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:	***2010.001a-dF***	(to be completed by ICTV officers)

Short title: : 3 new species in the new genus Trichomonasvirus, 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:

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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)	Before submitting this proposal to the ICTV-EC, we shared it with Reed B. Wickner, chair of the Totiviridae study group, who expressed his support. Co-proposer Said A. Ghabrial is a member of the Totiviridae study group.

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

We were asked by the EC to provide a new, unrooted tree for Fig. 2, and we have done that in this revision. The Fig. 2 legend has also been changed to reflect the new tree.

Date first submitted to ICTV:
Date of this revision (if different to above): July 2, 2010

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.

Code	***2010.001aF***	(assigned by ICTV officers)

To create 3 new species within:

Genus:	*Trichomonasvirus* (new)
Subfamily:	unassigned
Family:	*Totiviridae*
Order:	unassigned

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:

Trichomonas vaginalis virus 1
Trichomonas vaginalis virus 2
Trichomonas vaginalis virus 3

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.
- Provide accession numbers for genomic sequences
- Further material in support of this proposal may be presented in the Appendix, Module 9

Characterized dsRNA viruses of the protozoan parasite *Trichomonas vaginalis* have been assigned to the family *Totiviridae* based on several criteria including monosegmented genome, coding content and expression strategy (Fig. 1), and phylogenetic similarity (Fig. 2). Most of the reports concerning important basic features of these viruses have been generated by Wang, Alderete, Tai, and their colleagues (Wang & Wang, 1985, 1986; Wang et al., 1987; Khoshnan & Alderete, 1993, 1994, 1995; Tai et al., 1993, 1995; Khoshnan et al., 1994; Tai & Ip, 1995; Su & Tai, 1996; Liu et al., 1998; Bessarab et al., 2000; Alderete et al., 2003; Gerhold et al., 2009).

Proposed species *Trichomonas vaginalis virus 1*, *Trichomonas vaginalis virus 2*, and *Trichomonas vaginalis virus 3* differ from all other species in the family *Totiviridae* in that their respective isolates infect the protozoan parasite *T. vaginalis*. Other protozoan viruses in the family *Totiviridae* infect the parasites *Leishmania* (genus *Leishmaniavirus*), *Giardia* (genus *Giardiavirus*), or *Eimeria* (genus unassigned). The *T. vaginalis* viruses are phylogenetically distinct from each of these other protozoan viruses as well as from other members of the family (see Fig. 2).

Virus isolates encompassed by the 3 proposed species of *T. vaginalis* viruses are respectively designated Trichomonas vaginalis virus 1 (TVV1), Trichomonas vaginalis virus 2 (TVV2), and Trichomonas vaginalis virus 3 (TVV3). The prototypical isolates of each, for which full-length genome sequences have been reported to Genbank, are: TVV1-1 (from *T. vaginalis* strain T1, Tai & Ip, 1995; acc. no. U08999), TVV2-1 (from *T. vaginalis* strain T1, Bessarab et al., 2000; acc. no. AF127178), and TVV3-1 (from an unspecified *T. vaginalis* strain, acc. no. AF325840). In addition, there are 3 other isolates of TVV1 for which full-length protein-coding sequences have been reported to Genbank: TVV1-T5 (from *T. vaginalis* strain T5, Su & Tai, 1996; acc. no. U57898), TVV1-Ch (from *T. vaginalis* strain Changchun, Zhao et al., 2006; acc. no. DQ528812), and TVV1-IH-2 (from *T. vaginalis* strain IH-2, Kim et al., 2007; acc. no. DQ270032).

Table 1 compares the lengths of some basic genome and protein elements of virus isolates belonging to the 3 proposed species of *T. vaginalis* viruses. The genome length of each is similar, 4647–4844 bp. The plus strand of each includes a longer 5´ untranslated region, 287–359 nt, and a shorter 3´ untranslated region, 72–157 nt. Open reading frames (ORFs) for the capsid protein (CP) and the RNA-dependent RNA polymerase (RdRp) are similarly sized in each, 678–709 aa for CP and 756–767 aa for RdRp. In addition, based on the proposed slippery sequence for ribosomal frameshifting, the predicted size of the CP/RdRp fusion protein expressed by each is also similar, 1430–1443 aa.

