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Southern rice black-streaked dwarf virus: A new proposed *Fijivirus* species in the family *Reoviridae*

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For the past several years, a novel dwarf disease has been observed on rice (*Oryza sativa*) in some regions of Guangdong Province and Hainan Province, southern China. Infected plants showed stunting, dark leaf and small enations on stem and leaf back. Typical *Fijivirus* viroplasma containing crystalline arrayed spherical virons approximately 70—75 nm in diameter and tubular structures were detected in ultrathin sections by an electron microscope in parenchyma phloem cells of the infected plants. The virus was transmitted to rice seedlings by white-backed planthoppers, *Sogatella furcifera* (*Hemiptera*: *Delphacidae*), collected in the diseased fields. Analysis of dsRNA extracts from infected plants revealed ten linear segments, which were similar to the electrophoretic profile of *Rice black-streaked dwarf virus* (RBSDV). RT-PCR with a single primer which matched to a linker sequence ligated to both 3′ ends of the viral genomic dsRNAs resulted in amplification of genome segments 9 (S9) and 10 (S10) cDNA products. The complete nucleotide sequences of S9 and S10 were obtained from clones of the RT-PCR amplicon exhibited characteristic properties of *Fijivirus* including low GC content (34.5% and 35.6%), genus conserved 5′ and 3′ termini sequences and similar genome organization. Blast searches indicated that the sequences of S9 and S10 shared 68.8%—74.9% and 67.1%—77.4% nucleotide identities with those of viruses in the *Fijivirus* group 2, respectively. These values were similar to those among other viruses in the *Fijivirus* group 2 and considerably lower than those among RBSDV isolates. Phylogenetic trees based on S9 and S10 nucleotide sequences and their putative amino acid sequences showed that this virus represented a separate branch among other *Fijiviruses*. The virus was also detected by a nested RT-PCR assay in corn (*Zea mays*), barnyard grass (*Echinochloa crusgalli*), *Juncellus serotinus* and flaccidgrass (*Pennisetum flaccidum*) in and/or adjacent to the infected rice fields. It is proposed that this virus be considered as a new species, Southern rice black-streaked dwarf virus, in the group 2 of the genus *Fijivirus* in the family *Reoviridae*.

southern rice black-streaked dwarf virus, rice, *Fijivirus*, Reoviridae, genome sequence

Rice black-streaked virus (RBSDV) is a species of the genus *Fijivirus* in the family *Reoviridae*[1]. It has icosahedric particles approximately 70—75 nm in diameter that contain 10 genome segments of double-stranded RNAs (dsRNA), designated as S1—S10 in increasing order of electrophoretic mobility in polyacrylamide gels. Small brown planthopper, *Laodelphax striatellus*, is its main natural vector, and *Unkanodes sapporonus* and *Chilodephax albifacia* can also transmit the virus with a relative low efficiency[2—4]. In the genus *Fijivirus*, 8 species, *Fiji disease virus* (FDV), *Oat sterile dwarf virus* (OSDV), *Garlic dwarf virus* (GDV), *Nilaparvata lugens reovirus* (NLRV), *Mal de Río Cuarto virus* (MRCV), *Pangola stunt virus* (PaSV), *Maize rough dwarf virus* (MRDV) and RBSDV were recognized[1]. Among them,

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the last four species were clustered as a group, named group 2, for they shared similar biological and genomic characteristics and closely serological relationship, whereas other 4 species formed 4 groups uniquely[1,4].

RBSDV prevailed severely on rice and maize in eastern and northern parts of China during 1960s and 1990s[2,3]. However, the virus had not been recorded in southern China until recently, possibly due to the absence of vector insects. In 2001, a rice disease was observed in Yangjiang, Guangdong Province. The infected plants exhibited stunting, dark leaf and small enations on stem and leaf back that were similar to those caused by RBSDV but did not form any up-growing rootlets on the stem nodes. RT-PCR with a pair of primers based on available RBSDV S10 sequences amplified an 850-bp product and sequence obtained from PCR clones shared only 80% nucleotide identity with that of RBSDV. The virus was then determined as a new variant of RBSDV[5]. Since then, the disease spread rapidly and has been observed in several counties in Guangdong and Hainan provinces. The disease incidence was less than 2% in most fields, but occasionally, over 80% rice plants were infected. In this paper, we report the virus particle morphology, its natural plant hosts range, insect vectors, viral dsRNA electrophoretic profile and the complete sequences of viral genome segments 9 (S9) and 10 (S10). Based on these data, we propose here that this virus should be considered as a new species, Southern rice black-streaked dwarf virus (SRBSDV), in the genus *Fijivirus*.

