BF-1826
[1-9]

RESEARCH ARTICLE Indian Journal of Animal Research

Molecular Identification of Forensic Insects Collected from Jeddah, Saudi Arabia

Somia Eissa Sharawi[1], Maryam M. Al Dagal[1], Tariq S. Alghamdi[2],
Naser Ahmed Alkenani[1], Manal E. Shafi[1], Hanan S. Alyahya[1], Jazem A. Mahyoub[1]

10.18805/IJAR.BF-1826

ABSTRACT

Background: Forensic entomology utilizes insects for legal and criminal investigations, particularly in determining the time of death when traditional medical parameters become ineffective after 72 h. Insects play a crucial role in Post-Mortem Interval (PMI) estimation and provide insight into crime scene events.

Methods: This study focused on the collection and molecular identification of forensic insects in Jeddah, Saudi Arabia. Rabbit carcasses were used as human analogs and were placed in specialized cages across five locations. Insects were collected twice daily for two weeks and identified based on their morphological and molecular characteristics. DNA extraction, polymerase chain reaction (PCR)and sequencing were used for genetic analysis.

Result: Phylogenetic analysis revealed significant genetic similarities among the collected species, indicating low genetic diversity and a stable population structure. The key species identified were *Sarcophaga dux, Wohlfahrtia nuba, Sarcophaga harpax, Chrysomya albiceps, Chrysomya megacephala, Chrysomya marginalis, Atherigona orientalis, Musca domestica* and *Hydrotaea capensis*. These findings support the use of genetic analysis for accurate species identification in forensics. Additionally, this study highlights the potential impact of climate change on insect populations, suggesting future research directions in forensic entomology. This study contributes to the understanding of forensic insect ecology in the Kingdom of Saudi Arabia and enhances forensic investigation methods.

Key words: 16S Rrna, Forensic insects, Molecular identification.

INTRODUCTION

Forensic entomology is a forensic science that uses insects in legal and criminal investigations (Bartkowska *et al.,* 2023). Several medical parameters, including livor mortis, algor mortis, rigor mortisand vitreous fluid, can be used by pathologists to estimate the time of death (Guo *et al.,* 2023). Usually, these methods are used within a few hours after death, become invalid after 72 hand are rarely used thereafter (Al-Shareef and Al-Mazyad, 2016). From a long time, forensic insects have become the most preciseand in some cases the only, tool for determining the time of death (Vasconcelos *et al.,* 2023). They are frequently used to clarify murders and suicides, identify both criminals and victimsand provide information about the location of death (Bartkowska *et al.,* 2023). According to him, stabbing was reported near rice fields. All workers were asked by the investigator to lay down their working tools (sickles) on the ground the day after murder. The blow flies were drawn to a single sickle with invisible blood traces. When confronted, the owner of the tool confessed to his crime and “knocked his head on the floor.” In 1999, Benecke and Leclercq confirmed the observed preference of certain blow flies for blood by discovering *Calliphora vomitoria* on a corpse 6 h after postmortem, laying eggs in the deceased’s blood (Benecke, 2001). In addition to medical and legal experts, sculptors and painters have closely observed the decomposition of human bodies, considering the effects of feeding maggots (Benecke, 2001). Early documents depicting maggots on corpses date back to the Middle Ages, which involved woodcuts in the 15th century and intricately cut ivory carving in the 16th century (Benecke and Leclercq, 1999). Such artwork displays an insect-mediated pattern of body mass reduction, specifically the early skeletonization of the skull and the reduction of internal organs, with large portions of the skin remaining intact (Benecke, 2001). Orfila, a famous French medical doctor, observed a significant number of exhumations in 1831 (Orfila 1835). He recognized that maggots play a crucial role in corpse decomposition (Benecke, 2001). French Dr. Bergeret provided the first modern forensic entomology case report to estimate the post-mortem interval (PMI) in

[1]Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia.
[2]Department of Biology, Faculty of Sciences, Al-Baha University, Al-Baha, Saudi Arabia.

Corresponding Author: Somia Eissa Sharawi, Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia. Email: sesharawi@kau.edu.sa

How to cite this article: Sharawi,S.E., Dagal, M.M.A., Alghamdi, T.S., Alkenani, N.A., Shafi, M.E., Alyahya, H.S. and Mahyoub, J.A. (2024). Molecular Identification of Forensic Insects Collected from Jeddah, Saudi Arabia. Indian Journal of Animal Research. 1-9.doi:10.18805/IJAR.BF-1826
Submitted: 05-06-2024 Accepted: 25-09-2024 Online:20-12-2024

1855, in which the case dealt with blow fly pupae and larval moths (Bergeret, 1855). Despite Bergeret's profession as a hospital physician, his interest in cadaver studies was evident. For example, that the corpse he examined resembled those observed in other places (*e.g.,* in the hot and dry lands on the cemetery of the Capucins of Palerme or in Toulouse) (Benecke, 2001).

