Egypt. J. Vet. Sci. **Vol. 56,** No. 1, pp. 205-214 (2025)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Molecular Identification of the American Cockroach (*Periplaneta americana*) in the Kurdistan Region of Iraq (Dictyoptera: Blattidae)

Israa. K. Ahmed* and Shamal A. Al-Muffti
Biology Department, College of Science, University of Duhok, Duhok, Kurdistan Region, Iraq.

COCKROACHES, considered one of the oldest and most winged insects, are a common urban pest, mostly found in tropical regions. *Periplaneta americana*, a globally invasive pest, poses economic and health risks due to its common closeness to humans. The morphological identification of Periplaneta species is challenging due to their similarities, so the present work is designed to identify *P. americana* molecularly by mitochondrial cytochrome c oxidase subunit I (MT-COI) extraction technique, which supports morphological taxonomies. From May 2021 to mid-July 2023, 377 specimens were collected from 18 localities in four Kurdistan Region-Iraq governorates (Duhok, Erbil, Sulaymaniyah, and Halabja). Individual MT-COI extraction was performed on 28 specimens after morphological identification, then amplified with primers SHam-IS-F (CTGTTCCGGCACCTCTTTCT) and SHam-IS-R (CGGGCAACCAGGTTCACTAA) through polymerase chain reaction (PCR), and its products were visualized on a 1.5% agarose gel electrophoresis, then sequenced by Korea's Macrogen Co. After sequencing, Basic Local Alignment Searching Tools (BLAST) were used to compare the sequences to GenBank homologs. Finally, MEGA11 was used to evaluate the evolutionary relationships of the specimens and closely related species. The PCR method amplified 250 bp of the MT-COI gene, and the phylogenetic research and BLAST findings showed the cockroach CO1 gene resembled *P. americana*. The sequencing results were submitted to GenBank and identified under the following accession numbers for the first time in the Kurdistan Region, Iraq: OQ396670.1, OQ396671.1, OQ396672.1, OQ396673.1, OQ422974.1, OQ422975.1, OQ422976.1, OQ422977.1, OR229085.1, OR229158.1, OR229086.1, OR842570.1, OQ780866.1, OR842569.1, OR842571.1, OR293248.1, and OR842572.1

Keywords: American Cockroaches, Morphological taxonomy, Genetic diversity, Molecular identification, COI gene.

Introduction

The American cockroach belongs to the Blattoidea order within the class Insecta [1]. It is an important medical insect. Periplaneta genus has around 53 species, which are members of the Blattidae family [2]. Generally, it can survive in moist and filthy environments, including sewers (3). Moreover, it is one of the largest insect species [4], measuring on average 4 cm in length [5]. In recent years, several investigations have indicated sewer systems as the primary source of these insects [6]. Identification of *P. americana* and other *Periplaneta* has traditionally been difficult based on morphological characteristics because of their high similarity and high degree of polymorphism between adults and juveniles [7]. Therefore, the use of a quick and effective molecular identification technique, such as MT-DNA, would be important in supplementing the morphological taxonomy of their insects [8]. Wolstenholme and Clary (1985) were the first to

*Corresponding author: Israa. K. Ahmed, E-mail: kamalisraa52@gmail.com, Tel.: +964 750 742 0186
(Received 17/02/2024, accepted 06/04/2024)
DOI: 10.21608/EJVS.2024.270790.1856

report MT-DNA sequences. Since then, the field has seen great growth; until October 2022, the data was available for 1436 species of the Blatodea order in the GenBank database [9]. The insect MT-DNA is a double-stranded molecule that varies in size from 14,503 bp to 19,517 bp [10]. Certainly, they are one of the most informative markers in phylogenetics [11] and have been projected as a systematic taxonomy and evolutionary research tool for species identification [12]. Consequently, these properties make it useful for regular PCR amplification and molecular marker application for lower-level problems [13]. One interesting fact about this gene is that its sequences are 600–700 base pairs long [14]. This makes it the largest gene in the mitochondrial genome and more flexible than other mitochondrial genes [15]. It is excellent for species-level identification [16], and their bases are highly conserved and exhibit little interspecies variation [17,18]. It provides enough variation to distinguish among a variety of closely related species (19). The objective of this study is to use a combination of morphological, molecular, and phylogenetic approaches to accurately identify *P. americana* in the Kurdistan region of Iraq.

Material and Methods

Specimen Collection

The specimens were collected employing clean gloves to catch specimens by hand [20], and each one was preserved in a separate 25-ml Makarthy test tube with an aluminum cap (vials) that contained 96% ethanol [21]. They were collected from sewers, stores, streets, toilets, kitchens, and parks at the eighteen sampling locations as follows: Duhok governorate included Duhok Center, Zakho, Bardarash, Shekhan, Khelake, and Kalak. Erbil governorate included Erbil center, Khabat, Shaqlawa, and Soran. Sulaymaniyah governorate included Sulaymaniyah center, Chamchamal, Raniay, Chwar-Qurna, and Said-Sadiq. The Halabja Governorate included Halabja Center, Serwan, and Khormal.

