

Direct Injury, Myiasis, Forensics

First Report of Necrophagous Insects on Human Corpses in Riyadh, Saudi Arabia

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Abstract

Necrophagous species of insects provide useful complementary data to estimate the postmortem interval in forensic cases. Here, for the first time, we report on insect specimens collected from human corpses in Riyadh, Kingdom of Saudi Arabia. During the study, 14 beetle larvae were collected from the outdoor corpse (case report one) and five flies and seven beetles were collected from the indoor corpse (case report two). Sequencing was performed to study the mitochondrial DNA (mtDNA) as the prospective basis of an identification technique. The sequencing focused on a section of the cytochrome oxidase I encoding region of mtDNA. Two beetle species, *Dermestes frischii* (Kugelann) and *Dermestes maculatus* (De Geer) (Coleoptera: Dermestidae), and one fly species, *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae), were identified. These results will be instrumental in the implementation of a Saudi database of forensically relevant insects.

Key words: DNA barcoding, *Dermestes*, *Chrysomya*, Saudi Arabia

The study of necrophagous arthropods on human corpses plays an important role in forensic investigations. The importance of these insects in criminal investigations (a science denominated as forensic entomology), resides in the fact that they are the first to detect and to then locate a cadaver and are present in all stages of body decomposition (Byrd and Castner 2010). This means that they can be helpful in determining the time of death or postmortem interval (PMI), as well as any postmortem transfer and the presence of drugs (Introna and Campobasso 2000). Insects are found in various places and there are many differences between insects associated with human corpses in outdoor and indoor conditions (Byrd and Castner 2010). When using arthropods to estimate PMI, it is important to consider the factors which may affect the rates and patterns of insect invasion in the body, such as the environmental conditions, including temperature, humidity, and rainfall (Goff 1992).

Payne (1965) showed that many insects are associated with cadaver after death and their pattern of succession occurs in a sequence. Insects are attracted to a body immediately after death, and they colonize in a predictable manner. A corpse is a large food resource for a great many creatures, and supports a large and rapidly changing ecosystem as it decomposes. The cadaver progresses through a recognized sequence of decomposition stages, from fresh to skeletal, over time. During this decomposition, it goes through dramatic physical, biological, and chemical changes. Insects attracted to the cadaver differed according to the decomposition stage. Some are attracted directly by the corpse, as food source or an

oviposition medium, whereas others are attracted by the large aggregation of other insects they use as a food resource (Van den Oever 1976, Coe and Curran 1980, Henssge et al. 1995).

The family Calliphoridae constitute the most common type of insect evidence collected during criminal investigations (Keh 1985, Catts and Haskell 1990). They are often the first organisms to arrive at a corpse after death, attracted by the odor produced in the early stages of decomposition (Goddard and Lago 1985, Smith 1986, Wall and Warnes 1994), and their activity may accelerate the decay and disintegration of the body (Mann et al. 1990). Calliphoridae are common in The Kingdom of Saudi and are widely distributed throughout the country Arabia (Al-Ahmadi and Salem 1999, Al-Misned 2003, Dawah and Abdullah 2009). In addition, carrion beetles are important in terrestrial ecosystems, consuming dead mammals and promoting the recycling of organic matter into the ecosystem (Dekeirsschietter et al. 2013). The most common carrion beetle families include Cleridae, Dermestidae, Histeridae, Scarabaeidae, Silphidae, and Staphylinidae (Byrd and Castner 2010).

The key point for any taxonomic system is its ability to deliver accurate species identification, and in recent years one of the most popular methods of identification has been molecular taxonomy (Harvey et al. 2003). Several mitochondrial (mt) genes have been used in molecular taxonomy to identify the forensic species of the following genes: mt 16s rRNA gene (Li et al. 2010, Tang et al. 2012), mt cytochrome oxidase c subunit II gene (Ratcliffe et al. 2003, Ying et al. 2007), NADH gene (Zaidi et al. 2011), and mt

cytochrome oxidase c subunit I (mt COI) (Nelson et al. 2007, Aly and Wen 2013). According to Hebert et al. (2003), DNA barcoding accurately identified species in more than 95% of cases. The efficacy of DNA barcoding depends on selection of a suitable segment of DNA. Indeed, its mutation rate must be slow enough so that intra-specific variation is minimized but sufficiently rapid to highlight interspecific variation, it must be relatively easy to collect, and should have as few insertions or deletions as possible to facilitate sequence alignment (Hebert et al. 2003).

