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Complete genome sequence of sacbrood virus isolated from Asiatic honey bee *Apis cerana indica* in India

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Abstract We determined the complete genome sequence of a sacbrood virus (SBV) infecting Indian honey bee (*Apis cerana indica*) from Tamil Nadu, India named as AcSBV-IndTN1. The genome of AcSBV-IndTN1 comprised of 8740 nucleotides, encoding a single large ORF containing 2849 amino acids flanked by 5′ and 3′ untranslated regions. Results of phylogenetic tree analysis based on complete genomes of SBV isolates indicated that the virus isolates from India isolated from the Asiatic honey bee *A. cerana* (AcSBVs) formed a separate group along with six Vietnam isolates and three Chinese isolates. The AcSBV-IndTN1 isolate showed closer genetic relationship with other isolates from India. The second major group had both AcSBVs and AmSBVs (virus isolated from European honey bee, *Apis mellifera* SBV) of Korea, China and Vietnam. The third and a distantly related group had AmSBVs of Australia, UK, USA and Korea. The results obtained from phylogenetic analysis were further supported with evolutionary distance analysis. AcSBV-IndTN1 isolate open reading frame had 95–99% amino acid sequence similarity with other Indian isolates and 92–96% with AcSBVs and AmSBVs of other geographical locations. In addition, sequence difference count matrix ranged from 154 to 907 nt among all the SBV isolates. This suggests that the virus isolates have evolved significantly in different geographical locations but isolates on different hosts in a given location/country are closely related. The high similarity in the genome among the AcSBV and AmSBV isolates indicate possible cross-infections and recombination of SBV isolates in Asian continent where both the honey bee species are reared in close proximity. Gene flow between SBV population indicating that an infrequent gene flow occur between them. The pattern of molecular diversity in SBV population revealed that the occurrence of recent population expansion of SBV. To the best of our knowledge this is the first report of the complete nucleotide sequence of AcSBV from Tamil Nadu, India. This study provided an opportunity to establish the molecular evolution of SBV isolates and shall be useful in the development of diagnostics and effective disease control strategies.

Keywords *Sacbrood virus* (SBV) · *Apis cerana indica* · Reverse transcriptase polymerase-chain reaction · Complete genome · Honey bee

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Introduction

Apis cerana indica Fab., has been one of the important domesticated species utilized for commercial beekeeping in India. Among the honey bee (Order: *Hymenoptera* and Family: *Apidae*) viruses, the sacbrood virus (SBV) is one of the most severe threats to the health of *A. cerana* and *Apis mellifera* [3]. SBV is a picorna-like virus and belongs to the genus *Iflavirus* in the family *Iflaviridae* [6] with a

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single stranded positive sense RNA genome of approximately 8.8 kb [19]. SBV particles are 28–30 nm in diameter and non-enveloped. AcSBV disease was first observed in 1976 in Thailand on *A. cerana* causing 100% mortality [2]. In India, this disease first appeared in 1978 in North India and had virtually wiped out colonies of *A. cerana indica* [22]. The AcSBV is popularly known as the *Thai sacbrood virus* (TSBV) as it was believed to be introduced from Thailand into India. During 1991–1992, the catastrophic outbreak of the SBV disease resulted in the destruction of more than 90% of the then existing bee colonies in the South India causing a drastic drop in the honey production [5]. This disease has since then been a reason for colony loss in regions wherever *A. cerana indica* is reared. This virus causes quick dwindling and sometimes even perishing of the bee colonies. The disease spreads and becomes serious because of crowding, social insect interactions, mutual grooming, food sharing, exchange and communication [15]. Reverse transcriptase polymerase-chain reaction (RT-PCR) has been proved to be a sensitive molecular method to detect SBV directly in samples of diseased honey bees and their brood [1]. Partial sequences have been determined for some Indian isolates [18], but report on a complete genome sequence was lacking. Here, we report the complete genome of AcSBV isolate collected from Tamil Nadu state of India to compare with other isolates to know the evolutionary history of the virus. This information will assist in the development of molecular diagnostic tests and effective disease management strategies.

