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# Phylogenetic Analysis of Mosquito (Diptera: Culicidae) Species with Mitochondrial Cytochrome Oxidase Subunit 1 Gene Distributed in Kocaeli

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**Abstract**

Mosquitoes, which are in the Culicidae family and have blood-sucking properties, infect humans and animals with many diseases. The present study, it was aimed to detect the mosquito species spreading in Kocaeli province based on the DNA barcoding method. DNA isolation was performed using the samples of mosquitoes collected in the Kocaeli province between June 2017 and September 2018. Then, Polymerase Chain Reaction (PCR) and DNA sequence analysis were performed using universal primers of the mitochondrial COI gene. The sequences in FASTA format obtained with the Chromas program were compared with those of other mosquito species in the world through the NCBI-BLAST database. For phylogenetic analysis, the sequences were uploaded into the MEGA X program, and phylogenetic trees were created in the Maximum Likelihood method, Tamura-Nei Model (Tamura & Nei, 1993), Bootstrap 1000. Among mosquitoes collected in this study, 7 species belonging to *Aedes*, *Anopheles*, *Culiseta* and *Culex* genera were identified and characterized as *Aedes geniculatus* (n = 10), *Aedes albopictus* (n = 7), *Anopheles funestus* (n = 1), *Anopheles plumbeus* (n = 1), *Culiseta longiareolata* (n = 1), *Culex torrentium* (n = 1) and *Culex pipiens* complex *sp.* (n = 33). Within them, *Culex pipiens* complex *sp*. was found as the dominant species in Kocaeli. In conclusion, this study is the first molecular research of mosquito species spreading in Kocaeli which provides records to GenBank.

**Keywords:** Aedes, Anopheles, Culex, Culiseta, COI

## 1. Introduction

Mosquitoes are in the Culicidae family in the Nematocera suborder belonging to the Diptera order, and they are the only member within the family that haveblood-sucking properties [1]. Pathogens transmitted by mosquitoes to animals and humans are arbovirus, helminths, and protozoa.

63 mosquito species have been found so far in Turkey. 12 of these species belong to *Anopheles (An.)*, 16 to *Culex (Cx.)*, 5 to *Culiseta (Cs.)*, 26 to *Aedes (Ae.)*, 2 to *Coquillettidia*, 1 to *Orthopodomyia* and 1 to *Uranotenia* (2). In Turkey, two studies revealing the characterization of *Cx. pipiens* complex members by genotyping have drawn attention [2, 3]. Günay (2) accomplished the characterization and barcoding of mitochondrial COI gene region of species including *Cx.* (*barraudius*) *modestus*, *Cx. laticinctus*, *Cx. mimeticus*, *Cx. perexiguus*, *Cx. pipiens*, *Cx. pipiens* form *molestus*, *Cx. quinquefasciatus*, *Cx. theileri*, *Cx. torrent*, *Cx. tritaeniorhynchus,* and *Cx. hortensis* which had been determined that they belonged to the Culex genus by morphological analysis and distributed in the border of Turkey. In addition, Şahingöz Demirpolat et al. [4] investigated the *Cx. pipiens* complex and *Cx. torrentium* species based on the COI sequences in mosquito samples collected from the province of Kayseri. In the study performed by Öter and Tüzer [5], in total 992 of 1085 female mosquitoes were determined as *Cx. Pipiens*, 32 were *Cs. longiareolata*, *An. maculipennis (22)*, *An. claviger (18)*, *Cs. annulata (13)*, *Ae. vexans (3)*, *Cx. hortensis (3)*, and *Ochlerotatus (2)* based on morphological identification [5]. In the study performed by Çetin and Yanıkoğlu (2004), six mosquito species were identified based on morphological characters in Antalya [6]. These were *Cx. pipiens* Linnaeus, 1758, *Cx. martini* Medschid, 1930, *Cx. deserticola*

Kirkpatrick, 1924, *Ochleratatus caspius* Pallas, 1771, *An. superpictus* Grassi, 1899, and *Cs. longiareolata* Macquart, 1838. They pointed out *Cx. pipiens* as the dominant species. Demirci et al. [7] made a study of SNP for 11 different gene regions belonging *Cx. theileri* from northeastern Turkey and created the first record for that type.

Topluoğlu et.al. [8], in their study conducted in Sanlıurfa, collected the larvae and determined species as *Ansacharovi* (84%), which is a vector for malaria, and *Anopheles superpictus* (41%) based on morphological features. As of today, in the world, there are 531 *Anopheles* species of mosquitoes, 481 of them areknown officially while 50 have not been named yet [9]. So far, 10 species belonging to that group have been identified in Turkey [8]. Öter et al. [5] made the molecular identification of *Ae. albopictus* using the COI barcode gene in Thrace region. In a study on morphological species identification of mosquitoes seen prevalently in the Felahiye district of Kayseri, 32.1% of 305 mosquitoes were found to be *Ae. vexans* and 67.9% were *Cx. pipiens* [10].