The work reported here sought to study and document insect species associated with human corpses in Saudi Arabia by identifying the collected species molecularly based on mt COI gene.

Materials and Methods

Case Reports

Case report 1: A 62-yr-old man was found dead in a field in Al-Manakh district, Riyadh, where the ambient temperature was about 42°C. The corpse was taken to the Institute of Legal Medicine at King Saud Hospital on 28th July 2015. Based on police investigations, the time of death was estimated to have been 14 d before the discovery of the body. The corpse was hidden by some planks of wood. The part of the body that was in indirect contact with sunlight was hard, stiff, and leathery. The corpse was at the decaying stage.

Case report 2: A 75-yr-old man was found dead in his house in Al-Massif district, Riyadh, where the ambient temperature was about 27°C. The corpse was taken to the Institute of Legal Medicine at King Saud Hospital on the 18th August 2015. Based on police investigations, the time of death was estimated to have been 61 d before the discovery of the body. The corpse was found on the ground next to an air-conditioning unit. The corpse was completely skeletonized.

Insect Collection

Samples were collected from two corpses during autopsy procedures by the second author. Beetle larvae were collected from the first corpse, which was located outdoors position, while flies (adult and larvae) and beetles were collected from the second corpse, which was located indoors. Corpse one was found dead in a field in Al-Manakh district, Riyadh (24° 36'53.04" N, 46° 46'19.52" E), where the ambient temperature was about 42°C. Corpse two, meanwhile, was found dead in his house in Al-Massif district, Riyadh (24° 46'7.72" N, 46° 41'4.01" E), where the ambient temperature was about 27°C. Samples were preserved in 70% ethanol in vials labeled with the date, time of collection, and the stage of decomposition at the time of collection. By using specialized taxonomic keys, the collected insects were classified into family and species levels and confirmed by the experts in the Insect Museum, College of Food & Agriculture Sciences. Then all specimens were identified to the lowest possible taxonomic level using molecular identification techniques.

DNA Extraction and Polymerase Chain Reaction

Ethanol-preserved specimens were washed in distilled water before isolation, allowed to dry, and crushed in sterile 1.5-ml microcentrifuge tubes. Whole genomic DNA was extracted from individual specimens using QIAGEN DNeasy Blood & Tissue Kit (Catalogue # 69504), as per the manufacturer's instructions.

The polymerase chain reaction (PCR) was used to amplify the barcoding region of the mitochondrial cytochrome oxidase subunit I

gene (COI) from each specimen using the primer (Cyto 1F) Forward sequence (5'- GGTCACAAATCCATAAAGATATTGG-3') and Reverse sequence (Cyto 1R) (5'- TAAACTTCAGGGTGACCAA AAAATCA -3') (Folmer et al. 1994); Zaidi et al. (2011) and Salem et al. (2015) approved the efficiency of the mt COI gene in the discrimination of forensically important species.

Polymerase chain reaction was performed in 20-μl reaction volume, comprising 4.0 μl of Solis BioDyne 5× FIREPol Master Mix (Reagents: FIREPol DNA polymerase, 5× Reaction Buffer B [0.4 M Tris-HCl, 0.1 M (NH₄)₂SO₄, 0.1% w/v Tween-20], 12.5 mM MgCl₂ [1× PCR solution—2.5 mM MgCl₂], and 2 mM dNTPs of each [1× PCR solution—200 μM dATP, 200 μM dCTP, 200 μM dGTP, and 200 μM dTTP]), 0.6 μl of each primer, 2.0 μl of DNA template, and finally 12.8 μl of nuclease-free water.

Polymerase chain reactions were carried out in an Applied Biosystems Veriti Thermal Cycler and the thermal cycling conditions involved were: an initial denaturation of 15 min at 95°C, followed by 35 cycles of 45 s at 95°C, an annealing step for 45 s at 51°C, and an extension for 45 s at 72°C. This was followed by a final extension of 10 min at 72°C. Agarose gel electrophoresis was performed to confirm amplification and 1.5% agarose gel was used to separate the PCR products.

