ISSN: 2148-2837

MAKÜ Sag. Bil. Enst. Derg. 2018, 6(1): 26-32. **ARAŞTIRMA MAKALESİ/RESEARCH ARTİCLE**

MEHMET AKİF ERSOY ÜNİVERSİTESİ
SAĞLIK BİLİMLERİ ENSTİTÜSÜ DERGİSİ
"MAKÜ Sag. Bil. Enst. Derg."
http://edergi.mehmetakif.edu.tr/index.php/sabed/index

Genetic characterization of *Varroa destructor* (Family: Varroidae) prevalent in honeybees (*Apis mellifera*) in the province of Aydın in Turkey

Aydın Bölgesindeki Bal Arılarında (Apis mellifera) Bulunan Varroa destructor'un (Akar: Varroidae) Genetik Karakterizasyonu

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Abstract: The aim of the present study was to identify the haplotypes of the *Varroa destructor* mite which infects honeybees (*Apis mellifera*) in the province of Aydın in Turkey, using two different modified techniques for the mitochondrial Cox1 gene of the mite. In order to confirm the haplotype, two different primer pairs were selected. 376 bp DNA in size was amplified using the first primer pair. *Sac*I restriction enzyme was applied to the amplified products; however, this restriction enzyme did not cut the DNA. 570 bp DNA in size was amplified using the second primer pair. *Xho*I and *Sac*I restriction enzymes were used for the amplified products. Although, the *Sac*I restriction enzyme did not cut the DNA, the *Xho*I restriction enzyme cut the amplified DNA into two fragments (bands), with the sizes of 270 and 300 bp two bands 270 and 300 bp. While comparing the results, these bands were found specific for Korean haplotype of *V. destructor*. In conclusion, all of the 200 samples of *V. destructor* examined in this study were identified to be the Korean haplotype.

Key words: *Varroa destructor*, Genetic characterization, Aydın

Öz: Bu çalışmada; Aydın bölgesinde bal arılarında (*Apis mellifera*) görülen *Varroa destructor*'un mitokondriyal Cox1 geninin haplotiplerinin belirlenmesi amacıyla farklı iki teknik modifiye edilerek uygulanmıştır. Haplotip belirlenmesi amacıyla iki farklı primer çifti seçilmiştir. Birinci primer çiftiyle 376 bp büyüklüğünde DNA amplifiye edilmiştir. Amplifiye ürüne *Sac*I restriksiyon enzimi uygulanmış ancak bu restriksiyon enziminin DNA'yı kesmediği görülmüştür. İkinci primer çiftiyle 570 bp büyüklüğünde amplifiye DNA elde edilmiştir. Elde edilen amplifiye DNA'ya *Xho*I restriksiyon enzimi ve *Sac*I restriksiyon enzimleri uygulanmıştır. Ancak *Sac*I restriksiyon enziminin DNA'yı kesmediği, *Xho*I restriksiyon enziminin ise elde edilen genomik DNA amplifikasyonunda 270 ve 300 bp büyüklüğünde iki band oluşturduğu saptanmıştır. Sonuçlar karşılaştırıldığında; elde edilen bandların *V. destructor* Kore haplotipi için spesifik olduğu tespit edilmiştir. Sonuç olarak; *V. destructor*'un haplotipinin belirlenmesine yönelik yapılan bu araştırmada, incelenen 200 örneğin tamamının *V. destructor* Kore haplotipi olduğu saptanmıştır.

