Research Article

Culex (Diptera: Culicidae) Mosquitoes in Jazan Region, Saudi Arabia, and Their Molecular Identification

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Morphological characteristics have been the gold standard method to identify mosquito species. However, morphological identification has many limitations including lack of expertise and damaging of external characters due to improper specimen handling. Therefore, we used the polymerase chain reaction technique (PCR) as an integrated tool to identify Culex mosquito species to establish a more precise and reliable identification system related to their spatial distribution in Jazan region. We identified Culex mosquito species and subspecies using taxonomic keys, and then we used the polymerase chain reaction technique (PCR) as an integrated tool to confirm and refine the list of Culex mosquito species in the region. Phylogenetic trees were constructed for the identified species, and their distinctive clustering was compared with their reference’s species in the GenBank. We identified 7026 adult Culex mosquitoes belonging to 4 species. Culex tritaeniorhynchus was the predominant species (45%), followed by Cx. quinquefasciatus (32%), then Culex sitiens (20%), and Cx. pipiens (3%). The most infested areas by Culex in the region were Gizan and Sabya. The PCR achieved 100% success in identifying the four Culex mosquito species. We also report the molecular identification of Cx. quinquefasciatus and Cx. pipiens species for the first time in Jazan region while the molecular identification of Cx. tritaeniorhynchus and Cx. sitiens was reported for the first time in Jazan region and the whole Saudi Arabia. This study utilized for the first time PCR to identify Culex mosquito species in Jazan region. The PCR is a complementary and integrated taxonomy-based identification tool for mosquito species. This integration has the capacity to promote and enhance vector surveillance and control programs, as well as defining the genetic diversity of species in the region.

1. Introduction

Mosquito-borne diseases, i.e., malaria [1, 2], filariasis [3], Rift Valley fever [4, 5], West Nile virus [6], dengue, and others [7, 8], pose potential public health threats in Saudi Arabia. Several authors have investigated the distribution of mosquito species in Saudi Arabia [1–16]. Most of these studies reported the predominance of Culex mosquitoes over other species. Within Culex species reported by these studies, Culex quinquefasciatus, Culex tritaeniorhynchus, and Cx. pipiens were the most dominant Culex mosquitoes in Saudi Arabia and Jazan region.

The abundance and distribution of mosquito fauna are influenced by many factors including, but not limited to, host availability, climatic conditions, especially rain and temperature, human mobility and activities, and land cover [17]. These factors have a potential impact on the vectorial capacity of mosquitoes for disease transmission.

The accurate identification of mosquito vector species and knowledge of their biology, ecology, and geographical distribution are considered important factors for surveillance and control of vectors and mosquito-borne diseases [18].

Morphological identification is the gold standard and the conventional method to identify mosquito species depending on their external characters [19]. However, morphological identification is highly time consuming and requires experienced taxonomists.
Furthermore, improper handling of specimen damage is often occurred to some important characteristics such as bristles and scales leads to incomplete identification. Additionally, the similarity of shared morphological features by species complexes’ members makes identification based on taxonomic keys alone a difficult task [20].

Polymerase chain reaction (PCR) is becoming a popular technique for mosquitoes identification based on their DNA sequencing and the fact that every species has its own genetic identity [19].

Molecular techniques to differentiate between Cx. pipiens complex or and other Culex species, or to differentiate between the Cx. pipiens complex biotypes, are based on gel electrophoretic analysis of certain DNA fragments amplified by PCR [21]. PCR assay identifies Cx. pipiens, Cx. quinquefasciatus, and their hybrids based on the nucleotide sequence differences in the acetylcholinesterase gene Ace2 [22].

The aim of this study was to determine fauna of adult Culex mosquitoes (Diptera: Culicidae) and to produce precise and refine records of their species and their distribution in Jazan region based on morphological and genomic (molecular) identification. To the best of our knowledge, this is the first study in Jazan region to use molecular characteristics to identify Culex species and their genetic diversity.