Morphological Identification

The study was based on adult male and female specimens. Morphological traits (wings, antennae, abdomen segments, and their appendages) for both sexes are analyzed using identifying keys [22,23], and dissecting microscope (type Lambomed, U.S.A.). Further molecular characteristics were employed to assess the similarity of sequences and the phylogenetic relationship with the existing entries in GenBank.

Molecular Identification

Genomic DNA was extracted from the thorax of each specimen [24,25]. Tissue (25 mg) was homogenized in FATG1 buffer with Proteinase K overnight at 60°C. After incubation with FATG2 buffer at 70°C, DNA was extracted using ethanol, centrifugation, and a FATG Mini Column. The elution buffer (100 µl) retrieved the DNA and stored it at 4°C or -20°C. This extration was used as a basis for the amplification of the CO1 gene. A new species-specific primer (SHam-IS Forward and Reverse) was created in this study using the Primer3 website (26). To prepare the stock solution from this primer, each primer was reconstituted by adding 300 µl of PCR-grade water to the new lyophilized primer to prepare a 100 pmol/µl concentration. For the PCR reaction, a 10 pmol/µl final concentration was prepared by adding 10 µl of the primer stock solution to 90 µl of PCR-grade water, mixing thoroughly, and keeping at -20 °C. The PCR mixtures were 25µl in size and contained 1µM of primer (forward and reverse), 2µl of DNA template (2 ng/µl), and the final concentration of 1X AddBio Master Mix. The PCR was done using a thermal cycler (T100 BioRad, USA).

Agarose gel electrophoresis

The amplicon of the CO1 gene that was amplified by the PCR was observed through agarose electrophoresis. It was done to separate the amplified products using 1.5% agarose and 3 µl of Gel Red dye. Add 5 µl of each PCR product into an individual well within the agarose gel. The electrophoresis was carried out at 75 V for 1 hour using a power supply of 300 mA and an electrophoresis tank containing 1X Tris-Borate-EDTA (TBE) buffer. A 100-bp DNA marker in 6 µl was used as a standard molecular weight marker. The gel was examined under UV light using a gel documentation system for the determination of expected bands.

DNA sequencing and analysis

The PCR products had been sent to Sanger sequencing (an automated DNA sequencer) at Macrogen Corporation in Korea. To ensure sequencing results, we have compared the sequences obtained for our specimens with those accessed from the BLAST tool on the NCBI website (www.ncbi.gov) program (27). All the sequences generated through this investigation were submitted to the NCBI-GenBank database (http://www.ncbi.nlm.nih.gov/blast), and specific accession numbers were provided to each

specimen. Selected BLAST sequences were used to build a phylogeny tree using MEGA 11 software [28], employing the UPGMA method with 1000x bootstrapping.

Results

Three hundred and seventy-seven adults of *P. americana* (217 males and 160 females) were collected from the eighteen sampling locations in Kurdistan of Iraq during the period of the study, as shown in Table 1. The table presents data on the number of specimens obtained from various locations in four provinces of Kurdistan, Iraq. Specifically, 151, 68, 125, and 33 specimens were collected from Duhok, Erbil, Sulaymaniyah, and Halabja provinces, respectively.

Mt-DNA was extracted from 28 specimens based on their morphological features, as follows: five, six, eleven, and six specimens from Duhok, Erbil, Sulaymaniyah, and Halabja provinces, respectively. The species-specific primer SHam-IS (forward and reverse) successfully amplified a certain 250-bp amplicon of the COI gene in all cockroach specimens through PCR amplification. Top of FormThe gel electrophoresis images show 13 lanes (Fig.1) and 11 lines (Fig. 2),

From the 28 samples whose DNA was extracted and PCR products were complete, 17 samples had successful mitochondrial DNA-COI nucleotide sequencing. Based on BLAST results at the NCBI site, the CO1 gene sequences have the closest similarities with *P. americana;* each of these sequences is uniquely identifiable through accession numbers. The series of accession numbers in the GenBank database correspond to specific locales within Kurdistan where specimens were collected. Each accession number is tied to a precise location: the accession numbers (OQ396670.1), (OQ396671.1), (OQ396672.1), and (OQ396673.1) were recorded for the Duhok center and Zakho, Khelaki, and Bardarash regions, respectively, in Duhok province. Following accession numbers: (OQ422974.1), (OQ422975.1), (OQ422976.1), and (OQ422977.1) were recorded for Erbil center, and Khabat, Shaqlawa, and Soran districts, respectively, in the Erbil province; (OR229085.1), (OR229158.1), (OR229086.1), and (OR842570.1) for Sulaymaniyah center; and Chamchamal, Rania, and Saidsadiq districts, respectively, in Sulaymaniyah province. (OQ780866.1 and OR842569.1) for Halabja center; (OR842571.1 and OR293248.1) for Serwan district; and (OR842572.1) for Khormal in Halabja province. The accession numbers of the seventeen cockroach specimens are listed in Table 2.

