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Molecular Identification of Culex pipiens Linnaeus, (Diptera: Culicidae) in the Kurdistan Region of Iraq

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Abstract

THE present study aims to establish a phylogenetic tree among the sequenced mosquitoes and classify the *Culex* species discovered in the Kurdistan region of Iraq according to molecular biological evolution. They are using partial sequencing of the mitochondrion genome for the CO1 gene. This study investigates the evolutionary relationships and sequence comparisons of *Culex* species originating from different geographic regions. Compare these data points with analogous information from neighbouring nations available via the NCBI. The research was carried out from September 15, 2020, to November 20, 2021. A total of 291 adult female *Culex* were collected randomly from the four provinces. After conducting morphological analysis to identify the *Culex* mosquitoes, the complete genomic DNA of each mosquito was extracted. In the next step, specific species primers were used to increase the size of the DNA of the *COI* gene in the mitochondria. The outcomes of DNA sequencing have been submitted to GenBank to compare and contrast with analogous data from neighbouring countries that are accessible via the NCBI. In the Kurdistan region of Iraq, six species belonging to the genus *Culex* and one species from the genus *Ochlerotus* have been uploaded for the first time to GenBank. GenBank has given the following accession numbers for six species of the genus *Culex* and one species of the genus *Ochlerotus*: OQ028836 for *Culex pipiens*; OQ026450.1 for *Culex pipiens*; OP998245.1 for *Culex pipiens pipiens*; OP998245.1 for *Culex pipiens pipiens*; OR757439 for *Culex perixugus*; OR757439 for *Culex territans*; and OR740595 for *Ochlerotus caspius*.

Keywords: Mosquito, Culex species, Kurdistan, molecular biological evolution, GenBank.

Introduction

The common house mosquito, *Culex pipiens*, an insect that is well-known throughout the world for its role in this regard, can transmit serious diseases like avian malaria, yellow fever, and encephalitis. Information regarding evolutionary biology and phylogenetic analysis may be derived from knowledge of the mitochondrial genome [1]. Within the *Culex pipiens* mosquito complex, there are currently recognized taxa: *C. quinquefasciatus*, and *C. pipiens* form *pipiens*, *C. pipiens. form molestus*, many phylogenetic aspects within this complex have eluded resolution [2]. Mosquitoes are often classified at the species level by examining their exterior features. However, additional techniques for identification are frequently required especially when specimens are gathered in early developmental stages, maintained improperly, or when distinguishing across species groups is difficult. The mitochondrial Cytochrome Oxidase Subunit 1 (*COI*) gene is a valuable tool utilized for species identification based on morphology [3]. The hematophagous females of some species distribute a variety of illnesses that result in millions of deaths annually[4] Across the animal world, the molecular technique of mitochondrial Cytochrome Oxidase Subunit 1 (*COI*)-based DNA barcoding has been extensively used to distinguish several taxonomic Grouping [5], including mosquitoes, for example; [6, 7]. Mitochondrial markers are widely used because of their haploid inheritance pattern, lack of introns, limited exposure to recombination, and frequency (1000 copies per cell). Due to the availability of universal primers that allow for the recovery of the 5' end from the majority of animal phyla, if not all of them, *COI* is particularly well-liked [8] .To control vector-borne diseases, health authorities at the local or state levels conduct vector surveillance programs. As a result, the effectiveness of these programs

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depends on the availability of quick detection techniques [6]. So, the goal of this study was to identify and group the *Culex* mosquitoes in Iraq's Kurdistan region based on their molecular biological evolution by sequencing a part of their mitochondrial genome. In addition, from different geographical regions in four provinces of the Kurdistan region, sequence comparisons and evolutionary relationships among *Culex* species compare with similar data from neighboring countries available in the NCBI (National Center for Biotechnology Information).

Material and Methods

Field of study: This survey was carried out on September 15th, 2020, and continued until November 20th,

2021. During the period, immature stages were collected by the dipping method, while adult *Culex* by trap net were collected randomly in the four provinces of Kurdistan Region-Iraq. As shown in Figure.1 *Culex sp.* was sampled in randomized areas in four different locations in each Erbil and Duhok provinces and three locations in each Sulaymaniyah and Halabja provinces. The number of female *Culex* collected was 291 mosquitoes in the four provinces. There are one hundred thirty-five samples in Erbil, 82 in Duhok, 23 in Sulaymaniyah, and 52 in Halabja these locations, their Y (lat) and X (long), are shown in Table 1.

