MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	*2016.079a-dB*	(to be completed by ICTV officers)

Short title: To create one (1) new genus, *Pradovirus*, including three (3) new species in the subfamily *Autographivirinae*.
(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: July 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	*2016.079aB*		(assigned by ICTV officers)

To create 3 new species within:

Fill in all that apply.

Genus:	*Pradovirus* **(new)**
Subfamily:	*Autographivirinae*
Family:	*Podoviridae*
Order:	*Caudovirales*

- 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)
Xylella virus Prado *Xanthomonas virus f20* *Xanthomonas virus f30*	Xylella phage Prado Xanthomonas phage f20-Xaj Xanthomonas phage f30-Xaj	KF626667 KU595432 KU595433

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**

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

Code	*2016.079bB*	(assigned by ICTV officers)

To create a new genus within:

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.

Subfamily:	*Autographivirinae*
Family:	*Podoviridae*
Order:	*Caudovirales*

- If no family is specified, enter "**unassigned**" in the family box

Code	*2016.079cB*	(assigned by ICTV officers)

To name the new genus: *Pradovirus*

Code	*2016.079dB*	(assigned by ICTV officers)

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

Xylella virus Prado

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

"The plant-pathogenic bacterium *Xylella fastidiosa* is the causative agent of a number of economically important diseases, including Pierce's disease of grape, phony peach disease, periwinkle wilt, citrus variegated chlorosis, almond leaf scorch, oleander leaf scorch, and coffee leaf scorch." [5]. Xylella phage Prado was isolated on *X. fastidiosa* strain Temecula 1 but could plaque on several *Xanthomonas axonopodis* pv. citri isolates. Its mean capsid width is 69 nm. The genome is terminated by 616-bp direct terminal repeats [5]. The authors of the paper describing this phage consider it to be a member of the *Phikmvvirus* genus, however, Xylella phage Prado only shares 3% DNA homology with Pseudomonas phage phiKMV (BLASTN). Xanthomonas phages f20-Xaj and f30-Xaj are lytic for *Xanthomonas arboricola* pv. juglandis and have received very little in the way of scientific description [4].

BLASTN, CoreGenes (Table 1) [2], progressiveMauve alignment (Fig. 1) [1] and phylogenetic analyses (Fig. 2) [3] all indicate that the proposed genus, *Pradovirus*, is cohesive and distinct from the other genera of viruses. On average the genomes of this genus are 44.0 kb (60.9 mol% G+C), and encode 52 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. Retamales J, Vasquez I, Santos L, Segovia C, Ayala M, Alvarado R, Nuñez P, Santander J. Complete Genome Sequences of Lytic Bacteriophages of *Xanthomonas arboricola* pv. juglandis. Genome Announc. 2016 Jun 2;4(3). pii: e00336-16.

5. Ahern SJ, Das M, Bhowmick TS, Young R, Gonzalez CF. Characterization of novel virulent broad-host-range phages of *Xylella fastidiosa* and *Xanthomonas*. J Bacteriol. 2014 Jan;196(2):459-71.

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 three phages belonging to the genus *Pradovirus*

Phage	GenBank (RefSeq) Accession No.	Genome size (kb)	Genome (mol%G+C)	No. CDS	DNA (% sequence identity)*	% Homologous proteins **
Xylella phage Prado	KF626667 (NC_022987)	43.9	63.0	52	100	100
Xanthomonas phage f20-Xaj	KU595432	43.9	59.8	53	59	88.5
Xanthomonas phage f30-Xaj	KU595433	44.3	59.9	51	60	90.4

