

## ***Trichomonasvirus*: a new genus of protozoan viruses in the family *Totiviridae***

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Received: 11 August 2010 / Accepted: 30 September 2010 / Published online: 26 October 2010  
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**Abstract** The family *Totiviridae* includes a number of viruses with monosegmented dsRNA genomes and isometric virions that infect either fungi or a number of medically important protozoan parasites such as *Leishmania* and *Giardia*. A new genus, *Trichomonasvirus*, was recently approved for this family. Its name is based on the genus of its host organism, *Trichomonas vaginalis*, a protozoan parasite that colonizes the human genitourinary mucosa and is the most common non-viral sexually transmitted infection in the world. The type species of this new genus is *Trichomonas vaginalis virus 1*. Distinguishing characteristics of the new genus include infection of a human sexually transmitted parasite, stable mixed infection with more than one distinct *Trichomonasvirus* species, and sequence-based phylogenetic divergence that distinguishes it from all other family members.

### **Introduction**

Members of the family *Totiviridae* are characterized by icosahedral virions, ranging between 30 and 40 nm in diameter, which normally encapsidate monosegmented (i.e., nonsegmented) double-stranded RNA (dsRNA) genomes with overlapping open reading frames encoding a capsid protein (CP) and an RNA-dependent RNA polymerase (RdRp) [26]. In some members of this family (e.g., those from the genera *Giardiavirus*, *Leishmanivirus*, and *Totivirus*), the RdRp is expressed as a CP/RdRp fusion protein, either as a consequence of ribosomal frameshifting [15, 32] or as a direct fusion with CP [34]. In other members (e.g., those from the genus *Victorivirus*), it is expressed as a separate, nonfused protein [31].

The greater than 25 species in the family *Totiviridae* that have been recognized by the International Committee on Taxonomy of Viruses (ICTV) as of spring 2009 are known to mediate largely noncytopathic, persistent infections of a diverse range of fungi and protozoa. Four genera are currently recognized: *Giardiavirus*, *Leishmanivirus*, *Totivirus*, and *Victorivirus* [26]. Viruses that infect protozoa have been assigned to the former two genera, and those that infect fungi to the latter two.

*Trichomonas vaginalis* is a sexually transmitted, flagellated protozoan that colonizes the mucosal epithelium of the human genitourinary tract, most consistently the vagina in women and urethra in men, causing inflammatory disease and associated risks of reproductive problems [17]. Trichomoniasis is the most common curable sexually transmitted disease in the world, with estimated prevalence higher than that of syphilis, gonorrhoea, and chlamydia combined [24]. The prevalence of trichomoniasis in inner-city, sexually transmitted infection (STI) clinics in the USA approaches 25%, and trichomoniasis is estimated to

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account for almost 50% of all curable infections worldwide [47], or an estimated 170 million new cases each year [63]. In addition to the morbidity associated with symptomatic vaginitis (pruritus, irritation, and discharge) and/or urethritis, it has also been associated with a variety of other clinical outcomes, including low birth weight and premature delivery [13], and an increased risk of transmission and acquisition of human immunodeficiency virus, human papillomavirus, and cervical cancer [40, 64]. Recurrence of *T. vaginalis* infection, reflecting lack of a protective immune response in the human host, and resistance to the drug of choice metronidazole are other associated problems [47]. Virulence of the parasite is mediated by several factors including cysteine proteases, surface proteins, and the major surface lipophosphoglycan, the latter being responsible for selective upregulation of inflammatory mediators by the host cervical and vaginal epithelial cells [18]. Immunosuppressive effects on bystander immune cells have also been observed in vitro; however, their molecular basis remains poorly understood [17].

