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Genetic variability and evolution of rice stripe virus*

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Abstract: Rice stripe virus (RSV) is the type member of the genus *Tenuivirus*. RSV is known to have four segmented, single-stranded RNA molecules and causes rice stripe disease in the rice fields of China, Japan, and Korea. Based on the complete genomic sequences of the determined 6 RSV isolates (from Yunnan, Jiangsu, Zhejiang, and Liaoning Provinces, China) and 27 other RSV isolates (from Yunnan, Jiangsu, Anhui, Henan, and Shandong Provinces of China, also Japan and Korea) downloaded from GenBank, we provided a genotyping profile of RSV field isolates and described the population structure of RSV. All RSV isolates, except isolate CX, could be divided into two subtypes, one including 6 isolates from Yunnan Province, and the other including 26 isolates from different parts of China, Japan, and Korea, which were referred to as subtype II and subtype I, respectively. The amino acid distances between subtypes range from 0.053 to 0.085. RSV isolates in Yunnan Province were genetically differentiated from other parts of China, Japan, and Korea and showed infrequent gene flow. The RSV populations collected from other parts of China, Japan, and Korea were only composed of subtype I and showed very low genetic diversity. We speculated that isolate CX may be the result of recombination of isolates from two subtypes. Two potential recombination events were detected in RNA4 of isolate CX.

Key words: Rice stripe virus, Genetic variability, Genetic evolution
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1 Introduction

RNA viruses have high mutation rates, mainly due to their error-prone replication and fast rates of genome replication (Domingo and Holland, 1997; Roossinck, 1997; Elena and Sanjuán, 2007; Lauring and Andino, 2010; Sanjuán *et al.*, 2010). Thus in natural populations of RNA viruses, nucleotide variations commonly exist, leading to changes of virus host range, disease symptoms, and emergence of new viruses in nature. Analyzing the polymorphic pattern and selection pressure of these variations will help us to understand the phylogenetic relationships, epidemiological routes, population structures, and underlying evolutionary mechanisms of RNA viruses, and facilitate the development of effective control strategies for plant viral diseases.

Rice stripe virus (RSV) is one of the most damaging rice pathogens in China. It was first identified in a rice field in China in 1963 and has now been identified in 16 provinces of China, causing significant yield losses (Wei *et al.*, 2009; Xiong *et al.*, 2009). RSV is the type member of the genus *Tenuivirus*. It mainly infects rice and a few other species in the family Poaceae including maize, oats, and wheat. RSV is transmitted efficiently by the small brown planthopper (*Laodelphax striatellus*) in a persistent, circulative-propagative manner; it can propagate in the insect vector and is transmitted to its progenies by eggs (Koganezawa, 1975; Falk and Tsai, 1998).

The genome of RSV is composed of four single-stranded RNAs, designated as RNA1, RNA2,

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RNA3, and RNA4, in the order of decreasing sizes (Fig. 1) (Zhu *et al.*, 1991; 1992; Takahashi *et al.*, 1993; Toriyama *et al.*, 1994). RNA1 is negative sense and encodes a putative protein of approximately 337 kDa, which is probably the RNA-dependent RNA polymerase (RdRP) (Toriyama *et al.*, 1994). The other three RNA segments of RSV are all ambisense. RNA2 encodes a membrane-associated protein of 22.8 kDa (NS2) from the viral sense RNA (vRNA) and a polyglycoprotein of 94.0 kDa (NSvc2) from the viral complementary sense RNA (vcRNA) (Ramirez and Haenni, 1994). RNA3 encodes a 23.9-kDa RNA silencing suppressor (NS3) from the vRNA (Xiong *et al.*, 2009) and a nucleocapsid protein of 35.0 kDa (coat protein, CP) from the vcRNA (Kakutani *et al.*, 1991; Zhu *et al.*, 1991). RNA4 encodes a 21.5 kDa protein involved in disease symptom development from the vRNA (Toriyama, 1986) and a 32.5-kDa movement protein from the vcRNA (Xiong *et al.*, 2008).

Aetiology, pathogenesis, ecology, molecular biology, and control strategies of RSV have been studied in recent decades (Falk and Tsai, 1998), and the genetic diversity and population structure of RSV have been studied based on the five gene sequences of RSV, but not on the whole genomic level (Wei *et al.*, 2009). In this study, the sequences of 6 RSV isolates collected from rice fields in Liaoning, Jiangsu, Zhejiang, and Yunnan Provinces of China were determined. The genetic diversity and population structure of the 6 RSV isolates and other isolates with full sequences deposited in GenBank were analyzed. Our data showed that RSV isolates collected from China, Japan and Korea could be divided into two subtypes. All the subtype II isolates were collected from Yunnan Province of China, whereas subtype I isolates were from different parts of China, Japan, and Korea.

2 Materials and methods

2.1 Virus isolates

Rice infected plants with typical RSV symptoms were collected from rice fields in Liaoning, Jiangsu, Zhejiang, and Yunnan Provinces of China. RSV infection of each rice plant was confirmed by reverse transcription-polymerase chain reaction (RT-PCR) using RSV-specific primers and enzyme-linked immunosorbent assay (ELISA) using specific antibodies as previously described (Wang *et al.*, 2004). A virus sample from an individual rice plant was considered as one isolate.