In forensic entomology, the minimum post-mortem interval (PMI, min), or the time since the first insect colonization, is determined by determining the age at which insect stages develop on human remains and analyzing successive patterns, including pre-appearance (Matuszewski and Mêdra-Bielewicz, 2016), arrival, residency (Matuszewski *et al.,* 2011)and departure of insects from the carcass. Nonetheless, it can be applied to many more questions and areas. for instance, by providing valuable hints for cadaver relocation or crime scene manipulation (Matuszewski *et al.,* 2013). It has also been demonstrated that the gut and tissue contents of larvae and pupae can provide valuable information for the investigation of sexual crimes, including rape (Chamoun *et al.,* 2020). Specifically, the victim is discovered in an advanced state of decomposition (Clery, 2001). Alternatively, genotyping of human DNA can be used to identify the food source the deceased consumed during their lifetime (Di Luise *et al.,* 2008) or to detect drugs consumed by the deceased (Bugelli *et al.,* 2017). In entomotoxicological studies, fly tissues can be used to detect alcohols (such as ethanol), drugs (*e.g.,* barbiturates, benzodiazepines, opioidsand phenothiazine), metals (such as thioridazine, antimony, barium, cadmium, leadand mercury)and pesticides (e.g., malathion and parathion) (Gosselin *et al.,* 2011). However, genetics and toxicology can be determined using flies in different ways depending on the species, their developmental stages, feeding activities, the environment, insect sampling techniques, specimen numbers and frequencyand insect killing and preserving methods (Bambaradeniya *et al.,* 2023). Moreover, with climate change and the further spread of invasive species, insect infestations and new species will become more common in the futureand the assessment of neglect can be performed by analyzing the patient's fauna (Lutz *et al.,* 2021). Building on prior research, this study aimed to collect and conduct molecular identification of forensic insects in Jeddah, Saudi Arabia.

MATERIALS AND METHODS

Animal carcasses used

Five positions were used to collect forensic insects (Al-Hamdaniya 3, Al-Safa 8, Al-Samer 4, Al-Rehab 2and Abraq AL-Rughama 3). Domestic rabbits were purchased from the Amazon store for domestic animals at Hira Street in Jeddah city and were used as mammals closer to a human corpse. Forensic insects were collected from five different locations in Jeddah city (Al-Hamdaniya 3, Al-Safa 8, Al-Samer 4, Al-Rehab 2and Abraq AL-Rughama 3) using a dead rabbit (killed using formaldehyde)and there was no control group. Rabbit carcasses were distributed in special cages to collect forensic insects (Fig 1). Insect samples were collected every two days for two weeks and saved in formaldehyde 70% for further identification.

Experimental cages and collected insects

Special cages were designed to place rabbit carcasses. They were made of acrylic panels with dimensions of 100 cm × 50 × 50 cm^3. Two holes were made on the upper side of the box (lid) to install lantern traps (Fig 2). These are specialized traps for collecting flies that were obtained by direct purchase from a local market with some modifications, where the trap was cut from the bottom and a cloth basket was installed to facilitate insect collection. Insects were collected in small boxes and data on the day of collection were recorded (Fig 2). To avoid the entry of

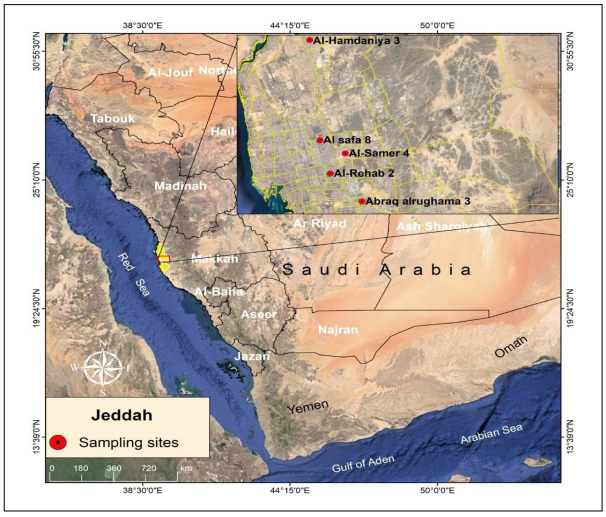

Fig 1: Locations of collecting forensic insects.

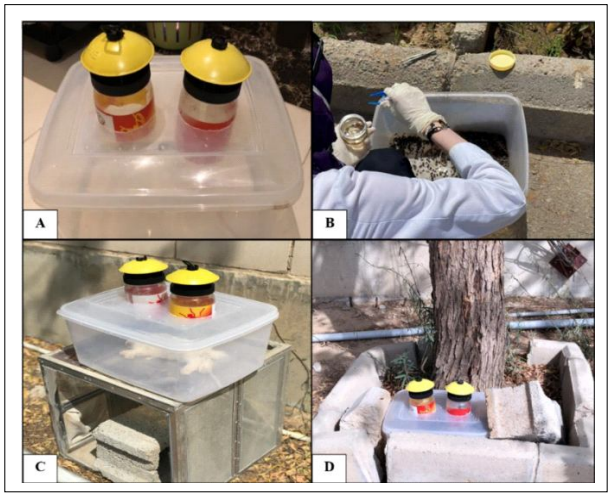

Fig 2: A) Acrylic cages. B) Collecting insects in small boxes. C) Cages used in the experiment. D) The modified final flight traps.

ants into the box, it was placed in a plastic basin filled with water. Samples were collected from previous cages and separated into nine different families based on morphological features into 9 different families (Fig 3). All samples were separated at the Dengue Mosquito Experimental Station (DMES) and sent to the Jeddah Governorate Municipality (JGM) for molecular identification. As indicated in the Fig 2.

Molecular identification

Thermocintific 200 prep kit was used for DNA extraction.The cytochrome c oxidase subunit 1 (cox1) gene was amplified using universal primers LCO1490 (5'-GGTCAA CAAATCATAAAGATATTGG-3') and HCO2198 (5'TAAACTT CAGGGTGACCAAAAAATCA-3'). In agarose gel electrophoresis: A 1% agarose powder solution was dissolved in 1X TAE buffer in the gell apparatus (MOLECULE-ON PS-M-300V Electrophoresis Power Supply, India). Agarose gel was prepared by adding a final concentration of 0.1 ìg/ml ethidium bromide (EtBr) from a 10 mg/ml stock solution in distilled water. The loading dye was added to the samples at a volume ratio of 5:1 according to the volume ratio of 5X loading dye to the sample. A horizontal gel apparatus was used to run the gel for 60-90 minutes at 127 volts. A UV transilluminator was used to visualize the DNA fragments and a Viber Lourmat Gel Imaging System was used to photograph the results. The molecular weights of the DNA fragments were determined using a DNA ladder.

