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# MITOCHONDRIAL CYTOCHROME OXIDASE I VARIATION IN ASIAN TIGER MOSQUITO (*AEDES ALBOPICTUS*): DETERMINATION OF THE DIFFERENT AND MULTIPLE INTRODUCTION SITUATIONS IN TÜRKIYE

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*Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894) is an invasive mosquito species that can transmit many arboviral diseases. In Turkiye, this species was found first in Thrace in 2011, then in the Eastern Black Sea in 2014, and in the Aegean regions in 2018. We analyzed the COI gene region of 554 samples from 13 different locations in the Black Sea, Aegean, and Marmara regions to determine the COI diversity and possible introduction origin of *A. albopictus* in Türkiye. Three haplotypes derived from two segregation sites were detected, and the Hd and $\pi$ values were 0.175 and 0.00029, respectively. Hap_1 was a common haplotype and was detected in all regions. Hap_2 was detected in the Black Sea and Marmara regions, while Hap_3 was rare, and recorded only in Aegean lineages. In pairwise FSTs of 13 geographical populations, the Marmara lineages were statistically different from the Black Sea and Aegean lineages (except the Mugla samples). AMOVA test results indicated significant differences between the three studied regions (df = 2, f = 0.01784). Although Turkish *Aedes albopictus* lineages appear to have originated from temperate Northern Italy lineages and Aegean lineages may have originated from a mix of Italy and Albania lineages, the results revealed multiple introduction events via various routes.

Keywords: Black Sea region, Cytochrome c oxidase subunit I (COI).

## INTRODUCTION

The Asian tiger mosquito *Aedes* (*Stegomyia*) *albopictus* (Skuse, 1894), is an invasive mosquito vector species that can transmit many arboviral diseases such as Zika virus (ZIKV), dengue virus (DENV), and chikungunya virus (CHIKV) (Paixão *et al.* 2018). This mosquito originated from tropical forests in Southeast Asia and has invaded almost every continent over the past decades (Knudsen 1995, Paupy *et al.* 2009, Rochlin *et al.* 2013, Ngoagouni *et al.* 2015). Its global expansion has been facilitated by increasing global trade (especially of used tires and lucky bamboo) and developing transportation opportunities between countries (Aranda *et al.* 2006, Benedict *et al.* 2007, Medlock *et al.* 2015, Pichler *et al.* 2019). In addition, the physiological and biological plasticity of the species may also contribute to its rapid expansion (Kamgang *et al.* 2011, Ngoagouni *et al.* 2015).

In Asia, two different climatic *A. albopictus* lineages were known: a temperate climate lineage originating from Japan and a dry climate lineage originating from China. The difference between the lineages is important and related to the invasion success in newly invaded areas, e.g., the Japan or temperate lineage is more likely to successfully colonize temperate regions (Benedict *et al.* 2007, Poelchau *et al.* 2013, Sherpa *et al.* 2018).

In Europe, *Aedes albopictus* was introduced in two locations: Albania in 1979 (Adhami & Murati 1987) and Italy in 1990 (Sabatiani *et al.* 1990). The species has started to expand across the whole continent since the 90s and has invaded almost all of Europe via various routes (Sherpa *et al.* 2018, Konorov *et al.* 2021). This species was recorded previously in the Balkans and Caucasus around Türkiye (Samanidou-Voyadjoglou *et al.* 2005, Giatropoulos *et al.* 2012, Konorov *et al.* 2021).

In the Balkans, *A. albopictus* was reported for the first time in northwest Greece in 2005, and the most possible pathway of invasion is considered responsible for ferry traffic from Albania and/or Italy to Greece (Samanidou-Voyadjoglou *et al.* 2005, Giatropoulos *et al.* 2012). The presence of *A. albopictus* in Bulgaria was known before 2005, but it was first reported at a scientific meeting in 2011 (Samanidou-Voyadjoglou *et al.* 2005, Oter *et al.* 2013). The species was first detected in Sochi/Russia (north Caucasus), in 2011 (Ganushkina *et al.* 2012). It was reported on almost the entire western Black Sea coast over the next few years, eventually being detected in Georgia and Türkiye in 2015 (Akiner *et al.* 2016, Konorov *et al.* 2021).