Materials and methods

Sample collection, RNA isolation and sequencing

The infected honey bee prepupae were collected from *A. cerana indica* colonies at the Apiary of Department of Agricultural Entomology, TNAU, Coimbatore and stored at − 20 °C until used for the studies. Healthy prepupae were also used as control. The total RNA was isolated from infected prepupae samples as per the method described [1]. First strand complementary DNA (cDNA) was synthesized from extracted RNA using a RevertAidTM First Strand cDNA Synthesis Kit Thermo Fisher Scientific, USA with an oligo(dT) primer according to the manufacturer's instructions. Nine sets of primer pair were designed based on the sequence of the SBV-UK genome and used to amplify overlapping PCR products of complete genome of SBV (Table 1). The resulting cDNA (2 µl) was amplified in 25 µl reaction mixture. PCR amplification was performed in a Veriti 96 well Thermal Cycler (Applied Biosystems, USA). The amplification conditions were as follows: 95 °C for 5 min, 40 cycles of 95 °C for 20 s, 49–53 °C for 30 s, 72 °C for 60 s and a final extension of 72 °C for 10 min. The amplified RT-PCR products were resolved by electrophoresis through 1.5% agarose gels, and the gel was documented. The amplified products were purified using a GenElute Gel Extraction Kit (Sigma Aldrich, USA), quantified and cloned into the pTZ57R/T cloning vector (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Two clones of each PCR product were sequenced in both directions.

Sequence analysis

The sequences were analyzed using BLAST (National Center for Biotechnology Information, USA) to identify related sequences and aligned using CLUSTALW [24]. Sequence identity matrix and sequence difference count matrix were calculated using Bioedit program version 7.05 [9]. Multiple alignments were used to infer the phylogenies and the evolutionary distance with the maximum-likelihood (ML) method implemented in MEGA 7 [12]. To obtain the ML tree topologies, 1000 bootstrap replicates were performed for each dataset. The details on information on the virus isolates which were subjected to phylogenetic analysis are given in the Table 2. The AcSBV-IndTN1 sequence determined in this study has been deposited in the NCBI GenBank database under accession no. KX663835 and used as a reference sequence for analysis. Ka/Ks value was calculated using the DnaSP version 5.10 [14] to analyze synonymous and non-synonymous mutations at nt level, which really affect the amino acid (aa) sequences of the protein. Genetic differentiation between the SBV populations was examined by three permutation-based statistical tests, Ks*, Z, and Snn [11]. The level of gene flow between populations were measured by estimating Fixation index (FST), Tajima's D, Fu and Li's D, Fu and Li's F tests, haplotype and nucleotide diversity using DnaSP version 5.10 [14].

Results and discussion

Nucleotide and amino acid analysis

The complete genome of AcSBV-IndTN1 comprised of 8740 nucleotides (nt), encoding a single large open reading frame (ORF) contained 2849 amino acids (aa) flanked by 5′ and 3′ untranslated regions (UTRs). The GC content of AcSBV-IndTN1 genome was 40.7 while AT content was 59.3% with AT/GC ratio of 1.459. The AcSBV-IndTN1 ORF region shared 95–98% nt similarity with other Indian isolates and 89–96% nt identity with isolates from other countries. AcSBV-IndTN1 isolate had 95–99% aa

Table 1 List of primer sequences used for AcSBV genome sequencing primers designed in the study to synthesis whole genome of AcSBV through RT-PCR

