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Evolution of the Complete Genome Sequence of Sacbrood Virus from Sichuan Province of China

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Abstract: The Chinese honeybee (*Apis cerana cerana*) sacbrood virus (CSBV) causes death of larvae and colony collapse, and could damage the beekeeping industry in China. We sequenced complete genomes of CSBV strains derived from the Maerkang area, Wenjiang area, and Wanyuan area of Sichuan province of China. The genome length of CSBV strains from Sichuan was 8863bp, and it contained one complete Open Reading Frame of a gene with 8544 bp that encoded a protein with 2848 amino acids. The (G+C) % and (A+T) % composition ranged from 40.6 to 40.7 and 59.3 to 59.4, respectively. A phylogenetic tree was constructed using the three CSBV strains and previously reported SBV and CSBV sequences from other regions. We found that viral strains clustered based on their region of origin and host species. The genetic sequences of the CSBV strain from Maerkang were 98.7% and 99.6% similar to CSBV strains from Wanyuan and Wenjiang, respectively. In addition, CSBV from Maerkang had 88.4%-95.2% sequence similarity to previously published genomes of CSBV or SBV from other areas. The *VP1* gene sequenced in our study had a 43 bp deletion compared to *VP1* sequences of CSBV from other regions in Asia. We detected 10 antigenic determinants on the VP1 protein of CSBV form Aba. Our study provides new insight into the diversity of CSBV strains in China and may help with identifying methods to prevent infection of honeybee colonies.

Keywords: Chinese sacbrood virus; *VP1*; *Apis cerana*; Phylogenetic analysis

1. Introduction

The sacbrood bee virus (SBV) is a highly contagious disease that can kill honeybee larvae[1]. SBV can infect the two widely distributed species of honeybees *Apis cerana* and *Apis mellifera*[2,3]. SBV infections of honeybee colonies have been reported across the world[4-6], including the United States [7], South America [8,9], Europe [2], Australia [10], South Africa[11], Asia [5,12-15], and New Guinea[16]. In China, SBV infections were first recorded in Guangdong in 1972, and since then, SBV has caused substantial economic losses to the Chinese beekeeping industry [17].

The genome sequence of SBV was first reported in 1999, and is 8.8 kb in length and contains one large ORF that encodes three structural proteins that form the viral capsid: VP1, VP2, and VP3 [7]. The since ORF in the SBV genome encodes structural protein genes when transcribed from the 5′ end, and encodes non-structural genes when transcribed from the 3′ end[18]. Compared with other viruses, SBVs have a unique minor capsid protein called Micp[19]. In general, SBV strains that share geographical origins or host species are more related to each other [3,20-23]; however, some Korean SBV strains that infect the same host do not appear to be closely related. This suggests that cross-infection of SBVs can occur between *Apis cerana* and *Apis mellifera* [24].

The Chinese honeybee is a subspecies of *A. cerana*, and is essential for the honeybee industry in the Maerkang area. The wild populations are found in remote mountainous regions of Sichuan province. However, recent introduction of invasive bee species have reduced the native population, and the Chinese honeybee is now listed on the National Endangered Animal Genetic Resources

Protection List [25]. In recent years, honeybees infected with CSBV were found in the Aba area of Maerkang city, leading to the sudden mass death of bees and subsequent economic loss[26]. Only a few CSBV genome sequences have been reported from China, and in this study, we sequenced three CSBV strains from Sichuan province to analyze their evolutionary relationship to other known CSBV strains. We aimed to identify methods for early diagnosis and treatment of CSBV infections in China.

2. Materials and Methods

2.1 Sample collection

CSBV samples were collected from three regions of Sichuan province, China: Maerkang, Wenjiang, and Wanyuan (Fig.1). Ten larvae or capped pupae of *A.cerana* from asymptomatic colonies were randomly collected, and the samples were stored at -80℃ in an ultra-low temperature freezer.

