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Marks caused by the scavenging activity of *Necrobia rufipes* (Coleoptera: Cleridae) under laboratory conditions

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ABSTRACT

Insects are an important group involved in carrion consumption and are thus of forensic interest. In the laboratory we studied the taphonomic marks that *Necrobia rufipes* (Cleridae) can produce. Pig trotters were exposed to adult beetles at 21 ± 3 °C and 12:12 h day/night cycle. We made observations and took photographs every 4–5 days for 12 months. Marks were noted after a month. We found scratches, pits, holes, and tunnels in several kinds of tissue such as integumental, connective and muscular. This work contributes preliminary data of significant application in biology, ecology, anthropology and forensics. Until now, no study has provided taphonomic information with *N. rufipes*.

1. Introduction

Human or animal remains can suffer changes which can result from different factors such as physical variables; environmental conditions; the stage of the corpse; the activity of scavenger animals, among others.[1] All these changes are studied by Forensic Taphonomy. Insects are an important group involved in carrion consumption and are thus of forensic interest. Denic et al.[2] described body wounds which had been initially confused with acid but were finally identified as the result of cockroach activity. Some species of Formicidae have been observed to produce marks and lesions potentiated by formic acid.[3] Several artifacts produced by ants are described in Byard[4] and Zanetti et al.[5] Termites have an osteophagic behavior and this was observed upon human remains in archeological tombs.[6,7] Britt et al.[8] pointed out that they can colonize the burial place and damage bone remains and thus influence taphonomic processes. These authors also suggested that tineid moths (Lepidoptera) can be involved in the deterioration of bones.

Skin, checkered, clown and burying beetles could probably cause artifacts in human body parts.[9] Ururahy-Rodrigues et al.[10] found that a scarab beetle species caused different post-mortem effects in the corpse and modified the discovery scene. These alterations can be confused with lesions or artifacts which may have actually caused death.

The study of the feeding habits and other biological aspects of scavenger species may represent a great contribution to forensic taphonomy. Mazzanti[11] indicated that making observations on the effects caused on skeletons by coleopteran can provide interesting contributions such as ecological and paleontological information.

The Cleridae family (Coleoptera) contains mostly predaceous species but some are scavengers and others have been found feeding on flower pollen.[12] *Necrobia rufipes* De Geer and *Necrobia ruficollis* Fabricius (Coleoptera: Cleridae) are species with an omnivorous habit which have been found associated with Egyptian mummies,[13,14] are pests of stored commodities and other products of rich protein contents, and have been found in forensic cases and succession experiments some of them conducted in Argentina,[15–18] thus they are important beetles in forensic entomology and stored product entomology.

Thus, the aim of this study was to conduct research into the artifacts that *N. rufipes* can produce on animal tissue when feeding and reproducing or completing its life cycle.

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2. Materials and methods

To perform this study, adults of *N. rufipes* were selected from a culture established in 2010. The colony was obtained from pig carcasses used in field succession experiments performed in Bahía Blanca, Argentina.[18] Fifteen adults were placed inside a 2 kg glass container filled with approximately 3 cm of sand. The neck of the container was greased with mineral oil to prevent insects from escaping. To allow ventilation and eliminate excess humidity and fungal growth, the opening was covered with a piece of voile mesh secured with a rubber band. Protection and a water source were provided by introducing a piece of cotton sprayed with distilled water. To evaluate taphonomic marks, pig trotters (n = 2) were boiled in a pot for 10 min and then exposed for 30 h to open-air temperature and humidity, sheltered from the rain and covered with a piece of voile material to protect them from scavengers, this procedure was followed for the reasons explained in Zanetti et al.[5]

The trotters were photographed for control purposes and then introduced to insects except in the control sample. Three replicates were carried out. Containers were maintained in a room at approximately 21 ± 3 °C and 12:12 h day/night cycle. Insect activity was observed and photographed every 4–5 days for 12 months. All

