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:	***2016.016a-dM***	(to be completed by ICTV officers)

Short title: Create one (1) new genus including one (1) new species
(e.g. 6 new species in the genus *Zetavirus*)

Modules attached (modules 1 and 11 are required)	**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 Study Group comments (if any) and response of the proposer:

Date first submitted to ICTV: July 18, 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	***2016.016aM***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Tilapinevirus***
Subfamily:	**N/A**
Family:	**Unassigned**
Order:	**N/A**

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)**
Tilapia tilapinevirus	tilapia lake virus (TiLV) isolate Til-4-2011	Segment 1: KU751814 Segment 2: KU751815 Segment 3: KU751816 Segment 4: KU751817 Segment 5: KU751818 Segment 6: KU751819 Segment 7: KU751820 Segment 8: KU751821 Segment 9: KU751822 Segment 10: KU751823

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 11

See "Reasons to justify the creation of a new genus section".

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2016.016bM***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	**N/A**
Family:	**N/A**
Order:	**N/A**

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	***2016.016cM***	(assigned by ICTV officers)

To name the new genus: *Tilapinevirus*

Assigning the type species and other species to a new genus

Code	***2016.016dM***	(assigned by ICTV officers)

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

Tilapia tilapinevirus	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:**

1

Reasons to justify the creation of a new genus:

Additional material in support of this proposal may be presented in the Appendix, Module 11

A novel virus, tilapia lake virus (TiLV), was recently identified as the etiological cause of massive losses of tilapia in Israel and Ecuador in 2009 (1). TiLV forms enveloped particles and has a decasegmented RNA genome. The full viral genome sequence, including 3′ and 5′ termini of each segment, has been determined and deposited in GenBank: each segment encodes one predicted protein (Table 1). Segment 1 encodes a protein with weak homology to the polymerase (PB1) subunit of orthomyxoviruses (≈17% amino acid identity, 37% segment coverage; FIG 1A). The other nine segments encode proteins without obvious homology to other sequences in the GenBank nucleotide and protein databases (mega blast/BLASTx/tBLASTn/tBLASTx). Thirteen nucleotides at the 5′ segment termini and 13 nucleotides at the 3′ segment termini are similar in all ten segments, an organization reminiscent of that of orthomyxoviruses (FIG 1B). The decasegmented nature of the TiLV genome was confirmed by Northern hybridization performed on tissue-cultured virus or infected tilapia (FIG 2). TiLV RNA was detected by *in situ* hybridization in livers and brains of diseased fish and in E-11 cell cultures experimentally infected with TiLV (FIG 3). Finally, the TiLV genome was determined of negative polarity/noninfectious (FIG 4). Based on the data presented here (1), TiLV's closest known relatives are orthomyxoviruses. However, TiLV is clearly not directly related to any of the currently classified orthomyxoviruses, therefore requiring the establishment of a novel genus, and further studies

ought to be performed before this genus can be included into the family.

Origin of the new genus name:

"Tilapine" and "virus"

Reasons to justify the choice of type species:

Currently, only one species is included in the 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.

Currently, only one species is included in the genus; consequently species demarcation criteria cannot be established at this point in time.

TABLE 1 Genomic characterization of 10 segments of TiLV isolated from tilapia in Israel

Segment no.	Segment length (nt)	GenBank accession no.	Predicted protein length (aa)	Protein mol mass (kDa)	pI	Identification by MS[a]
1	1,641	KU751814	519	57.107	7.99	–
2	1,471	KU751815	457	51.227	9.64	+
3	1,371	KU751816	419	47.708	7.99	+
4	1,250	KU751817	356	38.625	9.01	+
5	1,099	KU751818	343	38.058	8.46	+
6	1,044	KU751819	317	36.381	8.67	+
7	777	KU751820	195	21.834	9.98	+
8	657	KU751821	174	19.474	9.86	+
9	548	KU751822	118	13.486	6.49	+
10	465	KU751823	113	12.732	4.45	+

