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The prevalence of seven crucial honeybee viruses using multiplex RT-PCR and their phylogenetic analysis

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Abstract: Honey bee viruses may be of severe adverse effect on bee colonies. Till now, more than twenty honey bee viruses have been identified. The aim of this study was to investigate the prevalence of seven honey bee viruses, namely Israeli acute paralysis virus (IAPV), deformed wing virus (DWV), sacbrood virus (SBV), acute bee paralysis virus (ABPV), black queen cell virus (BQCV), Kashmir bee virus (KBV), and chronic bee paralysis virus (CBPV) using a multiplex reverse transcription polymerase chain reaction (mRT-PCR). A total of 111 apiaries were randomly selected and adult bees and larvae samples were obtained from seemingly healthy colonies located at Aegean region of west Turkey. The presence of black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), deformed wing virus (DWV), acute bee paralysis virus (ABPV), and sacbrood virus (SBV) was detected, while Israeli acute paralysis virus (IAPV) or Kashmir bee virus (KBV) couldn't be detected in studied colonies. The results showed the virus with the highest prevalence was DWV followed by BQCV, ABPV, SBV, and CBPV. PCR products for DWV, BQCV, ABPV, SBV, CBPV were sequenced and compared to the Genbank database. The Turkish strains demonstrated heterogeneous populations of DWV, BQCV, and relatively homogenous populations of ABPV, CBPV, and SBV. Based on these results, SBV and CBPV had formed different branch and genotypes when compared to previous studies. In conclusion, this study provides information about the distinctive honey bee viruses for future researches in Turkey, which holds the largest number of managed colonies in Europe.

Key words: *Apis mellifera*, deformed wing virus, black quenn cell virus, sacbrood virus, chronic bee paralysis virus, acute bee paralysis virus

1. Introduction

Turkey has tremendous beekeeping potential with 7.4 million colonies ranking just after China and India. Twenty-five percent of these colonies are located in the Aegean region, which has an important place in the world of beekeeping [1].

Until now, approximately twenty-four different viruses affecting Apis genus have been identified [2]; this number changes continuously with the identification of new viruses such as Apis Rhabdovirus-1, Apis Rhabdovirus-2 [3], and Varroa orthomyxovirus-1 [4]. It is known that a large part of honey bee viruses is of single-stranded RNA, while filamentous virus (AmFV) and Apis iridescent virus (AIV) have DNA genome. Apart from cronic bee paralysis virus that has an isometric shape, most of the other viruses have indistinguishable using particle morphology [5-7].

In general bee viruses belong to one of these two families known as Iflaviridae and Dicistroviridae. They are nonenveloped, single-stranded, and positive-sense RNA viruses [6]. Seven viruses namely Israeli acute paralysis virus (IAPV), deformed wing virus (DWV), sacbrood virus (SBV), acute bee paralysis virus (ABPV), black queen cell virus (BQCV), Kashmir bee virus (KBV), and chronic bee paralysis virus (CBPV) are thought to be primarily responsible for the majority of severe disease in apiaries around the globe [8,9]. IAPV, KBV, and ABPV belong to the genus *Aparavirus*, BQCV belongs to the genus *Cripavirus*, and both genera belong to the Dicistroviridae family. SBV and DWV belong to the genus *Iflavirus* of the Iflaviridae family, while CBPV is not classified yet [10,11].

Characteristic symptoms of DWV include shrunken wings that make flight impossible, decreased body size, and discoloration in adult bees [12–14]. SBV can infect either larvae or adult bee, but it causes larval death [14]. ABPV leads to typical symptoms in brood with discoloured larvae and paralysed adult bees [15]. BQCV causes mortality in queen larvae and pupae [16]. IAPV leads to rapidly progressing paralysis, trembling, inability to fly, darkening and loss of hair from the thorax and abdomen [7,17]. In experimental infections, KBV is extremely lethal to adults

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and larvae without clearly defined disease symptoms [16]. Characteristic symptoms of CBPV are paralysis and trembling, hairless and shiny, sometimes black individuals crawling at the hive entrance [5,18]. Although many bee viruses can cause fatal disease at the individual or colony level, they usually persist and spread harmlessly at low titres within and between bee populations. Difficult identification, unknown disease progress, the diagnosis, and management of disease progress of viral diseases cause difficulties comparing to other bee pathogens [7].

Many bee viruses are common throughout the world. Also, bee viruses have been the subject of different studies conducted in Turkey [19–23]. Turkey is located in both Europe and Asia, and it is situated at the crossroads of the Balkans, the Middle East, and the Mediterranean. Therefore, it is an important country in terms of the bilateral transmission of viruses in the area. Hence, in this study, we investigated seven honey bee viruses in local colonies in Turkey. In addition, a phylogenetic analysis was performed to provide information about the geographic distribution of different virus strains.