2. Materials and Methods

2.1. Study Area. Jazan area is about 22,000 km² and with 1.6 million population, lies between 16°–12 and 18°–25, latitude north, and located in the subtropical zone, southwestern of Saudi Arabia, with a coastal boundary of 250 km along the Red Sea and a 120 km border with the Republic of Yemen (Figure 1). This region includes over 3000 villages scattered along the area and about 100 islands located in the Red Sea, including the Farasan Islands. It is surrounded by the Red Sea from the west and by Arabic Republic of Yemen from the south and east and Asir region from the north ([23]; GASTAT 2017: https://www.stats.gov.sa/en/5655).

2.2. Mosquito Collection. CDC miniature light traps were used for the adult mosquitoes’ collection from different parts of Jazan region from February 2018 to December 2019 (Table 1). Ten light traps were installed once per month in each of the houses, animals’ shelters, wild vegetation, near wadies, sewerage plants, dams, and ponds from 1800 to 0600 hr.

For outdoor collections, a 2 kilogram block of dry ice (CO2) was wrapped in a Hessian bag above the trap. To minimize the mortality of the collected mosquitoes due to desiccation, damp cotton pads were kept in the collection cups. Collected mosquitoes were brought to the insectary of the Saudi Center for Disease Prevention and Control in Giza city for morphological and genetic identification.

2.3. Mosquito Morphological Identification. Female Culex pipens and Culex quinquefasciatus mosquitoes were morphologically identified and differentiated by using wing measurements of intersection of costa, subcosta, and bifurcation of R2 + 3 veins, as described by some taxonomic pictorial keys [24–26].

For the Females of Culex tritaeniorhynchus and Culex sitiens, relevant pictorial keys were used [24, 27, 28].

2.4. Molecular Identification of Culex Species. Previously identified Culex mosquitoes of individual species by morphometric methods were selected according to their location of capture. DNA was extracted from the stored homogenate using GeneJET Genomic DNA Purification Kit (Thermo Scientific) following the manufacturer’s recommendations: For every individual mosquito, legs were homogenized in a mortar and pestle (mini borosilicate glass chamber length 60 mm/pestle diameter 9.0 mm, 3.0 Ml, Fisherbrand) in 150 μL of digestion solution and then the homogenate was transferred to 1.5 tubes. Twenty microliters of Proteinase K Solution was added and mixed thoroughly by vortexing to obtain a uniform suspension and incubated in the thermo shaker for 30 minutes at 56°C, 1300 rpm. Twenty microliters of RNase A Solution which was added and mixed by vortexing and incubated for 10 min at room temperature, then 200 μL of lysis solution was added and mixed thoroughly by vortexing for 15 s until a homogeneous mixture is obtained. After adding 400 μL of 50% ethanol and mixed by vortexing the prepared lystate was transferred to a GeneJET Genomic DNA Purification Column inserted in a collection tube and centrifuged for 1 min at 8000 rpm. The GeneJET Genomic DNA Purification Column was placed into a new 2 mL collection tube and 500 μL of wash buffer 1 was added and centrifuged for 1 min at 8000 RPM, then the column was placed into a new collection tube and 500 μL of wash buffer 2 was added and centrifuged for 3 min at 13,000 rpm. DNA Purification Column was transferred to a sterile 1.5 mL microcentrifuge tube, and 200 μL of elution buffer was added to the center of the column membrane to elute genomic DNA. Incubated for 2 min at room temperature and centrifuged for 1 min at 8000 rpm.

The molecular identification of Culex species was performed by polymerase chain reaction technique (PCR) following the described procedure and using the primers provided in (Table 2) [22, 29].

The PCR was carried out in a total volume of 50 μL using Dream Tag Green Master Mix (Thermo Scientific) containing 25 μL master mix ready to use, 1 μL (10 pmol) of each reverse and species-specific forward primers, 5 μL of DNA template, and 18 μL nuclease-free water. The mix was subjected to initial denaturation at 94°C for 3 minutes, 30 cycles of denaturation (94°C, 60 s), primer annealing (51°C), primer extension (72°C, 60 s), and final extension for 5 minutes. In each run, negative and positive controls were included. The PCR products amplifications were analyzed by gel electrophoresis (1.5 agarose in Tris-Acetate EDTA buffer) staining with ethidium bromide. The visualization was carried out using Gel Doc XR Imaging System (Bio-Rad).