1 Materials and methods

1.1 Origin and maintenance of SRBSDV isolate

The rice plants infected with SRBSDV were collected from a field in Dongao city, Hainan province, southern China, during the autumn season of 2003. The virus was transmitted to seedlings of the seedlings of rice cv. Jiuyugui 99 by white-backed planthoppers, *Sogatella furcifera* (*Hemiptera: Delphacidae*), and maintained in a greenhouse.

1.2 Electron microscopy

Small pieces of infected rice leaf ribs were embedded in Spurr resin after fixed with glutaraldehyde and osmium tetroxide. Ultra-thin sections were obtained, stained with uranyl acetate and lead citrate, and viewed with a PHILLIPS-400 electron microscope.

1.3 RT-PCR for virus detection

Forty pairs of primers, 4 for each segment, were designed according to available sequences of RBSDV genome S1—S10 in GenBank database and tested. Total RNA was extracted from plant leaves or insect body using RNA Isolation Kit I (Sino-American Biotech., China) according to the manufacture's instruction. RT-PCR was performed with One-Step RNA PCR Kit (TaKaRa, Dalian, China) by the method described previously[5]. The RT-PCR products were analyzed by electrophoresis on a 1.2% agarose gel containing ethidium bromide and visualized in a UV transilluminator. The expected products were excised from the gels and purified with Qiagen Gel Extraction Kit (Qiagen, Germany), cloned into pDM18-T vector (TaKaRa). Plasmid DNA was prepared with PureLinkTM Quick Plasmid Miniprep Kit and sequenced (Invitrogen Inc., Guangzhou, China). The sequence obtained was then used to design specific primers for the SRBSDV detection in a nested RT-PCR assay.

1.4 Screen for natural plant host of SRBSDV

Leaf samples of crops and weeds in the families *Gramineae* and *Cyperaceae* were collected from the diseased rice fields and/or adjacent fields in Hainan and Guangdong provinces from August to October during 2007. Total RNA extracts were prepared and used in the nested RT-PCR for the SRBSDV detection as mentioned above. To confirm the detection results, some amplified products were directly sequenced. The symptoms of tested plants were also investigated and described.

1.5 Transmission by planthoppers

Transmission experiments were conducted in a greenhouse using white-backed planthopper, brown planthopper (*Nilaparavata lugens*) and small brown planthopper collected from rice fields. Adult insects of white-backed planthopper and brown planthopper were collected in Guangdong province and small brown planthopper from Jiangsu Province of eastern China, respectively, and reared over two generations on rice seedlings in a greenhouse at 20—25℃. Nonviruliferous planthopper populations were determined after testing the rice plants on which they fed and some hopper individuals as SRBSDV-free by the nested RT-PCR. Planthopper nymphs were placed on diseased source rice plants (cv. Jiuyugui 99) for an acquisition access period of 7—10 d. Rice seedlings at different growth stages and

maize seedlings at three-leaf stage were used for vector transmission tests. Each plant was caged with various number of viruliferous adult or 4—5th instar nymph hoppers for 3 d before insecticide was sprayed. The inoculated plants were tested by the nested RT-PCR for SRBSDV infection two weeks later.

1.6 Extraction and electrophoresis of viral dsRNA

Viral dsRNAs were extracted from naturally infected rice leaves using the method described by Dodds et al.[6]. The dsRNA preparation was digested by DNase I and RNase A (TaKaRa), then analyzed on a 1.0% agarose gel. Electrophoretic profile of SRBSDV dsRNA was compared with those of RBSDV dsRNA kindly provided by Dr. LI DaWei and YU JiaLing , China Agricultural University.