DNA Sequencing and Sequence Analysis

The PCR products were sequenced using the same primers as were used in the PCR with the sequencing kit Big Dye terminator V3.1 (Applied Biosystems, Foster City, CA). The results were analyzed using an ABI 3700 DNA Analyzer (Applied Biosystems, Foster City, CA).

Sequences were trimmed to ~650 bp and were then aligned with their respective reference species using BioEdit Sequence Alignment Editor version 7.2.5 (Hall 1999), and with ClustalW Multiple Alignment program with the maximum number of 1,000 iterations (Thompson et al. 1994).

Results

A total of 26 specimens of adult insects and insect larvae were collected randomly from the two human corpses, whereas only dead beetle larvae were collected from corpse one, which had been found outdoors. Adult flies and beetles, as well as beetle larvae (both adult and larvae) were collected from corpse two. Molecular identification of the collected insects was attempted based on the mitochondrial cytochrome oxidase subunit 1 gene. A 500 bp fragment of COI was successfully sequenced for most of the specimens ($n = 17$). According to the studied gene, three species were identified: from corpse one, *Dermestes frischii* (Kugelann) ($n = 2$), and from corpse two, *Dermestes maculatus* (De Geer) ($n = 10$) and *Chrysomya albiceps* (Wiedemann) ($n = 5$). Sequences were identified using the online BLAST search tool to ensure that there was no misidentification. Intraspecific and interspecific variations were analyzed by BioEdit Sequence Alignment version 7.2.5. Sequences were aligned between individuals of the same species to study the intra-specific variability, and between individuals of different species to study the interspecific variability. All sequences were aligned with a standard reference recoded in the gene bank for each species.

Specimens belonging to *Dermestes frischii* were the least abundant, accounting for 11.76% of the identified species, although both obtained sequences showed 99% identity with the reference sequence (GenBank KM578824.1) from the GenBank database, Table 1 and Fig. 1.

Dermestes maculatus represents the most abundant species among the identified specimens, which account for 58.82%. The sequence of all 10 specimens showed 99% identity with the reference used for this species (GenBank HM909035.1) from GenBank, Table 2 and Fig. 2.

>The third species, *Chrysomya albiceps*, accounted for 29.41% of the identified species, and all the sequences showed 99% identity with the reference species (GenBank KJ193726.1), Table 3 and Fig. 3.

The results of the sequences revealed high interspecific variability between *D. frischii* and *D. maculatus*, ranging from 8–9% (Table 4).

Discussion

The major aim of this study was to identify some of the important forensic insects in Saudi Arabia based on molecular data. To date, studies attempting the molecular identification of forensically important insects present in Saudi Arabia have been limited (Abouzeid 2014; Mashaly 2016a,b) and, to our knowledge, this is the first study to identify forensic insects in Saudi Arabia based on the sequence of the mt CO1 gene where amplified region of the gene confirmed the identification of insects belonging to two different orders.

The high temperature in Saudi Arabia may affect the number of species detected on human corpses in this study where only three

species were detected. Similar results were obtained by Chittaro et al. (2005) where they collected only four to five species during the famous hot summer wave in 2003.

In the current study fly and beetles were observed on human corpse while corpses were in their decomposition stage, and this agree with the view of Schoenly and Reid (1987) and Tantawi et al. (1996) where they marked a decrease in arthropod richness and their dispersal from the carrion in the last stage of decomposition to be restricted to fly and beetle orders.

Our results confirm the presence of two species belonging to the family Dermestidae. Smith (1986) considered Dermestidae to be one of the most forensically important Coleoptera families (Smith 1986). Santos et al. (2014) also agreed that species belonging to the genus *Dermestes* are of considerable forensic importance (Santos et al. 2014). The results of the current study identify species belonging to the genus *Dermestes* (*D. frischii* and *D. maculatus*) both

Table 2. Percentage of difference between collected individuals of species *Dermestes maculatus* and a reference for the same species from GenBank (HM909035.1)