Universal primers [30], LCO1490 and HCO2198 (Table 2), were used for the PCR amplification of a 710-bp fragment of the mitochondrial cytochrome oxidase subunit I
gene (COI) for the samples not identified by the species-specific primers follow the same previous PCR procedure.

2.5. Sequencing. Purification and standard sequencing for PCR products were performed by Macrogen Company (Seoul, Korea). Sequencing reactions were performed in an ABI PRISM® 3730XL Analyzer (96 capillary type) using an ABI PRISM® BigDye™ Terminator Cycle Sequencing Kit with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems), following the protocols supplied by the manufacturer. Single-pass sequencing was performed on each template using LCO1490 primer. Gel elution was performed using MG Gel Extraction SV (MD007) kit (MGmed), following the protocols supplied by the manufacturer.

The sequences were searched for sequence similarity through Basic local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/) [31] and compared to reference sequences of Culex species detected in BLAST and downloaded from GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

Evolutionary relationship of taxa tree was constructed for each of the Culex species using the Molecular Evolutionary Genetic Analysis (MEGA 5 software).

3. Results and Discussion

Results of the identification and counts of Culex species mosquitoes are shown in Table 1. Seven thousand twenty-six adult Culex mosquitoes were collected from 25 different areas of Jazan region during 2018 and 2019 (Figure 1).

Clearly, the number of mosquito specimens was relatively low compared to the period of study (around 2 years). This is may be attributed to the fact that effective and extensive vector control activities were taken place during the last 5 years in the region to combat malaria, dengue, and rift valley, the most prevalent vector-borne diseases in the region.

In this study, four Culex species were present in the region, namely; Culex tritaeniorhynchus, Cx. quinquefasciatus, Cx. sitiens, and Cx. pipiens. Their respective percentages of occurrence were 45%, 32%, 20%, and 3%.

Culex tritaeniorhynchus was the most abundant species encountered in 23 areas out of 25, followed by Cx. quinquefasciatus and Cx. sitiens which occurred in 14 areas, and Cx. pipiens in 4 areas.

These results are in accordance with the findings of Al Ahmad et al. [15] who indicated that Culex tritaeniorhynchus is a common widespread species in Saudi Arabia and has
been recorded from Jazan and other 14 provinces out of 15. Similar results were also reported by Alsheikhetal. [32], who found Cx. tritaeniorhynchus to be the most predominant species in the Red Sea coastal plain of Jazan region, followed by Cx. quinquefasciatus.

The findings are also in line with those of AlAhmadetal. and Bakretal. [14, 33] who reported the same four Culex species from Jazan region. Culex tritaeniorhynchus, Cx. quinquefasciatus, and Cx. sitiens have also been reported from different parts of Jazan region with the predominance of Culex tritaeniorhynchus over other Culex species [34].

In the present study, the most infested areas by Culex mosquitoes in Jazan region were Gizan (2160 specimens), Sabya (1267), Baish (753), and Aboareesh (429) (Table 1). This may be due to the nature of those areas, which are highly populated and relatively flat and permit for the formation of small stagnant water collections following rainfall and/or water pipe leakage in their urban areas [35]. The prevalence of the Culex species in those areas could be attributed also to their wide suitability to different breeding sites and variable extreme climatic factors prevailing there [11].

Three of the Culex species reported in the present study are considered important disease vectors, namely Culex tritaeniorhynchus, Cx. quinquefasciatus, and Cx. pipiens.

Characterizations of dominant mosquito vectors include well adaptation to a wide range of climatic conditions and habitats, high anthropophilic propensity, and variable adult resting behaviour [36].

