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DNA Barcoding and Phylogenetic Analysis of *Culex* and *Anopheles* Species in Siirt, Türkiye

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ABSTRACT

Mosquitoes play a critical role as disease vectors, making them significant in terms of both public health concerns and ecological balance. This study aims to identify mosquito specimens collected from Siirt city center and six different districts using morphological and molecular methods. A 658 bp fragment of the mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene region was used for molecular diagnosis. The findings revealed four mosquito species: *Culex theileri* Theobald, 1903, *Culex mimeticus* Noè, 1899, *Culex quinquefasciatus* Say, 1823, and *Anopheles superpictus* Grassi, 1899. Mitochondrial gene PCR products were sequenced, and the sequences were uploaded to the NCBI database for public access. Phylogenetic analysis was conducted using these sequences to investigate the genetic distances and evolutionary relationships among the mosquito species. In the phylogenetic analysis, *Chironomus kiiensis* was used as an outgroup. The analysis revealed that *C. quinquefasciatus* and *A. superpictus* were had the highest genetic distance (0.16), while the closest genetic distance was observed between *C. quinquefasciatus* and *C. theileri* (0.06). This study is presented as a preliminary investigation into the genetic diversity, evolutionary relationships, and population dynamics of mosquito species in Siirt Province. Further studies with a larger sample size and additional sequences are needed to establish more comprehensive phylogenetic relationships. The molecular findings contribute significantly to the systematic and ecological studies of mosquitoes.

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Introduction

The order Diptera is one of the most species-rich and ecologically diverse groups of insects, consisting of approximately 130 families (Yeates & Wiegmann, 1999). Among these families, the Culicidae family comprises around 3,500 species across 41 genera worldwide (Foster & Walker, 2019). In Türkiye, recent studies on mosquito identification have reported the presence of 62 species belonging to 7 genera (Kuçlu & Dik, 2018). Mosquitoes of the subgenus *Culex* belong to the genus *Culex* Linnaeus, which has a broad global distribution and currently includes 203 recognized species (Somboon et al., 2023). The classification of the Culicidae family is shown below (Harbach, 2007):

- **Kingdom:** Animalia
- **Phylum:** Arthropoda
- **Class:** Insecta
- **Order:** Diptera
- **Family:** Culicidae
    - **Subfamily:**
        - **Anophelinae**
        - **Culicinae**

The taxonomic classification of the Culicidae family is illustrated in Figure 1.

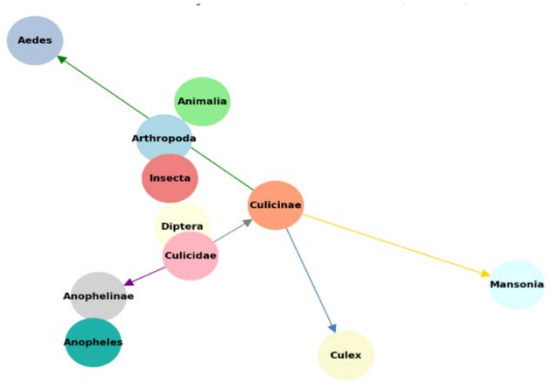

Figure 1. Taxonomic classification of the Culicidae family

Geographic region and ecological features are known to play a significant role in the distribution of mosquito species. Species that select various habitats show tolerance to environmental factors, while species that can breed in only a few habitat types tend to be less tolerant. Generally, species with high tolerance are more widely distributed and have higher population densities compared to other species (Eisen et al., 2008). Due to their specialized adaptation abilities, mosquitoes can thrive in a wide range of environments. Nearly every type of water source globally can serve as a breeding site for mosquitoes (Becker et al., 2010). As a result, mosquitoes are found worldwide and carry the diseases. They are absent only in specific regions of Antarctica, while especially coastal regions provide an ideal habitat for many mosquito species in Türkiye (Aldemir & Boşgelmez, 2006; Alten et al., 2000; Sengil et al., 2011).

Mosquitoes attract attention both in terms of health and economics due to their ability to survive for extended periods under unfavorable environmental conditions, rapidly reproduce in almost all aquatic habitats, and their presence in all zoogeographical regions (Smith et al., 2006). They have the potential to transmit pathogens that are significant to both human and animal health (Batovska et al., 2016). Mosquitoes, as biological vectors, transmit malaria, which is considered one of the most dangerous diseases, causing 500 million cases and approximately 2.5 million deaths globally each year (Hasan Muslu & Özbilgin, 2011). In Türkiye, malaria is most commonly found in southeastern provinces such as Diyarbakır, Batman, Siirt, Şanlıurfa, Mardin, and Şırnak, as well as in the Çukurova region. There is also a risk of malaria transmission from neighboring countries, such as Iran, Iraq, and Syria, where numerous cases of the disease occur (Smith et al., 2006).