Kuçlu and Dik (2018) determined the mosquito fauna in the Western Black Sea Region (Bartın, Bolu, Karabük, Düzce, Zonguldak, Kastamonu) and identified 13 mosquito species belonging to the genera *Aedes*, *Anopheles*, *Culex* and *Culiseta*. They also morphologically identified *Ae. caspius*, *An. maculipennis*, *Cx. theileri* and *Cx. pipiens* which were dominant species. In a study conducted by Sarıkaya et al. [12] on refugee migration routes involving 17 provinces, 6 genera and 22 species were identified namely *Anopheles*, *Aedes*, *Coquillettidia*, *Culex*, *Culiseta* and *Uranotaenia*.

When we investigated previous research in the northern region of Turkey, there were some considerable studies such as the molecular analysis of *Ae. albopictus* in the Western Thrace and the Eastern Black Sea region (Artvin, Hopa, Rize, Trabzon, Beğendik and İğneada) [5, 13], the detection of mosquito species seen in Istanbul [14], and the morphological determination of the mosquito species distributed in the Western Black Sea [11]. The distribution and species of mosquitoes have been determined from the study of these regions, from Western Thrace to the Eastern Black Sea Region, however, among these studies, there has been no study specifically focusing on Kocaeli situated in the Marmara region of Turkey.

The present study, has aimed to determine the species of mosquitoes at the molecular level based on the COI gene barkoding method in Kocaeli, where no detailed research has been conducted so far. We believe that this study would contribute additional information to the literature which is valuable for effective vector control management.

## 2. Materials and Methods

### 2.1. Collection of Samples

Mosquitoes were collected from 4 different locations in the Kocaeli province (Table 1) between June 2017 and September 2017 by selecting forest areas, resting places, and water edges with the help of mouth aspirators. The altitude values for the locations are 0-260 m for Izmit, 78-314 m for Derince, 223-232 m for Başiskele, and 464-823 m for Kartepe, respectively. The collected 54 adult mosquitoes were kept at -20$^{o}$C in 70% ethyl alcohol until DNA isolation.

### 2.2. DNA Isolation-Polymerase Chain Reaction-DNA Sequencing

DNA isolation was performed using the Macherey-Nagel (Genomic DNA from insects, NucleoSpin DNA Insect, Catalog number 740470.50) following the procedures recommended by the company. DNA concentrations of the samples were measured using the Qubit 2.0 Fluorometer kit (Invitrogen, America). Primers for COI gene were as follows: 5′-GGTCAACAAATCATAAAGATATTGG-3′ (forward) and 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ [15]. 5x FIREPol Master Mix (Solis BioDyne) was used to prepare the PCR reaction mixture. The PCR reaction mix consisted of 6µl 5x Master Mix, 0.5 µl 10 µM primer (sense), 0.5 µl 10 µM primer (antisense), 2 µl mold DNA. Bidistillated water was added to 30 µl for the PCR mixture of the gene region. PCR steps were involved: 5 min at 94°C (pre-denaturation), 35 cycles at 95°C for 60 s (denaturation), 60 s at 55 °C (annealing), 60 s at 72°C (extension), and 7 min at 72°C (final extension). The amplified COI gene PCR products were then for 30 minutes at 100 volts in 1,5% agarose gel electrophoresis and visualized with a UV transilluminator by using Safe-T staining (ethidium bromide alternative).

PCR products were purified by BM Lab using ExoSAP-ITTM PCR Product Cleanup Reagent (Thermo Fisher Scientific, USA) kit procedures. Sequence analysis was performed in the Macrogen Netherlands laboratory using the ABI 3730XL Sanger Sequencer (Applied Biosystems, Foster City, CA) and the forward and reverse primers of the COI gene.

### 2.3. Phylogenetic Analysis

The DNA sequences were visualized using the Chromas (Version: 2.6.5) program, and the sequences were recorded separately in the FASTA format with the Chromas program. The forward and reverse complement readings of the sequences were compared by aligning them with the ClustalW program [16]. The COI gene regions for all mosquito samples were deposited into the Genbank.
The similarities of the COI sequences of the same mosquito species with the sequences registered in the NCBI database (Table 2) were compared and used in

the phylogenetic tree. For evolutionary analysis, MEGA X [17] program was executed and modeling methods, genetic distance matrices, nucleotide compositions, nucleotide pair frequencies, substitution matrices for DNA barcode gene sequences of the samples were determined. Phylogenetic trees were created in the Maximum Likelihood method, Tamura-Nei Model [18], Bootstrap 1000.

## 3. Results

In this study, the coordinate and altitude information of the four districts in Kocaeli where mosquito samples were collected are shown in Table 1. The COI fragments belonging to the collected mosquitoes were analyzed by amplifying them three times, to obtain the highest quality sequence, and deposited in GenBank.

Phylogenetic analysis of the COI fragments for the sequences obtained from both the 4 populations of this study and other genera and mosquito species of Genbank enabled us to identify 7 species belonging to the genera *Culex*, *Aedes*, *Anopheles*, and *Culiseta* (Table 2). Findings for each species will be discussed separately.

