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Forensic Entomology in Jeddah: Molecular Identification of Emerging Insect Strains

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ABSTRACT

Background: The precise identification of forensic insects is one of the most important aspects in forensic entomology since it is the basis for determining post-mortem intervals (PMI) and providing other valuable forensic evidence in criminal investigations.

Methods: This study demonstrates the utility of DNA barcoding in accurately identifying new strains of forensic insects collected from the Jeddah Governorate.

Result: High similarity percentages ranging from 98.74% to 99.68% were observed, underscoring the precision of molecular identification. The identified species included members of the Diptera (flies) and Coleoptera (beetles) orders, commonly associated with forensic cases. Notably, *Phoridae* sp. (Forensic_JED_21) was identified with 98.74% similarity, highlighting their role in providing valuable post-mortem interval (PMI) estimations. *Physiphora alceae* was identified twice (Forensic_JED_22 and Forensic_JED_24) with similarities of 99.22% and 99.07%, respectively, demonstrating the robustness of DNA barcoding. *Philonthus discoideus* (Forensic_JED_23) and *Physiphora demandata* (Forensic_JED_25) were identified with 99.68% similarity, confirming the presence of these species in the Jeddah region. Phylogenetic analysis using MEGA11 placed these strains in well-defined clades, confirming their taxonomic accuracy and evolutionary relationships. These findings align with previous research on the genetic diversity of forensic insects, adding crucial data to forensic entomology databases. This study enhances the forensic entomology framework by providing accurate species identification, essential for legal investigationsand supports the development of region-specific forensic tools through the addition of new genetic data from Jeddah.

Key words: 16S rRNA, Forensic insects, From Jeddah Governorate, Molecular identification.

INTRODUCTION

Forensic entomology relies heavily on identification of forensic insects, which is essential in criminal investigations for determining post-mortem intervals (PMIs) and obtaining other essential evidence. It is mainly insects from the orders Diptera and Coleoptera that colonize decomposing remains, especially flies. Their predictable succession patterns on carrion make them invaluable in forensic investigations (Amendt *et al.,* 2011). Conventional methods for of identifying forensic insects often rely on morphological characteristics, which can be challenging and time-consuming, especially when dealing with immature stages or closely related species (Sperling *et al.,* 1994). It is also important to note that morphological identification requires significant taxonomic expertise and may not always provide the resolution required for precise species identification (Wells and Stevens, 2008). A revolutionary change has occurred in forensic entomology as a result of recent advances in molecular techniques. For the accurate identification of insect species, DNA barcoding, in particular, has proven to be an extremely powerful tool. This technique involves sequencing a short, standardized region of the mitochondrial DNA (mtDNA) cytochrome c oxidase subunit I (COI) gene, which has been shown to provide reliable species-level identification in a large number wide range of taxa (Hebert *et al.,* 2003). DNA barcoding can distinguish between species with high morphological similarity and can be applied to all life stages of insects, including eggs, larvae and pupae (Cywinska *et al.,* 2006).

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In forensic entomology in Saudi Arabia, specifically in the Jeddah Governorate, there is a need for comprehensive studies that utilize molecular techniques to identify local insect fauna associated with forensic cases. To improve the accuracy and applicability of forensic entomological methods, region-specific research is needed to study the unique assemblage of forensic insect species that exist in the region (Al-Qahtni *et al*., 2020).

The purpose of this study is to use molecular identification techniques to characterize new strains of forensic insects collected from the Jeddah Governorate. Our goal is to establish a reference library of genetic sequences for the local forensic insect fauna, enhancing the accuracy and reliability of species identification in forensic investigations through DNA barcoding. Besides adding to the global database of forensic insect DNA

barcodes, this study provides insight into the biodiversity and ecological dynamics of forensic insects in the Jeddah region. Molecular identification methods are incorporated into Saudi Arabia's forensic entomology practices, thereby filling a gap in current research and improving forensic accuracy. It will contribute to a broader understanding of forensic entomology in different ecological contexts and provide robust tools for legal investigations in the field of forensic science.

MATERIALS AND METHODS

DNA extraction of collected forensic insects

Insects were transferred to a 1.5 ml microcentrifuge tube and 180 ml of buffer ATL was added. After mixing 20 l of proteinase K by shaking, the tissue was incubated for one hour at 56°C until completely lysed. To the sample, 200 l of ethanol (96-100%) was added and mixed by vertexing for 15 seconds. The samples were transferred to the QIA amp Mini spin column (2 ml collection tube), centrifuged at 8000 rpm for 1 minuteand the filtrate was discarded. After adding 500 mL of buffer AW1, the sample was centrifuged for 1 minute at 8000 rpmand the filtrate was discarded. This was followed by centrifugation of 500 l of buffer AW2 at full speed (14,000 rpm) for 3 minutesand the collection tube was discarded. After adding buffer AE or distilled water to the sample, it was incubated at room temperature for 1 minute and centrifuged for 1 minute at 8000 rpm. All samples were stored at -70 or -80.