Based on their GenBank accession numbers, the ClustalW alignment created the phylogenetic tree in Figure 3. In addition to the 17 currently available sequences and the new record from the Kurdistan Region of Iraq, it also includes 14 previously reported sequences from various countries. The phylogenetic analysis showed that the accession numbers MW291029.1, HM424021.1, MK936745.1, JQ350707.1, and MT498807.1 came from India, Canada, the USA, South Korea and Colombia, in that order, and OR229085. 1 was recorded from Sulaymaniyah Center in Sulaymaniyah province and was grouped in one cluster, whereas the following accession numbers: MG587916.1, OL589367.1, LC619069.1, and MH686446.2, were recorded from Bangladesh, Vietnam, Japan, and Thailand, respectively, and OR293248, which was recorded from Serwan district in Halabja province, was grouped in another cluster in the same clade. OR229158.1 recorded from Chamchamal in Sulymaniyah province clustered with OR229086.1 recorded from Rania and they have common ancester. The following accession numbers: OQ422976.1 and OQ422977.1 were recorded from Shaqlawa and Soran, respectively; OQ422975.1 and OQ422974.1 were recorded from Khabat, districts, and Erbil Center, respectively, and were grouped in one cluster; and the following accession numbers: OR842571.1 and OR842572.1 were documented from the Serwan and Khormal districts in Halabja province. OQ780866.1 and OR842569.1 from Halabja Center and OR842570.1 from Saidsadiq are grouped together in one cluster, which is all in the same clade. The following accession numbers: OQ396672.1, OQ396673.1, OQ396670.1, and OQ396671.1 were recorded from the following regions in Duhok province: Khelaki village, Bardarash districts, Duhok Center, and Zakho district clustered in the same glade. JN900479.1 from Iran formed one single taxon as an outgroup.

Discussion

The study identified twenty-eight cockroach specimens using the COI gene. Our primer prevented PCR failures and consistently produced a 250-bp band across all specimens. Sequencing was conducted in one direction (forward only). Consequently, recorded the results of sequencing under seventeen accession numbers in GenBank.

According to [29-31], molecular research identifies new and cryptic species, which are difficult to identify depending only on morphological features. Morphological identification performed insufficiently compared to molecular markers [32]. Proper identification is the initial stage in pest management, and DNA-based techniques are the most effective approach for identifying insect species from every position. Therefore, the results demonstrated that COI-DNA determination was highly effective for almost every insect species [33]. Nowadays, the MT-COI gene has the advantage of avoiding confusion in the presence of polymorphism as well as throughout the different life stages and sexual forms of the species being examined. In contrast, traditional taxonomy has some limitations in this regard [34]. Numerous researchers have recently explored the molecular identification of the American cockroach through the COI gene, including [35] who conducted a study in Saudi Arabia where they observed that the universal primer (LCO 1490 and HCO 2198) produced a single band with a size range of 700–800 bp when examined with 1% agarose gel electrophoresis. [12] Identified *P. americana* for the first time in Bangladesh based on COI gene sequences. Hashemi-Aghdam et al. [15] stated that their PCR amplification generated a single 710 bp-sized amplicon for American cockroaches using a universal primer (LCO 1490 and HCO 2198) and also showed a significant intra-species variation within their populations. Furthermore, the findings by Cevahi and Duzlv [36]. indicated that a 384 bp amplification piece targets a region within the MT-COI gene of the cockroach species discovered in Kayseri city, aligning 99% with other cockroach species in Turkey. In another study by Pava-Ripoll et al. [37] in the United States and Canada, the specific primer was used to amplify the COI gene fragment in MT-DNA from *P. americana*, and a 179-bp amplicon band was generated. Other authors, Highlighted that nuclear DNA, which is stable in its variation, does not accurately reflect geographical evolutionary changes like mitochondrial DNA [38]. In other words, this gene has been effective in unraveling the evolutionary connections among Blattodea species. Our phylogenetic tree for 31 specimens based on COI showed that specimens from different countries for the same species were placed into separate clades. Species in different clades with genetic similarities may be linked. Despite clade diversity, similar DNA sequences indicate conserved features or evolutionary history. This may indicate genetic exchanges or shared heritage. [39] discovered three distinct and widely dispersed *P. americana* COI haplogroups. They concluded that all available COI barcodes formed a monophyletic lineage separated from congeneric species. Genetically, species in the same group are more closely related than those outside it. This shows a closer genetic resemblance and common evolutionary history among species. Closely related taxa commonly have the same morphological characteristics. Due to their evolutionary proximity, they have low degrees of genetic differentiation [40].