Table 2 shows amino acid identity scores for the *T. vaginalis* viruses. Fifty percent amino acid sequence identity was previously adopted for the family *Totiviridae* as an appropriate cut-off between species (Wickner et al., 2005) and applies in this case as well for pairwise comparisons of isolates belonging to the 3 proposed species of *T. vaginalis* viruses (<44% amino acid sequence identity between TVV1, TVV2, and TVV3). In addition, in the current case, there is >82% amino acid sequence identity in pairwise comparisons between the 4 different TVV1 isolates.

Other important differences between the 3 proposed species of *T. vaginalis* viruses remain largely unknown due to limited characterizations to date. However, it does appear that the 4 different TVV1 isolates express their respective RdRp regions following a +1 (or -2) ribosomal frameshift whereas the single TVV2 and TVV3 isolates express their respective RdRp regions following a –1 ribosomal frameshift.

MODULE 3: **NEW GENUS**

creating a new genus

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

Code	***2010.001bF***	(assigned by ICTV officers)

To create a new genus within:

Subfamily:	unassigned
Family:	*Totiviridae*
Order:	unassigned

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	***2010.001cF***	(assigned by ICTV officers)

To name the new genus: *Trichomonasvirus*

Assigning the type species and other species to a new genus

Code	***2010.001dF***	(assigned by ICTV officers)

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

Trichomonas vaginalis virus 1

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

Virus isolates belonging to each of the 3 proposed species of *T. vaginalis* viruses are phylogenetically divergent from Giardia lamblia virus (GLV), which represents the type species of genus *Giardiavirus*, to which Trichomonas vaginalis virus was previously assigned as a tentative species (Wickner et al., 2005) (see Fig. 2). At the same time, all isolates of *T. vaginalis* viruses show phylogenetic clustering consistent with their descent from a common ancestor and their assignment to a single new genus (see Fig. 2).

Table 1 additionally includes the values for GLV. In comparison with the values for *T. vaginalis* viruses, the values for GLV are consistently larger, and substantially so in most cases. These differences are consistent with the phylogenetic divergence of viruses in the genus *Giardiavirus* from those in the proposed genus *Trichomonasvirus*.

Table 3 shows representative alignment results from TBLASTN using RdRp sequences of *T. vaginalis* viruses as queries. Notably, other members of family *Totiviridae* (isolates of proposed genus *Trichomonasvirus* followed by isolates of genera *Leishmaniavirus*, *Victorivirus*, and *Totivirus*) receive highest scores in this type of analysis. GLV, however, is either absent (Table 3) or receives only very low scores with certain other *T. vaginalis* virus RdRp queries, below those of

isolates of the other *Totiviridae* genera (data not shown). These findings provide further strong evidence for divergence of the *T. vaginalis* viruses from GLV and support the taxonomic classification of the *T. vaginalis* viruses and GLV in separate genera. Table 3 and Fig. 2 also provide strong evidence that no other currently recognized genus in the family *Totiviridae* is appropriate to accommodate the *T. vaginalis* viruses and thus that the *T. vaginalis* viruses should most appropriately be placed into their own genus, *Trichomonasvirus*, as proposed here.

The host species *T. vaginalis* and *G. lamblia*, though both so-called excavate protists (Dacks et al., 2008), are in fact well differentiated from one another, including at the genome level (Carlton et al., 2007; Morrison et al., 2007), and thus it is not surprising that the viruses that respectively infect these 2 distinct hosts are also distinct.

Origin of the new genus name:

The proposed genus name, *Trichomonasvirus*, is based on the genus name of the protozoan host species, *Trichomonas vaginalis*. The name *Trichomonasvirus* parallels the other genus names for protozoan viruses in the family *Totiviridae*, *Leishmaniavirus* and *Giardiavirus*, in containing the full name of the host genus.