2. Materials and methods

2.1. Collection of bee samples and isolation of total RNA

The samples were randomly collected at a 95% confidence interval and 3% expected prevalence from apiaries in seven Aegean provinces (Aydın, Denizli, İzmir, Kütahya, Manisa, Muğla, Uşak) using the multistep sampling method [24] (Figure 1). A total of 111 apiaries, including 30 adult bees and 30 larvae from each apiary, were selected in this method. A pool consisting of 30 adult bees and 30 larvae from each apiary was created and homogenized with 9 mL Eagle Minimum Essential Medium (EMEM) (Sigma–Aldrich Corp., Dorset, United Kingdom) and then centrifuged at 3500 rpm and 4 °C for 30 min. The supernatant was stored at –80°C until analyzed.

Samples were collected between March 2017 and August 2017. There were no overt clinical symptoms in any of the collected bee samples. The presence of bacterial, parasitic, and fungal diseases was not checked.

For RNA extraction, 200 µL of supernatant was used. Total RNA extraction was carried out using High Pure Viral RNA Kit (Roche, Germany) in accordance with the manufacturer's instructions.

2.2. mRT-PCR amplification and nucleotide sequencing

The multiplex PCR method developed by Cagirgan and Yazici [25] was used in this study. The final optimum reaction mixture consisted of 18.5 µL water, 10 µL reaction buffer (Roche), 7 µL forward primer (0.4 µM each primers), 7 µL reverse primer (0.4 µM each primers), 1.5 µL $MgCl_2$ (2 mM), 5 U/µL Taq polymerase (Roche) and 5 µL RNA (samples), in a total volume of 50 µL.

The cycling conditions consisted of one cycle at 50 °C for 30 min for reverse transcription followed by initial denaturation at 95 °C for 7 min, followed by denaturation at 95 °C 20 s, annealing at 58 °C for 20 s, extension at 72 °C for 1 min, for 45 cycles, with a final extension at 72 °C for 10 min (Techne TC-412, United Kingdom). The results were analyzed using 2.5% agarose gel and staining with ethidium bromide in TAE buffer at 80 V for 80 min (Thermo, USA), the photographs was taken by Vilber Lourmat, France.

The PCR products were sequenced in Forward and Reverse by Microsynt (Balgach, Switzerland). Nucleotide sequence results were assembled and edited using DNADynamo DNA sequence analysis software. The consensus nucleotide sequences that were obtained were verified using the basic local alignment search tool (BLAST) at the National Central for Biotechnology Information (NCBI) [26].

2.3. Phylogenetic analysis

In order to determine phylogenetic relationships among strains of DWV, SBV, ABPV, BQCV, and CBPV viruses in Turkey, the following sequences were first identified: 484 nt sequences encoding a helicase gene of DWV, 317 nt sequences encoding a polyprotein protein of SBV, 719 nt sequences encoding a capsid protein of ABPV, 383 nt sequences encoding a capsid protein of BQCV and 1293 nt sequences encoding a putative RNA-dependent RNA polymerase (RdRP) of CBPV. The sequencing primers are provided in Table. The other sequences, which had been reported before, were obtained from GenBank. The multiple sequence alignments of the data were performed using the ClustalW algorithm using MEGA 6 software. Then, the best DNA/Protein models were identified to use for phylogeny. Phylogenetic trees were constructed using MEGA 6 software [32] with the maximum likelihood (ML) and neighbor-joining (NJ) methods with the Tamura-3 parameter. The best substitution models were selected for each tree, a bootstrap value of 1000 replicates for all methods.

Amino acid distances were calculated using the Jones–Taylor–Thornton method with gamma distribution (JTT+G), and a bootstrap value of 1000 replicates [32,33]. Similarity and identity of amino acid and nucleotide were determined using an online programme (imed.med.ucm.es/Tools/sias.html).

3. Results

3.1. Virus detection

For adult bees from 111 apiaries sampled, 22 were positive for DWV. In this study, DWV was the most frequently detected viral agent in the apiaries, occurring in 19.8% of those tested. BQCV was detected in 20 apiaries, and it was the second most prevalent viral agent with a prevalence