Sequence	HM909035.1	*1	*2	*3	*4	*5	*6	*7	*8	*9	*10
HM909035.1	–										
<i>D. maculatus</i> 1	1%	–									
<i>D. maculatus</i> 2	1%	1%	–								
<i>D. maculatus</i> 3	1%	1%	0%	–							
<i>D. maculatus</i> 4	1%	1%	1%	1%	–						
<i>D. maculatus</i> 5	1%	1%	1%	1%	1%	–					
<i>D. maculatus</i> 6	1%	1%	1%	1%	1%	0%	–				
<i>D. maculatus</i> 7	1%	1%	1%	1%	1%	1%	1%	–			
<i>D. maculatus</i> 8	1%	1%	1%	1%	1%	1%	1%	1%	–		
<i>D. maculatus</i> 9	1%	1%	1%	1%	1%	1%	1%	2%	2%	–	
<i>D. maculatus</i> 10	1%	1%	1%	1%	1%	1%	1%	1%	1%	1%	–

**D. maculatus*.

Table 1. Percentage of difference between collected individuals of species *Dermestes frischii* and a reference for the same species from GenBank (KM578824.1)[TQ3]

Sequence	KM578824.1	<i>D. frischii</i> 1	<i>D. frischii</i> 2
KM578824.1	–		
<i>D. frischii</i> 1	1%	–	
<i>D. frischii</i> 2	1%	1%	–

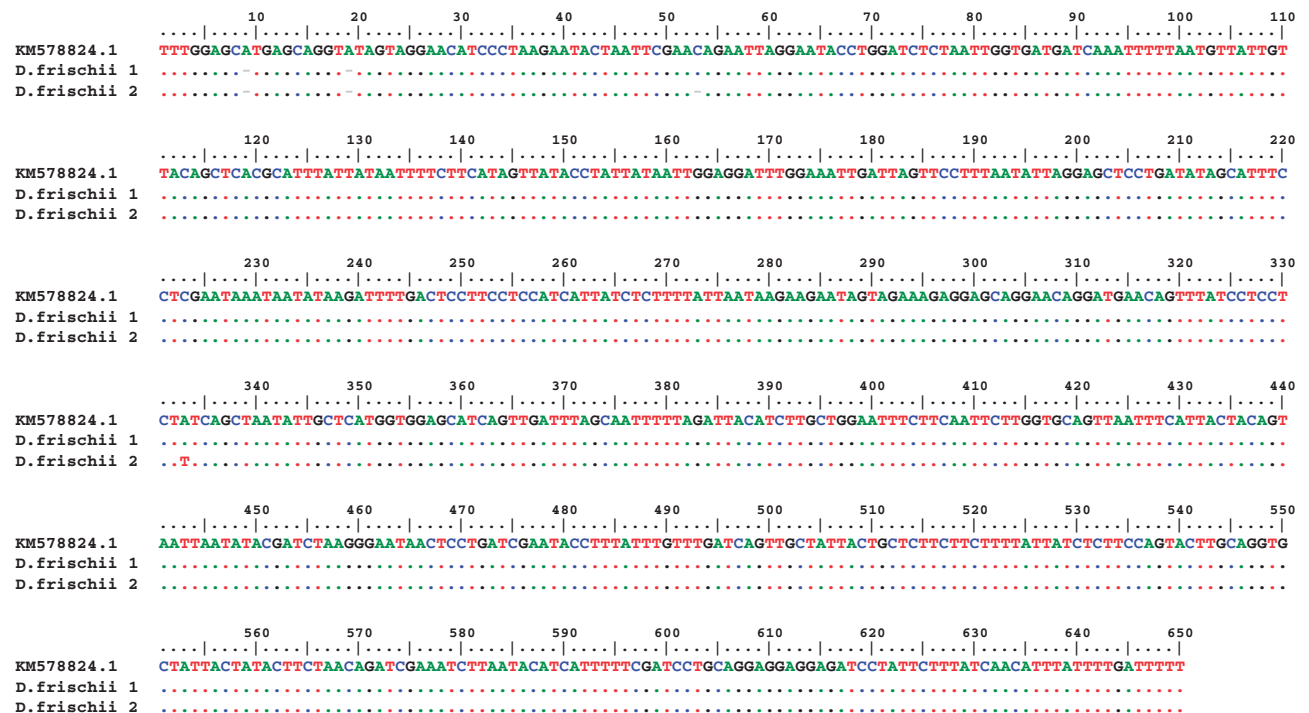


Fig. 1. Alignment of *D. frischii* sequences (1–2) with reference species sequence (KM578824.1) based on partial sequence of mitochondrial cytochrome oxidase subunit I (COI) gene.