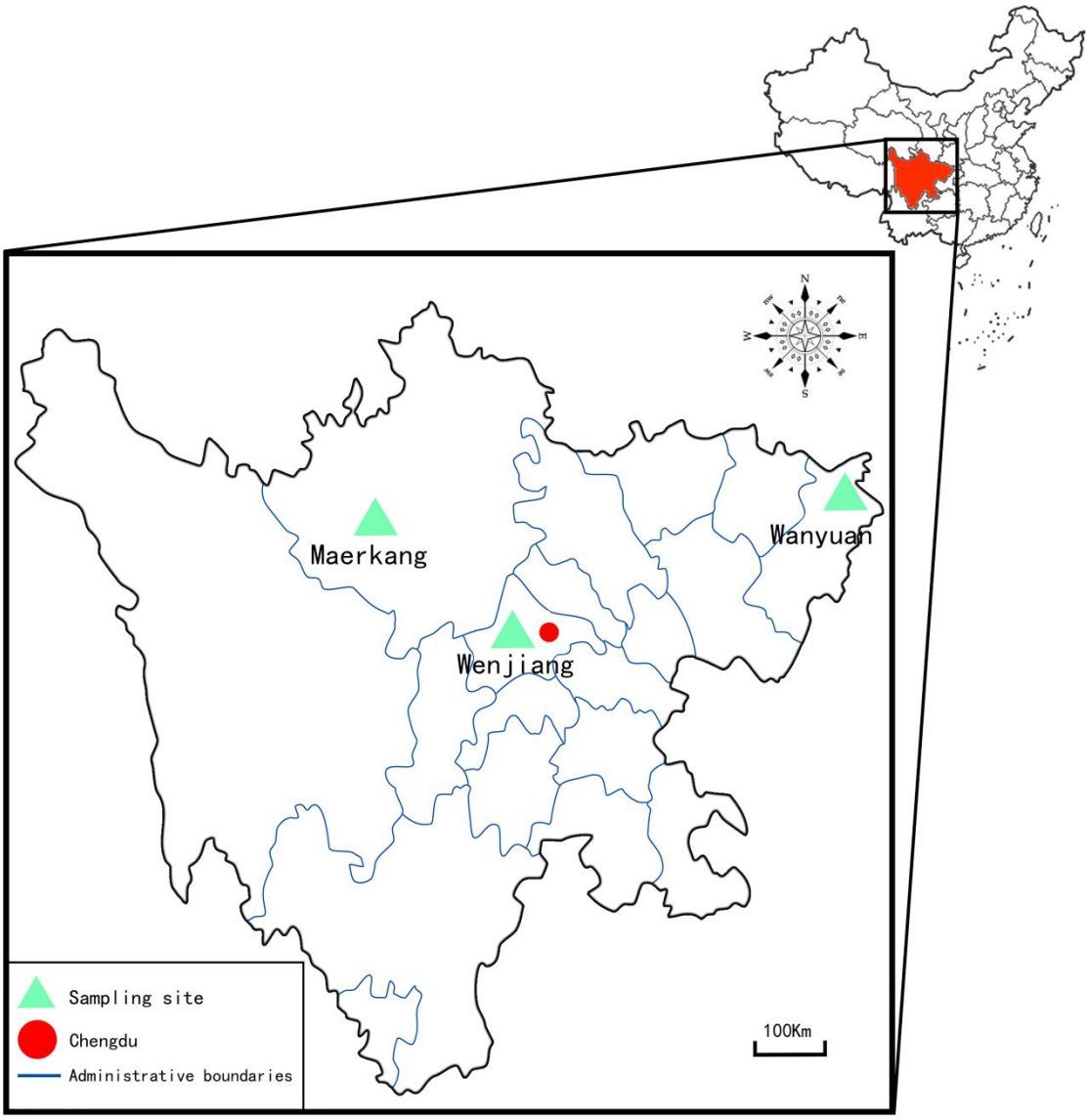

Fig.1.The map of Sampling location of sacbrood virus from Aba area, Wenjiang area and Wanyuan area of Sichuan province of China.

2.2 Extraction of viral RNA from infected samples

RNA was extracted from larvae or pupae using the TaKaRa MiniBEST Viral RNA/DNA Extraction kit ver.5.0 (Bao Bioengineering (Dalian) Co., Ltd., China). RNA samples were stored at -80℃.

2.3 RNA reverse transcription

The extracted RNA was reverse transcribed using the Tabarax kit from Takara Biomedical Technology (Beijing) Co., Ltd. using 2 ul of RNA, and samples were stored at -80 °C.

2.4 Amplification of cDNA, cloning and sequencing

The full-length cDNA of CSBV was amplified using primers listed in Table 1. PCR amplification were performed on a PE9700 PCR machine from Thermo Fisher Scientific lnc. Each PCR reaction contained 1.5μl of DNA template, 0.5μl of forward and reverse primers, and 22.5μl of PCR premix (Chengdu Qingke Biotech Co., Ltd.). The cycling conditions were as follows: denaturation at 98℃ for 4 min, 35 cycles consisting of denaturation at 98℃ for 30 s, 30 s at the optimized annealing temperature (Table 1), extension at 72°C for 30 s and a final elongation step at 72°C for 3 min.
The PCR products were separated by agarose gel electrophoresis. Bands of the correct length were purified from the agarose gel using Axygen Gel Recovery Kit (Beijing TIANGEN Biotech Co., Ltd.). The PCR product was ligated into the pMDl8-T Vector and transformed into competent DH5a. The recombinant plasmid was extracted from a positive clone and identified using PCR. Plasmids were also sent to Chengdu QingKe Biotechnology Co., Ltd. for sequencing.