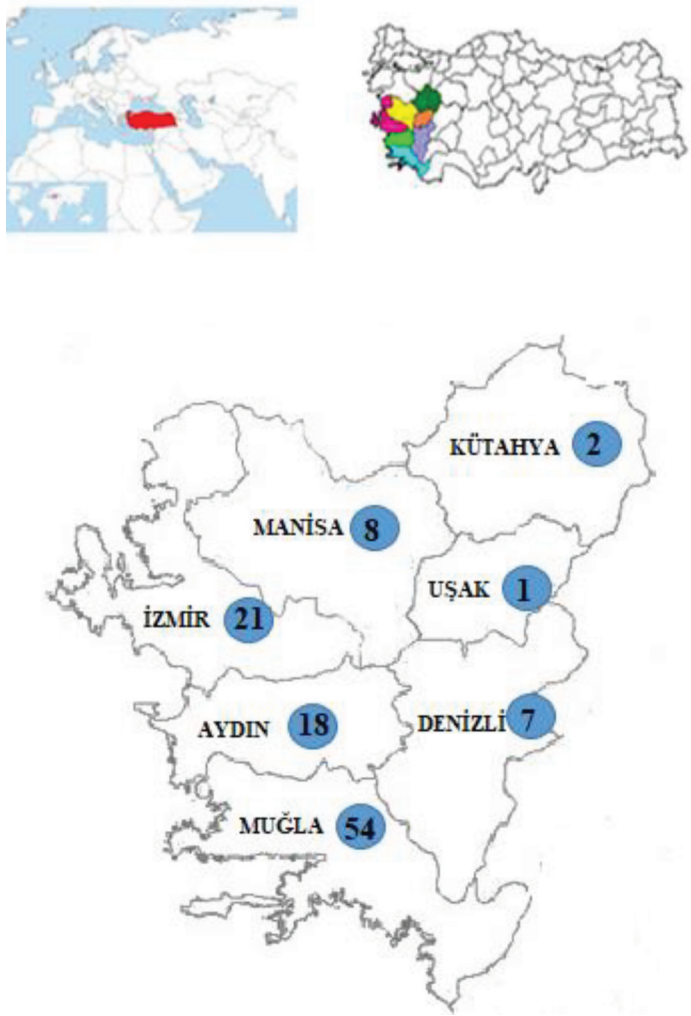

Figure 1. a) Turkey's geopolitical position. b) Provinces where samples are collected. c) Sample numbers collected from the provinces.

Table. Primers used in sequencing.

Viruses	Primer pairs	Amplified fragments (bp)	Reference Number
DWV HP-F **DWV HP-R**	*CCACATGGTCTGAATGGATGACG* *CACGACGCTTACTACACCAC*	618 bp	(27)
SBV-F **SBV-R**	*CGTAATTGCGGAGTGGAAAGATT* *AGATTCCTTCGAGGGTACCTCATC*	342 bp	(28)
ABPV CP-F **ABPV CP-R**	*TTATGTGTCCAGAGACTGTATCCA* *GCTCCTATTGCTCGGTTTTTCGGT*	900 bp	(29)
BQCV-F **BQCV-R**	*TGCAACCTCCTACGAACTCT* *TCCATGGCGACAGTTACATC*	514 bp	(30)
CBPV RNA1-F **CBPV RNA2-R**	*TCAGACACCGAATCTGATTATTG* *CCGGAGACAAAGGTCATCAT*	1525 bp	(31)

F: Forward primer; R: Reverse primer; DWV: Deformed wing virus; SBV: Sacbrood virus; ABPV: Acute bee paralysis virus; BQCV: Black quenn cell virus; CBPV: Chronic bee paralysis virus.

rate of 18%. Four out of 111 samples had ABPV, three had SBV, and two had CBPV. The prevalence of these viruses was 3.6%, 2.7% and 1.8%, respectively.

In terms of larvae, two viral agents, namely DWV and BQCV, were detected in 107 apiaries (no larvae obtained from four of the apiaries). DWV was detected in 11 out of 107 apiaries (prevalence ratio of 10.2%). BQCV was identified in only 3 of 107 apiaries (prevalence ratio of 2.8%). SBV, ABPV, and CBPV were not detected.

IAPV and KBV were not detected in either adult bees or larvae. Coinfection with 3 viruses (BQCV, SBV and DWV) was found in only one sample. Ten samples were positive for 2 viruses (eight with DWV and BQCV, one with ABPV and BQCV, and one with ABPV and CBPV).

In summary, DWV was detected in 17 of the adult bee samples, in 6 of the larvae samples, and in 5 samples of both adult bees and larvae. The overall prevalence of DWV was 25.2%. BQCV was detected in 20 of the adult bee samples and 3 of the larvae samples. The prevalence of BQCV was 20.2%.

3.2. Phylogenetic analysis

Phylogenetic analysis was carried out by sequencing 484 bp (6129–6613) of the gene coding for a helicase protein of DWV (MK431870, MK431871, MK431872, MK431873, MK431874), 383 bp (6614-6996) of a gene coding a structural polyprotein of BQCV (MK431878, MK431879, MK431880, MK431881, MK431882, MK431883), 796 bp (8526-9323) of a gene coding the capsid protein of ABPV (MK431884, MK431885, MK431886), 1293 bp (1991-3283) of a gene coding RNA1 RNA-dependent of CBPV (MK431887, MK431888), 317 bp (327-643) of a gene coding polyprotein of SBV (MK431875, MK431876, MK431877) for the purpose of describing the probable genetic relationship between Turkish viruses and those published in Genbank. In most cases, bootstrap analysis revealed high values of confidence (>70%), indicating that the present clustering is statistically supported.

The phylogenetic trees are given in Figures 2-6 for DWV, BQCV, SBV, ABPV, and CBPV, respectively. Turkish sequences of DWV are closely related to strains from Hungary, Poland, Slovenia, and Nepal, forming a sole cluster. The other subcluster consists of Germany, France, Italy, Austria, Sri Lanka, USA, UAE, and Canada. Korean strains form a distinct branch separated from other strains and comprise a subgroup among themselves.

BQCV strains are separated into two main groups, with all of the Korean BQCV genotypes (except Korea Am str3) and Hakkari (Turkish strains in another study) forming a common cluster, and all of the central European genotypes, the South African reference genotype, and the Korea Am str3 genotype forming a second cluster. Within this group, the six Turkish strains are further divided into two branches: one branch consists of the five Turkish strains, which had a similarity of 97%–99%, and the second branch consists of the Turkish Köyceğiz strains, which were 97% similar to strains from Poland (1,2,3), 94% similar to strains from Hungary (5,10) and Austria (2) and 92% similar to Korean Am str3. In this study, five Turkish strains from the same branch were 90% similar to the Turkish Köyceğiz strains.