Primer code	Sequence (5′–3′)	Position
AcSBV1 FP	GGTGCTTCGAGATTTACTTTGACGG	1–21
AcSBV2 RP	TAAGGCCACCGGATTTACTCGCAT	1520–1543
AcSBV3 FP	CTGGATCAATTGGGCCGAAGT	1399–1420
AcSBV4 RP	CATTCTAGAAGGCGGCATTATAGGT	2557–2581
AcSBV5 FP	GCGTAGACCAGTATTGTTGTT	2494–2512
AcSBV6 RP	TACCTGATTTCCCTCATCGT	3150–3169
AcSBV7 FP	CTCTGATGAGCACGCTCGAGTTCA	3100–3123
AcSBV8 RP	AGCACTGGACTGAGGAACAGTCA	4122–4144
AcSBV9 FP	CGGATGCTCAGCTTATTACCACAG	4008–4031
AcSBV10 RP	CAAACGCAAAGATCCCACTTCAG	4899–4912
AcSBV11 FP	CTAAGGAATGGTTGGTGGCGAAGT	4800–4823
AcSBV12 RP	AGTAATTTCCCTCTCTCGCATC	5794–5815
AcSBV13 FP	ATGTGGCTCGCTCTCTGATGCG	5778–5805
AcSBV14 RP	CCTCCTTAATGGCACGCACA	6532–6551
AcSBV15 FP	ATGGGACAGTGGCTTTATTACC	6501–6522
AcSBV16 RP	CTACATAAGGAAAACCCGCACT	7499–7520
AcSBV17 FP	TGAAACCCTTGGTGGTGAAACC	7401–7422
AcSBV18 RP	AACCAATATAGCATATATGAGACC	8706–8729

sequence similarity with other Indian isolates and 92–96% with the isolates from other countries (Table 2). The deduced aa sequences for part of the *VP1* protein in the eight Indian SBVs and forty-five SBVs from other countries were aligned (supplementary Fig. 1). Among Indian isolates, except for IndS2 and IndK3A isolates, all the AcSBV Indian isolates including isolate generated in this study lacked 10 continuous aa Sequence difference count matrix was ranged 154–907 nt among the SBV isolates used in this study (Table 2). A maximum of 907 nt differences was noticed in AmSBV-AUSS3 isolate while 154 nt difference was observed in AcSBv-IndII9 isolate. Further, protein sequence difference count matrix ranged from 34 to 217 aa for ORF. A minimum of 34 aa and maximum of 157 aa difference was noticed in IndII9 and ChiCQA isolates of AcSBV. Among the Indian isolates, a maximum of 134 aa difference was recorded in IndS2 isolates.

The isolate AcSBV-IndII9 had an evolutionary distance of 0.018 from the reference sequence (Table 2). Between the SBV isolates, the value of an evolutionary distance ranged from 0.018 to 0.110. Among AcSBV isolates, the Indian isolate (AcSBV-IndII9) and Vietnam isolate (AcSBV-Viet5) showed evolutionary distance of 0.018 and 0.087 respectively. The Korean AcSBV isolates had an evolutionary distance ranged 0.081–0.085. Among AcSBV-Vietnam isolates, the nearest isolate VietSBM2 and farthest isolate Viet5 showed an evolutionary distance of 0.057 and 0.087. In case of the Chinese isolates, CSBV-ChiLN isolate had minimum evolutionary distance (0.070) and AcSBV-ChiCQ1 isolate had maximum evolutionary distance of 0.081. The absolute values of an evolutionary distance between or within AmSBV isolates were recorded to be 0.067 for AmSBV-Viet 6 and 0.110 for AmSBV-AUS1, S3 and VN3. AmSBV isolates viz., USMD1, USMD2, AUS2, AUSWA2, AUSVN1 and AUSQLD had same value of evolutionary distance (0.109) from the reference sequence whereas AmSBV isolates belonging to Korea i.e., Kor19 and Kor2 showed 0.086 distance and Kor21 recorded the highest value of 0.108 compared to reference sequence. Isolates of Viet 4 and Viet 6 showed evolutionary distance of 0.082 and 0.067. The ratio of non-synonymous (Ka) to synonymous (Ks) nucleotide substitution rates (Ka/Ks) was calculated to understand the nt change, which affects the aa sequence of the protein. The values of Ka and Ks ranged from 0.0181 to 0.1056 and 0.0159 to 0.1189, respectively. Isolates AmSBV-AUSS3 and AmSBV-AUSVN3 had the highest Ka and Ks values (Table 2). In case of Indian isolates, the value of Ka/Ks ranged from 0.6131 to 1.2521. The isolates AcSBV-IndII2, IndII9, IndII10, AmSBV-UK and AmSBV-CRBrno had comparatively higher Ka/Ks values ranging 1.0098–1.2521 probably owing to high mutations both in nucleotide and protein level when compared to the reference isolate.