Anahtar sözcükler: *Varroa destructor*, Genetik karakterizasyon, Aydın

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Received: 21.06.2018 **Accepted:** 13.07.2018

How to cite this paper: Ayan A, Aldemir OS. 2018. Genetic characterization of *Varroa destructor* (Family: Varroidae) prevalent in honeybees (*Apis mellifera*) in the province of Aydın in Turkey. MAKU Journal of Health Sciences Institue. 6(1): 26-32.
doi: 10.24998/maeusabed.435383

Introduction

Honeybee health is an important topic in beekeeping by reason of *Apis mellifera* play an critical role in the pollination of agricultural crops (Ghazoul, 2005; Melin et al., 2014). Honeybees contribute to biodiversity conservation and food security. Honeybees provide source of income to beekeepers by sale of hive products (Jacobs et al., 2006). *Varroa destructor* is right now considered the largest threat to beekeeping worldwide and cause more damage, higher economic costs than all other known apicultural diseases (Boecking and Genersch, 2008). This mite is common all over the World (Dietemann et al., 2013; Fazier et al., 2010; Muli et al., 2014; Strauss et al., 2013) *V. destructor* is a factor for colony collapses in Europe and North America (Dainat et al., 2012; Shen et al., 2005). This mites suck the honeybee colonies hemolymph also weakens the honeybee colonies (Rosenkranz et al., 2010) it also acts as a vector for several viruses and spreads colonies (Amdam et al., 2004) especially *V. destructor* Korean and Japanese haplotypes were capable to infest *A. mellifera* populations (Anderson and Trueman, 2000). The Japanese haplotypes is seen low infestation levels in South and Central America, but the Korean haplotype is found high infestation levels in Europe (Strapazzon et al., 2009) Intensive beekeeping activities in Aydin region cause Varroosis problems and significant economic losses. For this reason, it is aimed to investigate the genetic characterization of *V. destructor* in Aydin region using molecular techniques.

Material and Methods

In this study, 200 adult female *V. destructor* mites were collected from queen bees, male bees and worker bees from the hives of the beekeeping enterprises in Aydin region from September 2014 to September 2015. The study protocol was reviewed and approved by the Animal Care and Use Committee of Adnan Menderes University (Number: 64583101/2014/177).

Therefore, especially Bozdoğan, Buharkent, Çine, Didim, Merkez, Germencik, İncirliova, Karacasu, Karpuzlu, Köşk, Kuşadası, Kuyucak, Nazilli, Paşayaylası, Söke, Sultanhisar, Yenipazar districts, which are rich in terms of apiculture, were preferred. The collected samples were kept at -20°C until they were brought to the laboratory. The *V. destructor* samples brought to the laboratory were subjected to DNA extraction using the format (Qiagen DNeasy Blood & Tissue Kit, 69504) as reported in the literature (Dietemann

et al., 2013). A randomly selected sample haplotype from beekeeping establishments was identified and used as the positive control. The positive control PCR product to be sequenced was sent to a special company for the purpose of synthesizing. In the total 50 µL reaction; 5 µL 10X PCR Buffer (Geneaid, New Taipei City, Taiwan), 3 mM MgCl 2 (Geneaid, New Taipei City, Taiwan), and 50 µL DNase / RNase-Free Distilled Water (Gibco Thermo Fisher Scientific, Waltham, MA USA), 1.5 µL Reverse primer (Iontek, Istanbul, Turkey), 1.25 units of Taq DNA polymerase (Geneaid, New Taipei City, Taiwan), 1 mM dNTP (Geneaid, New Taipei City, Taiwan) and 16 ng of DNA sample were used.