Table 1 The PCR primer of Chinese honeybee sacbrood virus

Primer name		Primer sequence(5'-3')	TM(℃)	Size of amplicons (bp)
SBV1[30]	F	GACCCTTTTCTTGTGAGTTTTA	53.3	600
	R	GTGTAGCGTCCCCCTGAATAGAT		
SBV2[30]	F	TATTCAGGGGGACGCTACA	61	1056
	R	TATTCCATCGGGGTTATTTG		
SBV3[30]	F	GGAGACGCGCATGGTAAAGA	50.7	714
	R	GCGCGGTAAATAAACACTCG		
SBV4[30]	F	AGGTAGATAATGTTAGTAGGGCG	56.7	299
	R	TAGTAATACACCTGGTTTCCCTC		
SBV5[30]	F	CAGAGTCTAAAATGAGAGTGAAAGTGAG	54.3	953
	R	TGGTATGCTGCTCCCGTAA		
SBV6[30]	F	TTACGGGAGCAGCACAACA	51.4	1047
	R	ATTTCCGATTTACCGATACC		
SBV7[30]	F	CGGTGCGTTATGAACCTTTT	50.8	1049
	R	AATGCGTAGATTGAGGTGCC		
SBV8[30]	F	GCGCAACTGGCACCTCAAT	52.3	920
	R	TTCCAAATATACTTCCCACTGC		
SBV9[30]	F	TGGCAGCTGCAGGTTTAGTGACGG	57.7	1134
	R	CCCGTCCCAAAGGGCTTCCTT		
SBV10[30]	F	TTTGGTAGCGGGGTGTAAG	46.5	1139
	R	CATTGCGTGGTATCATT		

SBV11[30]	F	TACGAATCGTGATTCGAT	55.3	400
	R	TAAACAAATCGGTATAAGAGTCC		

Note：F means the forward primer and R means the reverse primer

2.5 SBV sequences

The nucleotide sequences of all the fragments were assembled and aligned against CSBV-LN (HM237361) as the reference sequence using Chmmosoma 1.62 and DNAstar7. 2 [24]. We obtained the complete genome sequence of CSBV, and sequences of *VP1* from 24 SBV genome sequences from various countries or areas from NCBI (Table 2).

Table 2 The information about download sequences of sacbrood virus in GenBank

No.	Strain name	Short title	GeneBank number	Locating	Host
1	AcCSBV-BJ	BJ	KF960044.1	Beijing, China	Ac
2	AcCSBV-CQ	CQ	KJ716806.1	Chongqing, China	Ac
3	AcCSBV-FZ	FZ	KM495267.1	Fuzhou China	Ac
4	AcCSBV-GD	GD	AF469603.1	Guangdong China	Ac
5	AcCSBV-LN	LN	HM237361.1	Liaoning China	Ac
6	AcCSBV-SX	SX	KJ006692.1	Shanxi China	Ac
7	AmSBV-America MD1	America MD1	MG545286.1	America	Am
8	AcSBV-India K1A	India K1A	JX270796.1	India	Ac
9	AcSBV-India K5B	India K5B	JX270797.1	India	Ac
10	AcSBV-India K3A	India K3A	JX270798.1	India	Ac
11	AcSBVIndia II-2	India II-2	JX270795.1	India	Ac
12	AcSBV-India II-10	India II-10	JX270797.1	India	Ac
13	AmSBV-Kor1	Kor1	KP296800.1	Korea	Am
14	AcSBV-Kor2	Kor2	KP296801.1	Korea	Ac
15	AcSBV-Kor3	Kor3	KP296802.1	Korea	Ac

16	AcSBV-Kor4	Kor4	KP296803.1	Korea	Ac
17	AmSBV-Kor21	Kor21	JQ390591.1	Korea	Am
18	AmSBV-UK	UK	AF092924.1	United Kingdom	Am
19	AcSBV-Viet1	Viet1	KM884990.1	Vietnam	Ac
20	AcSBV-Viet2	Viet2	KM884991.1	Vietnam	Ac
21	AcSBV-Viet3	Viet3	KM884992.1	Vietnam	Ac
22	AmSBV-Viet4	Viet4	KM884993.1	Vietnam	Am
23	AcSBV-Viet5	Viet5	KM884994.1	Vietnam	Ac
24	AmSBV-Australian	Australian	KY465671	Australian	Am

Note:Ac stands for *Apis cerana*, Am stands for *Apis mellifera*

2.6 Sequence analysis

Alignment of nucleotide sequences was performed using Clustal X 1.8, and obvious mismatches were manually corrected. We analyzed sequence homology and composition using MEGA 5.0 [27]. The phylogenetic tree using the complete genome or *VP1* sequences of SBV was constructed using MEGA 5.0. We used the neighbor-joining method with Kimura's two-parameter model, and the bootstrap values were 1000 replicates [27]. PREDICTED ANTIGENIC PEPTIDES was used to model the surface protein antigen of CSBV-AB VP1 (http://imed.med. ucm.es/Tools/antigenic.pl).