Geographical isolation, environmental influences, and selection pressures all contribute to the possibility that species populations in various places have diverse biological traits and genetic diversity [41]. According to [12], the phylogenetic analysis revealed that species of the same family often grouped into major clades, and those of the same species from different countries clustered together. JN900479.1 from Iran formed one single taxon as an outgroup, thus revealing distant relationships and genetic variations with other *P.americana* sequences, whether in the Iraq or other countries.

Conclusion

The findings of this research highlight the effectiveness of the mtDNA-COI gene as a valuable and potent molecular identifier in identifying cockroach species, especially in cases where physical characteristics are not easily distinguishable. Additionally, this gene proves to be advantageous in comprehending the evolutionary relationships within this order.

Acknowledgment

All thanks and appreciation to the College of Science, University of Duhok, for their supporting.

Conflict of Interest

None

Funding statement

Self-funding

Author`s contribution

All researchers participated in designing the research. The first researcher carried out the practical aspect and statistical analysis. The second researcher completed the task of supervising, making tables, and writing.

TABLE 1. Total number of *P. americana* specimens collected from four provinces in the Kurdistan Region of Iraq from 2021 to 2023

Provinces	#	Locations	x (long)	y (lat)
Duhok	88	Duhok center	43.036	36.846
	41	Zakho	42.716	37.122
	3	Bardarash	43.552	36.497
	11	Shekhan	43.379	36.689
	1	Khelake	43.6060	36.498
	7	Kalak	43.631	36.269
Erbil	32	Erbil center	44.028	36.185
	34	Khabat	43.668	36.260
	1	Shaqlawa	44.313	36.413
Sulaymaniyah	1	Soran	44.542	36.689
	25	Sulaymanyiah center	45.448	35.539
	14	Chamchamal	44.880	35.519
	46	Raniay	44.910	36.238
	6	Chwar-Qurna	44.831	36.209
	34	Said sadiq	45.859	35.356
Halabja	13	Halabja center	45.996	35.177
	16	Serwan	45.937	35.251
	4	Khormal	46.046	35.297

*X = Longitude *Y = Latitude # = Number of specimens

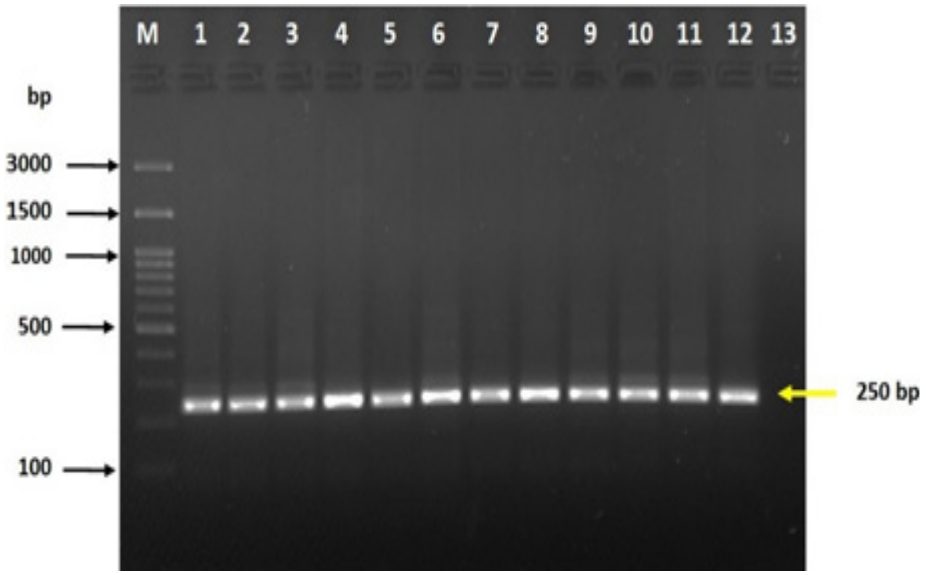

Fig. 1. Amplicon CO1 gene from P.americana, visualized using 1.5% agarose electrophoresis and run at 75 V for 1 hour using a power supply of 300 mA. Lane M=Marker (Molecular weight marker is a 3000 bp ladder; it means that each band with the next one has a 100 bp difference. Lanes 1–12 are positive samples, and lane 13 is negative control (without genomic DNA). The lengths of polymerase chain reaction products are approximately 250 bp.

