

Supporting Information

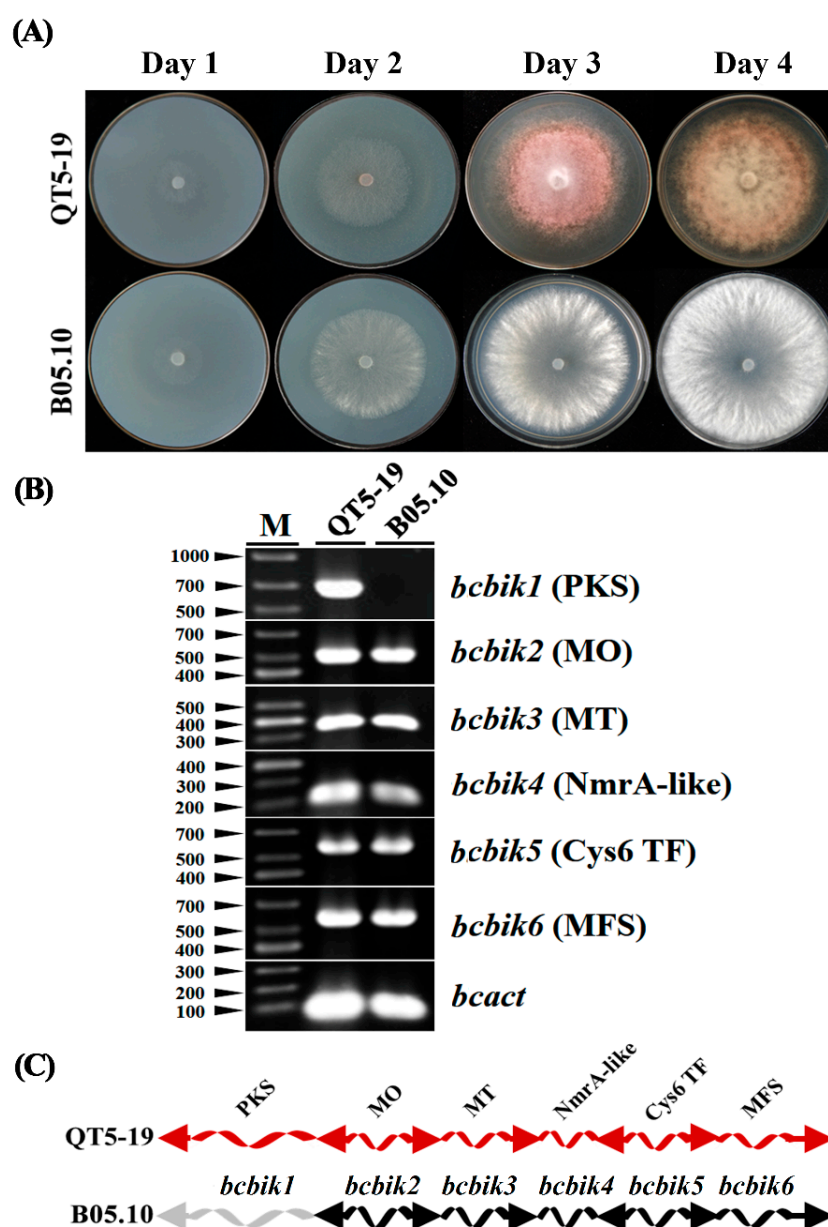
## A Novel Partitivirus in the Hypovirulent Isolate QT5-19 of the Plant Pathogenic Fungus *Botrytis cinerea*

Kamaruzzaman<sup>1</sup>, Guoyuan He<sup>1</sup>, Mingde Wu<sup>1</sup>, Jing Zhang<sup>1</sup>, Long Yang<sup>1</sup>, Weidong Chen<sup>2</sup> and Guoqing Li<sup>\*</sup>