Mosquito fauna and their distribution in Saudi Arabia had been widely investigated by many authors. For example, forty-nine mosquito species belonging to seven genera from

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**Table 1: Culex species in Jazan region.**

| Area                  | Cx. tritaeniorhynchus | Cx. quinquefasciatus | Cx. pipiens | Cx. sitiens | Total |
|-----------------------|------------------------|----------------------|-------------|-------------|-------|
| Gizan                 | 750                    | 904                  | 173         | 333         | 2160  |
| Al-shegairy           | 243                    | 102                  | 0           | 24          | 369   |
| Ahad Almasarha        | 0                      | 0                    | 0           | 29          | 29    |
| Sabya (Qaem jaaferi)  | 817                    | 0                    | 0           | 450         | 1267  |
| Sabya (Wadi sabya)    | 37                     | 0                    | 0           | 3           | 40    |
| Sam tah               | 0                      | 235                  | 7           | 0           | 242   |
| Damad                 | 53                     | 0                    | 0           | 204         | 257   |
| Baish                 | 406                    | 208                  | 25          | 114         | 753   |
| Aboareesh             | 119                    | 310                  | 0           | 0           | 429   |
| Al-tawal              | 13                     | 0                    | 0           | 65          | 78    |
| Al-mad haya           | 229                    | 0                    | 0           | 0           | 229   |
| Al-dar               | 67                     | 0                    | 0           | 0           | 67    |
| Al-ariddah (Batin asar)| 48                    | 93                   | 0           | 2           | 143   |
| Haroob                | 3                      | 61                   | 0           | 0           | 64    |
| Al-shegaig            | 84                     | 0                    | 0           | 0           | 84    |
| Haroob (Wadi wasa)    | 15                     | 0                    | 0           | 0           | 15    |
| Faifa                 | 96                     | 1                    | 0           | 0           | 97    |
| Al-golf               | 61                     | 32                   | 13          | 84          | 190   |
| Al-dhabia             | 0                      | 54                   | 0           | 2           | 56    |
| Al-horath             | 18                     | 0                    | 0           | 61          | 79    |
| Al-sehi               | 0                      | 109                  | 0           | 0           | 109   |
| Al-seidabi            | 16                     | 15                   | 0           | 11          | 42    |
| Al-seidabi (Wadi qessi)| 25                    | 0                    | 0           | 9           | 34    |
| Aiban                 | 8                      | 18                   | 0           | 0           | 26    |
| Belgazi               | 12                     | 103                  | 0           | 0           | 115   |
| Total                 | 3144                   | 2266                 | 218         | 1398        | 7026  |
| %                     | 45                     | 32                   | 3           | 20          |       |

**Table 2: Primers used for molecular identification of Culex species.**

| Culex species           | Consensus CPI6 R         | Culex pipiens complex PQIO F | Cx. restuans R6 F | Cx. salinarus S20 F | Cx. nigripalpus N901 F | Cx. nigripalpus NRI080 R | Cx. quinquefasciatus FCQ | Cx. quinquefasciatus RCQ | COI (LCO1490) | COI (HC02198) |
|-------------------------|--------------------------|-------------------------------|-------------------|---------------------|-----------------------|------------------------|---------------------------|--------------------------|----------------|---------------|
|                         | GCGGGTACCATGCTTTAATTTAGGGTA | CCTATGTCGCGGCTATACTA 698 bp   | CCAAAACCGGTACC7A 506 bp | TGAGAATACATACCTGCT 175 bp | ATACCCATGCGAAAGCATAC 404 bp | GTACCGGACCACAGCTT      | GGTCAACAAATCATAAGATATGG 740 bp | TAAACTTCCAGGGTGACAAAAATCA |
1956 to 2017 (18 Anophelines and 31 Culicines) have been listed [37].

Out of the 31 Culicines, 19 species were belonging to the genus *Culex* Linnaeus (1758): one species under the subgenus *Barraudius* Edwards, 1921, *Cx. (Barraudius) pusillius*; one species under subgenus *Culiciomyia* Theobald, 1907, *Cx. (Culiciomyia) nebulosus*; one species under subgenus *Oculeomyia* Theobald, 1907, *Cx. (Oculeomyia) bitaeniorychynchus*; two species under subgenus *Maillotia* Theobald, 1907, *Cx. (Maillotia) arbieeni* and *Cx. (Maillotia) salisburiensis*; and fourteen species under subgenus *Culex* Linnaeus, 1758: *Cx. (Culex) decens*, *Cx. (Culex) duttonii*, *Cx. (Culex) laticinctus*, *Cx. (Culex) mattinglyi*, *Cx. (Culex) mimeticus*, *Cx. (Culex) perexiguus*, *Cx. (Culex) pipiens*, *Cx. (Culex) quinquefasciatus*, *Cx. (Culex) simpsoni*, *Cx. (Culex) sinaiticus*, *Cx. (Culex) sitiens*, *Cx. (Culex) tritaeniorhynchus*, and *Cx. (Culex) univittatus*.