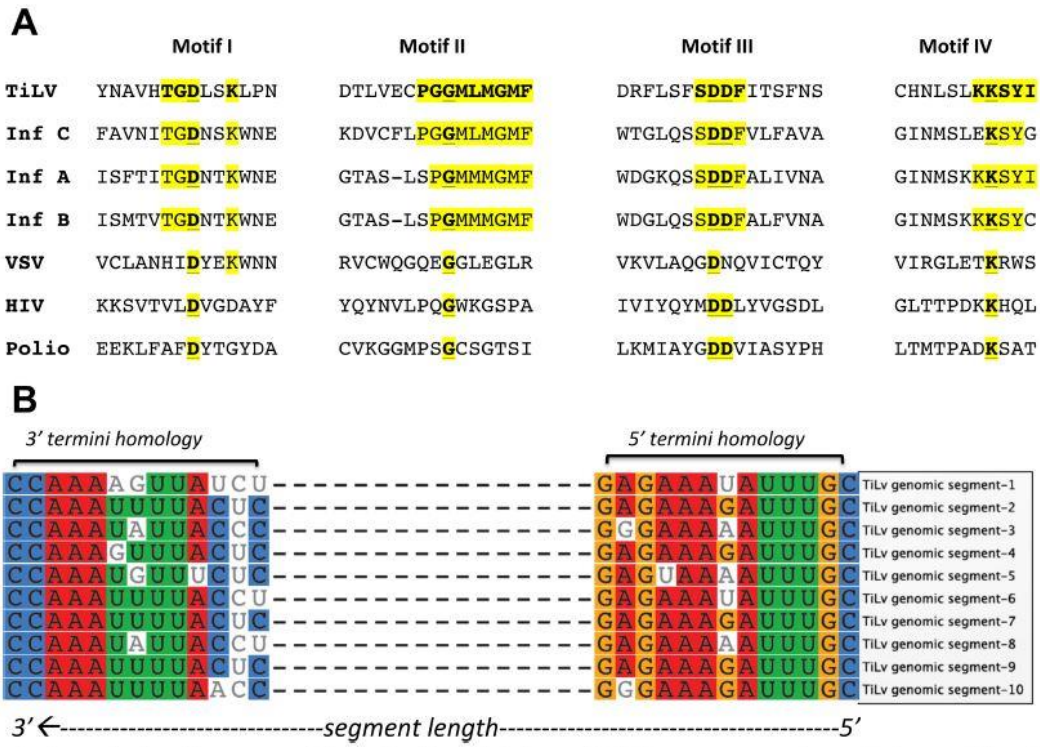

FIG 1 Genomic characterization of the segments of TiLV isolated from tilapia in Israel. (A) TiLV segment 1 putative protein shows weak homology to motifs conserved in RNA-dependent polymerases. Sequence comparison of TiLV's segment 1 predicted protein with motifs I to IV, conserved in polymerases of influenza virus strains C/JJ/50 (Inf C) (19), A/WSN/33 (Inf A) (34), and B/Lee/40 (Inf B) (35), vesicular stomatitis virus (VSV) (20), human immunodeficiency virus (HIV) (21), and poliovirus (Polio) (22). The relative motif positions are also shown. Invariant sequences in each motif are in boldface and underlined. TiLV sequences that show identity to one of the influenza virus sequences are highlighted in yellow. (B) Genomic segments of TiLV show conserved and homologous features at 5′ and 3′ termini.