Morphological identification

The fourth instar larvae of Culex pipiens were gathered and stored in glass vials with lock covers,

immersed in 70% ethyl alcohol. The vials were appropriately labelled and dispatched to the Entomology Laboratory of the Plant Protection Department at the College of Agricultural Engineering Sciences, Salahaddin University in Erbil, Iraq.The larvae were identified based on the shape and length of the siphon [9]. Adult *Culex* was caught using net traps. Some females were chosen and put in 70% ethanol for identification. Adults were recognized using morphological traits such as (wing venation) Costa and Subcostal intersection with R2+3 furcation in *Culex pipiens* with the help of the taxonomic key [9, 10]. Under a dissecting microscope, the wing of the female adult siphon in the fourth instar larvae is placed on a slide, examined, and photographed with a Canon camera (PowerShot SX50 HS12.1 MP). The measured proportions of the body parts are provided in points on a microscope micrometer calibration ruler with a dissecting microscope. Other females were put in 96% ethanol for molecular study.

Molecular identification:

Extraction of genomic DNA: Genomic DNA was extracted from whole adult female mosquitoes, preserved in 96% ethanol using (a tissue genomic DNA the Extraction Mini kit) from animal cells, animal tissues, blood, and bacteria provided by (FAVORGEN Biotechnology Core).

Determination Quantity of DNA Concentration:

The concentration and purity of Nucleic Acids (DNA) from each sample were evaluated using a Nano-Drop spectrophotometer, with measurements reported in units of ng/µL. A solution comprised of 2 µL of DNA and 2 µL of elution buffer was used for analysis. DNA concentration readings were detected after a five-minute incubation at room temperature.
A micro-volume spectrophotometer is used for measuring the quality and concentration of extracted DNA for each sample). DNA purity was assessed based on the optical density ratio at 260/280 nm. The ratio within the reading is 1.8 ± 5 is a DNA-acceptable purity for samples. This work has been done at the Scientific Research Center of Salaheddin University-Erbil.

Amplification by Polymerase Chain Reaction (PCR):

PCR amplification reactions were carried out in a 25 µl volume reaction mix according to the following protocol: 15 µl of Master mix. For this study, a new species-specific primer (SHam-Dl Forward and Reverse) was created by Dr. Shamal A. Al-Muffti, Department of Biology, College of Sciences, University of Duhok, Kurdistan Region, Iraq (shamal.al-muffti@uod.ac), using the Primer3 website (Rozen and Skaletsky 1999) to define the specific region of DNA that will be amplified during the PCR process. 1 µL of forward primer (5′TTTGGGGCTTGAGCTGGAA**3′**), 1µL of reverse primer (**3′**AAGCTCCAGCATGAGCTGTT**5′**) shown in Table 2., 5 µL of nuclease-free water, and 3 µL of DNA template are shown in Table 3.

The PCR products were observed on a 1.5% agarose gel stain in Gel Red (Biotium, USA). Amplified DNA products were run on 1.5% TEB agarose gel. The gel was stained with a safe stain in 3 µL after the mixture was cooled. After the gel red was allowed to solidify, 3 µL of amplified DNA fragments, and 5 µL of molecular 100-bp loading marker ladder provided by the company were independently added to wells made by a special comb and loaded in the gel, which was run under 75 volts for 60 seconds. The gel was viewed under UV On a UV transilluminator, photographs were taken with a digital photographic camera [12]. The COI partial gene was sequenced as the desired gene. Utilizing the ABI Prism Terminator Sequencing Kit from (Applied Biosystem.

DNA Sequencing

The PCR products had been sent to (Sanger sequencing an automated DNA sequencer) at Macrogen Corporation / Korea. The nucleotide sequences of 7 different DNA samples (2 samples of *Culex pipiens pipiens*, two samples of *Culex pipiens*,1 sample of *Culex perixigus*, 1 sample of *Culex teritans*, and 1 sample of *Ochlerotus caspius*) were aligned with existing sequences of DNA available in the GenBank databases using the BLAST programs on the NCBI (National Center for Biotechnology Information) site. The comparison was done by the mitochondrial gene.

Sequence alignment and phylogenetic analysis

The seven samples of the PCR product of *CO1* partial genes have been sequenced by the ABI Prism Terminator Sequencing Kit (Applied Biosystems) at Macrogen Molecular Company of Korea. The chromatograms of the partial gene from the seven samples of PCR products of *CO1* partial genes have been modified, and the accuracy of the base calls has been verified using the Finch TV program software. The *CO1* partial gene sequences were subjected to a homology search using NCBI-BLAST (http:/ /blast.ncbi.nlm.nih.gov/). Sequence alignment was then conducted to compare and align the query sequences in this study with other biological sequences from various countries, using Bio Edit and MEGA v.11.0.13. [13]. All the sequences produced in this investigation were submitted to NCBI-GenBank for deposition. The phylogeny tree was formed using the neighbor-joining method.