Reasons to justify the choice of type species:

The proposed type species *Trichomonas vaginalis virus 1* has been chosen to include the first isolate of *T. vaginalis* virus for which a full-length genome sequence was reported (Tai & Ip, 1995; Genbank acc. no. U08999). Full-length protein-coding sequences of 3 other isolates belonging to this species have been additionally reported to Genbank (acc. nos. U57898, DQ270032, and DQ528812). In contrast, the single isolates belonging to species *Trichomonas vaginalis virus 2* and *Trichomonas vaginalis virus 3*, for which full-length genome sequences have also been reported to Genbank (acc. nos. AF127178 and AF325840, respectively), remain less characterized.

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.

Fifty percent amino acid sequence identity was previously adopted for the family *Totiviridae* as an appropriate cut-off between species (Wickner et al., 2005) and also applies in this case for pairwise comparisons of isolates belonging to of the 3 proposed species of *T. vaginalis* viruses (Table 1; <44% identity between TVV1, TVV2, and TVV3). In addition, in the current case, there is >82% amino acid sequence identity in pairwise comparisons between the 4 different TVV1 isolates (Table 1).

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

References:

Alderete JF, Wendel KA, Rompalo AM, Erbelding EJ, Benchimol M, Chang TH. 2003. Trichomonas vaginalis: evaluating capsid proteins of dsRNA viruses and the dsRNA virus within patients attending a sexually transmitted disease clinic. Exp Parasitol 103:44-50.

Bessarab IN, Liu HW, Ip CF, Tai JH. 2000. The complete cDNA sequence of a type II Trichomonas vaginalis virus. Virology 267:350-9.

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Liu HW, Chu YD, Tai JH. 1998. Characterization of Trichomonas vaginalis virus proteins in the pathogenic protozoan T. vaginalis. Arch Virol 143:963-70.

Löytynoja A, Goldman N (2008) Phylogeny-aware gap placement prevents errors in sequence alignment and evolutionary analysis. Science 320:1632–1635

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Wickner RB, Wang CC, Patterson JL. 2005. Family Totiviridae. In: Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses. Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds), Elsevier/Academic Press, London, p 571-80.

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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. Coding diagrams for TVV1, TVV2, and TVV3. Open reading frames (ORFs) 1 (capsid protein (CP, red) and 2 (RNA-dependent RNA polymerase (RdRp), blue) are diagrammed for each virus. The first and last codons for each ORF are indicated. In each virus, the RdRp is thought to be expressed as a CP/RdRp fusion following ribosomal frameshifting as indicated. 5´ and 3´ untranslated regions (UTRs) are also labeled, along with the position numbers of the first and last nucleotides of each genome and ORF. The specific nucleotides and nucleotide position numbers shown in this figure are for the prototypical isolates TVV1-1 (Tai & Ip, 1995; Genbank acc. no. U08999); TVV2-1 (Bessarab et al., 2000; Genbank acc. no. AF127178); and TVV3-1 (Genbank acc. no. AF325840).