Sequencing reaction

The Macrogen Company in Korea performed Sanger sequencing. Subsequently, the obtained sequences were compared with the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST).

Phylogenetic analysis

Phylogenetic analysis of the collected forensic insects was carried out based on sequences obtained from reference sequences in the NCBI database through BLAST. Evolutionary distances were calculated using the maximum composite likelihood methodand bootstrap support values (1000 replicates) were used to construct the phylogenetic tree.

RESULTS AND DISCUSSION

In our study, the resulting PCR products ranged from 500 bp to 700 bp (Fig 4). The molecular identification of collected forensic insects from Jeddah, Saudi Arabia led to the discovery of 20 new species within nine families, all of which were submitted to the GenBank database (NCBI), as shown in Table 1.

Phylogenetic analysis

Phylogenetic analysis of forensic insect species collected from Jeddah, Saudi Arabia, revealed significant genetic similarities and distinct groupings within the Diptera order (Fig 5). The following sections summarize the findings based on the similarity percentages and accession numbers provided in Table 1.

Sarcophaga dux

The species *Sarcophaga dux* showed high genetic similarity between the two strains analyzed: Forensic_JED_1 exhibited 97.97% similarity (Accession No. PP150706.1) and Forensic_JED_2 exhibited a slightly higher similarity of 98.12% (Accession No. PP150707.1). These values indicated a very close genetic relationship, suggesting minimal genetic divergence within the local population of this species.

Wohlfahrtia nuba

Three strains of *Wohlfahrtia nuba* and two vouchers were analyzed: Forensic_JED_3, with 97.14% similarity (Accession No. PP150708.1) and Forensic_JED_4, with 97.67% similarity (Accession No. PP150709.1) and Forensic_JED_5, with 97.65% similarity (Accession No. PP150710.1) and Forensic_JED_7 (voucher): 97.65% similarity (Accession No. PP150712.1). The close similarity percentages among these samples indicated a stable genetic makeup within this species, with vouchers confirming their genetic identity.

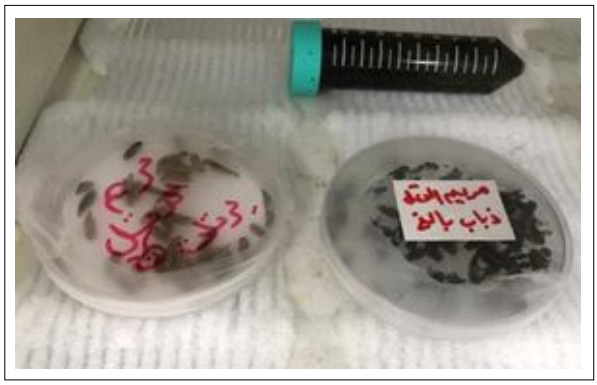

Fig 3: Collecting insects and saved in formaldehyde 70%.

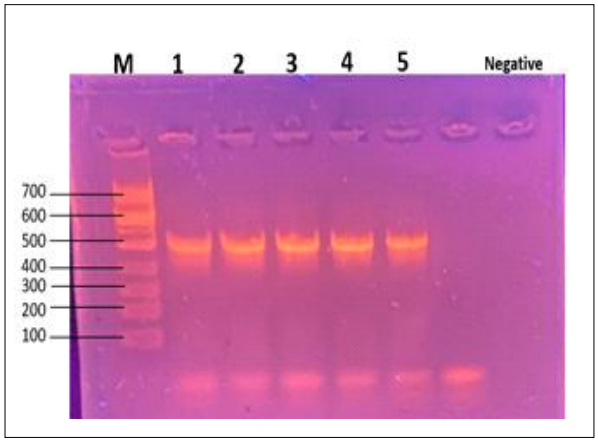

Fig 4: Agarose gel products.

Table 1: New strains of collected forensic insects.

Species	Order	Similarity	Accession number	Strain name
Sarcophaga dux	Diptera (Flies)	97.97%	PP150706.1	Forensic_JED_1
Sarcophaga dux	Diptera (Flies)	98.12%	PP150707.1	Forensic_JED_2
Wohlfahrtia nuba	Diptera (Flies)	97.14%	PP150708.1	Forensic_JED_3
Wohlfahrtia nuba	Diptera (Flies)	97.67%	PP150709.1	Forensic_JED_4
Wohlfahrtia nuba voucher	Diptera (Flies)	97.65%	PP150710.1	Forensic_JED_5
Sarcophaga harpax isolate	Diptera (Flies)	97.49%	PP150711.1	Forensic_JED_6
Wohlfahrtia nuba voucher	Diptera (Flies)	97.65%	PP150712.1	Forensic_JED_7
Chrysomya albiceps	Diptera (Flies)	99.38%	PP150713.1	Forensic_JED_8
Chrysomya albiceps	Diptera (Flies)	98.78%	PP150714.1	Forensic_JED_9
Chrysomya megacephala	Diptera (Flies)	99.38%	PP150715.1	Forensic_JED_10
Chrysomya megacephala	Diptera (Flies)	99.84%	PP150716.1	Forensic_JED_11
Chrysomya marginalis	Diptera (Flies)	99.22%	PP150717.1	Forensic_JED_12
Atherigona orientalis	Diptera (Flies)	99.68%	PP150718.1	Forensic_JED_13
Atherigona orientalis	Diptera (Flies)	98.92%	PP150719.1	Forensic_JED_14
Musca domestica	Diptera (Flies)	97.21%	PP150720.1	Forensic_JED_15
Hydrotaea capensis	Diptera (Flies)	99.53%	PP150721.1	Forensic_JED_16
Hydrotaea capensis	Diptera (Flies)	99.07%	PP150722.1	Forensic_JED_17
Musca domestica	Diptera (Flies)	98.20%	PP150723.1	Forensic_JED_18
Musca domestica	Diptera (Flies)	99.37%	PP150724.1	Forensic_JED_19
Musca domestica	Diptera (Flies)	99.69%	PP150725.1	Forensic_JED_20