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Results

Two hundred ninety-one female *C.pipiens* were collected from four provinces in the Kurdistan Region of Iraq (Table 4).

According to Table 5, which compares the four provinces, Erbil had the most *Culex s*pecimens, reaching 135 in total. The province of Sulaimaniyah has the fewest *Culex sp.*, with just 23, followed by Duhok with 82 and Halabja with 51. These results indicated that there are differences in the geographic distribution of *Culex species* in each of the four provinces, with the maximum number in Erbil province and the lowest number in Sulaimaniyah. These results are because in Erbil, the capital of Kurdistan Region Iraq, the rate of tourists and Travelers has increased nowadays and the number of migrating birds increased during the summer season. The nature of the environment in Erbil province's temperate climate was very comfortable for the *C. pipiens* life cycle. The results detected seven species (2 samples of *Culex pipiens pipiens*,2 samples of *Culex pipiens*,1 sample of *Culex perixigus*,1 sample of *Culex teritans*, and 1 sample of *Ochlerotus caspius*). The species-specific designed forward and designed species-specific reverse primers for the mitochondrial gene (*CO1*) Cytochrome Oxidase and their PCR product gave 362 bp, as demonstrated in Fig. 2.

Molecular Identification and Molecular Analysis

In the present study, the genomic DNA was extracted from insect samples (*C. pipiens*) (Tissue Genomic DNA Extraction Mini Kit) from animal cells, animal tissues, blood, and bacteria and provided by FAVORGEN Biotechnology Core. The extraction result was successful from the whole body of the adult stage of an insect. The DNA extraction protocol proved successful in achieving high-quality DNA. The isolated DNA was analyzed by electrophoresis on a 1% Agarose gel to evaluate its quality. The quality of the DNA samples was measured based on the presence of a single high molecular weight band in the gel image (Fig. 2).

Phylogenetic Analysis

Phylogenetic analysis is fundamental to understanding the evolutionary relationships between species. In this study, the genetic distances and branching patterns among various insect species are explored to elucidate their evolutionary history. The phylogenetic tree reveals distinct groupings among several insect species based on genetic differences. MEGA 11 programs of Phylogenetic analysis based on *COI* nucleotide sequences showed. The categorization of seven studied species of insects followed an expected pattern. Based on the analysis of sequence divergence similarity data and the generated phylogeny, it was shown that species within the same genera exhibited a high degree of closeness to each other.

The seven samples of insects were grouped in five clades with high similarity into the same genus and species and two clusters, as shown in Figure 4. Table 6 shows the identic number between species in the Kurdistan Region of Iraq and other species in neighboring countries. Figure 4. explains the phylogenetic relationships among six species of Genus *Culex* and one species of Genus *Ochlerotus* and relies on the nucleotide sequences of the partial mitochondrial genome. The maximum likelihood tree for mosquito species is shown in the five clades. Clade I; includes the *Culex quinqufaciatus* from Africa with accession number MT506038, *Culex pipiens* from Saudi Arabia with accession number MT199095.1; *C. pipiens* from Turkiye with accession number MK713990.1; *C. pipiens* from Slovenia with accession number OP715576.1; *C. pipiens. pipiens* in Erbil/ SHaqlawa/ Aquban with accession number OR715576.1. *C. pipiens pipiens* in Erbil / Shaqlawa /Aquban similar to *C. pipiens* from Solvenia the identic number 100%. This clade contains sibling species like *Culex pipiens pipiens* in Erbil / Shaqlawa / Aquban.

This indicates that the partial mitochondrion CO1 gene is a good marker for distinguishing sibling species of the *Culex pipiens* complex group, and this clade had a common ancestor. This clade makes a cluster with *C. pipiens* in Italy with accession number KM9226441; *C. pipiens* in Duhok/ Sumel with accession number OQ028836.1, similar to *Culex quiqufaciatus* from Africa, Identic number 97.48%, also similar to *Culex pipiens* in Saudi Arabia identic number 97.48%, and they have a common ancestor. Clade 2 includes *Culex pipiens* in Halabja/ Hawraman /Bayara with accession number OQ026450.1 similar to *Culex pipiens* in Turkey the identic number 100%; *Culex pipiens pipiens* in Halabja/ Chawg with accession number OP998245.1 with common ancestor similar to Solvenia in Europe. Identic number 96.77%, Clade 3 *Culex perexiguus in* Center Erbil /Shanader Park with accession number OR757439, similar to *Culex perexiguus* in Emarat with accession number MK170082.