Phylogenetic analysis

A phylogenetic tree was constructed using the complete genome sequences of AcSBV-IndTN1 and the previously reported complete SBV genome nt sequences from other countries retrieved from NCBI Genbank. The phylogenetic tree diverged into two main branches (Fig. 1). In the first

Table 2 Analysis of evolutionary distance, Ka/Ks ratio, and per cent sequence identity and sequence difference count matrix of open reading frame of AcSBVIndTN1 isolate with other SBV isolates

S. nos.	Isolate	Accession number	Evolutionary distance	Ka	Ks	Ka/ks	NT identity (%)	Sequence different count matrix	AA identity (%)	Sequence different count matrix
1	AcSBV-IndII2	JX270795	0.029	0.0298	0.0238	1.2521	97	248	98	69
2	AcSBV-IndK1A	JX270796	0.031	0.0294	0.0382	0.7696	97	265	97	75
3	AcSBV-IndK5B	JX270797	0.034	0.0321	0.0396	0.8106	97	286	98	71
4	AcSBV-IndK3A	JX270798	0.043	0.0374	0.0610	0.6131	95	394	97	96
5	AcSBV-IndS2	JX270799	0.050	0.0474	0.0597	0.7939	95	450	95	134
6	AcSBV-IndII9	JX270800	0.018	0.0181	0.0159	1.1383	98	154	99	34
7	AcSBV-IndII10	JX194121	0.038	0.0383	0.0346	1.1069	96	320	97	97
8	AcSBV-Kor	HQ322114	0.083	0.0796	0.0866	0.9191	92	709	95	147
9	AcSBV-VietSBM2	KC007374	0.057	0.0531	0.0688	0.7718	94	508	96	109
10	AcSBV-ChiFZ	KM495267	0.078	0.0747	0.0827	0.9032	92	692	94	170
11	AcSBV-ChiSXnor	KJ000692	0.075	0.0725	0.0789	0.9188	93	641	95	149
12	AcSBV-ChiBJ2012	KF960044	0.077	0.0742	0.0813	0.9126	92	657	94	164
13	AcSBV-ChiCQA	KC285046	0.079	0.0767	0.0827	0.9274	90	857	92	217
14	AmSBV-UK	AF092924	0.104	0.1022	0.1012	1.0098	90	855	95	135
15	AmSBV-Kor21	JQ390591	0.108	0.1015	0.1187	0.8550	90	889	95	155
16	AmSBV-Kor19	JQ390592	0.086	0.0818	0.0954	0.8574	91	738	95	155
17	AmSBV-Kor1	KP296800	0.106	0.1007	0.1124	0.8959	90	873	95	153
18	AmSBV-Kor2	KP296801	0.086	0.0827	0.0903	0.9158	92	726	95	151