### *3.1. Aedes geniculatus*

9 out of 10 samples were obtained from Kartepe (MH392201, MH392202, MH392203, MH392204, MH392205, MH463069, MK713997, MK713999, MK714000), and 1 of them was from Izmit (MK713998). When the G-C ratios of *Ae. geniculatus* were examined, it was found that the values varied between 31.2% and 32.3%. When considered together with the outgroups, the average G-C ratio was determined as 31.6% (17, 18). The average genetic distance among mosquitoes belonging to the genus *Aedes* was 0.017 (1.7%) (Data not shown here).
These results showed the values within the appropriate range of variation that could be seen among individuals representing the same species. The phylogenetic tree created at 0.0100 scale for *Ae. geniculatus* species is shown in Figure 1.

### 3.2. *Aedes albopictus*

Seven out of 17 *Aedes* genera mosquitoes were identified as *Ae. albopictus* as a result of the COI barcode gene examination (MK714010, MK714007, MK714003, MK713991, MK714006, MK714008, MK714009). When the G-C ratios of the samples were examined, it was determined that the values of *Ae. albopictus* varied between 30.7% and 32.5%. When considered together with the outgroups, the average G-C ratio was found as 32.3% (17, 18). The intra-species variation rate was determined as 0.16%. The lowest genetic distance was found to be 0, and the highest genetic distance was 0.0016. It is seen that different branches and clades are also separated from the 1000-repetitive bootstrap phylogenetic tree constructed with the sequences of other *Aedes* species obtained from GenBank (Figure 2).

**Table 1.** Mosquito species distributed in four different districts of Kocaeli (n: frequency, m: meter, Lon.: Longitude, Lat.: Latitude).

| **Districts** | | **İzmit** | **Derince** | **Başiskele** | **Kartepe** |
|---|---|---|---|---|---|
| **Geo. Details (Lon., Lat.)** | | 40.760071,29.928734<br>40.822020,29.924783 | 40.771936,29.816591<br>40.834515,29.903672 | 40.640383,29.938674<br>40.639618,29.938382 | 40.682336,30.136046<br>40.656733,30.146763 |
| **Altitude (m)** | | 0-375 | 78-315 | 223-232 | 464-823 |
| **Genus** | **species** | | | | |
| *Culex* | *pipiens complex sp (n=33)* | 26 | 5 | 2 | - |
| | *torrentium (n=1)* | - | - | 1 | - |
| *Aedes* | *geniculatus (n=10)* | 1 | - | - | 9 |
| | *albopictus (n=7)* | 2 | 3 | 2 | - |
| *Anopheles* | *funestus (n= 1)* | - | - | 1 | - |
| | *plumbeus (n=1)* | 1 | - | - | - |
| *Culiseta* | *longiareolata (n=1* | 1 | - | - | - |
| ***Total (n=54)*** | | 31 | 8 | 6 | 9 |

**Table 2.** Accession numbers of mosquito species obtained from NCBI for use in phylogenetic trees for the COI DNA barcode gene.

| Species | Accession numbers | Species | Accession numbers | Species | Accession numbers |
|---|---|---|---|---|---|
| *Ae. geniculatus* | KM258304.1 | *An. funestus* | MK300232.1 | *Cx. quinquefasciatus* | KF407473.1 |
| *Ae. japonicus* | FJ641869.1 | *An. funestus* | MH299888.1 | *Cx. pipiens molestus* | FN395171.1 |
| *Ae. notoscriptus* | MG242508.1 | *An. eiseni* | MF172271.1 | *Cx. torrentium* | KJ401313.1 |
| *Ae. albopictus* | MH817529.1 | *An. darlingi* | JF923693.1 | *Cx. pipiens pallens* | KC407754.1 |
| *An. funestus* | KJ522832.1 | *An. annulipes* | MG712534.1 | *Cx. hortensis* | KJ012068.1 |
| *An. funestus* | MK300231.1 | *An. plumbeus* | KM258215.1 | *Cx. pipiens* | KM258167.1 |

