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New record of sap beetle, *Nitidula flavomaculata* Rossi (Coleoptera: Nitidulidae) on an outdoor mummified human corpse, South of Iran

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Abstract
Beetles (Coleoptera) are recognized as important entomological clues in the forensic entomology field for the determination of post-mortem interval (PMI). The necrobiont nitidulid species of beetles are colonizers of human corpses in the advanced stages of decomposition which provide essential complementary data to estimate the PMI in forensic cases. We report the new finding of Palearctic sap beetle, *Nitidula flavomaculata* Rossi 1790, adults from a mummified human body located outdoor in a xeric mountain setting, north of Shiraz, the capital city of Fars province, Iran. This human corpse was a male discovered in December 2014. Adult beetles of *N. flavomaculata* were allowed to nourish, copulate and lay eggs in a rearing container at 23±1 °C. The time period from oviposition to the next F1 generation of egg-laying adults lasted 61 days. The species of beetle found in this case could be used in forensic investigations particularly during the cold season in future.

Keywords: Forensic entomology, Beetles (Coleoptera), *Nitidula flavomaculata*, Human cadaver, Iran

1. Introduction
Forensic entomology is the application of the study of cadaveric arthropods to medico-legal issues in the judicial system. The study of beetles is particularly important in forensic cases. This can be helpful in determining the time of death or post-mortem interval (PMI) and also obtain information about the place of death [1]. Insects are involved in carrion consumption and have thus forensic importance. They have a specific sequence to attack the bodies, as carrion beetles are usually found in the late stage of body decomposition [2].
The sap-feeding Nitidulidae beetles are a family of insects in the order of Coleoptera. This order is distinct from others by the presence of two pairs of wings; a pair of hard and thick protective forewings or “elytra”, concealing the second membranous pair of hind wings. The nitidulid adult beetles are variable in size (0.9-15 mm in length). Each antenna is usually 11-jointed with the distal three segments forming a club, which makes it easily recognizable [3].
This zoo-saprophagous family exhibits high species richness and lives in various habitats from tropical to subtropical and warm temperate regions. They have developed different feeding behaviors such as phytophagy, saprophagy, necrophagy, fruitivory, florivory, fungivory and granivory [4]. They thrive on different materials such as fungi, stored products and dead animal tissues [5]. In this family, three genera of *Nitidula* (Fabricius), *Omosita* (Erichson) and *Carpophilus* (Stephens) include species that are useful in forensic entomology [6].
Some nitidulid species are widely considered as important agents of the carrion eating community and have been reported on human corpses and cadavers of animals in the last stages of decay from different regions [4, 7-12]. Studies on the growth rates of forensic insects are commonly conducted under lab-based constant temperature and relative humidity. Determining the temperatures to which the different stages of beetles are exposed in their development is crucial. The data on the development rate of insect species at different temperatures could provide reliable evidence for the estimation of PMI in legal medicine.
The species of *Nitidula flavomaculata* has previously been reported from animal carcasses in Tehran and Lorestan provinces of Iran [3], but this species has never been reported on a human corpse in Iran so far. There is a lack of knowledge on the life cycle of Nitidulidae, particularly on species of forensic importance. The main aim of this study was to present life-table data on the sap beetle, *N. flavomaculata*. This is also a new report on natural detection of sap beetle, *N. flavomaculata*, on a male human corpse laid on a highland habitat north of the city of Shiraz, Fars province, south of Iran.

2. Materials and methods

2.1. Case study

A 44-year old man was found dead in a mountain field (known as Kohenjan, Sadi district, about 1582 m above sea level) in Shiraz (29°40'N, 52°35'E)**,** the capital city of Fars province, south of Iran on 7th December 2014. His weight was 40 kg. He was taken to Shiraz Institute of Legal Medicine on 9th December 2014. The cause and the time of death were unknown. Based on police investigations, his death was estimated to have occurred in September 2014. As a result of a likely suicidal attempt based on past medical history, medical and forensic investigations were followed up and samples were dispatched to toxicology and pathology laboratories. The mean ambient site temperature was reported to be 16 °C, relative humidity 65%, wind speed 4 m/s, no precipitation, and wind direction easterly at this time of the year.

2.2. Breeding experiment

Cadaveric insects were captured manually with fine forceps or a large fly-catch sweep net as appropriate. Upon arrival in the laboratory, a total of nine *N. flavomaculata* adults of both genders were put inside a plastic chamber (11×9 cm), filled with about 3 cm of dry wood chips and about 20 mg coarse sawdust mixed with sand to keep the container bottom dry, and nourished on semi-dry beef meat as a food source. To ensure protection and a source of humidity and water, a ball of cotton rinsed in distilled water was introduced into the chamber. The emerged beetles were then killed by deep shock freezing at -20 °C in a freezer for 1 h in a small closed cap vial and then identified using valid taxonomic keys [13-15].

The colonies were maintained for one generation in a thermostatic maggot (worm-like fly larvae) room at constant temperature (23 ± 1°C), 12:12 light/dark period, and a relative humidity of 62 ± 2%. Recording the time required for larval stage developments and pupation was implemented at every four-hour intervals. A preliminary life cycle period was thus worked out.

3. Results

Only two guilds of carrion-dwelling insects; adult beetles (no.: 9) in the family of Nitidulidae (Coleoptera) and fly maggots (no.: 13) of the Dipterous family Sarcophagidae (typically species of *Sarcophaga peregrina*); were collected from the brain, nasal cavity and body surfaces (thoracic area) during autopsy procedures by the first author at 10.45 am.