3. Results

3.1 Complete genome sequences of CSBV

The total length of the complete genome of three CSBV strains was 8863bp and sequences were deposited to NCBI (Maerkang strain: MK719542; Wenjiang strain: MN266898; Wanyuan strain: MN266899). The length of the open reading frame (ORF) was 8544bp, which encoded 2848 amino acids (Table 3). There were 1220 variable sites, including 462 singleton sites, and 758 Parism-informative sites when comparing CSBV strains from China (CSBV-AB, CSBV-WJ, CSBV-WY, CSBV-CQ, CSBV-FZ, CSBV-GD, CSBV-SX, CSBV-BJ, and CSBV-LN). From the three strains that were sequenced in this study, 128 singleton sites were found (CSBV-AB, CSBV-WJ, CSBV-WY) and Parism-informative site were not identified. Length of the *VP1* gene of the three CSBV strains from Sichuan was 975bp, and it encoded a protein with 325 amino acids. We found a 43 bp deletion in the Maerkang, Wenjiang and Wanyuan strains compared to strains from other countries or areas. We predicted surface protein antigen of VP1 and found that there were 10 predicted antigenic determinants (Table 4).

Table.3. cDNA sequence base composition and protein physicochemical propertie of complete genome of sacbrood virus from Aba area, Wenjiang area and Wanyuan area of Sichuan province of China

Name	Base composition						Molecular weight(kDa)	PI(PH=7)	Protein charge	Full length(bp)
	A	T	C	G	A+T(%)	C+G(%)				

CSBV-AB	2678	2588	1438	2159	59.40	40.60	2651.78	7.90	325.27	8864
CSBV-WJ	2678	2581	1450	2154	59.30	40.70	2651.67	7.89	320.84	8864
CSBV-WY	2678	2592	1444	2149	59.40	40.60	2651.49	7.89	317.69	8864

Table 4 Prediction of antigenic determinants of Chinese honeybee sacbrood virus from Aba area of Sichuan province of China.

No.	Start Position	Amino acid sequence	End Position
1	13	PSRSWPIDS	22
2	27	GYDPVKP	33
3	40	KRQSWDNPHRFLPA	54
4	62	EYSSVILPR	70
5	97	QSLDTQVSIKDILRRPVLLFNHVVLD	122
6	125	YTGFFIPIM	133
7	173	LRYTIIIHS	181
8	184	GHPIWTHVP	193
9	210	EYTKVPIFGCGL	221
10	226	IIPSVNPSICVEVP	239

3.2 Homology and phylogenetic analysis of CSBV

Genomes of CSBV-AB, CSBV-WJ, and CSBV-WY shared 0.951-0.882 nucleotide homology to other reported CSBV strains (Table 5). CSBV-AB strain was most similar to CSBV-FZ (0.951), and shared the lowest sequence similarity to AmSBV-America-MD1 (0.882). The phylogenetic tree of CSBV strains had two major clades (Fig. 2). One clade contained most of the Asian strains from Vietnam, China and India. The other clade contained strains from Europe, America, and Korea. CSBV strains in northern China (CSBV-BJ, CSBV-SX, CSBV-LN) clustered together within the clade containing strains from southern China (CSBV-AB, CSBV-WY, CSBV-WJ, CSBV-CQ, CSBV-FZ, CSBV-GD). Overall, CSBV strains appeared related based on their geographic origin and host species, with the exception of AmSBV (AmSBV-vite4), which clustered with other AcSBVs.