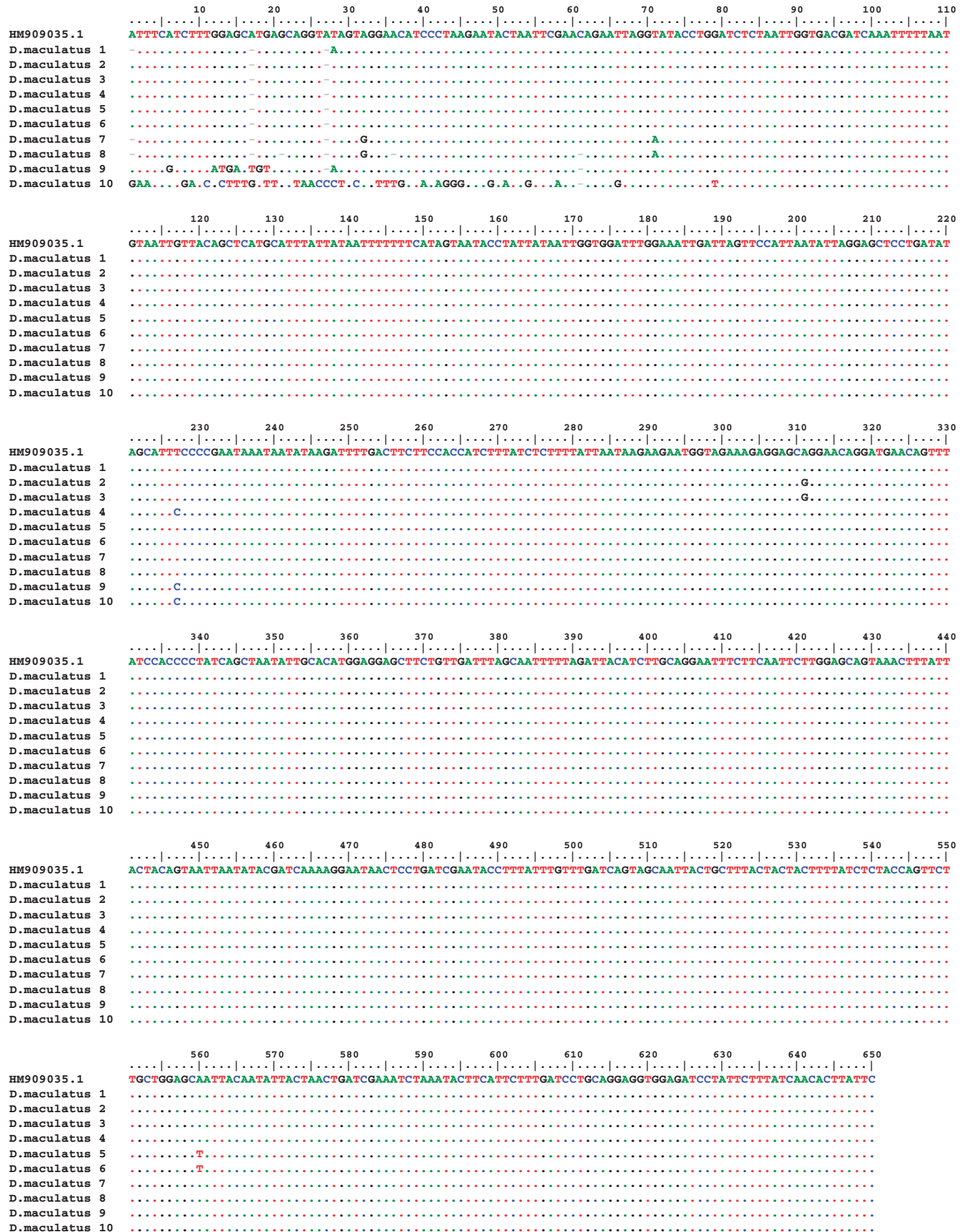