Many studies have been conducted on the presence of mosquitoes, the activity of key species, their seasonal abundance, bio-ecology, and population development in various regions of Türkiye (Bişkin et al., 2010; Çetin & Yanıkoğlu, 2004; Demirci, 2006; Ede & Öztemiz, 2021; Eren et al., 1996; Günay, 2015; Oter et al., 2013; Sona & Değer, 2015; Şakacı, 2021).

The Botan Valley in Siirt province contains numerous natural streams, both large and small, and features still bodies of both natural and artificial water. In recent years, large dams constructed in and around Siirt have also begun to retain water. Additionally, the prevalence of septic pits due to insufficient sewage systems, along with extensive irrigation channels and wetlands, has led to frequent mosquito complaints throughout the year. As a result, authorities in Siirt annually employ chemical control methods to reduce mosquito populations.

Mosquito species in Siirt province pose a potential threat to human and animal health as vectors of pathogens. Therefore, accurate identification of these species is essential for effective monitoring and control. The high degree of similarity among mosquito species makes distinguishing them based on morphological characteristics challenging (Walton et al., 1999). Identifying mosquitoes remains a systematic issue, as it often relies on morphological features that can be difficult to interpret without specialized taxonomic expertise and are frequently lost during collection and storage (Versteirt et al., 2015). Molecular methods provide clear identification where morphological methods are inadequate. DNA data obtained from molecular studies can overcome the limitations of morphological approaches, enabling the identification of sibling and cryptic species (Hebert et al., 2003). DNA barcoding is widely recognized as a valuable molecular tool for the rapid and accurate identification and assessment of species and biodiversity (Heber et al., 2016). It has garnered broad interest due to its effectiveness and accuracy in identifying species of mammals, birds, reptiles, amphibians, fish, and arthropods (Chaiphongpachara et al., 2022). Compared to other molecular methods (RAPD, RFLP, AFLP, etc.), DNA barcoding offers several advantages. The primer set used in DNA barcoding (Folmer et al., 1994) aims to amplify a short fragment of the *COI* gene from many animal species (Yatkın & Güz, 2018). The mitochondrial cytochrome c oxidase subunit I (*COI*) gene, a conserved gene and the first standardized genetic region used for animal DNA barcoding, is the most popular barcode marker (Adeniran et al., 2021). DNA barcoding is a complementary species identification method with the potential to overcome current limitations. This study aims to identify mosquito species (Culicidae) in Siirt province and support morphological identification through DNA barcoding using molecular methods.

Materials and Methods

Mosquito Sampling

The study was conducted monthly from April to September in 2023 and 2024 in the wetlands of Siirt province. Sampling was carried out in Siirt Central District, Tillo, Kurtalan, Baykan, Eruh, Şirvan, and Pervari. Sampling was performed in wetlands such as dams, stagnant waters, rivers, and streams of various sizes in and around Siirt to represent the entire region. Aquatic habitats were selected based on their differences in ecological features such as location, size, status, fauna, flora, and turbidity. GPS coordinates are provided in Table 1. The mosquito sampling sites were mapped using ESRI ArcGIS Desktop 18.08 (Figure 2).

A plankton net with a mesh size of 153 microns was used for mosquito sampling. As described by Özgökçe et al. (2007) the plankton net was thrown randomly into the water while walking approximately 100 steps along a 1-5 m shoreline at a depth of 0.5-1.5 m.