3.3 Homology and phylogenetic analysis of VP1

The *VP1* sequence from CSBV-AB, CSBV-WJ, and CSBV-WY had 87.2%-96.2% nucleotide homology with *VP1* from CSBV strains from other countries or areas. *VP1* of CSBV-AB was most

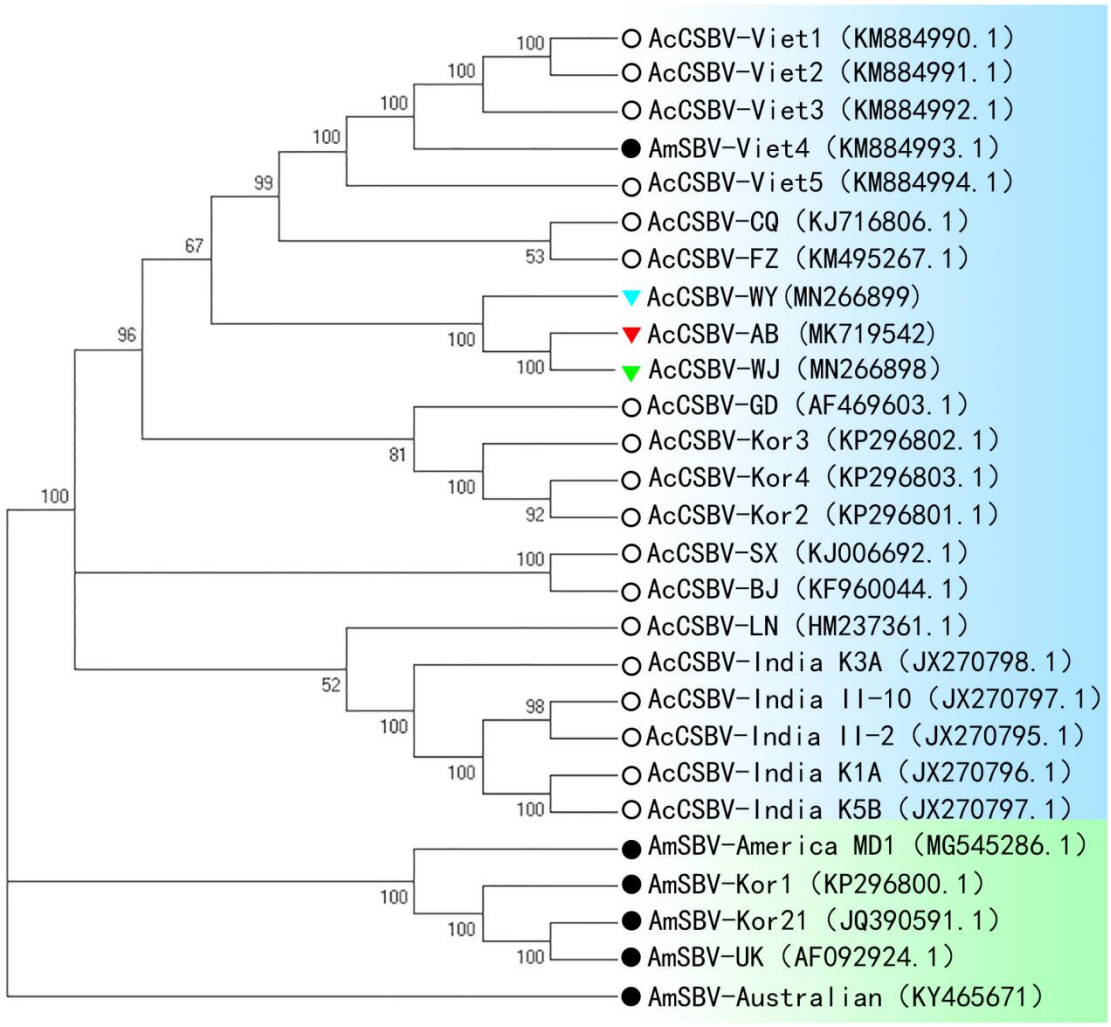

Fig.2. Phylogenetic tree of SBV based on the complete genome sequences. Divided into Asian strains and European strains, respectively, the red is CSBV-AB sequence, the green is CSBV-WJ, the blue is CSBV-WY sequence. The phylogenetic tree was constructed using MEGA 5.0 with the neighbor-joining method (NJ) with a bootstrap test of 1000 replicates. Numerals indicate bootstrap values from 1000 replicates. The hollow circle is *Apis cerana***, the solid circle is** *Apis mellifera.*

similar to CSBV-FZ (96.2%), and shared the lowest sequence homology to AmSBV-kor-1 (87.2%).The phylogenetic tree of *VP1* also had two main clades, similar to the phylogeny generated using whole genome sequences (Fig. 3). The strains from Maerkang, Wenjiang and Wanyuan were most closely related to each other, and were nested in a clade with CSBV trains from other areas of China and other near-by countries, such as CSBV-CQ strain, AcSBV-Viet1 strain and AcSBV-India II-2 strain. Interestingly, the two Korean strains (AmSBV kor-21, AmSBV kor-1) clustered with the American, Australian, and UK strains. AmSBV (AmSBV-vite4) clustered with other AcSBVs from Asia. Overall, the phylogenetic relationship of *VP1* was based on host species.