2.2 RT-PCR cloning and genomic sequencing

Total RNA of each rice plant was extracted from leaf tissues using a TRIzol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). RT-PCR reactions were performed using SuperScript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA) and Pfusion High-Fidelity DNA polymerase (New England Biolabs, Ipswich, MA, USA) with specific primer pairs: 5′-ACACAAA GTCCAGAGGAAAACAA-3′ and 5′-ACACATAG TCAGAGGAAAAA-3′ for RNA1; 5′-ACACAAA GTCCTGGGTATATAAGC-3′ and 5′-ACACAAAG TCTGGGTATAACTTCTT-3′ for RNA2; 5′-ACAC AAAGTCCTGGGTAAAATAG-3′ and 5′-ACACAA AGTCTGGGTAATAAAAT-3′ for RNA3; 5′-ACAC

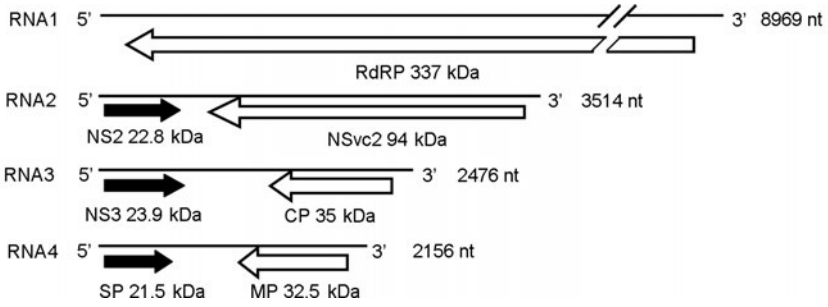


Fig. 1 Genome organization of RSV

Open reading frames (ORFs) were indicated as black arrows on the viral sense RNAs and white arrows on the viral complementary sense RNAs; direction of arrowheads indicated the direction of translation. RdRP: RNA-dependent RNA polymerase; CP: coat protein; SP: disease-specific protein; MP: movement protein; NS2 and NS3: non-structural proteins on the viral sense RNA2 and RNA3; NSvc2: non-structural protein on the viral complementary sense RNA2

AAAGTCCAGGGCATTTGT-3′ and 5′-ACACAA AGTCAGGGCATATCTT-3′ for RNA4. RT-PCR products were purified by using an AxyRep PCR cleanup kit (Axygen, Union, CA, USA). The purified cDNAs were added with 3′-overhang adenine using Taq plus DNA polymerase, inserted into pMD18-T vector (TaKaRa Biotechnology, Dalian, China), and transformed into *Escherichia coli* DH5α. Nucleotide sequencing was performed by the Invitrogen Shanghai Sequencing Department.

2.3 Phylogenetic analysis

Neighbor-Joining (NJ) method (Saitou and Nei, 1987) was used to infer the evolutionary history of RSV. Complete genomes, noncoding sequences, and coding sequences of RSV were used as the database for phylogenetic analysis conducted in MEGA5 (Tamura *et al.*, 2011), respectively. The maximum composite likelihood method (Tamura *et al.*, 2004) was used to compute the evolutionary distances. The tree stability was examined by a bootstrap test of 1000 replicates (Felsenstein, 1985).

2.4 Estimation of genetic distance

The average number of nucleotide substitutions between two randomly selected sequences in a population was referred to as genetic distance. Genetic distances of RSV isolates within and between subtypes were calculated by MEGA5 (Tamura *et al*., 2011). Analyses were conducted using the maximum composite likelihood model (Tamura *et al.*, 2004).

2.5 Estimation of selection pressure

d_n (the number of nonsynonymous substitutions per site) and d_s (the number of synonymous substitutions per site) are used to estimate selection pressure. The P values of models of $d_n \neq d_s$, $d_n > d_s$, and $d_n < d_s$ were estimated separately. Values of P less than 0.05 were considered significant at the 5% level. The variance of the difference was computed using the bootstrap method (500 replicates). Analyses were conducted using the Nei-Gojobori method (Nei and Gojobori, 1986). Evolutionary analyses were conducted in MEGA5 (Tamura *et al.*, 2011).

2.6 Genetic differentiation and gene flow

Two permutation-based statistical tests, Ks* and Z* (Hudson *et al.*, 1992; Hudson, 2000), were used to measure genetic differentiation between RSV population, and Fst (the interpopulational component of genetic variation or the standardized variance in allele frequencies across populations) was used to measure gene flow level. The statistical tests for them were performed by DnaSP 5.0 (Librado and Rozas, 2009). Fst value <0.33 suggests frequent gene flow, while Fst value >0.33 suggests infrequent gene flow.

2.7 Recombination analysis

The recombination analysis was performed by Recombination Detection Program (RDP) 3.44, (Martin *et al.*, 2010). The localization of potential recombination breakpoints, the possible recombination sequences, and the likely parental sequences were analyzed as previously described (Martin *et al.*, 2010), except that the Bonferroni-corrected P value was adjusted from 0.05 to 0.01.

3 Results

3.1 Genotype profile of RSV field isolates

Six RSV isolates were collected from six different rice fields in four provinces of China, and the whole genomes of them were determined (Table 1). Twenty-seven isolates with full genomic sequences of more than two genomic RNAs were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/) (Table 1). Note that isolates GC-JB, SC-CN, HZ, SD-JN2, and CX had one or two RNA sequences undetermined. Phylogenetic analyses of these 33 RSV isolates were performed. NJ trees were constructed by using datasets for four RSV genomic RNAs. It suggested that RSV field isolates fell into two clades except isolate CX (marked with ● in phylogenetic trees). All isolates collected from eastern China, Japan, and Korea formed one of the monophyletic clades, while all isolates collected from Yunnan Province except isolate CX formed the other, referred as subtypes I and II, respectively (Fig. 2). Isolate CX was almost equally distant from the two subtypes (Fig. 2).