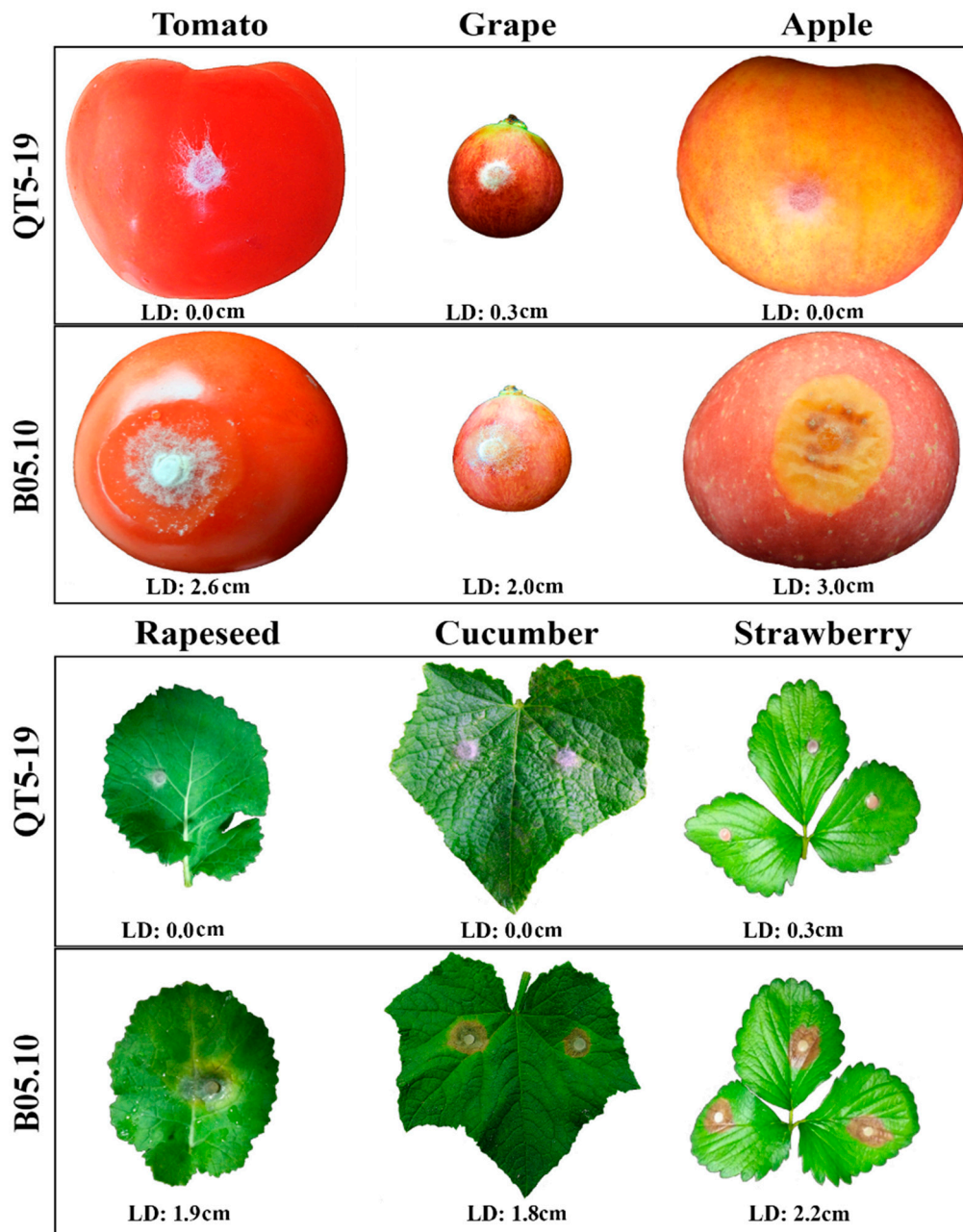
<sup>1</sup> The Key Laboratory of Plant Pathology of Hubei Province and The State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China.

<sup>2</sup> U. S. Department of Agriculture, Agricultural Research Service, Washington State University, Pullman, WA, 99164, USA.