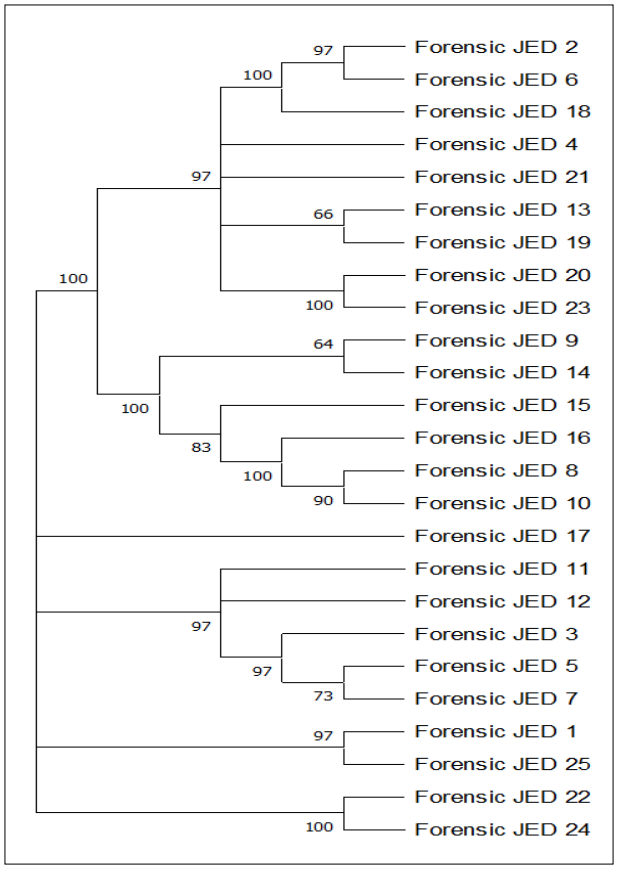

Fig 5: The phylogenetic analysis of collected forensic insects. The tree was generated by MEGA 11 software.

Sarcophaga harpax

The *Sarcophaga harpax* isolate showed a similarity of 97.49% (Accession No. PP150711.1), suggesting that it is genetically distinct but closely related to the other Sarcophaga species analyzed.

Chrysomya albiceps

Forensic_JED_8 with 99.38% similarity (Accession No. PP150713.1) and Forensic_JED_9 with 98.78% similarity (Accession No. PP150714.1).

Chrysomya megacephala

Forensic_JED_10 with 99.38% similarity (Accession No. PP150715.1)and Forensic_JED_11 with 99.84% similarity (Accession No. PP150716.1).

Chrysomya marginalis

Forensic_JED_12 with 99.22% similarity (Accession No. PP150717.1). These high similarity values within the *Chrysomya* genus indicate a very low genetic variation, reflecting a closely related population.

Atherigona orientalis

Forensic_JED_13 with 99.68% similarity (Accession No. PP150718.1)and Forensic_JED_14, with 98.92% similarity (Accession No. PP150719.1). High similarity percentages indicate a genetically homogenous population of *Atherigona orientalis.*

Musca domestica

Several strains of *Musca domestica* were analyzed, showing variable similarities: Forensic_JED_15 with

97.21% similarity (Accession No. PP150720.1)and Forensic_JED_18, with 98.20% similarity (Accession No. PP150723.1)and Forensic_JED_19, with 99.37% similarity (Accession No. PP150724.1)and Forensic_JED_20, with 99.69% similarity (Accession No. PP150725.1). The variation in similarity percentages suggests genetic diversity within the *Musca domestica* population.

Hydrotaea capensis

Forensic_JED_16 with 99.53% similarity (Accession No. PP150721.1)and Forensic_JED_17, with 99.07% similarity (Accession No. PP150722.1). High similarity values indicate a homogenous genetic population of *Hydrotaea capensis*.

In general, phylogenetic analysis revealed that most species exhibited high genetic similarities within their respective groups, indicating low genetic diversity and a stable population structure in the Jeddah region. These data support the effectiveness of genetic analysis for accurate species identification in forensic investigations.

