . (e.g. 6 new species in the genus . Modules attached	-	1961 virus,	2 🖂	three (3) $3 \boxtimes$	4	5	
(modules 1 and 10 are required)		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 <u>http://www.ictvonline.org/subcommittees.asp</u> . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)	ICTV B Subcommi	Bacterial ittee	and	Archaeal	Viruses
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## ICTV Study Group comments (if any) and response of the proposer:

Date first submitted to ICTV: Date of this revision (if different to above): July 2016

## **ICTV-EC** comments and response of the proposer:

# MODULE 2: NEW SPECIES

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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 2016.081aB (assigned by IC				TV office	ers)				
To crea	ate 2 no	ew species with	in:						
Genus: Una961virus (new)				<ul><li>Fill in all that apply.</li><li>If the higher taxon has yet to be</li></ul>					
Subfamily:			(		<ul> <li>created (in a later module, below) write</li> <li>"(new)" after its proposed name.</li> <li>If no genus is specified, enter</li> </ul>				
Fa	Family: <b>Podoviridae</b>								
(	Order:	Caudovirales	les			"unassigned" in the genus box.			
Name o	of new	species:	Representati 1 per species p		e: (only	GenBank sequence accession number(s)			
Helicobacter virus 1961P Helico		Helicobacter	bacter phage 1961P		JQ617284.1				
			obacter phage KHP40		AB731695.1				
Helicob	oacter v	virus KHP30	Helicobacter	phage KF	IP30	AB647160.1			

#### **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. Each of the proposed species differs from the others with 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	201	6.081bB	(assigned by ICTV officers)			
To create	a new	genus within:		Fill in all that apply.		
Subfa	mily:			• If the higher taxon has yet to be created		
Fa	mily:	Podoviridae		(in a later module, below) write "( <b>new)</b> " after its proposed name.		
С	Order:	Caudovirales		<ul> <li>If no family is specified, enter</li> <li>"unassigned" in the family box</li> </ul>		

naming a new genus

Code	2016.081cB	(assigned by ICTV officers)
To name tl	ne new genus: Una961virus	

Assigning the type species and other species to a new genus

Code	2016.081dB	(assigned by ICTV officers)		
To designat	te the following as the type sp	ecies of the new genus		
Helicobacte	er virus 1961P	Every genus must have a type species. This should be a well characterized species although not necessarily the first to be discovered		
are being mo	-	v species created and assigned to it (Module 2) and any that Please enter here the TOTAL number of species as 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

The phages belonging to this proposed genus are all temperate phages of *Helicobacter pylori* [4,5], and are related to a prophage, phiHP33 [6], of this bacterium. 1961P is the first podovirus isolated against *H. pylori*, and possesses an icosahedral head (71.1 nm) and a short tail (23 nm). 1961P has an unusually low buoyant density  $(1.35 - 1.40 \text{ g/cm}^3)$  and displays sensitivity to CHCl<sub>3</sub> indicative of the presence of structural lipids. More than 15 protein bands were observed by SDS-PAGE with six proteins identified by MALDI-TOF fingerprinting. The *attP* site is 8 bp (TTATCTTT), and *attB* lies between *metX* and *lpxD* on the host genome.

BLASTN, CoreGenes (Table 1) [2], progressiveMauve alignment (Fig. 2) [1] and phylogenetic analyses (Fig. 3) [3] all indicate that the proposed genus, *Una961virus*, is cohesive and distinct from the other genera of viruses. On average the genomes of this genus are 25.5 kb (35.7 mol% G+C), and encode 32 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. 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. 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. Luo CH, Chiou PY, Yang CY, Lin NT. Genome, integration, and transduction of a novel temperate phage of *Helicobacter pylori*. J Virol. 2012;86(16):8781-92.

5. Uchiyama J, Takeuchi H, Kato S, Takemura-Uchiyama I, Ujihara T, Daibata M, Matsuzaki S. Complete genome sequences of two *Helicobacter pylori* bacteriophages isolated from Japanese patients. J Virol. 2012;86(20):11400-1.

6. Lehours P, Vale FF, Bjursell MK, Melefors O, Advani R, Glavas S, Guegueniat J, Gontier E, Lacomme S, Alves Matos A, Menard A, Mégraud F, Engstrand L, Andersson AF. Genome sequencing reveals a phage in *Helicobacter pylori*. MBio. 2011;2(6). pii: e00239-11.

### 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) phage 1961P (with permission of publisher, ASM Press [4]).

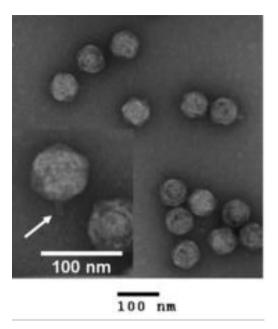
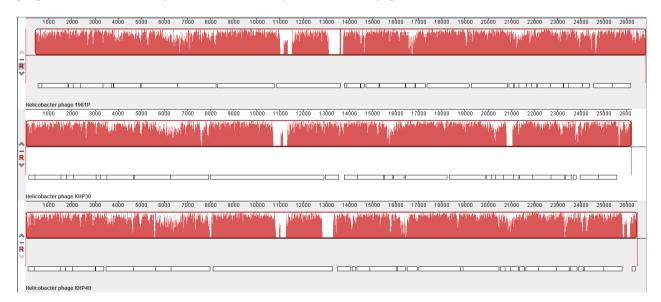


Table 1. Properties of the three phages belonging to the genus Una961virus.

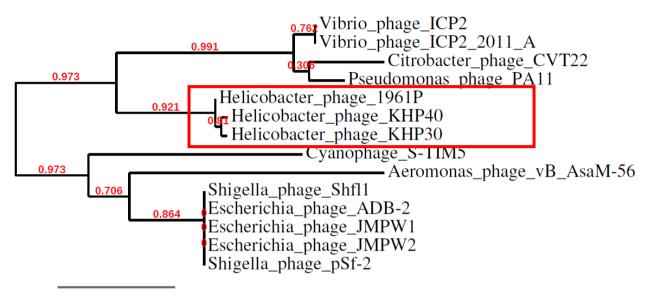
Helicobacter	RefSeq No.	GenBank	Genome	Genome	No.	DNA (%	%
phage		Accession	size	(mol%G+C)	CDS	sequence	Homologous
		No.	(kb)			identity)*	proteins **
1961P	NC_019512.1	JQ617284.1	26.84	35.5	33	100	100
KHP40	NC_019931.1	AB731695.1	26.45	35.8	32	82	84.8
KHP30	NC_019928.1	AB647160.1	26.22	35.8	30	84	97.9
***phiHP33	NC_016568.1	JF734911.1	24.65	37.3	27	50	63.4

\* Determined using BLASTN; \*\* Determined using CoreGenes [2]; \*\*\* related temperate prophage [6]

**Fig. 2.** progressiveMauve alignment [1] of the annotated genomes of members of the *Una961virus* genus – from top to bottom: 1961P, KHP30 and KHP40. 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 1961P-like viruses 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."



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