



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**

<b>Code assigned:</b>	<b>2014.001a-dF</b>	(to be completed by ICTV officers)		
<b>Short title:</b> A new mycovirus species, <i>Botrytis porri botybirnavirus 1</i> , in a new genus, <i>Botybirnavirus</i> (family unassigned) (e.g. 6 new species in the genus <i>Zetavirus</i> )				
<b>Modules attached</b> (modules 1 and 9 are required)	<b>1</b>	<b>2</b>	<b>3</b>	<b>9</b>

**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 <a href="http://www.ictvonline.org/subcommittees.asp">http://www.ictvonline.org/subcommittees.asp</a> . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)	Chairs of all SGs in the Fungal virus SC have seen the proposal prior to submission in 2014.
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**ICTV-EC or Study Group comments and response of the proposer:**

**ICTV-EC comments**

2014.001a-gF.N.v1.Botybirnaviridae: Decision: Ud. Proposers should be encouraged to consult with the SG(s) responsible for the viruses used in the phylogenetic tree (Fig. 2) as this does not appear to be convincing. Consider using a family name that differs from that of the genus.

**Response of proposers**

Following EC46, Dr. Ghabrial suggested to us to use pair-wise sequence comparison (PASC) analysis of full-length dsRNA1 of BpBV1 and closest related viruses (as shown in Fig. 2 based on RdRp phylogeny). Despite its limitations, results of PASC analysis (Table 1, Fig. 3) support the assignment of BpBV1 to a distinct species and genus; thus justifying the creation of a new family. In summary, classification of BpBV1 based on biological characteristics (e.g., genome size and organization, etc.) is consistent with results of genomic sequence and phylogenetic analysis. Family and genus names (Botybirnaviridae and Botybirnavirus) share the same root word similar to many precedences (e.g., *Totivirus* and *Totiviridae*). The names Botybirnaviridae and Botybirnavirus have been in the literature for 3 years and very recently, another Botybirnavirus was reported (Liu et al. 2015), therefore changing names now would create confusion.

**ICTV-EC comments**

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Subcommittee Chair approves this revised proposal.

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Date first submitted to ICTV: 11-1-2013  
Date of this revision (if different to above): 04-20-2015

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### ICTV-EC comments

At the summer 2015 EC meeting, the decision was that this proposal could move forward only if the family portion of the proposal were removed, i.e., leave genus *Botybirnavirus* unassigned to a family at present. This decision was reached because the designation of new family *Botybirnaviridae* still seems poorly supported by the presented evidence.

### Response of proposers

We have revised as requested.

### ICTV-EC comments

Subcommittee Chair approves this revised proposal.

Date of this revision 03-23-2016

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## MODULE 2: NEW SPECIES

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be "unassigned" within a subfamily or family. Wherever possible, provide sequence accession number(s) for one isolate of each new species proposed.

Code	<b>2014.001aF</b>	(assigned by ICTV officers)
<b>To create a new species within:</b>		
Genus:	<b><i>Botybirnavirus</i> (new)</b>	<b>Fill in all that apply.</b> <ul style="list-style-type: none"><li>• If the higher taxon has yet to be created (in a later module, below) write "<b>(new)</b>" after its proposed name.</li><li>• If no genus is specified, enter "<b>unassigned</b>" in the genus box.</li></ul>
Subfamily:	<b>Unassigned</b>	
Family:	<b>Unassigned</b>	
Order:	<b>Unassigned</b>	
<b>Name of new species:</b>	<b>Representative isolate:</b>	<b>GenBank sequence accession number(s)</b>
<i>Botrytis porri botybirnavirus 1</i>	GarlicBc-72	JF716350, JF716351