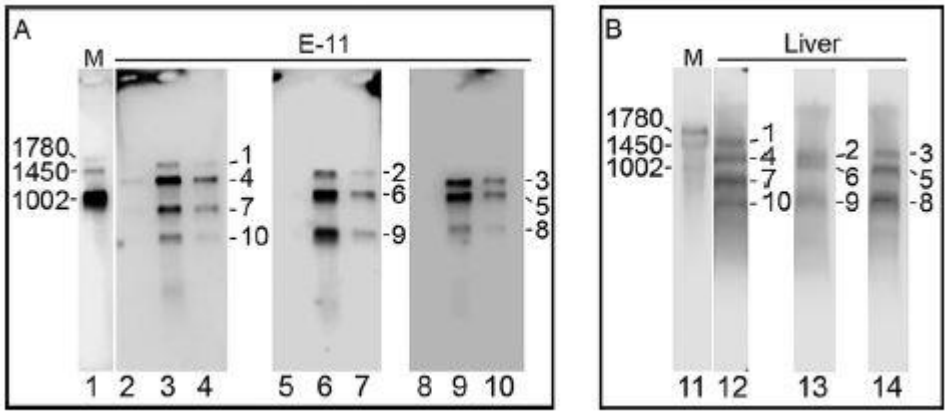

FIG 2 Northern hybridization analysis indicates that TiLV is a segmented RNA virus. (A) Total RNA extracted from E-11 cells 6 days postinfection with TiLV from brains of tilapia in Israel (lanes 3, 6, and 9), from virions that were pelleted from the culture supernatant (lanes 4, 7, and 10), or from noninfected E-11 cells (lanes 2, 5, and 8). (B) Total RNA extracted from livers of tilapia in Ecuador (lanes 12 to 14). The extracts were hybridized to probe mixtures representing segments 1, 4, 7, and 10 (probe mixture 1 [lanes 2 to 4 and 12]), segments 2, 6, and 9 (probe mixture 2 [lanes 5 to 7 and 13]), or segments 3, 5, and 8 (probe mixture 3 [lanes 8 to 10 and 14]) to prevent signal overlap from segments of similar sizes. Influenza A virus RNA (A/Moscow/10/99) hybridized with three probes representing hemagglutinin (HA) (1,780 nt), NA (1450 nt), and matrix (1,002 nt) sequences served as size references (M [lanes 1 and 11]). Size markers appear on the left sides of the panels and segment numbers on the right.

