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Original Article

PHYLOGENETIC ANALYSIS OF THE HONEY BEE SACBROOD VIRUS

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Abstract

Sacbrood virus (SBV) is one of the most common and harmful viruses to honeybees. It causes failure to pupate and death during larval stage, in adult bees it has an influence on their behavior and even shortens their life-span. In this study, we analyzed the phylogenetic relationships among the SBV isolates from all around the world, with from both *Apis cerana* and *Apis mellifera*. Phylogenetic trees were constructed based on three types of nucleotide sequences: complete genome sequence, VP1 gene and SB1-2 fragment of SBV. Moreover, genome recombination analysis was performed to assess the effect of genome recombination on the evolutionary relationship of some SBV isolates. The phylogenetic trees showed that although all the SBV isolates form two major groups, these two groups were not formed strictly according to their host specificity or geographical origin. These results indicate that both host specificity and geographic origin decide the genetic diversity of SBV strains.

Keywords: recombination, geographic origin, host specificity, nucleotide sequence, phylogenetic relationship, SBV

INTRODUCTION

Sacbrood virus (SBV) is one of the most common honey bee virus detected throughout the world, in almost all subspecies of honey bees (Allen et al., 1996; Ellis et al., 2005; Ma et al., 2010; Kim Cuc et al., 2008). This virus is passed to the honey bee eggs, then reproduces at the larval stage, and finally leads to death of the larvae (Ma et al., 2013; Ravoet et al., 2015). It also can infect adult bees, but obvious signs of disease are lacking in adults (Anderson et al., 1989; Bailey, 1969). It does have an influence on the behaviour of adult bees and even shortens their life-span (Bailey, 1969). Sacbrood virus is more likely to infect *Apis cerana* and often brings more serious damage to the *A. cerana* than to *Apis mellifera*. This is especially true in the spring and summer when the colony size is expanding rapidly and large numbers of susceptible larvae and young adults are available (Bailey, 1969; Ma et al., 2010). In China, the first record of SBV breakout was in the province of Guangdong, in 1972. This outbreak resulted in the collapse of more than 1 million colonies of *A. cerana* in China (Luo et al., 2013).

Sacbrood virus is a single-strand small RNA virus and belongs to the genus *Iflavirus* in the family *Iflavirus* (Ghosh et al., 1999). The virus has structural protein genes at the 5' end of its genome and the non-structural protein genes at 3' end (King et al., 2011). The complete genomic sequence of SBV was first reported by Ghosh et al. (1999). This sequence's 8.8kb geome contains a large open reading frame encoding structural proteins VP1, VP2, and VP3, to compose the virus capsid. Sacbrood virus can infect different honeybee species and has been found in different geographical areas, including the USA (Ghosh et al., 1999), South America (Antunez et al., 2005; Freiberg et al., 2012), Europe (Bakonyi et al., 2002; Tentcheva et al., 2004), Australia (Anderson et al., 1988), South Africa (Benjeddou et al., 2001), Asia (Bailey et al., 1982; Zhang et al., 2001; Ma et al., 2011; Choe et al., 2012; Nguyen et al., 2013), and New Guinea (Roberts & Anderson, 2014). The SBV specifically infecting *A. cerana* is named CSBV. There are now several studies on the evolutionary relationship of the SBV

isolates from around the world, but there is no concensus. Choe et al. (2012) and Ma et al. (2013), respectively, constructed phylogenetic trees based on SBV nucleotide sequences. Their results indicated that two major clades corresponding to different host formed in the trees. For this reason, the above-mentioned researchers believe that SBV can be classified into *A. cerana* genotype and *A. mellifera* genotype according to their host. But Grabensteiner et al. (2001) hold that SBV can be divided into different geographic types, according to origin. In recent years, with the increase of SBV nucleotide sequences deposited in GenBank database, we have been provided with the opportunity to systematically analyse the phylogenetic relationships among the different SBV isolates. Therefore, in this study we used the whole genome sequence, VP1 gene and SB1-2 fragment of SBV to carry out a comprehensive phylogenetic analysis of SBV isolates to elucidate their evolutionary relationship.

Keywords: geographic origin, host specifity, nucleotide sequence, phylogenetic relationship, recombination, SBV

MATERIAL AND METHODS

SBV sequences

Twenty-seven complete genomic sequences, 44 VP1 gene coding sequences, and 86 SB1-2 sequences from different SBV isolates were downloaded from the GenBank database. These SBV isolates used in our analysis have a wide range of geographical origins, and their hosts cover both *A. cerana* and *A. mellifera*. The SB1-2 sequence (a conservative fragment of SBV) was based on previously published research (Grabensteiner et al., 2001).