The Turkish ABPV strains (Köyceğiz and Datça) originated from Austria, Hungary, Poland and Germany and are clustered in one branch. The European strains were 94%–97% similar to Turkish strains and separate from the South African and US strains although they share the same origin. The sequences from Brazil, Uruguay, and Chile clustered in a branch (80%–81% similarity with Turkish strains) that diverged from the cluster of European, South African and US strains.

CBPV strains are divided into two main groups: Turkish strains (İzmir and Mugla) diverged from the cluster of European and Uruguay strains. İzmir and Muğla strains were 97% similar to each other. The analysis of the sequence pair distances of the 24 strains, not including the Turkish strains, were 91%–100% similar to each other, but were only 81%–85% similar to the Turkish strains.

SBV strains are divided into two main groups. Turkish strains including those used in the previous study (MH251271, MH251272, MH251273, MH251274) are clustered in one branch. Turkish strains were 99% similar to each other. Other subclusters separated into three branches consisting of European-Uruguay strains, Asian strains, and Korean strains. Turkish SBV strains were 89%–90% similar to European-Uruguay strains, 88%–90% similar to Asian strains and 87%–89% similar to Korean strains.

4. Discussion

This study is the first research of the prevalence of seven honey bee viruses in seemingly healthy bee colonies randomly chosen from 111 apiaries in the Aegean region of Turkey, using the mRT-PCR technique. In addition, a phylogenetic analysis was performed to identify the geographic distribution of different virus strains.

The results showed that the virus with the highest prevalence was DWV followed by BQCV, ABPV, SBV, and CBPV. The prevalence of DWV and BQCV were lower in larvae than in adult bees. Furthermore CBPV, ABPV, and SBV were found only in adult bees. The main reason may be attributed that adult bees may have taken the agent from the environment, while vertical transmission is less likely to play a role. Tentcheva et al. [34] found that the prevalence of viruses in adult bees is higher than that of larvae.

DWV (Deformed wing virus) is one of the most widely distributed honey bee viruses in the world [35].

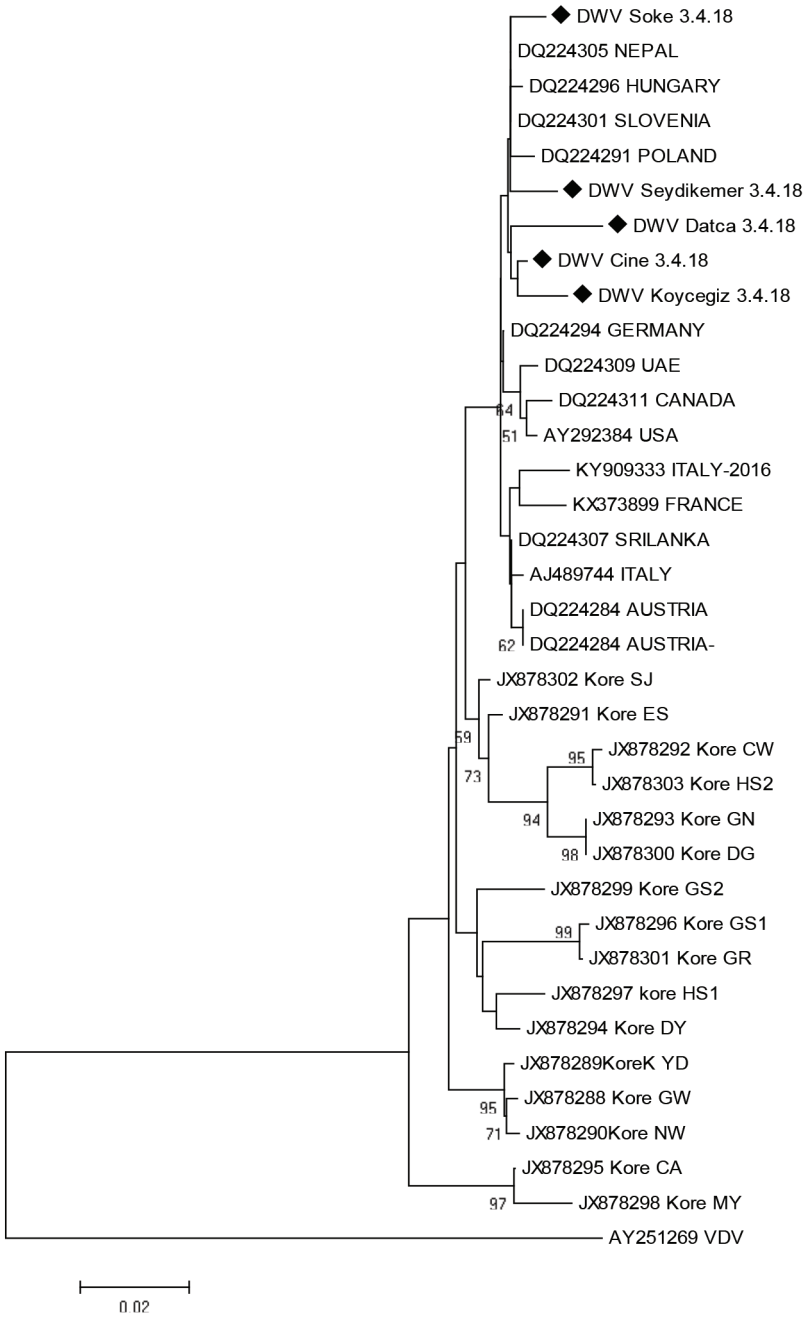

Figure 2. Phylogenetic tree of DWV strains from Turkey and other countries.

