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American Cockroach (*Periplaneta americana*): Genomic Analysis of Emerging New Strains

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ABSTRACT

Background: This study presents the first molecular characterization of *Periplaneta americana* from the Jeddah region of Saudi Arabia, utilizing DNA barcoding for accurate species identification.

Methods: DNA was extracted from 30 specimens using the Thermo Scientific GeneJET Genomic DNA Purification Kit and amplified with universal primers LCO 1490 and HCO 2198, producing a 700-800 bp band.

Result: Sequencing revealed two accession numbers, JQ267485.1 and KM576926.1, showing 96-100% and 92-96% similarity to *P. americana*, respectively. Phylogenetic analysis using MEGA7 confirmed the species' identification, adding novel genetic data to the global repository. The study aligns with previous findings on the genetic diversity of *P. americana*, highlighting the species' complexity and evolutionary relationships within Blattaria. The morphological identification of various life stages corroborates existing literature but emphasizes the need for detailed oothecae characterization in the region. This comprehensive approach, combining molecular and morphological methods, demonstrates the efficacy of DNA barcoding in pest identification and supports enhanced pest management strategies. By filling a critical gap in the genetic documentation of *P. americana* in Saudi Arabia, this research contributes valuable insights for future studies and control measures aimed at mitigating the impact of this urban pest.

Key words: American Cockroach, Jeddah, Molecular studies, *P. americana*, Saudi Arabia.

INTRODUCTION

American cockroaches (*Periplaneta americana*) (Linnaeus), order Dictyoptera, is an important insect in medical (Akbari *et al.,* 2015), they are the most notorious pests, found in kitchens (El-Sherbini and Gneidy, 2012). It is among the largest common cockroach species (Gary, 2015). Dictyoptera comprises about 5000 species under 398 genera in 28 families (Hanitsch, 1917). Out of 500, 30 species are considered as household pest (Chung *et al.,* 2005). Several cockroach pests live in/or around homes, and they are omnivorous scavengers (Akbari *et al.,* 2015). They survive in warm weather with high moisture conditions as well as in unfavorable environments for humans (*i.e*., sewers and other human-made habitats) (Jaramillo-Ramirez *et al.,* 2010).

P. americana can spread bacteria, fungi and other pathogenic microorganisms from infected areas (Czajka *et al.,* 2003), and cause allergies to humans (Kinfu and Erko, 2008). They play an important role in the transmission of different diseases in mechanical and biological ways (Kim *et al.,* 2016). *P. americana* spends most of its time in sewage and sewer pipe which usually contains a high density of pathogens (Basseri *et al.,* 2016). Also, they feed on garbage and they have large opportunities to disseminate human pathogens (Pai *et al.,* 2005). In addition, their nocturnal and filthy habits of eating their feces make them ideal carriers of numerous pathogenic microbes (Allen, 1987). Cockroaches spread pathogens through their cuticle (Mpuchane *et al.,* 2006b), because their nymphal cuticles go through ecdysis (Mpuchane *et al.,* 2006a). Therefore, they transfer pathogens in different ways such as vertical transmission which occurs when an infected mother passes on the pathogen or disease to her progeny (Jennifer, 2008). All these pathogens are used as dangerous organisms targeting animal or human populations.

Much of the work regarding insecticidal efficacy has been done on *B. germanica*, however, very little data is available concerning *P. americana*. Therefore, keeping in view the work carried out by various researchers, the present work was designed to identify collected *P. americana* using morphological and molecular techniques and to investigate the insecticidal efficacy of two groups of insecticides (conventional and non-conventional), with different classes on *P. americana* and study the susceptibility of different stages to these insecticides through laboratory bioassay using feeding and contact toxicity methods. Little is known about the infection of *P. americana* oothecae isolated bacteria as a safety method for controlling this phase, however, the possibility of finding a new method to

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control *P. americana* oothecae can bring new perspectives of controlling, since no insecticides are effective so far when applied topically on this phase of the pest and it is important to inhibit embryogenesis of *P. americana* to prevent them from hatching. The present work aims to identify *P. americana* by using morphological and molecular techniques.

