Template for Taxonomic Proposal to the ICTV Executive Committee
To create a new Genus in an existing Family

Code† **2007.066F** To create a new genus in the family* ***Totiviridae***

Code† **2007.067F** To name the new genus* ***Victorivirus***

Code† **2007.068F** To designate the species ***Helminthosporium victoriae virus 190S*** As the type species of the new genus*

Code† **2007.069F** To designate the following as species of the new genus*:

Chalara elegans RNA Virus 1
Coniothyrium minitans RNA virus
Epichloe festucae virus 1
***Gremmeniella abietina RNA virus* L1**
***Helicobasidium mompa totivirus* 1-17**
Helminthosporium victoriae virus 190S
***Magnaporthe oryzae virus* 1**
***Sphaeropsis sapinea RNA virus* 1**
***Sphaeropsis sapinea RNA virus* 2**

Code† To designate the following viruses as tentative species in the new genus:

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New Taxonomic Order

Order **None**
Family ***Totiviridae***
Genus ***Victorivirus***
Type Species ***Helminthosporium victoriae virus 190S***
Species in the Genus **See list above**

Argumentation to create a new genus:

The family *Totiviridae* encompasses a broad group of viruses characterized by isometric particles, ~40 nm in diameter, that contain a nonsegmented dsRNA genome coding for a capsid protein (CP) and an RNA-dependent RNA polymerase (RdRp). The accepted species constituting this family to date persistently infect either protozoa or fungi. At present, three genera are recognized: *Totivirus*, *Giardiavirus*, and *Leishmaniavirus*. Viruses currently placed in the genus *Totivirus* infect yeast, smut fungi, or filamentous fungi, whereas those in the latter two genera infect parasitic protozoa.

Three distinct RdRp expression strategies have been reported for species in the family *Totiviridae*: (i) those that express RdRp as a fusion protein (CP-RdRp) by ribosomal frameshifting, such as Saccharomyces cerevisiae virus L-A and the viruses that infect parasitic protozoa; (ii) those that express RdRp as a fusion with CP without the use of ribosomal frameshifting, such as the smut fungus virus Ustilago maydis virus H1 (the RdRp is putatively released from the fusion protein by a proteolytic mechanism); and of prime relevance to this proposal, (iii) those that synthesize RdRp as a separate nonfused protein by an internal initiation mechanism (e.g., a coupled termination–reinitiation mechanism), as shown for Helminthosporium victoriae virus 190S (HvV-190S) and proposed for all of the other viruses that infect filamentous fungi. Phylogenetic analysis of the CP or RdRp sequences reflects these differences, and separate phylogenetic clusters can be identified (Figs. 1 and 2). In particular for this proposal, HvV-190S and all of the other viruses that infect filamentous fungi are phylogenetically closer to each other than to the viruses infecting yeast and smut fungi (members of the genus *Totivirus*). The fact that independent alignments of CP and RdRp sequences give similar phylogenetic relationships (Figs. 1 and 2) supports the conclusion that the viruses infecting filamentous fungi should reside in a genus of their own, and should not be placed in the genus *Totivirus*. In addition, the viruses that infect filamentous fungi uniquely share a Pro/Ala/Gly-rich region near the C-terminus of CP; the C-terminal sequence of HvV-190S CP at positions 715 to 772, for example, is:
VPLPPAPGAAPPPPPGPPNGPPAGPPPSDDGSSNPAAPVPTAIHAPPAAAQADRAEGQ