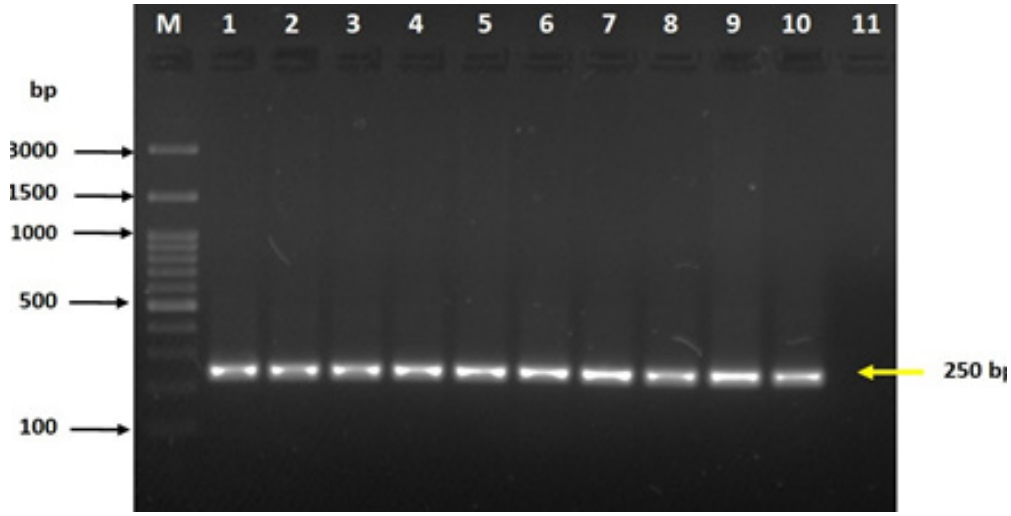

Fig. 2. Amplicon CO1 gene from P. americana, visualized using 1.5% agarose electrophoresis and run at 75 V for 1 hour using a power supply of 300 mA. Lane M=Marker (Molecular weight marker is a 3000 bp ladder; each band with the next one has a 100 bp difference. Lanes 1–10 are positive samples, and lane 11 is a negative control (without genomic DNA). The lengths of polymerase chain reaction products are approximately 250 bp.

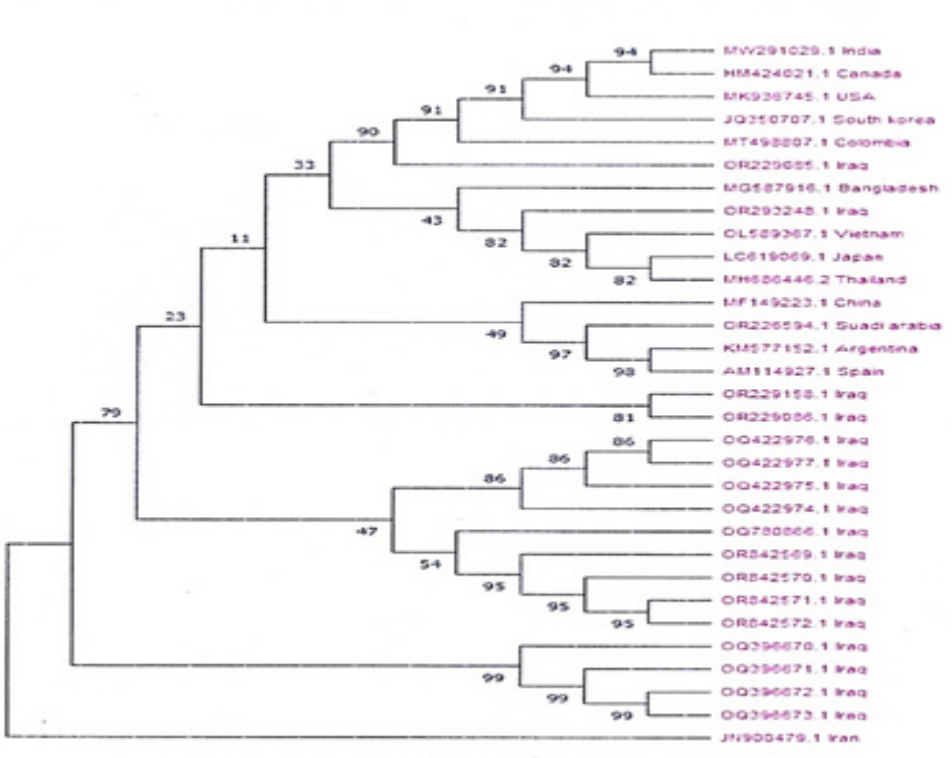

Fig. 3. Showing the best tree. Next to branches are the proportion of bootstrap test trees with connected taxa grouped. The evolutionary distances are in base substitutions per site and were calculated using the Maximum Composite Likelihood technique. Analysis included 31 nucleotides. Codon positions were 1st–3rd and Noncoding. For partial deletion, places with less than 100% site coverage were deleted, meaning no alignment gaps, missing data, or unclear bases were permitted. Total 195 locations in final dataset. In MEGA11, evolution was considered.