They have a common ancestor identic number 98%; Clade 4 *Culex territans* in Duhok/Bardarash with accession number OQ026176 have a close relationship with *Culex territans* in Canada with accession number KR765113, Identic number 100% with a common ancestor; Clade 5 which includes *Ochlerotus caspius* in Erbil/ Shaqlawa/ Aquban is smaller than *Ochlerotus caspius* in Iran, with accession number MK962483, the identic number 100%. This clade forms cluster 2 with clade 4 and has a common ancestor. Clusters 1 and 2 share a common ancestor. As shown in figure 6. The presented phylogenetic tree showcases the genetic relationships and divergence patterns among several *Culex* species. The branch lengths denote the evolutionary distances between these species, providing insights into their evolutionary history and genetic relatedness. Further studies can utilize this information to delve deeper into the evolutionary dynamics and ecological implications of these insect populations.

Discussion

This study applied a scientific technique called DNA barcoding to differentiate between different species of *Culex* mosquitoes from fourteen locations in the Kurdistan Region. Iraq. DNA markers, such as mitochondrion (*COI*), are used to distinguish between the *C. pipiens* bioforms. DNA-based markers were targeted in the assay to differentiate the *C. pipiens* bioforms. The mitochondrial *COI* gene identified *Culex* mosquitoes at the species level; it served as a good marker for discriminating among *Culex* species due to its conserved sequence. The identification of *Culex* mosquito species in the Kurdistan area, Iraq, was achieved by analyzing the mitochondrial *COI* gene. This gene demonstrated its capacity to accurately categorise different species due to its conserved sequence area that is shared among several taxa [14]. As an example, the form of C. pipiens, *Culex pipiens* enters a state of diapause and only lays eggs after consuming a blood meal primarily from bird hosts. It is primarily found in terrestrial environments where it is able to freely participate in reproduction [15]. In the present study, the *CO1* gene can distinguish between sibling species like *Culex pipiens pipiens* in Erbil /Shaqlawa /Aquban and is also able to detect *Culex pipiens pipiens* in Halabja Center/ Chawg.This study is comparable to other studies [6, 16, 17] they were reported that except *C. pseudostigmatosoma* and *C. nigripalpus*, all morphologically identified species were separated by their COI DNA barcodes. This study does not agree with [18] who was reported that the *CO1* marker is not a reliable marker for differentiating between the two *C. pipiens* bio forms in Sweden. Mosquitoes belonging to the *Culex* genus are common in all climate types in our country. In this study ,it was found two species of *Culex pipiens pipiens,* one from Erbil/ Shaqlawa/Aquban and the other from *C.pipiens pipiens* from Halabja Center/Chawg. Mosquitoes of the *C. pipiens* complex include species named as *C. pipiens* form *molestus*, *C. pipiens* form *pipiens* and *C. quinquefasciatus.* Fortunately, the *COI* barcode gene was able to accurately identify these closely related species, even if their physical characteristics made it challenging to distinguish between them. A phylogenetic analysis is conducted on the *COI* molecular markers of *Culex pipiens* from various places, including Kurdistan Region-Iraq and other parts of the world. The sequencing results and subsequent BLAST analysis confirm that the samples utilized in this investigation belong to the species *Culex pipiens*. Furthermore, with minimal or no deviation, all specimens utilized in this investigation were remarkably indistinguishable from those discovered in Africa, Saudi Arabia, Turkey, Slovenia, Italy, Emarat, Canada, and Iran. Moreover, the seven groups were 96.48, 96.77, 97%, 99 %, and 100 % identical to those found in neighboring countries. The diversity observed in this group can be attributed to mutations occurring within the sequences, potentially resulting from differences in sample locations. This also implies that the genetic variants present in the samples may result in varying rates of infection, and further investigation is required to have a clearer understanding of this phenomenon. In the BLAST application of the NCBI genome database, the % similarity rates of these sibling species were almost the same so the CO1 gene was able to detect two samples of *Culex pipiens pipiens* one in Erbil Shaqlawa /Aquban, and the other in Halabja Center/Chawg. Two samples of *Culex pipiens* seen by CO1 gene one from Duhok/Sumel.The other from Halabja/ Hawraman/Bayara also CO1 gene was able to identify *Culex* on a species level, like *Culex perexiguus* in Erbil/shanader park this species new record in Kurdistan Region Iraq smiller to *Culex perexiguus* in Emarat, *Culex territans* in Duhok / Berdarash also this species new record in Kurdistan region Iraq similar to *Culex territans* in Canada, *Ochlerotus caspius* in Erbil/Shaqlawa/Aquban first record in Kurdistan Region IRAQ similar to *Ochlerotus caspius* in Iran. Based on morphological and molecular research, it was shown *that Culex pipiens, C. pipiens pipiens, Culex perexiguus, Culex territans*, and *Ochlerotus caspius* were the most prevalent mosquito species in the Kurdistan Region -Iraq throughout the sample period. These findings are similar to those documented by [19]. In the Western Cape, South Africa, and other regions, the Pipiens Complex was found to be the most prevalent species of mosquitoes when they were being studied [20]. The *COI* phylogeny successfully distinguished between C. pipiens and *C. pipiens pipiens*, which is the sibling species of *Culex pipiens.* In contrast to the findings of [21], They were unable to distinguish between *C. quinquefasciatus* and *C. pipiens* using their *COI* barcode sequence. Nevertheless, studies have indicated that the genus *Culex* has a significant level of diversity, with the majority of its species posing challenges in terms of morphological identification. Therefore, combining morphological analysis with *COI*-gene-based barcoding provides a dependable method for identifying species. In summary, this study enhances our understanding of the molecular evolution of *Culex pipiens*, which might potentially be applied in biotechnology to better manage the virulence of this vector. Furthermore, this study involved the generation of *CO1* barcodes for *Culex pipiens* specimens obtained from several parts of Kurdistan. This study aims to demonstrate the significance and simplicity of this technique in distinguishing between different species within the Kurdistan Region of Iraq. Hence, conducting a comprehensive examination and analysis using DNA barcoding on additional mosquito species will assist scientists in acquiring a deeper understanding of the identification and molecular evolution of mosquito species. Furthermore, it will offer the chance to enhance the surveillance of mosquito-borne diseases, specifically dengue, West Nile virus, and filarial parasites.