Table 1. GPS Coordinates of Sampling Locations

No	Location	GPS Coordinates	
1	Siirt University	37°58'9.49"K	41°50'30.52"D
2	Siirt (Kezer Stream)	37°57'26.63"K	41°51'21.70"D
3	Siirt (Başur 1)	37°57'28.61"K	41°47'13.40"D
4	Siirt (Başur 2)	37°58'8.21"K	41°47'5.24"D
5	Siirt (Ziyaret 1)	38° 0'58.64"K	41°46'33.52"D
6	Siirt (Ziyaret 2)	38° 0'53.78"K	41°46'36.59"D
7	Baykan 1	38° 6'37.81"K	41°43'6.67"D
8	Baykan 2	38° 6'35.83"K	41°42'58.43"D
9	Kurtalan	37°55'50.45"K	41°42'41.04"D
10	Siirt Central 1	37°57'6.99"K	41°53'27.55"D
11	Siirt Central 2	37°51'35.14"K	41°53'19.58"D
12	Siirt Central 3	37°54'26.67"K	41°56'40.53"D
13	Eruh (Zarova Stream)	37°48'36.56"K	42°10'35.02"D
14	Şirvan 1	38° 1'19.14"K	41°56'4.03"D
15	Şirvan Dam (Stream)	38° 2'15.09"K	41°57'25.96"D
16	Şirvan Dam	38° 2'31.64"K	41°57'26.06"D
17	Şirvan Trout Facility	38° 3'13.15"K	42° 1'38.81"D
18	Şirvan 2	38° 3'50.04"K	42° 1'38.60"D
19	Şirvan Taşköy Trout Farm	37°58'49.42"K	42° 8'28.65"D
20	Siirt Limak Dam	37°56'13.89"K	42°14'22.87"D
21	Pervari Gölköy	37°56'38.91"K	42°25'6.20"D

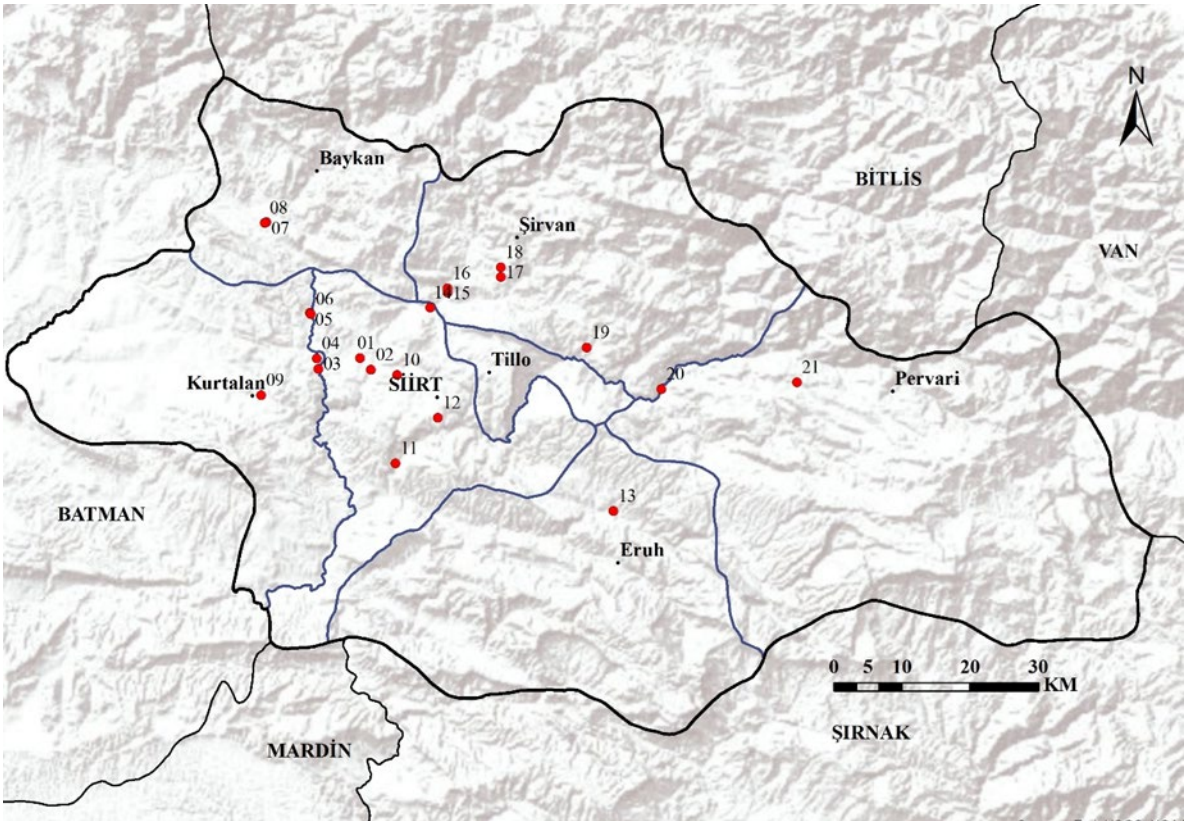

Figure 2. Mosquito sampling sites in and around Siirt Province.

The larvae collected in the net's sampling container were transferred into lidded plastic collection jars and transported to the laboratory under a cold chain. The net was manipulated by pulling and releasing the attached rope during the walk to ensure the net reached both surface and bottom areas. Additionally, mosquito larvae were collected from various water sources, such as shallow wetlands and pools, using plastic scoops, and were later brought to the laboratory in plastic containers. A large number of larval specimens were collected during the sampling process.