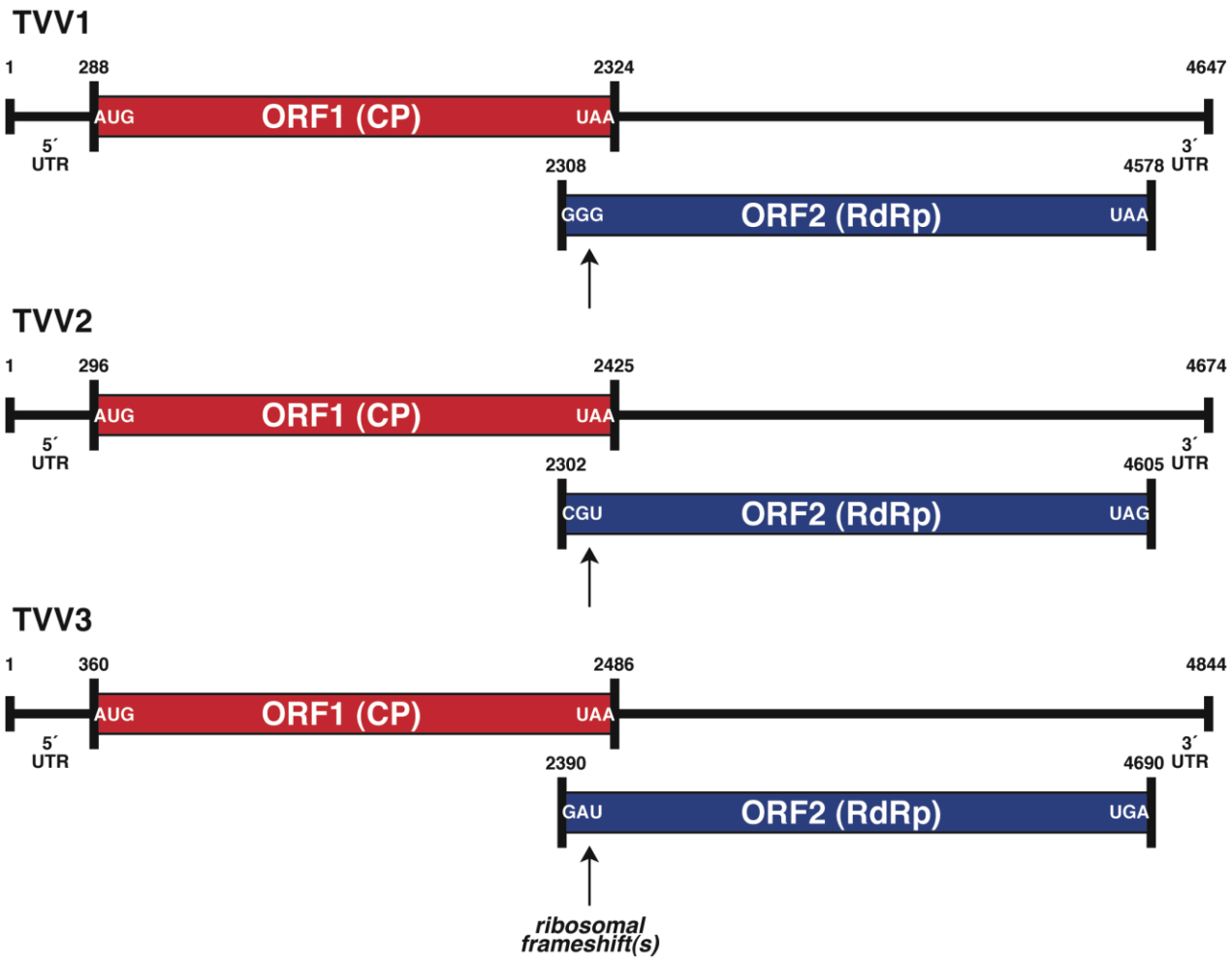

Fig. 2. Phylogenetic relationships among TVVs and other approved members of the family *Totiviridae*. Maximum likelihood trees were derived from the full-length, concatenated CP and RdRp ORF sequences of each analyzed virus. The multiple sequence alignment was generated using MUSCLE v3.7 without subsequent Gblocks curation; the phylogenetic tree was generated using PhyML v3.0; the confidence index for each branch (expressed as % in the labels) was determined as described by Anisimova and Gascuel (2006); and the tree was rendered using TreeDyn v198.3. All four steps were performed using the "A la Carte" option at http://www.phylogeny.fr/ [Dereeper et al., 2008]. The tree was additionally refined for presentation using FigTree v1.3.1 obtained from http://tree.bio.ed.ac.uk/software/figtree/. The tree is unrooted, and the scale bar indicates the number of substitutions per position in the alignment. See text for the Genbank accession no. of each TVV sequence (prototype strains TVV1-1, TVV2-1, and TVV3-1). Other members of the family *Totiviridae* included in the tree are (Genbank accession no. in parenthesis after each): LRV1-1, Leishmania RNA virus 1-1 (M92355); LRV2-1, Leishmania RNA virus 2-1 (U32108), LRV1-4, Leishmania RNA virus 1-4 (U01899); HvV190S, Helminthosporium victoriae virus 190S (HVU41345); CeRV1, Chalara elegans RNA virus 1 (AY561500); HmV1-17, Helicobasidium mompa totivirus 1-17 (AB085814); MoV1, Magnaporthe oryzae virus 1 (AB176964); SsRV1, Sphaeropsis sapinea RNA virus 1 (AF038665); CmRV, Coniothyrium minitans RNA virus (AF527633); EfV1, Epichloe festucae virus 1 (AM261427); GaRV-L1, Gremmeniella abietina RNA virus L1 (AF337175); SsRV2, Sphaeropsis sapinea RNA virus 2 (AF039080); ScV-LA, Saccharomyces cerevisiae virus LA (J04692); ScV-LBC, Saccharomyces cerevisiae virus LBC (U01060); UmV-H1, Ustilago maydis virus H1 (U01059); and GLV (L13218). Viruses are clustered as follows: genus *Trichomonasvirus* (magenta), genus *Leishmaniavirus* (green), genus *Victorivirus* (orange), genus *Totivirus* (cyan), and genus *Giardiavirus* (brown).

