Infection, Genetics and Evolution 68 (2019) 119–126

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Recombination in lineage 1, 3, 5 and 8 of porcine reproductive and respiratory syndrome viruses in China

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ARTICLE INFO

Keywords:
Porcine reproductive and respiratory syndrome virus (PRRSV)
NADC30-like
VR2332-like
QYYZ-like
JXA1-like
Recombination

ABSTRACT

Porcine reproductive and respiratory syndrome (PRRS) is one of the most important viral swine diseases, resulting in immense economic losses in Chinese pig industry. Currently, four major lineages: lineage 1 (NADC30-like), 3 (QYYZ-like), 5.1 (VR2332-like) and 8.7 (JXA1-like) of type 2 PRRSV (North American type) have been circulating in China based on classification system, which have caused concern about the potential of virus recombination. In the present study, a novel variant of PRRSV strain named FJLIUY-2017 was isolated from abortion rate (25%) in pregnant gilts in Fujian Province in China in 2017. To further our knowledge about the novel virus strain, we characterized the complete genome of FJLIUY-2017. Comparison to PRRS sequences in GenBank confirmed the absence of close relatives (< 92%), but indicated FJLIUY-2017 belonged to NADC30-like PRRSV. The full length of FJLIUY-2017 was determined to be 15017 nucleotides (nt), excluding the poly(A) tail, shared 86.2–86.6% identity with JXA1-like strains (JXA1, TJ and FJYR), 88.9–90.6% with NADC30-like PRRSVs (NADC30, FJZ03 and CHsx1401), 86.4–86.5% with VR2332-like (VR2332, RespPRRS MLV and BJ-4) and only 60.8% with LV (European type). Recombination analyses revealed genomic breakpoints in structural (ORF3, ORF4 and ORF7) and nonstructural (Nsp1, Nsp2, Nsp6, Nsp9, Nsp11 and Nsp12) regions of the genomes with evidence for recombination events between lineages 1, 3, 5.1 and 8.7. Taken altogether, the results of our study provide further confirmation that PRRSV is prone to undergo recombination events. Thus, it is critical to monitor PRRSV evolution in China and establish an effective strategy for the control of PRRS.

1. Introduction

Porcine reproductive and respiratory syndrome (PRRS) is a global viral swine disease causing significant economic losses in the pig industry worldwide (Wensvoort et al. 1992; Neumann et al. 2005; Holtkamp et al. 2013). The etiologic agent, PRRS virus (PRRSV), is a member of the *Arteriviridae*, a family of small, enveloped positive-sense, single-stranded RNA viruses (Cavanagh 1997). The PRRSV genome is approximately 15 kb in length, encoding at least 10 open reading frames (ORF) (Firth et al. 2011; Meulenberg 2000). ORF1a and ORF1b, which comprise about 75% of the genome, encode the viral non-structural proteins (Nsp), which are cleaved to produce at least 16 nonstructural proteins (Nsp1α, Nsp1β, Nsp2, Nsp2N, nsp2TF, Nsp3–6, Nsp7α, Nsp7β, Nsp 8–12) (Fang and Snijder 2010; Fang et al. 2012). ORFs 2 to 7 encode eight viral structural proteins (GP2a, E, GP3, GP4, GP 5a, GP5, M and N) (Meulenberg et al., 2000; Firth et al. 2011; Wu et al. 2005).

PRRSV strains were divided into two distinct genotypes, type 1 (European strains, EU) and type 2 (North American strains, NA). Based on global PRRSV classification systems, type 2 PRRSV was classified into nine monophyletic lineages, 1–9 (Shi et al. 2010). Now, an overwhelming majority of the type 2 PRRSV strains in China can be classified into lineage 1 (NADC30-like), lineage 3 (QYYZ-like), lineage 5.1 (VR2332-like), and lineage 8.7 (JXA1-like) (Gao et al. 2017; Guo et al. 2018; Han et al. 2017; Liu et al. 2017a; Liu et al. 2017b; Liu et al. 2017c; Zhou et al. 2018b). NADC30-like PRRSV is a groups of viruses that show the highest similarity with NADC30 strain emerged in 2012. Genetically, NADC30-like PRRSVs distinguished itself by three discontinuous deletions of a total of 131 amino acids (aa) in Nsp2 including 111-aa deletion at position 322–432, a 1-aa deletion at position 483, and a 19-aa deletion at position 504–522 which could distinguished themselves from other PRRSV strains (Zhao et al., 2015;

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https://doi.org/10.1016/j.meegid.2018.12.006
Received 17 September 2018; Received in revised form 28 November 2018; Accepted 4 December 2018

Table 1
List of primers used in this study.