Conclusion

The study conveniently sampled *Culex* spp. during hot and wet seasons in the Kurdistan Region of Iraq. The data confirm the presence of many mosquito species in this region. The results are significant because they have identified possible carriers of avian malaria. This research emphasizes the necessity for enhanced control methods of blood-feeding dipteran vectors in the Kurdistan Region-Iraq. *Ochlerotus caspius* is considered a significant pest due to its role as a vector for pathogen transmission and its ability to induce illnesses. Accurate identification and dispersion of this mosquito vector are crucial for effectively controlling the diseases it causes. This work yielded extensive insights into the molecular identification of *Ochlerotus caspius* in Erbil/Shaqlawa/Aquban. Hence, it is imperative to implement both traditional and innovative management strategies, along with a meticulous monitoring system, to monitor and prevent the spread of arboviral infections in Erbil/Shaqlawa/Aquban. The DNA barcoding and maximum likelihood (ML) tree analysis indicate that all the species examined are classified as *Ochlerotus caspius*, with a significant resemblance to specimens of the same species found globally. Additionally, it implies that the risk of variation in infection rates of *Ochlerotus caspius* in Erbil/ Shaqlawa/ Aquban in Kurdistan Region-Iraq is reduced due to the significant genetic similarity among the specimens.

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Funding statement

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

All ethical approvals were obtained from the College of Agricultural Engineering, Salahuddin University, Erbil.

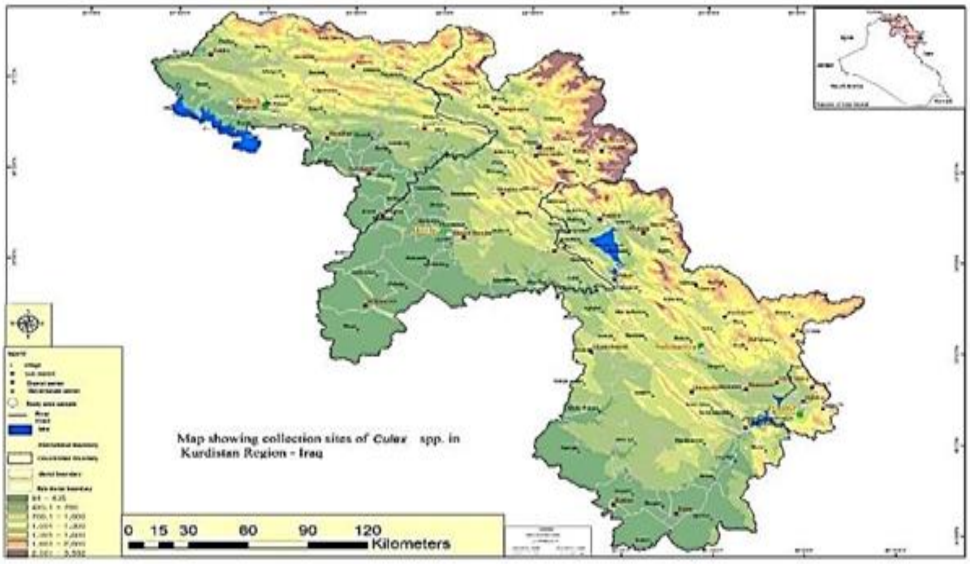

Fig. 1. The map of Kurdistan Region-Iraq shows cities where the samples of *Culex pipiens* mosquitoes were collected.