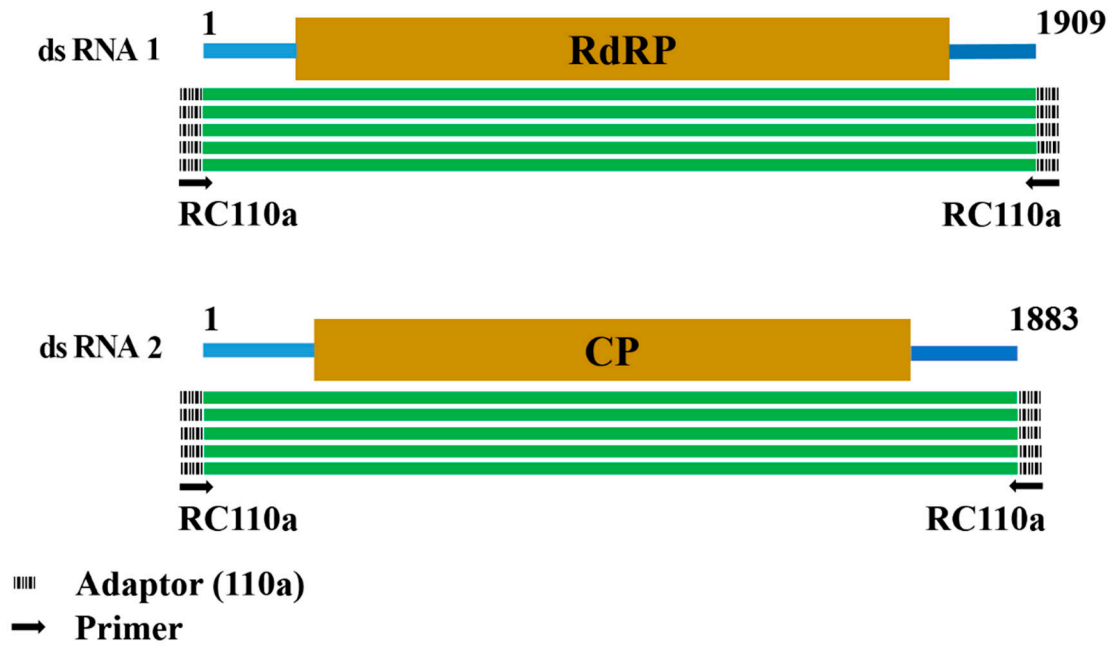
\* Correspondence: guoqingli@mail.hzau.edu.cn