The presence of long, linear dsRNA molecules in many strains of *T. vaginalis* was first reported in 1985, followed shortly by evidence of its association with virus-like particles [19, 53, 54]. Further screening of *T. vaginalis* strains revealed that the presence of viral dsRNA was relatively common, and that as many as three dsRNA segments of similar length (4,000–5,000 bp) could be identified in a single isolate, suggesting either the presence of a multi-segmented virus or the possibility of mixed infection by several different *Trichomonas vaginalis* viruses (TVVs) [20, 35]. Genome sequencing and phylogenetic analyses of several TVV genomes (Table 1) subsequently demonstrated homology to monosegmented dsRNA viruses of the family *Totiviridae* [10, 49, 51]. Based on relatedness of the *Trichomonas* and *Giardia* host organisms, TVVs were tentatively assigned to the genus *Giardiavirus*, of which *Giardia lamblia virus* is the well-characterized type species

[55, 57]. Further genome sequencing and phylogenetic analyses, however, have suggested that TVVs are not so closely related to *G. lamblia virus* (GLV) as to warrant grouping in the same genus [26, 39].

The phylogenetic divergence and other distinguishing characteristics of TVVs recently led to the proposal of a new genus in the family *Totiviridae* to accommodate them. The establishment of this new genus, named *Trichomonasvirus* based on the host organism and in parallel with the genus names for other protozoan viruses in the family (*Giardiavirus* and *Leishmaniavirus*), has been recently approved by the Executive Committee of the ICTV and is awaiting ratification at the next ICTV meeting, to be held in Sapporo, Japan, in September 2011.

### Taxonomic structure

Order:	Unassigned
Family:	<i>Totiviridae</i>
Genus:	<i>Trichomonasvirus</i>
Type species:	<i>Trichomonas vaginalis virus 1</i>
Other species:	<i>Trichomonas vaginalis virus 2</i> <i>Trichomonas vaginalis virus 3</i>

TVVs were first tentatively assigned to the genus *Giardiavirus* given that both hosts (*T. vaginalis* and *G. lamblia*) are related flagellates. However, these protozoa are generally now assigned to separate major clades—Parabasalia (order *Trichomonadida*) and Fornicata (order *Diplomonadida*), respectively—within the supergroup Excavata [1], and recent genome sequencing studies have confirmed that *T. vaginalis* and *G. lamblia* are indeed well differentiated at the genomic level [11, 43]. Moreover, genome sequences of their respective viruses have revealed that TVVs are phylogenetically divergent from GLV [26, 39]. These findings, along with phylogenetic clustering of the three

**Table 1** Properties of viruses in the new genus *Trichomonasvirus*

Virus name <sup>a</sup>	GenBank acc. no. <sup>b</sup>	Genome length (nt)	Coding region (nt)		Protein length (aa)	
			CP <sup>c</sup>	RdRp <sup>d</sup>	CP <sup>c</sup>	RdRp <sup>d</sup>
TVV1-1	U08999	4,647	288–2,324	2,308–4,578	678	756
TVV1-T5	U57898	4,648	286–2,322	2,306–4,576	678	756
TVV1-IH2	DQ270032	4,647	288–2,324	2,308–4,578	678	756
TVV2-1	AF127178	4,674	296–2,425	2,302–4,605	709	767
TVV3-1	AF325840	4,844	360–2,486	2,390–4,690	708	766

<sup>a</sup> Abbreviations of virus names include the strain designation (e.g., T5). The prototypical strain of each species has been assigned the strain designation “1”

<sup>b</sup> Viruses for which full-length genome sequences have been reported to GenBank; *acc* accession

<sup>c</sup> Beginning with the proposed AUG start codon in the CP ORF

<sup>d</sup> Based on the complete RdRp ORF, without regard to the proposed site of ribosomal frameshifting that leads to CP/RdRp fusion protein expression

