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:	***2013.002a-cF***	(to be completed by ICTV officers)

Short title: Addition of 10 new species to existing genera in family *Totiviridae*
(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:

Max L. Nibert (mnibert@hms.harvard.edu); Said A. Ghabrial (saghab00@email.uky.edu)

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)	*Totiviridae* Study Group

ICTV-EC or Study Group comments and response of the proposer:

Totiviridae Study Group (SG) members: Jeremy A. Bruenn, Øystein Evensen, Said A. Ghabrial, Tetsuya Mizutani, Max L. Nibert (Chair), Jean L. Patterson, Marilyn J. Roossinck, Drake C. Stenger, Ioannis Tzanetakis, Ching C. Wang, Reed B. Wickner, and Iñigo Zabalgogeazcoa. All *Totiviridae* SG members were sent a draft proposal, and several have provided comments, in response to which corrections have been made in the current proposal.

Please note that Said A. Ghabrial, Chair of the Fungal Virus Subcommittee, is an author of this proposal and also a member of the *Totiviridae* SG.

ICTV-EC comments;

Consider changing name of species *Beauveria bassiana RNA virus 1* to *Beauveria bassiana victorivirus 1*.

Response of proposers Nibert and Ghabrial: *Beauveria bassiana RNA virus 1* was renamed *Beauveria bassiana victorivirus 1*.

Date first submitted to ICTV: May 1, 2013
Date of this revision (if different to above):

MODULE 2.1: **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	***2013.002aF***	(assigned by ICTV officers)

To create 4 new species within:

Genus:	***Totivirus***
Subfamily:	
Family:	***Totiviridae***
Order:	

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:**
Scheffersomyces segobiensis virus L	**KC610514**
Tuber aestivum virus 1	**HQ158596**
Xanthophyllomyces dendrorhous virus L1A	**JN997472**
Xanthophyllomyces dendrorhous virus L1B	**JN997473**

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

Assignment to genus *Totivirus*

Reference strains of the proposed species *Scheffersomyces segobiensis virus L* (Taylor et al. 2013), *Tuber aestivum virus 1* (Stielow & Menzel, 2010), *Xanthophyllomyces dendrorhous virus L1A* (Baeza et al., 2012), and *Xanthophyllomyces dendrorhous virus L1B* (ibid.) all have similar genomic and coding characteristics to reference strains of the approved *Totivirus* species *Saccharomyces cerevisiae virus L-A* and *Saccharomyces cerevisiae virus L-BC (La)* (**Table 1**). The 4.5- to 4.7-kbp dsRNA genomes of all these viruses include two partially overlapping ORFs, the upstream one encoding the CP and the downstream one (in the -1 frame relative to CP), encoding the RdRp. The RdRp is expressed as a CP/RdRp fusion subsequent to –1 ribosomal frameshifting. In phylogenetic analyses of the CP/RdRp sequences, reference strains of the proposed species *Scheffersomyces segobiensis virus L, Tuber aestivum virus 1, Xanthophyllomyces dendrorhous virus L1A,* and *Xanthophyllomyces dendrorhous virus L1B* co-cluster with each other as well as with reference strains of the approved *Totivirus* species *Saccharomyces cerevisiae virus L-A* and *Saccharomyces cerevisiae virus L-BC (La)* (**Figure 1**), well apart from isolates of the other *Totiviridae* genera. Reference strains of the proposed species *Scheffersomyces segobiensis virus L, Tuber aestivum virus 1, Xanthophyllomyces dendrorhous virus L1A,* and *Xanthophyllomyces dendrorhous virus L1B* also comply with the following genomic and coding range values that are unique to genus *Totivirus* among isolates of the 5 current *Totiviridae* genera: (i) smallest genomes, (ii) shortest 5´ UTRs by a wide margin, and (iii) distinctive CP/RdRp lengths (**Table 2**). The combination of different findings thus strongly argues for assigning the proposed species *Scheffersomyces segobiensis virus L,*

Tuber aestivum virus 1, *Xanthophyllomyces dendrorhous virus L1A*, and *Xanthophyllomyces dendrorhous virus L1B* to genus *Totivirus*.

Distinguishing from other *Totivirus* species

Reference strains of the proposed species *Scheffersomyces segobiensis virus L*, *Tuber aestivum virus 1*, *Xanthophyllomyces dendrorhous virus L1A*, and *Xanthophyllomyces dendrorhous virus L1B* all show < 46% aa identity in their predicted full-length CP/RdRp sequences relative to each another and also relative to reference strains of the approved *Totivirus* species *Saccharomyces cerevisiae virus L-A*, *Saccharomyces cerevisiae virus L-BC (La)*, and *Ustilago maydis virus H1* (**Table 3**). These identity values are below the suggested threshold of 50% in family *Totiviridae*, supporting recognition of the new species. As indicated by the names of the proposed new species, their reference strains were obtained from distinct fungal host species. The one exception is that the reference strains of proposed species *Xanthophyllomyces dendrorhous virus L1A* and *Xanthophyllomyces dendrorhous virus L1B* were obtained from the same host species*;* nonetheless, these two viruses from *X. dendrorhous* meet the threshold for identification as distinct species, sharing only 43% identity in their CP/RdRp sequences (**Table 3**).