### 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

Botrytis porri botybirnavirus 1-GarlicBc-72 (BpBV1- GarlicBc-72) was obtained from hypovirulent strain GarlicBc-72 of *Botrytis porri*, the causal agent of garlic leaf blight. BpBV1-GarlicBc-72 was originally named as Botrytis porri RNA virus 1 (BpRV1) (Wu et al., 2012). The BpBV1- GarlicBc-72 genome comprises two dsRNAs, dsRNA-1 (6,215 bp) and dsRNA-2 (5,879 bp), and two open reading frames (ORFs), ORF I (dsRNA-1) and ORF II (dsRNA-2), were detected (Fig. 1). The protein encoded by the 3'-proximal coding region of ORF I shows sequence identities of 19 to 23% with RNA-dependent RNA polymerases (RdRp) encoded by viruses in the families *Totiviridae*, *Chrysoviridae*, and *Megabirnaviridae*. However, the proteins encoded by the 5'-proximal coding region of ORF I and by the entire ORF II lack sequence similarities to any reported virus proteins. Phylogenetic analysis showed that BpBV1-GarlicBc-72 belongs to a separate clade distinct from those of other known RNA mycoviruses (Fig. 2). Purified virions of ~35 nm in diameter encompass dsRNA-1 and dsRNA-2, and three structural proteins (SPs) of 70, 80, and 85 kDa, respectively. Peptide mass fingerprinting analysis revealed that the 80- and 85-kDa SPs are encoded by ORF I, while the 70-kDa SP is encoded by ORF II (Fig. 1). All these results suggest that BpBV1- GarlicBc-72 is a novel bipartite dsRNA virus, since it has several different features compared with all reported mycoviruses.

creating a new genus

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

Code	<b>2014.001bF</b>	(assigned by ICTV officers)
<b>To create a new genus within:</b>		
Subfamily:	<b>Unassigned</b>	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
Family:	<b>Unassigned</b>	
Order:	<b>Unassigned</b>	

## MODULE 2: NEW GENUS

naming a new genus

Code	<b>2014.001cF</b>	(assigned by ICTV officers)
<b>To name the new genus: <i>Botybirnavirus</i></b>		

Assigning the type species and other species to a new genus

Code	<b>2014.001dF</b>	(assigned by ICTV officers)
<b>To designate the following as the type species of the new genus</b>		
<i>Botrytis porri botybirnavirus 1</i>	Every genus must have a type species. This should be a well characterized species although not	

necessarily the first to be discovered

The new genus will also contain any other new species created and assigned to it (Module 2) and any that are being moved from elsewhere (Module 7b). Please enter here the TOTAL number of species (including the type species) that the genus will contain:

1

**Reasons to justify the creation of a new genus:**

Additional material in support of this proposal may be presented in the Appendix, Module 9

To create a novel genus for accommodating the novel mycovirus BpBV1- GarlicBc-72, as it shows a dramatic difference from all reported viral genera; See details below. Some viral families/genera were also created for accommodating only one species like *Megabirnaviridae/Megabirnavirus* and *Quadriviridae/Quadrivirus*. In this case, we propose to leave this new genus unassigned to a family for now, to allow additional analysis of related viruses.

The proposed new genus is distinguishable from other reported mycoviruses in the following features:

- (1) The size of its bipartite genome is ~12 kb, smaller than that of *Megabirnaviridae* (~17 kb) but larger than those of *Partitiviridae* (2.8 to 4.6 kb), *Picobirnaviridae* (~4 kb) and *Birnaviridae* (~6 kb).
- (2) The 5'-terminal sequence (500 bp) of the two dsRNA genome segments shows high level of identity (~95%) including the 5'-UTRs (404 bp) and partial coding region (96 bp).
- (3) In the two deduced proteins encoded by the two ORFs (ORF I and ORF II) (Fig. 1), only the 3'-proximal coding region of ORF I shows sequence identities of 19 to 23% with RdRps encoded by viruses in the families *Totiviridae*, *Chrysoviridae*, and *Megabirnaviridae*, whereas the proteins in the remaining coding regions lack sequence similarities to any reported virus proteins.
- (4) Two types of structural proteins (p85/80, p70) are detected in the viral particles, which are encoded by ORF I and ORF II respectively. (Fig. 1)
- (5) Phylogenetic analysis of the RdRp sequence showed that prototype BpBV1 of the proposed family belongs to a separate clade distinct from those of other known RNA mycoviruses. (Fig. 2)
- (6) Pair-wise sequence comparison (PASC) analysis on NCBI of full length sequence of BpBV-1 dsRNA-1 showed only low sequence similarities to related mycoviruses (*Ustilago maydis* virus H1, *Rosellinia necatrix* megabirnavirus 1/W779 segment L1, *Spissistilus festinus* virus 1, *Circulifer tenellus* virus 1) and members in family *Totiviridae* (see Table 1 and Fig. 3).