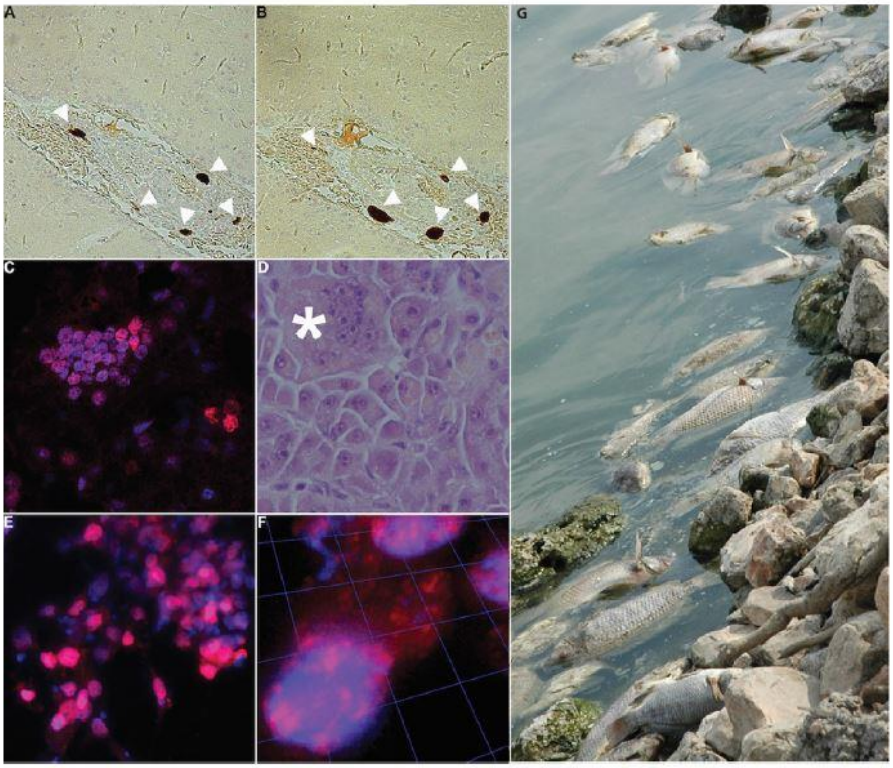

FIG 3 Detection of TiLV RNA in brain and liver of infected tilapia and infected E-11 cells by *in situ* hybridization and image of dead tilapia in Israel. (A and B) Brain sections of infected Nile tilapia hybridized with Affymetrix Cy3-conjugated probes (red) of various polarities to TiLV segment 1 to detect genomic RNA (A) or mRNA (B). White arrowheads indicate hybridization signal. (C) Liver sections hybridized with Cy3-conjugated (red) Stellaris probes to segment 3 to detect mRNA. Nuclei are stained with DAPI (blue). (D) Liver section stained with hematoxylin and eosin reveals multinucleated giant cells (asterisk). (E) TiLV-infected E-11 cells hybridized with Quasar 670-conjugated (red) Stellaris probe to segment 3 to detect TiLV mRNA. Nuclei are stained with DAPI (blue). (F) Images of confocal sections of cells in panel E were reconstituted into a 3D image. (G) Dead tilapia at a fish farm in Israel.

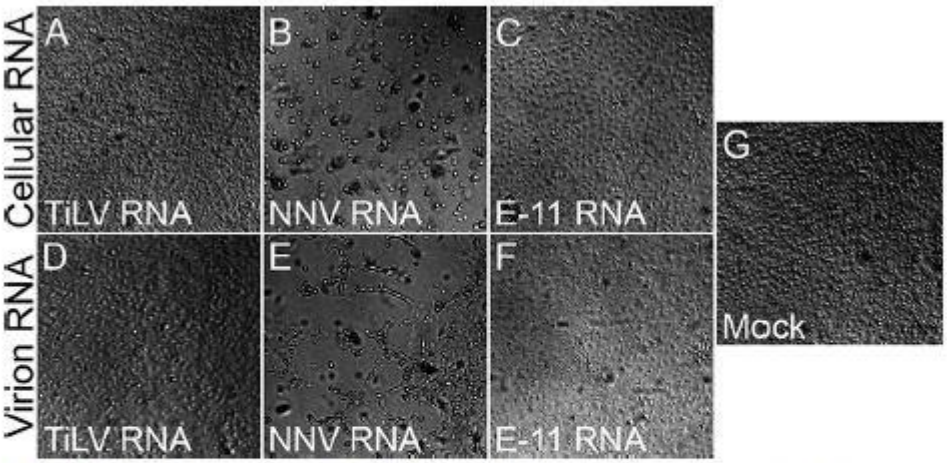

FIG 4 TiLV deproteinized RNA is not infectious. Naive E-11 cell cultures were transfected with deproteinized RNA, extracted from cultured cells ("Cellular RNA" [A to C]) or pellets of culture supernatants ("Virion RNA" [D to F]), from TiLV-infected ("TiLV RNA" [A and D]) or NNV-infected ("NNV RNA" [B and E]) E-11 cells, or from naive E-11 cells ("E-11 RNA" [C and F]). Transfection with no RNA ("Mock" [G]) was also included. Bright-field images of transfected cultures were taken at 8 days posttransfection.

References:

1. **Bacharach E, Mishra N, Briese T, Zody MC, Kembou Tsofack JE, Zamostiano R, Berkowitz A, Ng J, Nitido A, Corvelo A, Toussaint NC, Abel Nielsen SC, Hornig M, Del Pozo J, Bloom T, Ferguson H, Eldar A, Lipkin WI.** 2016. Characterization of a Novel Orthomyxo-like Virus Causing Mass Die-Offs of Tilapia. MBio **7:**e00431-00416.

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.