Fragment	Sequence	Nucleotides	Length of PCR products
Breakpoint 1102	5′-GAATTCATGACGTATAGGTGTTGGCT-3′ 5′- GTACTTGCCGGCAACACCATGCTTG-3′	1-1102[a]	1102 bp
Breakpoint 453	5′- CTTTCACATCCCCTGAGTGTG −3′ 5′- GTGCTTTCCTTGCTCTCTTTC -3′	910-1362[a]	453 bp
Breakpoint 456	5′- CTTCCTGAAAGAGTAAGGCCT-3′ 5′- TTCTTCAAGCACACCTGATCT −3′	1559-2014[a]	456 bp
Breakpoint 885	5′- CAAAGGTTCCGCTTGGCGACAT-3′ 5′- GCCACGGTGTCGGCAAAAGC-3′	5727–6622[a]	885 bp
Breakpoint 1388	5′- CAGAGCACGGTTGCGAGGATG −3′ 5′- GGCAATGCTGGTGAAAATGAGT −3′	7991-9329[a]	1338 bp
Breakpoint 348	5′-TAGGCAACTGCAGAGCCTATT −3′ 5′-GTGCCTAAAAACACCGAAGGG-3′	10345-10782[a]	348 bp
Breakpoint 458	5′-CCGGGGTTGTGTCATACTATC-3′ 5′- AAGCTGGTACCAGGTGAAATG −3′	10785-11242[a]	458 bp
Breakpoint 525	5′-AGCTTGCAAGTTATGCATCGT-3′ 5′-CAATGGACACCAGAAACTCCG-3′	11238–11762[a]	525 bp
Breakpoint 540	5′-TTCCGTTGTCCTCCAAGACAT-3′ 5′-ACTGCGAATAAGAGCTGCTGT-3′	12975–13514[a]	540 bp
Breakpoint 620	5′-TCCACTACTGTTAACGGCACA-3′ 5′- TTTTTTAATTTCGGCCGCATGG −3′	14398–115017[a]	620 bp
1[b]	5′-GGAGGGCCAAGTCTACTGCACACGA-3′ 5′-TGGTTGTGCTCAACCGCGT-3′	245-7464[a]	7220 bp
2[b]	5′-TCTCAGAGCTGGCGACCCT-3′ 5′-CAGGAAACAGCTATGACACCTGATCTCTAGAAACGTT(T)38–3′	7013-15017[a]	8005 bp

[a] position in genome of PCR products with respect to the FJLIUY-2017 genome.
[b] Kvisgaard et al. (2013) (Journal of Virological Methods 193: 697–705.

Table 2
PRRSV strains used in this study.

No.	Name	GenBank accession no.	Origin	No.	Name	GenBank accession no.	Origin
1	FJZ03	KP860909	China	12	QYYZ	JQ308798	China
2	FJYR	KT804696	China	14	SD17–38	MH068878	China
3	TJ	EU860248	China	15	JX143	EU708726	China
4	CH-1a	AY032626	China	16	BJ-4	AF331831	China
5	VR-2332	U87392	China	17	HENNAN-XINX	KF611905	China
6	JXA1-R [a]	R FJ548855	China	18	NADC30	JN654459	U.S.A
7	JXA1	EF112445	China	19	JL580	KR706343	China
8	HUN4	EF635006	China	20	CHsx1401	KP861625	China
9	GM2	JN662424	China	21	RespPRRS MLV	AF066183	U.S.A
10	FJSD	KP998474	China	22	FJZ03	KP860909	China
11	FJFS	KP998476	China	23	TJ	EU860248	China
12	SCcd17	MG914067	China	24	LV	M96262	Netherlands

Zhou et al. 2015; Sun et al. 2016; Li et al. 2017). Now, Multiple PRRSV types co-exist in Chinese swine herds resulting in immense economic losses in the swine industry and they have caused concern about the potential of virus recombination.

Currently, recombination events involving NADC30-like PRRSV strains and other PRRSV strains frequently occurred (Bian et al. 2017; Liu et al. 2017a; Liu et al. 2017b; Liu et al. 2017c; Wang et al., 2018; Zhao et al. 2015; Zhao et al. 2017; Zhang et al. 2016; Zhou et al. 2018b; Zhou et al. 2018c). In July 2017, we isolated a strain FJLIUY-2017 from pig farm in Fujian Province, China. Phylogenetic and molecular evolutionary analyses indicated that FJLIUY-2017 is a natural recombinant events among lineage 1 (NADC30-like), lineage 3 (QYYZ-like), lineage 5.1 (VR2332-like), and lineage 8.7 (JXA1-like).

2. Materials and methods

2.1. Virus isolation

Serum and tissue samples of pigs were collected from the farm with 25% abortion rate in pregnant gilts. Virus was initially isolated from the positive sera on Porcine alveolar macrophages (PAMs) as described as described previously (Balka et al. 2015). After 5 days of incubation, the culture supernatants were harvested when cytopathic effects appeared and were frozen at −80 °C. Real-time RT-PCR and anti-N protein of PRRSV monoclonal antibody analysis applied on the supernatants to detect PRRSV. The isolate was plaque-purified three times and then passaged three times in MARC-145 cells for complete genome sequencing.

2.2. RT-PCR

Total RNA was extracted from tissue of clinical samples (serum, lungs and lymph nodes) and virus using a viral nucleic extraction kit (TIANGEN, Beijing, China) following the manufacturer's instructions. cDNA was constructed by Superscript IV reverse transcriptase (Invitrogen, Carlsbad, CA, USA) according to the supplier's instructions. The complete genomic sequences of FJLIUY-2017 were amplified as described previously (Zhou et al., 2018a). The 5′ and 3′ end of the viral genome was generated using 5′/3′-rapid amplification of cDNA ends (RACE) methods (Takara, Dalian, China). We confirmed the full genome of FJLIUY-2017 using the PCR primers according to Kvisgaard et al. (2013) (Table 1). Additionally, we re-sequencing the flank regions around breakpoints from purified virus and clinical samples. Ten primer pairs (Breakpoint 1102, Breakpoint 453, Breakpoint 456, Breakpoint 885, Breakpoint 1388, Breakpoint 348, Breakpoint 458, Breakpoint 525, Breakpoint 540, Breakpoint 620) used to amplify and

Table 3
Detailed comparison of the full-length genomes of FJLiuY-2017 to other PRRSV reference strains.