19	AcSBV-Kor3	KP296802	0.081	0.0789	0.0818	0.9645	92	698	95	146
20	AcSBV-Kor4	KP296803	0.085	0.0820	0.0859	0.9545	91	729	95	147
21	AcSBV-VietLD	KJ959613	0.066	0.0632	0.0722	0.8753	96	568	96	116
22	AcSBV-VietHYnor	KJ959614	0.084	0.0804	0.0898	0.8953	91	733	95	148
23	AcSBV-Viet1	KM884990	0.084	0.0814	0.0868	0.9377	91	732	95	148
24	AcSBV-Viet2	KM884991	0.084	0.0814	0.0875	0.9302	91	734	95	149
25	AcSBV-Viet3	KM884992	0.085	0.0822	0.0853	0.9636	91	737	95	155
26	AmSBV-Viet4	KM884993	0.082	0.0790	0.0869	0.9090	92	719	95	141
27	AcSBV-Viet5	KM884994	0.087	0.0828	0.0937	0.8836	91	754	94	162
28	AmSBV-Viet6	KM884995	0.067	0.0647	0.0733	0.8826	93	583	96	124
29	AcSBV-VietBP	KX668139	0.072	0.0701	0.0742	0.9447	93	618	95	154
30	AcSBV-VietNA	KX668140	0.070	0.0662	0.0816	0.8112	93	622	95	135
31	AcSBV-VietBG	KX668141	0.073	0.0702	0.0767	0.9152	93	639	95	148
32	AcSBV-ChiCQ1	KJ716805	0.081	0.0789	0. 0812	0.9716	90	816	94	180
33	AcSBV-ChiCQB	KJ716806	0.080	0.0774	0.0826	0.9370	91	809	94	184
34	AmSBV-CRBrno	KY273489	0.106	0.1054	0.0979	1.0766	90	868	95	140
35	AmSBV-USMD1	MG545286	0.109	0.1046	0.1148	0.9111	90	896	95	145
36	AmSBV-USMD2	MG545287	0.109	0.1045	0.1146	0.9118	90	894	95	146
37	AmSBV-Aus1	KY887697	0.110	0.1051	0.1169	0.8990	89	907	95	154
38	AmSBV-Aus2	KY887698	0.109	0.1041	0.1161	0.8966	90	900	95	155
39	AmSBV-AusS3	KY887699	0.110	0.1056	0.1160	0.9103	89	907	95	151
40	AmSBV-AusWA2	KY465671	0.109	0.1032	0.1181	0.8738	90	897	95	153
41	AmSBV-AusWA1	KY465672	0.105	0.0996	0.1137	0.8759	90	873	95	149
42	AmSBV-AusVN3	KY465673	0.110	0.1048	0.1189	0.8814	90	898	95	154
43	AmSBV-AusVN2	KY465674	0.104	0.0982	0.1131	0.8682	90	861	95	152
44	AmSBV-AusVN1	KY465675	0.109	0.1038	0.1159	0.8955	90	901	95	156
45	AmSBV-AusTAS	KY465676	0.105	0.0993	0.1130	0.8787	90	872	95	153
46	AmSBV-AusSA	KY465677	0.104	0.0981	0.1123	0.8735	90	853	95	149