1.7 Cloning and sequencing of complete SRBSDV S9 and S10 genome segments

To obtain the complete sequences of SRBSDV S9 and S10, a sequence-independent PCR amplification strategy[7,8] was exerted. Briefly, dsRNA of S9 and S10 were gel purified from the total dsRNA extracted from leaves of the infected plants after electrophoresis. An oligodeoxyribonucleotide primer, P-linker (5′-PO_4-ttcctatgcagctgatcactctGTGTCAGTTCCAGTCACGACA-NH_2-3′), was ligated to the 3′ ends of both strands of viral S9 and S10 dsRNA using T_4 RNA ligase (TaKaRa). Unligated oligonucleotides were removed by spin column chromatography on cellulose nitrate CF-11. P-linker-tailed viral genomic dsRNA was denatured by heating to 90℃ for 5 min in the presence of primer P-ret (5′-tgtcgtgactggaactgacac-3′, complementary to the 3′ end of P-linker) and cooled rapidly on ice. cDNA was obtained with reverse transcripase XL (AMV) (TaKaRa) and clarified with chloroform:isoamyl alcohol (24∶1). PCR was then performed using a single primer, P-dsr (5′-ggaactgacacagagtgatcagctgcat-3′, complementary to the 5′ end of P-linker) by LA *Taq* DNA polymerase (TaKaRa). The annealing temperature was 65℃ and the extension time was 2 min for the reaction. The PCR products were gel purified with Gel Extraction Kit (Sangon, Shanghai, China) from a 1.0% agarose gel after electrophoresis and cloned into pMD18-T vector (TaKaRa). At least 3 clones for each segment were sequenced from both directions (Invitrogen). Nucleotide sequences were assembled and analyzed with Lasergen DNAStar software. Nucleotide composition, ORFs and their putative polypeptides were calculated with EditSeq software. The search for sequence homologies was conducted using the NCBI BLASTn program (http://www.ncbi.nlm.gov). Multiple sequence aligment analysis of SRBSDV S9 and S10 and different *Fijivirus* was performed using Clustal W algorithm. The phylogenetic trees were constructed with MegAlign and Neighbor-Joining method.

2 Results

2.1 Virus particle morphology and cytopathology in infected rice plant

In ultra-thin sections from infected rice plant, typical *Fijivirus*-like particles and cytopathological structures[3,9] were observed in the phloem cells. Numerous inclusions, named viroplasm, were present in infected cells. Most of icosahedric particles with 70—75 nm in diameter aggregated into crystalline array in the viroplasms although a few individual particles were found close to the cell wall in the phloem cells. Occasionally, tubules full of virus particles were also observed (Figure 1).

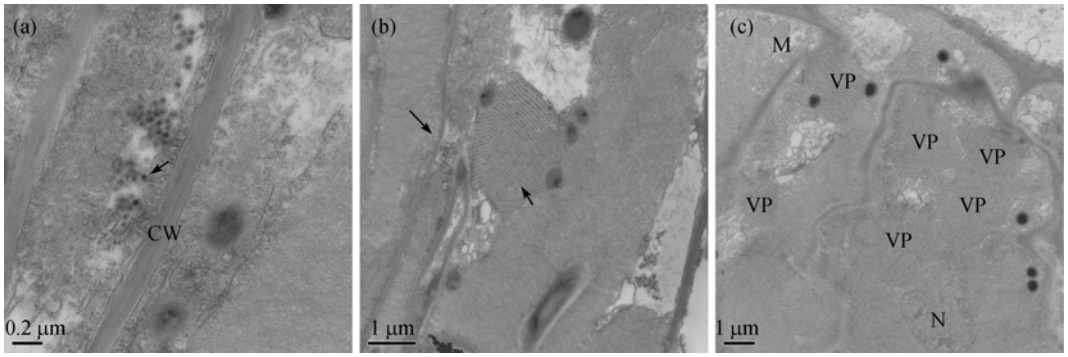

Figure 1 Ultra-thin sections of rice vein cells infected with SRBSDV. (a) Individual virus particles appeared in the cytoplasm near the cell wall (arrowed); (b) virus particles arrayed in row inside of transverse tubule (arrowed) and numerous virus particles aggregated into crystalline array in the viroplasms (arrowed); (c) viroplasms full with virus scattered in the cytoplasm. CW, cell wall; N, nucleus; VP, viroplasm; M, mitochondrion.

2.2 Specificity and sensitivity of nested RT-PCR for SRBSDV detection

Out of 40 pairs of primers tested, only one pair, PS10-1 (5′-ccgaccaacaatcactctgt-3′) and PS10-2 (5′-ggtcagttcgtattcatcgg-3′), amplified an 855-bp product from the total RNA extracted from rice leaves naturally infected with SRBSDV in the RT-PCR. The sequence obtained from the PCR clones shared about 80% similarity to those of different strains or isolates of RBSDV S10. A pair of inner primers, PS10-A (5′-tatTcAAaGttAtttcCGT-3′) and PS10-B (5′-aCatgaatAgtttcAAGT-3′), was then designed according to this sequence. These 2 primers contained 8 and 7 nucleotides unmatched with reported RBSDV S10, respectively (shown in capital). The two pairs of primers were tested in the nested RT-PCR for the SRBSDV detection in plants and insects. The result showed that the primers PS10-A and PS10-B were specific to SRBSDV, and it only amplified a 525-bp product from rice plants or planthoppers infected with SRBSDV (Figure 2). Sensitivity test showed that the nested RT-PCR could detect SRBSDV from 10^{-4} dilution of the total RNA extracted from 200 mg of leaf tissue and 10^{-1} dilution from a single white-backed planthopper (data not shown). All detection results were confirmed by the product direct sequencing.