**Table 2** Sequence comparisons of viruses in the new genus *Trichomonasvirus*

Virus <sup>a</sup>	Percent aa sequence identity in pairwise comparisons of the following TVVs: <sup>b</sup>					
	(A) For CP ORF					
	TVV1-IH2	TVV1-Ch	TVV1-T5	TVV1-1	TVV2-1	TVV3-1
TVV1-IH2	<b>100</b>	<b>87</b>	<b>86</b>	<b>88</b>	22	21
TVV1-Ch		<b>100</b>	<b>86</b>	<b>90</b>	23	21
TVV1-T5			<b>100</b>	<b>90</b>	22	20
TVV1-1				<b>100</b>	22	20
TVV2-1					<b>100</b>	32
TVV3-1						<b>100</b>
	(B) For RdRp ORF					
	TVV1-IH2	TVV1-Ch	TVV1-T5	TVV1-1	TVV2-1	TVV3-1
TVV1-IH2	<b>100</b>	<b>83</b>	<b>84</b>	<b>83</b>	28	28
TVV1-Ch		<b>100</b>	<b>84</b>	<b>89</b>	28	26
TVV1-T5			<b>100</b>	<b>84</b>	28	30
TVV1-1				<b>100</b>	28	27
TVV2-1					<b>100</b>	43
TVV3-1						<b>100</b>

<sup>a</sup> See Table 1 for explanation of abbreviations. Ch, Changchun

<sup>b</sup> Translated full-length CP (A) or RdRp (B) ORF sequences were globally aligned using EMBOSS Align at EMBL-EBI (<http://www.ebi.ac.uk/Tools/emboss/align/>) with default settings. Sequence identity values  $\geq 80\%$  are bolded

proposed species of TVVs, justify their assignment to a new genus in the family *Totiviridae*, apart from genus *Giardiavirus*.

*Trichomonas vaginalis virus 1* was chosen as the type species for the new genus because its prototypical strain *Trichomonas vaginalis virus 1-1* (TVV1-1) was the first virus for which full-length genome sequence data was reported [51] and because TVV1-1 and other TVV1 strains remain the best characterized and most commonly reported to date. Of the currently five full-length genomic sequences for TVVs in GenBank, three are from different strains of TVV1. The remaining two are from *Trichomonas vaginalis virus 2-1* (TVV2-1) and *Trichomonas vaginalis virus 3-1* (TVV3-1), representing the prototypical strains of the two other newly proposed species, *Trichomonas vaginalis virus 2* [10] and *Trichomonas vaginalis virus 3* [9], respectively (Tables 1, 2). In addition, full-length protein-coding sequences have been reported for a fourth strain of TVV1 [65] (Table 2).

An amino acid (aa) sequence identity of  $<50\%$  was previously adopted as a cut-off for identifying distinct species in the family *Totiviridae* [62]. By this criterion, pairwise comparison of the six TVV isolates with full-length protein-coding sequences justifies the proposal of three distinct TVV species, with interspecies aa sequence identities of  $\leq 32$  and  $\leq 43\%$  for CP and RdRp, respectively (Table 2). Reciprocally, the four reported strains of TVV1 have  $\geq 83\%$  aa sequence identity in pairwise comparisons of the two proteins (Table 2).

## Biological properties

Like most other members of the family *Totiviridae*, TVVs have not been shown capable of extracellular transmission and likely lack the molecular machinery for either exit or entry of their protozoan host. Rather, they are transmitted vertically during mitotic cell division, that is, during the asexual reproduction of *T. vaginalis* trophozoites by binary fission [47]. Transmission of TVVs during meiotic cell division and then by cell–cell fusion upon mating might also occur. Although mating by *T. vaginalis* has not yet been observed, evidence for its occurrence includes (1) chromosomal synapses typical of meiosis in a small subset of cultured *T. vaginalis* cells [16], (2) genetic variation among *T. vaginalis* strains sufficient to suggest meiotic recombination [30], and (3) the presence of a number of proteins uniquely required for meiosis encoded in the *T. vaginalis* genome [11, 42]. *T. vaginalis* does not form cysts, and transmission between human hosts is instead generally by transfer of trophozoites during sexual contact [47].