The presence of DWV has been reported in apiaries with collapsed colonies in Hatay province of Turkey [36]. The incidence of DWV infections in our Turkish samples was lower than that found in other European regions, including Austria, Croatia, Hungary, and Denmark (97%, 91%, 95.12%, 72%, 68%, respectively) [37–40]. In this study, DWV was found at 25.2% in Turkish apiaries. Our results were similar to Amiri et al. [40], Shumkova et al. [41], and Molineri et al. [42]. The most important reason for the lower prevalence in the present study is thought to be due to the fact that previous studies with higher prevalence conducted sampling from apiaries with disease symptoms or apiaries with colony losses, while studies with lower prevalence performed random sampling. In addition, considering that DWV is transmitted from Varroa, it suggests that effective Varroa control in some apiaries may contribute to the low prevalence of DWV.

The sequence and phylogenetic analyses of the five DWV strains obtained from this study were named Çine, Söke, Datça, Köyceğiz and Seydikemer, and were carried

Figure 3. Phylogenetic tree of BQCV strains from Turkey and other countries.

out by targeting the region which encodes the helicase gene of these strains. These results were similar to the results reported by Berenyi et al. [37] and Reddy et al. [27]. The Korean strains and European strains were divided into separate branches. For the RNA helicase region, there was 98%–100% shared similarity between Turkish genotypes, which shared 97–99% similarity with the USA reference strain, 94%–99% with genotypes from various countries, and 79%–81% with VDV-1. Turkish DWV strains were clustred with strains of central Europe (Poland, Hungary and Slovenia) and Asia (Nepal) (Figure 3). These similarities may have caused due to the geographical position of Turkey which is called as a transition zone between Asia and Europe and phylogenetic analysis was performed based on the nonstructural RNA-dependent RNA polymerase gene region. Nonstructural proteins are usually more conserved, so DWV viruses are similar to each other.