Other information

Table 1, **Table 3**, and **Figure 1** include Black raspberry virus F (GenBank accession no. EU082131) as a probable species (new) in genus *Totivirus*, but which has yet to be reported in a peer-reviewed paper and is thus not yet proposed for approval here. **Table 1**, **Table 2**, and **Figure 1** also include approved *Totivirus* species *Ustilago maydis virus H1*, which appears fairly distinct not only from the proposed species but also from the approved species *Saccharomyces cerevisiae virus L-A* and *Saccharomyces cerevisiae virus L-BC (La)*. The current assignment of *Ustilago maydis virus H1* to genus *Totivirus* may need to be reconsidered in a subsequent proposal.

MODULE 2.2: **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	***2013.002bF***	(assigned by ICTV officers)

To create 5 new species within:

Genus:	*Victorivirus*
Subfamily:	
Family:	*Totiviridae*
Order:	

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:**
Aspergillus foetidus slow virus 1	**HE588147**
Beauveria bassiana victorivirus 1	**HE572591**
Magnaporthe oryzae virus 2	**AB300379**
Rosellinia necatrix victorivirus 1	**AB742454**
Tolypocladium cylindrosporum virus 1	**FR750562**

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

Assignment to genus *Victorivirus*

Reference strains of the proposed species *Aspergillus foetidus slow virus 1* (Kozlakidis et al., 2013), *Beauveria bassiana victorivirus 1* (Herrero et al., 2012; originally named Beauveria bassiana RNA virus 1 by those authors), *Magnaporthe oryzae virus 2* (Maejima et al., 2008), *Rosellinia necatrix victorivirus 1* (Chiba et al., 2013), and *Tolypocladium cylindrosporum virus 1* (Herrero & Zabalgogeazcoa, 2011) all have similar genomic and coding characteristics to those of reference strains of the 9 approved *Victorivirus* species (**Table 4**) (Ghabrial & Nibert, 2009). The 4.9- to 5.4-kbp dsRNA genomes of all these viruses include two minimally overlapping or non-overlapping ORFs, the upstream one encoding CP and the downstream one encoding RdRp. The RdRp is expressed as a separate protein subsequent to a translational termination–reinitiation event; conserved RNA plus-strand elements relating to this mechanism include closely juxtaposed CP stop and RdRp start codons, and an upstream pseudoknot or long stem–loop structure (Li et al., 2011) (**Figure 2**). In phylogenetic analyses of the CP+RdRp sequences, the reference strains of proposed species *Aspergillus foetidus slow virus 1*, *Beauveria bassiana victorivirus 1*, *Magnaporthe oryzae virus 2*, *Rosellinia necatrix victorivirus 1*, and *Tolypocladium cylindrosporum virus 1* co-cluster with each other as well as with reference strains of the 9 approved *Victorivirus* species (**Figure 1**), well apart from isolates of the other *Totiviridae* genera. Reference strains of the proposed species *Aspergillus foetidus slow virus 1*, *Beauveria bassiana victorivirus virus 1*, *Magnaporthe oryzae virus 2*, *Rosellinia necatrix victorivirus 1*, and *Tolypocladium cylindrosporum virus 1* also comply with the following

genomic and coding range values that are unique to genus *Victorivirus* among isolates of the 5 current *Totiviridae* genera: (i) highest G+C content and (ii) distinctive combined CP+RdRp lengths (**Table 2**). The combination of different findings thus strongly argues for assigning the proposed species *Aspergillus foetidus slow virus 1, Beauveria bassiana victorivirus 1, Magnaporthe oryzae virus 2, Rosellinia necatrix victorivirus 1,* and *Tolypocladium cylindrosporum virus 1* to genus *Victorivirus.*

Distinguishing from other *Victorivirus* species

Reference strains of the proposed species *Aspergillus foetidus slow virus 1, Beauveria bassiana victorivirus 1, Magnaporthe oryzae virus 2, Rosellinia necatrix victorivirus 1,* and *Tolypocladium cylindrosporum virus 1* all show < 59% aa identity in their predicted full-length CP+RdRp sequences relative to each other and also relative to reference strains of the 9 approved *Victorivirus* species (**Table 5**). These pairwise aa-identity scores are close enough to the suggested threshold of 50% in family *Totiviridae* to warrant creation of the new species. As indicated by the names of the proposed new species, their reference strains were obtained from distinct fungal host species. The one exception is that the reference strain of proposed species *Magnaporthe oryzae virus 2* was obtained from the same host species as that of approved species *Magnaporthe oryzae virus 1;* nonetheless, these two viruses from *M. oryzae* meet the threshold for identification as distinct species, sharing only 33% identity in their CP+RdRp sequences (**Table 5**).

Other information

Table 4, **Table 5**, and **Figure 1** include Botryotinia fuckeliana totivirus 1 (GenBank accession no. AM491608) as a probable species in genus *Victorivirus*. Botryotinia fuckeliana totivirus 1 was previously identified as a probable species, but still has yet to be reported in a peer-reviewed paper and is thus still not suitable to be proposed for approval.