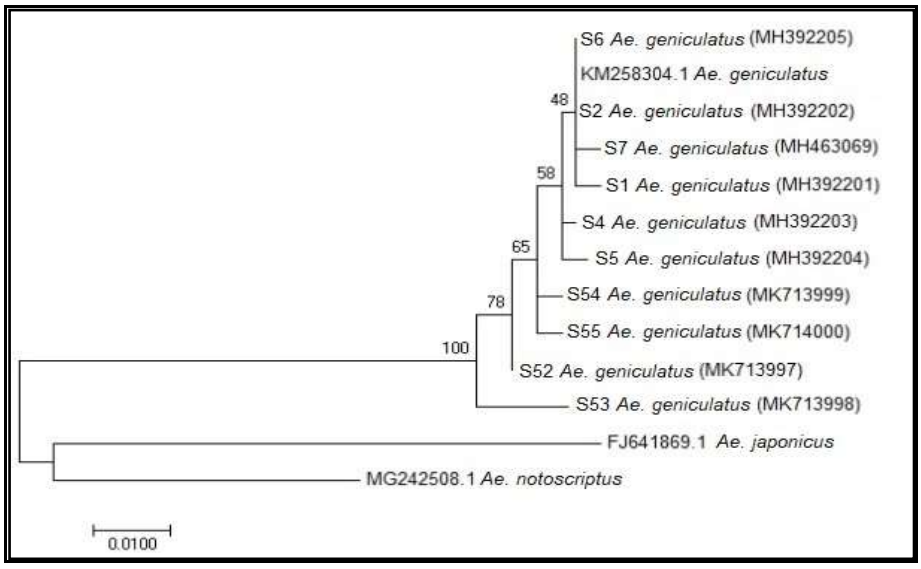


**Figure 1.** Molecular phylogenetic tree created by ML method for the COI gene region of *Aedes geniculatus*.

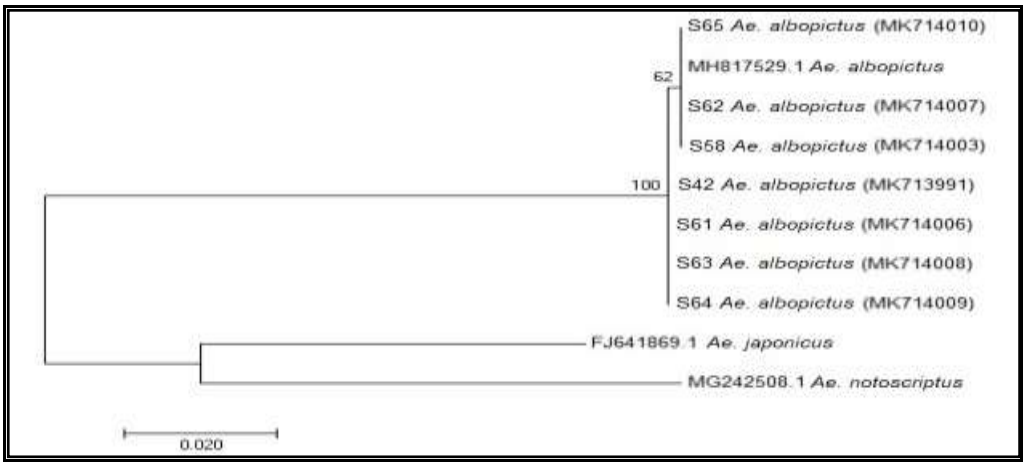


**Figure 2.** Molecular phylogenetic tree created by ML method for the COI gene region of *Aedes albopictus*

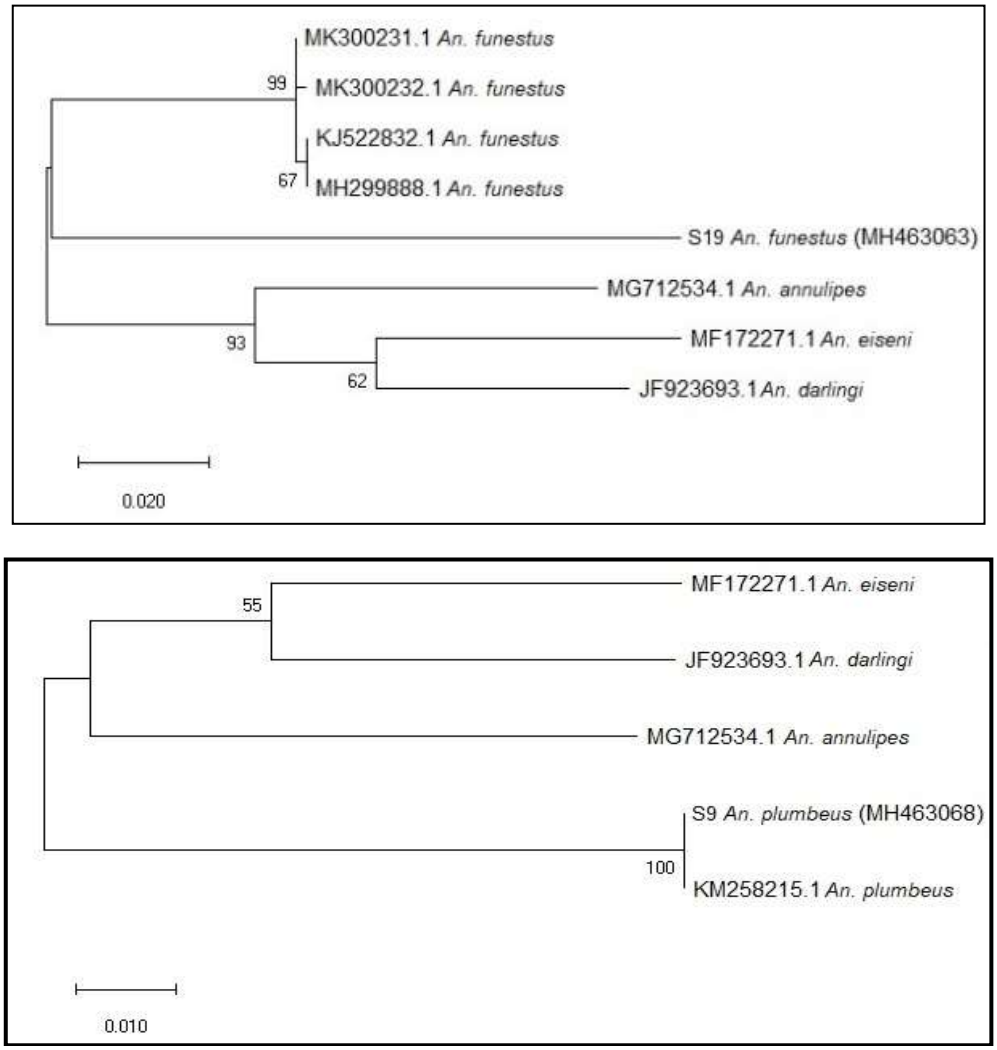


**Figure 3.** Molecular phylogenetic tree created by ML method for the COI gene region of *Anopheles funestus* (S19 MH463063) (upper) and *Anopheles plumbeus* (S9 MH463068) (below).