	VR2332	RespPRRS MLV	BJ-4	JXA1	TJ	FJYR	FJFS	GM2	QYYZ	NADC30	FJZ03	CHsx1401	SCcd17	SD17–38	LV
	Lineage 5.1 (VR-2332-Like)			Lineage 8.7 (JXA1-like)			Lineage 3 (QYYZ-like)			Lineage 1 (NADC30-like)					
	Pairwise % Identity to FJLiuY-2017 (nt/aa)														
Nucleotides															
Complete genome	86.5	86.5	86.4	86.6	86.6	86.2	84.8	84.7	84.6	90.6	89.4	88.9	89.3	88.8	60.8
5′ UTR	92.5	92.5	92.5	98.4	95.7	97.9	94.1	94.1	94.1	91.5	90.4	88.8	97.3	92.6	61.5
ORF1a	83.3	83.2	83.1	83.3	83.3	82.7	80.8	79.9	80.2	91.0	89.6	89.0	88.0	88.2	55.4
ORF1b	89.4	89.4	89.4	90.6	90.6	90.4	88.1	88.8	87.7	91.5	90.8	90.2	91.7	89.7	63.2
ORF2–7	90.2	90.3	90.2	88.5	88.6	88.3	90.3	90.2	90.7	88.0	87.0	87.0	88.1	88.6	65.6
3′ UTR	91.4	91.4	91.4	91.3	91.3	91.3	86.1	87.4	88.7	94.0	93.9	93.4	92.7	94.0	76.1
Amino acids															
NSP1α	95.8	96.4	96.4	99.4	99.4	98.8	95.2	97.0	97.0	97.6	94.6	95.8	97.0	94.6	66.3
NSP1β	77.9	77.0	77.0	74.7	75.1	75.1	74.7	71.4	71.4	91.2	89.4	86.6	83.4	87.6	45.1
NSP2	68.5	70.4	70.2	68.8	69.1	65.8	68.4	66.0	66.5	88.7	87.6	84.7	82.7	82.2	33.2
NSP3	91.3	91.5	91.3	90.1	90.4	90.4	87.2	87.2	87.2	98.2	96.0	97.1	93.5	96.2	58.4
NSP4	93.6	93.6	93.6	93.6	93.6	93.1	91.7	92.6	92.2	95.1	95.1	96.1	92.6	94.6	63.1
NSP5	91.8	91.8	91.8	91.8	91.8	91.8	88.2	88.8	88.8	95.3	92.9	94.1	90.6	95.3	69.4
NSP6	100	100	100	93.8	93.8	93.8	93.8	93.8	93.8	100	100	93.8	93.8	93.8	81.2
NSP7	90.0	90.0	88.8	95.8	95.0	95.4	91.9	90.0	92.7	84.9	84.2	83.8	95.0	88.4	45.5
NSP8	97.8	97.8	97.8	97.8	97.8	97.8	93.3	97.8	97.8	93.3	93.3	91.1	95.6	88.9	68.2
NSP9	97.2	97.2	96.9	97.8	98.1	97.4	95.5	96.7	96.4	96.7	95.8	95.6	98.1	95.8	74.4
NSP10	95.5	95.5	95.5	93.7	93.9	93.9	92.3	95.7	93.2	98.4	98.2	98.2	97.3	97.7	64.7
NSP11	94.2	94.6	94.6	97.3	96.9	96.4	94.2	94.6	93.7	97.3	95.5	94.2	94.2	96.4	78.0
NSP12	94.1	94.1	94.1	98.7	98.7	98.7	94.1	95.4	94.8	94.8	94.8	93.5	94.1	94.8	44.9
ORF2a/GP2	89.9	90.6	90.2	89.9	89.8	89.5	89.1	92.2	92.6	87.9	87.5	89.8	87.1	89.1	62.7
ORF2b/E	89.0	91.8	91.8	89.0	89.0	87.7	84.9	98.6	98.6	89.0	89.0	86.3	84.9	89.0	75.7
ORF3/GP3	92.1	91.7	92.1	85.8	86.2	85.8	85.0	83.5	84.3	87.0	85.0	86.2	89.4	90.6	57.3
ORF4/GP4	88.8	88.8	87.6	89.3	91.0	89.3	89.3	87.1	87.6	97.8	96.6	95.5	90.4	93.3	70.8
ORF5/GP5	81.0	81.0	81.0	82.5	82.5	83.0	90.5	90.5	92.0	85.5	83.0	84.0	84.4	84.4	55.1
ORF5a	82.6	82.6	82.6	78.3	78.3	73.9	97.8	95.1	95.7	80.4	82.6	80.4	81.9	83.9	46.5
ORF6/M	96.0	95.4	95.4	94.8	94.8	94.3	97.1	96.6	96.6	93.7	94.3	93.7	94.3	93.1	78.6
ORF7/N	91.1	91.9	91.9	90.2	90.2	90.2	91.1	86.2	90.2	91.1	90.2	86.2	88.6	87.8	61.7

subsequently sequence the flank regions around breakpoints 710, 1274, 1829, 6890, 9528, 11806, 11524, 12013, 13283, and 150140 (referenced to VR-2332) (Table 1). The PCR products were purified and cloned into pGEM-T Easy (Promega, Madison, WI, USA); at least three recombinant clones were sequenced by Ruibo Life Technologies Corporation (Beijing, China). The PRRSV isolate was designated FJLIUY-2017.