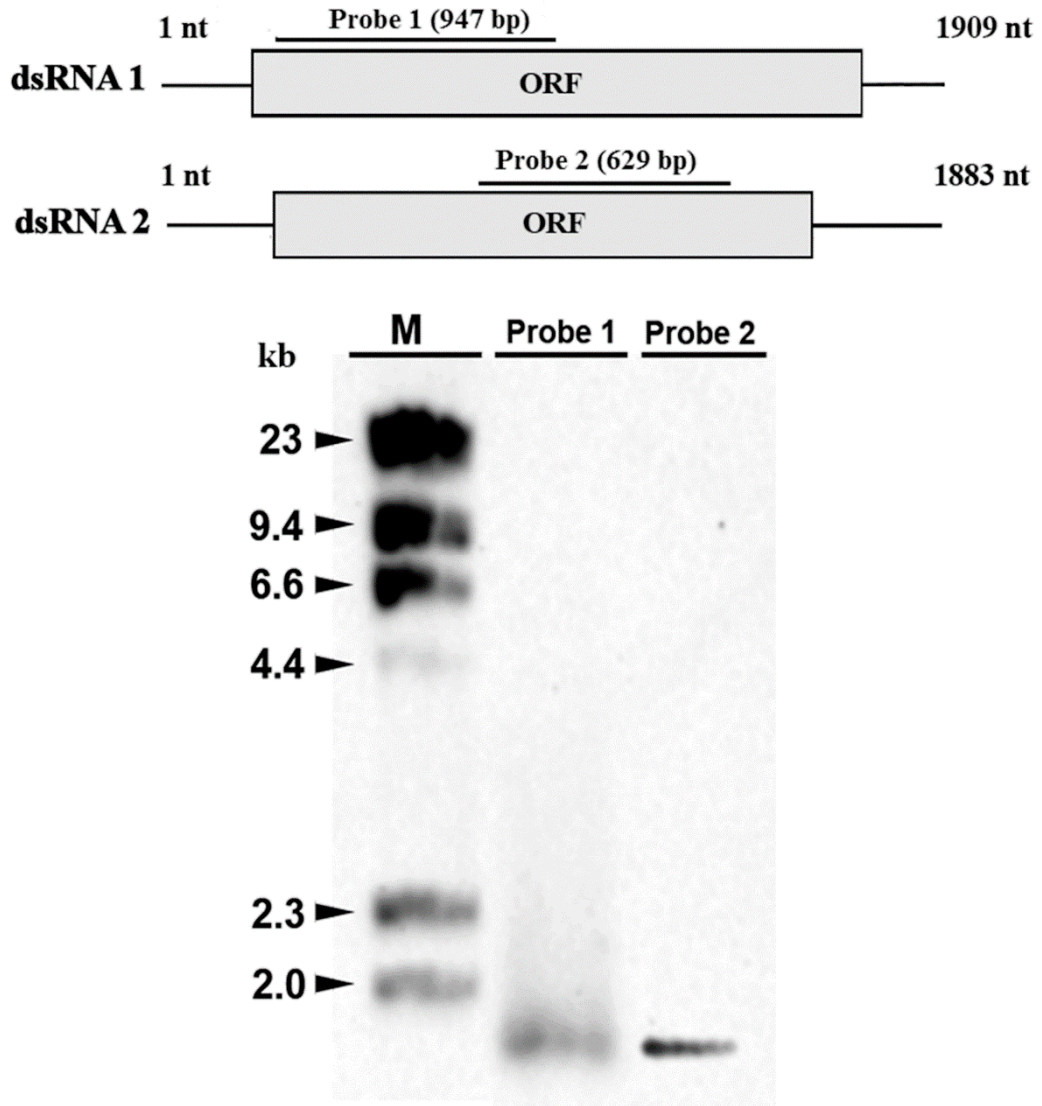




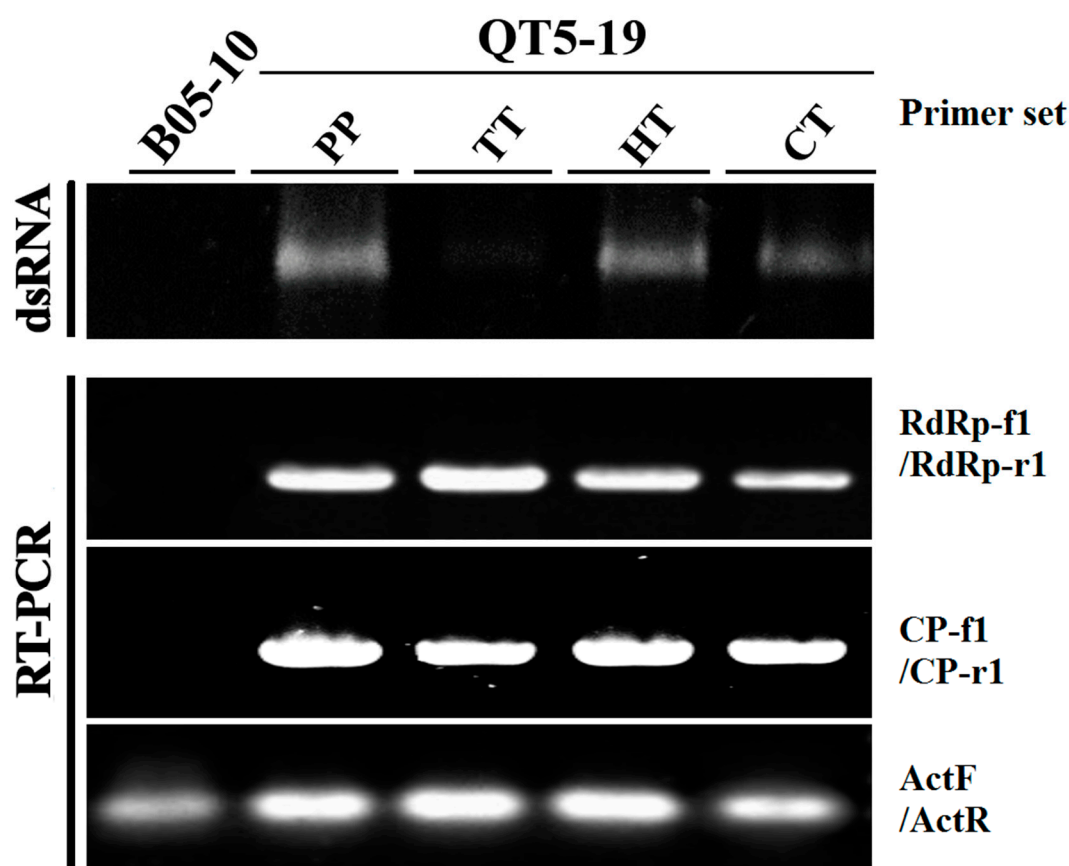
**Figure S3.** Pathogenicity of isolates QT5-19 and B05.10 of *B. cinerea* on fruits of tomato, table grapes and apple, and on leaves of cucumber, rapeseed and strawberry. Mycelial agar plugs (5 mm) of QT5-19 and B05-10 were placed on the surface of the fruits and leaves, which were kept at 20°C under humid conditions for 3 days. LD = lesion diameter (on average).



**Figure S4.** A schematic diagram showing the strategy for cDNA cloning of the full-length sequences of the two dsRNAs in *Botrytis cinerea partitivirus 2* (BcPV2). The adaptor/primers and their nucleotide sequences were listed in Table S1.



**Figure S5.** Northern-blotting detection of the dsRNAs extracted from the mycelia of *Botrytis cinerea* QT5-19. The position of the two DNA probes in the genome of BcPV2 was shown on the top.



**Figure S6.** Detection of BcPV2 in different isolates of *Botrytis cinerea* by dsRNA profiling and RT-PCR. Four derivatives of QT5-19 from protoplast regeneration (PP), thermal therapy (TH), hyphal tipping (HT) and chemical therapy (CT). In dsRNA profiling, the dsRNA extracts from different isolates were treated with