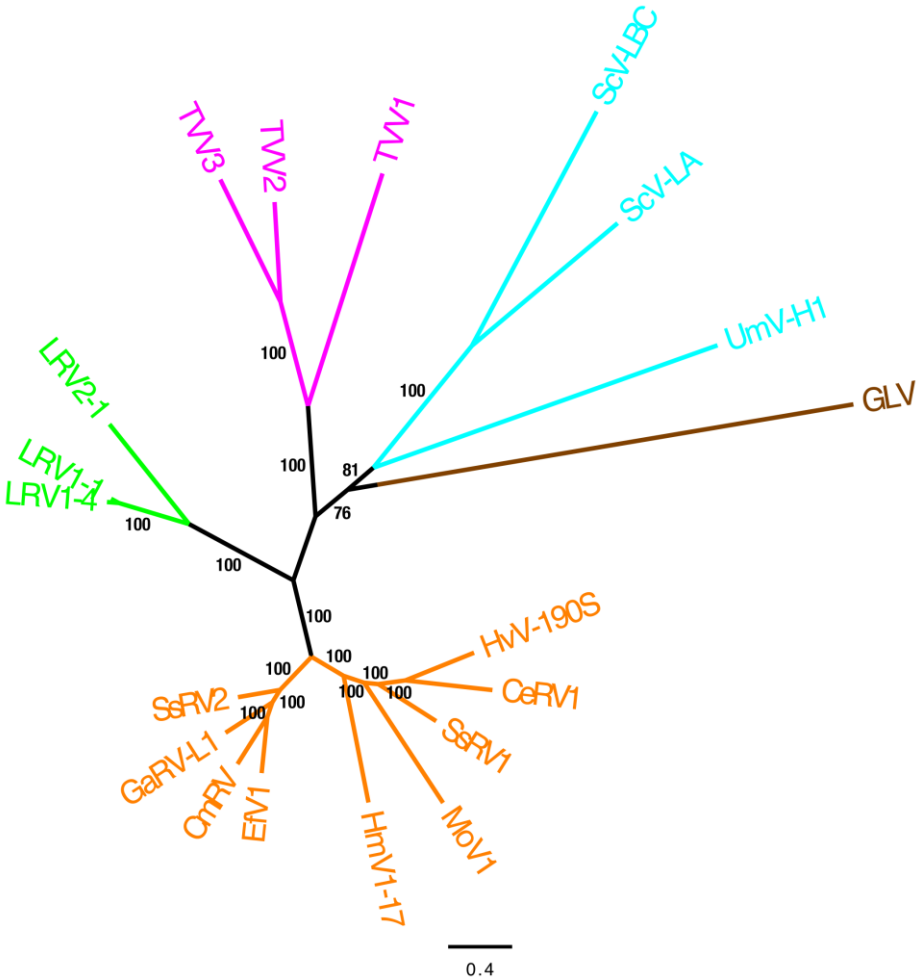

Table 1. Lengths of some basic elements of TVV1, TVV2, and TVV3

virus	genome (bp)	5'UTR (nt)	3'UTR (nt)	CP (aa)	RdRp ORF (aa)	CP/RdRp (aa)
TVV1	4647	287	72	678	756	1430
TVV2	4674	295	72	709	767	1436
TVV3	4844	359	157	708	766	1443
GLV	6277	366	302	886	1057	1870