In Türkiye, *A. albopictus* was reported in two districts in the western and eastern regions of the country at different times (Oter *et al.* 2013, Akiner *et al.* 2016, Demirci *et al.* 2021*a*). In 2011, *A. albopictus* was first found in the Thrace part of Türkiye, around Greece's border city, Edirne. Then, four years later, the species found on the eastern Black Sea coast included three provinces (Artvin, Rize, and Trabzon). Finally, in 2018, it appeared in the Aegean region in Izmir (Aliaga port). Recent research by different authors and our group has revealed that the species has spread along seaside cities in the Black Sea, Marmara, and Aegean regions, as well as some inland cities in central and east Anatolia (Oter *et al.* 2013, Demirci *et al.* 2021*a*, Yavasoglu 2021).In Europe, two distinct climatic lineages of *A. albopictus* exist: the Italian lineage, which descended from the American-derived temperate climate Japanese lineage, and the Albanian lineage, which descended from the Chinese lineage (Kamgang *et al.* 2011, Žitko *et al.* 2011, Ruiling *et al.* 2018). Previous genetic studies suggest that the origin of the Greek lineage was an admixture between Central Italy and Albania lineages, and the origin of *A. albopictus* populations from the eastern Black Sea region originated in the north Italian lineage (Sherpa *et al.* 2018). In the previous study from Türkiye using morphometric analysis, it

was determined that the Aliaga port (Aegean) population was separate from the Black Sea and Marmara populations (Demirci *et al.* 2021*b*).

Genetic studies have revealed that certain genetic markers can be used to infer population parameters. Mitochondrial DNA (mtDNA) is the most used marker in molecular studies to measure genetic drift and genetic differentiation in insects and is more sensitive than nuclear DNA (Avise 1994). Studies on mitochondrial phylogenies of *A. albopictus* in newly invaded areas revealed little variation among various *A. albopictus* populations (Urbanelli *et al.* 2000, Ayres *et al.* 2002, Kamgang *et al.* 2010). Cytochrome oxidase c subunit I (COI) has been widely used for identification, and to determine population structure and phylogeography of *A. albopictus* (Birungi & Munstermann 2002, Mousson *et al.* 2005, Usmani-Brown 2009, Delatte *et al.* 2011, Kamgang *et al.* 2011, Žitko *et al.* 2011, Porretta *et al.* 2012, Beebe *et al.* 2013, Shaikevich &Talbalaghi 2013, Zhong *et al.* 2013, Zawani *et al.* 2014). Significant sequence data can be obtained from almost every country where the species was recorded.

This study aimed to examine the COI variation of *A. albopictus* in three possible entry regions of Türkiye to understand the introduction events of *A. albopictus*.

## MATERIAL AND METHODS

Sampling – *Aedes albopictus* samples were collected from current distribution areas in the Black Sea (Artvin (94 samples), Rize (82 samples), Trabzon (60 samples), Giresun (21 samples), Ordu (25 samples)), Eastern Anatolia (Erzincan (21 samples), Elazığ (20 samples)), Marmara (Istanbul (47 samples), Kocaeli (44 samples), Sakarya (19 samples)), Thrace (Kırklareli (68 samples)), and Aegean (Izmir (44 samples), Mugla (10 samples)) regions between 2018 (June to September) and 2019 (May to October) in Türkiye. In total, 554 samples (including 67 collecting points) were collected from 13 provinces belonging to 4 geographical regions (Fig. 1) by two persons. Adults were collected by using BG Sentinel 2 ® (Biogents AG, Germany) (during the night) trap primarily. During the study 67 BG Sen-

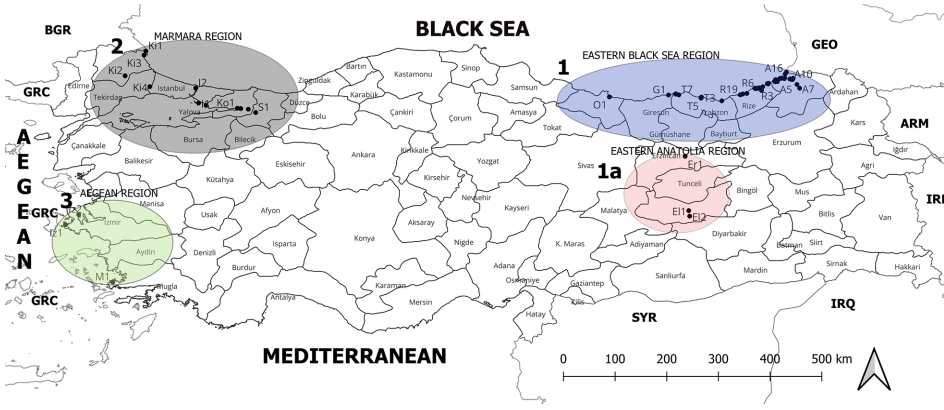


**Fig. 1.** Collection points of *A. albopictus* samples used in the study

tinel 2 ® traps (one trap per each location) used for the study areas. Larval sampling was performed by using larval dipping methods during daytime. Larvae are kept under laboratory conditions until adult emergence for reliable identification of the mosquito species. Identification of the species was performed under a computer-assisted Leica Microsystem EZ4® (Leica Microsystems, Wetzlar, Germany) stereo microscope using mosquito identification key developed by Schaffner *et al.* (2001). All samples were stored at -80 °C until molecular studies.