Likewise, 7 species of *Culex* had also been identified in Jazan region: (1) *Cx. pipiens* from Giza City, Alariddah, Baish, Ahad Almasarha, Samtah, Eldarab, Harooob, Bani Malik, and Farasan Islands; (2) *Cx. quinquefasciatus* from Giza City, Alariddah, Baish, Ahad Almasarha and Farasan Islands; (3) *Cx. tritaeniorhynchus* from Giza City, Alariddah, Baish, Ahad almasarha, Samtah, Eldarab, Harooob, and Bani Malik; (4) *Cx. sitiens* from Giza City, Alariddah, Baish, Ahad almasarha, Samtah, Wadi Jazan, and Farasan Islands; (5) *Cx. sinaiticus*; (6) *Cx. decens*; (7) *Cx. bitaeniorychynchus* [33].

On the other hand, another 10 species have been identified from different areas of Jazan region including *Cx. tritaeniorhynchus*, *Cx. laticinctus*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. sinaiticus*, *Cx. simpsoni*, *Cx. torrentium*, *Cx. sitiens*, *Cx. univittatus*, and *Cx. tigripes* [14].

Similarly, seven species were recorded from different parts of Jazan region including *Cx. tritaeniorhynchus*, *Cx. quinquefasciatus*, *Cx. sinaiticus*, *Cx. sitiens*, *Cx. duttonii*, *Cx. Arbieeni*, and *Cx. mimeticus* [34].

*Cx. (Culex) perexiguus* Theobald, 1903, and *Cx. pusillius* (Macquart) have been reported from the region [24, 38].

Consequently, 18 *Cx* species so far have been reported from Jazan region, namely: *Cx. tritaeniorhynchus*, *Cx. laticinctus*, *Cx. pipiens*, *Cx. quinquefasciatus*, *Cx. sinaiticus*, *Cx. univittatus*, *Cx. (Culex) perexiguus*, *Cx. pusillius*, *Cx. simpsoni*, *Cx. torrentium*, *Cx. sitiens*, *Cx. decens*, *Cx. bitaeniorychynchus*, *Cx. theleri*, *Cx. tigripes*, *Cx. duttonii*, *Cx. arbieeni*, and *Cx. mimeticus* [34].

*Culex* mosquitoes are proven or suspected vectors of *W. bancrofti*, vector of avian malaria, western equine encephalomyelitis, and St. Louis encephalitis. It was implicated as a vector of dog heartworm [45, 47].

The species is a primary vector of *Wuchereria bancrofti*, vector of human malaria worldwide including the Middle East countries, have been reported from the south-western districts of Saudi Arabia [6].

### 3.1. *Cx. quinquefasciatus* (Figure 2).

 Larvae of *Cx. quinquefasciatus* breed in water bodies with a high degree of organic pollution and close to human habitats. Females are nocturnal and enter houses and bite the man in preference to other mammals [45].

*Cx. quinquefasciatus* was found breeding all year round with peaks during winter in Taif, Jeddah, and Tabuk of Saudi Arabia [46]. In Jazan region, this species was collected from rocky pits and streams. It was found together with *Anopheles arabiensis* and *Cx. tritaeniorhynchus* at the ratio of 1:4:1:3 [34].

The species is a primary vector of *Wuchereria bancrofti*, vector of avian malaria, western equine encephalomyelitis, and St. Louis encephalitis. It was implicated as a vector of dog heartworm [45, 47].

*Cx. quinquefasciatus* and *Cx. pipiens*, which are the main vectors of bancroftian filariasis, *Wuchereria bancrofti* worldwide including the Middle East countries, have been reported from the south-western districts of Saudi Arabia [6].