Specific values are shown for the prototypical isolates TVV1-1 (Tai & Ip, 1995; Genbank acc. no. U08999), TVV2-1 (Bessarab et al., 2000; Genbank acc. no. AF127178), and TVV3-1 (Genbank acc. no. AF325840). Abbreviations: bp, base pairs; UTR, untranslated region; nt, nucleotides; CP, capsid protein; aa, amino acids; RdRp, RNA-dependent RNA polymerase; ORF, open reading frame; CP/RdRp, fusion protein expressed by ribosomal frameshifting. Length of the fusion protein is predicted based on the position of a proposed slippery heptamer in each virus. Values for the prototypical isolate of GLV (Wang et al., 1993; GenBank acc. no. L13218), from genus *Giardiavirus*, are also shown for comparison (see Module 3 text).

Table 2. Pairwise sequence identity scores for *T. vaginalis* viruses (TVVs)

A

		Amino acid sequence identity (%) in pairwise comparisons of CP from the following TVVs:					
		1-IH-2	1-Ch	1-T5	1-1	2-1	3-1
TVVs:	1-IH-2		87	86	88	22	21
	1-Ch			86	90	23	21
	1-5				90	22	20
	1-1					22	20
	2-1						32
	3-1						

B

		Amino acid sequence identity (%) in pairwise comparisons of RdRp from the following TVVs:					
		1-IH-2	1-Ch	1-T5	1-1	2-1	3-1
TVVs:	1-IH-2		83	84	83	28	28
	1-Ch			84	89	28	26
	1-5				84	28	30
	1-1					28	27
	2-1						43
	3-1						

Full-length CP (**A**) or RdRp (**B**) sequences were using EMBOSS ALIGN at EMBL-EBI (http://www.ebi.ac.uk/Tools/emboss/align/) with default settings. Abbreviations: 1-IH-2, TVV1-IH-2 from *T. vaginalis* strain IH-2; 1-Ch, TVV1-Ch from *T. vaginalis* strain Changchun; 1-5, TVV1-T5 from *T. vaginalis* strain T5; 1-1, TVV1-1 from *T. vaginalis* strain T1; 2-1, TVV2-1 from *T. vaginalis* strain T1; and 3-1, TVV3-1 from an unspecified *T. vaginalis* strain. See text for Genbank acc. nos. TVV1-1, TVV2-1 and TVV3-1 are the prototypical isolates of the 3 proposed species.

