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Genomic characterization of a novel bunyavirus FsBV2 from *Fusarium solani*

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Abstract

A novel bunyavirus, tentatively named Fusarium solani bunya virus 2 (FsBV2), was identified in the phytopathogenic fungus *Fusarium solani*. The complete sequence of the L segment of FsBV2 is 7,186 nucleotides (nt) in length and contains a single open reading frame (ORF) encoding an RNA-dependent RNA polymerase (RdRp) of 316 amino acids (aa). The RdRp contains six conserved bunyaviral motifs, including the SDD tripeptide. The M segment is 1,422 nt long and contains a single ORF encoding a YwqJ-like deaminase of 81 aa. The S segment is 1,301 nt long and contains a single ORF encoding a protein containing putative multifunctional flavoprotein (SiRFP) domain. BLASTp analysis showed that the RdRp of FsBV2 shared the highest amino acid sequence identity (48.73%) with that of Cheeloo tick associated virus 2. Phylogenetic analysis revealed that FsBV2 clusters with unclassified bunyaviruses. Based on its genome organization, protein sequences, and phylogenetic position, FsBV2 is proposed as a novel virus within the class Bunyaviricetes.

Introduction

Mycoviruses are viruses that replicate in fungi or oomycetes [1]. They often cause symptomless infections, but some mycoviruses can induce abnormal mycelial morphology and reduce host virulence [2,3]. Mycoviruses have shown significant potential for the biological control of plant pathogenic fungi. The first successful case of biological control of a plant disease using a mycovirus involved the use of Cryphonectria parasitica hypovirus 1 (CHV1; genus *Alphahypovirus*, family *Hypoviridae*), which attenuates virulence of the chestnut blight fungus *Cryphonectria parasitica* [4]. This landmark example highlights the promising research and application prospects of mycoviruses.

According to the latest 2025 classification by the International Committee on Taxonomy of Viruses (ICTV; https://ictv.global), the class *Bunyaviricetes* includes two orders (*Elliovirales* and *Hareavirales*), 16 families (*Cruliviridae*, *Fimoviridae*, *Hantaviridae*, *Peribunyaviridae*, *Phasmaviridae*, *Tospoviridae*, *Tulasviridae*, *Arenaviridae*, *Discoviridae*, *Konkoviridae*, *Leishbuviridae*, *Mypoviridae*, *Nairoviridae*, *Phenuiviridae*, *Tosoviridae*, and *Wupedeviridae*), as well as several unclassified viruses [5]. Members of this class possess multi-segmented negative-sense RNA genomes, typically comprising 2–8 segments. Most members exhibit the classic tripartite L, M, and S RNA segments, encoding the RdRp, envelope glycoprotein, and nucleocapsid protein, respectively [6].

Fusarium root rot is a soil-borne fungal disease of tobacco caused by mixed infections of various *Fusarium* species [7]. To date, numerous mycoviruses have been identified in the genus *Fusarium*. Osaki et al. used deep sequencing to identify 16 viral sequences from a single *Fusarium poae* strain, covering

families such as *Mitoviridae* and *Partitiviridae* [8]. Buivydaitė et al. screened 56 *Fusarium* strains and found that 46% carried mycoviruses [9]. Sato et al. reported Hadaka virus 1 (HadV1) in *Fusarium oxysporum* [10]. Jacquat et al. (2020) reported the first mycovirus from *Fusarium verticillioides* in maize and sorghum in Argentina [11]. Wang et al. (2016) analyzed Fusarium graminearum hypovirus 1 (FgHV1) using transcriptome sequencing [12]. With the iterative advancement of high-throughput sequencing, an increasing number of mycovirus resources have been discovered, greatly enriching our understanding of fungal viruses [13].