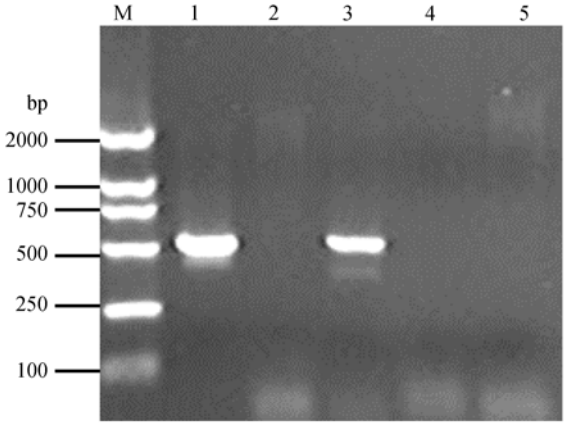

Figure 2 Specific detection of SRBSDV by a nested RT-PCR. M, Molecular marker; 1, rice infected with SRBSDV; 2, healthy rice; 3, planthopper infected with SRBSDV; 4, SRBSDV-free planthopper; 5, RBSDV dsRNA.

2.3 Natural plant host

Symptoms of stunting, leaf roll and/or dark green were observed on maize, barnyard grass and *Juncellus serotinus* grown in and/or adjacent to the diseased rice fields. Most plants exhibiting symptoms were SRBSDV positive by the nested RT-PCR. Some plants of flaccid grass were also SRBSDV positive, although they did not show any typical virus infecting symptoms during investigation period. Symptoms were not observed and the virus was not detected in *Leptochtva chinemis, Alopecurus aequalis, Eleusine indica* and *Digitaria sanguinalis* (Table 1).

2.4 Vector transmission

Results of the nested RT-PCR detection revealed that 60% of the white-backed planthoppers collected from the rice plants naturally infected with SRBSDV were the virus positive. However, the brown planthoppers from the same plants were SRBSDV negative.

Three different planthoppers, white-backed planthopper, brown planthopper and small brown planthopper, were tested for SRBSDV transmission. The results indicated that both white-backed planthopper and small brown planthopper could transmit the virus between rice seedlings (Table 2). The transmission effeciency of white-backed planthopper was higher than that of small brown planthopper. The younger the rice seedling was, the higher the transmission efficiency was shown. Furthermore, the former can transmit the virus from rice to maize, but the later failed.

2.5 Electrophoretic profile of viral dsRNA

Viral dsRNA extracted from infected rice leaves cannot be digested by RNase A and DNase I. Its electrophoretic profile was similar to that of RBSDV maize isolate. Therefore, SRBSDV probably has a genome containing 10 segments of dsRNAs, namely S1 to S10, corresponding to their immigration speed in agarose gel electrophoresis from slow to fast, though S2/3/4 and S8/9 are co-immigrated[4,10,11].

Table 1 Symptoms and RT-PCR results on crops and weeds in and/or adjacent to the diseased rice fields[a)]

Plant tested	*Zea mays*	*Echinochloa crusgalli*	*Juncellus serotinus*	*Pennisetum flaccidum*	*Leptochtva chinemis*	*Alopecurus aequalis*	*Eleusine indica*	*Digitaria sanguinalis*
symptom	dwf, dg	dwf, dg	dwf	ns	ns	ns	ns	ns
SRBSDV positive	80% (8/10)	100% (6/6)	100% (5/5)	60% (3/5)	0% (0/5)	0% (0/5)	0% (0/5)	0% (0/5)

a) Investigation date: August to October, 2007; investigation location: Jungzhong, Hainan and Guangzhou, Guangdong. dwf, dwaf; dg, dark green; ns, no symptom.