DNA isolation, PCR, and analysis of the sequence data – DNA isolation was performed from individual mosquitoes according to the manufacturer's recommended conditions using the Gene JET genomic DNA Extraction Kit (Thermo Scientific®). PCR was performed with using a pair of universal primers including LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG 3' and HC02198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer *et al.* 1994). PCR master mix was prepared in 50 µl reaction volume consisting of 3 µl 10 x PCR buffer, 1.5 µl 10 mM dNTP, 1 µl of each primer (20 µM), 0.5 µl Taq DNA polymerase (5U/µl), 3 µl 50 mM MgCl2, 35 µl of nuclease-free water deionized distilled water and 5 µl of template DNA. PCR conditions were: 95°C for 1 min for initial denaturation and 30 x [95°C for 1 min, 40°C for 30 s, 72°C for 1 min]; final elongation 5 min at 72°C followed by a 4°C hold on a T100™ Thermal Cycler (Bio-Rad, Hercules, CA). PCR products were electrophoresed on 1.5% agarose gel (Sigma, USA) in 1 x TAE buffer in horizontal agarose gel electrophoresis system (Bio-Rad, Hercules, CA, USA). 5 µl of PCR products and 1 µl of 6X DNA loading dye (bromophenol blue) (Thermo Scientific™) was mixed and load onto wells. Band sizes of the PCR products were determined using a 100 bp ladder (Thermo Scientific™). The gel was run for 30 min at 100 V. The gel was imaged on WİSEUV ® gel imaging device under UV light. The PCR products were produced about 650 bp bands. The positive product was sequenced at Macrogen Inc.

Data analysis – Mega 7 software was used to edit the raw DNA sequences (Kumar *et al.* 2016). Multiple sequence alignments were performed using the ClustalW section in Mega 7 software. All sequences were verified using the NCBI sequence database, and sequences of *A. albopictus* with 97% or less identity were removed from the study. Polymorphic sites (S), numbers of haplotypes (N), haplotype diversity (Hd), and nucleotide diversity ($\pi$) of *A. albopictus* geographical populations were assessed using DnaSP 5.0 software (Librado & Rozas 2009). Pairwise FSTs values of the genetic differentiation among the geographical populations, selective neutrality tests including Fu's Fs statistics and Tajima's D tests, and analysis of molecular variance analysis (AMOVA) were performed to determine whether there are differences between groups in Arlequin 3.5 (Excoffier & Lischer 2010) software. Network analysis was performed using Network 10.2 software (Bandelt *et al.* 1999). Also, all p values were corrected using Holm's correction method.

## RESULTS

### *Genetic diversities and haplotype distribution of* A. albopictus

A total of 554 COI (599 bp) nucleotide sequences were obtained from 13 geographical populations, and the populations were used for haplotype analysis. The sequences were composed of three haplotypes derived from two segregating sites (Haplotypes' genbank accession numbers: OP349658.1, OP349659.1, OP349660.1). The total haplotype and nucleotide diversity values of all geographical populations were 0.175 and 0.00029, respectively. The

**Table 1.** Genetic diversity of 13 geographical populations of *A. albopictus* using mtCOI gene sequences. The geographical region code given in parentheses according to the Figure 1.

| Geographical populations | No. individuals | No. segregation site | Nucleotide diversity | Haplotype diversity | No. haplotype | Tajima's D test | FS |
|---|---|---|---|---|---|---|---|
| Artvin (1) | 94 | 1 | 0.0017 | 0.102 | 2 | –0.56007 | –0.41083 |
| Rize (1) | 82 | 1 | 0.00016 | 0.094 | 2 | –0.63261 | –0.52429 |
| Trabzon (1) | 60 | 0 | 0 | 0 | 1 | 0.00000 | 0.00000 |
| Giresun (1) | 21 | 0 | 0 | 0 | 1 | 0.00000 | 0.00000 |
| Ordu (1) | 25 | 0 | 0 | 0 | 1 | 0.00000 | 0.00000 |
| Erzincan (1a) | 21 | 0 | 0 | 0 | 1 | 0.00000 | 0.00000 |
| Elazığ (1a) | 20 | 0 | 0 | 0 | 1 | 0.00000 | 0.00000 |
| Kırklareli (2) | 68 | 1 | 0.00066 | 0.395 | 2 | 1.11168 | 1.60084 |
| Istanbul (2) | 47 | 1 | 0.00057 | 0.342 | 2 | 0.69881 | 1.15613 |
| Kocaeli (2) | 44 | 1 | 0.00068 | 0.406 | 2 | 1.06453 | 1.44082 |
| Sakarya (2) | 19 | 0 | 0 | 0 | 1 | 0.00000 | 0.00000 |
| Izmir (3) | 44 | 1 | 0.00022 | 0.130 | 2 | –0.60390 | –0.30390 |
| Mugla (3) | 10 | 1 | 0.00033 | 0.2 | 2 | –1.11173 | –0.33931 |
| Total | 554 | 2 | 0.00029 | 0.175 | 3 | –0.00256 | 0.20150 |