MODULE 2.3: **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	***2013.002cF***	(assigned by ICTV officers)

To create 1 new species within:

Genus:	***Trichomonasvirus***
Subfamily:	
Family:	***Totiviridae***
Order:	

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:**
Trichomonas vaginalis virus 4	**HQ607522**

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

Assignment to genus *Trichomonasvirus*

The reference strain and two other reported strains of the proposed species *Trichomonas vaginalis virus 4* (Goodman et al., 2011a) all have similar genomic and coding characteristics to strains of the approved *Trichomonasvirus* species *Trichomonas vaginalis virus 1*, *Trichomonas vaginalis virus 2*, and *Trichomonas vaginalis virus 3* (**Table 6**) (Goodman et al., 2011b). The 4.6- to 5.0-kbp dsRNA genomes of all these viruses include two partially overlapping ORFs, the upstream one encoding CP and the downstream one encoding RdRp. The RdRp is expressed as a CP/RdRp fusion subsequent to –1 or –2 ribosomal frameshifting (Goodman et al., 2011a, b; Parent et al., 2013). Strains of the proposed species *Trichomonas vaginalis virus 4* are like those of the approved species *Trichomonas vaginalis virus 2* and *Trichomonas vaginalis virus 3* in having the RdRp ORF in the -1 frame relative to CP, whereas strains of the approved species *Trichomonas vaginalis virus 1* are distinct in having the RdRp ORF in the +1/–2 frame relative to CP. In phylogenetic analyses of the CP/RdRp sequences, the reference strain of the proposed species *Trichomonas vaginalis virus 4* co-clusters with the reference strains of the other *Trichomonasvirus* species (**Figure 1**), well apart from isolates of the other *Totiviridae* genera. Strains of the proposed species *Trichomonas vaginalis virus 4* also comply with the following genomic and coding range values that are unique to genus *Trichomonasvirus* among isolates of the 5 current *Totiviridae* genera: (i) distinctive genome lengths and (ii) distinctive CP/RdRp lengths (**Table 2**). The combination of different findings thus strongly argues for assigning the proposed species *Trichomonas vaginalis virus 4* to genus *Trichomonasvirus*

Distinguishing from other *Trichomonasvirus* species

The reference strain and two other sequenced strains of the proposed species *Trichomonas vaginalis virus 4* all show < 55% aa identity in their predicted full-length CP/RdRp sequences

relative to strains of the approved species *Trichomonas vaginalis virus 1, Trichomonas vaginalis virus 2,* and *Trichomonas vaginalis virus 3* (**Table 7**). These identity values are close enough to the suggested threshold of 50% in family *Totiviridae* to support recognition of the new species. In further support of this recognition is that the within-species values among strains of the approved species *Trichomonas vaginalis virus 1, Trichomonas vaginalis virus 2,* and *Trichomonas vaginalis virus 3* are ≥ 80%, ≥ 79%, and ≥ 90%, respectively, and similarly ≥ 91% among strains of the proposed species *Trichomonas vaginalis virus 4* (**Table 7**). Other distinctive features of *Trichomonas vaginalis virus 4* pertain to the genome lengths, etc., of its strains (**Table 6**). Strains of the proposed species *Trichomonas vaginalis virus 4* all have (i) longer genomes, (ii) higher G+C contents, (iii) lower A+G (purine) contents, (iv), longer 3´ UTRs, (v) longer CPs, and (vi) longer CP/RdRps than the strains of any of the approved *Trichomonasvirus* species. In addition, the 5´ UTRs of *Trichomonas vaginalis virus 4* strains have a distinctive length relative to those of the other *Trichomonasvirus* species.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Baeza M, Bravo N, Sanhueza M, Flores O, Villarreal P, Cifuentes V. 2012. Molecular characterization of totiviruses in *Xanthophyllomyces dendrorhous*. *Virol J* **9,** 140.

Chiba S, Lin YH, Kondo H, Kanematsu S, Suzuki N. 2013. A novel victorivirus from a phytopathogenic fungus, *Rosellinia necatrix* is infectious as particles and targeted by RNA silencing. *J Virol* [Epub ahead of print].

Ghabrial SA, Nibert ML. 2009. *Victorivirus*, a new genus of fungal viruses in the family *Totiviridae*. *Arch Virol* **154,** 373–379.

Goodman RP, Freret TS, Kula T, Geller AM, Talkington MW, Tang-Fernandez V, Suciu O, Demidenko AA, Ghabrial SA, Beach DH, Singh BN, Fichorova RN, Nibert ML. 2011a. Clinical isolates of *Trichomonas vaginalis* concurrently infected by strains of up to four *Trichomonasvirus* species (Family *Totiviridae*). *J Virol* **85,** 4258–4270.

Goodman RP, Ghabrial SA, Fichorova RN, Nibert ML. 2011b. *Trichomonasvirus*: a new genus of protozoan viruses in the family *Totiviridae*. *Arch Virol* **156,** 171–179.

Herrero N, Dueñas E, Quesada-Moraga E, Zabalgogeazcoa I. 2012. Prevalence and diversity of viruses in the entomopathogenic fungus *Beauveria bassiana*. *Appl Environ Microbiol* **78,** 8523–8530.

Herrero N, Zabalgogeazcoa I. 2011. Mycoviruses infecting the endophytic and entomopathogenic fungus *Tolypocladium cylindrosporum*. *Virus Res* **160,** 409–413.