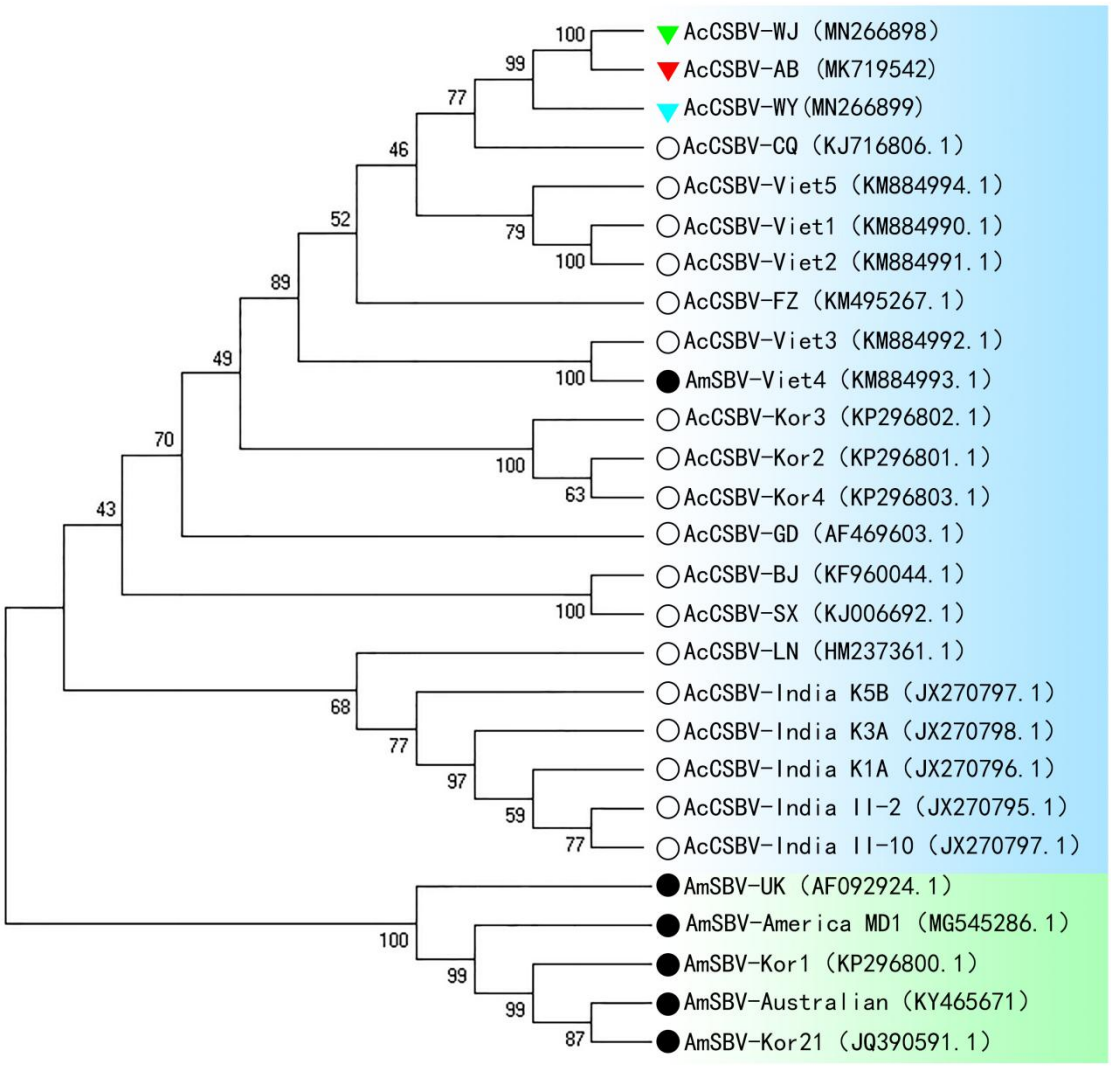

Fig.3. Phylogenetic tree of SBV based on the VP1 gene. Divided into Asian strains and European strains, respectively, the red is CSBV-AB sequence, the green is CSBV-WJ, the blue is CSBV-WY sequence. The phylogenetic tree was constructed using MEGA5.0with the neighbor-joining method (NJ) with a bootstrap test of 1000 replicates. Numerals indicate bootstrap values (%) from 1000 replicates. The hollow circle is *Apis cerana***, the solid circle is** *Apis mellifera.*

4. Discussion

CSBV was first reported in the Maerkang area of Sichuan province of China in 2015[26]. Here, we confirm this report by genetically testing CSBV strains from Sichuan. SBV can be spread by sharing of beekeeping equipment and human activity [28]. SBV can also infect *Eristalis tuna*, which can further spread CSBV to different areas through human activity[29]. Human trade activities may lead to spreading SBV to allopatric insect populations and farther away regions, and that is the likely source of infection for honeybees in the Aba area.