highest haplotype diversity and nucleotide diversity were observed in Kocaeli (Kce) geographical populations (0.406 and 0.00068, respectively). Trabzon (Trb), Giresun (Grs), Ordu (Ord), Erzincan (Erz), Elazığ (Elz), and Sakarya (Sky) geographical populations represented by a single haplotype. Haplotype and nucleotide diversity for these lineages found zero (Table 1). Tajima's D and Fu's FS tests conducted for neutrality analysis to detect the history of geographical population size. Most of the population's neutrality tests resulted in zero or negative values (except; Kırklareli, Kocaeli, and Istanbul) but all of the values were non-significant statistically (Table 1).

*The relationships among haplotypes of 13 geographical populations*

The relationship between haplotypes was analyzed using the median-joining method using Network 10.2 software. In the network, each circle represents one haplotype, and the circle's size is related to the frequency of occurrence of the haplotype. In addition, the distributions and frequencies of the three haplotypes in 13 geographical population lineages are given in parentheses. The frequencies of the three haplotypes were 501, 49, and 4, respectively. The main haplotype (Hap_1) was covered in all geographical populations and

about 90.43% of the total haplotypes. The Hap_2 was found in five geographical populations (Artvin (Art), Rize (Riz), Kırklareli (Krk), Kocaeli (Kce), and Istanbul (Ist) and constituted about 8.84% of the total haplotypes. The Hap_3 had the lowest proportion (0.72%) and it found only in Izmir (0.54%) and Mugla (0.18%) geographical populations, including the Aegean region (Fig. 2).

*Genetic relationships among geographical populations in Turkiye*

Genetic relationships among 13 geographical populations were determined using pairwise *Fst* distance. *Fst* distance values ranged between –0.05735 and 0.21395 (Table 2). The lowest *Fst* distance values were between Izmir and Mugla geographical populations and the pairwise differences value was statistically insignificant ($p > 0.05$). The highest *Fst* values were observed between Kocaeli and Izmir geographical populations pairs, and the value was found to be significant ($p < 0.005$) (Table 2).

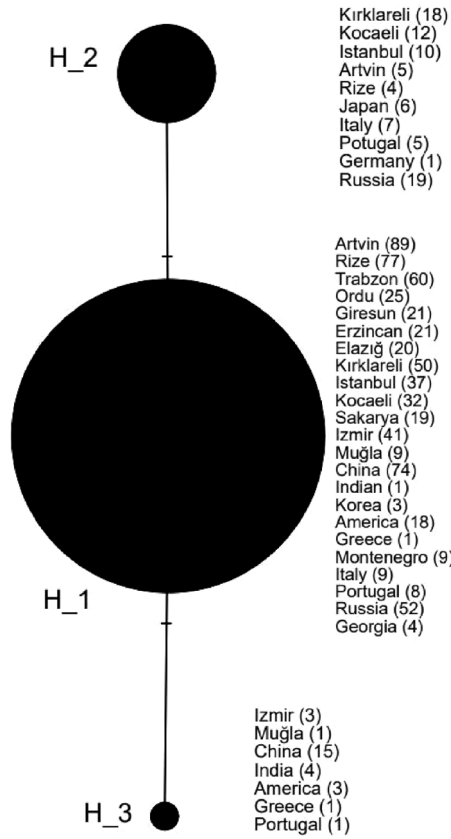


**Fig. 2.** Network of the COI haplotypes of *A. albopictus* and frequencies of haplotypes in geographic lineages

*Analysis of molecular variance (AMOVA) analysis*

*Aedes albopictus* geographical populations in Türkiye were grouped into three different groups according to the geographic region (Erzincan and Elazıg lineages grouped with the Black Sea) related to the possible invasion points (Fig. 1). The variance component proportion among regions and between geographical populations is large (81.54%), while that among geographical populations within regions is low (1.48%). The F-statistics, among regions and within geographical populations were FSC = 0.01784, and FCT = 0.16979 respectively, and both were statistically significant ($p < 0.05$). In contrast to this situation,

**Table 2**. Pairwise FSTs of 13 geographical populations using partial sequences of mitochondrial COI. Statistically significant values were marked in bold. The geographical region code given in parentheses according to the Figure 1.