We also performed phylogenetic analyses based on sequences of seven genes and the non-coding region of these RSV isolates by using datasets (Figs. 3 and 4). The trees were similar to these constructed by using datasets for genomic RNAs, but isolates CX showed quite peculiar: it fell perfectly in subtype I

when using datasets for non-coding sequences of RNA1, RNA2, RNA4, but fell perfectly in subtype II when using datasets for NS2, SP, and MP; it remained almost equally distant from two subtypes when using datasets for RdRP and NSvc2.

The mean genetic distance of RSV genomic RNAs between two subtypes, within subtype I and subtype II ranged from 0.0703 to 0.0863, 0.0169 to 0.0373, and 0.0256 to 0.0536, respectively (Table 2). The average numbers of nucleotide substitutions between two randomly selected genomic RNA sequences in subtype II were generally higher than those in subtype I, suggesting that RSV isolates in subtype II were more genetically diverse than those in subtype I. The mean genetic distance of RSV genes between two subtypes, within subtype I and subtype II ranged from 0.0529 to 0.0865, 0.0123 to 0.0256, and 0.0183 to 0.0387, respectively (Table 2). The mean genetic distances of RSV genes were generally lower than those of RSV genomic RNAs whether between or within subtypes, suggesting that open reading frame (ORF) sequences were more conservative than non-coding sequences. The mean genetic distances between isolate CX and subtypes I and II ranged from 0.0537 to 0.0779 and from 0.0212 to 0.0698, respectively.

Table 1 NCBI accessions and collected locations of RSV field isolates

Designation	NCBI accession number				Location
	RNA1	RNA2	RNA3	RNA4	
AD-JJ	GQ229086	GQ229099	FJ602684	FJ602697	Korea
BA1-JB	GQ229087	GQ229100	FJ602675	FJ602688	Korea
BA2-JB	GQ229088	GQ229101	FJ602676	FJ602689	Korea
CY-CN	GQ229089	GQ229102	FJ602682	FJ602695	Korea
GC-JB		GQ22910	FJ602679	FJ602692	Korea
GS-JB	GQ229091	GQ229104	FJ602677	FJ602690	Korea
IS-JB	GQ229092	GQ229105	FJ602678	FJ602691	Korea
JD-JN	GQ229093	GQ229106	FJ602681	FJ602694	Korea
MA-JN	GQ229094	GQ229107	FJ602680	FJ602693	Korea
SA-JN	GQ229095	GQ229108	FJ602685	FJ602698	Korea
SC-CN	GQ229096		FJ602683	FJ602696	Korea
WD-JN	GQ229097	GQ229110	FJ602686	FJ602699	Korea
YG-JN	GQ229098	GQ229111	FJ602687	FJ602700	Korea
HZ	AY186788	AY186789		AF513505	Jiangsu, China
T	NC_003755	NC_003754	NC_003776	NC_003753	Japan
SD-JN2			DQ108406	EF538684	Shandong, China
YBS07	EU931498	EU931499	EU931500	EU931501	Yunnan, China
CX	AY186787	AY186790		AY185501	Yunnan, China
YCX07	EU931494	EU931495	EU931496	EU931497	Yunnan, China
YQJ07	EU931502	EU931503	EU931504	EU931505	Yunnan, China
YWD07	EU931506	EU931507	EU931508	EU931509	Yunnan, China
Zhejiang	DQ333942	DQ333943	DQ333944	DQ333945	Zhejiang, China
ABB07	EU931522	EU931523	EU931524	EU931525	Anhui, China
HKF07	EU931518	EU931519	EU931520	EU931521	Henan, China
JY	EF141327	EF141328	EF141329	EF141330	Jiangsu, China
JYC07	EU931514	EU931515	EU931516	EU931517	Jiangsu, China
SJN07	EU931510	EU931511	EU931512	EU931513	Shandong, China
DaL08*	JQ927432	JQ927426	JQ927420	JQ927414	Yunnan, China
HuZ10*	JQ927433	JQ927427	JQ927421	JQ927415	Zhejiang, China
JiangY10*	JQ927434	JQ927428	JQ927422	JQ927416	Jiangsu, China
KunM08*	JQ927435	JQ927429	JQ927423	JQ927417	Yunnan, China
LiaoN08*	JQ927436	JQ927430	JQ927424	JQ927418	Liaoning, China
SuZ10*	JQ927437	JQ927431	JQ927425	JQ927419	Jiangsu, China