Morphological Identification

The collected larvae were kept in white plastic trays (25x30x5 cm) under controlled laboratory conditions (25-28°C, 12:12 light-dark cycle, 50-60% relative humidity). Individuals that reached the pupal stage were transferred to containers and monitored until they developed into adults. The adult mosquito species were identified according to Azari-Hamidian & Harbach (2009), Cranston et al. (1987), Darsie & Samanidou-Voyadjoglou (1997), and DuBose & Curtin (1965). Photographs of the mosquitoes were taken

using an Olympus SC61 stereo microscope with an Olympus SC50 camera and CellSens Entry software. All larval and adult mosquito samples identified morphologically were preserved in 96% ethanol in centrifuge tubes (one specimen per tube) and stored at -20°C for molecular analysis.

DNA Isolation and PCR Procedures

DNA isolation of the morphologically identified mosquito species was performed using the Invitrogen DNA PureLink™ Genomic DNA Mini Kit. Insects were ground thoroughly in liquid nitrogen with metal pestles, and total genomic DNA was extracted following the kit protocol. The quantitative and qualitative properties of the extracted DNA were evaluated using a Nano-400A spectrophotometer and agarose gel electrophoresis, respectively. The mitochondrial cytochrome c oxidase subunit I (*COI*) gene region was amplified using PCR (MiniAmp Plus Cycler) with universal primers LCO1490-F (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198-R (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al., 1994). The PCR cycle included 1 min pre-denaturation at 95°C, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and elongation at 72°C for 60 sec, with a final extension step at 72°C for 10 minutes. PCR products were analyzed using 1% agarose gel electrophoresis, prepared by dissolving agarose in 1x TAE buffer. The gel was run at 100 volts for around 45 minutes. To estimate the size of the PCR products, a 100 bp DNA ladder was included as a molecular weight marker. Following electrophoresis, the gel was stained with ethidium bromide for 20 minutes, and DNA fragments were observed under UV light. The PCR products were sent for sequencing.

DNA Sequence Analysis of PCR Products

The PCR products of the *COI* region were sequenced using the Sanger method by a commercial company. The sequence data were processed using software such as MEGA X for phylogenetic analysis (Kumar et al., 2018), where the alignment of sequences was performed, and genetic distances between the species were calculated. Additionally, ChromasPro (Version 2.1.10) (Technelysium Pty Ltd, South Brisbane, Australia) was used for sequence visualization and editing, BioEdit (Version 7.2.5) (Hall, 1999) was utilized for sequence alignment, and CLC Main Workbench (v6.7.1) (Matvienko, 2015) was employed for further data manipulation and analysis (Figure 3).

Phylogenetic Analysis

DNA sequences were visualized using ChromasPro (Version 2.1.10) and saved in FASTA format. The forward and reverse complement reads of the sequences were aligned and compared using BioEdit (Version 7.2.5) (Hall, 1999). For each insect species, *COI* gene sequences were uploaded in FASTA format to NCBI Nucleotide BLAST, and their similarities with sequences in the NCBI database were compared. The most similar sequences were noted with their GenBank accession numbers and used for the phylogenetic tree construction. Sequences from our study and *COI* gene sequences of insect species obtained from NCBI Nucleotide were uploaded to CLC Main Workbench (v6.7.1) (Matvienko, 2015) and MEGA X (Kumar et al., 2018) to determine modeling methods and distances. Phylogenetic trees were constructed using the Maximum Likelihood method.

Results and Discussion

In this study, three different mosquito species belonging to two genera that were previously unrecorded from Siirt Province are identified. The identified species are: *Culex mimeticus* Noè, 1899, *Culex theileri* Theobald, 1903, *Culex quinquefasciatus* Say, 1823, and *Anopheles superpictus* Grassi, 1899.

Culex mimeticus is a mosquito species belonging to the genus *Culex*, first described from specimens collected in Grassano, located in the southern part of Basilicata, Italy (Noè, 1899). It has been distributed from the southwestern Palearctic region eastward to the Eastern region (Somboon et al., 2021). *C. mimeticus* is a medium-sized species that can be easily distinguished from closely related species by the presence of three distinct yellowish areas on the costa of the wing and pale areas on other veins. In the fourth instar larvae of this species, seta 2-S is long and curved, seta 7-I is typically single and as long as 6-I. The distal pecten spines possess seven or more ventral denticles. The pre-clypeal seta is thick and significantly thicker than the inner and median branches of the setae. The siphonal trachea is narrow and less than half the width of the siphon (Azari-Hamidian & Harbach, 2009; Harbach, 1988) (Figure 4).