Morphological identification

The nine families we found in this study are described upon characteristic morphological keys according to (Alikhan *et al.,* 2018; Bharti and Singh, 2023; Khalaf and Mahmoud, 2023). There is a variability in the emerging time among different families, in which some of them have a faster rate of emerging within the first few days or even the first hours since discovering the carcasses at the crime scene. In contrast, other families have arrived at the latest stages of decomposition within the second week. In general, each species plays a crucial role in detecting post-mortem interval (PMI). The family *Calliphoridae* were identified as most species are shiny with metallic luster, generally green, blue, bronzeand black in color; the antenna in adult flies is three segments with hair or arista on the last segment (Bharti and Singh, 2023). The family *Sarcophagidae* has black and grey stripes on the thorax and a tessellated pattern on the abdomen, these flies do not appear metallic in appearance and the eyes are widely spaced (Bharti and Singh, 2023). The adult insects of family *Muscidae* measure approximately 7 to 10 mm in length, have a dull thorax and abdomen, the thorax is characterized by 4 black longitudinal stripes, pale abdomen sidesand multiple sterno-pleural bristlesand the scutellum's bottom is not hairy (Alikhan *et al.,* 2018). The apical section of the scutellum varies in color from reddish to brown (Ivorra *et al.,* 2021). In family *Fannidae*, the fronto-orbital plate and parafacial regions are covered in dense greyish pollinosity. The thorax is translucent yellow with black markings, while the wings are light brown with dark brown veins. The abdomen is black, elongated, depressedand flattenedand the legs are entirely black (Wei *et al.,* 2021). In males, white bordered eyes meet, female with one curled bristle on every side of the front, the 2nd vein is paler and more bent (Alikhan *et al.,* 2018). Family *Ulidiidae* (Acalyptrate) flies, mainly found in neotropical regions, typically ranging from small to medium in size, measuring between 2 to 14 mm, are easily identifiable by their uniquely patterned wings, from which the family derives its common name (Wallace, 2021). However, not all members possess this traitand it is not exclusive to them. Their head morphology varies but is generally higher than long. The chaetotaxy of the thorax also varies, as does the wing appearance, often featuring yellow or brown patterns such as stripes, bars, or spots. Female flies have modified terminalia, forming an oviscape and ovipositor (Wallace, 2021). They exhibit a color spectrum from yellow to black, with occasional blue or green iridescence. Additionally, their wings commonly display spotting (Marchiori, 2023). The family *Phoridae* (humped flies) is small flies that are gray to bluish brown, black, or dark yellow in color and are characterized by what resembles a hump-backed body, also they are distinguished by their presence on corpses and ability to jump and run (Khalaf and Mahmoud, 2023). It has just one antenna segment, narrow radial veins that end at the wing borderand long rigid legs, wings with distinct venation, noticeably thickened toward the fore border, with no cross veins, the veins run parallel to one another. Additionally, it causes inadvertent intestinal myiasis and is forensically significant (Alikhan *et al.,* 2018). Family *Cleridae*, most species in this family are adorned with bristly hairs and display vibrant colors on their bodies. Adults typically measure between 3 to 12 mm in length. Their head is often broader than the pronotum (the neck area), which in turn is narrower than the bases of the wings. This creates a distinctive narrowing between the head and the wing attachment points. The types of antennae found among these beetles vary (Byrd and Tomberlin, 2019). They are primarily predatory insects. For instance, the larvae of Trichodes rely entirely on grasshopper eggs or bee cells for sustenance (Ahmed *et al.,* 2023). Family *Dermestidae* are frequently referred to as skin beetles and consume bones and dried skin, also colonize cadavers during the dry phase of decomposition (Bharti and Singh, 2023). Dermestids are typically petite beetles, spanning from 2 to 12 mm in length. Their bodies are often rounded or ovaland they are adorned with scales that can create unique and vivid patterns. The larvae, which measure between 5 to 15 mm, typically feature dense tufts of long hair. This information is sourced from (Byrd and Tomberlin, 2019). Family *Staphylinidae* (rove beetle) have slender bodies and conspicuously short elytra which are square-shaped pads that emerge from the thorax, six to seven abdominal segments are exposed, as the membranous hindwings remain folded under and entirely concealed except during flight (Bharti and Singh, 2023). Adult beetles within this family exhibit considerable variation in size, spanning from 1 to 25 mm. However, many species attracted to carrion share a distinctive shape, facilitating easy identification at the family level. This arrangement gives the rove beetle the appearance of being divided into four sections. The head, thoraxand elytra constitute the first three sections, each approximately equal in size. The fourth section comprises

the exposed abdomen, which is roughly equivalent in size to the combined mass of the first three sections (Byrd and Tomberlin, 2019).

Molecular identification

Over the decades, multiple molecular techniques have been extensively used to identify numerous significant forensic insect species. Despite the advantages and disadvantages of these techniques, they are considered the most potent tools applied by many scientists. In this study, two *Physiphora alceae* species were authenticated for the first time in Jeddah city and documented in the GeneBank. They are now associated with accession numbers of PP150727.1 and PP150729.1. Compared to a study conducted by Tuccia *et al.* (2021), two *Physiphora alceae* were discovered in a decapitated corpse in Northern Italy and submitted under accession numbers MH686505 and MH686506. On the other hand, two *Sarcophaga dux* species were observed and documented in the GenBank with the accession numbers of PP150706.1 and PP150707.1. While Wang *et al.* (2023) also identified this species in Hainan Island of China and recorded in GenBank under accession no OQ519760. Species *Wohlfahrtia nuba* were registered in the GenBank under accession no PP150708.1 and PP150709.1 and *Wohlfahrtia nuba voucher* with accession numbers of PP150710.1 and PP150712.1. In relation to a study conducted by Simin *et al.,* (2024) in Serbia and Western Balkans region which they reported the first molecular evidence of *Wohlfahrtia* species: *Wohlfahrtia magnifica* submitted to GenBank under accession numbers (MT027108 - MT027114). Two species *Chrysomya albiceps* were submitted in the GenBank under accession no PP150713.1 and PP150714.1. In comparison to a study conducted by Tembe *et al.,* (2021) in South Africa in which they addressed a new strain of *Chrysomya albiceps isolate S-F2* with accession number MZ476266.1. Two species *Chrysomya megacephala* were submitted in the GenBank under accession no PP150715.1 and PP150716.1. In comparison to two *Chrysomya megacephala* were recognized by Shinde *et al.* (2021) from the Nagpur region of Maharashtra, India with accession numbers MT502110 and MT502109. While Wang *et al.,* 2023 also identified this species in Hainan Island of China and recorded in GenBank under accession no OQ519766. One species *Chrysomya marginalis* were submitted in the GenBank under accession no PP150717.1. In comparison to a study conducted by Tembe *et al.* (2021) in South Africa in which they addressed a new strain of *Chrysomya marginalis isolate S-B1* with accession number MZ476261.1. Two species *Atherigona orientalis* were approved in the GenBank with accessions no PP150718.1 and PP150719.1. In relation to a study conducted by Ouma *et al.* (2023) in Kenya in which they registered multiple strains of *Atherigona orientalis* with accession numbers (OQ835541– OQ835545 and OQ832304). Four *Musca domestica* strains were assigned in this study with accession numbers PP150720.1, PP150723.1, PP150724.1, PP150725.1. Compared to research published by Samerjai *et al.,* (2020) in Thailand were two *Musca domestica* strains submitted in the GenBank under accession numbers MH765542 and MH765543. In addition to another research in Hainan Island of China by Wang *et al.,* (2023) they recognized the same species with accession no OQ519776.