Two primers with different sequences were used for the amplification of the Cox1 gene of *V. destructor*. 5′-TACAAAGAGGGAAGAAGCAGCC-3′ Forward and 5′-GCCCCTATTCTTAATACATAGTGAAAATG-3′ Reverse primers (Solignac *et al.,* 2005) and COXF [5'GG(A/G)GG(A/T)GA(C/T)CC(A/T)ATT(C/T)T(A/T)TATCAAC3'] Forward and COXRa [5'GG(A/T)GACCTGT(A/TA(A/T)AATAGCAAATAC3'] Reverse primers (Strapazzon et al., 2009) were synthesized at a commercial company. The reaction was carried out on an AB Applied Biosystems Veriti automated thermal cycler. The steps of the reaction are as follows: preliminary denaturation at 94°C for 4 min, denaturation in each of the cycles at 94°C for 1 min, annealing at 50°C for 1.30 min, elongation at 72°C for 1.30 min and the last elongation phase consisting of 35 cycles at 72°C for 10 minutes. Then, 1.5% agarose gel was prepared. The PCR products in the agarose gel were subjected to electrophoresis for 1 hour in a 90-volt linear current. After this process, "UV transilluminator, UVP EC3 ChemiHR 410 Imaging System" images were obtained in the gel imaging device. The resulting bands were evaluated by their comparison with DNA markers. *V. destructor* was used to identify the Japanese and Korean haplotypes using the SacI restriction enzyme with recognition points 5'…GAGCTC…3' 3'…CTCGAG…5' and the *Xho*I digestion enzyme 5'…GTCGAG…3' 3'…GAGCTC…5' as described in the study of Anderson and Fuchs (1998). R0156S-0501212 New England Biolabs *Sac*I restriction enzyme and R0146S-0581507 New England Biolabs *Xho*I restriction enzyme were used, and 2% agarose gel images were obtained.

ISSN: 2148-2837
MAKÜ Sag. Bil. Enst. Derg. 2018, 6(1): 26-32.

Results

The genomic DNA amplifications obtained using different primers and different methods are presented in Figure 1-4.

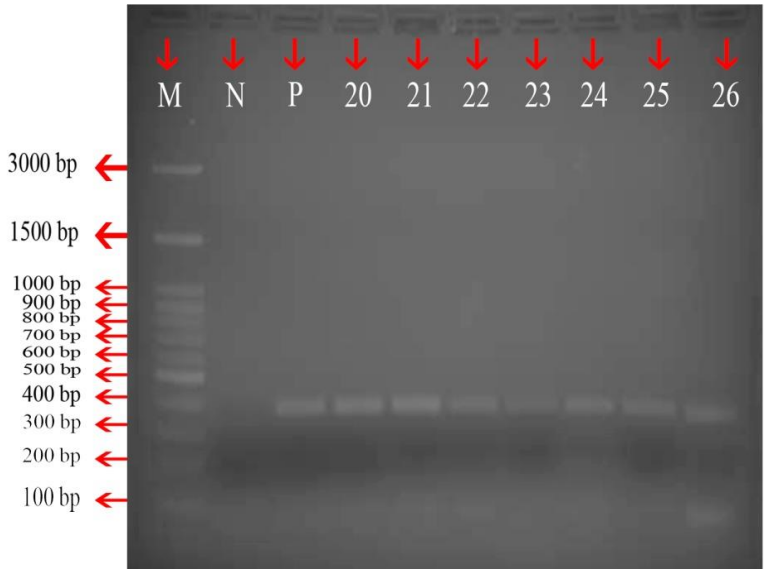

Figure 1. *Varroa destructor* Cox1 gene region of mtDNA 1.5% gel electrophoresis image of some of the samples in the PCR process (approximately 376 bp)