Virus isolation and sequencing

A total of 144 *Fusarium* strains were isolated from tobacco plants showing root rot symptoms collected from different tobacco-growing areas in Hunan Province, China [14]. Total RNA was extracted from all 144 strains and subjected to high-throughput sequencing. Sequence comparison with the NCBI non-redundant (nr) database using BLASTx revealed three contigs with sequence similarity to viruses of the class *Bunyaviricetes* [15,16], and would be a partial gnome of a bunyavirus. We temporarily named this bunyavirus as Fusarium solani bunya virus 2. To obtain the complete genome of FsBV2, seven pairs of primers (Supplementary Table S1) were designed based on these three contigs. RT-PCR amplification was performed on an aliquot of the total RNA used for sequencing, and the products were ligated to PC3-T7loop adapters (5'-p-GGATCCCGGGAATTCTAGATACACTTACTACTAGTAGTAGTTA-OH-3') using T4 RNA ligase. First-strand cDNA synthesis was carried out at 16°C for 16 h using a first-strand cDNA synthesis kit (TaKaRa, Dalian, China). Six pairs of terminal primers (Supplementary Table S1) designed based on the assembled sequences of contigs 692, 51237, and 60111, together with the PC2 primer (5'-CCGAATTCCCGGGATCC-3') complementary to the PC3-T7loop adapter, were used for nested PCR amplification. Amplified products were gel-purified, cloned into the pMD18-T vector, and sequenced bidirectionally. The consensus sequence was determined from three independent biological replicates.

The complete genome of FsBV2 was assembled by integrating RACE-derived termini, RT-PCR, and HTS data. Open reading frames (ORFs) were identified using DNAMAN v9.0 (Lynnon Biosoft), and conserved domains were annotated using the motif search tool online (https://www.genome.jp/tools/motif/). Sequence similarity analysis was performed by BLASTp searches against the NCBI RefSeq database. Multiple sequence alignments and phylogenetic reconstructions based on RdRp and nucleocapsid protein sequences were performed using PhyloSuite software to elucidate evolutionary relationships with related viruses [17].

Sequence properties

Specific primers for FsBV2-L, FsBV2-M, and FsBV2-S were designed based on contigs 692, 51237, and 60111, and the full-length sequences were validated (Fig. 1A and Fig. 1B). Homology comparisons revealed that FsBV2-L showed sequence similarity to viruses of the class *Bunyaviricetes*, with the highest identity

(48.73%) to Cheeloo tick associated virus 2 (GenBank: XUK79380.1), indicating that FsBV2 represents a novel bunyavirus.

FsBV2 possesses three segments: FsBV2-L, FsBV2-M, and FsBV2-S, with lengths of 7,186, 1,422, and 1,301 nt, respectively (GenBank: PZ526015, PZ526016, PZ526017). Using T7-loop cloning, the complete nucleotide sequences of FsBV2-L, FsBV2-M, and FsBV2-S were obtained. The 5' and 3' termini of the three segments were conserved, and the 5' and 3' ends of each segment were complementary (Fig. 2B). The FsBV2-L segment contains a single complete ORF encoding a 316-aa RdRp, the FsBV2-M segment contains a single complete ORF encoding an 81-aa YwqJ-like deaminase, and the FsBV2-S segment contains a single complete ORF encoding a 49-aa multifunctional flavoprotein (SiRFP) (Fig. 2A). Multiple alignment of the RdRp of FsBV2 with those of other bunyaviruses revealed the presence of six conserved motifs (Fig. 2C). A phylogenetic tree constructed using the RdRp amino acid sequences of FsBV2 and viruses from the two orders and 16 families of the class *Bunyaviricetes*, as well as unclassified related viruses (Supplementary Table S2), showed that FsBV2 clusters with unclassified bunyaviruses (Fig. 2D). Therefore, FsBV2 is considered a novel virus within the class *Bunyaviricetes*, and it is proposed to establish a new family to accommodate these unclassified bunyaviruses.

Conclusions

This study reports the molecular characterization of FsBV2 for the first time and provides evidence supporting its classification as a novel virus within the class *Bunyaviricetes*. Future studies are needed to determine whether FsBV2 causes phenotypic changes in its host or exerts other effects on host biology.