Table 2 Results of SRBSDV transmission tests between rice and maize by three species planthoppers[a)]

Host plants and their growth stage	No. of hopper inoculated	White-backed planthopper		Small brown planthopper		Brown planthopper	
		Adult	Nymph	Adult	Nymph	Adult	Nymph
Rice							
3－4 leaf	1	44.0% (4/8)	–	–	–	–	–
	2	75.0% (6/8)	–	–	–	–	–
	3	100% (8/8)	87.5% (7/8)	87.5% (7/8)	–	0% (0/8)	0% (0/8)
	4	100% (8/8)	100% (8/8)	100% (4/4)	–	–	–
7－8 leaf	3	100% (8/8)	–	–	–	–	–
Tilling	3	100% (8/8)	–	–	–	–	–
Earing	3	66.7% (4/6)	–	–	–	–	–
Maize							
3－4 leaf	3	75.0% (6/8)	–	0% (0/10)	–	0% (0/10)	–

a) virus source: SRBSDV infecting rice plants at tilling stage showing typical symptoms; access period: 7—10 d; transmission period: 3 d; virus detection: nested RT-PCR. –, not tested.

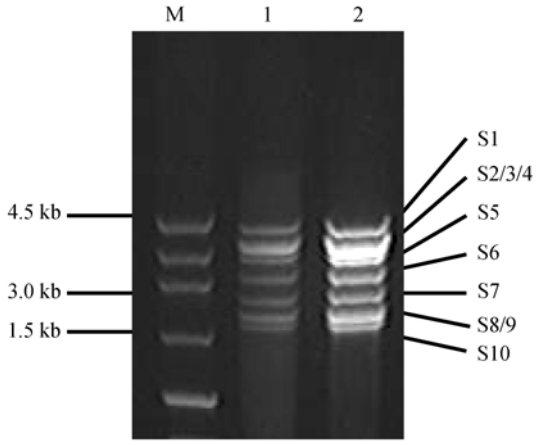

Figure 3 Comparison of electrophoretic profile of SRBSDV and RBSDV genomic dsRNAs. M, Molecular marker; 1, viral dsRNA extracted from rice leaves infected with SRBSDV; 2, dsRNA of RBSDV maize isolate.

2.6 Analysis of SRBSDV S9 complete genomic sequence

The nucleotide sequence of SRBSDV S9 was obtained using sequence-independent amplification, and deposited in GenBank as accession number EU523359. It exhibited the common properties of the genome segment counterparts of Fijiviruses[10−23]. Complete SRBSDV S9 had a total length of 1900 bp and a (G+C) content of 34.53%, which fell within the values obtained in other Fijiviruses from group 2 (30%－37%). The 5′ and 3′ untranslated regions (UTRs) were 51 and 111 bp, respectively. It contained 2 ORFs. ORF1 in the 5′ partial region had a length of 1047 bp. ORF2 in the 3′ partial region had a length of 633 bp. A 64-bp intergenic region presented between ORF1 and ORF2 (Table 3). SRBSDV S9 5′ and 3′ termini sequences, 5′-aagtttttttaagcctg-g……ccggcttacagctgatgtc-3′, showed typical *Fijivirus* character, containing 8 nucleotides at 5′ end and 11 nucleotides at 3′ end conserved in group 2 of *Fijivirus* (shadowed). Next to the conserved nucleotides is an eight-nucleotide imperfect inverted repeats (underlined).

Comparison of the SRBSDV S9 with RBSDV S9 and MRDV S8 revealed 74.2%－74.9% identity at nucleotide level. This value is much lower than those among different RBSDV isolates (>90.5%) and between RBSDV S9 and MRDV S8 (>85.7%), but slightly higher than those among RBSDV S9, MRDV S8 and MRCV S9 (68.4%－68.8%) (Table 4). The amino acid identity comparison between SRBSDV S9 ORF1 and ORF2 with counterpart ORFs showed that the identity were 67.1%－77.4% and 61.6%－73.5%, similar to those among RBSDV, MRDV and MRCV (Table 5).

Phylogenetic tree (Figure 4) based on S9 nucleotide sequence showed that SRBSDV formed a distinct branch with Fijiviruses in the genus *Fijivirus*.

2.7 Analysis of SRBSDV S10 complete genome sequence

SRBSDV S10 complete nucleotide sequence was obtained and deposited in GenBank as accession number EU 523360. This segment contained 1798 bp, identical to MRCV S10, but 2 and 4 bp shorter than those of RBSDV S10 and MRDV S10, respectively. SRBSDV S10, like its S9, also showed the common properties of Fijiviruses counterparts (Table 6).