2.3. *Complete genomic sequence analysis*

To identify the evolutionary relationship of FJLIUY-2017, 24 representative PRRSV strains in GenBank were utilized in sequence alignments and phylogenetic analyses (Table 2). Multiplex sequence alignments were performed using CLUSTAL X (version 1.83). The phylogenetic trees were constructed by neighbor-joining in MEGA 6.0, using maximum composite likelihood and bootstrap confidence values from 1000 replicates.

2.4. *Recombination analyses*

To test for recombination, the alignment was screened using RDP v.4.80 (Martin et al., 2010) as described in Ramos et al. (Ramos et al. 2013). Potential recombination events were tested by seven different algorithms (RDP, GeneConv, BootScan, MaxChi, Chimera, SiScan, and 3Seq) with Bonferroni correction and the highest acceptable *p* value 0.01. Recombinant events were confirmed by Bootscan analysis in SIMPLOT software (Lole et al. 1999) within a 200-bp window sliding along the genome alignment (20-bp step size). To further confirm these putative recombination events, a series of phylogenetic trees for each of the sequence regions identified during the analysis were generated (Boni et al. 2010).

3. Results

3.1. *Complete genomic sequence analysis*

Comparison to PRRS sequences in GenBank confirmed the absence of close relatives (< 92%), but indicated FJLIUY-2017 belonged to NADC30-like PRRSVs. In order to evaluate the genomic characteristics of FJLIUY-2017 strain, the genome of the novel PRRSV isolate was compared with that of the different lineage viruses including JXA1-like PRRSVs (JXA1, FJYR and TJ), VR2332-like PRRSVs (VR-2332, RespPRRSV MLV and BJ-4), NADC30-like PRRSVs (NADC30, CHsx1401 and FJZ03) and QYYZ-like PRRSVs (FJFS, QYYZ and GM2) (Table 3).

The complete genome sequence of FJLIUY-2017 (MG011718) strain was 15017 nt in length, excluding the poly (A) tail at the 3′ end. Genome alignments revealed that FJLIUY-2017 isolate shared 86.2–86.6% identity with JXA1-like PRRSV strains (JXA1, TJ and FJYR), 88.9–90.6% with NADC30-like PRRSV strains (NADC30, FJZ03, and CHsx1401), 84.6–84.8% with QYYZ-like PRRSV strains (FJFS, GM2 and QYYZ), 86.4–86.5% with VR2332-like strains (VR2332, RespPRRS MLV and BJ-4), and only 60.8% with LV, indicating that FJLIUY-2017 strain belonged to the type 2 PRRSV (Table 3).

The results also showed that ORF1a, ORF1b and the 3′-UTR of FJLIUY-2017 shared 89.0–94.0% nucleotide homology with NADC30-like PRRSVs, which was higher than the homology shared with JXA1-like PRRSVs, VR2332-like PRRSVs and QYYZ-like PRRSVs. ORF2–7 of FJLIUY-2017 shared 90.2–90.7% nucleotide homology with QYYZ-like PRRSVs, which was higher than the homology shared with JXA1-like PRRSVs, VR-2332-like PRRSVs and NADC30-like PRRSVs. The 5′-UTR of FJLIUY-2017 was 188 nt in size and shared 95.7–98.4% nucleotide identity with the JAX1-like PRRSVs (JXA1, HLJA1 and NT1), which was higher than that with other representative strains. ORF1a and ORF1b encoding 16 nonstructural proteins of PRRSV, among these