| | Art (1) | Riz (1) | Trb (1) | Grs (1) | Ord (1) | Erz (1a) | Elz (1a) | Krk (2) | Ist (2) | Kce (2) | Sky (2) | Izm (3) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Art (1) | – | | | | | | | | | | | |
| Riz (1) | –0.011 | – | | | | | | | | | | |
| Trb (1) | 0.0302 | 0.0291 | – | | | | | | | | | |
| Grs (1) | 0.0042 | 0.0020 | 0.0000 | – | | | | | | | | |
| Ord (1) | 0.0091 | 0.0071 | 0.0000 | 0.0000 | – | | | | | | | |
| Erz (1a) | 0.0027 | 0.0005 | 0.0000 | 0.0000 | 0.0000 | – | | | | | | |
| Elz (1a) | 0.0027 | 0.0005 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | – | | | | | |
| Krk (2) | **0.1588** | **0.1571** | **0.2411** | **0.1660** | **0.1759** | **0.1633** | **0.1633** | – | | | | |
| Ist (2) | **0.1122** | **0.1128** | **0.2205** | **0.1344** | **0.1457** | **0.1313** | **0.1313** | –0.0108 | – | | | |
| Kce (2) | **0.1868** | **0.1852** | **0.2932** | **0.1891** | **0.2026** | **0.1855** | **0.1855** | –0.0189 | –0.0125 | – | | |
| Sky (2) | –0.000 | –0.0028 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | **0.1577** | **0.1249** | **0.1780** | – | |
| İzm (3) | **0.0483** | **0.0462** | **0.0611** | 0.0169 | 0.0237 | 0.0149 | 0.0149 | **0.1920** | **0.1569** | **0.2139** | 0.0106 | |
| Mgl (3) | 0.0578 | 0.0579 | 0.2421 | 0.0802 | 0.1018 | 0.0745 | 0.0745 | **0.1363** | 0.1012 | **0.1427** | 0.0625 | –0.0573 |

**Table 3.** Analysis of molecular variance (AMOVA) among geographical regions. Statistically significant values were marked in bold.

| Source of variation | d.f | Sum of squares | Variance components (% of variation) | F-statistics | |
|---|---|---|---|---|---|
| Among regions | 2 | 5.217 | 0.01622 (16.98%) | FSC | **0.01784** |
| Among geographical lineages within regions | 10 | 1.343 | 0.00141 (1.48%) | FST | 0.18459 |
| Within geographical lineages | 540 | 42.070 | 0.07791 (81.54%) | FCT | **0.16979** |
| Total | 552 | 48.629 | 0.09554 | | |

FST values among geographical populations within regions were found to be 0.18459 and insignificant ($p < 0.05$) (Table 3).

*Genetic relationships among geographical lineages of Turkiye and different geographical lineages in the world*

Geographical or regional lineages from continental Europe or other continents Genbank sequences were searched for comparison using the keywords "*Aedes albopictus* COX1" or "*Aedes albopictus* COI" from the GenBank. The study excluded sequences with ambiguous bases and/or unknown geographic origin, as well as those with insufficient length. A total of 2179 COI sequences were obtained, resulting in 105 different haplotypes. Our result was compared with lineages from 14 countries (including Asia, Africa, the USA, West Europe, the Balkans, Turkiye, Caucasia, and the Middle East regions) in different geographic regions using pairwise FSTs to determine the possible origin of the three regional lineages in Türkiye. The Marmara lineage was different from the Aegean and Black Sea lineages, and this difference was found to be statistically significant ($p < 0.05$). The differences between Turkish lineages and Asian lineages were found to be statistically significant, and the lowest differences values were observed between Türkiye (Black Sea, Marmara, and Aegean) and China lineages (0.09347, 0.06432, and 0.03210, respectively). The difference between African (Morocco and Congo) lineages and Turkish lineages was insignificant (except Marmara and Congo pairs). The difference between Marmara and Congo pairs was quite low, and the value was 0.03665 ($p < 0.05$). The difference between USA lineages and Turkish lineages was found to be statistically significant, and the lowest difference was seen between Aegean populations and USA lineage (0.04107, $p < 0.05$). West European populations were statistically different from Turkish lineages, except for German samples. German samples were found to be closely related to all three regional lineages in Türkiye. Furthermore, the differences between the Montenegrin and Black Sea lineages were found to be statistically significant ($p < 0.05$). The difference between the other pairs was very low and sta-

**Table 4.** Pairwise FSTs between three regional lineages in Türkiye and different parts of the world using partial sequences of mitochondrial COI. Statistically significant values were marked in bold.