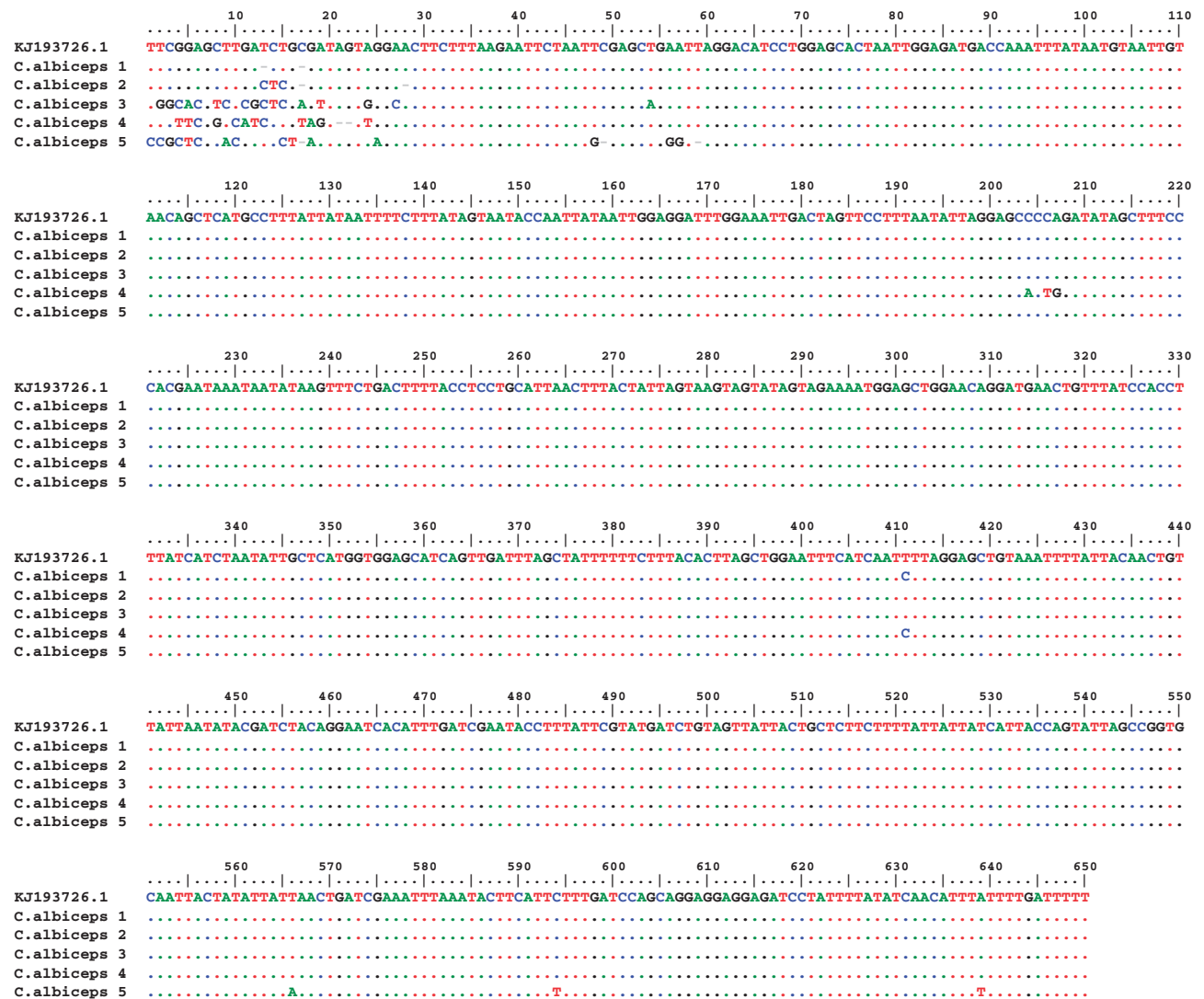