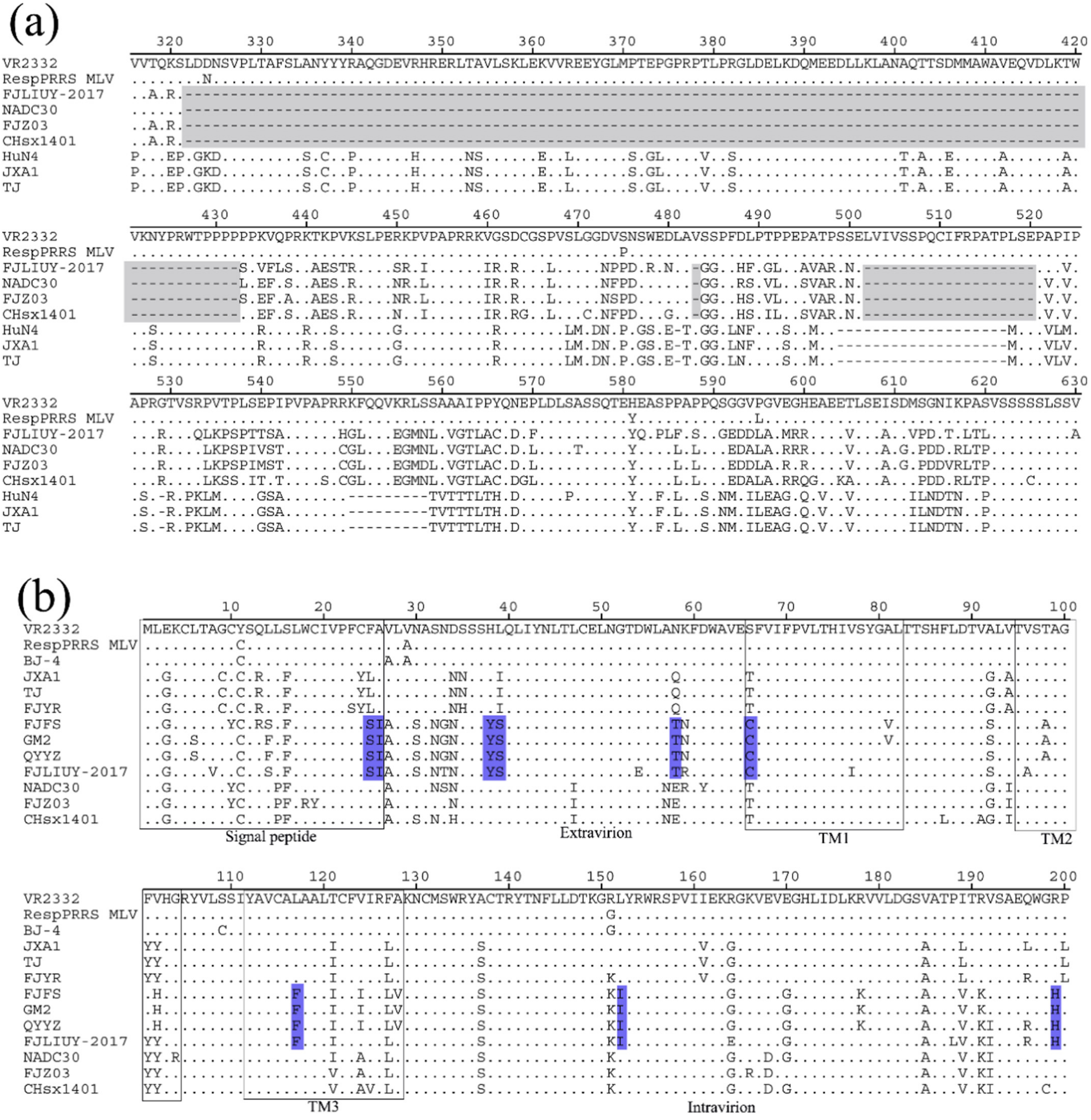

Fig. 1. Analysis and comparison of amino acid mutations in Nsp2 (a) and GP5 (b) compared to reference viruses. (a) In comparison to VR2332, a 131-aa deletions (322–432, 483, and 502–520) is highlighted in dark gray. (b) The signal peptide and transmembrane (TM) domains in GP5 are highlighted by gray rectangles. Important amino acid changes between FJLIUY-2017 and QYYZ-like PRRSVs in GP5 are indicated with dark blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

nsps, Nsp1β and Nsp2 are the most variable protein products. ORFs 2a to 7 encode the PRRSV structural proteins, GP2, GP3, GP4, GP5, and GP5a exhibited the most variance among the structural proteins (Table 3).

3.2. Amino acid analysis of NSP2 and ORF5

Nsp2 is the most variable non-structural protein of PRRSV, and contains different patterns of deletions and insertions, as compared to prototype VR2332. In the present study, the Nsp2 gene of the FJLIUY-2017 was 2547 nt in length and encoded 849 aa, with a discontinuous 131-aa deletion (111 + 1 + 19-aa) compared with the Nsp2 of the type 2 prototype strain VR2332, which is identical to that of NADC30-like strains (Fig. 1A).

GP5 is the major envelope protein encoded by ORF5 and is the most variable PRRSV protein. Sequences alignments of GP5 revealed that FJLIUY-2017 shares 82.5–83.0%, 81.0%, 90.5–92.0%, and 83.0–85.5% amino acid identity with JXA1-like, VR-2332-like, QYYZ-like, and NADC30-like strains, respectively (Table 3). GP5 has six B-cell epitopes (AR1–15, AR27–35, AR37–51, AR149–156, AR166–181, and