MATERIALS AND METHODS

Molecular identification of *P. americana*

P. americana samples were collected from five different places in Jeddah city. All genomic DNA extraction experiments were conducted in the molecular identification experimental unit, in the Dengue Mosquito Experimental Station (DMES), belonging to the Department of Biological Sciences, Faculty of Sciences, King Abdul-Aziz University, Jeddah, Saudi Arabia. The place is intended to be a working and training unit in insect molecular identification and help university postgraduate students perform their specialized research. Equipment, tools and instruments were kindly supplied by the author of this work.

Genomic DNA extraction

Thermo Scientific GeneJET Genomic DNA Purification Kit #K0721 was used for *P. americana* DNA extraction, with some modifications. *P. americana* body (antenna, legs, and wings), was cut into small pieces, and collected into three tubes of 1.5 ml Eppendorf tube. Samples were resuspended in 180 µl of digestion solution and sterilized white sand. 20 µl of Proteinase-K solution was added and mixed thoroughly by vortexing to obtain a uniform suspension. Samples were incubated at 56°C for one hour until the tissue was completely lysed and no particles remained. 20 µl of RNase was added, mixed by vortexing then incubated for 10 min at room temperature. Then, 200 µl of lysis solution was added and mixed thoroughly by vortexing for 15 seconds until a homogeneous mixture was obtained. After that, 400 µl of 50% ethanol was added and mixed by vortexing. The lysate solution was then transferred to a GeneJET Genomic DNA Purification column inserted in a collection tube. The column was centrifuged for 1 min at 8000 rpm. The collection tube containing the flow-through solution were then discarded. 500 µl of wash buffer I were added, centrifuged for 1 min at 10,000 ×g. The flow-through were discarded and placed the purification column back into the collection tube. Then, 500 µl of wash buffer II were added to the GeneJET Genomic DNA Purification column, centrifuged for 3 min at maximum speed (12,000 ×g). Exactly 200 µl of elution buffer were added to the centre of the GeneJET Genomic DNA Purification column membrane to elute genomic DNA, samples were incubated for 2 min at room temperature and centrifuged for 1 min at 10000 rpm. Finally, the purification column was discarded. The eluted DNA was measured with Nano Drop. Purified DNA was stored at -20°C.

Primer sequence

For molecular identification of *P. americana*, Universal primers (LCO1490 and HCO2198) were used in this study (Table 1, 2 and 3).

Agarose gel electrophoresis

For estimation size estimation, 10 µl of molecular weight marker were loaded in the first well. The gel was run at 127 V for one hour, which was attached firmly and connected to the power supply (MOLECULE-ON PS-M-300V Electrophoresis Power Supply, India). 1% of agarose powder was weighed and dissolved in 1X TAE buffer by placing the suspension in a microwave oven for heating. A final concentration of 0.1 µg/ml ethidium bromide (EtBr) from 10 mg/ml stock in distilled water was added to the agarose, then agarose was poured into the gel tray and the comb was placed at one end. The gel was left for one hour to solidify before the comb was removed. The gel was then placed into an electrophoresis tank and submerged with 1X TAE buffer as a running buffer. The 5X loading dye was added to the samples to be loaded according to the volume ratio 5:1 of sample to dye. The gel was run in a horizontal gel apparatus at 127 volts for 60-90 min. DNA fragments were visualized on a UV transilluminator and photographed by using a Viber Lourmat Gel Imaging System. DNA ladder was used to determine the molecular weights of DNA fragments (Sharawi, 2023).

Sequencing reaction

DNA sequencing was carried out in Macrogen Company, Korea. Deduced nucleotide and amino acid sequence data were compared with the National Centre for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST).