DNase I and S1 nuclease before loading for agarose gel electrophoresis. In RT-PCR, the actin gene *bcact* amplified by the primers ActF/ActR was used as control. The oligonucleotide sequences of the primers were shown in Supplementary Table S1.

**Table S1.** List of the oligonucleotide primers or adaptor used in this study.

Primer name	Sequence (5' to 3')	Size of the product
To confirm the taxonomic status of isolates 08168, B05.10, QT5-19, RoseBc-3 and XN-1 as <i>B. cinerea</i>		
Bc-f	CAGGAAACACTTTTGGGGATA	327 bp
Bc-r	GAGGGACAAGAAAATCGACTAA	
To detect and clone the ITS region		
ITS1	TCCGTAGGTGAACCTGCGG	539 bp
ITS4	TCCTCCGCTTATTGATATGC	
To synthesize cDNAs of BcPV2		
110a	(PO4)-TATCTTATCGGCGTGTCCCCC-(NH2)	1930 bp for dsRNA1
RC110a	GGGGACACGCCGATAAGATA	1904 bp for dsRNA2
To detect the cDNA insert in the plasmid pMD18-T		
M13F-47	CGCCAGGGTTTTCCCAGTCACGAC	1930 bp for dsRNA1
M13R-48	AGCGGATAACAATTTACACAGGA	1904 bp for dsRNA2
To detect BcPV2/QT5-19 in RT-PCR		
RdRp-f1	GTTCCGACCTCATCGTCGTT	970 bp
RdRp-r1	TGCTTCGGAGGTAAGGGTTG	
CP-f1	CCGTTCCCTTTACACGCCAG	796 bp
CP-r1	ACGTGTGACAGTAGACGCTG	
To prepare the DNA probes used in Northern blotting for detection of the BcPV2 dsRNAs		
RdRp-f2	GATCAATCGACGTACCAAA	946 bp