**Origin of the new genus name:**

The spelling "Boty" originates from the Latin name of the host fungus "*Botrytis*" of BpRV1, as BpBV1 has been detected in *B. porri* and *B. squamosa*. The spelling "-birna" represents the bipartite dsRNA genome of this virus.

**Reasons to justify the choice of type species:**

BpBV1-GarlicBc-72 is the reference strain of *Botrytis porri botybirnavirus 1* and it is the only well-characterized virus strain. In the phylogenetic tree constructed using RdRp sequences (Fig. 2), BpBV1- GarlicBc-72 belongs to a separate clade distinct from those of other known RNA mycoviruses.

**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.

MODULE 9: **APPENDIX**: supporting material

additional material in support of this proposal

**See Annex below.**

**References:**

1. Pearson MN. and Bailey AM. 2013. Viruses of *Botrytis*. *Advances in Virus Research*. 86: 249-272.
2. Spear A, Sisterson MS, Yokomi R, Stenger DC. 2010. Plant-feeding insects harbor double-stranded RNA viruses encoding a novel proline alanine rich protein and a polymerase distantly related to that of fungal viruses. *Virology* 404:304 –311.
3. Wu MD, Jin FY, Zhang J, Yang L, Jiang DH, Li GQ. 2012. Characterization of a novel bipartite double-stranded RNA mycovirus conferring hypovirulence in the phytopathogenic fungus *Botrytis porri*. *Journal of Virology*. 86: 6605-6619.
4. Liu L, Wang Q, Cheng J, Fu Y, Jiang D and Xie J (2015) Molecular characterization of a bipartite double-stranded RNA virus and its satellite-like RNA co-infecting the phytopathogenic fungus *Sclerotinia sclerotiorum*. *Front. Microbiol.* 6:406. doi: 10.3389/fmicb.2015.00406

**Annex:**

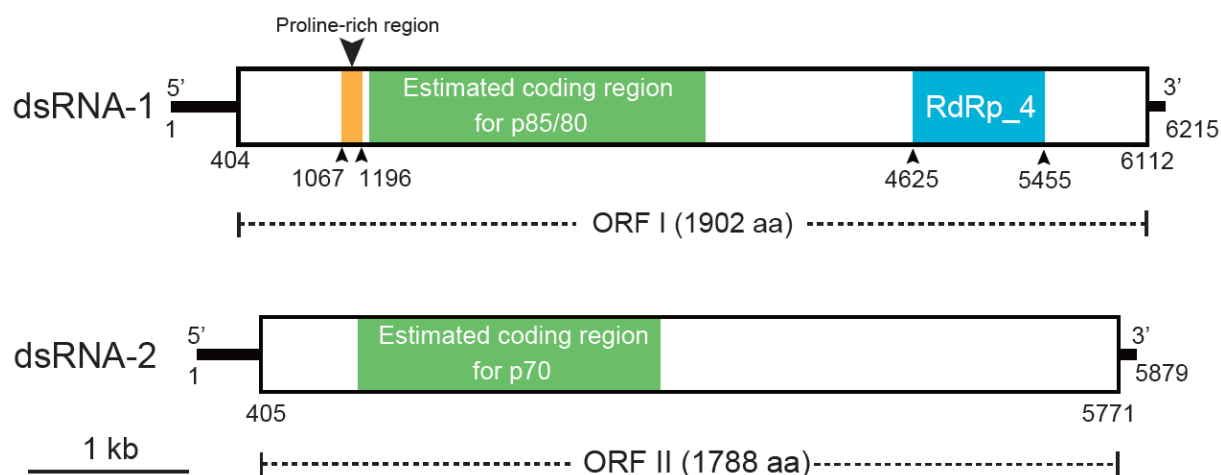
Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate 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.