Table 2 continued

S. nos.	Isolate	Accession number	Evolutionary distance	Ka	Ks	Ka/ks	NT identity (%)	Sequence different count matrix	AA identity (%)	Sequence different count matrix
47	AmSBV-AusQLD	KY465678	0.109	0.1030	0.1162	0.8864	90	890	95	154
48	AmSBV-AusNT	KY465679	0.101	0.0973	0.1038	0.9373	90	835	95	146
49	CSBV-ChiJL	KU574661	0.077	0.0749	0.0788	0.9505	92	676	95	153
50	CSBV-ChiSXYL	KU574662	0.078	0.0747	0.0807	0.9256	92	652	95	137
51	CSBV-ChiLN	HM237361	0.070	0.0685	0.0695	0.9856	93	633	94	176
52	SBV-Chi	AF469603	0.072	0.0691	0.0758	0.9116	93	626	94	163

AcSBV, *A cerana Sacbrood virus*; AmSBV, *A mellifera Sacbrood virus*; CSBV, Chinese *Sacbrood virus*; Aus, Australia; Chi, China; CR, Czech Republic; Ind, India; Kor, Korea; Viet, Vietnam; UK, United Kingdom; US, United states

main branch, except AmSBV-Viet6 and Viet-4, AmSBV-Kor2 and Kor-19, all other AmSBV isolates from Australia, Czech Republic, United Kingdom and United states were clustered together and formed as one group (I). The second main branch subdivided into two sub branches. Most of the isolates belong to Korea, Vietnam, China are grouped into sub branch IIa. AcSBV-IndTN1 and all the seven complete genome sequences of Indian isolates (unpublished) along with five Vietnam isolates (VietSBM2, VietNA, VietBG, VietBP, VietLD) and three Chinese isolates (ChiSXnor, ChiSXYL, ChiBJ2012) were grouped together to form a sub-branch IIb. The AcSBV-IndTN1 isolate showed closer genetic relationship with other isolates from India. This data reinforces the finding that SBV can cross-infect between *A. cerana* and *A. mellifera* species [7, 13, 21]. The Korean isolates were more diverse and were distantly related to Indian isolates. The phylogenetic analysis clearly grouped the isolates based on geographical locations rather than the host on which they were found. For instance, the AcSBV-VietLD was close in genetic makeup with AmSBV-Viet6 which was recorded on different hosts but close geographic location. Similarly, AcSBV-Viet1 to Viet3 were closely related to AmSBV-Viet4 which have different host insects. The close genetic relationship of SBV isolates in a geographic location irrespective of the host insect, highlights the possibility of cross infection of SBV isolates between *A. cerana indica* and *A. mellifera* and the management criteria to be followed to keep the disease under check. The high similarity between the AcSBV-IndS2 and the AmSBV-IndHP isolates may be due to the cross-infections. Similar cross-infection has been reported by Li et al. [13]. The phylogenetic variation is consistent with nucleotide similarity among the isolates.

Population dynamics

Genetic differentiation between populations was examined by three permutation-based statistical tests, Ks*, Z, and Snn [11]. These statistical tests revealed a higher divergence between the Indian isolates and the subpopulations from the Australia, China, Korea, Vietnam and USA (Table 3). FST values among all the isolates were above 0.33, indicating an infrequent geneflow occurring between them. The pattern of molecular diversity was evaluated using Tajima's D, Fu, and Li's D* and F* statistical tests at segregating sites, and haplotype and nucleotide diversity at all sites (Table 4). These statistics are expected to have negative values for background selection, genetic hitchhiking, and demographic expansion which also indicate that a population has maintained low frequency polymorphism [10, 23]. Except the isolates of India and USA, all other groups across the world, had negative values of Tajima's D, Fu, and Li's D* and F*, indicating that the population expansion of SBV was a recent phenomenon. The haplotype diversity values of all geographic location pairs were equal to one, while the nucleotide diversity values were low (Table 4). Overall, the deviations of the ORF from the neutral equilibrium were analyzed, within/between geographical groups, the results of which were consistent with a model of recent population expansion.

Naturally, viruses infecting and circulating in the honeybee populations for a long time can lead to an exchange of viruses among the host populations, and as a consequence, the viruses have evolved more or less independently. Both mutation and recombination are important forces driving the evolution of honey bee RNA viruses [16, 17], but their relative contribution to SBV evolution remains unexplored. This hypothesis of cross infection has also been addressed in several previous studies [4, 7, 8, 13, 20, 25]. SBVs attacking honey bees of a geographic region are more closely related with one another than with other geographic locations irrespective of the host insects *A. mellifera* and *A. cerana indica*. This finding assumes significance since in India, different bee species are reared in the same apiary and there is possibility of cross infection by SBV isolates. Hence, it is imperative