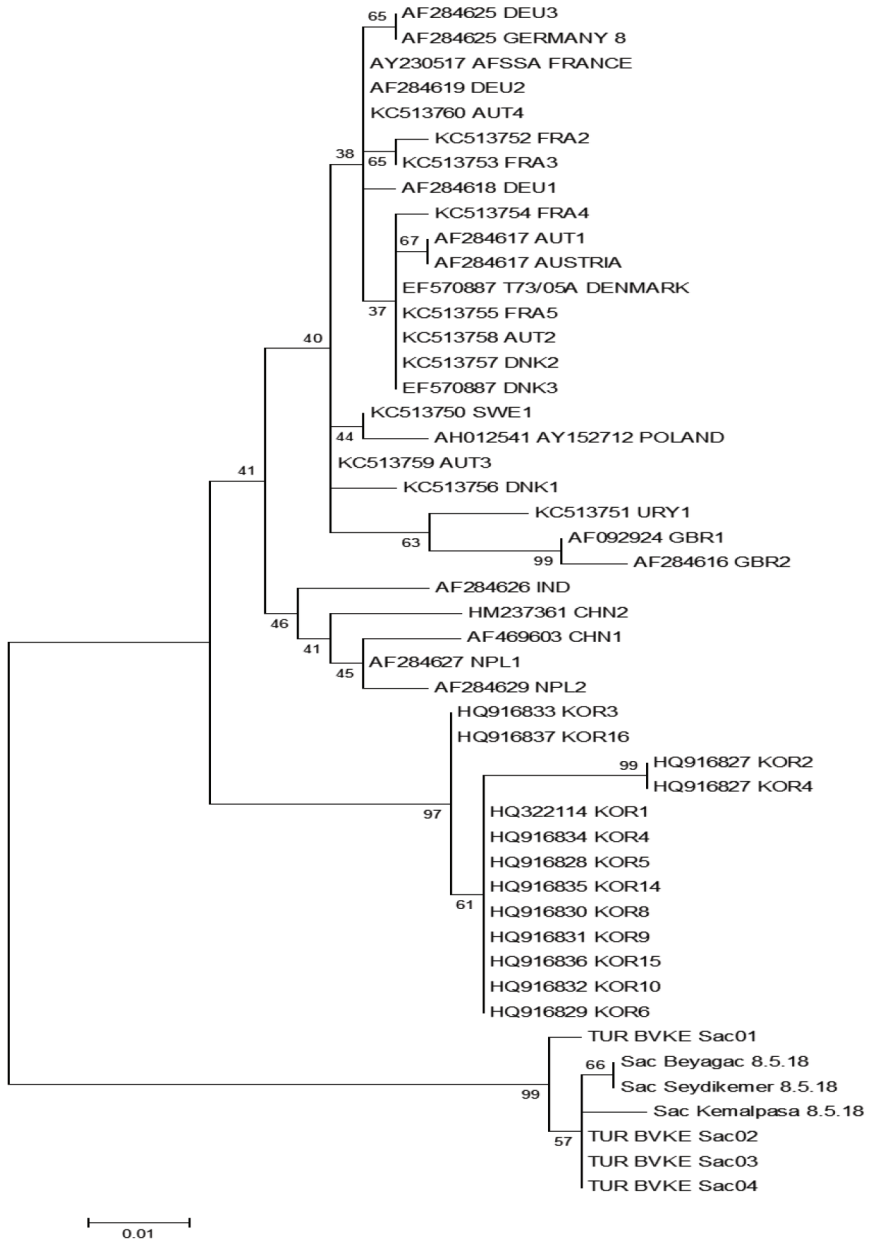

Figure 4. Phylogenetic tree of SBV strains from Turkey and other countries.

There are several phylogenetic analysis studies based on gene regions encoding the structural protein and RNA-dependent RNA polymerase gene for DWV [37,41,43,44]. The DWV genotypes investigated in Austria showed similarity levels of 98%, regardless of the geographic origins of the viruses [37]. According to the phylogenetic tree based on the polyprotein gene region, the Bulgarian DWV isolates was grouped with six UK branches, three in Spanish, and two branches in Turkish isolates [41]. Yang et al. [43] demonstrated the differences between US, Japanese, and Chinese strains. The study conducted by Karapınar et al. [44] reported that Turkish strains were 98%–100 % similar to strains from UK, Denmark, and Italy.

Previous studies have demonstrated the high prevalence of BQCV (black queen cell virus) in Turkey and European countries [23,34,38,44–46]. BQCV was the most prevalent virus in 32.2% of 90 apiaries in Hakkari province of Turkey [22]. In this study, BQCV was the second most common virus detected in apiaries with a prevalence of 20.7%. Our study is compatible with the study by Gumusova et al [19] in the Black Sea Region. Rodriguez et al. [47] found that the prevalence was 82% in 21 apiaries in the spring of 2010, when large colony losses were experienced. The same

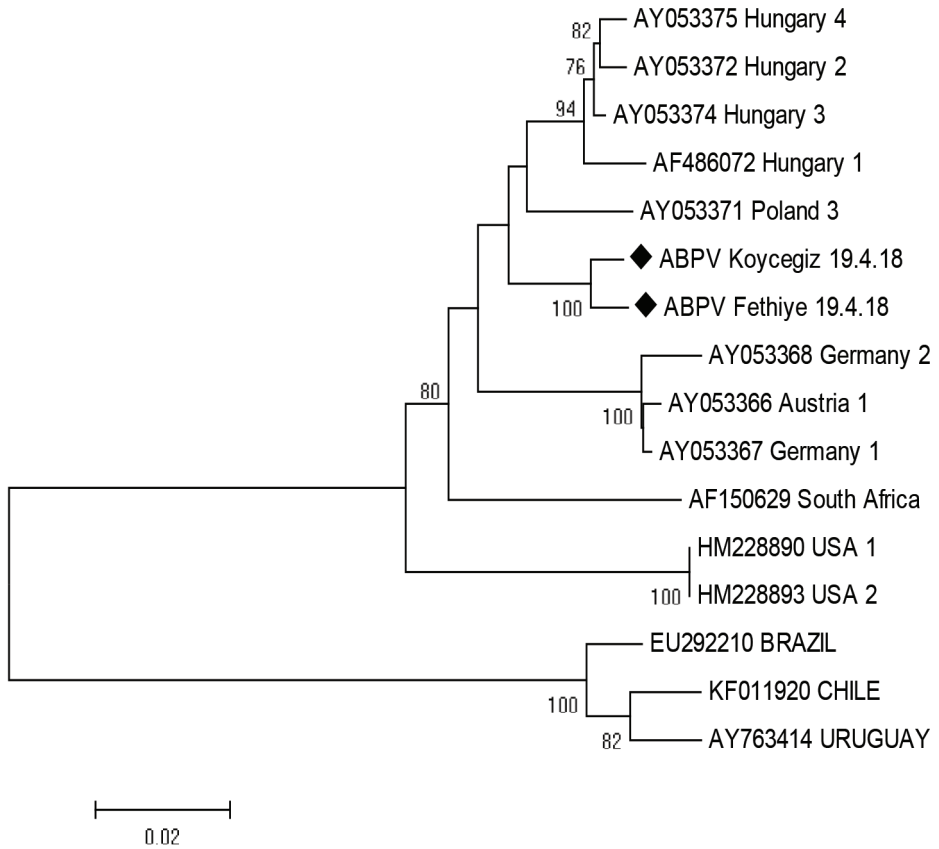

Figure 5. Phylogenetic tree of ABPV strains from Turkey and other countries.

group of researchers reported the prevalence of BQCV to be 10% in a study conducted in 60 apiaries by random sampling in the following years [48]. Another study was carried out as a result of random sampling in Bulgaria, the prevalence was found to be low [41].