Sequence analysis

Multiple alignment of nucleotide sequence was performed using Clustal X 1.8, and obvious mismatches were manually corrected. Phylogenetic trees based on sequences of complete genome, VP1 gene, and the SB1-2 fragment, were constructed by the MEGA 4.1 software using the maximum likelihood model of the neighbor-joining (NJ) method with a bootstrap test of 1000 replicates.

The analysis of the recombination based on sequences of complete genome, VP1 gene, and SB1-2 fragment of SBV, was carried out with the RDP, GENECONV, BootScan, MaxChi, Chimaera, and SiScan methods implemented in RDP ver. 4.44 software. To improve the reliability of the results, only those recombination events detected by two or more methods with $p<0.01$ were regarded as genuine recombination signals.

RESULTS

Phylogenetic analysis

Phylogenetic trees based on the complete genome sequences, VP1 gene, and SB1-2 fragment of SBV were constructed (shown in Fig. 1, 2, 3, respectively). In all the three phylogenetic trees, two major branches were formed. The SBV isolates from the *A. cerana* in Asia tended to clustered together, while isolates from *A. mellifera* in Western countries formed another clade. In the Asia clade, the isolates from India (in Fig. 3 isolates from Nepal were included) and the isolates from Korea each formed their own subclade. But isolates from China and Vietnam distributed into at least two subclades, suggesting population differentiation among SBV strains from these two countries.

Interestingly, the AmSBV-Kor19 isolate, which was mixed into the *A. cerana* group in Fig. 1 and Fig. 2, was clustered with other *A. mellifera* SBV isolates in Fig. 3, suggesting that recombination might have happened in this SBV isolate.

Analysis of genome recombination

The locations of the AmSBV-Kor19 isolate in the three phylogenetic trees were not consistent. Moreover, AmSBV-Kor19 and two other isolates: AmSBV-Viet4 and AmSBV-Viet6, were hosted by *A. mellifera* while they were clustered into the *A. cerana* group in Fig. 1 and Fig. 2. We suspect that these SBV isolates should derive from the genome recombination of other isolates distributed in the same region. Thus, we focused on detecting the evidence of genome recombina-