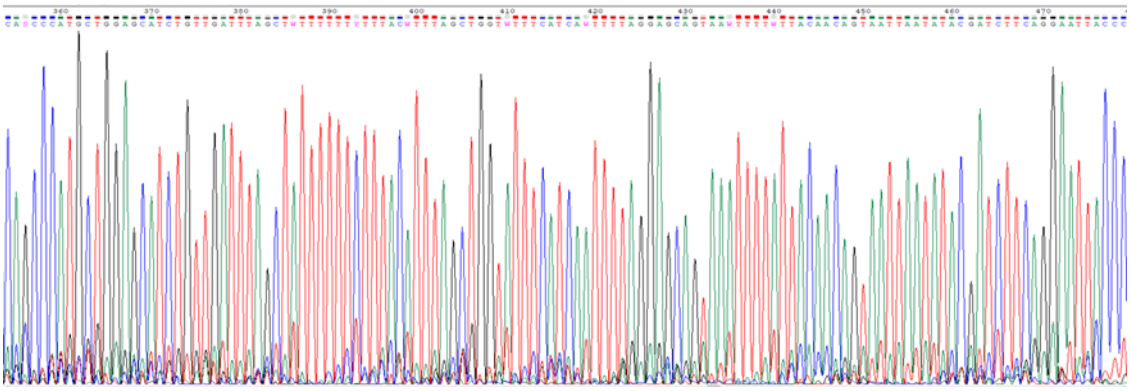

Figure 3. Chromatogram Image of DNA Sequence from Mosquito Species

Figure 4. Image of *Culex mimeticus* larva under the microscope

Figure 5. Image of *Culex theileri* larva under the microscope

Culex mimeticus prefers to breed in wetlands and stagnant water bodies. It can also be observed in urban and rural areas, making it a widespread species across a wide range of habitats (Harbach, 2018). This species may have the potential to act as a vector for some diseases, but further research is needed to determine whether it poses a health risk as significant as other *Culex* species. *C. mimeticus* has been identified as a previously unrecorded species in the region based on morphological and molecular analyses conducted in Siirt Province. The definitive identification of the species has been carried out using molecular analyses and DNA barcoding methods. This species has been found particularly abundantly in stagnant ponds located in the Şirvan and Pervari districts. The presence of *C. mimeticus* in these breeding sites is significant for understanding mosquito diversity and potential health risks in the region. The morphological and molecular findings indicate that this species contributes to the ecosystem dynamics in Siirt Province and plays a critical role in assessing health risks.

Culex theileri is a mosquito species belonging to the *Culex* genus, identified in this study. This species exhibits a broad distribution across the Afrotropical, Southern Palearctic, and Northeastern regions. It has been reported in several European countries including Portugal, Spain, France, Italy, Yugoslavia, Greece, Hungary, Bulgaria, and Ukraine (Demirci et al., 2012). Morphologically, it can be easily distinguished by the presence of postspiracular and prealar scales, along with white anterior banding on all tibiae and the fore and middle femora. The basal yellow banding of the abdominal tergites typically manifests as triangular patches towards the posterior. In the fourth instar larvae of this species, the siphonal tuft arises near the posterior midline of the siphon and consists of 4–11 branches. The siphonal trachea is broad, measuring more than half the width of the siphon. The distal pecten spines are very large and curved. Seta 1-C is dark, relatively thick, and never sharply tapered or filamentous (Azari-Hamidian & Harbach, 2009; Harbach, 1988) (Figure 5). Female individuals engage in blood-feeding behavior and often target humans (Theobald, 1901). *Culex theileri* prefers to breed in wetland areas and stagnant water bodies, and it can also be observed in urban and rural environments. The species is particularly prevalent in humid and warm climates (Harbach, 2018). Like other *Culex* species, it may act as a vector for various pathogens. In fact, Azari-Hamidian & Omrani (2022) reported that *C. theileri* serves as a vector for avian Plasmodium, *Dirofilaria immitis*, West Nile virus, Japanese encephalitis virus, and some insect-specific flaviviruses across multiple countries. *Culex theileri* has been identified as a new record for Siirt Province based on both morphological and molecular analyses (Figure 5). This species has been primarily found in stagnant water bodies, pools, and ponds within the province. Morphological analyses highlight the physical characteristics of *C. theileri*, while molecular methods, including DNA barcoding, confirm its accurate identification and genetic details. Its presence in Siirt offers valuable insights into the region's ecosystem dynamics and mosquito diversity, making these findings crucial for ecological research and the evaluation of health risks.