Kozlakidis Z, Herrero N, Coutts RH. 2013. The complete nucleotide sequence of a totivirus from *Aspergillus foetidus*. *Arch Virol* **158,** 263–266.

Li H, Havens WM, Nibert ML, Ghabrial SA. 2011. RNA sequence determinants of a coupled termination–reinitiation strategy for downstream open reading frame translation in Helminthosporium victoriae virus 190S and other victoriviruses (Family *Totiviridae*). *J Virol* **85,** 7343–7352.

Maejima K, Himeno M, Komatsu K, Kakizawa S, Yamaji Y, Hamamoto H, Namba S. 2008. Complete nucleotide sequence of a new double-stranded RNA virus from the rice blast fungus, *Magnaporthe oryzae*. *Arch Virol* **153,** 389–391.

Parent KN, Takagi Y, Cardone G, Olson NH, Ericsson M, Yang M, Lee Y, Asara JM, Fichorova RN, Baker TS, Nibert ML. 2013. Structure of a protozoan virus from the human genitourinary parasite *Trichomonas vaginalis*. *MBio* **4**(2).

Stielow B, Menzel W. 2010. Complete nucleotide sequence of TaV1, a novel totivirus isolated from a black truffle ascocarp (*Tuber aestivum* Vittad.). *Arch Virol* **155,** 2075–2078.

Taylor DJ, Ballinger MJ, Bowman SM, Bruenn JA. 2013. Virus-host co-evolution under a modified nuclear genetic code. *Peerj* **1,** e50.

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.

Abbreviations used for reference strains of species in the figure and tables are:

Genus *Totivirus*

ScV-L-A, *Saccharomyces cerevisiae virus L-A*
ScV-L-BC (La), *Saccharomyces cerevisiae virus L-BC (La)*
UmV-H1, *Ustilago maydis virus H1*
SsV-L, *Scheffersomyces segobiensis virus L* (proposed)
TaV1, *Tuber aestivum virus 1* (proposed)
XdV-L1A, *Xanthophyllomyces dendrorhous virus L1A* (proposed)
XdV-L1B, *Xanthophyllomyces dendrorhous virus L1B* (proposed)
BRV-F, Black raspberry virus F (probable)

Genus *Victorivirus*

- CeRV1, *Chalara elegans RNA Virus 1*
- CmRV, *Coniothyrium minitans RNA virus*
- EfV1, *Epichloe festucae virus 1*
- GaRV-L1, *Gremmeniella abietina RNA virus L1*
- HmTV1-17, *Helicobasidium mompa totivirus 1-17*
- HvV190S, *Helminthosporium victoriae virus 190S*
- MoV1, *Magnaporthe oryzae virus 1*
- SsRV1, *Sphaeropsis sapinea RNA virus 1*
- SsRV2, *Sphaeropsis sapinea RNA virus 2*
- AfSV1, *Aspergillus foetidus slow virus 1* (proposed)
- BbVV1, *Beauveria bassiana victorivirus 1* (proposed)
- MoV2, *Magnaporthe oryzae virus 2* (proposed)
- RnVV1, *Rosellinia necatrix victorivirus 1* (proposed)
- TcV1, *Tolypocladium cylindrosporum virus 1* (proposed)
- BfTV1, Botryotinia fuckeliana totivirus 1 (probable)

Genus *Giardiavirus*

- GLV, *Giardia lamblia virus*

Genus *Leishmaniavirus*

- LRV1-1, *Leishmania RNA virus 1-1*
- LRV1-4, *Leishmania RNA virus 1-4*
- LRV2-1, *Leishmania RNA virus 2-1*

Genus *Trichomonasvirus*

- TVV1, *Trichomonas vaginalis virus 1*
- TVV2, *Trichomonas vaginalis virus 2*
- TVV3, *Trichomonas vaginalis virus 3*
- TVV4, *Trichomonas vaginalis virus 4* (proposed)

Other

- EbRV1, Eimeria brunetti virus 1 (probable)
- IMNV, Penaeid shrimp infectious myonecrosis (probable)
- AhV, *Atkinsonella hypoxylon virus* (family *Partitiviridae*)
- WCCV1, *White clover cryptic virus 1* (family *Partitiviridae*)

Figure 1. Maximum-likelihood phylogenetic analysis of the CP+RdRp sequences of *Totiviridae* family members, highlighting the position of proposed species (red) within existing genera. Alignments were conducted using Clustal Omega version 1.1.0 as implemented at http://www.ebi.ac.uk/Tools/msa/clustalo/ with default settings. Trees were generated with PhyML 3.0 as implemented at http://www.hiv.lanl.gov/content/sequence/PHYML/interface.html using the LG substitution model, empirical equilibrium frequencies, program-estimated invariant-proportion value (0.007) and gamma-shape value (2.008), and 4 rate categories. The starting tree was obtained by BioNJ and optimized by both branch length and tree topology. Tree improvement was performed according to the best of nearest neighbor interchange and subtree pruning and regrafting. Branch support values (%) were estimated by the approximate likelihood ratio test (aLRT) with SH-like criteria; branches with support values ≥ 90% are unlabeled, and those with support values < 50% are collapsed to form polytomies. Two partitivirus sequences (AhV and WCCV1) were included as an outgroup, on which the tree is rooted. Two probable species of totiviruses that have not yet been assigned to genera are represented by strains EbRV1 and IMNV. Color and shading are explained in the figure. The putative host of each virus is shown at right: F, fungus; Pr, protozoan; A, arthropod; and P, plant.