**Table 1 Summary results of a PASC search for the full length sequence of *Botrytis porri* botybirnavirus 1 (dsRNA-1), *Ustilago maydis* virus H1, *Rosellinia necatrix* megabirnavirus 1/W779 (segment L1), *Spissistilus festinus* virus 1, *Circulifer tenellus* virus 1.**

PASC searched viruses	BLAST-based alignments			Global alignments		
	Ranking	Identity	Virus name (GenBank accession no.)	Ranking	Identity	Virus name (GenBank accession no.)
BpBV1	1	12.96%	Trichomonas vaginalis virus 2 (NC_003873.1)	1	52.21%	Giardia lamblia virus (AF525216.1)
	2	12.61%	Beauveria bassiana victorivirus NZL/1980 (NC_024151.1)	2	51.48%	Giardia lamblia virus (NC_003555.1)
	3	12.47%	Trichomonas vaginalis virus 2 (HQ607514.1)	3	50.34%	Ustilago maydis virus H1 (NC_003823.1)
	4	11.99%	Ustilago maydis virus 1 (KF791041.1)	4	50.13%	Giardia canis virus (DQ238861.1)
	5	11.82%	Ustilago maydis virus H1 (NC_003823.1)	5	49.54%	Piscine myocarditis virus ALV-708 (NC_015639.1)
	6	11.8%	Saccharomyces cerevisiae virus L-BC (La) (NC_001641.1)	6	48.69%	Drosophila melanogaster totivirus SW-2009a (NC_013499.1)
	7	11.04%	Helicobasidium mompatotivirus 1-17 (NC_005074.1)	7	46.25%	Leishmania RNA virus 2-1 (NC_002064.1)
	10	10.33%	Spissistilus festinus virus 1 (GU979419.1)	38	41.73%	Spissistilus festinusvirus 1 (GU979419.1)
	15	10.04%	Circulifer tenellus virus 1, (GU979420.1)	40	41.3%	Circulifer tenellus virus 1 (GU979420.1)
	38	7.71%	Rosellinia necatrix megabirnavirus 1/W779, segment L1 (AB512282.1)	57	37.68%	Rosellinia necatrix megabirnavirus 1/W779, segment L1 (AB512282.1)
CtV1	1	42.22%	Spissistilus festinus virus 1 (GU979419.1)	1	58.59%	Spissistilus festinus virus 1 (GU979419.1)
	2	16.07%	Rosellinia necatrix victorivirus 1 (NC_021565.1)	2	50.59%	Tianjin totivirus (NC_017084.1)
	3	13.82%	Coniothyrium minitans RNA virus (NC_007523.1)	3	47.66%	Rosellinia necatrix megabirnavirus 1/W779, segment L1 (AB512282.1)
	4	13.07%	Ustilago maydis virus 1 (NC_020997.1)	4	47.63%	Armigeres subalbatus virus SaX06-AK20 (NC_014609.1)
	5	12.91%	Tolypocladium cylindrosporium virus 1 (NC_014823.1)	5	47.21%	Penaeid shrimp infectious myonecrosis virus (KF836757.1)
	6	12.9%	Sphaeropsis sapinea RNA virus 1 (NC_001963.1)	6	46.6%	Penaeid shrimp infectious myonecrosis virus (KJ636783.1)
	7	12.81%	Gremmeniella abietina RNA virus L1 (NC_003876.1)	7	46.53%	Penaeid shrimp infectious myonecrosis virus (KJ636782.1)
	12	12.11%	Ustilago maydis virus H1 (NC_003823.1)	8	46.51%	Penaeid shrimp infectious myonecrosis virus (EF061744.1)
	17	10.97%	Rosellinia necatrix megabirnavirus 1/W779, segment L1 (AB512282.1)	13	41.33%	Botrytis porri botybirnavirus 1, segment 1 (JF716350.1)
	19	10.04%	Botrytis porri botybirnavirus 1, segment 1 (JF716350.1)	15	39.78%	Ustilago maydis virus H1 (NC_003823.1)
Sfv1	1	42.22%	Circulifer tenellus virus 1 (GU979420.1)	1	58.57%	Circulifer tenellus virus 1 (GU979420.1)
	2	15.11%	Magnaporthe oryzae virus 1 (NC_006367.1)	2	48.37%	Penaeid shrimp infectious myonecrosis virus (EF061744.1)
	3	14.49%	Coniothyrium minitans RNA virus (NC_007523.1)	3	47.69%	Tianjin totivirus (NC_017084.1)
	4	13.84%	Magnaporthe oryzae virus 2 (NC_010246.1)	4	47.51%	Penaeid shrimp (KJ636782.1)
	5	13.68%	Botryotinia fuckeliana totivirus 1 (NC_009224.1)	5	47.42%	Penaeid shrimp infectious myonecrosis virus (KJ636783.1)
	6	12.89%	Rosellinia necatrix victorivirus 1 (NC_021565.1)	6	46.91%	Penaeid shrimp infectious myonecrosis virus (KF836757.1)
	7	12.29%	Helminthosporium victoriae virus 190S (NC_003607.2)	7	46.87%	Armigeres subalbatus virus SaX06-AK20 (NC_014609.1)
	18	10.59%	Ustilago maydis virus H1 (NC_003823.1)	8	46.67%	Rosellinia necatrix megabirnavirus 1/W779, segment L1 (AB512282.1)