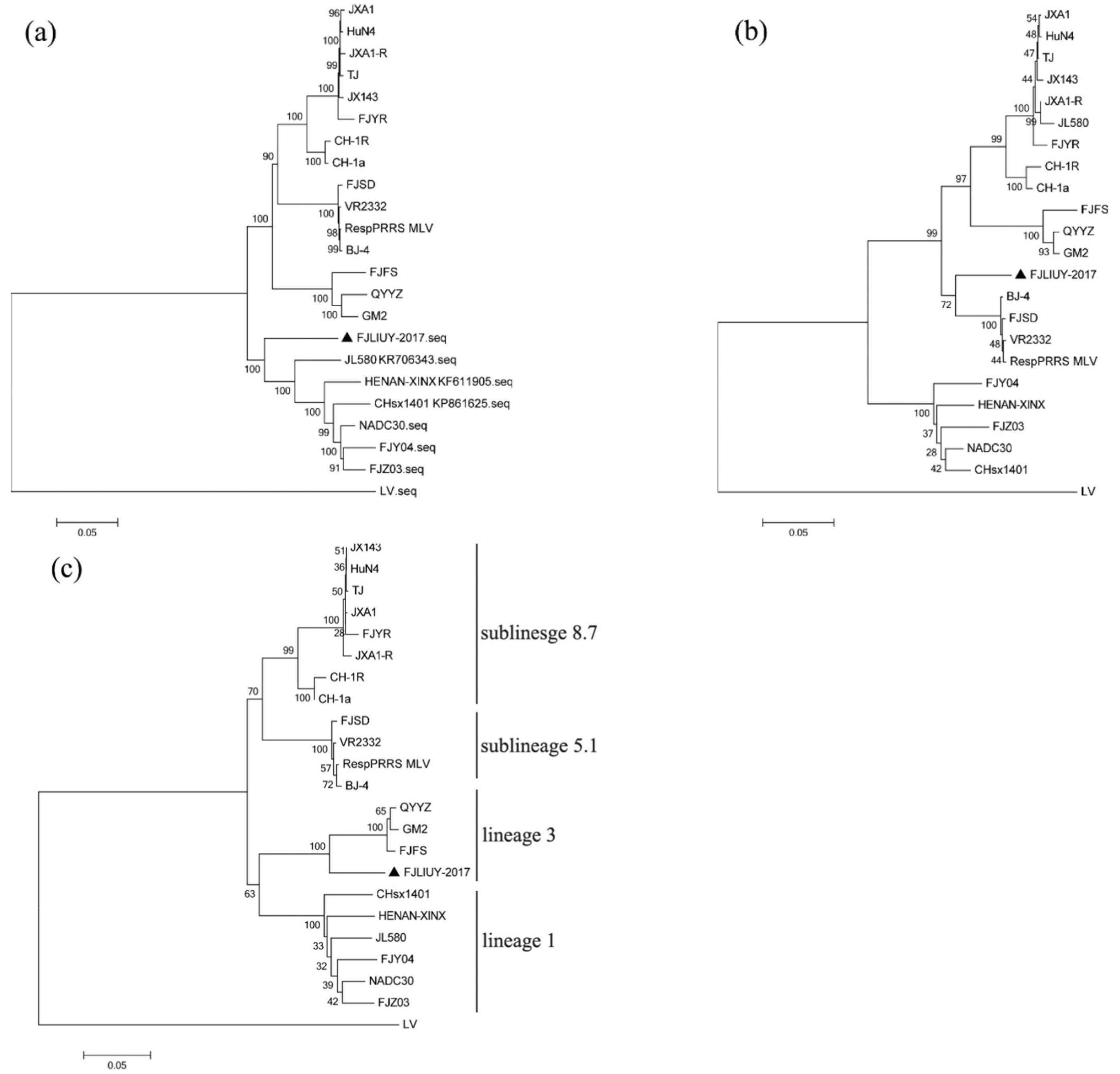

Fig. 2. Phylogenetic tree based on the full length (a), ORF3 (b) and ORF5 genes (c) of the FJLIUY-2017 and reference viruses. Reliability of the tree was assessed by bootstrap analysis of 1000 replications. Our representative isolate FJLIUY-2017 were marked with the black triangle (▲).

AR192–200), the N-terminus ARs (AR1–15 and AR27–35) of the protein were very variable among all strains. In addition, there are 9 amino acids distributed in GP5 that were only identified in QYYZ-like strains (S^{25}, I^{26}, Y^{38}, I^{39}, C^{66}, T^{58}, F^{117}, I^{152} and H^{199}) (Fig. 1B).

3.3. Phylogenetic analysis and recombination analysis

The phylogenetic trees showed that FJLIUY-2017 clustered as NADC30-like PRRSVs, VR2332-like PRRSVs and QYYZ-like PRRSVs based on the full-length genome, ORF3 and ORF5, respectively (Fig. 2). These findings indicated that FJLIUY-2017 strain was a mosaic.

To test for possible recombinant events within FJLIUY-2017 strain, we performed recombinant detection using SimPlot v3.5.1 and RDP 4.80 software, the complete genome of FJLIUY-2017 was chosen as query sequence. The recombination analysis revealed four inter-lineage recombination events among lineage 1 (NADC30-like), lineage 3 (QYYZ-like), lineage 5.1 (VR2332-like) and lineage 8.7 (JXA1-like) were identified: these data were supported by similarity plots and statistically incongruent phylogenetic trees (Fig. 2).

From the similarity plot, eleven recombination breakpoints within the FJLIUY-2017 genome were identified, which were located in Nsp1 (nt 710), Nsp1 (nt 1274), Nsp2 (nt 1829), Nsp6 (nt 6890), Nsp9 (nt 9528), Nsp11 (nt 11086),Nsp11 (nt 11524), Nsp12 (nt 12013),ORF3 (nt 13282), ORF4 (nt 13857) and ORF7 (nt 15140) (Table 4). The eleven breakpoints separated the genome of FJLIUY-2017 into 12 regions. The five regions between the breakpoints (nt 710–1273, nt 1830–6889, nt 9529–11085, nt 11525–12012, and nt 13283–13856) are closely related to the NADC30-like strains, one region (nt 12013–13282) is

Table 4

Recombination breakpoints identified in FJLIUY-2017and associated parental strains.