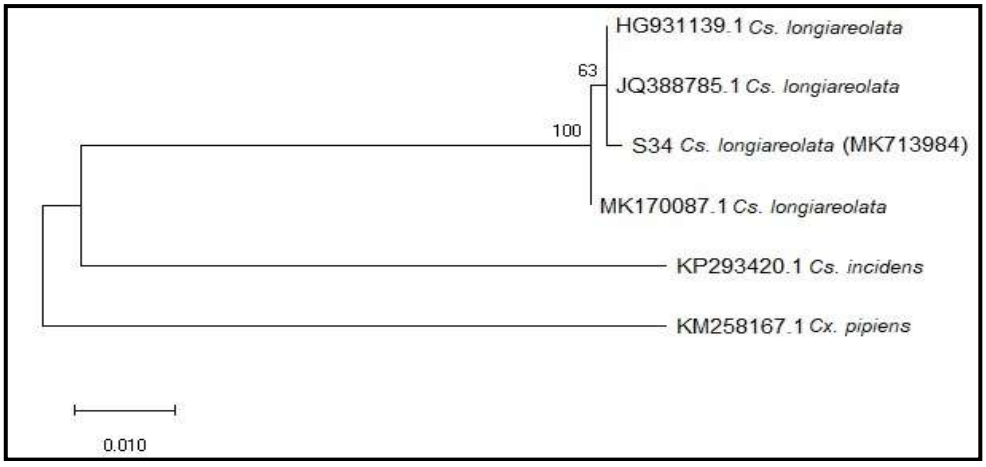


**Figure 4.** Molecular phylogenetic tree created by ML method for the COI gene region of *Culiseta longiareolata.*

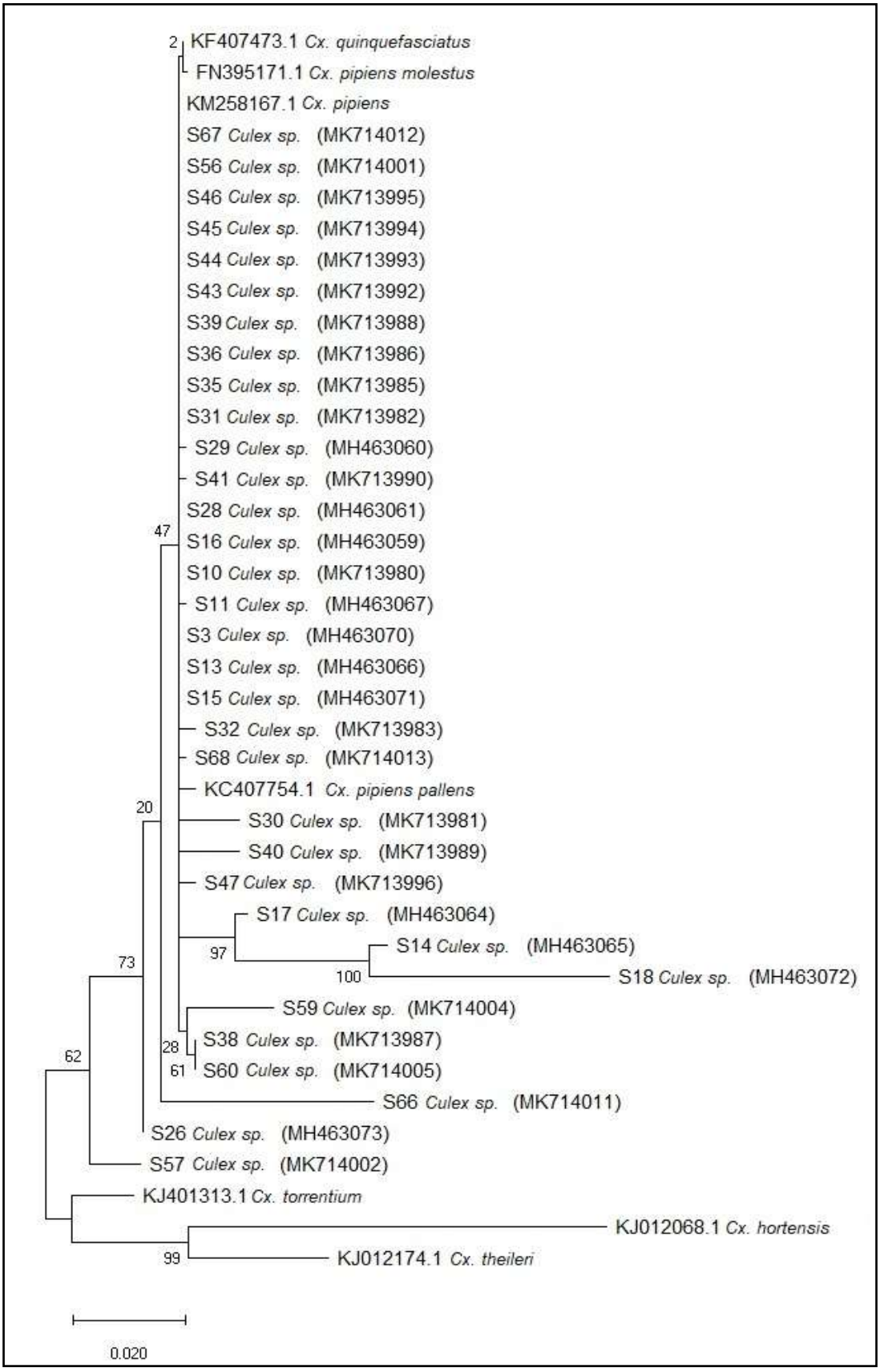


**Figure 5.** Molecular phylogenetic tree created by ML method for the COI gene region of *Cx. pipiens* complex *sp.*

### 3.3. *Anopheles funestus* and *Anopheles plumbeus*

One of the samples in Izmit was identified as *An. plumbeus* while in Başiskele *An. funestus* was found. When the G-C rates of *An. plumbeus* were examined, its ranged varied between 29.79% and 32.37%. When considered together with the outgroups, the average G-C ratio was determined as 31.09% [17, 19]. On the other hand, *An. funestus* was determined to vary between 29.86% and 34.99% in terms of G-C content [17, 18]. Phylogenetic trees obtained at 1000 bootstrap values are shown in Figure 3.

### 3.4. *Culiseta longiareolata*

As a result of the COI gene analysis one sample from Izmit was determined as *Cs. longiareolata* (MK713984). When the sequences belonging to other *Cs. longiareolata* species of the world were obtained from GenBank to be compared with the samples of our study, it was determined that the conserved region in the COI gene region was 99.69% and the variable region was 0.31%. In the pairwise genetic distance matrix created based on the Tamura 3-parameter model, the average genetic distance between mosquitoes belonging to the genus *Culiseta* was found to be 0.0554 (5.54%). The lowest genetic distance of *Cs. longiareolata* species was found to be 0, and the highest genetic distance was 0.0031 (0.31%). The phylogenetic tree created in 0.010 scale is shown in Figure 4.