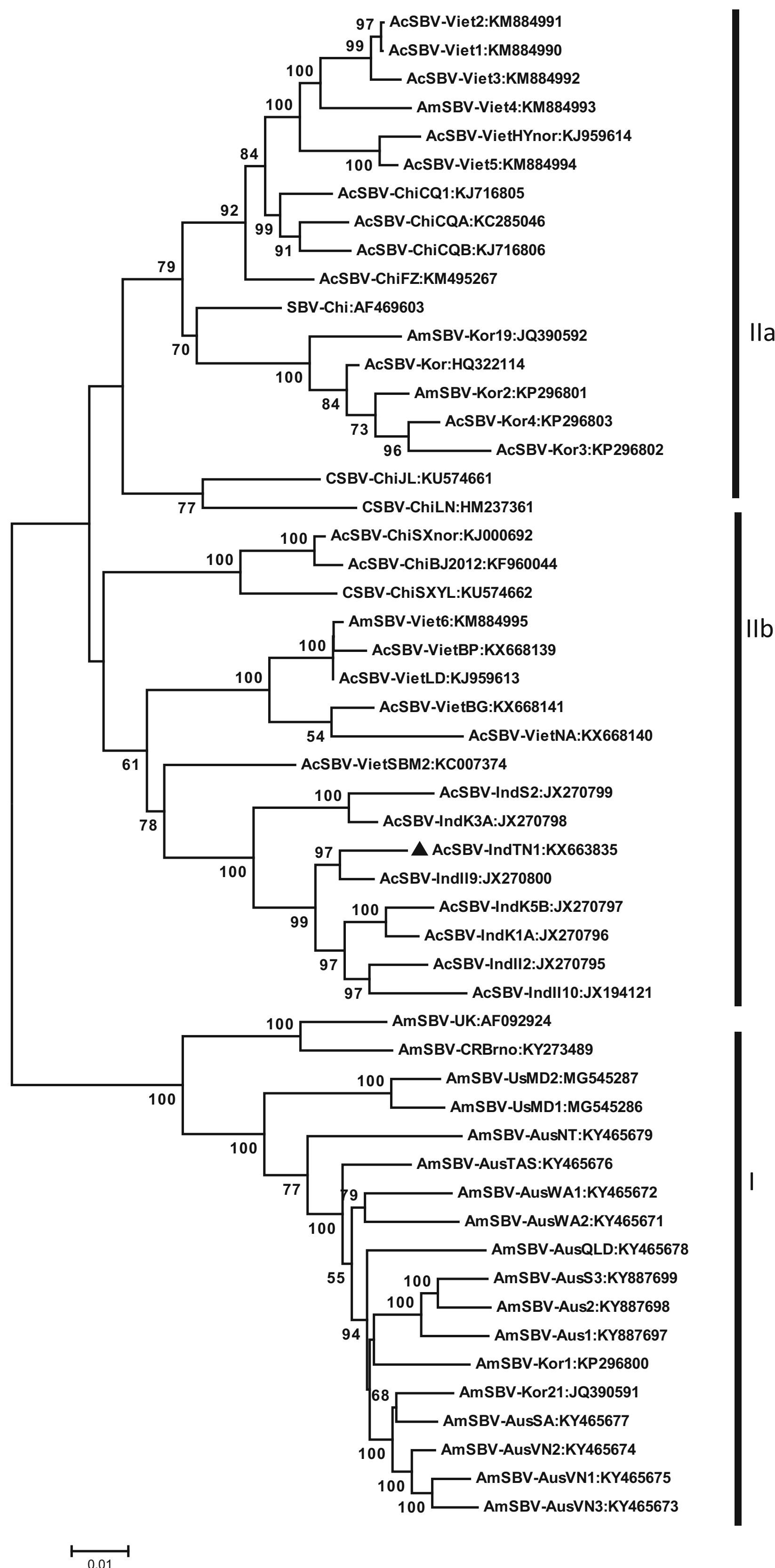

Fig. 1 Phylogenetic analysis of nucleotide sequence of complete genome of SBV isolates from different regions using MEGA 7.0 software. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Bootstrap scores above 50% (1000 replicates) are placed at the tree nodes. The scale bar represents the number of nucleotide substitutions per site. The evolutionary distances were computed using the maximum composite likelihood method and are in the units of the number of base substitutions per site. For the detailed of isolates, refer Table 2

Table 3 Genetic differentiation measurement for host and geography of SBV population