### 3.2. *Cx. pipiens* (Figure 3).

 Larvae of *Cx. pipiens* breed in various places ranging from highly polluted sewage and cesspits to clear pools and containers. It breeds in stagnant water in shaded or unshaded habitats. Females bite the man outdoors and indoors [41].

*Cx. pipiens* larvae were reported all year round in Taif, Jeddah, and Tabuk. The peak was at autumn while high density was in summer when temperature is high [46].

*Cx. pipiens* is a primary vector of periodic Bancroftian filariasis. It has been naturally infected with West Nile and Sindbis viruses in Israel, while it was infected with Rift Valley Fever virus and West Nile virus in Egypt [41].

*W. bancrofti* has been identified within foreign workers from five South-East Asian countries in Abha, southwest of the Saudi Arabia. *Cx. pipiens* may act as a potential vector of *Bancroftian filariasis* in Saudi Arabia [48].

*Culex pipiens* complex has occurred in different climatic and habitat zones, particularly temperate and temperate-humid regions of the world [44].

It is always difficult and problematic to differentiate morphologically between adults of *Cx. quinquefasciatus* and *Cx. pipiens* especially females. Nonetheless, taxonomists used to differentiate between the two species by wing veinations where the intersection of the vein subcosta with costa is before the level of furcation of R1+2 in the case of *Cx.*
Culex quinquefasciatus (Figure 2), while this intersection is at or beyond the level of furcation of $R_{1+2}$ in the case of Cx. pipiens (Figure 3) [24]. However, this is readily achieved by molecular characteristics.

Molecular identification of both Cx. pipiens (Figures 4 and 5) and Cx. quinquefasciatus (Figures 6 and 7) confirmed their initial morphological identification. Phylogenetic trees were constructed for Cx. pipiens and Cx. quinquefasciatus, and their distinctive clustering was compared with their reference’s species in the GenBank as shown in Figures 5 and 7. It is worthy to mention that the molecular characteristics of Cx. pipiens when compared with the records of the gene bank showed its similarity to those of Egypt, Kenya (100%) and very close to those of Iran (99.85%) (Table 3). While the molecular identification revealed one species of Cx. pipiens that is similar to the Turkish species (100%), and one subspecies of Cx. pipiens quinquefasciatus (Q primers 500 bp) is similar to the Sri Lankan subspecies (100%) (Table 3).

In Baljurashi Province of Saudi Arabia, the results of molecular identification of Cx. pipiens using PCR technique revealed that the Cx. pipiens cf-3 strain was the accurate definition after initial morphological identification of the species [49]. Likewise, the molecular characteristics of Cx. quinquefasciatus samples from Yanbu Province of Saudi Arabia indicated that they were very close to those found in Pakistan, Brazil, and India (GenBank references; KF406862.1, MK575480.1, MH538709.1, and MH538707.1.) [50].

Culex sitiens (Figure 8(a)). The main morphological features found in Cx. tritaeniorhynchus are the presence of a median pale ring in the proboscis that extends proximally to the ventral surface of the proboscis (Figure 9(b)), and the fore and midfemora are entirely black (Figure 10(b)), whereas the furcation of $R_{2+3}$ is proximal to the furcation of $M_{1+2}$ (Figure 11(b)).

Larvae of Cx. tritaeniorhynchus are found in many habitats that are sunlit and contain vegetation like the temporary, semi-permanent, and permanent ground water habitats, low-salinity tidal marshes, streams, ground pools, and swamps. Females feed primarily on pigs and cattle, but in their absence will feed on men [27]. It has been reported that larvae of Cx. tritaeniorhynchus preferred aquatic habitats with wet muddy substrate and low total dissolved salts (TDS). Those areas are found in eastern Sarwat Mountain range near the cities in Ahd almasarha, Eleidabi, Sabya, Al-Aridjah, and Abuareesh [51].

Cx. tritaeniorhynchus was found in all types of breeding sites in the Red Sea coastal plain of Jazan region (man-made pools, especially turbid ones, rain pools, dams, rock pools, and domestic water tanks). It was also noticed to share breeding sites with Anopheles arabiensis, the main malaria vector in the region [23].