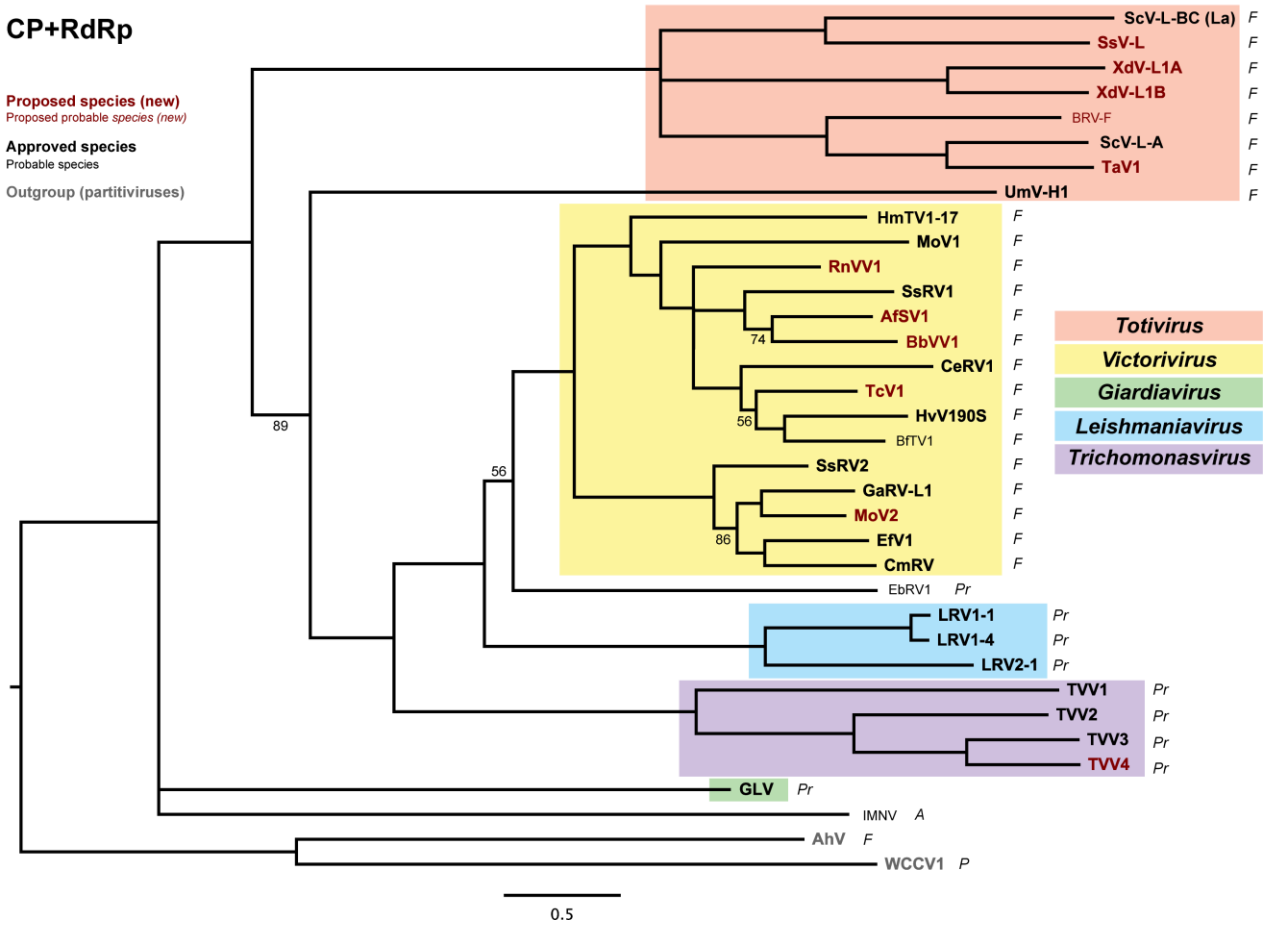
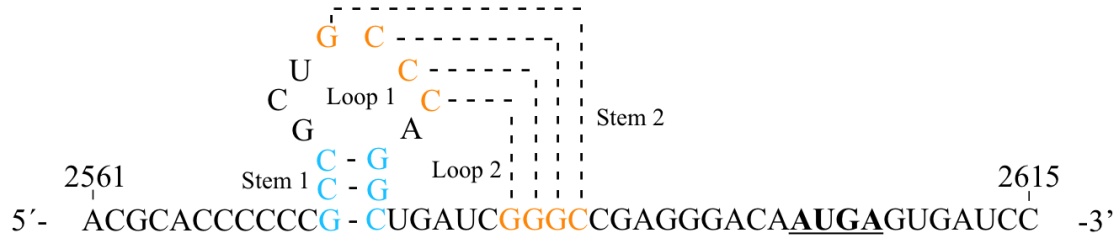


Figure 2. Translational termination–reinitiation region of *Victorivirus* genus members. (A) Plus-strand RNA sequence of HvV190S showing the AUGA termination–reinitiation motif and upstream pseudoknot or long stem–loop (Li et al., 2011). (B) Similar motifs in other approved, proposed, and probable *Victorivirus* genus members. Virus names are color-coded according to taxonomic status as described in Tables 4 and 5. AfSV1 and SsRV2 are the only viruses with a predicted long stem–loop just upstream of the termination–reinitiation motif, instead of pseudoknot. Sequences are aligned to the CP stop codon.