RESULTS AND DESSCUSSION

Molecular identification of *P. americana*

P. americana DNA extraction was prepared using Thermo Scientific GeneJET Genomic DNA Purification Kit #K0721 and examined on 1% agarose gel. PCR amplification was then carried out using universal primers, as shown in the materials and methods. The resulted 700-800 bp was examined on 1% agarose gel electrophoresis as in Fig (1). The PCR products were purified and send to Macrogen for sequencing. The phylogenetic relationships of *P. americana*

Table 1: Nucleotide sequences of the primers for PCR amplification.

Primer name	Nucleotide sequence (Sharawi, 2023)	Size of PCR product
LCO1490	5′- GGTCAACAAATCATAAAGATATTGG - 3′	700-800 bp
HOC2198	5′- TAAACTTCAGGGTGACCAAAAAATC - 3′	

isolate and closely related species were analyzed using the Multisequence Alignment Program (MEGA7) and the results are presented in phylogenetic tree, as in Table 4 and Fig (2). DNA barcoding promises to be a useful tool to identify pest species assuming adequate representation of genetic variants in a reference library (Beeren *et al*., 2015). In our finding, we used barcode region of mt-DNA (LCO 1490 and HCO2198) gene of DNA extracted from 30 cockroach specimens (Table 5), along with the development of a PCR method. The PCR generates a single band between 700-800 bp-sized in all cockroach specimens, followed by direct sequencing. Two accession numbers were found from DNA sequencing (JQ267485.1 and KM576926.1) with 96-100% and 92-96%, respectively of the similarities were related to *P. americana*. Anyway, the molecular identification studies of *P. americana* are not much studied in Saudi Arabia in general and Jeddah governorate in particular. Many researchers have studied the molecular identifications of different cockroaches. Beeren *et al*. (2015) found that three deeply-divergent, widely-distributed *P. americana* COI haplogroups. Xiao *et al.* (2012) sequenced the complete mitogenomes of two cockroaches, reconstructed the molecular phylogeny and attempted to infer the phylogenetic position of termites in Blattaria more reliably and he found that complete mt-DNA nucleotide sequences of *P. americana* is 15,025 bp in size and they found that *P. americana* shares only 75% sequence identity with *B. germanica*, which is lower than that with the two genome-sequenced termites, *i.e*., 79% to *Z. nevadensis* and 80% to *M. natalensis*. Ma *et al.* (2017) determined the complete mitochondrial genomes of two cockroach species, *Periplaneta australasiae* and *Neostylopyga rhombifolia*, 15,605 bp and 15,711 bp in length, respectively. So, this study first time confirm the molecular characters of *P. americana* from Jeddah region of Saudi Arabia.

Molecular identification through DNA barcoding has proven to be a valuable tool for accurately identifying pest species, given an adequate reference library (Beeren *et al*., 2015). In our study, we utilized the barcode region of mt-DNA, specifically the LCO1490 and $HCO_2$198 genes, extracted from 30 *P. americana* specimens. The PCR method developed for this study produced a single band of 700-800 bp in all specimens, which was subsequently sequenced. The sequencing results yielded two accession numbers, JQ267485.1 and KM576926.1, with 96-100% and 92-96% similarity, respectively, to *P. americana*. Our findings

Table 2: PCR mixture.

Primer	PCR mixture
LCO1490 and $HCO_2$198	Total volume of 25 µl containing: -1 µl DNA- -1 µl of each primer (Foreword and Reverse) -12.5 µl Master Mix -9.5 µl sterile dH_2O

Table 3: PCR conditions.

Primer	PCR conditions (Sharawi, 2023)
LCO1490 and HCO2198	-Initial denaturation: 5 min at 94°C -Denaturation: 1 min at 94°C -Annealing: 1 min at 58°C -Extension: 2 min at 72°C -Number of cycles: 35 -Final extension: 10 min at 72°C By a thermal cycler (Thermo Scientific)