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Figure legend

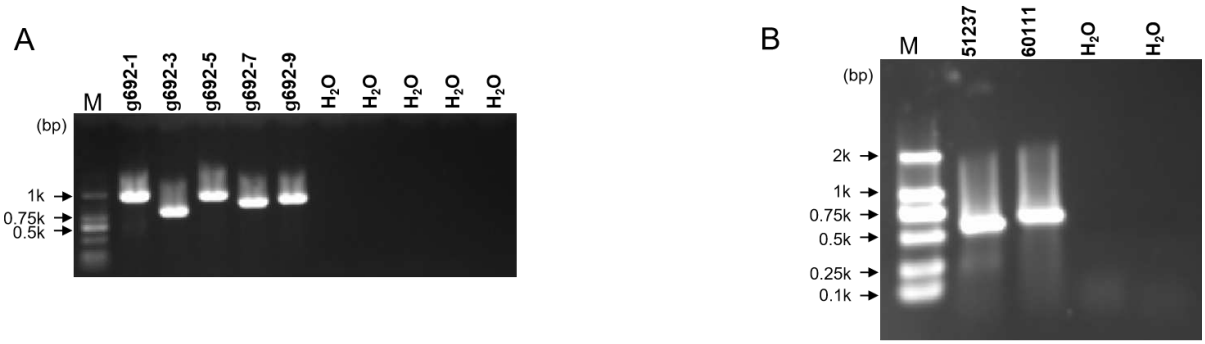
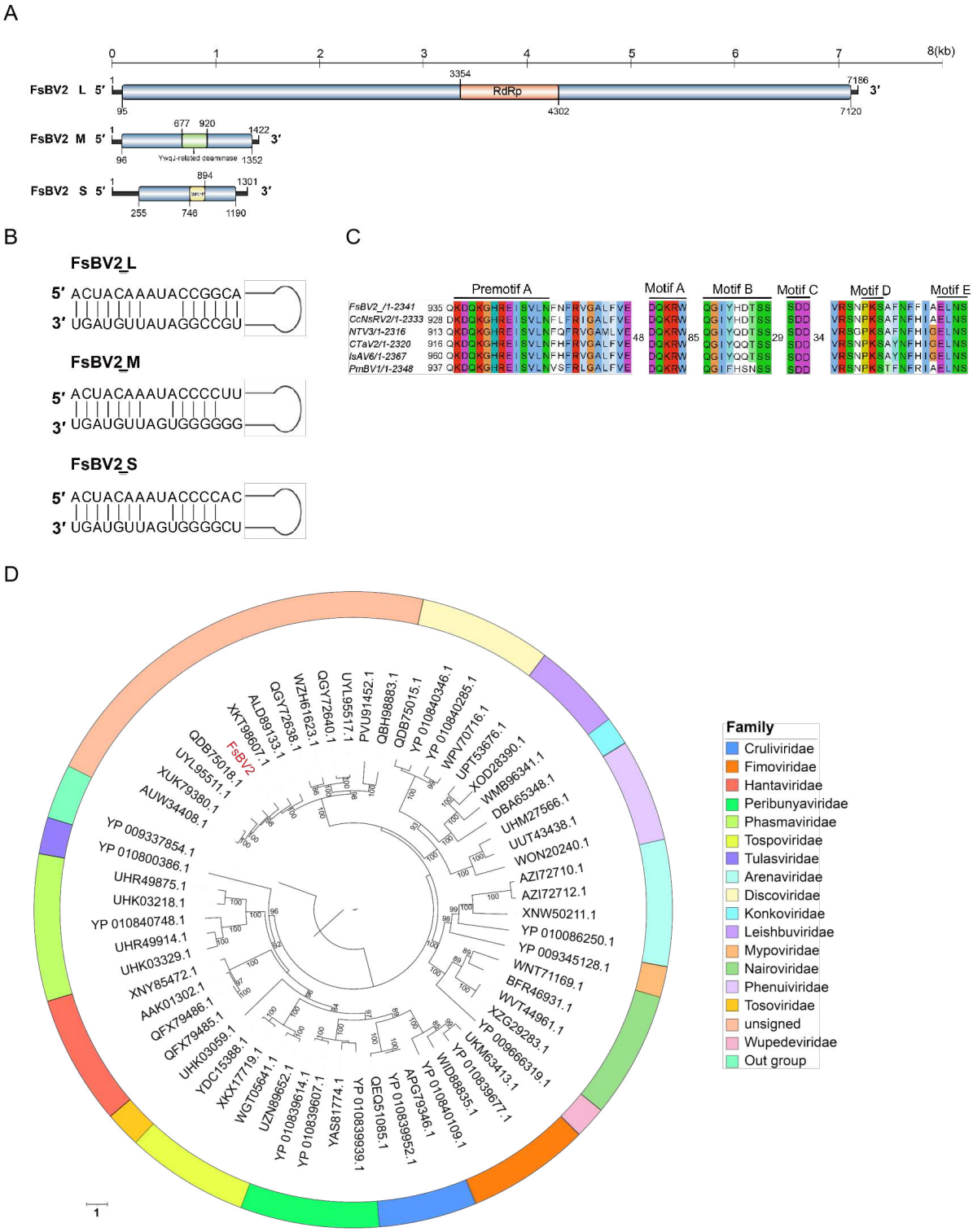