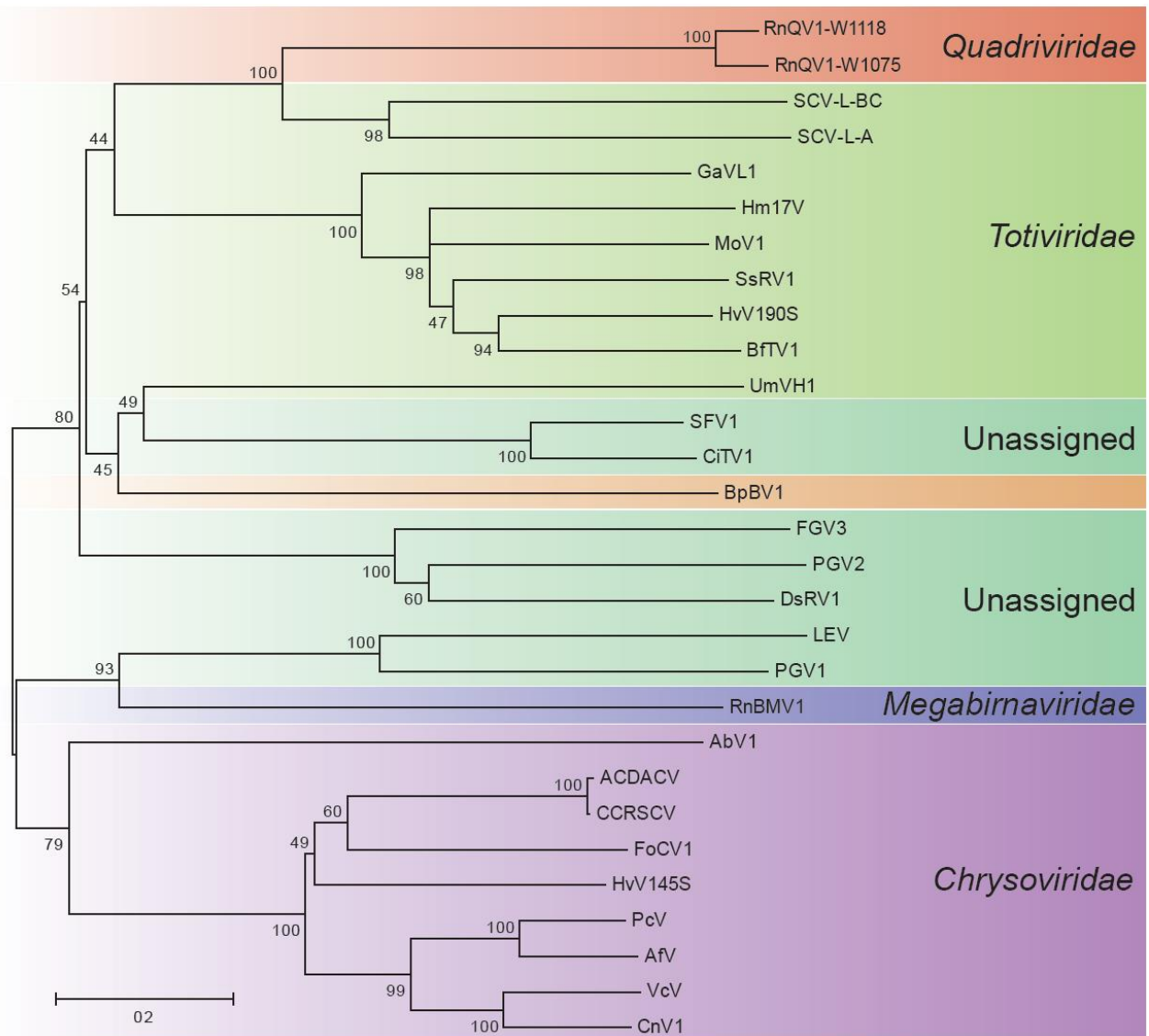
	19	10.33%	Botrytis porri botybirnavirus 1, segment 1 (JF716350.1)	11	41.76%	Botrytis porri botybirnavirus 1, segment 1 (JF716350.1)	
	23	9.57%	Rosellinia necatrix megabirnavirus 1/W779, segment L1 (AB512282.1)	13	41.2%	Ustilago maydis virus H1 (NC_003823.1)	
RnMV1/W779	1	10.97%	Circulifer tenellus virus 1 (GU979420.1)	1	47.67%	Circulifer tenellus virus 1 (GU979420.1)	
	2	9.57%	Spissistilus festinus virus 1 (NC_013999.1)	2	46.66%	Spissistilus festinus virus 1 (GU979419.1)	
	3	9.39%	Phlebiopsis gigantea mycovirus dsRNA 1 (NC_013999.1)	3	45.28%	Penaeid shrimp infectious myonecrosis virus (KF836757.1)	
	4	8.48%	Saccharomyces cerevisiae virus L-A (KC677754.1)	4	44.77%	Penaeid shrimp infectious myonecrosis virus (KJ636783.1)	
	5	8.47%	Xanthophyllomyces dendrorhous virus L1A (JN997474.2)	5	43.87%	Tianjin totivirus (NC_017084.1)	
	6	8.39%	Magnaporthe oryzae virus 2 (NC_010246.1)	6	43.76%	Phlebiopsis gigantea mycovirus dsRNA 1 (NC_013999.1)	
	7	8.37%	Beauveria bassiana victorivirus NZL/1980 (NC_024151.1)	7	43.49%	Armigeres subalbatus virus SaX06-AK20 (NC_014609.1)	
	8	7.97%	Sphaeropsis sapinea RNA virus 1 (NC_001963.1)	8	43.42%	Penaeid shrimp infectious myonecrosis virus (KJ636782.1)	
	12	7.71%	Botrytis porri botybirnavirus 1, segment 1 (JF716350.1)	15	37.71%	Botrytis porri botybirnavirus 1, segment 1 (JF716350.1)	