A



B

Virus	RNA Sequence	MFE
BbVV1	CCCUGUCGCAAUUGCCGGUGCUGCCCCACCCGGAGGGGCCGAACCCCGAGUAAUGG	-14.0
BfTV1	UACCCCUCCGCGUUCCCCUGCUGCCCCAGGUAACGGGGCCGGACACGAUAUGAGUG	-13.6
CeRV1	GUAGUAUCUUCUUCUGUGGCUGCCCCCACUAAUGAGGGGCCGAAACGAUGUCUAGA	-14.3
CmRV	AGGUGGUGACGUUCACGGUGCCGCCGGAGCCUUACCGGCUCCACAAGUAUGAUCG	-16.0
EfV1	UCCUCCCAUGCCUGAGGCUGCCACCGGUGCCGAACCGGUGCCCCAAGCAUGAUUG	-21.4
GaRV-L1	CGCCCACCUGCCAGCCGAAGCUACUGCUGCUGAAGCAGUGCCCGCUCAAUGAUUG	-16.5
HmTV1-17	GGCUGAAGCCAUGCAACAGGACGCAGCCCUGCAAGCUGCGGGGCUCAAUGAAGG	-20.2
HvV190S	CAUCCACGCACCCCCCGCCGCUGCCCAGGCUGAUCGGGCCGAGGGACAAUGAGUG	-17.0
MoV1	AGGGCCGACCCCCGAACCCCACGGCGCGGCGCCUAGCCUGCACGAAUAGAUAUGG	-13.8
MoV2	AGGCAAUAACAACGAGCAGGCCGCCGGCGCCCAGCCGGCCCCCGCUCAAUGAUUG	-23.4
RnVV1	CACCCCCUGUUGCGGUUGCCGCUGCCCCGGCUGAACGGGCCGCCGAACAGUAAUG	-24.8
SsRV1	GCCCCCCCUGUAGCACCCGGCCCCGCCCAGCCUGACGGGCCCGCCAAUGAAUAAG	-16.2
TcV1	CACUGCUCCCCCUGUUGCCGACCCCCAGGCUAUGGUGGGCGACCAGAUCUAAAUG	-16.6
AfSV1	CUGCAGCUGAUGAGGAUGAUAACCAACCCCCUCAACCAGUUGCGAUCGGUAACGGAAUUAAUGG	-15.9
SsRV2	UGCCGCUGAAGGCGCCGACGGUAAAGUACCGCCGGCGCCCCGCAAUGAGUAACG	-22.5

Table 1. Properties of strains of approved, proposed, and probable *Totivirus* species

Virus	Genome (bp)	G+C (%)	A+G (%)	5´ UTR (nt)	3´ UTR (nt)	CP (aa)	CP/RdRp (aa)
UmV-H1*	6099	51.9	53.8	539	97	na	1820
ScV-L-A	4579	45.7	52.5	29	33	680	1505
ScV-L-BC (La)	4615	42.5	54.9	23	54	697	1512
SsV-L†	4590	37.6	53.6	40	48	694	1500
TaV	4587	42.4	53.5	30	31	681	1522
XdV-L1A	4655	44.7	56.3	31	59	681	1535
XdV-L1B	4619	45.3	56.5	32	25	683	1534
BRV-F‡	5077	45.5	56.2	108	68	769	1645
range¶	4579-4655	37.6-45.7	52.5-56.5	23-40	25-59	680-697	1500-1535

* Approved species *Ustilago maydis virus H1* has several divergent properties from other *Totivirus* genus members, including one long ORF encompassing both CP and RdRp.
† Proposed species (new) in bolded red.
‡ Proposed probable species (new).
¶ Values for *Ustilago maydis virus H1* (approved) and Black raspberry virus F (probable) not included.

Table 2. Summary of properties of strains of the 5 current *Totiviridae* genera, including values of proposed species

Genus	Genome (bp)	G+C (%)	A+G (%)	5´ UTR (nt)	3´ UTR (nt)	CP (aa)	CP/RdRp (aa)
Totivirus*	4579-4655¶	37.6-45.7	52.5-56.5	23-40	25-59	680-697	1500-1535
Victorivirus†	4975-5359	52.6-63.0	43.0-51.7	199-574	43-137	741-789	1576-1616
Giardiavirus	6273-6277	49.7-50.0	50.4-50.5	366	295-299	886-887	1871-1900
Leishmaniavirus	5241-5284	46.1-46.4	53.1-53.4	340-447	47-50	714-741	1616-1620
Trichomonasvirus‡	4671-4944	44.5-49.3	46.2-49.7	295-363	63-161	678-746	1429-1481

* Values for *Ustilago maydis virus H1* (approved species) and Black raspberry virus F (probable species) not included in these ranges. See Table 1 for more details.
† Values for Botryotinia fuckeliana totivirus 1 (probable) not included in these ranges. See Table 4 for more details. The CP/RdRp value represents CP+RdRp for this genus.
‡ See Table 6 for more details.
¶ Shading highlights nonoverlapping ranges among these genera.