The sequence and phylogenetic analyses were performed according to the gene region that synthesizes the BQCV structural protein. Six BQCV strains namely Çine, Datça-1, Datça-2, Köyceğiz, Karpuzlu and Ödemiş were identified in our study, which corresponds with the studies of Tapastzi et al. [30] and Noh et al. [49]. Therefore, although the five strains were located in the same branch and showed similarities, the Köyceğiz strain was put into a different branch than the other Turkish strains. The reason that the Köyceğiz strain was put into a different branch could be recombination with different genotypes of viruses from the Picornaviridae family due to factors such as queen bee supply from different countries. Recombination between foot-and-mouth disease virus serotypes were defined as common issues with typical breakpoint distribution of "cold spots" and "hot spots" amongst the structural genes [50]. Similar recombination paths were revealed in other picornaviruses too [51]. The Dicistroviridae family is related to the Picornaviridae family; both of them are due to be classified into the order Picornavirales. Therefore, it would be thought that recombinations can be possible between BQCV genotypes as well. This would explain why the Korean Am 3 and Hakkari strains are located in different branches [30,49]. Tapastzi et al. [30] reached the same conclusion regarding the Polish strain, which was similarly divided into different branches. BQCV Turkish strains (*Tr*BQCVs) had differences in phylogenetic classification for both the helicase and capsid gene. *Tr*BQCVs were subdivided into different clusters, including one group for the helicase gene and two groups for the capsid gene. Similar to this work, it was demonstrated heterogeneous in populations and *Tr*BQCVs were closer to some central European viruses [21]. Also, Karapinar et al. [44] reported no difference among Turkish, European, and, Asian strains (Korean, Chinese, Japanese, and Thai). On the other hand, similar findings were reported in Hakkari strain which was put into a separate branch.

Some studies conducted in Europe have found a high prevalence of SBV (sacbrood virus) [34,37,39,52]. The presence of SBV has been reported in colonies of *Apis mellifera* L. in Hakkari province of Turkey [22]. In this study, the prevalence of SBV was 2.7%. Forgach et al. [38]

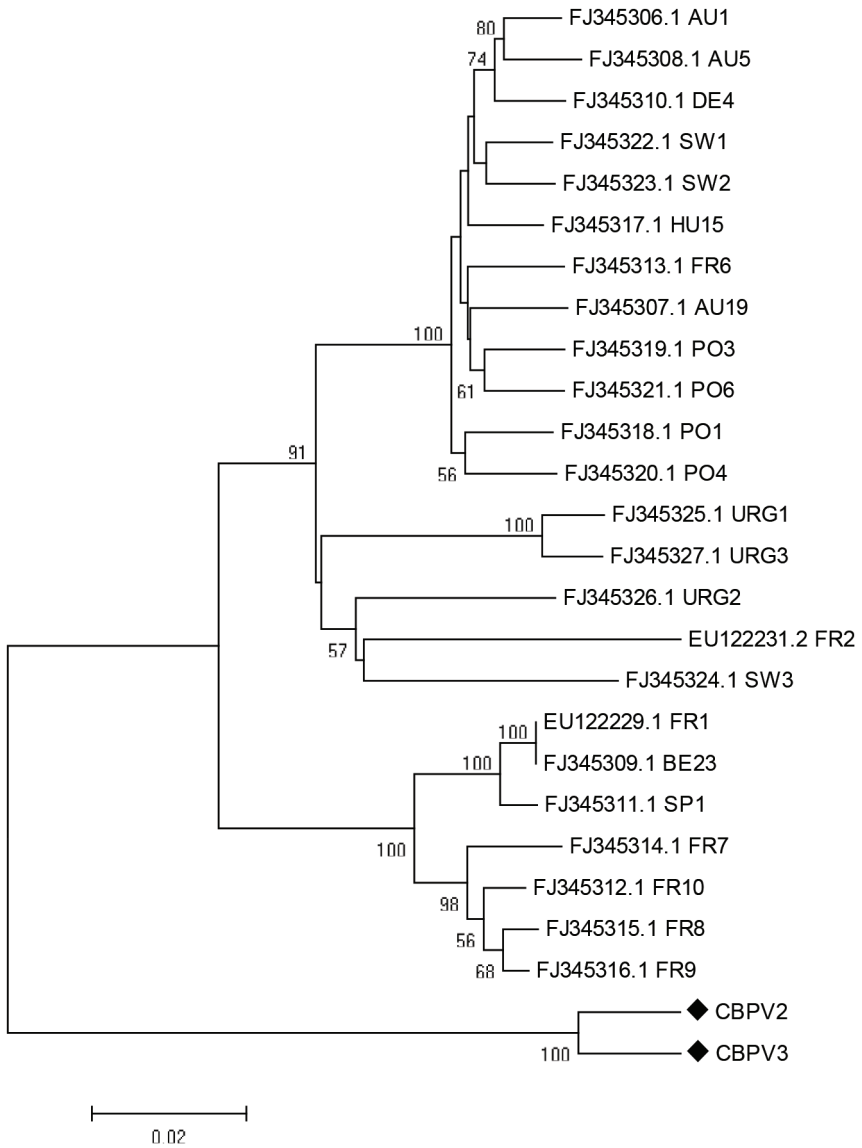

Figure 6. Phylogenetic tree of CPBV strains from Turkey and other countries.

found prevalence of SBV at 2% of 52 apiaries; and Baker and Schroeder [53] at 1.4% of 23 apiaries, which were similar to the present study.