Fig. 2. Alignment of *D. maculatus* sequences (1–10) with reference species sequence (HM909035.1) based on partial sequence of mitochondrial cytochrome oxidase subunit I (COI) gene.

Table 3. Percentage of difference between collected individuals of species *Chrysomya albiceps* and a reference for the same species from GenBank (KJ193726.1)

Sequence	KJ193726.1	<i>C. albiceps</i> 1	<i>C. albiceps</i> 2	<i>C. albiceps</i> 3	<i>C. albiceps</i> 4	<i>C. albiceps</i> 5
KJ193726.1	-					
<i>C. albiceps</i> 1	1%	-				
<i>C. albiceps</i> 2	1%	1%	-			
<i>C. albiceps</i> 3	1%	1%	1%	-		
<i>C. albiceps</i> 4	1%	1%	1%	1%	-	
<i>C. albiceps</i> 5	1%	2%	2%	1%	2%	-

**Fig. 3.** Alignment of *C. albiceps* sequences (1–5) with reference species sequence (KJ193726.1) based on partial sequence of mitochondrial cytochrome oxidase subunit I (COI) gene.

known commonly as larder beetles. Larder beetles primarily develop on human cadavers in outdoor locations in areas with a dry climate, and the number of dermestid species on a single corpse never exceeds three (Charabidze et al. 2014). Furthermore, beetles belonging to genus *Dermestes* were dominant in this study, and this may be because the corpses were in the decomposition stage where beetles used to be the predominant insects on cadaver. This observation is

agreed with (Rodriguez and Bass 1983, Early and Goff 1986, Grassberger and Frank 2004).

Dermestes maculatus is the most abundant species in the present study, and it was also the most abundant beetle collected by Mashaly (2016b) in Riyadh, Saudi Arabia. The presence of *D. maculatus* in association with human remains was reported in all continents, including Asia (Lee et al. 2004), Africa (Galal et al. 2009),

Table 4. Percentage of difference among species of same genus: *D. maculatus* (Horizontal) and *D. frischii* (Vertical)

Sequence	*1	*2	*3	*4	*5	*6	*7	*8	*9	*10
<i>D. frischii</i> 1	8%	8%	8%	8%	8%	8%	8%	8%	8%	9%
<i>D. frischii</i> 2	8%	8%	8%	8%	8%	8%	8%	8%	8%	9%

**D. maculatus*.

Europe (Schroeder et al. 2002), North America (Valdes-Perezgasga et al. 2010), and South America (Battan et al. 2012). The second species belonging to genus *Dermestes* collected in this study was *D. frischii*, which account for 10.5% of collected specimens. Besides, Keshavarzi et al (2015) reported *D. frischii* on human corpse in south Iran (Keshavarzi et al. 2015). Moreover, Yones et al (2010) reported *D. frischii* adults on human left over parts at all decomposition stages (fresh, bloat, and dry stage), but larval first appearance was in the decay stage of the body decomposition (Yones et al. 2010).

The present study showed that *C. albiceps* account for 29.41% of the identified species. Galal et al. (2009) reported the presence of *C. albiceps* on exposed human left over parts in Egypt, and also explained as one of the most important carrion breeding fly in Egypt by Adham et al. (2001) and Attia (2002). It was the dominant dipteran forensic species in the study of Galal et al. (2009) and similar domination was stated by Grassberger and Frank (2004).

The intraspecific variability between individuals of the same species collected in this study and with a reference of the same species from GenBank was 0–2%, in accordance with the standard set by Hebert et al. (2003). According to the standard established by Hebert et al. (2003), sequence divergence between different species is expected to be greater than 3%, while the variations among individuals of the same species is less than 3%. The same results were obtained by Nakano and Honda (2015). The interspecific variations in this study were 8–9%, which agree with the standard established by Hebert et al. (2003).

Eight specimens could not be sequenced in this study, possibly due to DNA degradation. The same issue was faced by Mazzanti et al. (2010), who could not identify 22.6% of forensic insects based on CO1 and CO11 genes. Also, Campobasso et al. (2001) indicated that the insect's food may become prominent in the DNA sample and its contents could interfere with successful extraction of larval DNA. Mitochondrial DNA genes, such as CO1, are known to be more variable than nuclear genes, and thus, it is not surprising that sequence variation within and among species is present (Boehme et al. 2012). In this study the same gene (CO1) is used to identify within and between species where results showed an intraspecific variability ranging between 0–2% while the interspecific variations between *D. frischii* and *D. maculatus* were 8–9%.

In conclusion, we report, for the first time in Riyadh, Saudi Arabia, insects from two human corpses *Dermestes frischii*, *Dermestes maculatus* (Coleoptera), and *Chrysomya albiceps* (Diptera). Also, this study supported the efficiency of mt CO1 gene sequencing within forensically important insects collected from Saudi Arabia.

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