Figure 1. RT-PCR detection of FsBV2

A: RT-PCR detection of FsBV2-L using FsBV2-specific primers (Supplementary Table S1). Agarose gel electrophoresis showed amplification of fragments contig692-1 (1,892 bp), contig692-3 (1,136 bp), contig692-5 (1,852 bp), contig692-7 (1,465 bp), and contig692-9 (1,617 bp) from the backup total RNA. H_2O served as blank control. Lane M, molecular weight marker (2-kb DNA ladder). B: RT-PCR detection of FsBV2-M and FsBV2-S using specific primers (Supplementary Table S1). Agarose gel electrophoresis showed amplification of contig51237 (548 bp) and contig60111 (631 bp) fragments from the backup total RNA. H_2O was used as blank control. Lane M, molecular weight marker (2-kb DNA ladder).


A
0
1
2
3
4
5
6
7
8(kb)
FsBV2 L 5′
1
3354
RdRp
7186
3′
95
4302
7120
FsBV2 M 5′
1
677
920
1422
3′
96
YwqJ-related deaminase
1352
FsBV2 S 5′
1
894
1301
3′
255
748
1190
B
FsBV2_L
5′ ACUACAAAUACCGGCA
3′ UGAUGUUAUAGGCCGU
FsBV2_M
5′ ACUACAAAUACCCCUU
3′ UGAUGUUAGUGGGGGG
FsBV2_S
5′ ACUACAAAUACCCCAC
3′ UGAUGUUAGUGGGGCU
C
Premotif A
Motif A
Motif B
Motif C
Motif D
Motif E
FsBV2_/1-2341
CcNsRV2/1-2333
NTV3/1-2316
CTaV2/1-2320
IsAV6/1-2367
PmBV1/1-2348
D
FsBV2
QDB75018.1
UYL95511.1
XUK79380.1
AUW34408.1
YP 009337854.1
YP 010800386.1
UHR49875.1
UHK03218.1
YP 010840748.1
UHR49914.1
UHK03329.1
XNY85472.1
AAK01302.1
QFX79486.1
QFX79485.1
UHK03059.1
YDC15388.1
XKX17719.1
WGT05641.1
UZN89652.1
YP 010839614.1
YP 010839607.1
YAS81774.1
YP 010839939.1
QEQ51085.1
APG79346.1
YP 010833952.1
YP 010840109.1
WID88835.1
YP 010839677.1
UKM63413.1
YP 009666319.1
XZG29283.1
WVT44961.1
BFR46931.1
WNT71169.1
YP 009345128.1
YP 010086250.1
XNW50211.1
AZI72712.1
AZI72710.1
WON20240.1
UUT43438.1
UHM27566.1
DBA65348.1
WMB96341.1
XOD28390.1
UPT53676.1
WPV70716.1
YP 010840285.1
YP 010840346.1
QDB75015.1
QBH98883.1
PVU91452.1
UYL95517.1
QGY72640.1
WZH61623.1
QGY72638.1
ALD89133.1
XKT98607.1
1
Family
Cruliviridae
Fimoviridae
Hantaviridae
Peribunyaviridae
Phasmaviridae
Tospoviridae
Tulasviridae
Arenaviridae
Discoviridae
Konkoviridae
Leishbuviridae
Mypoviridae
Nairoviridae
Phenuiviridae
Tosoviridae
unsigned
Wupedeviridae
Out group