The other significant mosquito species identified in our study is *C. quinquefasciatus*. This species is primarily found in tropical and subtropical regions worldwide (Becker et al., 2010). Morphologically, *C. quinquefasciatus* exhibits somewhat light brown scutum scales that are relatively long and sparse. The subcostal vein typically intersects with the costa before the R_{2+3} fork, prealar scales are absent, and the basal bands of abdominal tergites range from whitish to cream in color. In the fourth instar larvae of this species, the siphon is generally widest in the middle and tapers more towards the apex compared to the base. The siphon width at the apex is approximately half the width of the base. Seta 1-III and 1-IV are single (Azari-Hamidian & Harbach, 2009; Harbach, 1988) (Figure 6). *Culex quinquefasciatus* generally prefers to breed in stagnant water bodies, wetlands, channels, and other water sources. It can also be found in urban and rural areas, particularly near human settlements. This species is more prevalent in warm and humid climates (Harbach, 2018). Furthermore, *C. quinquefasciatus* is known as a vector for microfilarial parasites (Benelli et al., 2017) and has been identified as a primary vector for the Saint Louis encephalitis virus and West Nile virus (Samy et al., 2016). This makes this species significant from a public health perspective. *Culex quinquefasciatus* has been detected in stagnant and polluted water bodies as well as irrigation pools in Siirt Province Morphological and molecular analyses conducted in Siirt have identified this species as a new record for the region.

Figure 6. Image of *Culex quiquefasciatus* larva under the microscope

Figure 7. Image of *Culex quiquefasciatus* larva under the microscope

Table 2. BLASTn results of the obtained sequences in the NCBI GenBank

No	NCBI Accession Number	Species	NCBI- BLAST	Similarity
1	PQ631176	*Culex theileri*	KF407830.1 -*Culex theileri*	%99.05
2	PQ631175	*Culex mimeticus*	MW961280.1-*Culex mimeticus*	%99.03
3	PQ631177	*Culex quinquefasciatus*	NC014574.1 -*Culex quinquefasciatus*	%95.62
4	PQ631174	*Anopheles superpictus*	MT993498.1 -*Anopheles superpictus*	%99.52

Finally, *A. superpictus* has been identified. This species, a Palearctic mosquito from the genus *Anopheles*, is commonly found across the Middle East, Mediterranean region, Africa, and parts of Asia (Hanafi-Bojd et al., 2018). *Anopheles superpictus* is a large, pale species characterized by broad yellow scales in the middle of the scutum. It lacks upper proepisternal setae, and there are one or two yellow spots at the base of the costa. Notably, there is no dark spot at the tip of the cubitus bifurcation, and the fringes at the wing tip are mostly yellow, except for a small dark area between R_2 and R_3. In the fourth instar larvae of this species, prothoracic seta 1 is small and slightly sclerotized with tubercular structures. Prothoracic setae 1 and 2 are not fused, and the base of the dorsal apotome is marked with dark spots (Azari-Hamidian & Harbach, 2009) (Figure 7). *Anopheles superpictus* particularly prefers to breed in wetlands and stagnant water bodies. Agricultural fields, channels, and irrigation systems are among the breeding sites for this species. Additionally, it is common in warm and humid climates (Harbach, 2018). *Anopheles superpictus* is known as a carrier of malaria parasites and plays a role in the transmission of *Plasmodium* species, making it a significant target for malaria control programs (Aytekin et al., 2009). In our study, *A. superpictus* was notably detected in wetlands, stagnant ponds, and irrigation systems. This finding provides crucial insights into the diversity of mosquito species in the region and is critical for assessing potential health risks.

The identification of mosquito species through classical taxonomy based on physical characteristics is often challenging and time-consuming. Furthermore, morphological traits exhibit minimal variation among species (Aung et al., 2023). In this context, the DNA barcoding method is beneficial in supporting these identifications, as it aids in accurately distinguishing between species. Genetic analyses performed on samples from the Culicidae family collected in Siirt Province involved the amplification of a 658 bp fragment of the mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene using PCR. The sequence data related to the species that underwent morphological identification were obtained through DNA barcoding and compared with other species in the database via BLASTn in the NCBI GenBank. The results of the comparisons are presented in Table 2.

Based on the results of the sequence analysis, four different mosquito species have been identified in Siirt Province. These include *C. theileri, C. mimeticus*, *C. quinquefasciatus* and *A. superpictus* . The PCR products for these four mosquito species were examined for quality and quantity through agarose gel electrophoresis, confirming that the size of each PCR product was 658 bp..