Two *Hydrotaea capensis* strains were assigned in this study with accession numbers PP150721.1 and PP150722.1. Also, *Hydrotaea capensis voucher* was identified by Giordani *et al.,* (2019) in Italy under accession number MH921578. One *Phoridae sp.* strain deposited in the GenBank with accessions no PP150726.1. A study performed by Bukowski *et al.* (2022) in the southern Atlantic Forest of United States they mentioned *Phoridae sp. Voucher* with accession numbers OM549317, OM595416.1, OM706958. Two *Physiphora alceae* submitted with accession numbers PP150727.1 and PP150729.1. In relation to Dähn *et al.* (2024) who submitted *Physiphora alceae* strain with accession no PP110220 in Western Palearctic. One *Philonthus discoideus* recorded in the GenBank under accession number PP150728.1. As well as it was identified by Hendrich *et al.* (2015) in Germany with accession no. KM442117. One *Sarcophaga harpax isolate* submitted with accession number PP150711.1. As well as Kim *et al.* (2014) identified it in China *Sarcophaga harpax isolate Ha1* and *2* and submitted in the GenBank with accession numbers JX861474 and JX861475. Lastly, one *Physiphora demandata* submitted in the GenBank under accession number PP150730.1. Compared to a study performed by Hebert *et al.* (2016) and recognized *Physiphora demandata voucher* in the GenBank under accession number KR764557.

Phylogenetic analysis

The phylogenetic analysis of forensic insects collected from Jeddah, Saudi Arabia, provides valuable insights into the genetic relationships and diversity of species significant in forensic investigations. By examining the molecular similarities and accession numbers of various strains, we can infer their potential utility in forensic entomology. *Sarcophaga dux* (Order: Diptera) was identified among the collected specimens, with two strains (Forensic_JED_1 and Forensic_JED_2) showing high similarity percentages of 97.97% and 98.12%, respectively. These high similarity values suggest a close genetic relationship within the species, indicating low genetic variability. This finding aligns with previous studies that have highlighted the importance of *Sarcophaga* species in forensic investigations due to their predictable colonization patterns on decomposing remains, which aids in the estimation of post-mortem intervals (PMI) (Singh *et al.,* 2018). *Wohlfahrtia nuba*, another species in the Diptera order, exhibited moderate genetic diversity with similarity percentages ranging from 97.14% to 97.67% across four strains (Forensic_JED_3,

Forensic_JED_4, Forensic_JED_5 and Forensic_JED_7). This variation might be due to environmental or geographical factors influencing genetic diversity. *Wohlfahrtia nuba* is known for its forensic relevance in arid regions, as its presence and development rates are well-documented, contributing to accurate PMI estimates (Martinez *et al.,* 2016). *Chrysomya* species, specifically *Chrysomya albiceps* and *Chrysomya megacephala*, demonstrated very high genetic similarities. Strains of *Chrysomya albiceps* (Forensic_JED_8 and Forensic_JED_9) showed similarities of 99.38% and 98.78%, while *Chrysomya megacephala* (Forensic_JED_10 and Forensic_JED_11) exhibited similarities of 99.38% and 99.84%. These high similarities indicate strong genetic conservation, making these species reliable for PMI estimation due to their consistent colonization patterns and rapid development (Williams and Villet, 2014). *Atherigona orientalis* and *Musca domestica* also displayed high genetic similarity among their respective strains. *Atherigona orientalis* strains (Forensic_JED_13 and Forensic_JED_14) had similarities of 99.68% and 98.92%, while *Musca domestica* strains (Forensic_JED_15, Forensic_JED_18, Forensic_JED_19and Forensic_JED_20) showed similarities ranging from 97.21% to 99.69%. These results are consistent with other research highlighting the ubiquity and forensic importance of these species due to their rapid life cycles and widespread distribution (Grzywacz *et al.,* 2017). *Hydrotaea capensis* strains (Forensic_JED_16 and Forensic_JED_17) exhibited high genetic consistency with similarities of 99.53% and 99.07%, respectively. *Hydrotaea capensis* is significant in forensic contexts as it is often associated with the late stages of decomposition, aiding in PMI estimation during advanced decay (Tomberlin *et al.,* 2012).

CONCLUSION

In conclusion, the phylogenetic analysis of forensic insects from Jeddah, Saudi Arabia, reveals significant genetic similarities within species, suggesting their potential reliability for forensic applications. The high genetic similarity among strains of *Chrysomya*, *Musca*and *Atherigona* species reinforces their value in forensic investigations due to their consistent colonization patterns and developmental rates. Further research is needed to explore environmental factors influencing the genetic diversity of these species and validate their use in forensic casework across different regions.

ACKNOWLEDGEMENT

The present study was supported by the authors.

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.

Conflict of interest

The authors 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 or preparation of the manuscript.

REFERENCES

Ahmed, Z., Lalika, H., Khatri, I. (2023). First record of the genus *Trichodes herbst* (Coleoptera: Cleridae): Expansion range from Pakistan. Journal of Entomological Society of Iran. 43(4): 417-420.

Alikhan, M., Al Ghamdi, K., Mahyoub, J. A., Alanazi, N. (2018). Public health and veterinary important flies (order:Diptera) prevalent in Jeddah Saudi Arabia with their dominant characteristics and identification key. Saudi Journal of Biological Sciences. 25(8): 1648-1663.