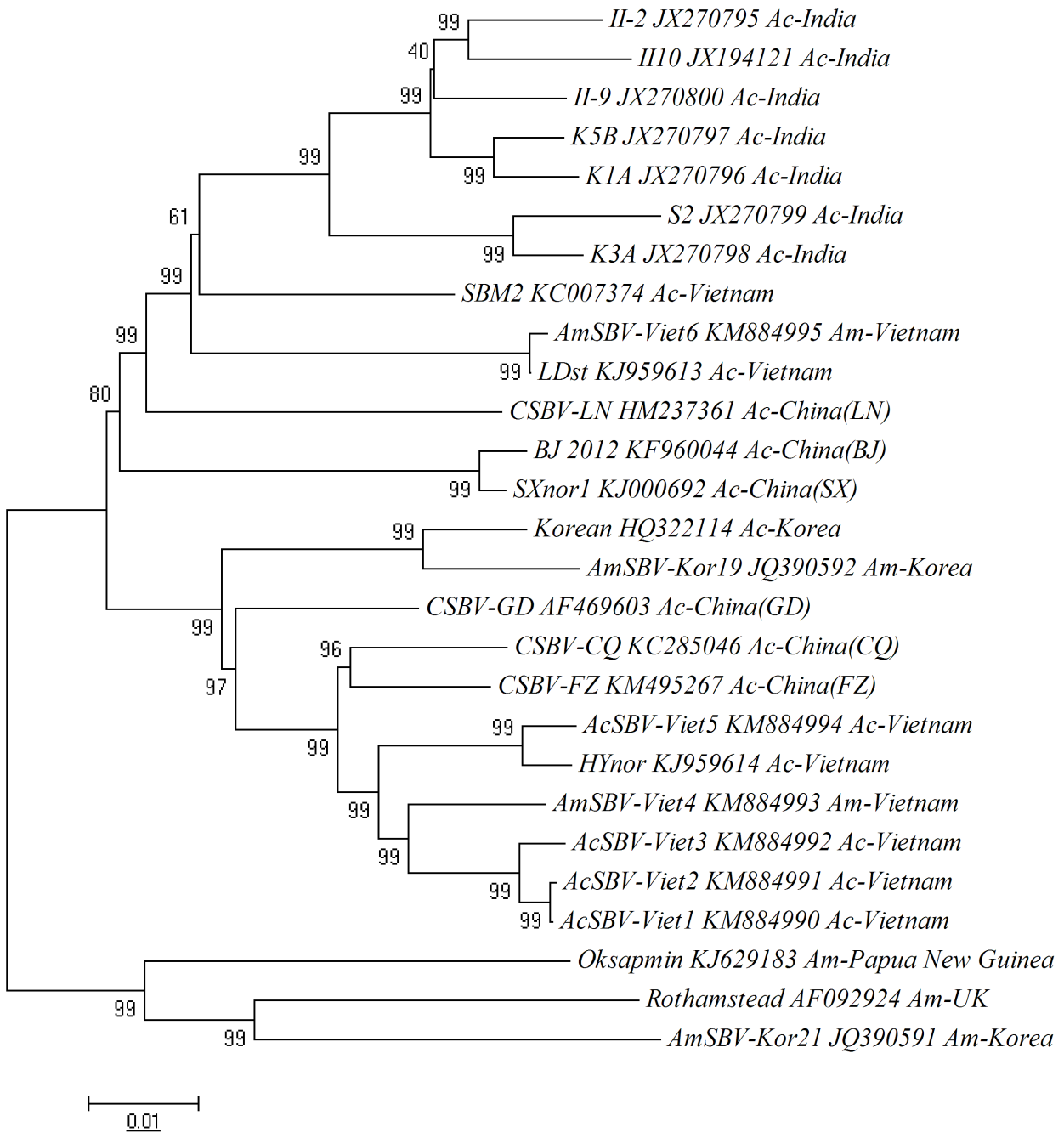

Fig. 1. Phylogenetic tree of SBV based on the complete genome sequences. The phylogenetic tree was constructed using MEGA 4.1 with the neighbour-joining method (NJ) with a bootstrap test of 1000 replicates. Numerals indicate bootstrap values (%) from 1000 replicates

tion events in these sequences.

When the complete genome sequences was used for recombination analysis, two different pieces of evidence for recombination were detected in isolate AmSBV-Viet4 (Tab. 1), one was located in 654-1270 bp with parental isolates AcSBV-Viet2 and CSBV-FZ, another was located in 609-2005 bp with parental isolates AcSBV-Viet1 and Korean. These parental isolates were from *A. cerana* in the same region. This could mean that the *A. mellifera* SBV isolate AmSBV-Viet4 might be derived from the recombination of other isolates distributed in the same region. For AmSBV-Kor19, recombination signals were detected in regions 1-1367 and 5080-5563 with parental isolates of Korean and AmSBV-Kor21. While no recombination event was detected in isolate AmSBV-Viet6.