The complete nucleotide sequences of HvV-190S and eight other putative members of the family *Totiviridae* infecting filamentous fungi have been reported and the sequences deposited in GenBank, with HvV-190S being the biochemically and molecularly best characterized of these (Table 1). Sequence analysis has shown that the genomic plus strand of HvV-190S, like that of the other viruses, comprises two large overlapping open reading frames (ORFs) (Fig. 3). The 5´-proximal ORF (ORF 1) codes for CP, whereas the 3´-proximal ORF (ORF 2), which in HvV-190S is in the -1 frame relative to ORF 1, codes for RdRp, including the eight consensus RdRp motifs of dsRNA viruses. The 5´ end of the HvV-190S genomic plus strand is uncapped and highly structured and contains a relatively long (289 nucleotides) 5´ nontranslated region with two minicistrons. The structural features of the 5´ nontranslated region suggest that the CP-encoding ORF 1 (with its AUG present in suboptimal context according to the Kozak criteria) is translated via a cap-independent mechanism. The UGA codon at position 2606–2608 of the HvV-190S genomic plus strand was verified by site-directed mutagenesis to be the authentic stop codon for ORF 1. The RdRp-encoding downstream ORF 2 of HvV-190S is in a -1 frame with respect to ORF 1, and its predicted start codon (nucleotide positions 2605–2607) overlaps the stop codon of ORF 1 (nucleotide positions 2606–2608) in the tetranucleotide sequence 2605-AUGA-2608 (Fig. 3). HvV-190S RdRp is detectable as a separate, virion-associated component, consistent with its independent translation from ORF 2. The tetranucleotide AUGA overlap region, or very similar features, is characteristic of the overlap region of all of the putative members of the family *Totiviridae* infecting filamentous fungi.

Origin of the proposed genus name

Victori: from the specific epithet of *Helminthosporium victoriae,* the host of the proposed type species.

Argumentation to choose the type species in the genus

Helminthosporium victoriae virus-190S (also referred to in the literature as Helminthosporium victoriae 190S virus) is the member of the proposed genus that has been characterized most comprehensively to date.

Species demarcation criteria in the genus

To include a species in the proposed genus, we required that a putatively complete genome sequence has been deposited and released in GenBank. The amino acid identity in pair-wise comparisons of either the CP or RdRp gene product of the nine species is no more than 60%. In addition, each of these species was isolated from a different filamentous fungus, with the exception of one pair. This pair, SsRV1 and SsRV2, is stably co-maintained in the same fungal host and adheres to the 60% maximum identity criterion. Abbreviations for species names are repeated from the original publications.

Additional considerations in assigning membership in the new genus

-The full-length CP and RdRp gene products of isolate Gremmeniella abietina RNA virus L2 (Tuomivirta & Hantula, 2005; GenBank AY615210) show more than 96% amino acid identity with those of isolate GaRV-L1 (Tuomivirta & Hantula, 2003; GenBank AF337175). We therefore consider these two isolates to be strains of the same species, designated in our list as GaRV-L.

-The published genome sequence of CeRV1 has a stop codon dividing the RdRp open reading frame into two halves, which remain in frame and are separated only by the stop codon. This stop codon seems almost certain to reflect a sequencing error.

-More limited sequence information in GenBank has led us not to list the following isolates as species or tentative species, even though the available evidence suggests that they might qualify as new species in this genus once their sequence analyses and reportings are complete: Chalara elegans RNA Virus 2 (Park et al., 2005; GenBank AY556461) and Fusarium graminearum dsRNA mycovirus 2 (GenBank AY372188).

List of Species in the created genus – (GenBank accession number)

Chalara elegans RNA Virus 1 – (AY561500)
Coniothyrium minitans RNA virus – (AF527633)
Epichloe festucae virus 1 – (AM261427)
Gremmeniella abietina RNA virus L1 – (AF337175)
Helicobasidium mompa totivirus 1-17 – (AB085814)
Helminthosporium victoriae virus 190S – (HVU41345)
Magnaporthe oryzae virus 1 – (AB176964)
Sphaeropsis sapinea RNA virus 1 – (AF038665)
Sphaeropsis sapinea RNA virus 2 – (AF039080)

List of Tentative Species in the created genus – (GenBank accession number)

References

Caston, J. R., Luque, D., B. l. Trus, , Rivas, G., Alfonso, C., Roca, R., Gonzalez, J. M, Carrascosa, J. L., Annamalai, P. and Ghabrial, S. A. (2006). Three-dimensional structure and stoichiometry of *Helminthosporium victoriae190S* totivirus. Virology 347, 323–332.

Cheng, J, Jiang, D., Fu, Y., Li , G., Peng, Y., and Ghabrial, S. A. (2003). Molecular characterization of a dsRNA totivirus infecting the sclerotial parasite *Coniothyrium minitans*. Virus Res. 93, 41–50.

Huang, S., Ghabrial, S.A. (1996). Organization and expression of the double-stranded RNA genome of *Helminthosporium victoriae* 190Svirus, a totivirus infecting a plant pathogenic filamentous fungus. Proc. Natl. Sci. U.S.A. 93, 12541–12546.