| Regional lineages (Number of samples) | Number of haplotype | Geography | Black Sea | Marmara | Aegean |
|---|---|---|---|---|---|
| Black Sea (322) | 2 | Türkiye | 0.0000 | | |
| Marmara (177) | 2 | | **0.0485** | 0.0000 | |
| Aegean (54) | 2 | | 0.0002 | 0.0456 | 0.0000 |
| India (7) | 3 | Asia | **0.6112** | **0.3231** | **0.3833** |
| Japan (27) | 7 | | **0.4306** | **0.2196** | **0.1847** |
| Korea (38) | 20 | | **0.7237** | **0.5655** | **0.4316** |
| China (143) | 54 | | **0.0934** | **0.0643** | **0.0321** |
| Pakistan (249) | 9 | | **0.8470** | **0.7913** | **0.7583** |
| Iranian (4) | 2 | | **0.5845** | **0.2324** | **0.6874** |
| Moroco (3) | 1 | Africa | –0.1886 | –0.1401 | 0.0000 |
| Congo (29) | 2 | | 0.0199 | **0.0366** | 0.0228 |
| USA (92) | 32 | USA | **0.1274** | **0.0846** | **0.0410** |
| Portugal (16) | 2 | W Europe | **0.6036** | **0.2028** | **0.4806** |
| Germany (11) | 1 | | –0.0386 | 0.0003 | 0.0000 |
| Austria (4) | 2 | | **0.8521** | **0.5893** | **0.8728** |
| Italy (14) | 3 | | **0.8304** | **0.5555** | **0.6856** |
| Montenegro (12) | 2 | Balkans | **0.1186** | 0.0437 | 0.1607 |
| Greece (3) | 1 | | –0.1886 | –0.1401 | 0.0000 |
| Georgia (4) | 1 | Caucasia | –0.1325 | –0.0881 | 0.0000 |
| Russia (69) | 4 | | **0.2469** | **0.0692** | **0.1265** |

tistically insignificant. Our results indicated a significant difference between the Russian and Turkish lineages, although the Georgian and Turkish lineage differences were insignificant. In Türkiye, all three regional lineages were statistically different from Middle East lineages (Table 4).

## DISCUSSION

Public health concerns about the spreading patterns of invasive Aedes species and mosquito-borne diseases such as dengue, zika, and chikungunya have increased (Schaffner *et al.* 2013). Genetic studies can provide information about the population situation in invaded areas as well as the spreading pattern of the species. Therefore, the results of genetic studies contribute

information relevant to preventing diseases of public health importance and control of the species (Ruiling *et al.* 2018), prediction of new distribution areas of the species and so precaution against possible *Aedes albopictus*-borne diseases. Furthermore, revealed introduction routes can give information about other potentially invasive species of (e.g. *Aedes koreicus*, *A. japonicus*, *A. atropalpus*) possible routes.

Haplotype diversity (Hd) and nucleotide diversity ($\pi$) are the two most important parameters that provide information about populations. The total haplotype and nucleotide diversity (0.00029 and 0.175) obtained in the study were generally found to be quite low, as expected. Several independent studies documented this situation and reported low-level mtDNA variation according to molecular markers such as Cytb, COI, and ND5 (Kambhampati *et al.* 1991, Kambhampati & Rai 1991, Urbanelli *et al.* 2000, Kamgang *et al.* 2011, Žitko *et al.* 2011, Zé-Zé *et al.* 2020). According to the results, the main haplotype showed one nucleotide difference from the others, while the second and third haplotype nucleotide differences were two. Moreover, haplotype and nucleotide diversity were zero in Trabzon, Giresun, Ordu, Erzincan, Elazığ, and Sakarya populations in Türkiye. Fang *et al.* (2018) determined low nucleotide and haplotype diversity of the COI gene region in Taiwan, and the values were 0.00215 and 0.75325, respectively. Zé-Zé *et al.* (2020) found nucleotide and haplotype diversity, respectively, of 0.00149 and 0.6895 in Algarve populations and 0.00043 and 0.2747 in Oporto populations of Portugal (invasion areas). The low nucleotide and haplotype diversity in our results can be explained by the bottleneck effect. According to Fang *et al.* (2018), the rapid expansion of populations following the bottleneck effect may result in haplotype losses related to the time required to accumulate nucleotide diversity in *A. albopictus* China populations. Although neutrality tests mostly indicated the expansion of our studied populations, these results were statistically non-significant (P > 005).

The polymorphism of our studied lineages was low, and our results produced only three haplotypes with MtDNA (COI) data. Previous studies have shown genetic variation in *A. albopictus* populations is higher in local populations, while genetic variation is lower in invasive populations (Birungi & Munstermann 2002, Mousson *et al.* 2005, Maia *et al.* 2009, Kamgang *et al.* 2011). Our results are consistent with previous data. While Fang *et al.* (2018) found 42 haplotypes using the COI gene region in the local population of China, Zé-Zé *et al.* (2020) determined only five haplotypes using the same gene region in Portugal (the invasive region). Similarly, Žitko *et al.* (2011) determined the presence of four haplotypes using the East-Adriatic populations COI gene region. The reason for low genetic variation may be due to different kinds of selection pressure, such as the heavy use of insecticides and temperature, humidity, and elevation. Although it is possible to explain haplotype losses in native areas with these parameters, it is a more accurate approach

to describe haplotype losses in new invasion areas with the bottleneck effect. Fang *et al.* (2018) indicated this situation, and they explained the low Hd and $\pi$ degrees of some Chinese *A. albopictus* populations with selection pressure and the bottleneck effect.