### 3.5. *Culex species complex*

As a result of COI analysis, from mosquito species collected, 26 in Izmit, 5 in Derince, and 2 in Başiskele were determined as *Cx. pipiens* complexes. In addition, 1 sample in Başiskele was identified as *Cx. torrentium*. When the G-C rates of 33 *Cx. pipiens* complex *sp.* samples were examined, it was found that they varied between 30.70% and 32.25%. When considered together with the outgroups, the average G-C ratio was determined as 31.25% (Tamura & Nei, 1993; Kumar et al., 2018). Sequence data were depozited in GenBank (MH463070, MK713980, MH463067, MH463066, MH463065, MH463071, MH463059, MH463064, MH463072, MH463073, MH463061, MH463060, MK713981, MK713982, MK713983, MK713985, MK13983, MK713985, MK13983, MK713993, MK713994, MK713995, MK713996, MK714001, MK714002, MK714004, MK714005, MK714011, MK714012, MK714013). KM258167.1 (*Cx. pipiens*), KF407473.1 (*Cx. quinquefasciatus*), FN395171.1 (*Cx. pipiens molestus*), KJ401313.1 (*Cx. torrentium*), KC407754.1 (*Cx. pipiens pallens*), KJ012068.1 (*Cx. hortensis*) and KJ012174.1 (*Cx. theileri*) were chosen as the outgroups to the phylogenetic tree. The average genetic distance between mosquitoes belonging to the genus Culex was found to be 0.0137 (1.37%). The phylogenetic tree created at 0.020 scale is shown in Figure 5.

### 3.6. *Culex torrentium*

When 1 *Cx. torrentium* (MH463062) obtained from Başiskele was evaluated together with the outgroups selected from GenBank, it was found that there were differences in 2 nucleotides (0.31%) on a region with 634 base pairs long, and the genetic distance between mosquitoes of the same breed was 0.018 (1.8%). The phylogenetic tree created at 0.0050 scale is shown in Figure 6.

