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:	***2016.049a-dB***	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Tin2virus*, including three (3) new species in the family *Siphoviridae*.
(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**

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	***2016.049aB***	(assigned by ICTV officers)

To create 3 new species within:

Genus:	***Tin2virus* (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.

Name of new species:	**Representative isolate: (only 1 per species please)**	**GenBank sequence accession number(s)**
Tsukamurella virus TIN2	Tsukamurella phage TIN2	KR011062.1
Tsukamurella virus TIN3	Tsukamurella phage TIN3	KR011064.1
Tsukamurella virus TIN4	Tsukamurella phage TIN4	KR011063.1

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. 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	***2016.049bB***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:		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
Family:	***Siphoviridae***	
Order:	***Caudovirales***	

naming a new genus

Code	***2016.049cB***	(assigned by ICTV officers)

To name the new genus: *Tin2virus*

Assigning the type species and other species to a new genus

Code	***2016.049dB***	(assigned by ICTV officers)

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

Tsukamurella virus TIN2	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

Tsukamurella phages TIN2, TIN3, and TIN4 were isolated by enrichment on the activated sludge bacterium *Tsukamurella inchonensis* strain DSMZ in Victoria, Australia. These siphoviruses are also lytic for *Tsukamurella paurometabola*. Morphologically, their isometric capsids measure 57 to 69 nm in diameter and the tails range from ca 450 to 471 nm in length [4]. The closest relative to Tsukamurella phage TIN2 is gordonia phage GMA7 [5], but, at this time, we have decided against inclusion of these viruses in a subfamily.

BLASTN, CoreGenes (Table 1) [2], progressiveMauve alignment (Fig. 2) [1], and phylogenetic analyses (Fig. 3) [3] all indicate that the proposed genus, *Tin2virus*, is cohesive and distinct from other genera. On average, the genomes of members of this genus are 76.5 kb in length (59.2mol% G+C), and encode 108 proteins and 1 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. Dyson ZA, Tucci J, Seviour RJ, Petrovski S. Three of a Kind: Genetically Similar *Tsukamurella* Phages TIN2, TIN3, and TIN4. Appl Environ Microbiol. 2015 ;81(19):6767-72.

5. Dyson ZA, Tucci J, Seviour RJ, Petrovski S. Lysis to Kill: Evaluation of the Lytic Abilities, and Genomics of Nine Bacteriophages Infective for *Gordonia* spp. and Their Potential Use in Activated Sludge Foam Biocontrol. PLoS One. 2015 ;10(8):e0134512.

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. **Virion morphologies of Tsukamurella phages TIN2, TIN3, and TIN4.** (A)

Tsukamurella phage TIN2; (B) phage TIN3; (C) phage TIN4. Scale = 50 nm.