* Six isolates sequenced by this work

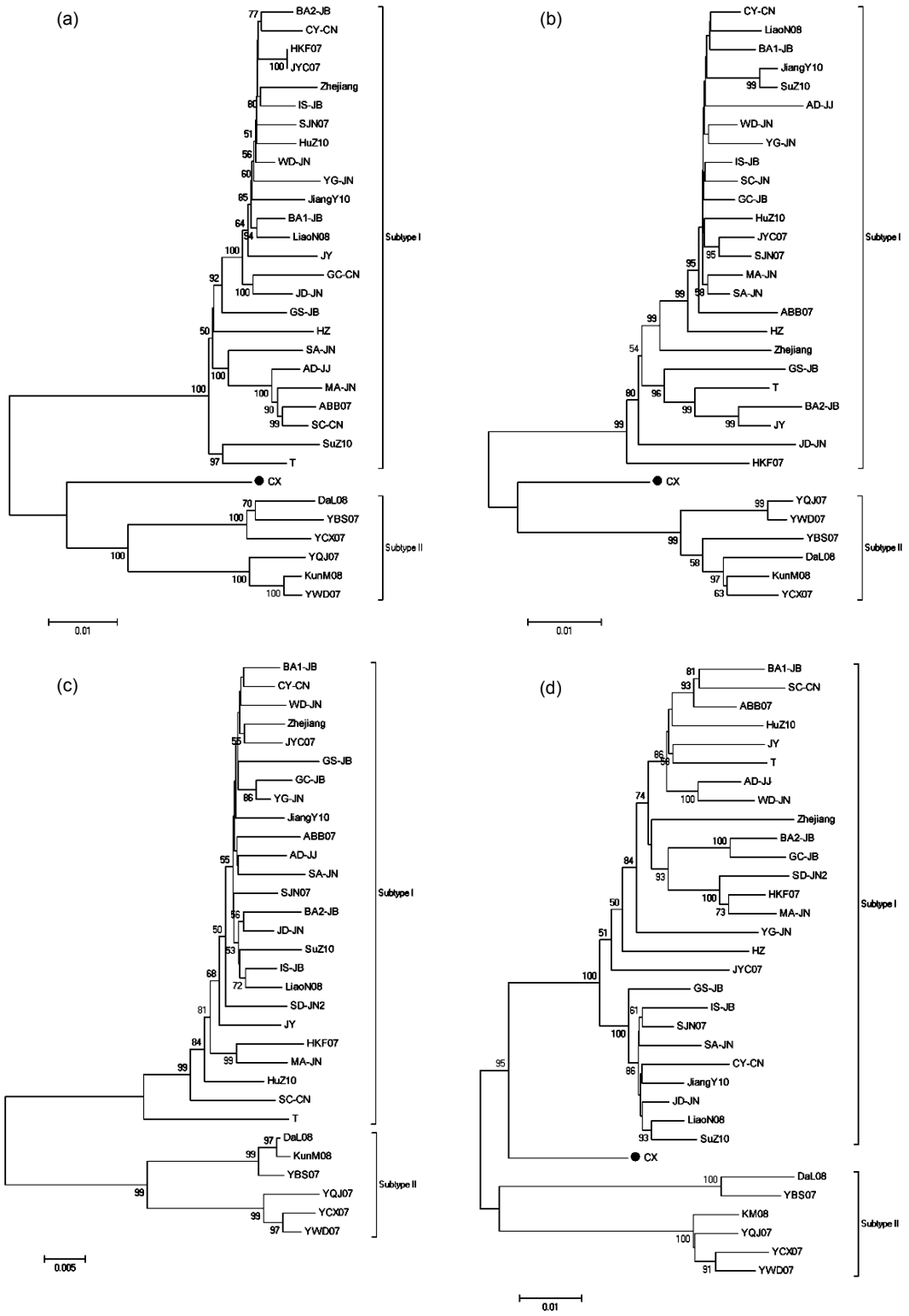
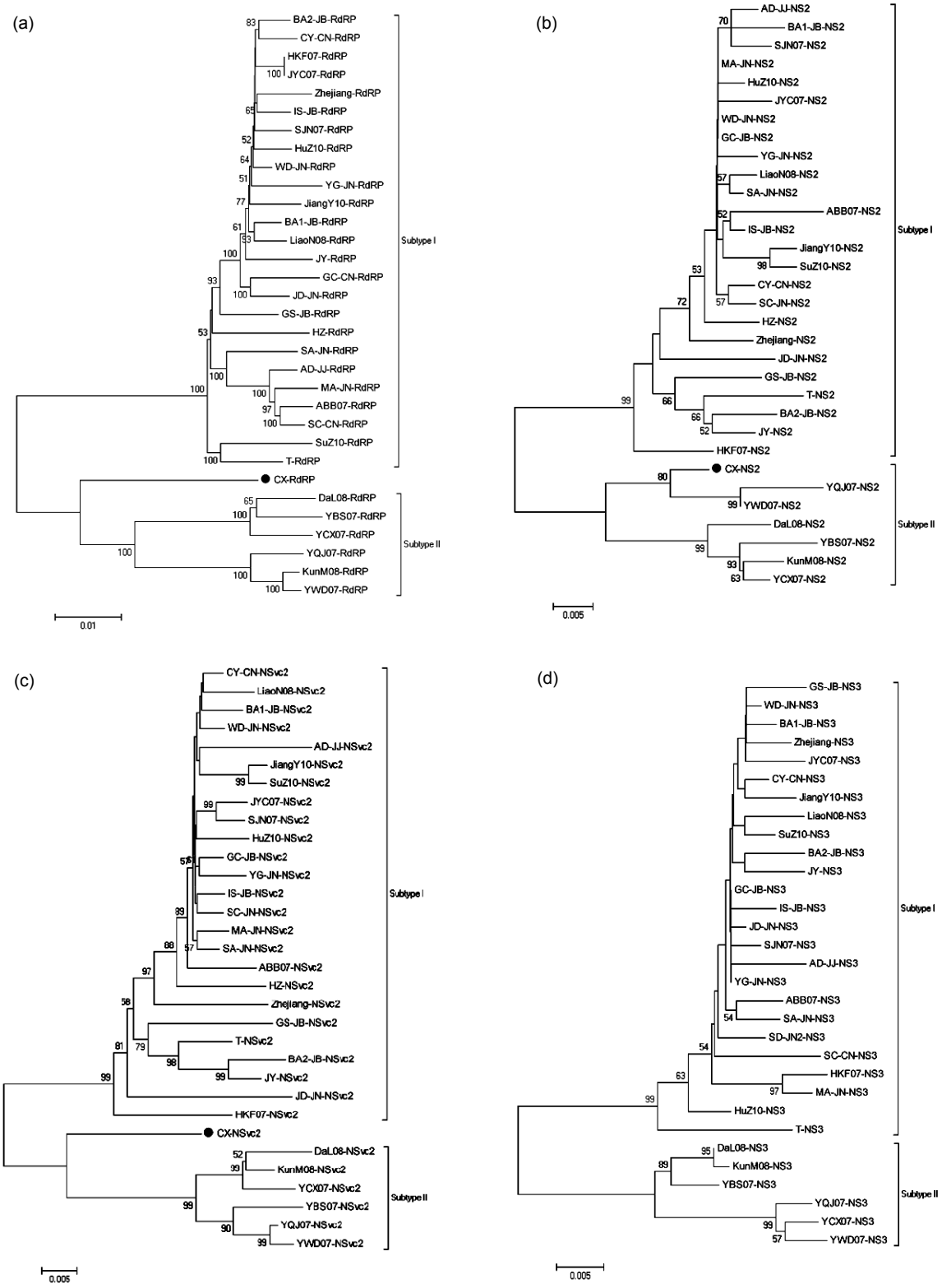