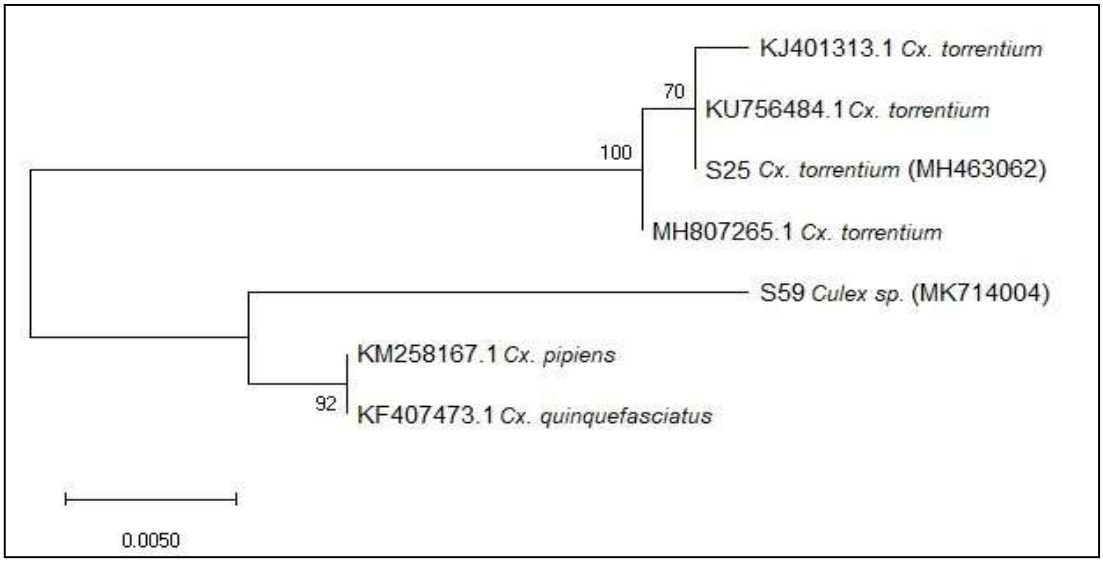


**Figure 6.** Molecular phylogenetic tree created by ML method for the COI gene region of *Cx. torrentium*.

## 4. Discussion and Conclusion

Considering that *Aedes*, *Anopheles*, and *Culex* mosquitoes are vectors for many important diseases, detailed information is needed on distribution and epidemiology of these mosquito species [4]. Studies on mosquitoes in Turkey have been generally based on morphology. Despite this, there have also been studies at the molecular level on the detection of mosquito species in recent years [2, 5, 6, 10, 20].

Mosquitoes belonging to the *Culex* genus are common in all climate types in our country. In our study, 34 out of 54 mosquitoes consist of two species belonging to the genus Culex. One of them belonged to *Cx. torrentium* and the others to the *Cx. pipiens* complex mosquitoes. Mosquitoes of the *Cx. pipiens* complex include species named as *Cx. pipiens* form *molestus*, *Cx. pipiens* form *pipiens* and *Cx. quinquefasciatus*. Unfortunately, these sibling species, whose identifications were difficult regarding morphology, could not be detected separately with the COI barcode gene in our study either. In the BLAST application of the NCBI genome database, the % similarity rates of these sibling species were almost the same. In our opinion, the COI barcode gene is not strong in distinguishing *Cx. pipiens* complex species. Thus, in the study by Laurito et al. [21] on Culex species in Argentina and Brazil using the COI gene, they pointed out that they were able to define species at the rate of 69%, but the remaning could be misidentified or not be identified. Şahingöz Demirpolat et al. [4] sampled 1052 female mosquitoes in their field study and analyzed 315 of them morphologically with diagnostic keys. As a result, they determined that 311 samples showed *Cx. pipiens* band profiles with ACE-2 and CQ11 microsatellite analysis. The remaining 4 samples were found to be hybrids of *Cx. pipiens* form *pipiens* and *Cx. pipiens* form *molestus* by microsatellite analysis (4).

In a study for molecular identification of the *Cx. pipiens* subspecies, the number of TG dinucleotide repeats in the microsatellite CQ11 region were compared [22]. Since there is no *Cx. torrentium* species in North America, the technique used in the study was effective in separating for *Cx. pipiens* s.s., *Cx. pipiens* form *molestus*, and *Cx. quinquefasciatus*. However, another study conducted in England reported that it would be insufficient to diagnose the above-mentioned species in the genus *Culex* with the COI DNA barcode gene and CQ11 region, and therefore it was shown that these genes could not be used for screening in Europe [23].

According to Morçiçek et al. [24], although *Cx. pipiens* and *Cx. quinquefasciatus* are two different species in terms of physiology and behavior, they found the interspecific genetic sequence difference was 0.2%. However, it has been suggested that in most Diptera species, greater than 2% sequence divergence in the COI gene is a threshold level for species limitation [25, 26].

Although there are some specific limitations in the use of DNA-based methods in the identification and classification of species, it has significant advantages compared to the use of conventional taxonomic methods. This sensetive method can be used in routine labs, and can make a more accurate and powerful diagnosis. The COI is the slowest changing region of all mitochondrial protein-encoding genes and is generally a good molecular tool in evolutionary genetic and interspecific and intraspecific variability studies [24]. However, as can be seen in this study, the COI gene was not effective in distinguishing *Cx. pipiens* sibling species.

*Cx. pipiens,* and *Cx. torrentium* are two morphologically similar sister species. When the *Cx. torrentium* (S25-MH463062) from our study was compared with the *Cx. torrentium* species from the study by Günay et al. [27] (KJ012236, KJ012242, and KJ012238) the similarity rate was found to be 100%. Likewise with *Cx. torrentium* samples recorded in data banks from different countries (KJ401313.1 (Denmark), HF562557.1 (Germany), KU756484.1 (Austria), and MH807265.1 (Austria)) the similarity rates were found to be quite distinctive and between 99.84% and 100%. With the present study, the first records for the *Cx. pipiens* complex and *Cx. torrentium* species in Kocaeli were created with the COI barcode gene.