Polymerase chain reaction

For the PCR technique, universal primers forward primer 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and reverse primer 1492R (5′-TACGGYTACCTTGTGTTACGACTT-3′) were used in this study. Bacteria were identified by the 16S rRNA gene through PCR amplification using two universal primers.Forward primer 27F (5′-AGAGTTTGATCMTGGC TCAG-3′) and reverse primer 1492R (5′-TACGGYTACCTTG TTACGACTT-3′) of the 16S rRNA gene were amplified in a thermocycler (VeritiTM 96-well Thermal Cycler, Applied Biosystems, US). Each amplification was performed in 25 µl of 1× GoTaq_Green Master Mix (Promega, USA), including 2 µl of DNA template and 1 µl of forward and reverse primer (10 pmol). The amplification was done by heating the sample at 95°C for 5 min and 35 cycles of 94°C for 30 s, 50°C for 30 sand 72°C for 45 s, followed by a final extension step of 72°C for 10 min. The temperature was then set at 4°C for an infinite time as a final step.

Gel electrophoresis

To estimate the size of the molecules, 10 µl of a molecular weight marker was loaded in the first well. The gel was run for one hour, at 127 V in the electrophoresis power supply. By placing the suspension in a microwave oven for heating, 1X TAE buffer was used to dissolve 1% of the agarose powder. Ethidium bromide (EtBr) at a concentration of 0.1 g/ml was added to the agarose, then the gel tray was filled with agaroseand the comb was inserted at one end. The gel was removed from the comb after one hour and placed in an electrophoresis tank with 1X TAE buffer as running buffer. A 5:1 volume ratio of dye to sample was used to add the 5X loading dye to the samples. The gels were run for 60-90 minutes at 127 volts in horizontal gel apparatus. The Viber Lourmat Gel Imaging System was used to visualize and photograph DNA fragmentsand the molecular weight of DNA fragments was determined using DNA ladders. In TAE buffer (40 mM Tris-Acetate, 1 mM EDTA, pH 8.0), two microliters of DNA amplicon were electrophoresed at 100 V for 25 minutes for amplification of the DNA fragment. DNA ladders of 100 bp (Promega, USA) were used as markers.

Sequencing reaction

DNA sequencing was performed by Macrogen Company, Korea. BLAST was used to compare the sequences of the sample with those from the National Center for Biotechnology Information (NCBI) database.

Statistical analysis

LSD (least significant difference) was used to compare averages using SAS (1988). Laboratory toxicity results of bacteria against mosquito larvae were evaluated by LDP Line Software following Bakr (2005) guidelines.

RESULTS AND DISCUSSION

This study showed that DNA barcoding could be used to accurately identify forensic insects collected from Jeddah Governorate based on their molecular identities, which demonstrates the utility of DNA barcoding for the identification of forensic insects. Table 1 summarizes the identified species, their order, similarity percentages, accession numbersand assigned strain names. The sequencing results showed high similarity percentages ranging from 98.74% to 99.68%, indicating accurate species identification (Table 1 and Fig 1). These species belong to the Diptera (flies) and Coleoptera (beetles) orders, which are often found in forensic cases. Forensic investigations rely on DNA barcoding to identify forensic insectsand the results show the effectiveness of the technique. The high percentages similarity (over 98%) affirms the accuracy of the molecular identification process, supporting the reliability of DNA barcoding as reported in previous studies (Hebert *et al.,* 2003; Wells and Stevens, 2008). Phoridae sp. (Forensic_JED_21) was identified with a similarity of 98.74%. Phoridae, commonly known as scuttle flies, are frequently encountered in forensic cases due to their presence on decomposing remains (Disney, 2008). Their identification is crucial as they can provide valuable PMI estimates. *Physiphora alceae* was identified twice (Forensic_JED_22 and Forensic_JED_24) with similarities of 99.22% and 99.07%, respectively. This species is a member of the Diptera order, which is often the first to colonize decomposing bodies, making them essential for forensic analysis (Amendt *et al.,* 2011). *Physiphora alceae* has consistently been identified using DNA bar coding

Table 1: The new stains of collected forensic insects.