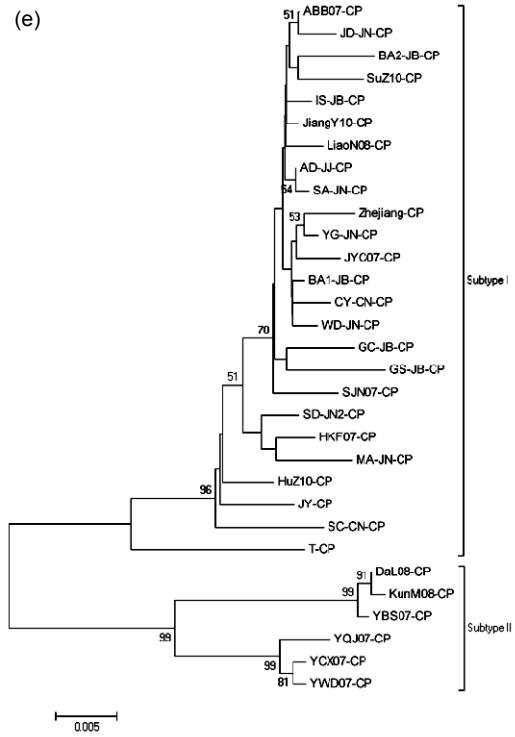
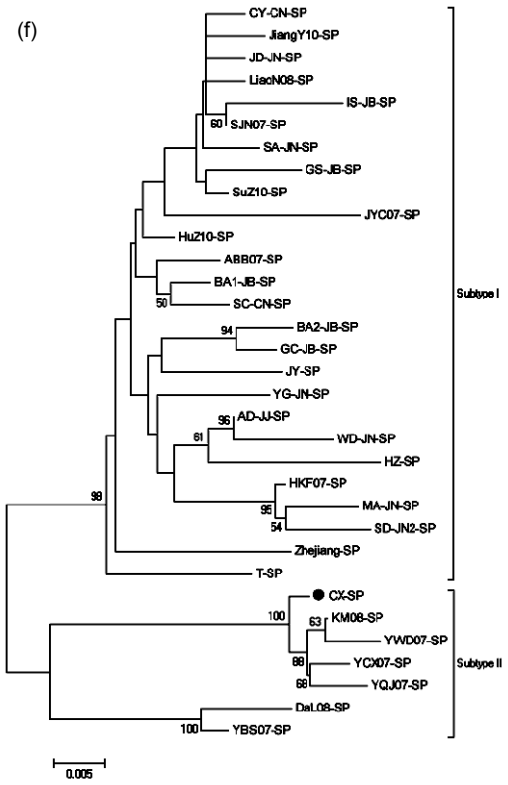


