



First survey of forensically important insects from human corpses in Shiraz, Iran



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ABSTRACT

The presence of insects on human cadavers has potential judicial value in medicolegal cases. This research emphasized the important role of insects in postmortem decomposition. It was conducted to investigate the composition and abundance of insects from human corpses during autopsies in legal medicine. It was implemented in the city of Shiraz, south Iran. Insects associated with human corpses were carefully collected and put into labelled vials. They were then identified using valid taxonomic keys. Fifteen outdoor (67%) and indoor discovered cadavers were examined. All but one was covered at the time of discovery. From these several species of entomofauna played important roles in the minimum postmortem interval (minPMI) estimate. Insects included the orders of Diptera and Coleoptera. Overall, 14 different species of arthropods were identified. Within Diptera, 2 families of Sarcophagidae and Calliphoridae were present in 73% of the cases with *Calliphora vicina* Robineau-Desvoidy and *Chrysomya albiceps* Wiedemann accounting for about half of the cases. The latter family members, Calliphoridae, were more frequently (52%) collected in autumn and winter. Only 4/15 outdoor cadavers had beetles. Four species of Coleopterans; namely *Dermestes frischii* Kugelann, *Nitidula flavomaculata* Rossi, *Creophilus maxillosus* Linnaeus and *Saprinus chalcites* Illiger; were recorded for the first time from 3 corpses in Iran. The presence and diversity of different insects on human corpses could contribute to the advancement of forensic entomology knowledge and the refined estimates of minPMI in medicolegal cases.

1. Introduction

Forensic medical entomology focuses on provision of evidence from interactions between insects and deceased or abused humans. It pertains to the use of insects and other arthropods found on human cadavers in order to estimate the minimal time elapsed since death, or the minimum postmortem interval (minPMI). This is the period of time between insects' colonization of human corpses and their discovery by the investigators.¹ The advantage of arthropods' manipulation in legal research, a science denominated as forensic entomology, resides in the fact that insects as the first ones to detect and find a cadaver are present at all stages of body decomposition.² Insect specimens sampled at death scenes could thus be used to determine the minPMI, season of dying, presence of toxins, or corpse relocation.^{2–4}

After death a human or animal cadaver gradually undergoes five different (fresh, bloat, decay, post-decay, and skeletal) basic phases of change.⁵ Two dominant insect groups including Diptera (flies) and Coleoptera (beetles, the largest arthropod group) are also the most important orders in forensic entomology.² Their identification and life cycle characteristics have some privileges in medicolegal applications. Numerous different species of adult female flies normally find and lay their eggs (oviposit) or larvae on fresh carcasses as they require moist nutritious crevices to breed successfully.⁶ The other species-rich order, the carrion-colonising beetles, is mostly associated with the late stages of decomposition.⁷ This group is most often under-represented for various reasons.⁸

Insects colonize the corpse in a predictable regularity; as Calliphoridae (or blowflies) family are found in the early stages of body

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decay.² They are thus useful in the estimation of the minPMI. Forensic dipterologists, wherever possible, utilize evidence from early corpse colonizers, which comprise carrion-colonising species of fleshflies (Diptera: Sarcophagidae) and blowflies.^{6,7} Fleshflies could provide minPMI estimates as they lay live larvae (larviparous) as maggots (worm-like fly larvae) which start feeding immediately on the corpse and contribute to its putrefaction and decomposition.

Every forensic entomology case has its own very specific natural history which merits careful consideration and explanation.⁹ Moreover, the use of the entomological method to determine the approximate time elapsed since death consists of two main methods; the estimate based on the oldest available insects developed on the body (minPMI) and one based on the succession patterns.¹⁰ Depending on species and ambient temperature, this latter development needs up to several weeks if the weather is cold enough.

Insects are found in almost all habitats at different times. There are numerous disparities between insects associated with human or animal remains in outdoor/indoor locations,^{11–13} open/forest habitats,³ high/low elevations,¹⁴ cold/warm seasons,^{4,15} shaded/sunlit,¹⁶ buried/exposed,^{17,18} intact/injured,¹⁹ and small or large cadavers.^{20,21} These changes could also be coupled with the geo-climatic differences.²² When considering insects to estimate minPMI, it is crucial to note that a wide range of abiotic variables including temperature, humidity, precipitation, wind speed, drugs, and toxins can affect the rate of insect invasion on the body and the rate of postmortem decay.^{23,24}

Reports on animal and human carcasses have revealed that insect species composition and development vary according to the biogeographic region,^{25–28} the ambient microclimatic conditions, and the ecological features of the death scene.²⁹ In Iran, the emergence and subsequent development of forensic entomology as a crucial medico-legal discipline has been neglected and hampered primarily by the lack of intersectional collaboration among disciplinary force, legal medicine, and entomology divisions. The main aim of the present study was to carry out a forensic entomology survey on insects from 15 human corpses at autopsies in Shiraz, Iran. To the best of authors' knowledge, this is the first report of its kind on insects collected from human corpses in south Iran.