Isolate	Breakpoint position in alignment		Major parent	Minor parent	*P*-value						
	Beginning	Ending			RDP	GENECONV	Bootscan	MaxChi	Chimera	SiScan	3Seq
FJLIUY-2017	1	710	NADC30	JXA1	6.05×10^{-33}	1.909×10^{-21}	1.856×10^{-33}	1.012×10^{-10}	3.228×10^{-12}	8.441×10^{-16}	1.151×10^{-13}
	1274	1829	NADC30	JXA1	3.01×10^{-37}	8.578×10^{-22}	5.298×10^{-39}	8.145×10^{-16}	2.228×10^{-17}	1.964×10^{-14}	2.02×10^{-13}
	6890	9528	NADC30	JXA1	1.292×10^{-56}	NS	2.844×10^{-29}	1.146×10^{-29}	1.322×10^{-33}	2.189×10^{-26}	5.903×10^{-8}
	11086	11524	NADC30	JXA1	3.408×10^{-18}	5.336×10^{-10}	8.078×10^{-19}	5.305×10^{-8}	8.520×10^{-9}	1.268×10^{-4}	1.515×10^{-13}
	12013	13282	NADC30	BJ-4	2.452×10^{-45}	1.965×10^{-42}	6.668×10^{-42}	8.511×10^{-17}	9.335×10^{-18}	9.665×10^{-3}	1.515×10^{-13}
	13857	15140	NADC30	QYYZ	2.648×10^{-35}	9.957×10^{-6}	1.212×10^{-29}	1.419×10^{-13}	3.518×10^{-13}	5.852×10^{-8}	2.02×10^{-13}

closely related to VR-2332-like strains, The four regions between the breakpoints (nt 1–709, nt 1274–1829, nt 6890–9528 and nt11806–11524) is closely related to JXA1-like strains and one region (nt 13857-15140) is closely related to QYYZ-like strains. Collectively, the above results suggested that FJLIUY-2017 is a mosaic recombinant strain between a NADC30-like, a JXA1-like, a QYYZ-like and a VR-2332-like strain (Fig. 3).

4. Discussion

PRRS has been causing significant economic loss to the swine industry in China since 1995. Importantly, the prevalence and transmission of novel PRRSV NADC30-like is now a severe threat to Chinese pig population. Notably, new lineage 3 PRRSVs also emerged in China (Lu et al. 2015; Liu et al., 2017a,b,c). Now, four different lineage PRRSVs co-existed in Chinese swine herds, and they have caused concern about the potential of virus recombination.

Recombination is critical for viral evolution, conditioning virulence and viral immune evasion (Liu et al. 2011; Mengeling, 2002; Murtaugh et al. 2010; Wang et al. 2015; van Vugt et al. 2001). NADC30-like PRRSVs increased the complexity of PRRS in the field since 2013. Notably, NADC30-like viruses characterized by frequently recombination with other strains, many recombinant strains between NADC30-like (lineage 1) and JXA1-like PRRSV (lineage 8)/VR2332-like (lineage 5)/QYYZ-like (lineage 3) PRRSVs were reported in China, including JL580, 14LY01-FJ, 14LY02-FJ, 15LY01-FJ, 15LY02-FJ, HNjz15, FJ1402, HNhx and HNyc15 (Zhao et al. 2015; Liu et al. 2017a; Zhang et al. 2016; Li et al. 2016; Wang et al., 2018). Surprisingly, some virulent strains including FJXS15, TJnh1501, 15HEN1 and 15SC3 were natural recombinant between commercial HP-PRRSV vaccine strains and NADC30-like PRRSVs (Bian et al. 2017; Liu et al. 2017b; Zhao et al. 2015; Zhao et al. 2017). Recently, PRRSV strains, SCcd17 and SDhz1512, SCcd16 and SCya17, SD17-38 were reported to originate from recombination events among three lineages (Lineage 8, 5 and 1 or Lineage 8, 3, and 1) (Chen et al. 2018; Zhou et al. 2018b; Zhou et al. 2018c). These data indicated that the recombination events in China were relatively complicated after NADC30 introduction from the United States. In the present study, a novel PRRSV variant FJLIUY-2017, was isolated in 2017 in Fujian province, and its genomic characteristics was analyzed. The results revealed that the FJLIUY-2017 isolate is a multiple recombinant strain among NADC30-like, VR-2332-like, QYYZ-like and JXA1-like strains and shared a very low genome identity with the SCcd17 and SD17–38 (88.8–89.3%), indicating PRRSVs in China have undergone rapid evolution in recent years.

Comparison to PRRS sequences in GenBank or representative strains confirmed the absence of close relatives (< 92%), but indicated FJLIUY-2017 belonged to NADC30-like PRRSVs (lineage 1), which contains NADC30 and NADC30-like PRRSVs in China. The genomic regions with the highest variation were found in Nsp1β, Nsp2, ORF2, ORF3, ORF4, ORF5 and ORF5a and the lowest variation were found in Nsp1a, Nsp8–12, and ORF6 (Table 3). FJLIUY-2017 had 131aa deletions within Nsp2, as compared to the reference strain VR-2332, and the deletion pattern was consistent with NADC30, which indicated FJLIUY-2017 belonged to NADC30-like PRRSV. In addition, GP5 antigenic regions in FJLIUY-2017 were similar to related lineage 3 viruses, but different from other lineage viruses. In addition, there are 12 amino acids distributed in GP5 that were thought to be unique to QYYZ-like strains.