Fig. 2 Phylogenetic analyses of four RSV genomic RNAs conducted in MEGA5

(a) RNA1; (b) RNA2; (c) RNA3; (d) RNA4. Neighbor-Joining (NJ) method was used to infer the evolutionary history. The bootstrap consensus tree inferred from 1 000 replicates was taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). The number next to the branches represented the percentages of replicate trees, in which the associated taxa clustered together in the bootstrap test (1 000 replicates). The branch lengths were drawn corresponding to the evolutionary distances, which were computed using the maximum composite likelihood method


(a)
BA2-JB-RdRP
CY-CN-RdRP
HKF07-RdRP
JYC07-RdRP
Zhejiang-RdRP
IS-JB-RdRP
SJN07-RdRP
HuZ10-RdRP
WD-JN-RdRP
YG-JN-RdRP
JiangY10-RdRP
BA1-JB-RdRP
LiaoN08-RdRP
JY-RdRP
GC-CN-RdRP
JD-JN-RdRP
GS-JB-RdRP
HZ-RdRP
SA-JN-RdRP
AD-JJ-RdRP
MA-JN-RdRP
ABB07-RdRP
SC-CN-RdRP
SuZ10-RdRP
T-RdRP
CX-RdRP
DaL08-RdRP
YBS07-RdRP
YCX07-RdRP
YQJ07-RdRP
KunM08-RdRP
YWD07-RdRP
Subtype I
Subtype II
0.01
(b)
AD-JJ-NS2
BA1-JB-NS2
SJN07-NS2
MA-JN-NS2
HuZ10-NS2
JYC07-NS2
WD-JN-NS2
GC-JB-NS2
YG-JN-NS2
LiaoN08-NS2
SA-JN-NS2
ABB07-NS2
IS-JB-NS2
JiangY10-NS2
SuZ10-NS2
CY-CN-NS2
SC-JN-NS2
HZ-NS2
Zhejiang-NS2
JD-JN-NS2
GS-JB-NS2
T-NS2
BA2-JB-NS2
JY-NS2
HKF07-NS2
CX-NS2
YQJ07-NS2
YWD07-NS2
DaL08-NS2
YBS07-NS2
KunM08-NS2
YCX07-NS2
Subtype I
Subtype II
0.005
(c)
CY-CN-NSvc2
LiaoN08-NSvc2
BA1-JB-NSvc2
WD-JN-NSvc2
AD-JJ-NSvc2
JiangY10-NSvc2
SuZ10-NSvc2
JYC07-NSvc2
SJN07-NSvc2
HuZ10-NSvc2
GC-JB-NSvc2
YG-JN-NSvc2
IS-JB-NSvc2
SC-JN-NSvc2
MA-JN-NSvc2
SA-JN-NSvc2
ABB07-NSvc2
HZ-NSvc2
Zhejiang-NSvc2
GS-JB-NSvc2
T-NSvc2
BA2-JB-NSvc2
JY-NSvc2
JD-JN-NSvc2
HKF07-NSvc2
CX-NSvc2
DaL08-NSvc2
KunM08-NSvc2
YCX07-NSvc2
YBS07-NSvc2
YQJ07-NSvc2
YWD07-NSvc2
Subtype I
Subtype II
0.005
(d)
GS-JB-NS3
WD-JN-NS3
BA1-JB-NS3
Zhejiang-NS3
JYC07-NS3
CY-CN-NS3
JiangY10-NS3
LiaoN08-NS3
SuZ10-NS3
BA2-JB-NS3
JY-NS3
GC-JB-NS3
IS-JB-NS3
JD-JN-NS3
SJN07-NS3
AD-JJ-NS3
YG-JN-NS3
ABB07-NS3
SA-JN-NS3
SD-JN2-NS3
SC-CN-NS3
HKF07-NS3
MA-JN-NS3
HuZ10-NS3
T-NS3
DaL08-NS3
KunM08-NS3
YBS07-NS3
YQJ07-NS3
YCX07-NS3
YWD07-NS3
Subtype I
Subtype II
0.005





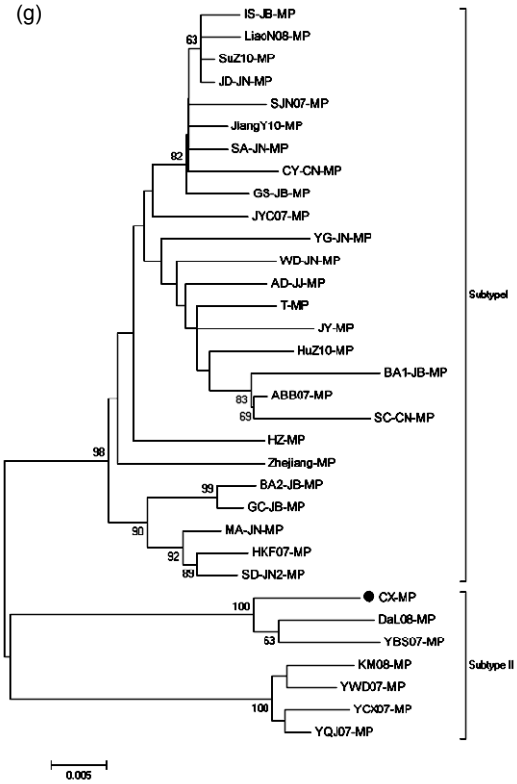


Fig. 3 Phylogenetic analyses of seven RSV genes conducted in MEGA5

(a) RdRP; (b) NS2; (c) NSvc2; (d) NS3; (e) CP; (f) SP; (g) MP. Phylogenetic analyses used the statistical method as in Fig. 2

3.2 Genetic differentiation of Yunnan isolates from other isolates

To compare RSV isolates from Yunnan Province (subtype II) and other places (subtype I), the genetic differentiation of genomes between them was tested using two statistical tests, Ks* and Z* (Hudson *et al.*, 1992; Hudson, 2000). Both *P* values of Ks* and Z* tests between two subtypes were <0.001, which provided significant evidence of genetic differentiation between two subtypes (Table 3). We also used Fst to estimate the extent of genetic differentiation of genomes, which is also an estimate of gene flow. The values of Fst between two subtypes were all greater than 0.33 (Table 3), suggesting infrequent gene flow between RSV population in Yunnan Province and in places other than Yunnan Province.

3.3 Detection of strong negative selection pressures on all RSV genes

The selection pressure on the genes of RSV was estimated by codon-based test using the Nei-Gojobori method (Nei and Gojobori, 1986). The *P* values of models of $d_n \neq d_s$ and $d_n < d_s$ of all the seven genes were <0.001 (Table 4), suggesting that negative pressures