Icho, T., Wickner, R.B., 1989. The double-stranded RNA genome of yeast virus L-A encodes its own putative RNA polymerase by fusing two open reading frames. J. Biol. Chem. 264, 6716–6723.

Nomura, K., Osaki, H., Iwanami, T., Matsumoto, N., Ohtsu, Y., 2003. Cloning and characterization of a totivirus double-stranded RNA from the plant pathogenic fungus, *Helibasidium mompa* Tanaka. Virus Genes 23, 219–226.

Park,Y., James,D. and Punja,Z.K. (2005). Co-infection by two distinct totivirus-like double-stranded RNA elements in Chalara elegans (Thielaviopsis basicola). Virus Res. 109, 71–85.

Preisig, O., Wingfield, B.D., Wingfield, M.J., 1998. Coinfection of a fungal pathogen by two distinct double-stranded RNA viruses. Virology 252, 399–406.

Romo, M., Leuchtmann, A. and Zabalgogeazcoa, I. (2007). A totivirus infecting the mutualistic fungal endophyte *Epichloë festucae*. Virus Res 124, 38-43.)

Soldevila, A., Ghabrial, S.A., 2000. Expression of the totivirus Helminthosporium victoriae190S Virus RNA-dependent RNA polymerase from its downstream open reading frame in dicistronic constructs. J. Virol. 74, 997–1003.

Tuomivirta, T. T. and Hantula, J. (2003). Two unrelated double-stranded RNA molecule patterns in *Gremmeniella abietina* type A code for putative viruses of the families *Totiviridae* and *Partitiviridae*. Arch. Virol. 148, 2293–2305.

Tuomivirta, T. T. and Hantula, J. (2005). Three unrelated viruses occur in a single isolate of *Gremmeniella abietina* var. *abietina* type A.Virus Res.110, 31–39.

Yokoi, T.,Yamashita, S. and Hibi, T. (2007). The Nucleotide Sequence and Genome Organization of *Magnaporthe oryzae* Virus 1. Archiv. Virol. (in press).

Figure Legends.

Fig. 1. Neighbor-joining phylogenetic tree constructed based on the RdRp conserved motifs and flanking sequences. The RdRp sequences were derived from aligned deduced amino acid sequences of members of the family *Totiviridae* using the program CLUSTAL X. Motifs 1 through 8 and the sequences between the motifs, as previously designated by Ghabrial (Virus Genes 16, 119-131, 1998) were used. See Table 1 for virus name abbreviations and GenBank accession numbers. Other members or tentative members of the family *Totiviridae* included in the tree are: LRV1, Leishmania RNA virus 1-1 [M92355]; LRV2, Leishmania RNA virus 2-1 [U32108], LRV4, Leishmania RNA virus 1-4 [U01899]; TVV1, Trichomonas vaginalis virus 1 [U08999]; TVV2, Trichomonas vaginalis virus II [AF127178]; TVV3, Trichomonas vaginalis virus 3 [AF325840]; ScV-L-A, Saccharomyces cerevisiae virus L-A [J04692]; ScV-L-BC, Saccharomyces cerevisiae virus L-BC [NC_001641]; UmV-H1, Ustilago maydis virus H1 [NC_003823]; GLV, Giardia lamblia virus [L13218]; and EbRV1, Eimeria brunetti RNA virus 1 [AF356189]. The phylogenetic tree was generated using the program PAUP*. Bootstrap numbers out of 1,000 replicates are indicated at the nodes. The tree was rooted with the RdRp of the penaeid shrimp infectious myonecrosis virus (IMNV; NC_002701), an unclassified virus tentatively assigned to the family *Totiviridae*, which was included as an outgroup

Fig. 2. Neighbor-joining phylogenetic tree constructed based on the complete amino acid sequences of the capsid proteins of viruses in the family *Totiviridae*. The CP multiple sequence alignment was performed with the program CLUSTAL X and the phylogenetic tree was generated using the program PAUP*. See Table 1 for virus name abbreviations and GenBank accession numbers. Other members or tentative members of the family *Totiviridae* included in the tree are: LRV1, Leishmania RNA virus 1-1 [M92355]; LRV2, Leishmania RNA virus 2-1 [U32108], LRV4, Leishmania RNA virus 1-4 [U01899]; TVV1, Trichomonas vaginalis virus 1 [U08999]; TVV2, Trichomonas vaginalis virus II [AF127178]; TVV3,