Cx. tritaeniorhynchus is a primary vector of Japanese B encephalitis in the oriental region [27] and Rift valley virus in Jazan region of Saudi Arabia with a biting preference for humans and sheep [4]. In the present study, the molecular identification of Cx. tritaeniorhynchus using the universal primer COI (Figure 12), along with the constructed phylogenetic trees and their distinctive clustering, was compared with their reference’s species in the GenBank (Figure 13) and showed that it is quite similar to the species of USA, Japan, and China (100% similarity, Table 3).

3.3. Culex tritaeniorhynchus (Figure 8(b)). The main morphological features found in Cx. tritaeniorhynchus are the presence of a median pale ring in the proboscis that extends proximally to the ventral surface of the proboscis (Figure 9(b)), and the fore and midfemora are entirely black.
Figure 5: Evolutionary relationship of taxa of *Culex pipiens* identified in Jazan region using MEGA 5.

Figure 6: PCR amplified 500 bp DNA bands using *Cx. quinquefasciatus* specific primers. Lane 1: 100 bp ladder, lane 2: positive control, lane 3: negative control, lane 4: negative sample, lanes 5–15 positive samples.

Figure 7: Evolutionary relationship of taxa of *Culex quinquefasciatus* identified in Jazan region using MEGA 5.
artificial containers in coastal, urban, and suburban areas [41].

It can also be found in almost every stagnant brackish waters with full sunlight [52]. Females feed primarily on pigs and birds, but will also bite men. It is an implicated vector of Japanese B encephalitis [41].

In Jeddah, Saudi Arabia, the density of Cx. sitiens was directly related to temperature. Its peak of density is in

Table 3: Similarity of Culex species in Jazan Region related to gene bank references.

| Culex species                              | Gene bank accession no. | Similarity (%) | Country   |
|--------------------------------------------|-------------------------|----------------|-----------|
| Culex tritaeniorhynchus                    | KJ012245.1              | 100            | USA       |
|                                            | AB738247.1              | 100            | Japan     |
|                                            | MF179221                | 100            | China     |
| Culex pipiens                              | MT199095                | 100            | Egypt     |
|                                            | MK300250                | 100            | Kenya     |
|                                            | IQ958371                | 99.85          | Iran      |
| Culex quinquefasciatus                     | MK713993.1              | 100            | Turkey    |
| Culex pipiens quinquefasciatus (Q primers 500 bp) | AF089002.1             | 100            | Sri Lanka |
| Culex sitiens                              | MN552296                | 100            | Guinea    |
|                                            | MK300241                | 100            | Kenya     |
|                                            | MF179212                | 100            | China     |

Figure 8: Culex sitiens (a) and Culex tritaeniorhynchus (b).

Figure 9: Proboscis of Culex sitiens (a) and Culex tritaeniorhynchus (b).
summer, and it is highly abundant in spring, while it breeds throughout the year [46].

*Cx. sitiens* molecular identification in the present study using the universal primer COI (Figure 12), along with the constructed phylogenetic trees and their distinctive clustering, when compared with their reference’s species in the GenBank (Figure 14) revealed that it is similar to the African species of Guinea and Kenya (100%) and China (100%) (Table 3).

All mosquito species identified morphologically in this study have also been molecularly identified, assuring absolute compatibility between taxonomic and molecular identification, confirming that PCR is a complementary tool for the identification of mosquito species.
4. Conclusion

In this study, *Culex tritaeniorhynchus* was the predominant species in Jazan region, followed by *Cx. quinquefasciatus*, then *Cx. sitiens*, and *Cx. pipiens*. The most infested areas by *Culex* mosquitoes in the region were Gizan and Sabya.

The results showed the potentiality of integrating the morphological and molecular identification to refine the list of *Culex* mosquito species and accurately document their geographical distribution in the Jazan region. This integration has the capacity to promote and enhance vector surveillance and control programmes, as well as defining the genetic diversity of species in the region.

Data Availability

The data used in this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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