TABLE 2. COI-MTDNA Sequence Accession Numbers and Their Respective Regions

Provinces	Regions	Accession No.
Duhok	Duhok Center	OQ396670.1
	Zakho district	OQ396671.1
	Khelaki village	OQ396672.1
	Bardarash district	OQ396673.1
Erbil	Erbil center	OQ422974.1
	Khabat district	OQ422975.1
	Shaqlawa ditrict	OQ422976.1
	Soran district	OQ422977.1
Sulaymaniyah	Sulaimaniyah center	OR229085.1
	Chamchamal district	OR229158.1
	Rania district	OR229086.1
	Saidsadiq district	OR842570.1
Halabja	Halabja center	OQ780866.1
	Halabja center	OR842569.1
	Serwan district	OR842571.1
	Serwan district	OR293248.1
	Khormal district	OR842572.1

References

1. Zhao, Y., Yang, A., Tu, P. and Hu, Z. Anti-tumor effects of the American cockroach, Periplaneta americana. *Chinese Medicine,* **12**(1),1-6(2017).
2. Beccaloni, G.W. Cockroach Species File (Version 5.0/5.0) Available from: http:// cockroach. speciesfile. org/HomePage/Cockroach. HomePage. aspx (accessed 20 December 2021). 2014.
3. Jaramillo-Ramirez, G.I., Cárdenas-Henao, H., González-Obando, R. and Rosero-Galindo, C.Y. Genetic variability of five Periplaneta americana L.(Dyctioptera: Blattidae) populations in southwestern Colombia using the AFLP molecular marker technique. *Neotropical Entomology,* **39**,371-378(2010).
4. Li, S., Zhu, S., Jia, Q., Yuan, D., Ren, C., Li, K., Liu, S., Cui, Y., Zhao, H., Cao, Y. and Fang, G. The genomic and functional landscapes of developmental plasticity in the American cockroach. *Nature Communications*, **9**(1), 1008 (2018).
5. Borah, N. and Hazarika, L.K. Biology and morphometrics of Periplaneta americana. *J. Entomol. Zool. Stud,* **7**, 1206-1210 (2019).
6. Khodabandeh, M., Shirani-Bidabadi, L., Madani, M. and Zahraei-Ramazani, A. Study on Periplaneta americana (Blattodea: Blattidae) fungal infections in hospital sewer system, Esfahan City, Iran, 2017. *Journal of Pathogens*, **2020**, Article ID 4296720 (2020).
7. Che, Y., Gui, S., Lo, N., Ritchie, A. and Wang, Z. Species delimitation and phylogenetic relationships in ectobiid cockroaches (Dictyoptera, Blattodea) from China. *PLoS One*, **12**(1), e0169006(2017).
8. Ma, J., Liu, J., Shen, Y., Fan, Z, Yue. B. and Zhang, X. Population genetic structure and intraspecific genetic distance of Periplaneta americana (Blattodea: Blattidae) based on mitochondrial and nuclear DNA markers. *Ecology and Evolution*, **9**(22),12928-39(2019).
9. Şeyda, B.E. and Pektaş, A.N. DNA Barcoding of Commercial Cockroaches in Turkey. *Cumhuriyet Science Journal*, **44**(1), 28-35(2023).
10. Lewis O.L., Farr, C.L. and Kaguni, L.S. Drosophila melanogaster mitochondrial DNA: completion of the nucleotide sequence and evolutionary comparisons. *Insect Molecular Biology,* **4**(4), 263-78 (1995).

11. Xiao, B., Chen, A.H., Zhang, Y.Y., Jiang, G.F., Hu, C.C. and Zhu, C.D. Complete mitochondrial genomes of two cockroaches, Blattella germanica and Periplaneta americana, and the phylogenetic position of termites. *Current genetics*, **58**,65-77 (2012).

12. Rain, F.F. and Aslam, A.F.M. The first DNA barcode of medically important cockroaches in Bangladesh. *AsPac J. Mol. Biol. Biotechnol.*, **31**(2), 80-90 (2023).

13. Kelly, R.P. and Sarkar, I.N., Eernisse DJ, Desalle RO. DNA barcoding using chitons (genus Mopalia). *Molecular Ecology Notes*, **7**(2),177-183 (2007).

14. Hebert, P.D., Ratnasingham, S. and De Waard, J.R. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London. *Series B: Biological Sciences*, **270** (suppl_1), S96-9(2003).

15. Hashemi-Aghdam, S.S., Rafie, G., Akbari, S. and Oshaghi, M.A. Utility of mtDNA-COI barcode region for phylogenetic relationship and diagnosis of five common pest cockroaches. *Journal of Arthropod-Borne Diseases*, **11**(2),182(2017).

16. Bergsten, J., Bilton, D.T., Fujisawa, T., Elliott, M., Monaghan, M.T., Balke, M., Hendrich, L., Geijer, J., Herrmann. J. and Foster, G.N., Ribera, I. The effect of geographical scale of sampling on DNA barcoding. *Systematic Biology*, **61**(5),851-69(2012).