Trichomonas vaginalis virus 3 [AF325840]; ScV-L-A, Saccharomyces cerevisiae virus L-A [J04692]; ScV-L-BC, Saccharomyces cerevisiae virus L-BC [NC_001641]; UmV-H1, Ustilago maydis virus H1 [NC_003823];GLV, Giardia lamblia virus [L13218]; and EbRV1, Eimeria brunetti RNA virus 1 [AF356189]. The phylogenetic tree was generated using the program PAUP*. Bootstrap numbers out of 1,000 replicates are indicated at the nodes. The tree was rooted with the RdRp of the penaeid shrimp infectious myonecrosis virus (IMNV; NC_002701), an unclassified virus tentatively assigned to the family *Totiviridae*, which was included as an outgroup

Fig. 3. Genome organization of Helminthosporium victoriae virus 190S, the type species of the newly proposed genus *Victorivirus*. Two large overlapping open reading frames (ORFs) with the 5' ORF encoding a capsid protein (CP) and the 3' ORF encoding an RNA-dependent RNA polymerase (RdRp). Note that the termination codon of the CP ORF overlaps the initiation codon of the RdRp ORF in the tetranucleotide sequence AUGA.

Template for Taxonomic Proposal to the ICTV Executive Committee
Creating Species in an existing genus

Code† **2007.069F** To designate the following as species in the genus:

Victorivirus

belonging to the family° : ***Totiviridae***

Chalara elegans RNA Virus 1
Coniothyrium minitans RNA virus
Epichloe festucae virus 1
Gremmeniella abietina RNA virus L1
Helicobasidium mompa totivirus 1-17
Helminthosporium victoriae virus 190S
Magnaporthe oryzae virus 1
Sphaeropsis sapinea RNA virus 1
Sphaeropsis sapinea RNA virus 2

† Assigned by ICTV officers
° leave blank if inappropriate or in the case of an unassigned genus

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New Taxonomic Order

Order	
Family	***Totiviridae***
Genus	***Victorivirus***
Type Species	***Helminthosporium victoriaevirus 190S***
Species in the Genus	**(See list above)**
Tentative Species in the Genus	
Unassigned Species in the family	

ICTV-EC comments and response of the SG

Species demarcation criteria in the genus

Each of the nine species listed under the proposed new genus (genus *Victorivirus*) was isolated from a different filamentous fungus, with the exception of SsRV1 and SsRV2, which are stably co-maintained in the same fungal host. The amino acid identity in pair-wise comparisons of either the CP or RdRp gene product of the nine species is no more than 60%. Abbreviations for species names are derived from the original publications.

Argumentation to justify the designation of new species in the genus

Members of the family *Totiviridae* that infect fungi are currently classified in the genus *Totivirus*. At present, there are four species recognized in the genus *Totivirus*, these are:

Helminthosporium victoriae virus 190S [U41345]
Saccharomyces cerevisiae virus L-A [J04692]
Saccharomyces cerevisiae virus L-BC [U01060]
Ustilago maydis virus H1 [NC_003823]

Of these, Helminthosporium victoriae virus190S (HvV-190S) infects a filamentous fungus whereas the other three infect yeast and smut fungi. Eight putative totiviruses with similar properties to HvV-190S have been reported from various species of filamentous fungi (see references 2, 4-7, 9-11), but have yet to be recognized by ICTV as species in the family *Totiviridae*. The fact that the nine viruses that infect filamentous fungi are clearly distinct from the three totiviruses that infect yeast and smut, justifies the creation of a new genus (genus *Victorivirus*) to accommodate them (as described under the "Argumentation to create a new genus" for the proposal (code "2007.066F.04") to create a new genus in an existing family.