Genetic analyses utilizing a region approximately 650 base pairs in length from the *COI* gene region revealed the evolutionary relationships and genetic distances among these mosquito species. In the phylogenetic tree, *Chironomus kiiensis* (Diptera) was used as an outgroup to ensure the accuracy of the analyses. The genetic distance of this species from the other mosquito species served as a reference point for the rooting of the phylogenetic tree. The sequences from NCBI that showed the highest similarity in the BLAST comparison were visualized along with their accession numbers (Figure 8), allowing for an understanding of the closeness of the species to one another.

The phylogenetic tree illustrates the genetic relationships and evolutionary distances among the analyzed species, including *C. theileri*, *C. quinquefasciatus*, *C. mimeticus*, and *A. superpictus*, with *C. kiiensis* used as the outgroup. Close genetic relatedness is observed among the *Culex* species, while *Anopheles superpictus* occupies a more distant branch, indicating its genetic divergence from the *Culex* genus. Branch lengths represent genetic distances, while the bootstrap values, indicated in red, reflect the reliability of the respective branches.

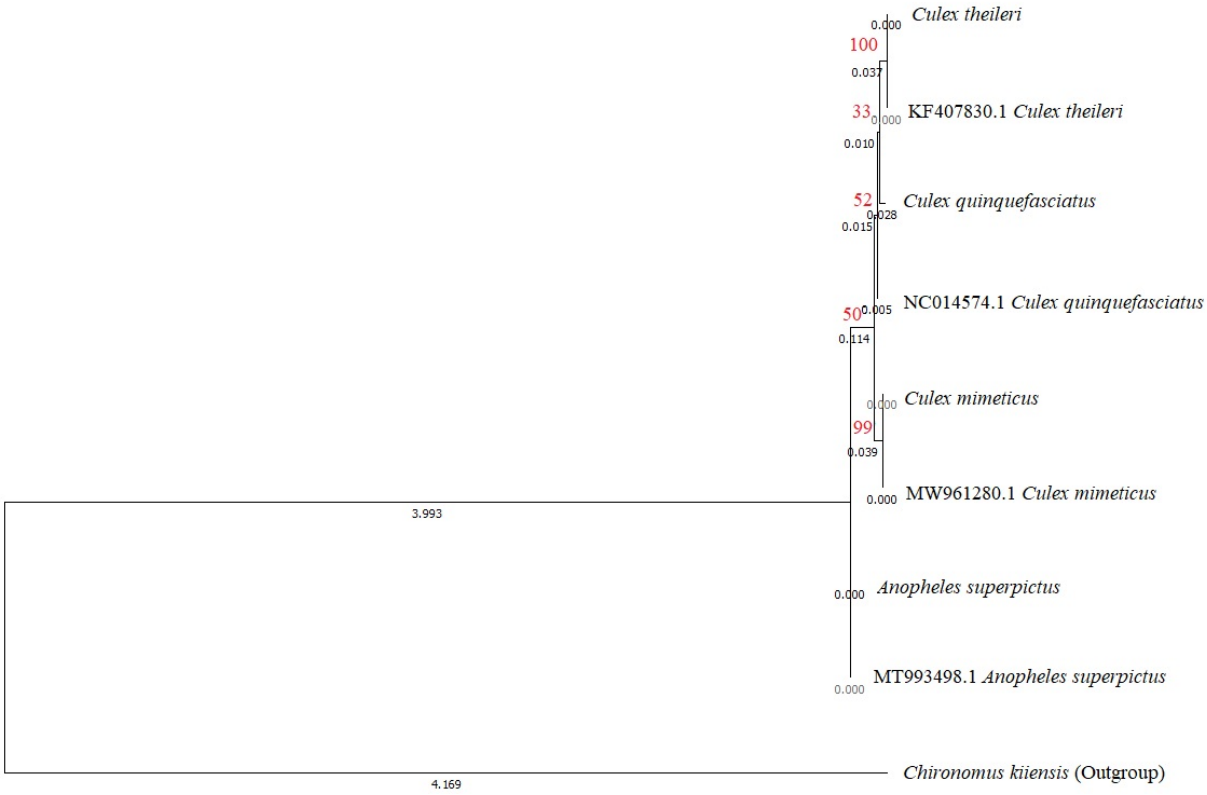

Figure 8. Phylogenetic relationships among mosquito species and closely related taxa (Maximum Likelihood).