	40	3.87%	Ustilago maydis virus H1 (NC_003823.1)	16	37.35%	Ustilago maydis virus H1 (NC_003823.1)	
	UmV1	1	100%	Ustilago maydis virus H1 (NC_003823.1)	1	100%	Ustilago maydis virus H1 (NC_003823.1)
		2	12.76%	Saccharomyces cerevisiae virus L-A (NC_003745.1)	2	51.8%	Giardia lamblia virus (NC_003745.1)
3		12.17%	Helicobasidium mompa totivirus 1-17 (NC_005074.1)	3	50.37%	Botrytis porri botybirnavirus 1, segment 1 (JF716350.1)	
4		12.11%	Circulifer tenellus virus 1 (GU979420.1)	4	49.52%	Giardia canis virus (DQ238861.1)	
5		11.82%	Botrytis porri botybirnavirus 1, segment 1 (JF716350.1)	5	49.19%	Eimeria brunetti RNA virus 1 (NC_002701.1)	
6		11.25%	Gremmeniella abietina RNA virus L1 (NC_003876.1)	6	47.44%	Giardia lamblia virus (NC_003555.1)	
7		10.94%	Saccharomyces cerevisiae virus L-A (M28353.1)	7	47.28%	Drosophila melanogaster totivirus SW-2009a (NC_013499.1)	
8		10.94%	Beauveria bassiana victorivirus NZL/1980 (NC_024151.1)	45	41.7%	Spissistilus festinus virus 1 (GU979419.1)	
12		10.59%	Spissistilus festinus virus 1 (GU979419.1)	55	39.76%	Circulifer tenellus virus 1 (GU979420.1)	
45		3.87%	Rosellinia necatrix megabirnavirus 1/W779, segment L1 (AB512282.1)	58	37.36%	Rosellinia necatrix megabirnavirus 1/W779, segment L1 (AB512282.1)	