Table 3. Pairwise identity scores for CP/RdRp sequences of strains of approved (bold), proposed (red), and probable (italics) *Totivirus* species*

Virus	**UmV-H1**	**ScV-L-A**	**ScV-L-BC (La)**	**SsV-L**	**TaV**	**XdV-L1A**	**XdV-L1B**	*BRV-F*
UmV-H1	100%	17%	17%	18%	17%	17%	15.2%	17%
ScV-L-A		100%	25%	25%	45.7%†	25%	25%	33%
ScV-L-BC (La)			100%	29%	25%	21%	25%	23%
SsV-L				100%	26%	23%	23%	25%
TaV					100%	24%	26%	31%
XdV-L1A						100%	43%	25%
XdV-L1B							100%	23%
BRV-F								100%

* Determined using EMBOSS 6.3.1 needleall for pairwise global alignments (Blosum62 matrix, gap opening penalty 10, gap extension penalty 0.5).
† Highest and lowest scores are highlighted with shading.

Table 4. Properties of strains of approved, proposed, and probable *Victorivirus* species

Species	Genome (bp)	G+C (%)	A+G (%)	5´ UTR (nt)	3´ UTR (nt)	CP (aa)	RdRp* (aa)
CeRV1	5310	52.6	47.9	328	64	770	871
CmRV	4975	59.2	48.7	61§	100	775	829
EfV1	5109	60.3	47.0	270	58	766	827
GaRV-L1	5133	56.9	47.3	275	53	776	825
HmTV1-17	5207	55.3	51.7	199	107	788	845
HvV190S	5179	58.1	48.3	289	67	772	835
MoV1	5359	57.9	43.0	574	43	746	832
SsRV1	5163	61.7	45.7	250	73	776	838
SsRV2	5202	63.0	46.7	295	67	789	825
AfSV1†	5194	57.9	47.3	373	76	741	839
BbVV1	5228	55.3	47.5	443	52	742	834
MoV2	5193	61.9	48.9	274	63	788	830
RnVV1	5329	61.7	46.5	372	137	763	842
TcV1	5196	61.0	43.5	326	70	758	840
BfTV1‡	5261	54.7	49.9	336	114	765	788
range¶	4975-5359	52.6-63.0	43.0-51.7	199-574	43-137	741-789	825-871

* *Victorivirus* genus members express their RdRp as a separate protein, not in fusion with CP
† Proposed species or probable species (new) in red.
‡ Probable species.
¶ Values for Botryotinia fuckeliana totivirus 1 (probable) not included.
§ This sequence appears to be truncated at its 5´ plus-strand end and is thus not included in the range.

Table 5. Pairwise identity scores for CP+RdRp sequences of strains of approved (bold), proposed (red), and probable (italics) *Victorivirus* species*

virus	**CeRV1**	**CmRV**	**EfV1**	**GaRV-L1**	**HmTV1-17**	**HvV190S**	**MoV1**	**SsRV1**	**SsRV2**	**AfSV1**	**BbVV1**	**MoV2**	**RnVV1**	**TcV1**	*BfTV1*
CeRV1	100%	33%	31.7%	33%	35%	44%	35%	41%	33%	43%	43%	32%	43%	47%	45%
CmRV		100%	53%	54%	33%	34%	32%	35%	51%	35%	35%	54%	35%	35%	33%
EfV1			100%	52%	32%	34%	33%	35%	53%	34%	34%	52%	34%	35%	33%
GaRV-L1				100%	33%	36%	32%	34%	54%	36%	35%	58.4%	35%	35%	34%
HmTV1-17					100%	37%	34%	36%	33%	38%	38%	34%	38%	36%	37%
HvV190S						100%	38%	43%	36%	46%	45%	37%	48%	52%	53%
MoV1							100%	37%	32%	39%	39%	33%	41%	38%	37%
SsRV1								100%	35%	48%	47%	33%	45%	45%	45%
SsRV2									100%	35%	36%	55%	36%	37%	34%
AfSV1										100%	53%	35%	49%	48%	47%
BbVV1											100%	35%	46%	48%	48%
MoV2												100%	37%	32%	54%
RnVV1													100%	50%	48%
TcV1														100%	35%
BfTV1															100%

* Determined using EMBOSS 6.3.1 needleall for pairwise global alignments (Blosum62 matrix, gap opening penalty 10, gap extension penalty 0.5) of concatenated CP+RdRp sequences.
† Highest and lowest scores are highlighted with shading.