*Ae. geniculatus*, which we recorded in the NCBI genome database, showed a similarity between 99-100% compared with the those from other countries. For *Ae. geniculatus,* which can be adapted to the waters accumulating in tree hollows during pre-adult periods [2] and has been registered previously in the Antalya, Bursa, Rize, Samsun, Edirne and Kırklareli provinces in Turkey [2, 28, 29], our study also determined it at the molecular level based on the COI gene region in Kocaeli and stored to NCBI-Nucleotide.

When the records in GenBank were compared, the similarity rate of the 7 *Ae. albopictus* we identified was between 99.8-100%. *Ae. albopictus*, known as the Asian Tiger Mosquito, has the potential to transmit approximately 32 viruses such as Dengue fever, Zika virus, Japanese encephalitis, Yellow fever, Western equine encephalitis, Venzuella equine encephalitis [29]. Öter et al. [30] was the first to identify the *Ae. albopictus* species based on the DNA barcoding method in Kashan and Ipsala from the Thrace Region in Turkey. It was also detected in the Eastern Black Sea [31], the Black Sea coasts of the Thrace region and some districts of the European side in Istanbul, and registered to GenBank [2, 29]. In a study based on morphology, it was reported that *Ae. albopictus* specimen was found for the first time in Izmit-Kocaeli in August 2018 [32].

In our study carried out between June 2017 and September 2018, we also detected the same type of mosquito sample by using the COI barcode gene in 3 different locations in Kocaeli and recorded its information in the gene database. In a study conducted by Tuna Türkozan [13] using mitochondrial ND5 and COI genes, this mosquito species was detected in a large region including the Eastern Black Sea and the Thrace region. Then, in our study, it was also detected in a small region between mentioned areas and its first record at the molecular level was created by us. Therefore, it can be stated that *Ae. albopictus* is a mosquito species spreading along the Black Sea coast based on the data obtained from both this study and a study reported by Türkozan (2020).

*An. plumbeus* specimen with accession number MH463068 was 100% similar to the Belgian specimen, *An. plumbeus* with access number KM258215.1. *An. funestus* specimen, accession number MH463063 was slightly weaker supported by NCBI-registered species, at approximately 88%. It showed 87.81% similarity with the Kenya sample with the access number MK300231 and 87.71% with the American sample with the access number KJ522832.1. Unfortunately, we could not find any sample registered in GenBank from Turkey for *An. funestus*, so we did not have the opportunity to make a comparison. *An. plumbeus* has been reported to be found in forest areas up to 1200 m above sea level [33]. In our study, *An. plumbeus* was obtained at 225 m above sea level, while *An. funestus* was at 341 m in the forest area.

*Cs. longiareolata*, a vector for brucellosis, avian influenza and West Nile encephalitis, can be found in many areas such as swamps, septic tanks, and drainage channels, although it is found in similar habitats to *Culex* mosquitoes. Although it is known to be zoophilic, it is rarely fed with human blood [34]. According to the analysis results of *Cs. longiareolata* from NCBI BLAST, it was found to be 99.69%-99.85% similar to samples with access numbers JQ388785.1, MK170087.1, and HG931139.1. In a thesis study conducted by Günay [2] in 2015, mosquitoes belonging to the *Cs. longiareaolata* species of the Allotheobaldia subgenus obtained from different cities were studied and 13 haplotypes within this species were determined. The first COI registration at Genbank for *Cs. longiareolata,* which was also found in Kocaeli, was achieved with this study.

In this study, the mitochondrial COI barcode gene was used. This barcode gene has high discriminatory power for *Aedes*, *Anopheles*, and *Culiseta* species, whereas it is effective for only one *Cx. torrentium* within *Culex* species. It was not possible to distinguish the sibling species belonging to the *Cx. pipiens* complex by using this DNA barcode gene. Knowing which barcode genes should be used for the definitive identification of vector mosquitoes at the molecular level and species basis will enable us to reach practical information more easily. For this reason, to researchers who wish to study the phylogenetics of mosquito species, we suggest that they can try the following nuclear genes: IGS (Intergenic Spacer), ITS1 (Internal Transcribed Spacer 1), mitochondrial protein-coding regions ND1 (NADH dehydrogenase 1), ND2 (NADH dehydrogenase 2), COII (Cytochrome Oxidase Subunit 2), and cytb (cytochrome b apoenzyme).

As a result, from a total of 54 mosquito samples collected in Kocaeli between June 2017 and September 2018, 7 species belonging to 4 genera were identified with a molecular analysis based on the mitochondrial COI barcode gene. With this study, the first molecular records for the mosquito species in Kocaeli were created. The limitation of the study might be sample size which was relatively small.

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**Author's Contributions**

**Fikriye Polat:** Drafted and wrote the manuscript, performed the experiment and result analysis.
**Serkan Dede:** Collection of mosquitoes, experimental study, DNA extraction, PCR.

**Ethics**

This study does not present any ethical concerns.

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