Al-Shareef, L.A., Al-Mazyad, M.M. (2016). Insect faunal succession on decaying rabbit carcasses in urban area at Jeddah city, Kingdom of Saudi Arabia. Am. J. Sci. 12: 78-88.

Bambaradeniya, T. B., Magni, P. A., Dadour, I. R. (2023). A summary of concepts, procedures and techniques used by forensic entomologists and proxies. Insects. 14(6): 536.

Bartkowska, A., Mieczan, T., Płaska, W. (2023). Colonization of Artificial Substrates by Invertebrate Macrofauna in a River Eco system-Implications for Forensic Entomology. International Journal of Environmental Research and Public Health. 20(4): 2834.

Benecke, M. (2001). A brief history of forensic entomology. Forensic Science International. 120(2): 2-14.

Benecke, M., Leclercq, M. (1999). Ursprünge der modern angewandten rechtsmedizinisch-kriminalistischen Gliedertierkunde bis zur Wende zum 20. Jahrhundert. Rechtsmedizin.9: 41-45.

Bergeret, M. (1855). Infanticide, momification naturelle du cadavre. Ann Hyg Publique Med Leg. 4: 442-452.

Bharti, M., Singh, D. (2023). Insects in Forensic Investigations. In Insects as Service Providers. Singapore: Springer Nature Singapore. (pp. 165-182).

Bugelli, V., Papi, L., Fornaro, S., Stefanelli, F., Chericoni, S., Giusiani, M., Vanin, S., Campobasso, C. P. (2017). Entomotoxicology in burnt bodies: A case of maternal filicide-suicide by fire. International Journal of Legal Medicine. 131(5): 1299-1306.

Bukowski, B., Ratnasingham, S., Hanisch, P. E., Hebert, P. D. N., Perez, K., deWaard, J., Tubaro, P. L., Lijtmaer, D. A. (2022). DNA barcodes reveal striking arthropod diversity and unveil seasonal patterns of variation in the southern Atlantic Forest. PloS One. 17(4): e0267390.

Byrd, J.H., Tomberlin, J.K. (2019). Forensic entomology: the utility of arthropods in legal investigations, 3rd edition. Boca Raton: CRC Press. pp 1- 620.

Chamoun, C. A., Couri, M. S., Garrido, R. G., Moura-Neto, R. S., Oliveira-Costa, J. (2020). Recovery andidentification of human Y-STR DNA from immatures of Chrysomya albiceps (Diptera: Calliphoridae). Simulation of sexual crime investigation involving victim corpse in state of decay. Forensic Science International. 310: 110239.

Clery, J.M. (2001). Stability of prostate specific antigen (PSA)and subsequent Y-STR typing, of Lucilia (Phaenicia) sericata (Meigen) (Diptera: Calliphoridae) maggots reared from a simulated postmortem sexual assauit. forensic science international,120(1): 72-76.

Dähn, O., Werner, D., Mathieu, B., Kampen, H. (2024). Large-Scale Cytochrome C Oxidase Subunit I Gene Data Analysis for the Development of a Multiplex Polymerase Chain Reaction Test Capable of Identifying Biting Midge Vector Species and Haplotypes (Diptera: Ceratopogonidae) of the Culicoides Subgenus Avaritia Fox,1955. Genes.15(3): 323.

Di Luise, E., Magni, P., Staiti, N., Spitaleri, S., Romano, C. (2008). Genotyping of human nuclear DNA recovered from the gut of fly larvae. Forensic Science International: Genetics Supplement Series. 1(1): 591-592.

Giordani, G., Grzywacz, A.,Vanin, S. (2019). Characterization and Identification of Puparia of Hydrotaea Robineau-Desvoidy, 1830 (Diptera: Muscidae) From Forensic and Archaeological Contexts. Journal of medical entomology. 56(1): 45-54.

Gosselin, M., Wille, S. M., Fernandez, M.delM., Di Fazio, V., Samyn, N., De Boeck, G., Bourel, B. (2011). Entomotoxicology, experimental set-up and interpretation for forensic toxicologists. Forensic science international. 208(3): 1-9.

Grzywacz, A., Hall, M. J., Pape, T., Szpila, K. (2017). Muscidae (Diptera) of forensic importance-an identification key to third instar larvae of the western Palaearctic region and a catalogue of the muscid carrion community. International journal of legal medicine. 131: 855-866.

Guo, Y., Li, L., Liao, M., Wang, J., Wang, Y. (2023). Thick quilt may severely impact the estimation of postmortem interval using forensic entomology-based methods-two case reports. Journal of Forensic and Legal Medicine. 95: 102501.

Hebert, P. D., Ratnasingham, S., Zakharov, E. V., Telfer, A. C.,Levesque-Beaudin, V., Milton, M. A., Pedersen, S., Jannetta, P., deWaard, J. R. (2016). Counting animal species with DNA barcodes: Canadian insects. Philosophical transactions of the Royal Society of London. Series B, Biological sciences. 371 (1702): 20150333.

Hendrich, L., Morinière, J., Haszprunar, G., Hebert, P. D., Hausmann, A., Köhler, F., Balke, M. (2015). A comprehensive DNA barcode database for Central European beetles with a focus on Germany: adding more than 3500 identified species to BOLD. Molecular ecology resources.15(4): 795-818.

Ivorra, T., Martinez-Sanchez, A., Rojo, S. (2021). Review of *Synthesiomyia nudiseta* (Diptera: Muscidae) as a useful tool in forensic entomology. International Journal of Legal Medicine. 135(5): 2003-2015.

Khalaf, M. Z., Mahmoud, S. K. (2023). Biological techniques and forensic evidences. Kut University College.