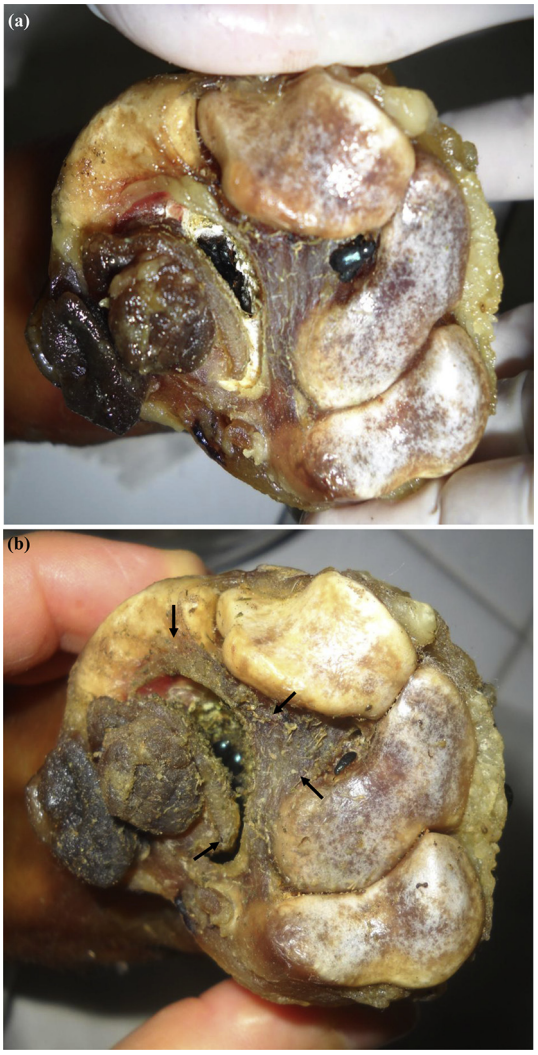

Fig. 1. Insect activity on cadaveric substrate. (a) Cadaveric substrate at the beginning of the experience. (b) Cadaveric substrate after 7 months of *N. rufipes* activity at adult stage (arrows indicate the tissue consumption in the joint area).

Fig. 2. Marks on pig hoof. (a) Pig hoof without marks (control). (b) Pig hoof eaten by checkered beetles after 2 months of insect activity (arrow). The black horizontal marker equals 1 cm.

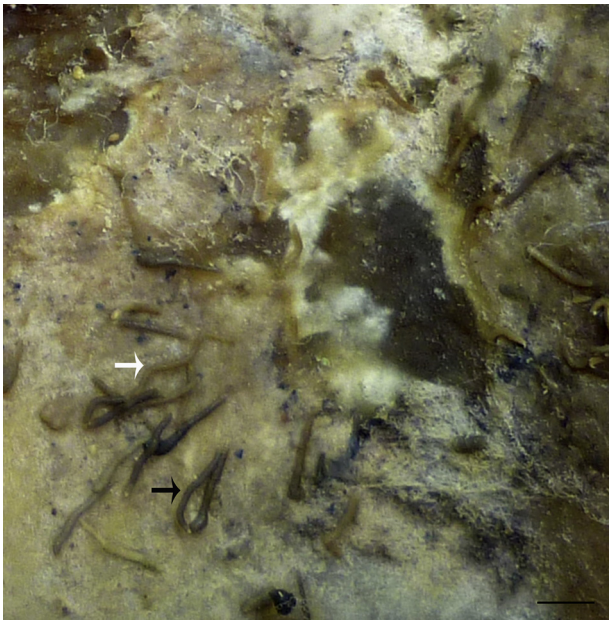

Fig. 3. Fecal pellets of *N. rufipes* over the trotter. Oldest pellets (black arrow) were darker than youngest pellets (white arrow). The black horizontal marker equals 1 cm.