Part of the unclothed body being in contact with direct sunlight was rendered hard and leathery or mummified (Figure 1). The skin was stiff and dry, and the viscera remained intact. The skin was so hard that even wild animals could not use it. Following the identification of the victim, it was found that he had escaped from home in September 2014. The body underwent natural mummification due to the prevailing climatic conditions. The cause of death could have been starvation or electrolyte imbalance, as the gastrointestinal tract was empty.

Fig 1: A-The mummified male human corpse; B: A pair of beetle species, *Nitidula flavomaculata*, showing their relative size.

The sap-feeding species of *N. flavomaculata* adults collected on the human corpse in winter could be distinguished from other species by the yellow stripe on the elytra (Figure 2). The latter were long enough to conceal most of the abdominal segments; only the last 2 abdominal segments were exposed (Figure 3). This species was 4-5 mm in length, its antennae with a three-segmented club (Figure 4), five segmented tarsus of which segments 1-3 were usually dilated and segments 4 and 5 were small. The confirmation of this species was done based on its habitus exhibiting a pronotum with yellow-orange thick band on lateral side and elytra with U-shaped light yellow maculae reaching lateral edges on anterior parts of the adult (Figure 3) by Dr. Andrzej Lason, a leading researcher on Nitidulidae beetles.

Fig 2: The breeding of adult *Nitidula flavomaculata* Rossi inside a container.

Fig 3: The habitus of adult *Nitidula flavomaculata* Rossi.

Fig 4: *Nitidula flavomaculata* adult in lateral view. Note the club-shaped antenna, tarsus and elytra.

In this study, no puparia or eggs were seen on the human corpse. The life cycle duration of *N. flavomaculata* was worked out for only one generation. This life cycle estimate was performed under controlled laboratory conditions as stated

above. The life cycle from one adult stage generation to the next one was 61 days. The first larval stage appeared 16 days after the male and female adults were put together. Larval development to the stage of pupa lasted 34 days, and the development time from pupa to the emergence of F1 generation adults was 11 days.

4. Discussion

Carrion beetles could be crucial sources of evidence in medico-legal investigations [16]. The identification of the cadaveric insects in every season of the year on a human corpse is important in forensic entomology [17]. Species of *Nitidula* genus are usually reported during the active decomposition stages: decay and dry stages [11, 18, 19]. Some species of *Nitidula* genus (such as *Nitidula carnaria*) breed exclusively in open xeric habitats and are thus considered as indicators of corpse relocation once they are found on corpses in, for instance, forest habitats [20]. The seasonality shown by *N. flavomaculata* in this region of Iran suggests this species as a forensic entomologic marker of the season when a human corpse was found. This seasonality could thus serve as a strong potential indicator in forensic investigations [20]. More research is, however, required to confirm these two concepts. In addition, active decomposition in large biomass cadavers (35-40 kg), as in the present human corpse case, compared to smaller bodies, is less efficient and more biomass is left out after its termination; but much more useful for late-arriving insects, such as carrion beetles [21].

The presence of sap-feeding species of beetle, like *N. flavomaculata*, on a human corpse has rarely been reported. This beetle is considered as an insect of forensic significance in the world and it has previously been reported from human corpses in line with the present study [8, 12]. It is reported as a late-arriving insect and seasonally oriented to cold winter months. The predominance of nitidulid beetles during winter season is also supported by previous studies [22]. Furthermore; this carrion beetle is highly associated with open xeric habitats which make it an ideal candidate marker in verification of medico-legal investigations.

This species of sap beetle is commonly distributed throughout the Turanic-Mediterranean basin extending easterly to Turkey and southern parts of Iran [23], where many infectious diseases prevail [24-26]. According to Adair and Kondratieff, the presence of sap beetle *N. flavomaculata* on a human corpse was also reported in the cold season and in the late stages of decay and decomposition in parallel to our study [8]. It is a common carrion colonizer living on the dry bones of large terrestrial mammalian species in the advanced stages of decomposition. Thus, this species of sap beetle may be utilized to estimate the time of death in the future during the cold season.

The study on the life history patterns of insects with forensic importance is crucial and plays a significant role in forensic entomology for PMI determination [27]. According to Zanetti and colleagues, the development rate for *Nitidula carnaria*, a beetle species of forensic importance, was 59.7 ± 2.4 days at 25 °C and a 54% relative humidity from adult stage at the start to the one at F3 generation [6]. Another recent study on the life cycle development of the same species at 15 °C and a 60% mean humidity, the larvae and pupae developed in 55 ± 3 and 12 ± 2 days, respectively [4]. Under field conditions, the life cycle studies is formidable as many confounding environmental factors effective on survival and development rates could lead to bias in results [2]. The present investigation was carried out under thermostatically controlled laboratory conditions in a maggot breeding room (maggotarium). The length of life cycle in *N. flavomaculata* was found to be 61 days at 23 °C at a 62% relative humidity. One of the limitations in the present study was the low number of collected sap beetles from the human corpse which could partly be due to its mummification.

For estimation of PMI using this sap beetle in Iran, it is important to study the rates of development of instars and adult stages in this species. Future research will be needed on the biology of *N. flavomaculata*, in order to determine the exact length of each life cycle stages, which will be of great value to the further improvement of the PMI estimation and other forensic implications. Forensic entomology is a neglected field of study in Iran and in order to make baseline studies, more research is needed. Eventually, steps should be taken to advance on forensic entomology investigations in Iran.

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