17. Ali, M.A., Gyulai, G., Hidvegi, N., Kerti, B., Al Hemaid, F.M., Pandey, A.K. and Lee, J. The changing epitome of species identification–DNA barcoding. *Saudi Journal of Biological Sciences*, **21**(3),204-31(2014).

18. Rach, J., Bergmann, T., Paknia, O., DeSalle, R., Schierwater, B. and Hadrys, H. The marker choice: Unexpected resolving power of an unexplored CO1 region for layered DNA barcoding approaches. *PloS one*, **12**(4), e0174842(2017).

19. Lin, X., Stur, E. and Ekrem, T. Exploring genetic divergence in a species-rich insect genus using 2790 DNA barcodes. *PloS one*, **10**(9), e0138993 (2015).

20. Paul, S., Khan, A.M., Baqui, M.A. and Muhibullah, M. Evaluation of the common cockroach Periplaneta americana (L.) as carrier of medically important bacteria. *The Journal of Communicable Diseases*, **24**(4), 206-10(1992).

21. Moreau CS. A practical guide to DNA extraction, PCR, and gene-based DNA sequencing in insects. *Halteres*, **5**(32–42),11(2014).

22. Abul-Hab, J. A list of arthropoda of medical and veterinary importance recorded from Iraq. *Bull Biol Res Cent*, **12**(1),9-40(1980).

23. Robinson W.H. Urban insects and arachnids: a handbook of urban entomology. *Cambridge University Press*, (2005)

24. Paydar, S., Ucar, E., Yari, P., Safavi, S.M., Kahrizi, D., Nateqi, M., Mirmoayedi, A. and Yari, K. A simplified and optimized protocol for total DNA extraction from insect species: applicable for studying genetic diversity and PCR-based specimen identification via partial amplification of cytochrome oxidase I (COI) gene. *Cellular and Molecular Biology*, **64**(12),22-5(2018).

25. AL-Obaidi, O.R., Asaad, M.M. and Hameed, H.N. Study Of Isolating The Mitochondrial DNA And The S28 Gene Of Periplaneta Americana In Two Areas Of Samarra City And Making A Genetic Variation For It. *European Journal of Molecular & Clinical Medicine*, **7**(11), 2505-12(2020).

26. Rozen, S. and Skaletsky H. Primer3 on the WWW for general users and for biologist programmers. *Bioinformatics methods and protocols*, 365-86(1999).

27. Muffti, S.A., Genetic diversity analysis of Anopheles species in Kurdistan Region-Iraq, using molecular biology techniques [Ph.D. thesis]. Baghdad: University of Baghdad; (2008).

28. Tamura K, Stecher G, and Kumar, S. MEGA11: molecular evolutionary genetics analysis version 11. *Molecular biology and evolution*, **38**(7),3022-7(2021).

29. Hebert, P.D., Stoeckle, M.Y., Zemlak, T.S., and Francis, C.M. Identification of birds through DNA barcodes. *PLoS biology*, **2**(10), e312(2004).

30. Perring, TM., The Bemisia tabaci species complex. *Crop protection*, **20**(9), 725-37(2001).

31. Toda, S and Murai, T. Phylogenetic analysis based on mitochondrial COI gene sequences in Thrips tabaci Lindeman (Thysanoptera: Thripidae) in relation to reproductive forms and geographic distribution. *Applied Entomology and Zoology*, **42**(2),309-16(2007)

32. Taha, K.M., Khdr, D.M. and Kareem, K.Y. Identification and differentiation of poultry meat and products using PCR-RFLP technique. *Mesopotamia Journal of Agriculture*, **49**(1), 34-42(2021).

33. Muhammad H.M, Mawlud, D.H, Taha, K.M. and Mawlood, N.A. Molecular identification of some aphid species (homoptera; aphididae) based on rflp-pcr technique. *Mesopotamia Journal of Agriculture*, **50**(4),107-116(2022).

34. Asokan, R.,, Kumar N.K., Kumar, V. and Ranganath, H.R. Molecular differences in the mitochondrial cytochrome oxidase I (mtCOI) gene and development of a species-specific marker for onion thrips, Thrips tabaci Lindeman, and melon thrips, T. palmi Karny (Thysanoptera: Thripidae), vectors of tospoviruses (Bunyaviridae). *Bulletin of Entomological Research*, **97**(5), 461-70(2007).

35. Sharawis, S. and Assagafa, J.M.Y.A. Morphological and molecular identification of the American cockroaches (Periplaneta americana) in Jeddah province (Dictyoptera: Blattidae). *International Journal of Entomology Research*, **6**(5), 31-36 (2021)

36. Cevahi, F. and Duzlu, O. Cockroaches (Insecta: Blattaria) Common in Kayseri Region Phylogenetic Characterization. In: 21st Parasitology Congress. Kayseri: Erciyes University Faculty of Veterinary Medicine Department of Parasitology, 341-342(2019).