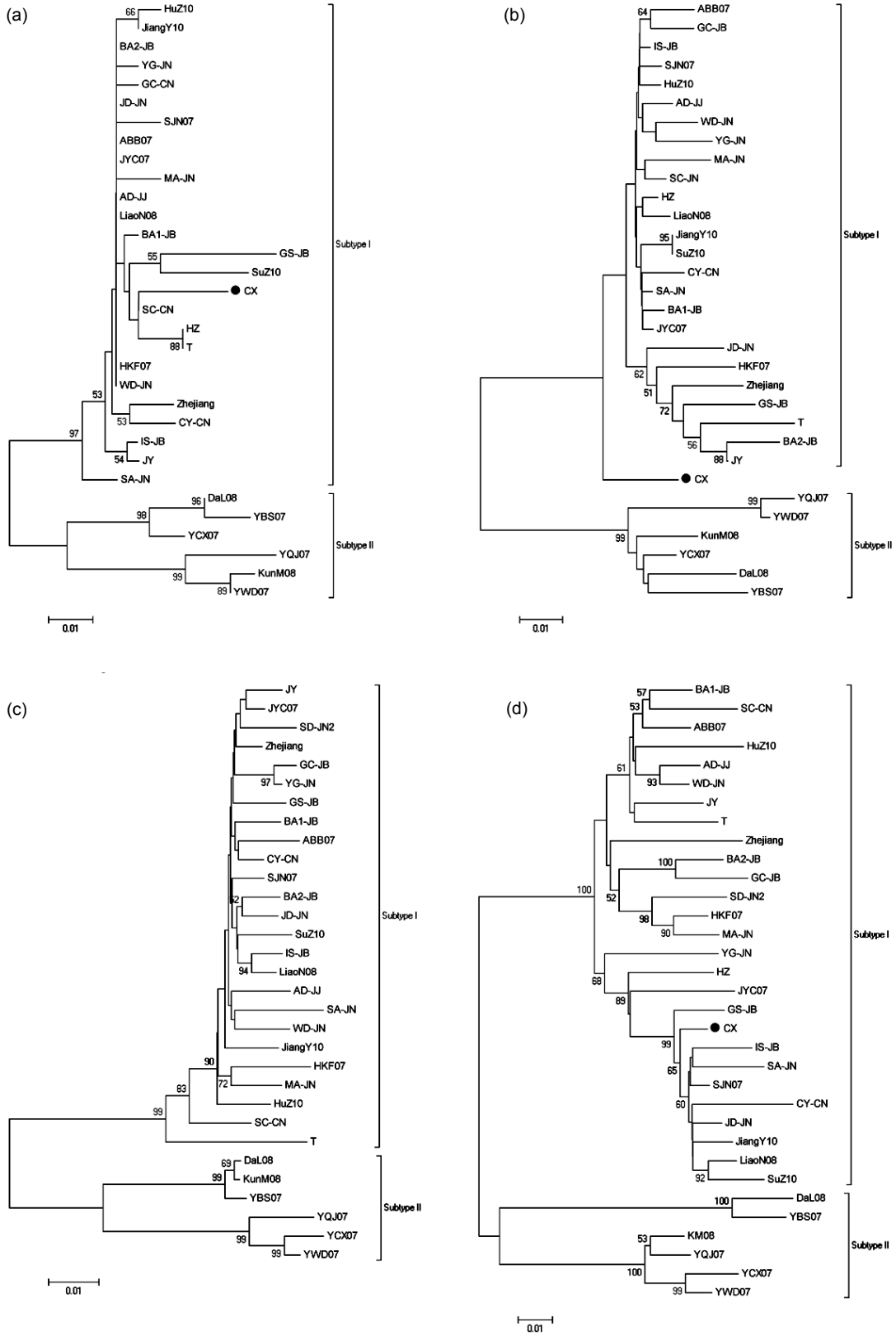


Fig. 4 Phylogenetic analyses of non-coding sequences of four RSV genomic RNAs conducted in MEGA5
(a) RNA1; (b) RNA2; (c) RNA3; (d) RNA4. Phylogenetic analyses used the statistical method as in Fig. 2

Table 2 Genetic distances between subtype I and subtype II and within the same subtype

Sequence	Genetic distance		
	Within subtype I	Within subtype II	Between subtype I and subtype II
RNA1	0.0209±0.0007	0.0383±0.0013	0.0863±0.0027
RNA2	0.0241±0.0012	0.0257±0.0016	0.0780±0.0043
RNA3	0.0169±0.0010	0.0256±0.0023	0.0703±0.0052
RNA4	0.0373±0.0021	0.0536±0.0037	0.0844±0.0054
RdRP	0.0211±0.0008	0.0380±0.0015	0.0865±0.0032
NS2	0.0184±0.0035	0.0270±0.0053	0.0645±0.0110
NSvc2	0.0248±0.0014	0.0226±0.0021	0.0763±0.0053
NS3	0.0136±0.0017	0.0183±0.0037	0.0545±0.0081
CP	0.0123±0.0017	0.0184±0.0034	0.0529±0.0076
SP	0.0256±0.0033	0.0303±0.0054	0.0562±0.0076
MP	0.0220±0.0024	0.0387±0.0045	0.0545±0.0055

Table 3 Genetic differentiation between subtype I and subtype II

Genome	P value[#]		Fst[Δ]
	Ks*	Z*	
RNA1	0.0000	0.0000	0.49108
RNA2	0.0000	0.0000	0.52272
RNA3	0.0000	0.0000	0.51652
RNA4	0.0000	0.0000	0.38509

[#] P<0.001, which was considered as significantly rejecting the null hypothesis that there is no genetic differentiation between two subtypes. [Δ] Fst is a coefficient of the extent of genetic differentiation and provides an estimate of the extent of gene flow. Value of Fst >0.33 suggested infrequent gene flow

Table 4 Estimation of selection pressure on the RSV genes

Gene	Unneutral H1: $d_n \neq d_s$		Positive H1: $d_n > d_s$		Negative H1: $d_n < d_s$	
	$d_n - d_s$	P value*	$d_n - d_s$	P value	$d_n - d_s$	P value*
RdRP	−41.428	0.000	−40.645	1.000	40.874	0.000
NS2	−8.289	0.000	−7.974	1.000	8.160	0.000
NSvc2	−19.296	0.000	−19.970	1.000	19.299	0.000
NS3	−7.509	0.000	−6.998	1.000	7.705	0.000
CP	−9.728	0.000	−9.762	1.000	9.522	0.000
SP	−9.025	0.000	−8.724	1.000	8.901	0.000
MP	−11.444	0.000	−11.814	1.000	12.646	0.000

* P<0.001, which was considered as significantly rejecting the null hypothesis (H0) that there is a neutral selection, to be replaced by alternative null hypothesis (H1)

were detected on all RSV genes. In addition, the estimated $d_n - d_s$ for the RdRP was −41.428 and was the lowest among all the RSV genes (Table 4), which suggested that the RdRP of RSV was under quite strong negative selection pressure.