Earlier research found that SBV had an extra 52bp region in the *VP1* gene compared to CSBV [24]. Reddy hypothesized that the extra 52bp may confer host specificity of CSBV and SBV. We found a new 43bp deletion in the strains from Sichuan province, and this deletion may be related to

Table 5 Nucleotide homology of the VP1 gene and complete genome sequences of sacbrood virus

No	Name	AB	WY	WJ	SX	GD	CQ	LN	BJ	FZ	India II-10	India II-2	India K3A	India K1A	India K5B	Kor 3	Kor 4	Kor2 1	Kor 1	Kor 2	Viet 1	Viet 2	Viet 3	Viet 5	Viet 5	UK	US MD1	Australia
1	AB		97.5	99.8	92.1	93.4	97.2	92.3	91.7	96.1	91.5	89.9	92.7	91.5	92.3	93	93.3	87.3	87.1	93.4	95.6	95.4	93.7	93.6	95.6	87.9	86.4	87.8
2	WY	98.7		97.6	92.4	92.8	96.8	92.3	92.2	95.3	91.4	89.8	92.4	91.5	92.3	92.8	93	86.8	86.6	93.1	95.4	95.2	93.7	93.8	95.4	87.7	86	87.6
3	WJ	99.6	98.7		92.2	93.5	97.3	92.1	91.8	96	91.7	90.2	92.8	91.6	92.4	93.1	93.4	87.2	86.9	93.5	95.8	95.5	93.8	93.7	95.8	87.8	86.3	87.7
4	SX	91.8	91.7	91.7		93.4	93	93.2	93.99	93.1	92.8	91.6	93.6	93.5	93.9	93.2	93.2	88.7	88.7	93	91.8	91.6	91.8	92.1	91.3	89.3	88	88.5
5	GD	94	93.8	94	93.5		94	93.1	93.2	93.7	92.2	91.5	93.9	93	94.1	93.7	94	88.8	88.8	93.8	93.2	93	92.5	92.6	92.8	88.8	87.5	88.4
6	CQ	95.2	95	95.1	92.8	96.1		92.8	93	96.4	92.4	91.4	93.8	92.8	93.6	94.6	94.6	88.1	88.1	94.8	96.3	96.1	94.4	94.7	96.6	88.8	87.4	88.3
7	LN	93.9	93.8	93.8	93.2	93.5	92.9		93.3	94	94.6	93.1	94.6	94.3	94.5	92.9	92.9	89.6	89.6	92.8	91.8	91.6	92.1	92.5	91.8	89.4	88.9	89.7
8	BJ	91.6	91.6	91.5	99.3	93.3	92.7	93.1		93	92.9	91.7	93.5	93.3	93.8	93	93	88.9	88.8	92.9	91.5	91.2	91.5	91.7	91	89.2	88.1	88.6
9	FZ	95.2	94.9	95.1	92.9	96.1	97.3	93.4	92.8		92.8	91.5	93.6	92.4	93.2	93.4	93.4	88.6	88.4	93.3	95.7	95.4	93.4	93.2	95.5	88.9	87.9	88.6
10	India II-10	91	91	91	91.8	92.4	91.5	92.6	91.6	91.9		96	94.4	96.8	96.1	92.3	92.3	88.1	88.5	92.2	91.1	90.9	91.2	91	91	88.6	88	88