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

Fig. 1. progressiveMauve alignment [1] of the annotated genomes of members of the *Pradovirus* genus – from top to bottom: Xanthomonas phages f20-Xaj, f30-Xaj and Xylella phage Prado. 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). Please note that the genomes are not co-linear.
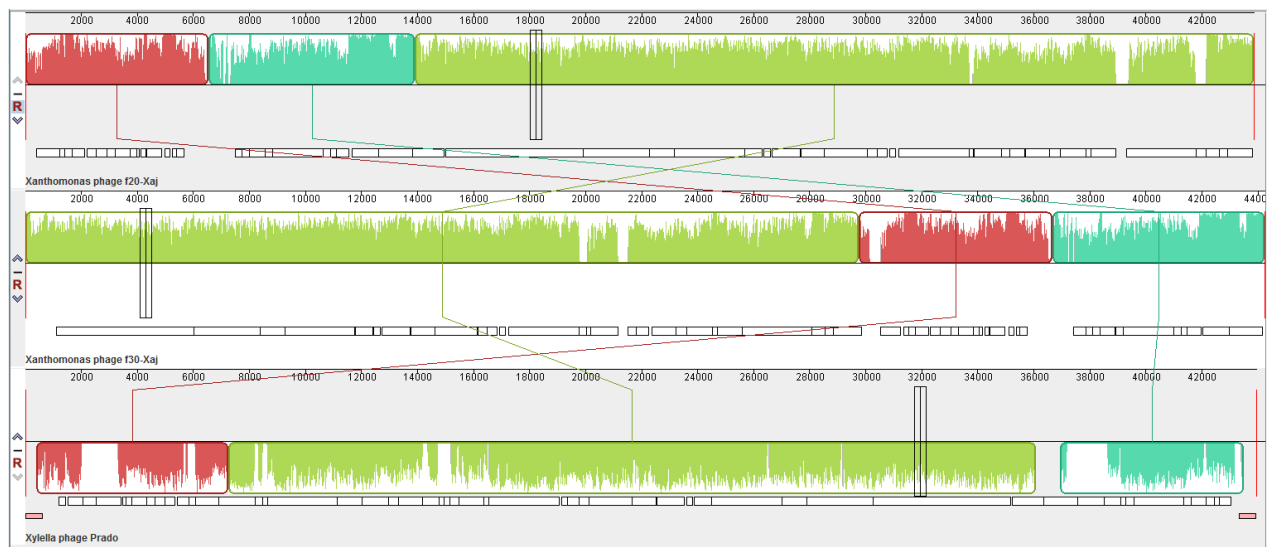
Fig. 2. Phylogenetic analysis of (A) DNA packaging protein, (B) major capsid protein and (C) RNA polymerase of Prado-like viruses and homologous proteins from a variety of other phages constructed using "one click" at phylogeny.fr [3]. "The "One Click mode" targets users that do not wish to deal with 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." Member of the genus *Pradovirus* are boxed in **red**.
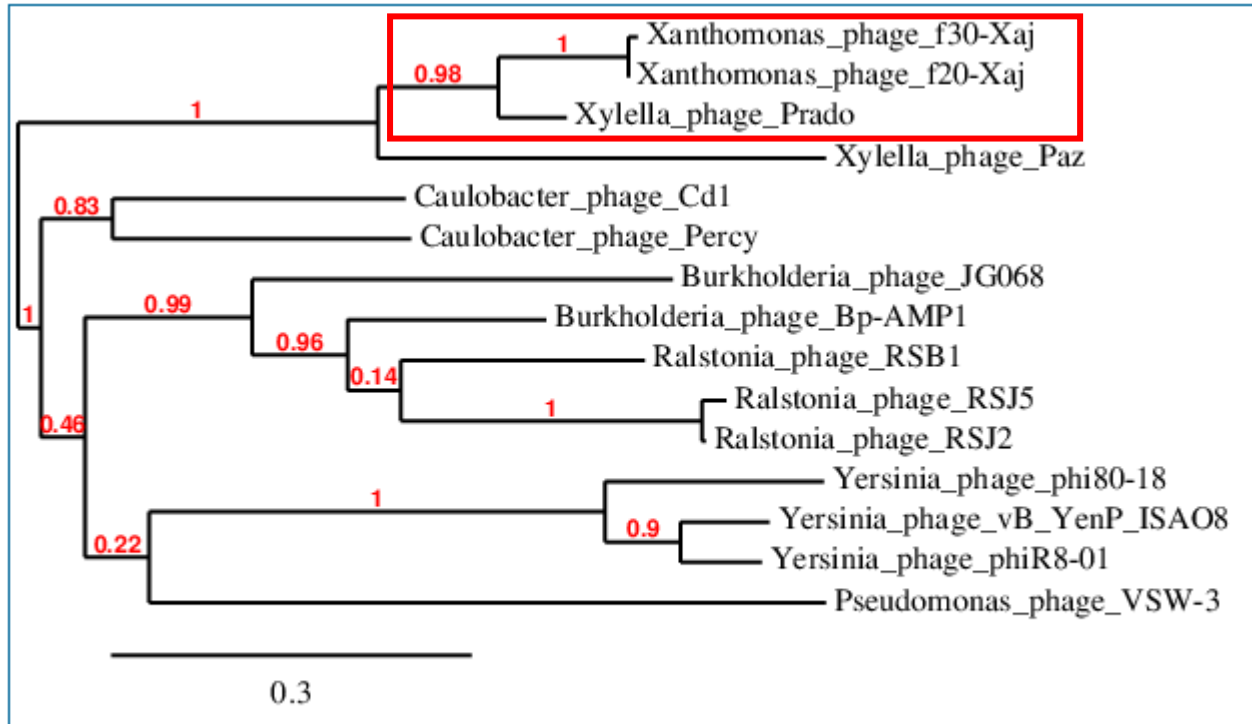
A. DNA packaging proteins