TVVs are considered to be well adapted to *T. vaginalis* such that they cause few if any deleterious effects in the protozoan and maintain a largely noncytopathic, persistent infection. In our own experience, TVVs can remain stably associated with the *T. vaginalis* host for 6 months or more of serial passages in vitro with little or no changes in the parasite proliferative cycle (RN Fichorova and ML Nibert, unpublished data). The literature, however, reports

occasional examples of TVV-associated cytopathology in *T. vaginalis* [8, 12, 46]. Thus, there may be particular circumstances in which the détente achieved by this virus–host pair is broken.

Despite the usual lack of overt harm to *T. vaginalis*, TVV infection has been demonstrated to alter cell-surface expression of a highly immunogenic *T. vaginalis* protein, P270 [2]. *T. vaginalis* strains infected with the virus are heterogeneous with respect to the presence of the surface immunogen and display phenotypic variation upon cultivation, whereas those without virus infection display a stable negative phenotype [56]. In one study, expression of the surface immunogen in the presence of TVV infection was increased at the mRNA level [36], and in another study, upregulation of P270 resulted in diminished ability to cause contact-dependent cytotoxicity of HeLa cells [2]. In a crude mouse model of pathogenicity, which has limited physiologic relevance to the natural mucosal infection, subcutaneously injected TVV-infected *T. vaginalis* demonstrated that one freshly isolated TVV-positive strain caused weaker abscess formation relative to seven TVV-negative and two other TVV-positive *T. vaginalis* strains [39]. Collectively, these sparse data suggest that TVV infection may impair the clearance of the *T. vaginalis* infection and allow its longer persistence in the host. Further basic research is needed to elucidate all aspects of virus–parasite–human interactions.

TVV infection has also been shown to alter cysteine proteinase expression profiles in *T. vaginalis* [46]. Cysteine proteinases are known virulence factors of *T. vaginalis*, responsible for degradation of a number of key proteins in vaginal immune defenses, such as proteins of the complement cascade [3] and immunoglobulins [45], and also facilitate cytoadherence [6]. It is thus possible that TVV modulation of *T. vaginalis* proteinase expression may in turn increase its survival in the human host. Once again, more studies are needed to correlate TVV infection with *T. vaginalis* pathogenicity and clinical outcome.

Much remains unknown about the specifics of the cell biology of TVV infections, including the subcellular localization of their replication and assembly complexes. An association of some virus-like particles with the Golgi apparatus in TVV-infected *T. vaginalis* has been described [8], but the significance of this observation remains unclear.

The extent to which related dsRNA viruses may infect other trichomonads is unclear. A recent study identified VLPs in isolates of *Tritrichomonas foetus*, a bovine pathogen, but required treatment with various cytoskeleton-affecting drugs to visualize the VLPs through electron microscopy [29]. On the other hand, a recent examination of isolates of *Trichomonas gallinae*, a significant fowl pathogen, revealed no evidence of viruses by electron microscopy or RNA purification [25] but did not include

similar drug treatments as in the *Tritrichomonas foetus* study.

## Virions and replication

TVVs have isometric virions between 30 and 40 nm in diameter, as visualized by negative staining and transmission electron microscopy [8, 10, 54]. The capsids appear single layered and thin, without obvious protrusions, and have buoyant densities between 1.33 and 1.39 g/cm<sup>3</sup> on CsCl gradients. Higher-resolution structures remain to be determined.

From genome-based predicted protein sequences, the CPs of different TVVs are similar in size, ranging from 678 to 709 aa in length (Table 1), or roughly 75 to 79 kDa in molecular mass. These values are similar to those of the CPs of several other members of the family *Totiviridae* [26]. The RdRp open reading frames (ORFs) are also similar in size among different TVVs, ranging from 756 to 767 aa in length (Table 1), or roughly 86 to 88 kDa in molecular mass. These values are similar to those of the CP and RdRp ORFs of several other members of the family *Totiviridae* [26]. Given the overlapping ORFs of the CP and RdRp in TVVs, the RdRp in each is believed to be expressed as a fusion protein with the CP (see below).