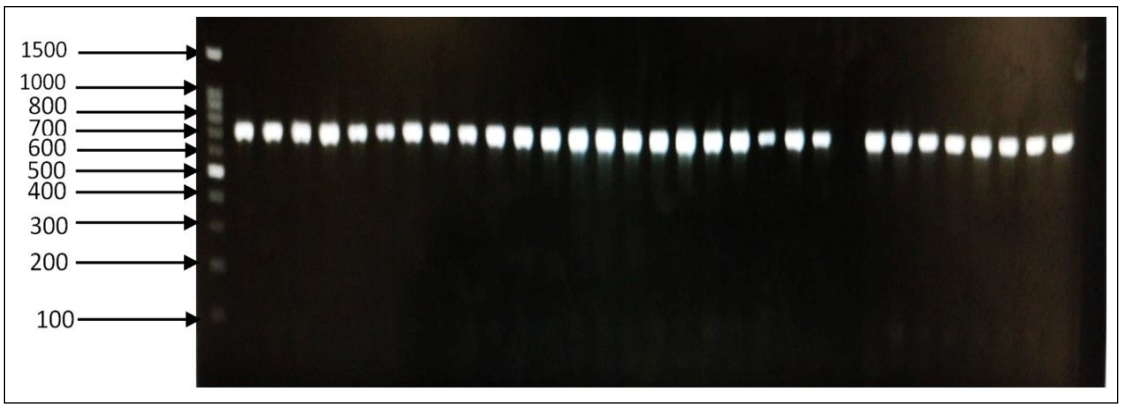


Fig 1: Complies GenBank accession numbers of the highest sequence similarity as well as the closest neighbor (s) to the mtDNA gene partial sequence of *P. americana*.

Table 4: Similarity percentages to the nearest neighbor(s) of the isolate.

P. americana isolates	Nearest neighbor(s)	Accession number	Similarities
	P. americana	JQ267485.1	96-100%
	P. americana	KM576926.1	92-96%

indicate a high degree of similarity to *P. americana* corroborating the effectiveness of the molecular approach in identifying this species. Despite the successful identification, it is noteworthy that molecular studies on *P. americana* in Saudi Arabia, particularly in the Jeddah governorate, are limited. This study contributes novel molecular data from this region, filling a significant gap in the genetic documentation of *P. americana*. Previous research has also highlighted the genetic diversity within *P. americana* Beeren *et al.* (2015) identified three deeply-divergent, widely-distributed COI haplogroups within *P. americana*, demonstrating the genetic complexity of this species. Similarly, Xiao *et al.* (2012) sequenced the complete mitogenomes of two cockroach species and inferred the phylogenetic position of termites within Blattaria. They reported that the complete mt-DNA sequence of *P. americana* is 15,025 bp in size and shares only 75% sequence identity with *Blattella germanica*. This lower identity compared to termites (79% with *Zootermopsis nevadensis* and 80% with *Macrotermes natalensis*) suggests significant genetic divergence within the Blattaria order. Additionally, Ma *et al.* (2017) determined the complete mitochondrial genomes of *Periplaneta australasiae* and *Neostylopyga rhombifolia*, finding them to be 15,605 bp and 15,711 bp in length, respectively. These studies collectively underscore the extensive genetic variability and complexity within cockroach species, highlighting the importance of molecular identification in understanding their phylogeny and evolutionary relationships. Our study, for the first time, confirms the molecular characteristics of *P. americana* from the Jeddah region of Saudi Arabia, adding to the global genetic data on this species. The high similarity rates observed in our sequences suggest a close genetic relationship with previously identified *P. americana* specimens, reinforcing the utility of DNA barcoding in pest