RdRp-r2	AAGAGCAGATTGACCGAC	
CP-f2	ACGACTCCCGTTCCTTT	
CP-r2	TTCTTCAGTTTCGGTTGG	629 bp
To detect expression of <i>bcik1</i> in QT5-19 and B05.10		
Bcbik1-f	GTTCGCAGACGAGCTGAAA	
Bcbik1-r	TCGATCTTTGGTCAGGCTCT	679 bp
To detect expression of <i>bcik2</i> in QT5-19 and B05.10		
Bcbik2-f	TGGCGATGCAGCTTGTAAGA	
Bcbik2-r	GACTCGCAAACATCCGC	503 bp
To detect expression of <i>bcik3</i> in QT5-19 and B05.10		
Bcbik3-f	TTGCGTCTATGGTTACCGCT	
Bcbik3-r	AAGGCTTCGGCTAGCTTCAC	348 bp
To detect expression of <i>bcik4</i> in QT5-19 and INRA 1750		
Bcbik4-f	TTCATCTACGCCGTACGCTC	
Bcbik4-r	TGTGGCACCTCGAGTTCTTC	212 bp
To detect expression of <i>bcik5</i> in QT5-19 and B05.10		
Bcbik5-f	GGATGCCATCGCTTCTCTGA	
Bcbik5-r	TCACACCTGACCAACGTGAG	570 bp
To detect expression of <i>bcik6</i> in QT5-19 and B05.10		
Bcbik6-f	ATCTGCCTGCTGCTTACGAT	
Bcbik6-r	AAGATGGTGGTGAAGCCAAC	572 bp

Note: The primer pair ActF/ActR was designed based on the actA gene in *Botryotinia fuckeliana* (GenBank Acc. No. AJ000335.1). The primer set for RT-PCR detection of the expression of *bcik1* was adopted from Schumacher and colleagues (2013) (Schumacher *et al.*, 2013. *PLoS ONE* 8, e53729). The primer sets for RT-PCR detection of *bcik2*, *bcik3*, *bcik4*, *bcik5*, *bcik6* were designed based on the DNA sequences of these genes under the GenBank accession numbers HE802545, HE802546, HE802547, HE802548, HE802549, respectively.