The genome of each TVV is a single dsRNA molecule, approximately 4,600 to 4,900 bp in length. It is unknown at this time whether the genome is 5'-capped at either end. As many as three dsRNA molecules of roughly similar size may be isolated from a single strain of *T. vaginalis* in association with purified virus particles [35]; however, sequencing information from these molecules suggests co-infection with distinct TVVs, rather than multisegmented viruses. In addition, smaller dsRNA molecules ~500 to ~1,700 bp in length may be isolated from some strains of *T. vaginalis* infected with TVVs, which likely represent satellite dsRNAs [10, 37, 38, 50] (see below).

RNA transcription by TVVs is expected to be like that by other members of the family *Totiviridae*: asymmetric (producing only plus-strand transcripts), end-to-end (producing full-length plus-strand transcripts that mimic the genomic plus strand), and conservative (retaining both parental strands, plus and minus, within the transcribing virus particles) [23, 27, 44, 59]. A single round of minus-strand synthesis using a full-length plus-strand RNA as template is thought to generate the duplex RNA genome in newly assembling virions.

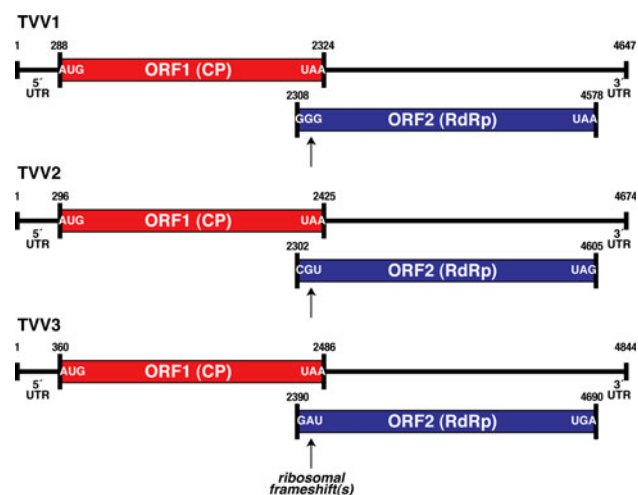
## Genomic and coding properties

Full-length nucleotide sequences of five TVV strains representing the three distinct species have been deposited in

GenBank (Table 1). Each virus has a dsRNA genome ranging in length between 4,647 and 4,844 bp.

The genome plus strand of each TVV encodes at least two long ORFs (Fig. 1). The upstream ORF (ORF1) encodes the CP, whereas the downstream ORF (ORF2) encodes the RdRp [9, 10, 39, 49, 51, 65]. The two ORFs are in different reading frames, which overlap by 16–123 nt. The RdRp of each virus is thereby thought to be expressed as a CP/RdRp fusion protein following either a +1 (TVV1) or –1 (TVV2, TVV3) ribosomal frameshift. Putative slippery heptanucleotides for promoting ribosomal frameshifting have been identified (TVV1, TVV2) [10, 39, 49, 51, 65] or can be predicted (TVV3) in the overlapping segments of the CP and RdRp ORFs (Fig. 2). The previous reports of TVV sequences have failed to identify RNA pseudoknots or other RNA structures that have been commonly associated with ribosomal frameshifting sites in other viruses, etc. [28]. Expression of a 160,000-M<sub>r</sub> CP/RdRp fusion protein (sequence-predicted molecular mass 160 and 162 kDa, respectively) has been shown by western blotting for both TVV1 and TVV2 [10, 41].