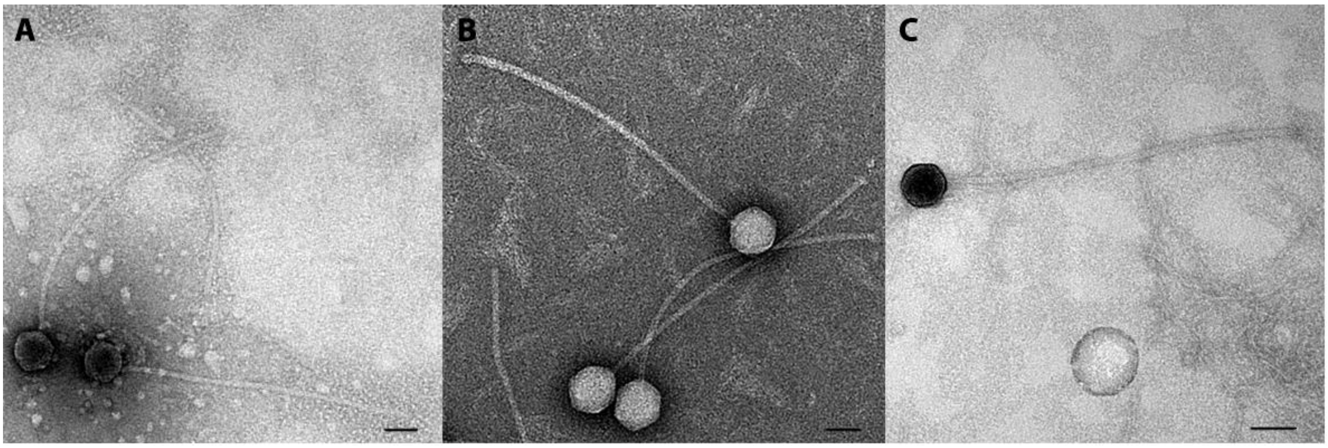

Table 1. Properties of the three phages belonging to the genus *Tin2virus*.

Tuskamurella phage	GenBank Accession No.	Genome length (kb)	Genome (mol% G+C)	No. CDS	DNA (% sequence identity)*	% Homologous proteins **
TIN2	KR011062.1	76.94	58.92	107	100	100
TIN3	KR011064.1	76.27	59.29	109	80	90.6
TIN4	KR011063.1	76.27	59.29	109	80	90.6

* Determined using BLASTN; ** Determined using CoreGenes [2]

Fig. 2. progressiveMauve alignment [1] of the genomes of members of the *Tin2virus* genus – from top to bottom: Tsukamurella phages TIN2, TIN3, and TIN4. 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 Tsukamurella phage TIN2-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."

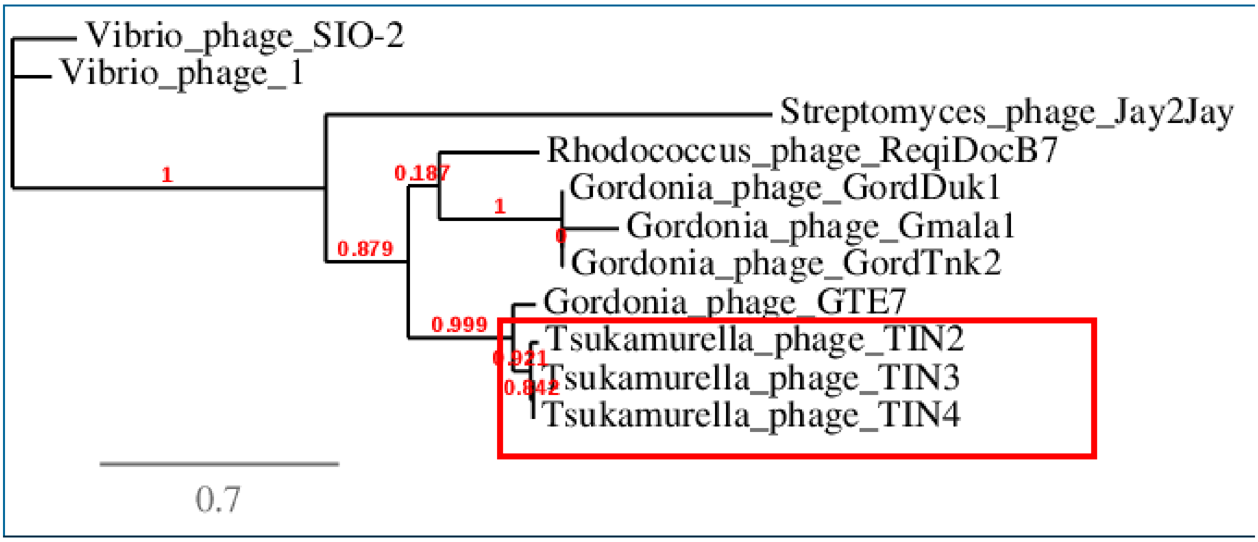

Figure 1: *Phylogenetic tree (the branch length is proportional to the number of substitutions per site).*