SRBSDV S10 showed 77.3%－79.1% nucleotide identity with RBSDV S10 and MRDV S10, this figure is much lower than the value among different RBSDV isolates (>93.5%) and between RBSDV and MRDV (87.7%). The nucleotide identity of SRBSDV S10 with MRCV S10 was 72.5%, which was slightly higher than

Table 3 Properties of SRBSDV S9 and homologous segments of determined Fijiviruses

Virus (isolate) and its genome segment	Accession No. in GenBank	Complete length (bp)	(G+C)(%)	UTR(bp)			Length of coded product	
				5′	3′	GIR	ORF1	ORF2
SRBSDV S9	EU523359	1900	34.53	51	111	64	348	210
RBSDV-r-Zhj S9	NC_003731	1900	33.37	51	111	64	348	210
RBSDV-r-Jpn S9	AB011403	1900	34.11	51	111	64	348	210
RBSDV-m-Hub S9	AF536564	1900	33.47	51	111	64	348	210
MRDV S8	L76561	1900	34.32	51	111	64	348	210
MRCV S9	DQ023312	1870	33.74	51	111	64	338	210
FDV S9	NC_007156	1843	32.83	49	102	57	336	209
OSDV S10	AB011027	1761	34.64	50	149	29	316	195
NLRV S9	D49700	1640	33.23	52	38	56	291	207

Table 4 Nucleotide identity between SRBSDV S9 and related Fijiviruses

	RBSDV-r-Zhj	RBSDV-r-Jpn	RBSDV-m-Hub	MRDV	MRCV	FDV	OSDV	NLRV
SRBSDV S9	74.2	74.4	74.0	74.9	68.8	51.3	40.5	35.9
RBSDV-r-Zhj S9		90.5	98.8	86.3	68.6	50.3	40.1	37.8
RBSDV-r-Jpn S9			90.5	85.7	68.8	50.4	39.2	36.9
RBSDV-m-Hub S9				86.4	68.8	50.1	39.7	37.7
MRDV S8					68.4	50.8	40.7	38.5
MRCV S9						51.9	40.4	38.2
FDV S9							42.8	39.8
OSDV S10								44.6

Table 5 Amino acid identities of polypeptides coded by SRBSDV S9 ORF1 and ORF2 with related Fijiviruses[a)]

	SRBSDV	RBSDV-r-Zhj	RBSDV-r-Jpn	RBSDV-m-Hub	MRDV	MRCV	FDV	OSDV	NLRV
SRBSDV S9		77.4	77.1	77.1	77.4	67.1	37.1	26.3	16.7
RBSDV-r-Zhj S9	72.0		93.1	98.0	89.1	65.6	36.4	25.6	15.4
RBSDV-r-Jpn S9	73.0	98.6		92.6	89.1	65.3	37.4	26.0	15.4
RBSDV-m-Hub S9	72.0	99.1	97.6		89.1	64.7	36.4	25.6	15.4
MRDV S8	73.5	94.3	93.4	95.3		62.5	36.7	26.7	14.6
MRCV S9	61.6	63.0	63.0	63.0	63.5		40.3	28.0	17.8
FDV S9	37.8	36.7	36.7	36.7	37.2	33.5		28.0	16.6
OSDV S10	18.0	17.4	16.9	17.4	16.9	16.9	23.3		21.1
NLRV S9	15.0	16.8	16.2	16.8	17.4	18.6	21.6	25.0	

a) Upper-right shows the results of ORF1, down-left shows the results of ORF2.

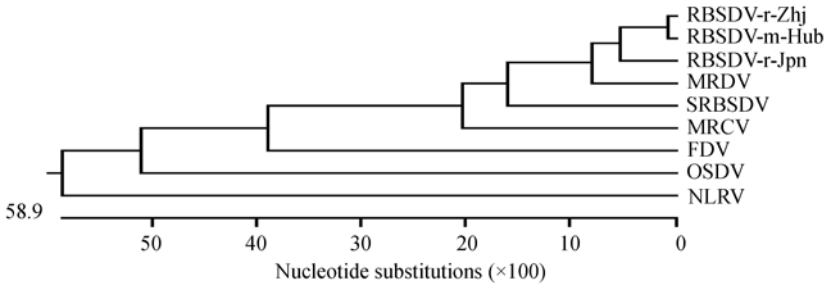

Figure 4 Polygenetic tree based on complete S9 nucleotide sequence of SRBSDV and other related Fijiviruses.

those among RBSDV, MRDV and MRCV (72.1%—73.1%). The comparison result of the deduced amino acid sequence of SRBSDV S10 was similar to that of S9 at nucleotide level (Table 7).