In the single reported TVV2 genome sequence, third and fourth long ORFs (encoding >200 aa each) have been noted in a central portion of the remaining reading frame [10]. The putative proteins encoded by these ORFs are very basic (pI = 11) and rich in Ser and Thr residues, with no



**Fig. 1** Coding diagrams for TVV1, TVV2, and TVV3. Open reading frames (ORFs) 1 [capsid protein (CP, red in online version of figure)] and 2 [RNA-dependent RNA polymerase (RdRp), blue in online version of figure] are diagrammed for each virus. The first and last codons for each ORF are indicated; in the case of ORF1, the first codon is considered to be that of the proposed AUG start codon. 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 as well as in Fig. 2 are for the prototypical strains TVV1-1 [51], TVV2-1 [10], and TVV3-1 [9]

significant similarity to protein sequences in GenBank, and there is no gel evidence for their expression [10]. Among the reported TVV1 and TVV3 sequences, additional ORFs of similarly long length are not present in the remaining reading frame, although one or two ORFs of >100 aa each can be found in each strain. The significance of any of these additional ORFs in TVV1, TVV2, and TVV3 remains open to question. The genomic minus strands of the TVVs similarly contain only short ORFs in all three reading frames, consistent with their expected lack of availability for translation due to sequestration in viral capsids throughout the replication cycle.

The 5' and 3' untranslated regions (UTRs) of TVVs range from 285 to 359 nt and 69 to 154 nt, respectively, and demonstrate little sequence identity between species. Secondary structure predictions of the 5' and 3' ends of the plus strands of TVV1 have suggested the presence of a large 3' stem-loop structure, but no analogous 5' stem loop [49, 51]. Similar analyses for TVV2 have revealed stem-loop structures at both ends of the plus strand [10]. Such RNA secondary structures may act as signals for RNA replication and/or packaging.

Some isolates of *T. vaginalis* infected with TVVs are also associated with smaller dsRNA molecules, ranging in size from ~500 to ~1,700 bp [10, 37, 38, 50]. Experiments suggest that these dsRNAs can be packed into TVV capsids independently from genomic dsRNA [50], can serve as specific templates for the viral RdRp [38], do not encode their own RdRp [37, 50], and have little sequence homology with the TVV genome [37], consistent with satellite dsRNAs. Sequencing of several satellites has revealed them to have only short ORFs in all three reading frames of both strands, suggesting a lack of protein-coding ability [37, 50]. While the biological significance of the satellite dsRNAs associated with TVVs is unclear, dsRNA satellites are commonly associated with fungal viruses in the family *Totiviridae* [26, 61, 62].

## Phylogenetic relationships

The full-length protein-coding sequences for six TVV strains that have been deposited into GenBank (Table 2) allow phylogenetic comparisons among these viruses as well as with other members of the family *Totiviridae*. From such analyses, it is clear that TVVs constitute a monophyletic cluster distinguishable from all other viruses in the family (Fig. 3), including from members of the genus *Giardiavirus*, to which TVVs had been previously assigned on a tentative basis before the recently approved proposal to segregate them into their own new genus, *Trichomonasvirus*. Moreover, the TVVs appear to be more closely related to protozoan viruses in the genus *Leishmanivirus*

**Fig. 2** Regions of ribosomal frameshifting in TVV1, TVV2, and TVV3. Virus is labeled at the top of each sequence block, along with the position numbers of the displayed nucleotides from the genomic plus-strand sequence. The nature of the putative frameshifting event in each is also shown at *top*. The putative slippery heptanucleotide involved in signalling the ribosomal frameshift event in each virus is underlined in its genomic sequence. For each virus, beneath each of the three displayed lines of genomic sequence, the amino acid translation for all three frames is shown. Any stop codons in each frame are shown as *filled circles*. The genomic sequences were chosen for display such that the long ORF for CP (*red* in online version of figure) appears in the middle frame. In TVV1, the long ORF for RdRp (*blue* in online version of figure) therefore appears in the bottom frame (+1 frameshift), whereas in TVV2 and TVV3, it appears in the top frame (−1 frameshift)