For instance, the relationship between individuals of *C. theileri* is strongly supported with a bootstrap value of 100%, whereas the relationship for *C. quinquefasciatus* is less robust, with a bootstrap value of 52%. Additionally, the outgroup, *C. kiiensis*, demonstrates a more distant evolutionary relationship to the other species. The values presented as "0.000" in the figure denote the minimal genetic distance at specific nodes, suggesting either identical or nearly identical genetic sequences between those taxa. These values are indicative of high genetic similarity rather than complete absence of divergence. Overall, this tree highlights close evolutionary relationships within the *Culex* genus while emphasizing the distinct divergence of *A. superpictus*. The molecular results illustrate the genetic differences and evolutionary relationships among the mosquito species identified in Siirt Province. The phylogenetic tree indicates that species within the *Culex* genus are genetically closer to each other, while *A. superpictus* occupies a more genetically distant position within this group. Furthermore, the low genetic distance between *C. theileri* and *C. quinquefasciatus* supports the notion of an evolutionary relationship between these two species (Figure 8).

The findings of this study shed light on the genetic diversity and evolutionary relationships of mosquito populations in Siirt Province. Mosquitoes, particularly species belonging to the *Culex* and Anopheles genera, possess significant potential as disease vectors. The four species identified in our study (*C. theileri*, *C. mimeticus, C. quinquefasciatus,* and *A. superpictus*) pose risks to human health and contribute to ecosystem balance. These results are crucial for understanding regional population dynamics and strategically planning mosquito control efforts.

DNA barcoding is a widely recognized molecular biological approach due to its effectiveness and accuracy in identifying species across mammals, birds, reptiles, amphibians, fish, and arthropods (Chaiphongpachara et al. 2022). DNA barcoding relies on the amplification of a highly conserved and standardized short DNA region (approximately 400-800 base pairs using PCR for species-level taxonomy (Yang et al., 2018). In recent years, molecular techniques have gained increasing importance for the accurate and rapid identification of species. For this purpose, molecular techniques involving various genetic markers such as *COI*, *COII*, *Cyt b*, *ITS1*, and *ITS2* have been proposed as complementary tools to morphological species identification (Adeniran et al., 2021). The *COI* gene region employed in molecular analyses is a commonly preferred method for determining genetic differentiation among various insect species (Hebert et al., 2003). The utilization of the 658 bp segment of *COI* ensures that our study aligns with existing literature and facilitates international comparisons. The PCR products obtained, measuring 658 bp, confirm the successful amplification of the target region and accurate species identification. The *COI* gene used in our study is reported to be one of the most conserved mitochondrial genes, offering significant advantages for taxonomic studies (Roe & Sperling, 2007). In this context, numerous studies utilizing DNA barcoding with the *COI* gene have conducted identifications and phylogenetic analyses of mosquitoes (Adeniran et al., 2021; Chaiphongpachara et al., 2022; Daravath et al., 2015; Hernández-Triana et al., 2019).

The results of the phylogenetic analyses reveal the genetic relationships among *Culex* and *Anopheles* species, highlighting their evolutionary divergence. Notably, the

minimal genetic distance (0.000) between individuals of *C. theileri* indicates a high level of genetic similarity within this species. Similarly, the close relationship between *C. mimeticus* and *C. quinquefasciatus* (genetic distance of 0.039) suggests a shared evolutionary history, offering valuable insights into their potential ecological interactions and habitat overlap. Similarly, the position of *A. superpictus* on a more distant lineage compared to *Culex* species suggests that these two genera have followed different evolutionary pathways (Harbach, 2007).

Conclusion

In summary, this study provides valuable insights into the mosquito species in Siirt Province, documenting new records and verifying species identification through morphological and *COI* gene analyses. These findings enhance the existing knowledge on regional mosquito diversity and provide a solid baseline for future taxonomic and molecular studies. Documenting new records in this region contributes to a more comprehensive understanding of the biodiversity in Türkiye, supporting future research focused on regional species identification and distribution.

Declarations

Author Contribution Statement

Halil Dilmen: Project administration, supervision, conceptualization, methodology, review and editing, and molecular analysis.

Behcet İnal: Laboratory work, data collection, investigation, and molecular analysis.

Mehmet Salih Özgökçe: Data evaluation and analysis.

Mustafa Cemal Çiftçi: Field sampling, species identification, and investigation.

Hilmi Kara: Molecular analysis.

Sedriye Çatkın: Field sampling and data collection.

Meryem Özer Dilmen: Laboratory work and data collection.

Gülcihan Koyunçu: Field sampling and data collection.

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Conflict of Interest

No potential conflict of interest was reported by the author(s).

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