Species	Order	Similarity	Accession number	Strain name
Phoridae sp.	Diptera (Flies)	98.74%	PP150726.1	Forensic_JED_21
Physiphora alceae	Diptera (Flies)	99.22%	PP150727.1	Forensic_JED_22
Philonthus discoideus	Coleoptera (Beetles)	99.68%	PP150728.1	Forensic_JED_23
Physiphora alceae	Diptera (Flies)	99.07%	PP150729.1	Forensic_JED_24
Physiphora demandata	Diptera (Flies)	99.68%	PP150730.1	Forensic_JED_25

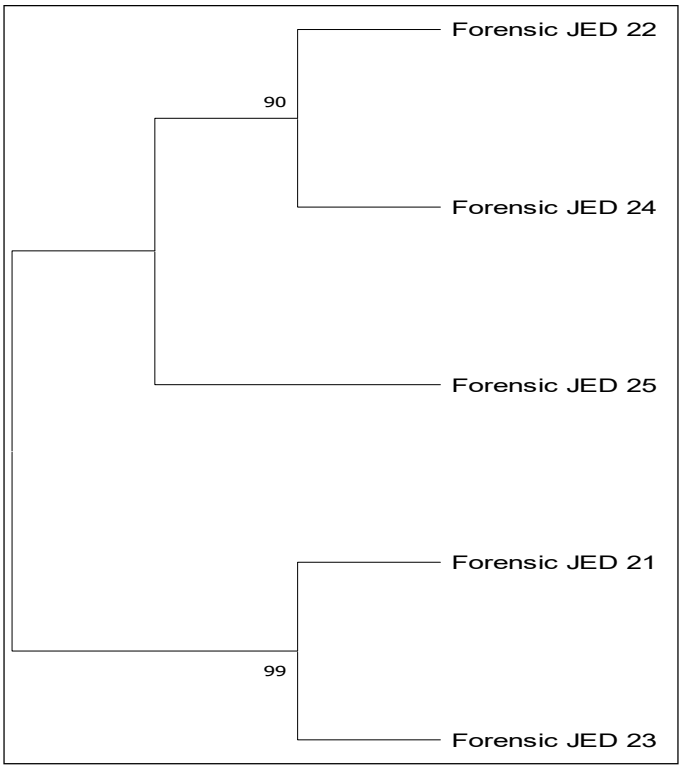

Fig 1: Phylogenetic analysis of new strains. The data was conducted using MEGA11 software.

techniques across a variety of samples, demonstrating the robustness of this method. According to the Forensic_JED_23, *Philonthus discoideus* (Forensic_JED_23), belongs to Coleoptera. Beetles are integral to the decomposition process, especially in later stagesand their identification can provide insights into the succession patterns of insects on remains (Smith, 1986). *Physiphora demandata* (Forensic_JED_25) was identified with a similarity of 99.68%, further confirming the presence of *Physiphora* species in the Jeddah region. The close genetic similarity between *Physiphora alceae* and *Physiphora demandata* underscores the importance of precise molecular tools in distinguishing closely related species.

Phylogenetic analysis using Multisequence Alignment Program (MEGA11) placed these strains in distinct clades with their relatives, as illustrated in the phylogenetic tree constructed. This placement confirms the taxonomic accuracy and evolutionary relationships of the identified strains. These findings are consistent with previous research demonstrating the genetic diversity and complexity within forensic insect species (Beeren *et al.,* 2015; Xiao *et al.,* 2012). This identification adds to the growing database of forensic entomology in the Jeddah Governorate and provides crucial information for forensic investigations. As a result of integrating molecular identification methods, this study contributes to the development of forensic entomology, providing reliable and accurate species identifications that can be used in legal investigations. By capturing genetic data from the Jeddah region, a significant gap in forensic insect fauna documentation is filledand region-specific forensic tools can be developed.

CONCLUSION

This study confirms the utility of DNA barcoding in forensic insect species with high similarity percentage between 98.74% - 99.68%. The identified species, which include members of the orders Diptera and Coleoptera are important for forensic investigations due to their association with decomposing remains. Notably, the identification of *Phoridae* sp., *Physiphora alceae, Philonthus discoideus*

and *Physiphora demandata* underscores the effectiveness and robustness of DNA barcoding. The phylogenetic analysis further validated the taxonomic accuracy and evolutionary relationships of these species. These findings are consistent with previous research on the genetic diversity within forensic insects, contributing valuable data to forensic entomology databases. By filling a critical gap in the genetic documentation of forensic insects in the Jeddah region, this study supports the development of region-specific forensic tools and enhances the overall framework for legal investigations. The integration of molecular identification methods thus proves essential for accurate species identification in forensic contexts.

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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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