**TVV1, nt 2222–2401 (+1)**

**agg**aatgaccatctgcagtagacagcgtagacacatcaacggccatcgacatcatcacaacagtt  
 R N D H L Q Y S V H I N G H R H H H N S  
**G M T I C S T A Y T S T A I D I I T T**  
 E • P S A V Q R T H Q R P S T S S Q Q  
**ctttgg**tccttacgctccgtaggtagcctttttgagtaaagctgtacgttggtggcc  
 L W S L T P P R R I P F • V K L Y V V A  
**F G P L R L R V G S L F E** • S C T L W  
 L V P Y A S A • **D P F L S K A V R C G**  
**ccg**taataccatccgctcaagcatcacttcaatatcaattaccttagtattctcaaacaca  
 P • Y H P S S I T S I S I T L V F S N T  
 R N T I R Q A S L Q Y Q L P • Y S Q T Q  
**V I P S V K H H F N I N Y L S I L K H**

**TVV2, nt 2281–2460 (−1)**

**tca**aggactcgagtttgctgacggttccaacggacggtcacgcggaagatccttggtggt  
 S R T R V C • R F Q R T V T R K D P C G  
**Q G L E F A D V S N G R F S R G K I L V**  
 K D S S L L T F P T D G H A E R S L W  
**cg**taactcaccctcaagacatgcgctgcctaccagggccccagcttcgcgccaaagcc  
 R Q L T P Q D M R C L P G P Q L R A K A  
**V N S P L K T C A A Y Q G P S F A P K**  
 S T H P S R H A L P T R A P A S R Q S  
**ggg**cagcgccatgtggaacgaataaaccaatgcgggggcagcgtcatctatccgcgctt  
 G Q R H V E R I N Q C G G S V I Y P R L  
**G S A M W N E** • T N A G A A S S I R A  
 A A P C G T N K P M R G Q R H L S A L

**TVV3, nt 2342–2521 (−1)**

**cg**attcagaaggcatagaattctacgacaaatccaacggctctctataagggccggtgtaa  
 R F R R H R I L R Q I Q R S L • G P C Q  
**D S E G I E F Y D K S N G L Y K G R V**  
 I Q K A • N S T T N P T V S I R A V S  
**tg**tccctgtttctggatttgccatcccagggtcgcgcgatcagggccctcgcttgagggt  
 C P C F W I C H P R S R V S G P S L A G  
**V L V S G F A I P G R A Y Q G P R L Q**  
 S L F L D L P S Q V A R I R A L A C R  
**ag**caacaatggccgcccagatctaagcgacgtcccggcgacaggaagtctgtccaacctc  
 S N N G R P D L S D V P A T G S L S N L  
**A T M A A Q I** • A T S R R Q E V C P T  
 Q Q W P P R S K R R P G D R K S V Q P

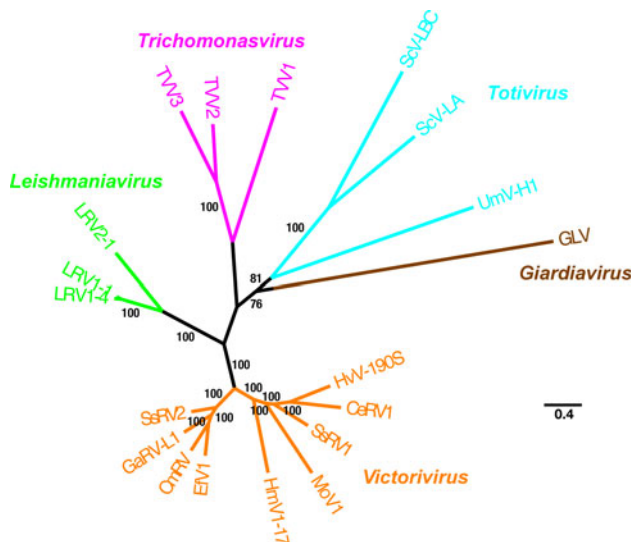
and to fungal viruses in the genus *Victorivirus* than to other protozoan and fungal viruses in the respective genera *Giardiavirus* and *Totivirus*.