3.4 Two potential recombination events in RNA4 of isolate CX

The potential recombination events were detected by RDP3.44 (Martin *et al.*, 2010). Among the sequences of 31 analyzed isolates, two potential recombination events were detected in RNA4 of isolate CX. The possible recombination breakpoint position and parental sequences of these two potential recombination events were detected. Potential recombination event 1 showed recombination between an unknown isolate and isolate YBS07 (1206–2074 nucleotides) (Fig. 5). Potential recombination event 1 was detected with a high degree of confidence by seven detection methods: GENECONV (average P value, 2.464×10^{-7}), RDP (average P value, 2.600×10^{-5}), Chimaera (average P value, 2.844×10^{-5}), BOOTSCAN (average P value, 3.137×10^{-6}), SiScan (average P value, 4.449×10^{-7}), Max Chi (average P value, 1.117×10^{-5}), and 3Seq (average P value, 5.882×10^{-16}). Potential recombination event 2 showed recombination between an unknown isolate and isolate YCX07 (572 to 2075 nucleotides) (Fig. 5). Also potential recombination event 2 showed a high degree of confidence with seven detection methods:

GENECONV (average P value, 1.1315×10^{-7}), RDP (average P value, 5.504×10^{-7}), BOOTSCAN (average P value, 1.063×10^{-8}), Chimaera (average P value, 2.340×10^{-4}), SiScan (average P value, 2.876×10^{-8}), Max Chi (average P value, 5.699×10^{-5}), and 3Seq (average P value, 6.591×10^{-7}) (Fig. 5). The data shown by RDP3.44 strongly suggested that isolate CX may derive from two potential recombination events among isolate YCX07, isolate YBS07, and an unknown isolate.

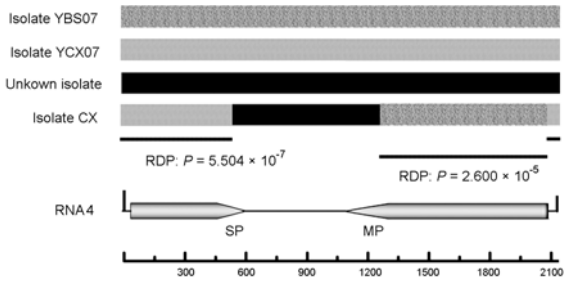


Fig. 5 Schematic representation of the recombinant region of isolate CX

The possible breakpoints are presented, as are the Bonferroni-corrected P values, which indicate that the region does not have a recombinant origin

4 Discussion

In this study, we constructed the phylogenetic trees of RSV isolates collected from different areas in China, Japan, and Korea using full genomic RNA sequences as datasets. Overall, these isolates fell into two monophyletic clades, namely subtypes I and II (Fig. 2). All the isolates from Yunnan Province were in subtype II and all the isolates from other places were in subtype I. We also found that RSV isolates from Yunnan Province were more various and differed from other parts of China. We speculate that the climate and geography of Yunnan Province are not suitable for *Laodelphax striatellus*, the vector of RSV, to migrate long distances.

However, a peculiar isolate (CX) was found to be fluctuant between two subtypes. The isolate CX formed an individual subclade in the phylogentic trees based on RdRP and NSvc2, and fell into the same clade for NS2, SP and MP with the other isolates from Yunnan Province in subtype II (Fig. 3), but fell into the same clade with the isolates in subtype I based on RNA1 and RNA4 non-coding sequences (Fig. 4), suggesting that isolate CX may be derived from either recombination or reassortment of segments between the two subtypes. And the RDP analysis suggested that RNA4 of isolate CX may be derived from two potential recombination events among isolate YCX07, isolate YBS07 and an unknown isolate. Isolate YCX07 and isolate YBS07 were two isolates from Yunnan Province, and thus the unknown isolate was most likely from a geographical origin other than Yunnan Province.

Our data showed that the RSV population collected from Japan, Korea, and some provinces (except Yunnan) of China was only composed of subtype I and showed very low genetic diversity, indicating that the outbreak of RSV in Korea in recent years was not due to the emergence of new strong virulent isolates or invasion of isolates from other districts.

Though RSV populations in Yunnan and other districts were genetically differentiated and showed infrequent gene flow (Table 3), the genetic distance between them was still very low (0.0703 to 0.0863 for RNA segments and 0.0529 to 0.0865 for genes). And all the genes of RSV were under strong negative selection (Table 4). These findings were similar to Wei *et al.* (2009)'s work, in which some RSV isolates collected from Yunnan Province fell into subtype I when using gene sequences as database, while none of these were found in our work. This may be due to the fact that only seven isolates were used in this study with whole genome sequences. With more whole genome sequences of RSV isolates from Yunnan Province and other districts, the population structure and evolution of RSV will be elucidated more accurately.

Compliance with ethics guidelines

Ling-zhe HUANG, Li-xia RAO, Xue-ping ZHOU, and Jian-xiang WU declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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