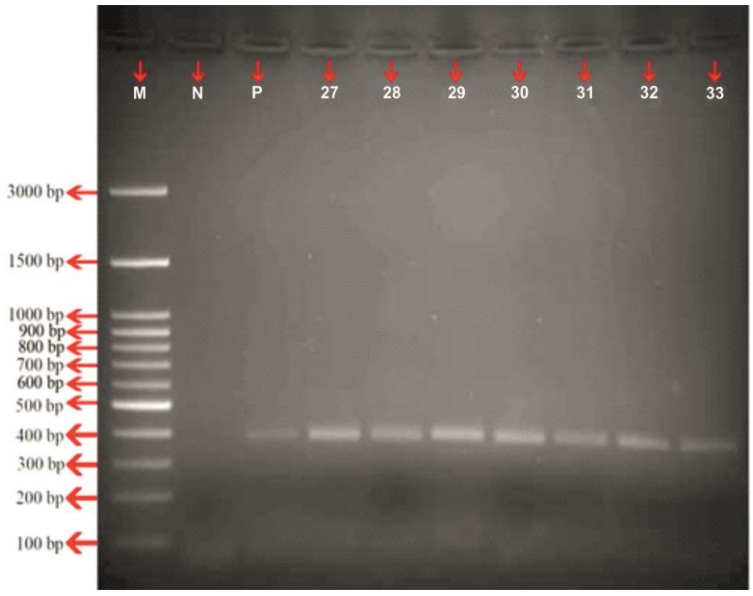

Figure 2. Varroa destructor restriction profiles of Cox1 region of mtDNA was digested with endonucleases SacI (S) 2 % Gel electrophoresis image of the samples in the Restriction fragment length polymorphism (RFLP) process

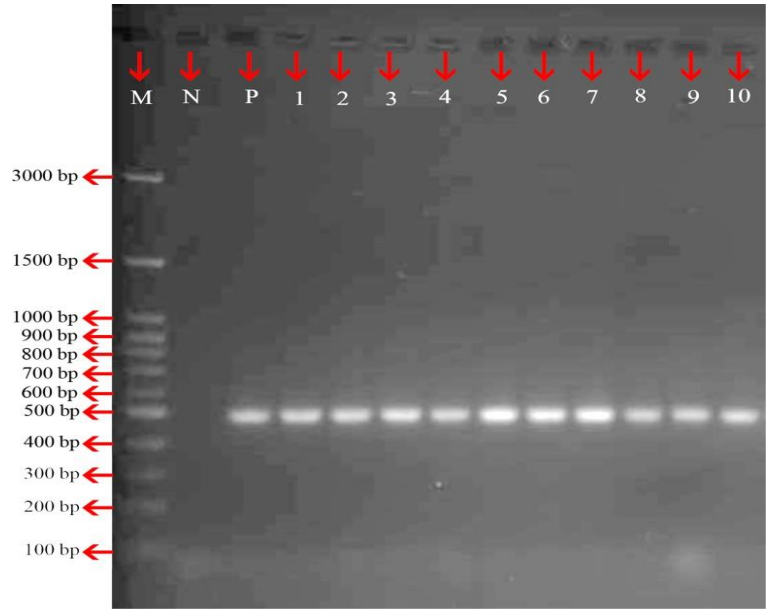

Figure 3. *Varroa destructor* Cox1 gene region of mtDNA 1.5 % gel electrophoresis image of some of the samples PCR process (approximately 570 bp)

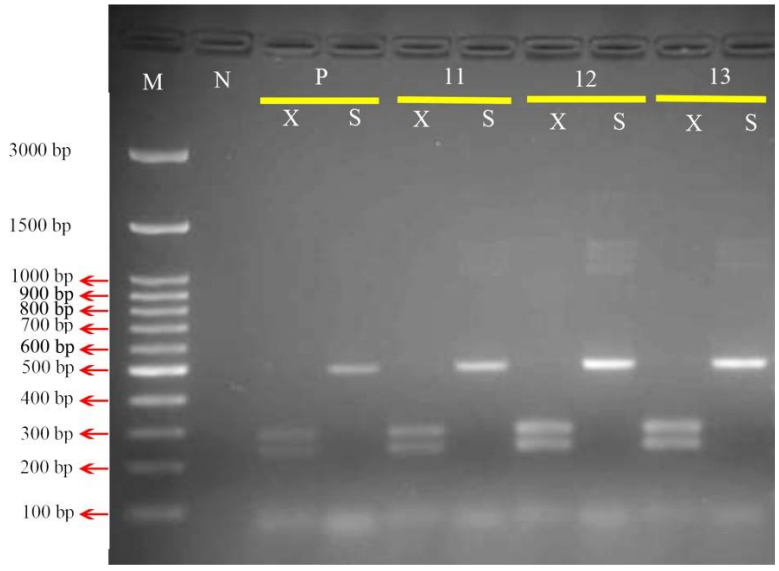

Figure 4. Varroa destructor restriction profles of Cox1 region of mtDNA was digested with endonucleases *Sac*I (S) ve *Xho*I (X) 2% Gel electrophoresis image of the samples in the Restriction fragment length polymorphism (RFLP) process