TABLE 2. Numbers of specimens of *Culex pipiens* collected from the different locations in the four provinces in Kurdistan Region-Iraq during 2020-2021.

No.	Province	Location	No. of *Culex* spp.	*Culex* spp.	Y (Lat)	X (long)
1	**Erbil**	Center of Erbil Shanader Park	21	*Culex. spp,*	36.17648	44.04073
		Soran-Berzewa	38	*Culex spp*	36.68887	44.54231
		Koysinjaq	38	*Culex spp*	36.09252	44.57872
		Shaqlawa-Aquban	38	*Culex spp*	36.41321	44.29752
2	**Duhok**	Sumel	17	*Culex spp*	36.88274	42.8029
		Duhok-center	35	*Culex spp*	36.82208	43.04981
		Bardarash	17	*Culex spp*	36.49714	43.55231
		Akre	13	*Culex spp*	36.7638	43.83633
3	**Sulaymaniyah**	Bakrajo-center	11	*Culex spp*	35.53897	45.44791
		Dokan	10	*Culex spp*	35.90719	44.99988
		Ranya	2	*Culex spp*	36.237554	40.91007
4	**Halabja**	Chawg center	21	*Culex spp*	35.22906	46.0059
		Bayara	20	*Culex spp*	35.31907	46.0059
		Tawela	10	*Culex spp*	35.2076	46.08124

Species-specific Designed primer for the amplification of the mitochondrion *CO1* gene

The presence of water, ensures that the reaction will take place in a liquid environment. Most people and laboratories prefer sterile, deionized water [11].

TABLE 2. Primer was used to confirm the identification of common house mosquitoes, *C.pipiens* pipiens.

Gene	Primer name	Primer Sequence 5' – 3'	Length	Ta (°C)	GC%	Reference
COI	Sham-Dl-F	TTTTGGGGCTTGAGCTGGAA	20	60	55.00	This study
	Sham-Dl-R	AAGCTCCAGCATGAGCTGTT				

TABLE 3. The chemical reactions for cycles of PCR amplification.

Master mix components	one Sample / µL
Master Mix	15 µl
Forward primer	1 µL (10 p mol / 1 µM)
Reverse primer	1 µL (10 p mol / 1 µM)
Nuclease Free Water	5 µl
DNA Template	3(25- 48) ng/µl
Total volume	25 µl

The PCR thermal protocol was a single cycle at (95 °C) for 05:00 min., followed by 30 cycles at (95 °C) for 30 seconds, 58°C for annealing for 30 seconds,72 °C for extension for 30 seconds, and a final elongation step at for 72 °C minutes in one cycle. As shown in Table 4.

TABLE 4. Thermocycler program for the COI gene

Steps	(COI) gene (°C)	Minutes: Second	Cycle (s)
Initial denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	58	00:30	
Extension	72	00:30	
Final extension	72	07:00	1

TABLE 5. Total numbers of specimens of *Culex* species collected from four provinces in Kurdistan Region-Iraq during 2020-2021

Provinces	No. of *Culex* species
Erbil	135
Duhok	82
Sulaymaniyah	23
Halabja	51
Total	291