Previously studies showed that most of the breakpoints of recombination events were located in Nsp2 or Nsp9 between NADC30-like PRRSVs and other strains in China (Zhang et al. 2016; Li et al. 2017; Liu et al. 2017b; Bian et al. 2017; Zhao et al. 2015; Zhao et al. 2017; Wang et al., 2018; Zhou et al. 2018b). In the present study, breakpoints of the recombinant strain FJLIUY-2017 were located in Nsp1, Nsp2, Nsp6, Nsp9, Nsp11, ORF2 and ORF7, indicating Nsp2 or Nsp9 is the hot region for breakpoint of recombination events and

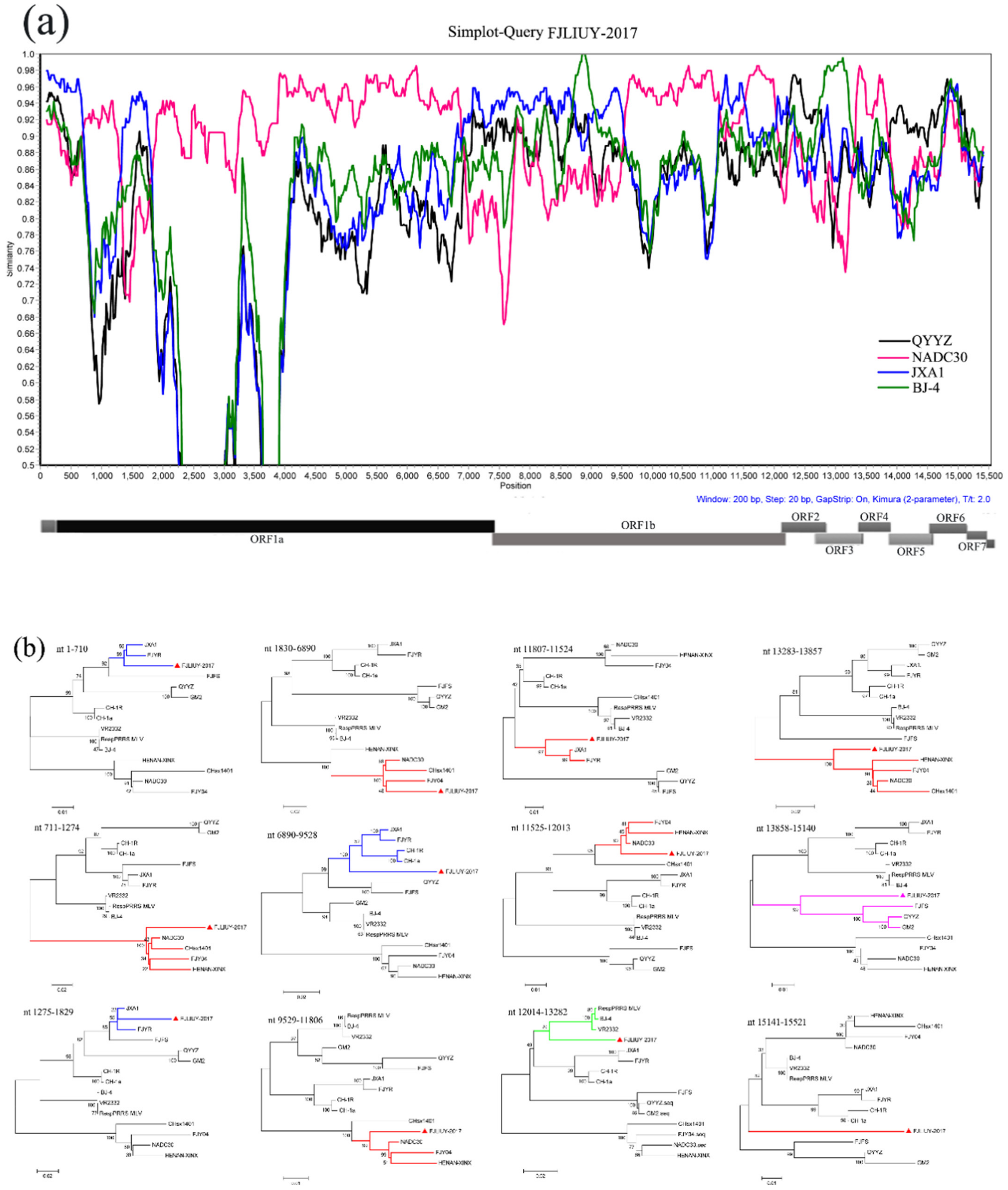

Fig. 3. Characterization of the eleven statistically supported recombinant events between FJLIUY-2017 and representative PRRSV lineages. (a) Similarity plot and bootscan analyses of FJLIUY-2017 by SimPlot. The complete genome of FJLIUY-2017 was chosen as query sequence. The y-axis indicates the percentage similarity between the parental sequences and the query sequence. (b) Phylogenetic trees based on different regions of FJLIUY-2017.

whether these events are associated with virulence require further study. On the other hand, it suggests NADC30 or NADC30-like PRRSVs gained genetic diversity by frequency of recombination with local PRRSV strains in China.

Recombination events between field PRRSV strains/vaccine-like strains and NADC30-like PRRSVs could result in greater PRRSV pathogenicity and increasing the complexity of PRRS in the field, such as strain JL580, 14LY01-FJ, 14LY02-FJ, 15LY01-FJ, 15LY02-FJ, FJ1402, FJXS15 and TJnh150. Additionally, most studies showed that many filed strains only occurred two or three lineages of type 2 PRRSV, resulting in generating new strains or unexpected behavior in virus through recombination. Considering the FJLIUY-2017 originate from recombination events among four lineages (Lineage 1, 3, 5 and 8) circulating in China, further effort should be deserved to study virulence, immunogenicity and diagnosability providing more extensive knowledge on potential importance of evolution and more efficient control of PRRSV.

Acknowledgements

This study was supported by Leading Project Foundation of Science Department of Fujian Province (2018N0022), Fujian Natural Science Foundation, Fujian Province, China (2016J01168), and Major project of Science and Technology Program of Fujian Province, China (2014NZ0002-3).

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