In this study, we determined that Hap_1 was the dominant haplotype in all populations. The Hap_1 may be related to the possible origin of species, including active or passive invasion. Because higher-frequency alleles in parent populations are more likely to be selected in the bottleneck effect (Sokal & Rohlf 2012). It was determined that Hap_1 is a common haplotype recorded on almost every continent in previous studies like ours (obtained from Genbank samples) (Table 5). Black Sea lineages probably originate from Georgia and possibly originate from the North Italian lineage. Sherpa *et al.* (2018) also indicated that Türkiye's eastern Black Sea lineages originated from North Italy. Furthermore, the presence of *Aedes albopictus* along the Russian and Georgian borders was previously supported. Kutateladze *et al.* (2016) reported that *A. albopictus* is very dominant in Batumi, Georgia's border province with Türkiye. Hap_2 was described in Turkish (the Black Sea and Marmara populations) and Russian samples (according to the Genbank samples) in the Black Sea coastal area. But this haplotype could not be detected in the Georgian samples (Ganushkina *et al.* 2016, Fedorova *et al.* 2018). This situation may explain the insufficient number of studied samples from Georgia (according to the Genbank samples, only 11 samples). Similarly, the Marmara regional lineages consisted of Hap_1 and Hap_2. Although Hap_1 was found to be the dominant haplotype in this region, the frequency of Hap_2 was relatively higher than in the eastern Black Sea region. The AMOVA test results revealed a significant difference ($p < 0.05$) between the Black Sea, Marmara, and Aegean Sea regions (Table 3). Furthermore, the pairwise FSTs analysis showed differences between Marmara, Eastern Black Sea, and Marmara, Aegean regional lineage pairs (Table 4). While significant differences were determined between Izmir and other geographical populations, no significant differences were determined between Mugla populations. On the other hand, the presence of Hap_3 in this region may indicate that the Izmir and Mugla populations share a common ancestor.

It is possible that *A. albopictus* geographical populations in the Marmara region were more likely invaded via Greece or Bulgaria. Oter *et al.* (2013) mentioned this situation in the first report of the *A. albopictus* invasion in Türkiye's Thrace region. In addition, we found *A. albopictus* populations in small villages (Begendik and Limankoy) along the Turkish and Bulgarian border areas in 2016. This situation is not fully explained and is related to the lack of Genbank samples from Greece and Bulgaria (Table 5). Although Istanbul and Kocaeli are bigger than other cities in the Marmara region, the invasion situation for these cities may be different from that in other parts of Türkiye and

the Thrace part of the Marmara region. These cities' lineages may have been invaded by ships from other ports in Europe and other continents because the two cities included the biggest port areas in Türkiye.

The genetic structure of the geographical populations of the Aegean region is different from other regions. Although Hap_1 was dominant in other regions, Hap_2 could not be found in this region. Hap_3 only inhabited this region, and its frequency was low. It explains clearly that the Aegean lineages were distinct from the Marmara and Black Sea lineages. In addition, the presence of *A. albopictus* in the Aegean region was found later than in other regions; first around the Aliaga harbor and then in Mugla. These two kinds of situations suggest that there were multiple invasions, possibly via ships orig-

**Table 5.** *Aedes albopictus* Genbank COI sequences identical to the haplotype retrieved from the Turkey population.

| | | | |
|---|---|---|---|
| Asian | China | Hap_1 | KR349279-83; KR349285; KR349287; KX266638-39; KX266642-45; KX266647-55; KX266657-67; KX266669; KX266673-74; KX266677; KX266681; KX266683-84; KX266686-88; KX266690-94; KX266696-707; KX266709; KX266714-16; KX266719-20; KX266723-26; KX886284; KX886286; KX886298; KX886325; KX886341; KY765450; KY765475-76; KY971592-93 |
| | | Hap_2 | |
| | | Hap_3 | KX266699; KX886304; KX886306-07; KX886316; KX886320; KX886338; KY765468; KY765472-73; KY765479; KY765481; KY765483-84; KY765492 |
| | Japan | Hap_1 | |
| | | Hap_2 | LC054323-25; KX809765 |
| | | Hap_3 | |
| | India | Hap_1 | KR817732 |
| | | Hap_2 | |
| | | Hap_3 | KC970276; MK284525; KJ410335; KJ410333 |
| | Korea | Hap_1 | MG871392-94 |
| | | Hap_2 | |
| | | Hap_3 | |
| USA | America | Hap_1 | KC690955; KC690952-53; KC690950; KC690945; KC690935; KC690932; KC690921; KC690918; KC690916; KC690914; KC690912; KC690910; KC690904; KC690901; KC690897-99 |
| | | Hap_2 | |
| | | Hap_3 | KC690951; KC690940-41; |