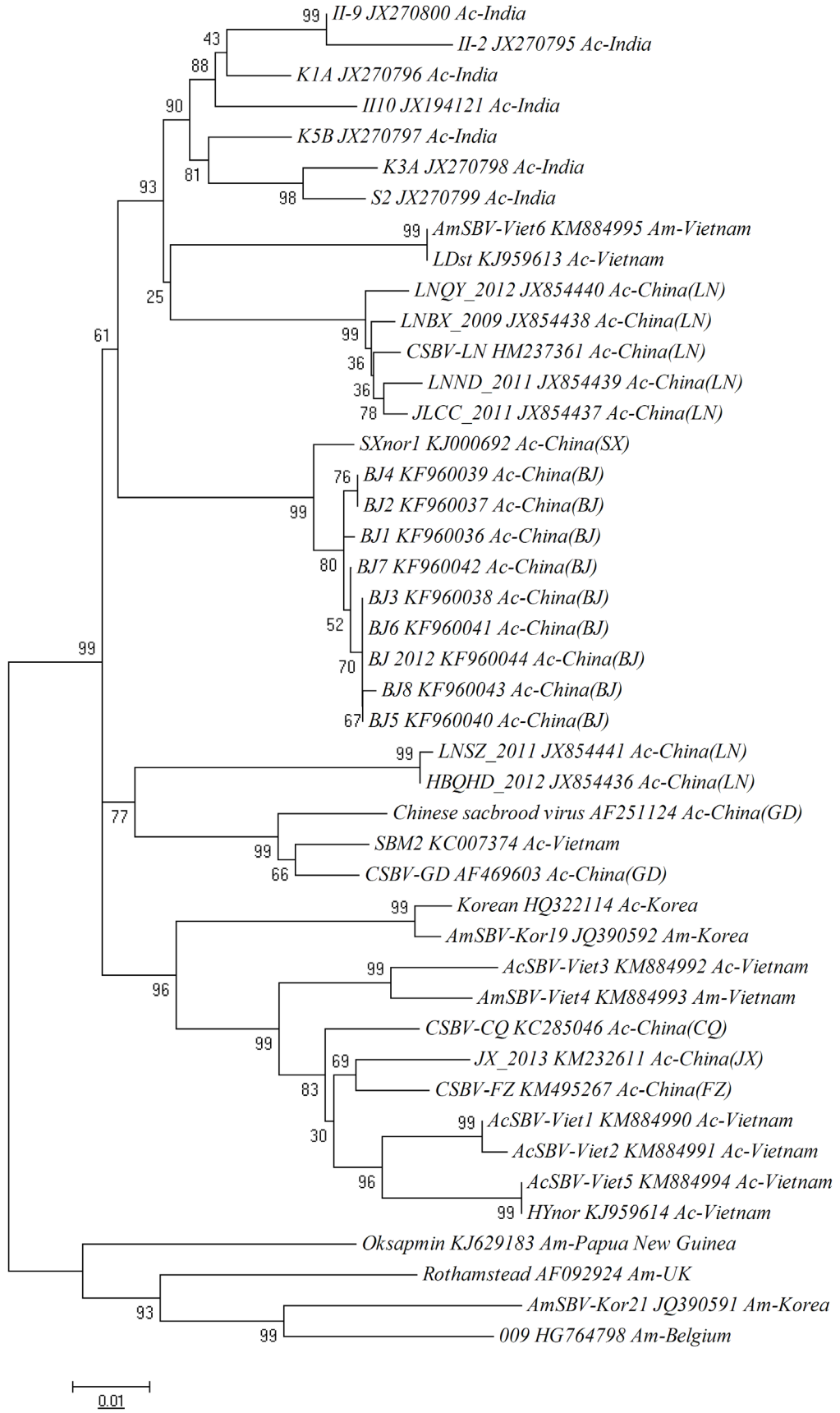

Fig. 2. Phylogenetic tree of SBV based on the VP1 gene. The phylogenetic tree was constructed using MEGA 4.1 with the neighbour-joining method (NJ) with a bootstrap test of 1000 replicates. Numerals indicate bootstrap values (%) from 1000 replicates