Isolates	Parameters				
	Ks* (*P* value)	Z (*P* value)	Snn (*P* value)	FST	Nm
All AcSBV and all AmSBV	6.134112 (0.0000)	350.47143 (0.0000)	0.85417 (0.0000)	0.34276	0.48
Indian AcSBV and other AmSBV	6.01090 (0.0000)	138.72120 (0.0000)	0.96667 (0.0000)	0.50538	0.24
India and Aus	5.62117 (0.0000)	46.81189 (0.0000)	1.00000 (0.0000)	0.66567	0.13
India and China	5.83538 (0.0000)	37.63124 (0.0010)	1.00000 (0.0010)	0.40213	0.37
India and other CSBV	5.79238 (0.0020)	18.57440 (0.0020)	1.0000 (0.0090)	0.32965	0.51
India and Korea	5.79894 (0.0000)	32.84091 (0.0000)	1.00000 (0.0000)	0.45083	0.30
India and US	5.65358 (0.0180)	14.46429 (0.0180)	1.00000 (0.0720)	0.74581	0.09
India and Vietnam	5.77774 (0.0000)	57.57190 (0.0000)	1.00000 (0.0000)	0.40626	0.37
India and all others	6.25862 (0.0000)	595.54594 (0.0000)	1.0000 (0.0000)	0.34997	0.46

Ks*, Z, and Snn represent the most powerful sequence-based statistical tests for genetic differentiation and are recommended for use in cases of high mutation rate and small sample size [11]. The Z statistic value results from ranking distances between all pairs of sequences. Snn the frequency with which the nearest neighbors of sequences are found in the same locality; FST, coefficient of gene differentiation or fixation index, which measures inter-population diversity; Nm can be interpreted as the effective number of migrants exchanged between demes per generation

Table 4 Neutrality tests, haplotype, and nucleotide diversity of SBV population

Host and geography	Tajima's D	Fu and Li's D	Fu and Li's F	Haplotype diversity	Nucleotide diversity
All	− 0.75177	− 0.81112	− 0.9446	1.000	0.07711
India and CZ	− 0.97406	− 0.98500	− 1.10488	1.000	0.04971
India and Vietnam	− 0.05609	− 0.10154	− 0.10246	1.000	0.05982
India and US	− 0.27790	0.33689	0.20656	1.000	0.05948
India and UK	− 0.95882	− 0.96735	− 1.08569	1.000	0.04947
India AcSBV and all AmSBV	− 0.73071	− 0.90271	− 1.00068	1.000	0.07368
India and Korea	− 0.24809	0.05296	− 0.03671	1.000	0.00675
India and CSBV	− 0.82193	− 0.78566	− 0.90779	1.000	0.00512
India and China	− 0.54423	− 0.45027	− 0.55615	1.000	0.06069
India and Aus	− 0.13926	− 0.59369	− 0.53195	1.000	0.06837

Tajima's D test compares the nucleotide diversity with the proportion of polymorphic sites which are expected to be equal under selective neutrality. Fu and Li's D* test is based on the differences between the numbers of singletons (mutations appearing only once among the sequences) and the total number of mutations. Fu and Li's F* test is based on the differences between the number of singletons and the average number of nucleotide differences between pairs of sequences

to keep the two species of bees separately in different apiaries separated by a safe isolation distance of at least a few kilometres to prevent accidental cross infection of drones of either species that freely move between the colonies as well as foraging workers that visit the same flowers.

In conclusion, we report that complete genome of SBV isolate infecting *A. cerana* from Tamil Nadu, India has been sequenced and compared with 52 complete genome isolates from India and other countries. The nt and aa diversity ranged from 89 to 98% and 92 to 99% respectively. We observed an infrequent geneflow between the isolates used in this study. Further we prepared a model of recent population expansion of SBV isolates as they lack nt diversity within the groups. Since cross-infection of SBV between *A. melifera* and *A. cerana* is highly suspected, we recommend that the rearing them in separate apiaries with safe isolation distance.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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