The complete nucleotide sequences of HvV-190S and the eight putative members of the proposed new genus (genus *Victorivirus)* have been reported and the sequences deposited in GenBank (Table 1). HvV-190S, being the first and best-characterized member at the biochemical and molecular levels, is designated as the type species of the new genus. Sequence analysis has shown that the genomic plus strand of HvV-190S, as well as that of the other eight viruses, comprises two large overlapping open reading frames (ORFs) (Fig. 3). The 5´-proximal ORF (ORF 1) codes for CP, whereas the 3´-proximal ORF (ORF 2), which in HvV-190S is in the -1 frame relative to ORF 1, codes for RdRp, including the eight consensus RdRp motifs of dsRNA viruses. The UGA codon at position 2606–2608 of the HvV-190S genomic plus strand was verified by site-directed mutagenesis to be the authentic stop codon for ORF 1. The RdRp-encoding downstream ORF 2 of HvV-190S is in a -1 frame with respect to ORF 1, and its predicted start codon (nucleotide positions 2605–2607) overlaps the stop codon of ORF 1 (nucleotide positions 2606–2608) in the tetranucleotide sequence 2605-AUGA-2608 (Fig. 3). The tetranucleotide AUGA overlap region, or very similar features, is characteristic of the overlap region of all of the eight putative members of the genus *Victorivirus* that infect filamentous fungi.

List of created Species in the genus

Chalara elegans RNA Virus 1
Coniothyrium minitans RNA virus
Epichloe festucae virus 1
Gremmeniella abietina RNA virus L1
Helicobasidium mompa totivirus 1-17
Helminthosporium victoriae virus 190S
Magnaporthe oryzae virus 1
Sphaeropsis sapinea RNA virus 1
Sphaeropsis sapinea RNA virus 2

References

1. Caston, J. R., Luque, D., B. l. Trus, , Rivas, G., Alfonso, C., Roca, R., Gonzalez, J. M, Carrascosa, J. L., Annamalai, P. and Ghabrial, S. A. (2006). Three-dimensional structure and stoichiometry of *Helminthosporium victoriae190S* totivirus. Virology 347, 323–332.
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3. Huang, S., Ghabrial, S.A. (1996). Organization and expression of the double-stranded RNA genome of *Helminthosporium victoriae* 190Svirus, a totivirus infecting a plant pathogenic filamentous fungus. Proc. Natl. Sci. U.S.A. 93, 12541–12546.
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11. Yokoi, T.,Yamashita, S. and Hibi, T. (2007). The Nucleotide Sequence and Genome Organization of *Magnaporthe oryzae* Virus 1. Archiv. Virol. (in press).