Table 5: Strain names and accession numbers of collected *P. americana.*

Strain name	Accession number
SUB13595453 AC_1	OR225969
SUB13595616 AC_2	OR225971
SUB13602400 AC_3	OR227138
SUB13572344 AC_4	OR227136
SUB13603085 AC_5	OR227146
SUB13602823 AC_6	OR226594
SUB13602984 AC_7	OR226596
SUB13602984 AC_8	OR226597
SUB13602984 AC_9	OR226598
SUB13602984 AC_10	OR226599
SUB13603027 AC_11	OR227139
SUB13603027 AC_12	OR227140
SUB13603027 AC_13	OR227141
SUB13603027 AC_14	OR227142
SUB13603027 AC_15	OR227143
SUB13603264 AC_16	OR227164
SUB13603264 AC_17	OR227165
SUB13603264 AC_18	OR227166
SUB13603264 AC_19	OR227167
SUB13603173 AC_20	OR226737
SUB13603173 AC_21	OR226738
SUB13603173 AC_22	OR226739
SUB13603173 AC_23	OR226740
SUB13603173 AC_24	OR226741
SUB13603173 AC_25	OR226742
SUB13603173 AC_26	OR226743
SUB13603173 AC_27	OR226744
SUB13603173 AC_28	OR226745
SUB13603173 AC_29	OR226746
SUB13603173 AC_30	OR226747

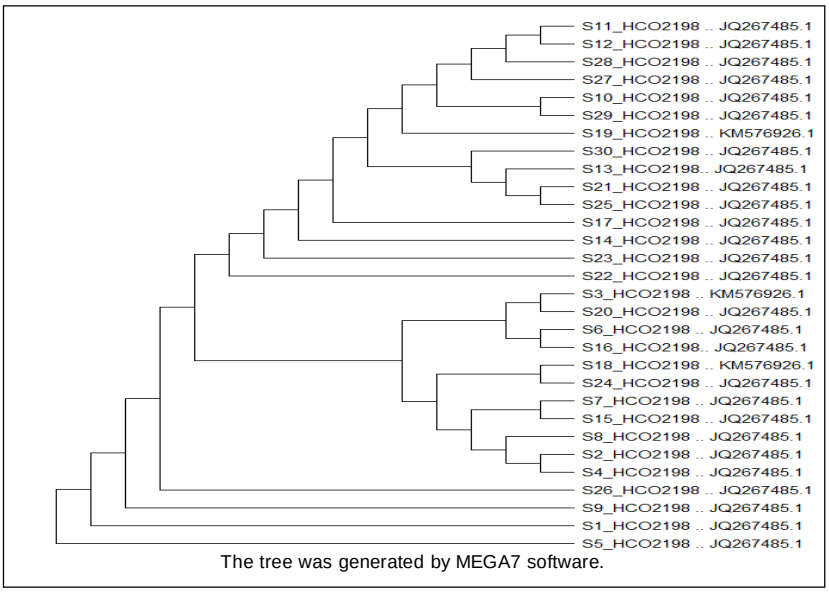


Fig 2: Phylogenetic relationship among *P. americana* DNA isolate and the most closely related species.

identification. Overall, the molecular identification approach used in this study provides a reliable and accurate method for identifying *P. americana*, which can be instrumental in pest management strategies. By contributing to the genetic database of *P. americana* in Saudi Arabia, this study supports future research and control measures aimed at mitigating the impact of this pervasive urban pest.

CONCLUSION

This study provides the first molecular characterization of *P. americana* from the Jeddah region of Saudi Arabia, contributing novel genetic data to the global database for this species. Morphological identification of various life stages, including adults, nymphs, and oothecae. However, there is still a need for detailed morphological characterization of oothecae with clear photographs and identification keys for this region. Our molecular approach, utilizing the barcode region of mt-DNA (LCO 1490 and HCO2198), confirmed *P. americana* with high similarity rates to known sequences (JQ267485.1 and KM576926.1). These findings demonstrate the effectiveness of DNA barcoding for accurate pest identification, specifically for *P. americana*. By addressing a significant gap in the genetic documentation of *P. americana* in Saudi Arabia, this study enhances understanding of its genetic diversity, providing valuable insights that support future pest management strategies. This wording streamlines the points and emphasizes the contribution to genetic documentation and pest control. The integration of molecular and morphological methods provides a comprehensive framework for identifying and managing this urban pest.

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Disclaimers

The views and conclusions expressed in this article are solely those of the author and do not necessarily represent the views of their affiliated institutions. The author is responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal Care and Handling Techniques were approved by the University of Animal Care Committee.

Conflict of interest

The author declares that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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