Among TVVs, TVV2 and TVV3 strains appear more closely related to each other than either is to TVV1 strains (Fig. 3; also see Table 2). This parallels a basic difference in coding strategy in that all four reported strains of TVV1 express their RdRps following a +1 ribosomal frameshift, whereas both TVV2 and TVV3 strains do so following a −1 frameshift. The TVV1 strains also seem to lack a predicted stem-loop structure at their 5′ termini that the TVV2 and TVV3 strains contain. The four TVV1 strains show limited divergence (see Table 2), and sequences of additional

TVV2 and TVV3 strains are needed to determine if they each also constitute such a well-delimited group.

### Clinical relevance

*T. vaginalis* is the most common nonviral STI in the world and is the causal agent of trichomoniasis [63]. The severity of morbidity varies broadly, from asymptomatic to severely symptomatic vaginitis, and complications affect nearly every aspect of reproductive health including risks of pelvic inflammatory disease and tubal infertility, premature membrane rupture, preterm delivery, low birth weight,



**Fig. 3** 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 [5]; 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/> [14]. The tree was additionally refined for presentation using FigTree v1.3.1 obtained from <http://www.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 (prototypical 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); HmV190S, *Helminthosporium victoriae* virus 190S (HVV41345); 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 and labeled as follows: genus *Trichomonasvirus* (magenta in online version), genus *Leishmaniavirus* (green in online version), genus *Victoriavirus* (orange in online version), genus *Totivirus* (cyan in online version), and genus *Giardiavirus* (brown in online version)

post-hysterectomy cuff cellulitis, and viral STIs including human immunodeficiency virus and human papillomavirus [17]. Given the widespread prevalence of TVV infections (see below) and the demonstration that TVV infections can modulate host translation and expression of immunogenic proteins [2], it is possible that underlying TVV infections may modulate the pathogenesis of *T. vaginalis* infections

and possibly underlie differences in symptomatology, drug resistance, parasite load, transmissibility, etc. There is precedence in the literature for endobiotic viruses modulating the pathogenicity or behavior of their parasitic hosts; for example, a virus infecting *Cryptosporidium parvum* has been shown to increase the fecundity of its protozoan host (levels of oocysts shed in feces) during *C. parvum* infection of dairy calves [33].

While a few reports have suggested that *T. vaginalis* infection with TVV may result in attenuated cytopathogenicity and acute inflammatory response [39], others suggest the possibility of hypervirulence through upregulation of a major surface immunogen [2] and upregulation of host proteinase expression [46]. A study of adolescents with *T. vaginalis* infection and no other STIs showed that the presence of vaginal discharge, dysuria, dyspareunia, and cervical erythema, but not pruritus, vulvar or vaginal erythema, were significantly associated with TVV infection diagnosed by gel electrophoresis of nucleic acids, suggesting its clinical relevance [22]. More studies are needed to correlate TVV-infected *T. vaginalis* with symptomatology or pathogenicity in infected humans.

The prevalence of TVV in different *T. vaginalis* clinical isolates varies. Rates as high as 82 and 75% were found in clinical isolates from South Africa [58] and Baltimore [60], respectively, whereas rates closer to 50% were noted in isolates from Cuba (55%) [21], the USA (50%) [48], and various geographic locations (44%) [52]. In each of the listed studies, TVVs were detected by gel electrophoresis of nucleic acids. Opportunities for improved diagnostics of TVV infection include immunodetection methods with TVV-specific antibodies [4] and nucleic acid microarrays to detect TVV RNAs [7].

**Acknowledgments** Work on TVV in the Fichorova and Nibert Labs has been supported by a Harvard Catalyst Pilot Grant (RNF and MLN) funded through NIH 5UL1 RR025758-02. In addition, work on *T. vaginalis* in the Fichorova Lab has been supported by grants NICHD 2R21HD054451 (RNF) and NIAID 5R01AI079085-01A2 (RNF).

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