In Figure 1 Solignac et al. (2005) primers were used, and a 376 bp band was amplified for the *V. destructor* Cox1 gene findings in the 200 samples examined. The amplified 376 bp band is specific for *V. destructor*. In Figure 2, the amplified band 376 bp in size in the genomic DNA amplification obtained using the *Sac*I restriction enzyme is specific for *V. destructor* and forms *V. destructor* Korean haplotype.

In Figure 3 Strapazzon et al. (2009) primers were used, and a 570 bp band was amplified for the *V. destructor* Cox1 gene findings in the 200 samples examined. The amplified 570 bp band is specific for *V. destructor.* In Figure 4, the *Sac*I and *Xho*I restriction enzymes were used, but only the *Xho*I restriction enzyme cut the amplified genomic DNA. The *Sac*I restriction enzyme did not cut the amplified genomic DNA. In the genomic DNA amplification obtained using the *Xho*I restriction enzyme, two bands 270 and 300 bp in size were acquired. The obtained bands are specific for *V. destructor* Korean haplotype.

According to these results, when considering the band numbers and sizes obtained in the study conducted for the genetic characterization of *V. destructor*, it has been observed that all of the 200 samples examined are *V. destructor* Korean haplotype and in none of the samples, the Japanese haplotype has been detected.

Discussion

V. destructor is an invader species and rapidly spread in *A. mellifera* colonies, having a great impact across the globe. Different *Varroa* genotypes appear to be important agents in the population aliveness of the *V. destructor* (De Guzman et al., 1998; Strapazzon et al., 2009). In many parts of the world (Akinwande et al., 2012; Ayan et al., 2017a; Ayan et al., 2017b; Beaurepaire et al., 2015; Chemurot et al., 2016; Fazier et al., 2010 Gajic et al., 2013; Maggi et al., 2012; Muñoz et al., 2008; Navajas et al., 2010; Rasolofoarivao et al., 2013; Solignac et al., 2005; Strapazzon et al., 2009; Warrit et al., 2004), studies have been carried out to determine which haplotypes *Varroa destructor* has.

Beaurepaire et al. (2015) have found that the *V. destructor* obtained from *Apis mellifera* in the cities of Lipa, Dien Bien and Son La is the Korean haplotype. Chemurot et al. (2016) have determined *V. destructor* South Korean haplotypes with sequence results in Uganda. Fazier et al. (2010) have found Korean haplotype in honeybee colonies likely *A. mellifera scutellata*, and possibly *A. mellifera scutellata* hybrids Kenya. Akinwande et al. (2012) have found that *V. destructor* is the Korean haplotype in *A. mellifera* colonies in

southwest Nigeria. Gajic et al. (2013) have found Korean haplotypes, Serbia 1 haplotypes, Peshter 1 haplotypes in Serbia. Maggi et al. (2012) have determined *V. destructor* Korean haplotypes in Argentina. Muñoz et al. (2008) have found *V. destructor* Korean haplotype and Japanese haplotype in Guadalajara city of Spain while Portugal, the Balearic island and Canary island have been found *V. destructor* Korean haplotype.

Warrit et al. (2004) have examined *V. destructor* mites from Black Sea province of Turkey. All samples have been reported to be the Korean haplotype. Ayan et al. (2017a) reported *V. destructor* Korean haplotypes in *A. mellifera* in the province of Van in Turkey province of Van. Ayan et al. (2017b) have found *V. destructor* Korean haplotypes in *A. mellifera* in in Siirt city of Turkey.

In this study, the Korean haplotype of *V. destructor* has been found but Japanese haplotype has not been found in Aydin province of Turkey. The results may help to develope new control strategies in Aydın

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