11	India II-2	91.2	91	91.1	92.1	92.6	91.8	92.7	91.9	92	97.3		93.2	96.2	95.8	91.5	91.5	87.5	87.9	91.4	90	89.8	90.5	90.3	89.8	88.2	87.3	87.6
12	India K3A	91.3	91.3	91.3	92.3	92.7	91.9	92.8	92.1	92.2	94.7	95.1		95	96.4	94	94	88.7	89.1	93.8	92.4	92.2	93.1	92.7	92.8	89.8	88.3	88.7
13	India K1A	91.2	91.1	91.2	92.2	92.8	91.9	92.9	92.2	92.2	96.9	97.3	95.8		98.1	93.2	93.1	89.1	89.6	93	91.7	91.5	92.2	92.2	91.7	90.1	88.5	89.1
14	India K5B	91.5	91.3	91.4	92.4	93.2	92.2	93	92.2	92.4	96.5	97.4	95.5	98.6		93.7	93.7	89.3	89.5	93.6	92.3	92.1	93.1	93	92.3	89.9	88.5	89.3
15	Kor3	92.7	92.4	92.7	92.6	95.2	94.5	92.9	92.9	94.4	91.6	91.8	92.1	91.9		99.8	88.9	88.9	99.7	92.8	92.6	94.2	92.9	92.9	89.2	88.2	88.8	
16	Kor4	93	92.8	93	92.4	95.2	95.2	92.1	92.2	95.2	91.2	91.4	91.7	91.4	91.8	98		88.9	88.9	99.9	92.8	92.6	94.2	92.9	92.9	89.2	88.2	88.8
17	Kor21	88.6	88.5	88.6	89.2	90.2	89.6	89.5	89	89.7	88.6	88.7	88.9	88.9	88.9	89.4	89.5		97.6	88.8	87.6	87.3	88.3	89.2	86.8	92	93.6	98.3
18	Kor1	88.8	88.7	88.7	89.3	90.1	89.6	89.4	89.2	89.5	88.8	89	89	89.1	89.2	89.5	89.5	97.1		88.8	87.1	86.8	87.9	88.9	86.6	92.1	94.4	97.6
19	Kor2	92.8	92.5	92.7	92.5	95.4	94.7	92.2	92.3	94.8	91.1	91.3	91.5	91.4	91.7	97.4	98.4	89.3	89.3		92.7	92.4	94.3	93	93	89.1	88	88.6
20	Viet1	94.6	94.4	94.6	92.5	95.4	96.8	92.5	92.3	96.9	91.1	91.3	91.4	91.5	91.7	94	95	89.1	89	94.4		99.8	96	95.9	96.9	88.9	87.2	87.8
21	Viet2	94.6	94.3	94.6	92.4	95.4	96.8	92.4	92.2	96.8	91.1	91.2	91.4	91.4	91.7	93.9	94.9	89	88.9	94.3	99.9		95.8	95.6	96.9	88.7	87.2	87.6
22	Viet3	94.2	94	94.2	92.4	95.3	96.4	92.4	92.2	96.4	91.1	91.3	91.5	91.4	91.8	94.2	95.1	89	89	94.5	99.3	99.3		98	94.6	88.4	87.9	88.4

23	Viet5	94.3	94	94.2	92	95.1	96.6	92.2	91.8	96.5	91	91.1	91.3	91.3	91.6	94	95.5	88.8	88.7	94.4	97.1	97.1	96.6	94.5	88.8	88.4	89.4
24	Viet4	94	93.9	94	92.6	95.5	96.4	92.7	92.4	96.1	91.5	91.7	91.8	91.8	92.2	94.1	95.1	89.3	89.2	94.3	97.4	97.3	97.6	96.5	88	86.5	87.1
25	UK	88.9	88.9	88.8	89.7	90.4	89.9	89.8	89.5	90	89	89.2	89.2	89.2	89.3	89.6	89.7	98.3	97.3	89.5	89.3	89.3	89.3	89.1	89.5	91.5	92
26	US MD1	88.4	88.4	88.4	89	89.9	89.5	89.2	89.9	89.5	88.4	88.7	88.6	88.8	88.9	88.9	88.9	94.6	94.7	88.8	88.8	88.8	88.8	88.6	89.1	95	93.7
27	Austraila	88.8	88.7	88.8	89.7	90.3	89.4	89.8	89.5	89.7	89.1	89.2	89.2	89.4	89.5	89.1	89.3	92.8	92.6	89.4	89.1	89	88.9	88.9	89.1	93.1	92.5

Note: The lower triangle is the homology of the CSBV genome. The upper triangle shows the homology of the VP1 gene

the specificity of this CSBV strain for infecting the Sichuan subspecies of *A. cerana*, which coincide closely with the views of previous studies [24]. Further studies would be needed to understand whether this region can contribute towards the different host specialization of SBV strains [20,22,24].

Phylogenetic analyses of viral whole genome sequences can improve our understanding of how SBVs are spread. In this study, we sequenced CSBV strains from Sichuan province and found that they were genetically more similar to strains from southern China. We found that CSBV strains were clustered by geographical origin and species, which was consistent with previous studies [20,22,24]. However, the AmSBV-viet4 strain that infects A. mellifera was more closely related to strains that infect A. cerana from southern China and neighboring areas, which suggested that SBV may be spreading in both A. cerana and A. mellifera populations. Similar results have been reported for SBV strains in Korea [24,30]. Our results suggest that the introduction of invasive bee species could lead to cross-species infection of SBV in different countries and regions. This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

5. Conclusions

This study was the first to complete the sequencing and evolutionary analysis of the CSBV strain in sichuan, China, to determine the evolutionary relationship between the strain and SBV strains in other regions, and to find a 43bp unique base deletion that may be related to specific infection. Provides new insight into the diversity of CSBV strains in China and may help with identifying methods to prevent infection of honeybee colonies.

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