2. Materials and methods

2.1. Study area

The county town of Shiraz (29°32' N, 52°34' E) is located at 1484 m above sea level in Fars province on the south central region of Iran. It has a subtropical hot semi-arid climate with a mean annual temperature of 17 °C, relative humidity of 41% and precipitation rate of 323 mm. Its hilly landscape is corrugated with Zagros mountain ranges which run from northwest to southeast of country. Climate data were obtained from the closest meteorological stations and from multiple measurements done on the site.

2.2. Corpse collection and autopsy

All forensic entomology cases discovered in Shiraz were transferred within 24–48 h of discovery from death scenes to Shiraz Institute of Legal Medicine from May 2014 to June 2015. Human corpses were laid in the morgue at a mean temperature of 4 °C from finding to autopsy. They were carefully examined for the presence of any insects or their remnants at autopsy by a medical entomologist. All corpses were re-examined and pathologically validated by a pathologist. The personal features of each corpse, including gender, age, cover, location and cause of death, date of discovery, postmortem stage, etc. were recorded. A few cases in which the time and cause of death were not determined, medical and forensic investigations were followed up and samples were dispatched to toxicology and pathology labs.

2.3. Insects' collection, identification and breeding

At autopsy, cadaver-associated insects were caught with fine forceps, an aspirator, a camel-hair paint brush, a spatula, or a large fly catch sweep net as appropriate. Based on the abundance of insects, some (about 10% if > 100 specimens present) or all (if < 100 specimens present) of both small and large sized larvae, pupae, or adult male and female of different flies or beetles, were isolated from each corpse. Some fly larvae were killed by immersion in hot (> 80 °C) water for 20 s, treated in 75% ethanol and preserved accordingly. Beetles were frozen at –20 °C and then kept in ethanol. For subsequent identification to the lowest possible taxon, each insect was viewed under standard binocular microscope using valid taxonomic keys of the Palaearctic region.^{7,30–33}

Actively crawling dipteran larvae were removed from each corpse, put in a transparent glass jar covered with a soaked (1% mycostatin in distilled water) piece of cloth fastened with a rubber band to prevent fungal growth and animal escape.²⁹ Immature insects were reared to adult stage in the lab to assert their exact identity. Each jar was supplied with up to 5 g of semi-dry chicken muck or minced beef as a food source on sterilized sawdust powder at the bottom of the container to keep it dry. A small cotton wool rinsed in distilled water was placed inside a small plate to avoid desiccation and spillage. These colonies were kept for at least one generation in a thermostatically adjusted maggotarium (maggot breeding room) which was maintained at constant temperature (21 ± 1 °C), a relative humidity of 52 ± 2%, and a 12 h (L:D) photoperiodicity.

Live beetles were likewise isolated from each corpse, placed separately in a clear glass jar covered with a damp piece of cloth to inhibit fungal growth and insect escape. Minced mutton or beef was used as a food source. They were normally maintained at a constant temperature of 25 °C, a relative humidity of 80 ± 1%, and a 16:8 h (L:D) photoperiodicity. A piece of cork and sufficient amount of coarse sawdust were provided on the floor of each container to prevent wetting.

All containers were labeled accordingly, stored in a dark dry space and type species were kept in school's entomological museum.

2.4. Data analysis

With regard to data analysis, only descriptive statistics including percentages and means were considered. The data were explicitly grouped according to the basic indicators to supply their abundances and percentages.

3. Results

Overall, fifteen corpses (2 females and 13 males) infested with arthropods were investigated in this study (Table 1). Almost half of them were in decaying phase ($n = 7$) including a rare mummified corpse. The age range was from 23 to 83 years old. The weight range was from 23 to 77 kg. All except one corpse was covered at the time of discovery. The cause of death was known in almost three quarters of cases. Homicide (plus one suicide case) and narcotic drug abuse accounted for 40% of cases, while natural and accidental causes were recorded in one third of cases. Most (60%) of these corpses were found outdoors. Forensic cadavers were mostly (66%) examined during spring and summer.

The present data indicated that 842 insect specimens were totally recovered from 10 outdoor and 5 indoor forensic cases (Table 2). The corpse body region from which insects were sampled were recorded. These insects predominantly belonged to the two orders of Diptera and Coleoptera; encompassing six families, nine genera (including three unidentified species genera), and 14 different species of medically-important arthropods on male and female corpses at different ages. Beetles were found on only four corpses (26.7%) while dipterans were present on most (80%) corpses. Insects from dipteran genera were most often (86.7%) collected from the head area (e.g. eye, ear, mouth, etc.) of the

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