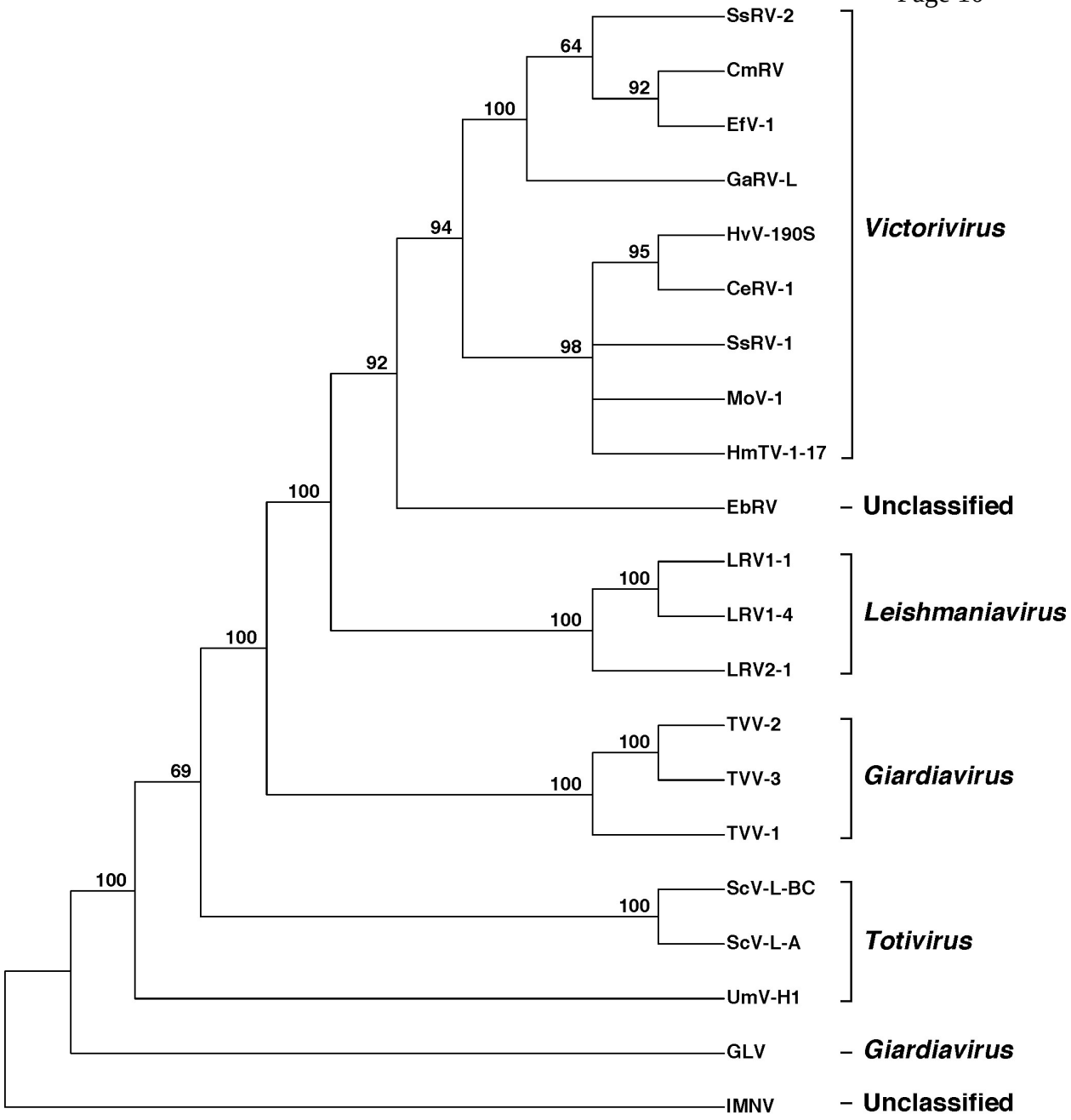
Annexes:

Table 1. Members of the newly proposed genus *Victorivirus*

Virus name	GenBank accession no.	Genome length (nt)	CP coding region (nt)	RdRp coding region (nt)	CP length (aa)	RdRp length (aa)
CeRV1[a]	AY561500	5310	329-2641	2634-5246[a]	770	871
CmRV	AF527633	4975[b]	62[b]-2389	2386-4875	775	829
EfV-1	AM261427	5109	271-2571	2568-5051	766	827
GaRV-L	AF337175	5133	276-2606	2603-5080	776	825
HmTV-1-17	AB085814	5207	200-2566	2563-5100	788	845
HvV-190S	HVU41345	5179	290-2608	2605-5112	772	835
MoV-1	AB176964	5359	575-2815	2818-5316	746	832
SsRV-1	AF038665	5163	251-2581	2574-5090	776	838
SsRV-2	AF039080	5202	296-2665	2658-5135	789	825

[a] Sequence has a stop codon dividing the RdRp ORF into two halves, probably due to a sequencing error.