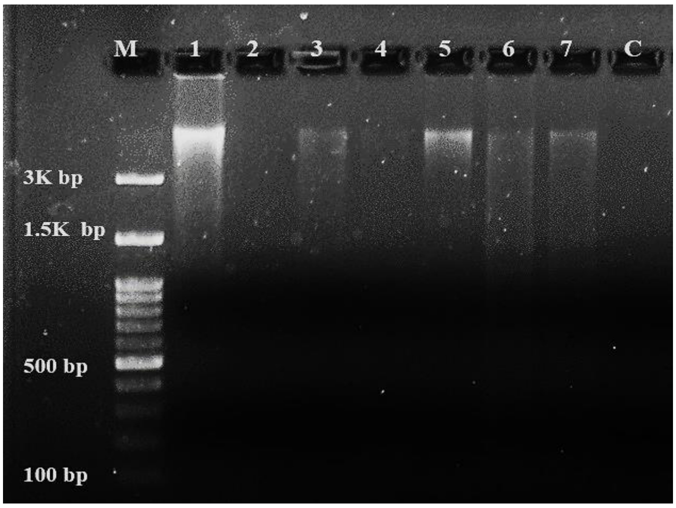

Fig. 2. An agarose gel electrophoresis was performed using a 1% concentration to display the genomic DNA that was extracted from the female adult. of *Culex. Pipiens.* The recording concentration was 25-48 ng/µ and purity was 1.8 ± 5 for each sample using a Nano-Drop spectrophotometer (ng/µ). The PCR product was subjected to electrophoresis and observed using a 1.5% Agarose gel. The amplification of the *CO1* region produced a uniform fragment size of up to 362 bp (Fig. 3).

TABLE 6. Percentage distribution of Culex species based on partial mitochondria (COI) gene according to blast in GenBank of NCBI.

Samples	Culex sp. Identified	Accession Numbers	Query Cover %	Identic Number %	Genbank Accession Number	Country
1-Duok/sumel	C. pipiens	OQ028836	**99** **99**	**97.48** **97.48**	**MT506038** **MT199095**	Saudy arabia Africa
2-Halabja/Hawraman/ Bayara	C. pipiens	OQ026450	**100**	**100**	**MK713990**	Turkiye
3-Halabja/Center/ Chawg	C.pipiens pipiens	OP998245	**98**	**96.77**	**KM922644**	Italy
4-Erbil/Shaqlawa/ Aquban	C.pipiens pipiens	OR733251	**100**	**100**	**OP715576**	Solvenia
5-Erbil center/ Shanader park	Culex perexiguus	OR757439	**100** **100**	**97** **97**	**MK170082**	Emarat
6- Duhok/ Bardarash	Culex territans	OQ026176	**100**	**100**	**KR765113**	Canada
7- Erbil/Shaqlawa/ Aquban	Ochlerotus caspius	OR740595	**100%**	**100%**	**962483MK**	Iran

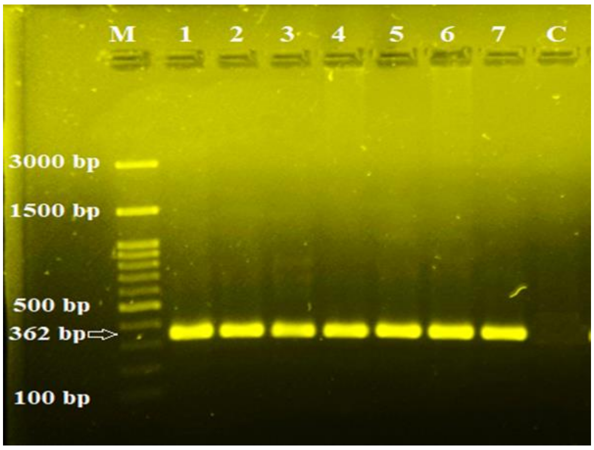

Fig. 3. Agarose gel electrophoresis 1.5% of each band the PCR product of the *CO1* gene, the first lane is Marker (molecular weight Marker is a 3000 bp ladder; each band 100bp difference), Samples one to seven consist of PCR products of 362 base pairs in size. The final lane (C) serves as a negative control and does not display any visible band. Six species of *Culex* and one species of *Ochlerotus* have been submitted to GenBank. As following had recorded by the mitochondria (*COI*) gene as 1-*Culex pipiens* in Duhok/ Sumel with the accession number OQ028836; 2-*Culex pipiens* in Halabja /Hawraman/Bayara with accession number OQ026450.1; 3-*Culex pipiens pipiens Halabja* center/Chawg with accession number OP998245.1; 4- *Culex pipiens pipiens* in Erbil/Shaqlawa/Aquban with accession number OR733251; 5- *Culex perexiguus in* Center Erbil /Shanader Park with accession number OR757439; 6 – *Culex territans* in Duhok/Bardarash with accession number OQ026176 ;7- *Ochlerotus caspius* in Erbil/Shaqlawa/Aquban with accession number OR740595.As shown in Table 6. The seven recorded species of mosquitoes were aligned in the phylogenetic tree in Figure 4.