The phylogenetic tree based on SRBSDV S10 complete nucleotide sequence (Figure 5) and putative protein amino acid sequence (data not shown) revealed that the relationship between this virus and RBSDV or MRDV is more distant than that of the two accepted species. Here the results again supported the idea that SRBSDV should be considered as a species different from those already described in genus *Fijivirus*.

Table 6 Comparison of SRBSDV S10 with other related Fijiviruses

Virus (isolate)	Accession No. in GenBank	Complete length (bp)	(G+C)(%)	UTR (bp)		Length of coded product (aa)
				5′	3′	
SRBSDV S10	EU523360	1798	35.6	21	103	557
RBSDV-r-Zhj S10	NC_003733	1801	36.59	21	103	558
RBSDV-r-Jpn S10	D00606	1800	36.28	20	103	558
RBSDV-m-Hub S10	AF227205	1801	36.37	21	103	558
MRDV S10	L76560	1802	36.63	22	103	558
MRCV S10	AY607586	1798	34.71	22	102	557
FDV S10	NC_007162	1819	34.14	23	128	556
OSDV S8	AB011025	1874	33.88	19	88	589
NLRV S8	NC-003653	1802	35.29	6	107	563

Table 7 Nucleotide and putative amino acid identities among SRBSDV S10 and other Fijiviruses[a)]

	SRBSDV	RBSDV-r-Zhj	RBSDV-r-Jpn	RBSDV-m-Hub	MRDV	MRCV	FDV	OSDV	NLRV
SRBSDV S10		78.5	77.3	78.8	79.1	72.5	56.4	45.4	39.6
RBSDV-r-Zhj S10	83.4		93.8	97.1	87.7	72.1	58.4	45.3	39.0
RBSDV-r-Jpn S10	82.3	96.2		93.4	87.7	72.3	58.8	45.1	39.1
RBSDV-m-Hub S10	84.3	98.8	96.6		88.1	72.5	58.6	45.7	38.7
MRDV S10	83.5	92.0	90.9	92.9		73.1	57.4	44.9	39.4
MRCV S10	71.9	71.9	70.7	72.3	71.2		58.2	45.2	39.4
FDV S10	47.6	49.6	49.1	49.6	49.3	49.1		46.5	42.7
OSDV S8	33.1	32.2	31.4	32.4	32.4	32.7	32.8		48.2
NLRV S8	16.9	17.8	17.1	17.5	17.3	17.1	18.8	24.4	

a) Upper-right shows nucleotide identity, down-left shows putative amino acid identity.

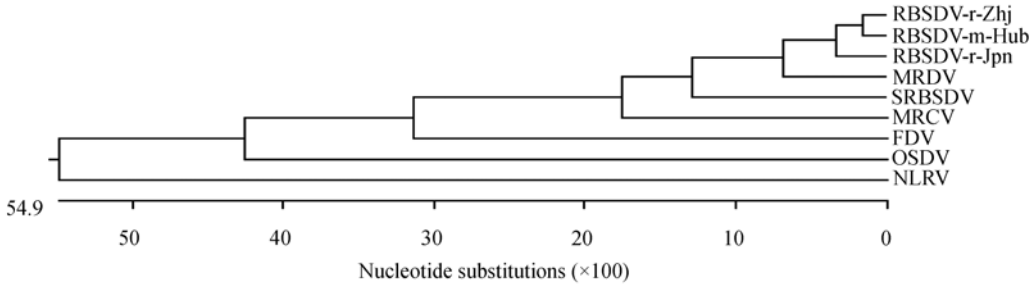

Figure 5 Polygenetic tree based on complete S10 nucleotide sequence of SRBSDV and other related Fijiviruses.

3 Discussion

Based on symptoms, host range, vector transmission, and the S9 and S10 genome segment sequences, the virus infecting rice in southern China should be considered as a new species in *Fijivirus* group 2. A tentative name, Southern rice black-streaked dwarf virus (SRBSDV), was suggested for the following facts: (i) this virus was firstly found in southern region of China; (ii) the symptoms on rice caused by this virus were similar to those caused by RBSDV; and (iii) the distribution area of its major natural vector, white-backed planthopper, extends further south than the small brown planthopper, the vector of RBSDV, in the northern hemisphere.

It is no doubt that SRBSDV should be classed as a member of the genus *Fijivirus*, based on its particle morphology, cytopathology, host range, transmission vectors and genome properties. Although there is not yet defined species demarcation for the genus *Fijivirus*, a new species taxon can be determined by comparing it with other determined species.