Table 3. TBLASTN alignment results using full-length TVV1-1 RdRp ORF as query

Sequences producing significant alignments:	Score (Bits)	E Value
gb|U08999.1|TVU08999 Trichomonas vaginalis virus T1	**1579**	**0.0**
gb|DQ528812.1| Trichomonas vaginalis virus	1404	0.0
gb|U57898.1|TVU57898 Trichomonas vaginalis virus	1341	0.0
gb|DQ270032.1| Trichomonas vaginalis virus	1339	0.0
gb|FJ997643.1| Trichomonas vaginalis virus isolate NYH286	357	1e-95
gb|AF127178.1|AF127178 Trichomonas vaginalis virus II	**295**	**7e-77**
gb|AF325840.1|AF325840 Trichomonas vaginalis virus 3	**291**	**9e-76**
gb|U32108.1|LRU32108 Leishmania RNA virus 2-1	**177**	**1e-41**
gb|AF039080.1|AF039080 Sphaeropsis sapinea RNA virus 2	**172**	**5e-40**
gb|AF337175.1| Gremmeniella abietina RNA virus L1	**172**	**8e-40**
emb|AM261427.1| Epichloe festucae virus 1	**170**	**2e-39**
gb|AY615210.1| Gremmeniella abietina RNA virus L2	170	3e-39
gb|M92355.1|LV1RDRP Leishmania RNA virus 1-1	**166**	**3e-38**
gb|U01899.1|LRU01899 Leishmania RNA virus 1-4	**166**	**5e-38**
gb|EU289895.1| Aspergillus mycovirus 178 clone 178b	162	8e-37
gb|S62744.1|S62744 {clone C001} [Trichomonas vaginalis virus	162	9e-37
dbj|AB300379.1| Magnaporthe oryzae virus 2	**159**	**5e-36**
gb|AF356189.1|AF356189 Eimeria brunetti RNA virus 1	**147**	**3e-32**
gb|AF527633.1| Coniothyrium minitans mycovirus	**146**	**3e-32**
dbj|AB176964.1| Magnaporthe oryzae virus 1	**146**	**4e-32**
gb|AY561500.1| Thielaviopsis basicola dsRNA virus 1	**139**	**4e-30**
gb|AY556461.1| Thielaviopsis basicola dsRNA virus 2	138	1e-29
gb|AF038665.1| Sphaeropsis sapinea RNA virus 1	**137**	**2e-29**
gb|U41345.2|HVU41345 Helminthosporium victoriae virus 190S	**134**	**3e-28**
dbj|AB085814.1| Helicobasidium mompa No.17 dsRNA virus	**129**	**4e-27**
emb|AM491608.1| Botryotinia fuckeliana totivirus 1	**129**	**6e-27**
gb|J04692.1|SCSCOATRNA Saccharomyces cerevisiae virus L-A	**102**	**7e-19**
gb|M28353.1|SCSL1A Saccharomyces cerevisiae virus L-A	**102**	**7e-19**
gb|CP000501.1| Pichia stipitis CBS 6054 chromosome 7	101	2e-18
ref|XM_457518.1| Debaryomyces hansenii CBS767	100	5e-18
gb|AF296439.1| Penicillium chrysogenum virus	97	5e-17
emb|AM111098.1| Ophiostoma minus totivirus	92	7e-16
gb|EU289896.1| Aspergillus mycovirus 1816	88	2e-14
emb|AJ781397.1| Cherry chlorotic rusty spot associated chrysovirus	86	5e-14
emb|AJ781166.1| Amasya cherry disease associated chrysovirus	86	9e-14
gb|EU082131.1| Black raspberry virus F	83	5e-13
gb|AF297176.1| Helminthosporium victoriae 145S virus	80	4e-12
emb|X94361.1| Agaricus bisporus virus 1 (ABV1) L1 dsRNA	79	8e-12
gb|U01060.1|SCU01060 Saccharomyces cerevisiae virus La	**75**	**1e-10**
gb|EU495331.1| Ribes virus F isolate Corvallis	68	2e-08
gb|EF568774.1| Diplodia scrobiculata RNA virus 1	67	4e-08
gb|EF152346.1| Fusarium oxysporum chrysovirus 1	63	7e-07
emb|AM111097.2| Phlebiopsis gigantea mycovirus	60	6e-06
dbj|AB275288.1| Helicobasidium mompa V670	59	1e-05
emb|X92203.2| Cucurbit yellows-associated virus	59	1e-05
gb|U01059.1|UMU01059 Ustilago maydis virus UmVH1	**57**	**3e-05**
emb|AM111096.2| Phlebiopsis gigantea mycovirus	52	0.001
emb|X96870.1| Equine Rhinovirus type 1	52	0.001
gb|DQ272578.1| Equine rhinitis A virus strain PERV-1	52	0.001
gb|AF154271.1|AF154271 Foot-and-mouth disease virus	51	0.002
gb|AY593847.1| Foot-and-mouth disease virus SAT 2 isolate	51	0.002

Analysis was performed using TBLASTN (protein sequence query against the translated nucleotide sequence databases) with default settings as implemented at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The 50 highest-scoring matches are shown. Specific isolates of family *Totiviridae* that appear in Fig. 2 are bolded and color-coded by genus in the same manner here.