Kim, Y. H., Shin, S. E., Ham, C. S., Kim, S. Y., Ko, K. S., Jo, T. H., Son, G. H., Park, S. H., Hwang, J. J. (2014). Molecular identification of *necrophagous muscidae* and *sarcophagidae* fly species collected in Korea by mitochondrial cytochrome C oxidase subunit I nucleotide sequences. The Scientific World Journal. 2014:275085.

Lutz, L., Zehner, R., Verhoff, M. A., Bratzke, H., Amendt, J. (2021). It is all about the insects: a retrospective on 20 years of forensic entomology highlights the importance of insects in legal investigations. International Journal of Legal Medicine. 135(6): 2637- 2651.

Martinez, H., Taveras, R., Nuñez, F. (2016). Forensic importance of Wohlfahrtia nuba in arid regions. Journal of Forensic Sciences. 61(3): 728-730.

Matuszewski, S., Bajerlein, D., Konwerski, S., Szpila, K. (2011). Insect succession and carrion decomposition in selected forests of Central Europe. Part 3: Succession of carrion fauna. Forensic science international. 207(3): 150-163.

Matuszewski, S., Mądra-Bielewicz, A. (2016). Validation of temperature methods for the estimation of pre-appearance interval in carrion insects. Forensic Science, Medicineand Pathology. 12: 50-57.

Matuszewski, S., Szafałowicz, M., Jarmusz, M. (2013). Insects colonising carcasses in open and forest habitats of Central Europe: search for indicators of corpse relocation. Forensic science international. 231(3): 234-239.

Marchiori, C.H. (2023). Diversity of the Ulidiidae Family (Insecta: Diptera). Qeios.

Matuszewski, S., Bajerlein, D., Konwerski, S., Szpila, K. (2011). Insect succession and carrion decomposition in selected forests of Central Europe. Part 3: Succession of carrion fauna. Forensic science international, 207(3): 150-163.

Orfila, M.J.B., Lesueur, C.A. (1835). Handbook for the use at legal exhumations from corpses of every age found at the free air, in the water, in cesspoolsand in the manure, trans. v. E.W. Güntz. Barth. Leipzig. 292-294.

Ouma, L.O., Muthomi, J.W., Kimenju, J.W., Beesigamukama, D., Subramanian, S., Khamis, F.M., Tanga, C.M. (2023). Occurrence and management of two emerging soil-dwelling pests ravaging cabbage and onions in Kenya. Scientific Reports. 13(1): 18975.

Samerjai, C., Sukontason, K. L., Sontigun, N., Sukontason, K., Klong-Klaew, T., Chareonviriyaphap, T., Kurahashi, H., Klimpel, S., Kochmann, J., Saeung, A., Somboon, P., Wannasan, A. (2020). Mitochondrial DNA-Based Identification of Forensically Important Flesh Flies (Diptera: Sarcophagidae) in Thailand. Insects. 11(1): 2.

Shinde, A. M., Mahakalkar, A. L., Sapkal, H. P., Abd Al GAlil, F. M., Al Mekhlafi, F. A., Wadaan, M. A. (2021). Molecular identification of a forensically relevant blowfly species (Diptera: Calliphoridae) from the Nagpur region of Maharashtra, India. Entomological Research. 51(6): 315-320.

Simin, S., Tomanović, S., Sukara, R., Stefanov, M., Savović, M., Gajić, B., Lalošević, V. (2024). Long Time No Hear, Magnificent Wohlfahrtia Morphological and Molecular Evidence of Almost Forgotten Flesh Fly in Serbia and Western Balkans. Microorganisms. 12(2): 233.

Singh, B., Green, B. P., Benjamin, R. C. (2018). The role of Sarcophaga species in forensic investigations. Journal of Medical Entomology. 55(2): 195-202.

Tembe, D., Malatji, M. P., Mukaratirwa, S. (2021). Molecular identification and diversity of adult arthropod carrion community collected from pig and sheep carcasses within the same locality during different stages of decomposition in the KwaZulu-Natal province of South Africa. PeerJ. 9: e12500.

Tomberlin, J.K., Byrd, J.H., Wallace, J.R., Benbow, M.E. (2012). Hydrotaea capensis: A potential forensic indicator in late-stage decomposition. Journal of Forensic Sciences. 57(5): 1233-1236.

Tuccia, F., Giordani, G., Cattaneo, C., Mazzarelli, D., Vanin, S. (2021). First record of Physyphora alceae (Preyssler, 1791) (Diptera, Ulidiidae) from a forensic case in Northern Italy: description of immature stages, DNA barcoding and phylogenetic analysis. The European Zoological Journal. 88(1): 1071-1083.

Vasconcelos, S. D., Silva, A. M., Barbosa, T. M. (2023). Differential colonization of ephemeral resources by Sarcophagidae Diptera in the Brazilian Caatinga and its implications for forensic entomology in arid environments. Journal of Arid Environments. 214: 104996.

Wallace, C. (2021). An illustrated identification key to the genera of Ulidiidae (Diptera: Tephritoidea) of the United States and Canada. Canadian Journal of Arthropod Identification. 45: 94 pp. doi:10.3752/cjai.2021.45.

Wang, Y., Li, L., Hu, G., Kang, C., Guo, Y., Zhang, Y., Wang, J. (2023). Development of Necrobia ruficollis (Fabricius) (Coleoptera: Cleridae) under Different Constant Temperatures. Insects. 13(4): 319.

Wei, S., Wang, M., Zhang, D. (2021). One new species of Fannia (Diptera, Fanniidae) from Yunnan, China with a key to the Fanniafuscinata-group in China. Biodiversity data Journal. 9: e72444.

Williams, K. A., Villet, M. H. (2014). Forensic application of Chrysomya species. African Entomology.22(1): 45-49.