37. Top of Form Pava-Ripoll, M., Miller, A.K. and Ziobro, G.C. Development of a Multiplex Polymerase Chain Reaction (PCR) Assay for the Potential Detection of Insect Contaminants in Food. *Journal of Food Protection*, **86**(8),100120 (2023).

38. Hickerson M.J and Cunningham, C.W. Contrasting quaternary histories in an ecologically divergent sister pair of low-dispersing intertidal fish (Xiphister) revealed by multilocus DNA analysis. *Evolution*, **59**(2),344-60(2005)

39. Von Beeren, C., Stoeckle, M.Y., Xia, J., Burke, G. and Kronauer, D.J. Interbreeding among deeply divergent mitochondrial lineages in the American cockroach (Periplaneta americana). *Scientific Reports*, **5**(1),8297 (2015)

40. Zhang, L., Lu, N.T, Zhou, X.M, Chen, D.K, Knapp, R., Zhou, L., Guo, L., Luong, T.T., Sun, H., Gao, X.F. and Zhang, L.B. A plastid phylogeny of the Old World fern genus Leptochilus (Polypodiaceae): Implications for cryptic speciation and progressive colonization from lower to higher latitudes. *Molecular phylogenetics and evolution*, **1**(134),311-22(2019)

41. Khidr, S.K., Hardy, I.C., Zaviezo, T. and Mayes, S. Development of microsatellite markers and detection of genetic variation between Goniozus wasp populations. *Journal of Insect Science*, **14**(1),43 (2014).

التحليل الجزيئي للصرصر الأمريكي (*Periplaneta americana*) في إقليم كردستان العراق (Dictyoptera: Blattidae)

اسراء كمال احمد و شمال عبد الله المفتي
قسم علوم الحياة - كلية العلوم - جامعة دهوك - دهوك - كردستان - العراق.

الخلاصة

تعتبرالصراصر واحدة من أقدم الحشرات وأكثرها انتشاراً، تعتبر آفة حضرية شائعة، وتوجد في الغالب في المناطق الاستوائية.يعتبر الصرصر الأمريكي *Periplaneta americana*آفة متوغلة عالمياً، ويشكل مخاطر اقتصادية وصحية بسبب اقترابه الشائع من البشر. يعتبر التعرف المورفولوجي على أنواع الصراصر التابعة لجنس Periplaneta تحديًا بسبب تشابهها، لذا تم تصميم العمل الحالي لتحديد *P. americana* جزيئيًا باستخدام تقنية استخلاص جين الأكسدة السايتوكرومي C للميتوكوندريا (MT-COI)، والتي تدعم التصنيفات المورفولوجية. تم جمع 377 عينة من 18 موقعًا في أربع محافظات في إقليم كردستان العراق (دهوك، أربيل، السليمانية، وحلبجة) من مايو 2021 حتى منتصف يوليو 2023. تم إجراء استخلاص MT-COI الفردي على ٢٨ عينة بعد التصنيف المورفولوجي، ثم تم تضخيمها باستخدام البادئات (SHam-IS-F (CTGTTCCGGCACCTCTTTCT وSHam-IS-R (CGGGCAACCAGGTTCACTAA) من خلال تفاعل البلمرة المتسلسل (PCR)، وتم تحليل نتائجها من خلال الهلام الكهربائي من الأغاروز بتركيز 1.5٪، ثم تم دراسة وتحليل تسلسلها من قبل شركة Macrogen Co. في كوريا. بعد التسلسل، تم استخدام (Basic Local Alignment (BLAST لمقارنة التسلسلات بمثيلاتها في GenBank. وأخيراً، تم استخدام برنامج MEGA11 لتقييم العلاقات التطورية للعينات والأنواع ذات الصلة بشكل كبير. تم تضخيم الجين MT-COI بواسطة تقنية PCR وتم الحصول على باندات بقياس ٢٥٠ bp ، وأظهرت نتائج BLAST أن جين CO1 للصراصرفي هذه الدراسه يشابه *P. americana*. تم ادخال نتائج التسلسل إلى GenBank وتحديدها تحت الأرقام التسلسلية التالية لأول مرة في إقليم كردستان، العراق: OQ396670.1، OQ396671.1، OQ396672.1، OQ396673.1، OQ422974.1، OQ422975.1، OQ422976.1، OQ422977.1، OR229085.1، OR229158.1، OR229086.1، OR842570.1، OQ780866.1، OR842569.1، OR842571.1، OR293248.1، و OR842572.1.

الكلمات الدالة: الصرصر الأمريكي، التصنيف المورفولوجي، التنوع الوراثي، التحديد الجزيئي، جين الأكسدة السايتوكرومي