[b] Possibly has a deletion in the 5′ UTR, suspected due to the smaller size and the sequencing strategy.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CmRV
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MoV-1
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EbRV
Unclassified
LRV1-1
LRV1-4
LRV2-1
Leishmaniavirus
TVV-2
TVV-3
TVV-1
Giardiavirus
ScV-L-BC
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UmV-H1
Totivirus
GLV
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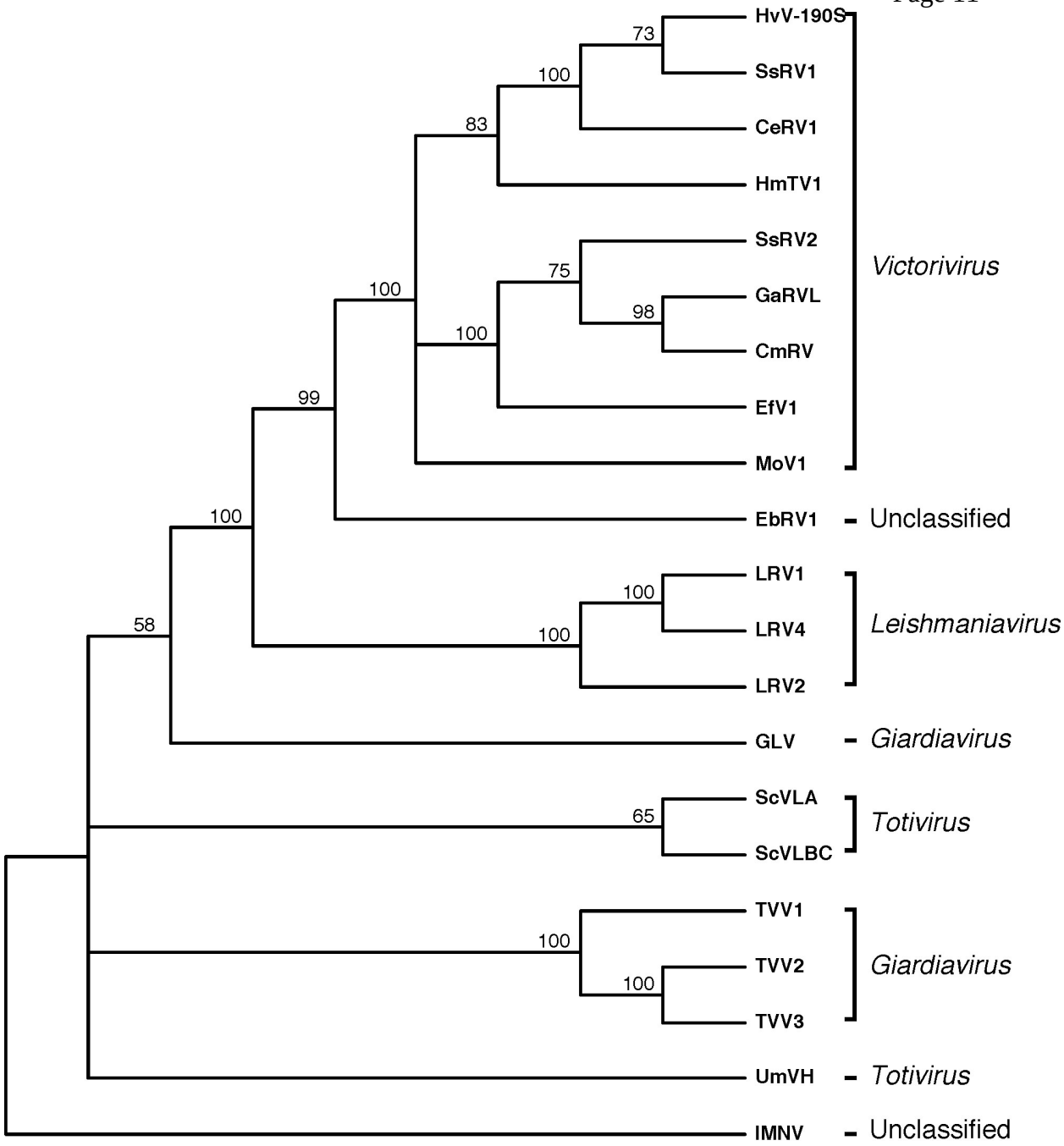
Fig. 1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LRV4
LRV2
GLV
ScVLA
ScVLBC
TVV1
TVV2
TVV3
UmVH
IMNV
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83
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Victorivirus
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Totivirus
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Totivirus
Unclassified

Fig. 2

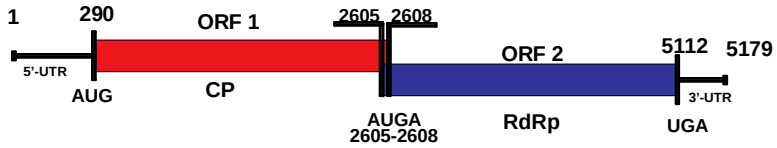

Fig. 3. Genome organization of Helminthosporium victoriae virus 190S, the type species of the newly proposed genus *Victorivirus*. Two large overlapping open reading frames (ORFs) with the 5' ORF encoding a capsid protein (CP) and the 3' ORF encoding an RNA-dependent RNA polymerase (RdRp). Note that the termination codon of the CP ORF overlaps the initiation codon of the RdRp ORF in the tetranucleotide sequence AUGA.