Figure

2. Genome organization and phylogenetic analysis of FsBV2 within the class *Bunyaviricetes*

A: Schematic representation of the tripartite RNA genome of FsBV2. FsBV2-L contains one putative ORF encoding a conserved RdRp. FsBV2-M contains one putative ORF encoding a predicted YwqJ-like deaminase. FsBV2-S contains one putative ORF encoding a predicted multifunctional flavoprotein. B: Complementary 5' and 3' terminal sequences of FsBV2. C: Multiple sequence alignment of the RdRp of FsBV2. D: Maximum-likelihood phylogenetic tree constructed from RdRp sequences of FsBV2 and other representative of the class *Bunyaviricetes*. Bootstrap support values (%) based on 1,000 replicates are shown at nodes. Branch lengths are drawn to scale corresponding to genetic distance. Reference viruses are identified by genus and GenBank accession numbers. The scale bar represents 0.5 substitutions per site. FsBV2 is indicated in red.

Table S1. Primiers are used to detect and obtain the terminal sequences of FcMV1

Primer Name	Primer Sequence (5" to 3")
FsBV2-L-1-F	CCAGTCGTGTTGAGCCAGC
FsBV2-L-1-R	CCGTATGCCTACTCAAGTTGG
FsBV2-L-2-F	CAAGCCCAGTCTGCCTTTG
FsBV2-L-2-R	CCAGCGTGCATACTCTAGA
FsBV2-L-3-F	CTGCTTTCTTACCTGCCAAG
FsBV2-L-3-R	CAGAAAGGGCATAGGGAAATT
FsBV2-L-4-F	CCCCATCTCTTTTGATCTGAG
FsBV2-L-4-R	CTTATTGCTCAGTGACAATGG
FsBV2-L-5-F	CATTCCCCTTGATATCGAAGC
FsBV2-L-5-R	CCCGATCACCATTTACCATCC
FsBV2-M-1-F	CTGTGCTGAAATCGGTAAGA
FsBV2-M-1-R	CTGAATCGGATGGTGCAAT
FsBV2-S-1-F	CATGAGCGCTCTATCATTGA
FsBV2-S-1-R	CAGAATGTGATTTGCTGGAAC
FsBV2-L-5' -1	GCTGGCTCAACACGACTGG
FsBV2-L-5' -2	CTCTTGAAGGCGCATACCC
FsBV2-L-3' -1	GGATGGTAAATGGTGATCGGG
FsBV2-L-3' -2	CCTAAAGTACCGGGTTCAGT
FsBV2-M-5' -1	CTTACCGATTTCAGCACAGA
FsBV2-M-5' -2	GTTGACACTGATAAGGGCCGT
FsBV2-M-3' -1	CAGAAGTGATGAGTCTAACGC
FsBV2-M-3' -2	GATTGCACCATCCGATTCAG
FsBV2-S-5' -1	CCATCAATGATAGAGCGCTC
FsBV2-S-5' -2	CTCGACGGTGGTGAAGCTAG
FsBV2-S-3' -1	CAGGTTGACGAAAGAGCTATC
FsBV2-S-3' -2	CGGAGTGGATAAAGTAGCAAT
PC2	CCGAATTCCCGGGATCC