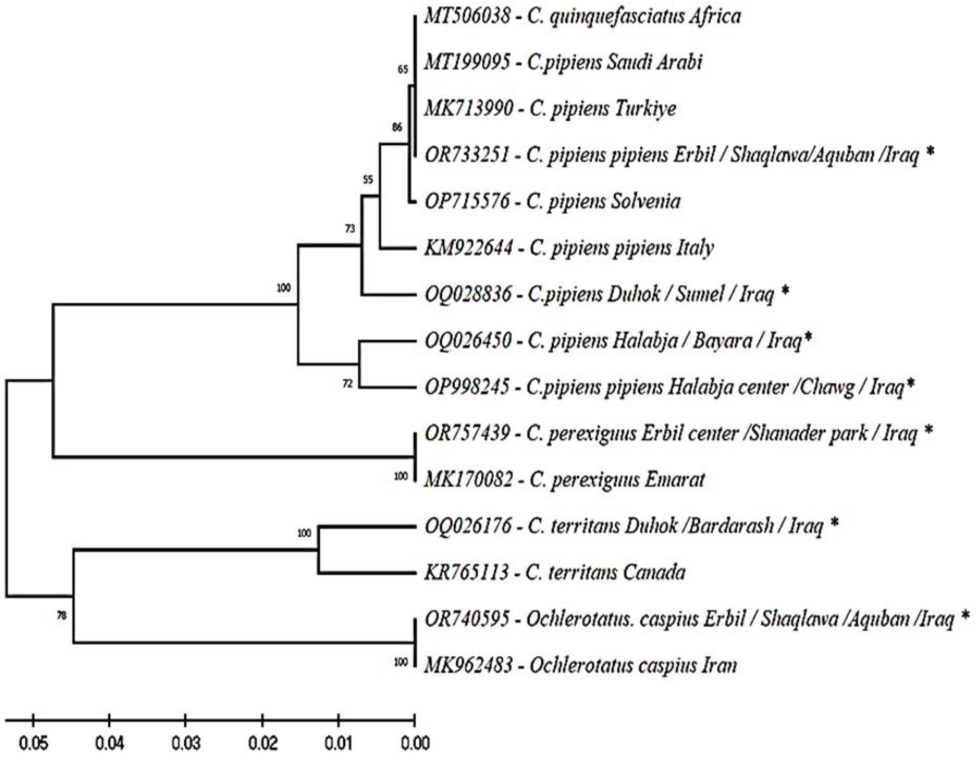

Fig.4. The phylogenetic positioning of *Culex pipens* in the seven samples from Iraq's Kurdistan region was determined using the Maximum Likelihood method based on the Tamura-Nei model in Mega 11 software. The analysis included bootstrap analysis and compared the *CO1* partial gene sequences of the samples with similar sequences available in GenBank.

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التعريف الجزيئي لبعوض Culex pipiens Linnaeus (Diptera: Culicidae) في منطقة كردستان العراق

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الملخص

هدفت الدراسة الحالية إلى إنشاء شجرة تطورية بين البعوض المسلسل وتصنيف أنواع البعوض من نوع Culex المكتشفة في منطقة كردستان العراق وفقًا للتطور البيولوجي الجزيئي. يتم استخدام التسلسل الجزئي لجينوم الميتوكوندريا لجين CO1. تبحث هذه الدراسة في العلاقات التطورية ومقارنات التسلسل لأنواع البعوض Culex التي تنشأ من مناطق جغرافية مختلفة. تُقارن هذه البيانات مع المعلومات المماثلة من الدول المجاورة المتاحة عبر NCBI. أُجريت الأبحاث من ١٥ سبتمبر ٢٠٢٠ إلى ٢٠ نوفمبر ٢٠٢١. تم جمع ما مجموعه ٢٩١ من إناث البعوض البالغين من نوع Culex بشكل عشوائي من المحافظات الأربع. بعد إجراء تحليل مورفولوجي لتحديد بعوض Culex، تم استخراج الحمض النووي الجينومي الكامل لكل بعوضة. في الخطوة التالية، تم استخدام برايمرات محددة لزيادة حجم الحمض النووي لجين COI في الميتوكوندريا. تم تقديم نتائج تسلسل الحمض النووي إلى GenBank للمقارنة مع البيانات المماثلة من الدول المجاورة المتاحة عبر NCBI. في منطقة كردستان العراق، تم تحميل ستة أنواع تنتمي إلى جنس Culex ونوع واحد من جنس Ochlerotus لأول مرة على GenBank. وقد أعطى GenBank أرقام الوصول التالية لستة أنواع من جنس Culex ونوع واحد من جنس Ochlerotus: OQ028836 لـ Culex pipiens ، OQ026450.1 لـ Culex pipiens ، OP998245.1 لـ Culex pipiens pipiens؛ OP998245.1 لـ Culex pipiens pipiens؛ OR757439 لـ Culex perixugus ، OR757439 لـ Culex territans و OR740595 لـ Ochlerotus caspius.

الكلمات الدالة: البعوض، نوع Culex كردستان، التطور البيولوجي الجزيئي، بنك الجينات.