Table 6. Properties of strains of approved and proposed *Trichomonasvirus* species

Virus	Genome (bp)	G+C (%)	A+G (%)	5´ UTR (nt)	3´ UTR (nt)	CP (aa)	CP/RdRp (aa)
TVV1-1*	4647‡	44.8%	48.9%	287‡	69	678	1429
TVV1-T5	4648‡	45.0%	48.4%	285‡	72	678	1429
TVV1-IH2	4647‡	45.5%	48.7%	287‡	69	678	1429
TVV1-UR1	4684	45.8%	49.1%	326	68	678	1429
TVV1-UH9	4678	44.8%	49.7%	324	63	678	1429
TVV1-OC3	4684	45.5%	49.2%	326	67	678	1429
TVV1-OC4	4680	45.1%	49.6%	324	65	678	1429
TVV1-OC5	4680	45.4%	49.3%	322	67	678	1429
TVV1-C344	4657‡	44.5%	49.2%	287‡	79	678	1429
TVV2-1*	4674	45.7%	46.9%	295	69	709	1436
TVV2-UR1	4674	45.0%	47.5%	296	68	709	1436
TVV2-OC3	4674	44.9%	47.1%	297	67	709	1436
TVV2-OC5	4671	45.1%	47.0%	295	66	709	1436
TVV2-C76	4689	46.0%	47.2%	304	69	711	1438
TVV2-C351	4686	45.8%	46.6%	304	69	710	1437
TVV3-1*	4844	47.8%	47.4%	359	154	708	1443
TVV3-UR1	4845	47.4%	47.7%	362	151	708	1443
TVV3-OC3	4846	48.3%	47.1%	363	151	708	1443
TVV3-OC5	4842	47.7%	47.4%	359	152	708	1443
TVV4-1*†	4943	49.3%	46.4%	337	161	746	1481
TVV4-OC3	4944	49.3%	46.2%	338	161	746	1481
TVV4-OC5	4942	48.7%	46.5%	337	160	746	1481
range	4671-4944	44.5-49.3	46.2-49.7	295-363	63-161	678-746	1429-1481

* Reference strain, including proposed reference strain for *Trichomonas vaginalis virus 4*.

† Strains of proposed species (new) in bolded red.

‡ These sequences appear to be truncated at the 5´ plus-strand end of each and are thus not included in the ranges.

Table 7a. Pairwise identity scores for CP/RdRp sequences of strains of approved (bold) and proposed (red) *Trichomonasvirus* species*

virus	**TVV1-1**	**TVV1-T5**	**TVV1-IH2**	**TVV1-Cc**	**TVV1-UR1**	**TVV1-UH9**	**TVV1-OC3**	**TVV1-OC4**	**TVV1-OC5**	**TVV1-C344**
TVV1-1	100%	87%	85%	90%	83%	86%	85%	85%	83%	96%
TVV1-T5		100%	85%	85%	85%	86%	86%	86%	84%	85%
TVV1-IH2			100%	85%	84%	86%	86%	86%	83%	83%
TVV1-Cc				100%	83%	85%	84%	85%	82%	87%
TVV1-UR1					100%	85%	84%	85%	94%	81%
TVV1-UH9						100%	94%	95%	84%	85%
TVV1-OC3							100%	93%	83%	84%
TVV1-OC4								100%	83%	84%
TVV1-OC5									100%	**80.5%†**
TVV1-C344										100%
TVV2-1										
TVV2-UR1										
TVV2-OC3										
TVV2-OC5										
TVV2-C76										
TVV2-C351										
TVV3-1										
TVV3-UR1										
TVV3-OC3										
TVV3-OC5										
TVV4-1										
TVV4-OC3										
TVV4-OC5										

* Determined using EMBOSS 6.3.1 needleall for pairwise global alignments (Blosum62 matrix, gap opening penalty 10, gap extension penalty 0.5). Table sections a, b, and c can be consecutively joined left to right to view the complete table.
† Lowest score for strains within each species (shading) are highlighted in bold.

Table 7b. Pairwise identity scores for CP/RdRp sequences of strains of approved and proposed *Trichomonasvirus* species

virus	**TVV2-1**	**TVV2-UR1**	**TVV2-OC3**	**TVV2-OC5**	**TVV2-C76**	**TVV2-C351**
TVV1-1	26%	27%	27%	26%	25%	25%
TVV1-T5	26%	26%	27%	25%	24%	25%
TVV1-IH2	27%	26%	27%	26%	25%	26%
TVV1-Cc	26%	25%	26%	26%	25%	26%
TVV1-UR1	26%	26%	27%	25%	24%	26%
TVV1-UH9	25%	26%	26%	25%	25%	25%
TVV1-OC3	25%	27%	27%	26%	25%	26%
TVV1-OC4	25%	26%	25%	25%	24%	25%
TVV1-OC5	25%	26%	26%	26%	24%	25%
TVV1-C344	26%	26%	26%	25%	26%	24%
TVV2-1	100%	90%	90%	94%	88%	87%
TVV2-UR1		100%	93%	90%	80%	79%
TVV2-OC3			100%	90%	80%	**79.0%**
TVV2-OC5				100%	84%	82%
TVV2-C76					100%	81%
TVV2-C351						100%
TVV3-1						
TVV3-UR1						
TVV3-OC3						
TVV3-OC5						
TVV4-1						
TVV4-OC3						
TVV4-OC5						

* Determined using EMBOSS 6.3.1 needleall for pairwise global alignments (Blosum62 matrix, gap opening penalty 10, gap extension penalty 0.5).

† Lowest score for strains within each species (shading) are highlighted in bold.