The three strains identified in this study were 99% similar to the nucleotide sequences (NCBI Access No: MH251271, MH251272, MH251273, MH251274) of the 4 strains previously obtained from Aegean region and clustered a separate branch. Four different genotypes have been reported for SBV, one of which is the Turkey genotype [20]. Nucleotide sequences and genetic variations of SBV have been discussed in previous studies with strains originating from different countries [54–57]. Grabensteiner et al. [54] reported the existence of three different groups: European, Far East, and South African genotypes. In another study, Kukielka and Sanchez-Vizcaino [55] reported that there are three different genotypes: the European genotype with strains from Spain, France and Austria, a different genotype from Uruguay and the United Kingdom, and the Chinese genotype in strains from China. In this study, genotyping was performed using the reference from Blanchard et al. [57]. According to Blanchard et al. [57], the strains obtained from France, Germany, Sweden, Denmark, England, and Uruguay formed the European-South American genotype, the strains obtained from China, Nepal, and India formed the Asian genotype, and the strains obtained from Korea formed the Korean genotype. However, strains from Turkey did not match the genotypes from previous

studies. They formed a completely different branch and a separate group in the phylogenetic tree. The genetic diversity of SBV was investigated previously [43,54] and a phylogenetic analysis based on the structural polyprotein gene also showed a geographical distribution of the SBV strains [58]. A study previously conducted by Yildirim et al. [58] revealed the Turkish SBV group according to the structural and nonstructural gene.

ABPV (acute bee paralysisvirus) is frequently encountered in European countries [34,37,38,52,53,59]. In Turkey, BQCV was previously reported at 2.2% by assessing 270 samples from four locations in the Hakkari province [60]. In this study, the prevalence of ABPV was found to be 3.6%. An earlier study in Black Sea Region did not detect ABPV [19].

The nucleotide sequences of ABPV strains obtained in this study show 99% similarity with each other. Bakonyi et al. [59] claims that German and Austrian strains formed a group and Hungarian strains formed another group while Polish strains showed variability. This study corresponds with the study carried out by Rodriguez et al. [48]. Therefore, the Turkish strains were classified among the European strains but in a sub-branch. The reason for the inclusion of ABPV with the other European strains is the fact that the capsid protein of this agent has a protected region [59].

In the previous study carried out in Turkey by Gumusova et al. [19], CBPV (chronic bee paralysis virus) prevalence was 25 %. In our study, prevalence was 1.8%. Therefore, the results of our study are quite different from the study by Gumusova et al. [19]. In the study carried out by Tentcheva et al. [34], the prevalence was 28% in adult bees, but CBPV could not be detected in larvae. Forgach et al. [38] could not identify the agent in 52 apiaries in Hungary. Again, in studies carried out in Europe, the prevalence of CBPV is low [37,39,52], and all the results are similar to our study.

The nucleotide sequences of the 2 identified CBPV strains were 97% similar to each other. In present study corresponds with the study of Blanchard et al. [31], which classified strains obtained from different countries as A, B, C, and D. Group A includes France, Spain and Belgium, Group B includes Poland, Austria, Denmark, Hungary, and Switzerland and parts of France, Group C includes Uruguay and Group D includes France, Switzerland, and Uruguay. Even among strains from the same country, similarity was reported to be low. Turkey forms a separate branch, and the strains are 83%–85% similar to each other. Therefore, Turkish strains can be considered to be Group E. The extensive variation between CBPV strains can be explained by the fact that CBPV is a segmented virus, and there are genetic changes.

KBV and IAPV were not detected in this study. In Turkey, Tozkar et al. [23] claims that they were unable to detect KBV in their study, and Ozkırım and Schiesser [61] identified IAPV in 10 of 71 apiaries. Studies have reported that KBV in France and Denmark [34,52], but KBV has not been detected in Austria, Hungary and Croatia [37-39]. Therefore, KBV is thought to be exotic for Europe [37].

In summary, there are many studies on the prevalence, genetic diversity and phylogeny of honey bee viruses around the world. However, there are a limited number of studies on honey bee viruses in Turkey. Turkey is in an important geographical location and ranks first in Europe in terms of the number of colonies and honey production. It is also an important country in terms of geopolitics because it is a transition region that serves as a bridge between Asia and Europe. Therefore, it is crucial to understand the honey bee viruses present in Turkey and their phylogenies.

This study underlines the presence of five viruses in the Aegean Region, Turkey: chronic bee paralysis virus, sacbrood virus, acute bee paralysis virus, black queen cell virus, and deformed wing virus. Turkish strains of ABPV, BQCV, and DWV showed high percentage of sequence similarity to other strains obtained other countries, but Turkish strains of SBV and CBPV had formed different branch and genotypes when compared to previous studies. Further investigation of Turkish strains of honey bee viruses is necessary to reveal their geographical distribution, pathogenesis, and genetic variability.

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

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