**Fig. 1 Schematic diagrams of the genome organization of BpBV1 dsRNA-1 and dsRNA-2**



The coding strand of dsRNA-1 is 6,215 bp long and comprises one large ORF, designated ORF I, which encodes a polyprotein of 1,902 aa. The coding strand of dsRNA-2 is 5,788 bp long and also comprises one large ORF, designated ORF II, which encodes a polypeptide of 1,788 aa. p70, p80, and p85 are the structural proteins of BpBV1 inferred from peptide fingerprinting analyses.

**Fig. 2** Phylogenetic analysis of BpBV1 and 28 selected RNA viruses presented in an NJ tree inferred from the RdRp sequences.



The neighbor-joining (NJ) tree was constructed by using the CLUSTAL W program in the MEGA 5.0 software. The number labeled at each node indicates the bootstrap percentage (N = 1000).

Abbreviated virus names and GenBank accession number for viral RdRp: UmVH1, *Ustilago maydis* virus H1 (NC\_003823); ScV-L-A, *Saccharomyces cerevisiae* virus L-A (J04692); ScV-L-BC, *Saccharomyces cerevisiae* virus L-BC (U01060); HvV145S, *Helminthosporium victoriae* virus 145S (AF297176); PcV, *Penicillium chrysogenum* virus (AF296339); FoCV1, *Fusarium oxysporum* chrysovirus 1 (EF152346); AfV, *Aspergillus fumigatus* chrysovirus (FN178512); CnV1, *Cryphonectria nitschkei* chrysovirus (GQ290650); ACDACV, *Amasya cherry disease-associated chrysovirus* (NC\_009947); VcV, *Verticillium dahliae* chrysovirus (HM004067); AbV1, *Agaricus bisporus* virus 1 (X94361); CCRSCV, *Cherry chlorotic rusty spot-associated*



chrysovirus (AJ781397); Hm17V, *Helicobasidium mompa* no. 17 dsRNA virus (AB085814); SsRV1, *Sphaeropsis sapinea* RNA virus 1 (NC\_001963); MoV1, *Magnaporthe oryzae* virus 1 (AB176964); GaVL1, *Gremmeniella abetina* RNA virus L1 (AF337175); BfTV1, *Botryotinia fuckeliana* totivirus 1 (AM491608); HvV190S, *Helminthosporium victoriae* virus 190S (U41345); RnBMV1, *Rosellinia necatrix* megabirnavirus 1 (AB512282); SFV1, *Spissistilus festinus* virus 1 (GU979419); CiTV1, *Cirulifer tenellus* virus 1 (GU979420); PGV1, *Phlebiopsis gigantea* mycovirus dsRNA 1 (AM111096); FgV3, *Fusarium graminearum* dsRNA mycovirus 3 (NC\_013469); DsRV1, *Diplodia scrobiculata* RNA virus 1 (NC\_013699); PGV2, *Phlebiopsis gigantea* mycovirus dsRNA 2 (AM111097); LEV, *Lentinula edodes* mycovirus HKB (AB429554); BpBV1, *Botrytis porri* botybirnavirus 1 (JF716350); RnQV1, *Rosellinia necatrix* quadrivirus 1 (AB620063)

**Fig. 3** The frequency distribution of pairwise identities from the complete genome sequence comparison of 63 totiviruses. Red arrowheads indicate the position of the highest pairwise identity of BpBV1 through the PASC search in the viral family *Totiviridae*.