Figure 1: *Phylogenetic tree.*
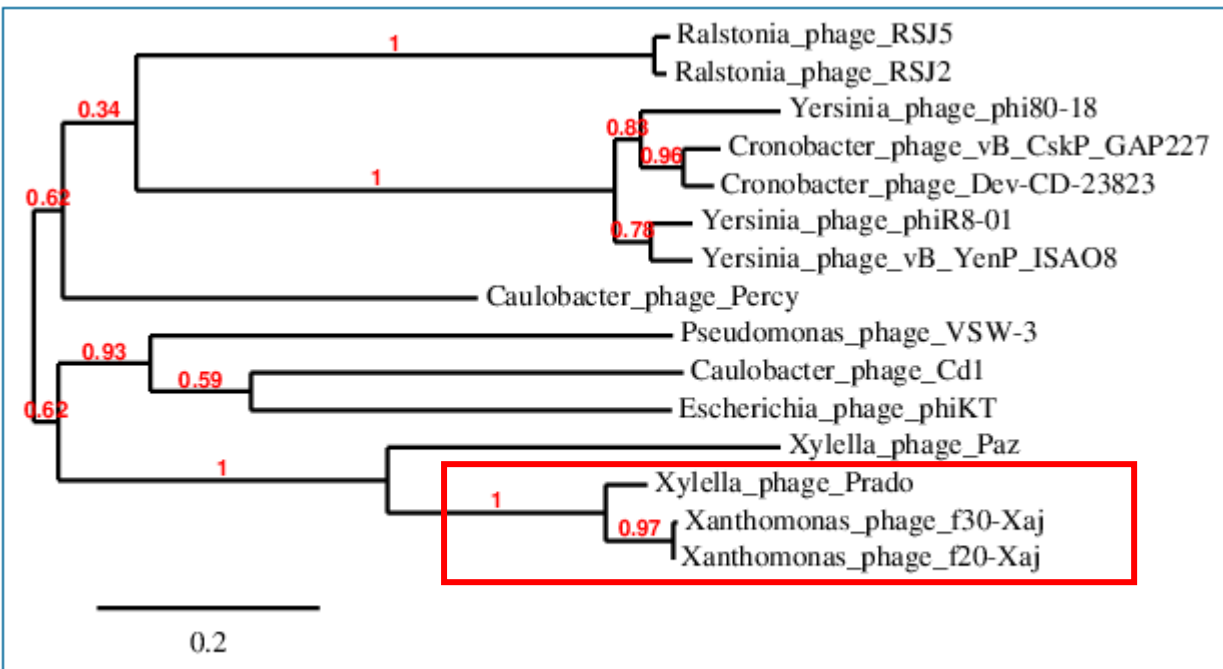
B. Major capsid proteins

Figure 1: *Phylogenetic tree.*

C. RNA polymerases

Figure 1: *Phylogenetic tree.*