Genomic sequence difference was considered as the most important criteria for defining specific taxa between closely related viruses[10,12−17]. For the 4 species currently accepted in the *Fijivirus* group 2, complete sequences of all 10 genome segments of RBSDV and MRCV and S6, S7, S8, S10 of MRDV are available[10,18−20]. In this study, SRBSDV S9 and S10 shared relative low nucleotide identities, approximately 75% and 80%, with viruses in the genus Fijiviruses group 2, whereas these values were 68%—86% and 72%—88%,

respectively, among RBSDV, MRDV and MRCV. Comparison indicated that S10 encoded a putative major outer capsid protein. This protein plays an important role in virus pathogenicity and vector transmission, and is one of the most conserved segments in Fijiviruses. S9 encoded for two non-structure proteins, which are probably involved in viral particle formation[10,11,21]. In theory, viral dsRNA genome has the potential for much variation. In fact, studies on RBSDV gave contrary results. All RBSDV isolates from rice and maize or from different geographical regions showed high conservation, the nucleodite identities among them were >90% for S9 and S10 (Tables 4 and 7). In addition, a study conducted by Giménez-Pecci et al.[22] showed that >96% nucleotide identities for S1, S7, S9 and S10 among 4 MRCV isolates from different regions in Argentina. Jiang et al.[23] also revealed that 25 FDV isolates from Australia, Papua New Guinea and Malaysia had >85% nucleotide identities for S3 and S9. Therefore, it is reasonable to consider SRBSDV as a new species in the *Fijivirus* group 2.

Transmission vectors and their transmission characters are another important criteria in species classification of Fijiviruses[3,4]. It was accepted by many researchers that the selection pressure from vector insect has an important role on the Fijivirus evolution[24,25]. All Fijiviruses can multiply in the body of vector hoppers, except GDV, whose vector is still unknown[4]. These viruses can be recognized as insect viruses. Forth more, NLRV replicates only in its insect host, whereas rice just functions as a transmission vector[12]. In natural condition, all Fijiviruses transmission vectors belonged to planthoppers (family Delphacidae). Genus *Perkinsiella* for FDV, genus *Javesella* for OSDV, genus *Delphacodes* for MRCV, genus *Laodelphax* for RBSDV and MRDV, and genus *Sogatella* for PaSV [4]. In experimental condition, planthoppers in other genus can transmit other Fijivirus, but with a low efficiency[1,26]. In this study, we confirmed that white-backed planthopper, which cannot transmit RBSDV, can efficiently transmit SRBSDV not only between rice seedlings but also from rice to maize. In artificial inoculation test, small brown planthopper can transmit SRBSDV between rice seedlings with a relative low efficiency, but not from rice to maize. Field investigation revealed that the disease was always connected with white-backed planthopper and the nested RT-PCR detection also gave a high SRBSDV positive rate (60%) in the nature population of white-backed planthopper in diseased rice fields. As a conclusion, SRBSDV has an insect vector different from RBSDV and other Fijiviruses.

SRBSDV and other known Fijiviruses were also different in their geographical distributions. RBSDV has been found in Japan, Korea and northern, eastern and center parts of China. MRDV occurred in Europe and Israel. MRCV was exclusively found in Argentina and the nearby regions. PaSV affects some plant species in genus *Digitaria* in Australia and some islands in the Pacific Ocean[4]. SRBSDV reported here is the first *Fijivirus* that occurrs in southern China.

SRBSDV classification should be further confirmed by complete nucleotide sequences of eight other genome segments. In this study, 40 sets of primers matched with RBSDV genome segments (4 for each segment) were tried to amplify SRBSDV fragment with RT-PCR. Most primers gave negative result except one which was based on RBSDV S10. These results implicated that segment 10 probably is the most conversation segment between these two viruses. SRBSDV S9 and S10 were successfully obtained with a sequence-independent PCR strategy, but not other eight segments, possibly due to the fact that other segments are longer and/or have complicated secondary structures. Alternative methods should be invoked to get those segment sequences.

In the past several years, SRBSDV has rapidly spread throughout southern China and caused severe damage on rice and maize in some regions. Great attention should be paid to its potential damage on rice and maize because its vector, white-backed planthopper, is a typical immigration pest in China and many other Asia countries[27,28]. The planthopper has a relative wide plant host range including most of important *Gramminea* crops, which are also SRBSDV potential plant host. The southern China is on its immigration path and one of its overwintering regions[27,29]. The epidemics of this planthopper may cause break out of SRBSDV in southern China and/or other Asia countries.

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