**Table S2.** List of partitiviruses for multiple sequence alignment and phylogenetic analysis.

Name	GenBank Acc. No.		Genus	
	dsRNA 1	dsRNA 2		
Botrytis cinerea Partitivirus 2 (BcPV2)★	MG011707	MG011708	<i>Alphapartitivirus</i>	
Beet cryptic virus 1(BCV1)	ACA81389	ACA81390		
Carrot cryptic virus (CCV)	ACL93278	ACL93279		
Ceratobasidium partitivirus (CPV)	AOX47571	AOX47604		
Cherry chlorotic rusty spot associated partitivirus (CCRSaPV)	CAH03668	CAH03669		
Chondrostereum purpureum cryptic virus 1 (CpCV1)	CAQ53729	CAQ53730		
Dill clover cryptic virus 1 (DCV1)	KF484726	AGY36137		
Diuris pendunculata cryptic virus (DpCV)	AFQ95555	AFY23215		
Flammulina velutipes browning virus (FvBV)	BAH56481	BAH56482		
Helicobasidium mompa partitivirus V70 (HmPVV70)	BAC23065	-		
Heterobasidion partitivirus 1 (HetPV1)	ADV15441	ADV15442		
Heterobasidion partitivirus 12 (HetPV12)	AHL25151	AHL25152		
Heterobasidion partitivirus 13 (HetPV13)	AHL25153	AHL25154		
Heterobasidion partitivirus 15 (HetPV15)	AHL25162	AHL25163		
Heterobasidion partitivirus 3 (HetPV3)	ACO37245	ACO37246		
Powdery mildew-associated partitivirus (PmAPV)	AOF47283	AOF47284		
Rhizoctonia solani partitivirus 2 (RsPV2)	AGY54938	AGY54939		
Rosellinia necatrix partitivirus 2 (RnPV2)	BAM78602	BAK53192		
Sclerotinia sclerotiorum partitivirus S (SsPV-S)	ACT55329	ACT55330		
Soybean leaf-associated partitivirus 2 (SLAPV2)	ALM62247	ALM62248		
Vicia cryptic virus (VCV)	EF173389	EF173390		
White clover cryptic virus 1 (WCCV1)	AAU14888	AAU14889		
Atkinsonella hypoxylon partitivirus (AhV)	AAA61829	AAA61830		<i>Betapartitivirus</i>
Botrytis cinerea Partitivirus 1 (BcPV1)	-	AGQ21570		
Cannabis cryptic virus (CaCV)	AET80948	AET80949		
Dill cryptic virus 2 (DCV2)	AGJ83771	AGJ83772		
Fusarium poae virus 1 (FpV1)	NP_624349	NP_624348		
Grapevine partitivirus (GPV)	AFX73019	-		
Heterobasidion partitivirus 2 (HetRV2)	ADL66905	ADL66906		
Heterobasidion partitivirus 8 (HetRV8)	AFW17810	AFW17811		
Pleurotus ostreatus virus 1 (PoV1)	AAT07072	AAT06080		
Rhizoctonia solani virus 717 (RsV-717)	AAF22160	AAF40300		
Rosellinia necatrix partitivirus 1 (RnV1)	BAD98237	BAD98238		
Sclerotinia sclerotiorum partitivirus 1 (SsPV1)	AFR78160	AFR78159		
White clover cryptic virus 2 (WCCV2)	AGJ83763	AGJ83764		
Botryotinia fuckeliana partitivirus 1 (BfPV1)	YP_001686789	YP_001686790	<i>Gammapartitivirus</i>	
Discula destructiva virus 1 (DdV1)	AAG59816	AAK13165		
Discula destructiva virus 2 (DdV2)	AAK59379	AAK59380		
Fusarium solani virus 1 (FsV1)	BAA09520	BAA09521		
Ophiostoma partitivirus 1 (OPV1)	CAJ31886	CAJ31887		
Penicillium stoloniferum virus F (PsV-F)	AAU95758	AAU95759		
Penicillium stoloniferum virus S (PsV-S)	AAN86834	AAN86835		
Verticillium dahliae partitivirus 1 (VdPV1)	AGI52210	AGI52209		
Beet cryptic virus 2 (BCV2)	ADP24757	AGQ49466	<i>Deltapartitivirus</i>	
Fig cryptic virus (FCV)	CBW77436	CBW77437		
Fragaria chiloensis cryptic virus (FcCV)	AAZ06131	ABC73696		
Pepper cryptic virus 1 (PCV1)	AEJ07890	AEJ07891		



Pepper cryptic virus 2 (PCV2)	AEJ07892	AEJ07893
Persimmon cryptic virus (PeCV)	CCH50609	CCH50610
Raphanus sativus cryptic virus 2 (RsCV2)	YP_001686783	YP_001686784
Raphanus sativus cryptic virus 3 (RsCV3)	YP_002364401	YP_002364402
Rose cryptic virus 1 (RoCV1)	YP_001686786	YP_001686787

---

Note: The symbol “-” indicates that accession number is not available in GenBank.