Table S1. Primiers are used to detect and obtain the terminal sequences of FcMV1

PC3-T7 loop	GGATCCCGGGAATTCGGTAATACGACTCACTATATTTTTAT AGTGAGTCGTATTA-OH

Table S2. List of viruses used for multiple alignments and phylogenetic analyses

Accession	Virus
QEQ51085.1	European_shore_crab_virus_1
YP_010839952.1	Chinese mitten crab virus 1
APG79346.1	Wenling crustacean virus 9
WID88835.1	Jujube yellow mottle-associated virus
YP_010840109.1	Emaravirus camelliae
UKM63413.1	Actinidia chlorotic ringspot-associated virus
YP_010839677.1	blackberry leaf mottle-associated virus
QFX79486.1	Orthohantavirus jejuense
AAK01302.1	Orthohantavirus hantanense
QFX79485.1	Orthohantavirus jejuense
XNY85472.1	Orthohantavirus chocloense
YP_010839614.1	Nepuyo virus
YP_010839607.1	Apeu virus
YP_010839939.1	Triniti_virus
YAS81774.1	Oropouche_virus
UHR49875.1	Fushun_phasmavirus_1
UHR49914.1	Sanya_sesamia_inferens_phasmavirus_
UHK03329.1	Hangzhou_frankliniella_intonsa_phasmavirus1
UHK03218.1	Hangzhou_zicrona_caerulea_phasmavirus_1
YP_010840748.1	Guagua_virus
WGT05641.1	Capsicum_chlorosis_virus
XKX17719.1	Limonium_orthotospovirus_1
UZN89652.1	Orthotospovirus_iridimaculaflavi
YDC15388.1	Orthotospovirus_tomatomaculae
YP_010800386.1	Tulasnella_bunyavirales-like_virus_1
YP_010086250.1	Dante_Muikkunen_virus_1
AZI72712.1	Peto_jauhoksi_virus
AZI72710.1	Hipoen_jatkoon_virus
XNW50211.1	Mecsek_Mountains_virus
YP_010840346.1	Plasmopara_viticola_lesion_associated_mycobunyavirales-like_virus_8
YP_010840285.1	Penicillium_discovirus
WPV70716.1	Dothistroma_septosporum_bunya-like_virus_2
QDB75015.1	Coniothyrium_diplodiella_negative-stranded_RNA_virus_1

Table S2. List of viruses used for multiple alignments and phylogenetic analyses

DBA65348.1	Epipactis_associated_konkovirus_2
WMB96341.1	Leptomonas_pyrrhocoris_leishbunyavirus_2
BEK62765.1	Culex_leishbunyavirus_3
UPT53676.1	Bactrocera_correcta_trypanosomatid_leishbunyavirus
XOD28390.1	Leishmania_braziliensis_leishbuvirus_1
YP_009345128.1	Hubei_myriapoda_virus_5
WVT44961.1	Iricinus_orthonairovirus
WNT71169.1	Sulina_virus
BFR46931.1	Iwanai_Valley_virus
XZG29283.1	Beiji_nairovirus
CAI5383902.1	Brown_algae_phenui-like_virus
WON20240.1	Severe_fever_with_thrombocytopenia_syndrome_virus
UUT43438.1	Citrus_virus_A
UHM27566.1	Sanya_phenuivirus_1
YP_010784671.1	Tomato_chlorotic_spot_virus
UHK03059.1	Guiyang_Tospo-like_virus_1
XKX17719.1	Limonium_orthotospovirus_1
UKB93130.1	Turtle_fraservirus_1
ALD89133.1	Rhizoctonia_solani_negative-stranded_virus_4
AUW34408.1	Ixodes_scapularis_associated_virus-6
PVU91452.1	Smittium_simulii
QBH98883.1	Yunnan_manyleaf_rhizome_virus
QDB75018.1	Cladosporium_cladosporioides_negative-stranded_RNA_virus_2
QGY72638.1	Plasmopara_viticola_lesion_associated_mycobunyavirales-like_virus_1
QGY72640.1	Plasmopara_viticola_lesion_associated_mycobunyavirales-like_virus_3
UYL95511.1	Nanning_tick_virus_3
UYL95517.1	Heihe_tick_virus_1
WZH61623.1	Sonemauk_virus
XKT98607.1	Pestalotiopsis_mangiferae_bunyavirus_1
XUK79380.1	Cheeloo_tick_associated_virus_2
YP_009666319.1	Wuhan_Millipede_Virus_2
YP_009337854.1	Shahe_yuevirus-like_virus_1

Viral sequence

FsBV2-L:

TACTACAAATACCGGCAACAATAGTTATCGCAAATGAACCCTTTTCGACACTCGAACTCGCATCGAA

FsBV2-M:

ACTACAAATACCCCTTTTAGCAAACTATTCAAAACTTCACCGAGGAGATAACCGAAATTGAACCCTGA

FsBV2-S:

ACTACAAATACCCCACAGAGACAAAACGAACAATGCACATTGCCTAAACCCGCTGAGATGAACGCCAC