| | | | |
|---|---|---|---|
| Balkans | Greece | Hap_1 | MN005054-55 |
| | | Hap_2 | |
| | | Hap_3 | MN005056 |
| | Monte-negro | Hap_1 | MK505609; MK505606; MK505594; MK505592; MK505589; MK505583; MK505580; MK505575; MK505570; |
| | | Hap_2 | |
| | | Hap_3 | |
| | Croatia | Hap_1 | HQ906848 |
| | | Hap_2 | HQ906851; HQ906849 |
| | | Hap_3 | HQ906850 |
| W Europe | Italy | Hap_1 | JX679374; JX679376; JX679378; JX679380; KX383929; KX383923; KX383921; KX383916; KX383933 |
| | | Hap_2 | JX679373; JX679375; JX679379; JX679382-86 |
| | | Hap_3 | |
| | Portugal | Hap_1 | MF990905; MN513361; MN513352-55; MN513357; MN513359 |
| | | Hap_2 | MK995326; MN513367-68; MN513362; MN513360 |
| | | Hap_3 | MK995332.1 |
| | Germany | Hap_1 | |
| | | Hap_2 | JQ388786 |
| | | Hap_3 | |
| Caucasia | Russia | Hap_1 | MH817490-92; MH817495-97; MH817498-99; MH817501-07; MH817502-07; MH817511; MH817513; MH817515-16; MH817518; MH817525-28; MH817530-36; MH817539-42; MH817544-58 |
| | | Hap_2 | MH817529; MH817522-24; MH817519; MH817517; MH817514; MH817512; MH817510; MH817509; MH817505; MH817500; MH817493-94; JX679382-86; |
| | | Hap_3 | |
| | Georgia | Hap_1 | MG198597-60 |
| | | Hap_2 | |
| | | Hap_3 | |

inating from other continents or mainland Europe, and human movement between the countries via ferries. *A. albopictus* may have invaded partly from the Aegean islands, where its existence is known (Badieritakis *et al.* 2018). Demirci *et al.* (2021*b*) indicated the differences between Aegean and Black Sea lineages according to the geometric morphometric data. This finding relates

to phenotypic diversity and may be related to a variety of factors, including biological, ecological, and genetic. Our results showed the difference between the Aegean and other regions with molecular data and supported the geometric morphometric data. According to the Genbank data, Hap_3 was not found in the first introduction areas in European and Caucasian samples. It was also found rarely in some European countries (Croatia, Greece, and Portugal). Chinese, Indian, and USA samples draw a similar pattern to our study. Hap_1 is the main and Hap_3 is the second haplotype for these lineages in these areas according to the Genbank data. This situation reveals that the lineages found in the Aegean region entered independently from the Marmara and Black Sea lineages and could probably be mixed with different lineages but are closer to the Chinese lineage.

Our results revealed that *A. albopictus* in Türkiye may have multiple invasions from different origins according to the haplotype diversity in the regions. Pairwise FSTs results indicated that all regional lineages showed different degrees of differences between Asian, European, American, and African lineages. Although differences showed with other continental lineages, our regional lineages results did not show differences with Moroccan, German, Greek, and Georgian Genbank samples. Although the invasion may have originated in Europe, our regional lineages appear to be more closely related to temperate Asian lineages (except Aegean lineages). Aegean lineages possibly have mixed ancestry with both Albanian and Italian ancestry. Elazig and Erzincan populations in the interior probably have sporadic links to the used tire trade from the Black Sea region.

In conclusion, invasive vector species introduction and spread in any country is a key factor for arboviral transmission. Introduction of the *A. albopictus* into Türkiye may have been through multiple routes and vector competence of the species varies according to the geographic origin. Lourenco-De-Oliveira *et al.* (2003) indicated linkage vector competence of the species with the geographic origin. Türkiye is the bridge between Asia and Europe or Middle East to Eastern part of Europe and many refugees originating from arboviral disease endemic areas use this route to reach continental Europe. The presence and spread of *A. albopictus* in Türkiye is an important public health threat not only for the introduction of emerging arboviruses but also for autochthonous West Nile virus circulation. In these aspects, the introduction of national entomological surveillance programs and applying efficient vector control measures are critical factors in reducing the risk of future autochthonous arboviral disease.

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