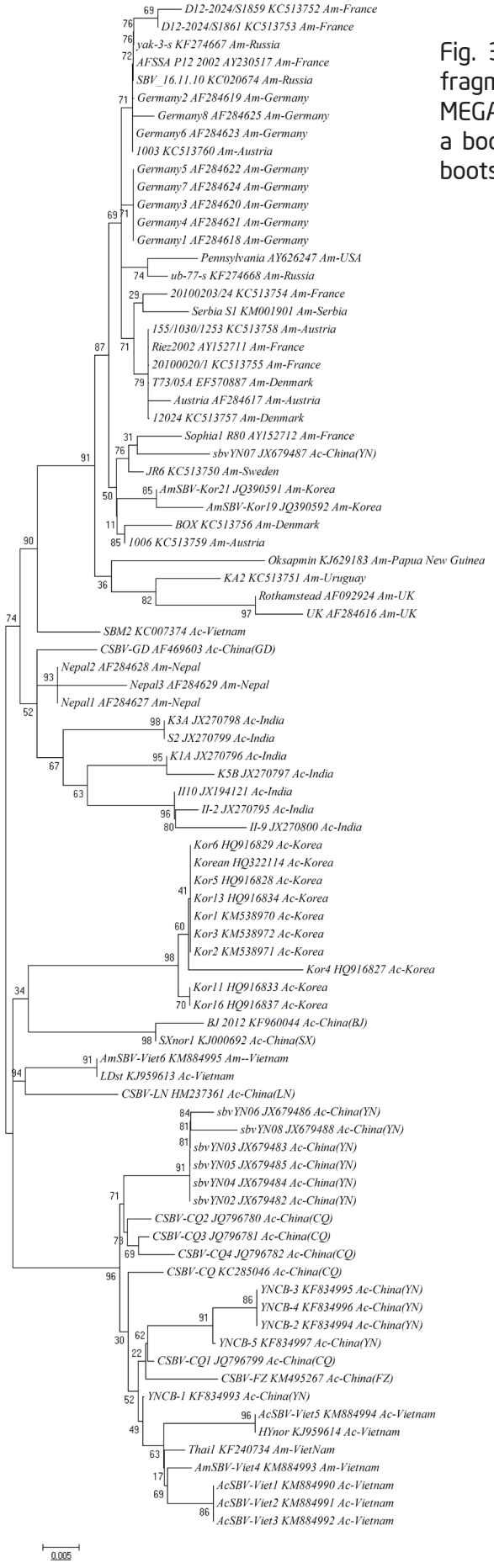

Fig. 3. Phylogenetic tree of SBV based on the SB1-2 fragments. The phylogenetic tree was constructed using MEGA 4.1 with the neighbour-joining method (NJ) with a bootstrap test of 1000 replicates. Numerals indicate bootstrap values (%) from 1000 replicates

When only the VP1 gene was used for recombination analysis, the results showed that only in AmSBV-Viet4 was there identified a recombination event at region 574-1028 with possible parental isolates of K3A and AcSBV-Viet2 (Tab.1). As for the SB1-2 fragments, no evidence for genome recombination was obtained within these three isolates.

DISCUSSION

Sacbrood virus was first reported in honeybees in the United States in 1913 (White, 1913). Since then, it became one of the most common bee diseases in the world. This disease often brought significant damage to *A. cerana*. At present, studies about SBV are a hotspot in honeybee research.

In this study, phylogenetic trees based on three different types of SBV sequences have been constructed. The results indicated that, in general, the SBV isolates form two major clades in the trees. But we found these two major clades can hardly be identified just by host specificity or geographic region. For example, in Figures 1 and 2, it seems that SBV isolates from the same host tend to clustered together, but the AmSBV-Viet6 and AmSBV-Viet4 isolates from Vietnam, and the isolate AmSBV-Kor19 from Korea which are from *A. mellifera*, were mixed into the *A. cerana* branches with a high bootstrap value. Although it is possible that the AmSBV-Kor19 and AmSBV-Viet4 isolates came from genome recombination of *A. cerana* SBV isolates according to the results of the recombination analysis, no genome recombination was detected in AmSBV-Viet6. Moreover, in the subclades, those isolates from the same geographical region tend to be clustered in the same clades. On the other hand, it is also not accurate to identify the two major clades

Table 1
Recombination events detected in the nucleotide sequences of isolates AmSBV-Kor19 and AmSBV-Viet4

Nucleotide sequence	Recombinant isolates	Parental isolates		Break point	Detection methods#
		Major	Minor		
Whole genome sequence	AmSBV-Viet4	AcSBV-Viet2	CSBV-FZ	654-1270	RDP, GENECONV, Bootscan, Maxchi, Chimaera, SiSscan, 3Seq
	AmSBV-Viet4	AcSBV-Viet1	Korean	609-2005	RDP, Bootscan, Maxchi, Chimaera, 3Seq
	AmSBV-Kor19	Korean	AmSBV-Kor21	1-1367 5080-5563	RDP, GENECONV, Bootscan, Maxchi, Chimaera, SiSscan, 3Seq
VP1	AmSBV-Viet4	K3A	AcSBV-Viet2	574-1028	GENECONV, Maxchi, Chimaera, SiSscan, 3Seq

Analysis of the recombination was carried out with the RDP, GENECONV, BootScan, MaxChi, Chimaera, and SiScan methods implemented in RDP ver. 4.44 software.

according to just geographic origin, because in all the three trees the Korean *A. mellifera* isolate AmSBV-Kor21 was clustered with the *A. mellifera* isolates from European countries. Therefore, these results indicated that both host specificity and geographic region influence the phylogenetic relationship of the SBV strains. Our results indicated that the AmSBV-Viet4 isolate from Vietnam which is from *A. mellifera* may come from the recombination of the *A. cerana* SBV isolates distributed in the same region, and the Korean isolate AmSBV-Kor19 may come from the recombination of a *A. cerana* SBV isolate and a *A. mellifera* isolate, suggesting that SBVs infecting *A. cerana* can also infect *A. mellifera*. In other words, cross-infection of SBVs might exist between *A. cerana* and *A. mellifera*. To summarise, we discovered that both host specificity and geographic origin determine the genetic diversity of SBV isolates.

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