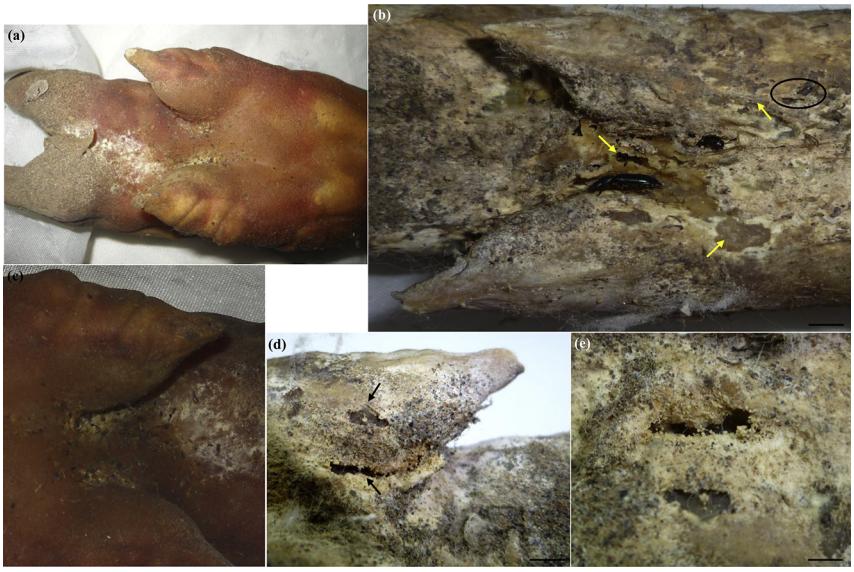

Fig. 4. Marks made by adults and larvae of *N. rufipes* in cadaveric tissue after 7 months. (a) Ventral surface of the trotter (control). (b) Ventral surface of the trotter with depressions and holes made by insects (arrows). Circle indicates the presence of a larva. (c) Ventral surface of the phalanges (control). (d) and (e) Depressions and holes on the ventral side of a phalange (arrows). The black horizontal markers equals 1 cm.

the life cycle occurred in the same container. Photographs were taken with a NIKON COOLPIX L20 camera and the program Adobe Photoshop CS version 8.0.1. was used to create TIFF files images.

3. Results

Necrobia rufipes, did not make effects so quickly on pig tissue, they were observed after a month. Clerids, both in adult and in larval stages, made undulations and pits in skin and connective tissue, generally starting in skin.

In one of the trotters, the adults entered in the cadaveric tissue through the postmortem wound made by the butcher cut and also consumed part of the connective tissues of the articulation (Fig. 1a, b). After 12 months, the trotter was cut longitudinally to observe if in the interior cadaveric beetles produced artifacts, but these were not observed. The spaces between the phalanges of the trotters, as

Fig. 5. Pupal chambers of *N. rufipes*. (a) Sandy chambers. (b) Sandy chambers built and glued against the wall container (arrows). (c) Chamber made with cotton fibers (the arrow indicates the pupa inside the chamber).

well as their ventral skin and hooves, were used as a refuge and food source by beetles (Figs. 2 and 4a). From the moment that the insects started to feed, fecal pellets and shavings from eaten tissue could be seen accumulating on different parts of the trotters, soil substrate and cotton (Fig. 3a, b).

In the replicates, the adults did not enter in the cadaveric tissue through the postmortem wound made by the butcher cut, but grooves, holes and tunnels were found in skin and connective tissue (Fig. 4a–d). Tunnels appeared to resemble the path that larvae went through at feeding. Last larval instar built superficial and deep chambers with the sandy substrate, and with the cotton fibers for the pupa (Fig. 5).

None of the effects found in the trotters exposed to cadaveric beetles were seen in the control samples (Fig. 6a, b).

Some of the artifacts described in this section (grooves, holes and tunnels) were also observed in colony cultures reared on muscular tissue (beef) (Fig. 7).

4. Discussion

The activity of *N. rufipes* beetles produced undulations, small pits, holes and tunnels in skin and connective tissues. Some of these marks were observed under *Dermestes maculatus* activity.[5] The artifacts produced by clerids appeared more slowly and in a lesser number than that made by dermestids, which fed and started to produce marks between the second and third day of the experiment compared with the month or more needed by clerids, may be this was to the size of the clerids' progeny. In cases where these beetles colonized a cadaver, the presence of their marks would indicate that the cadaver was colonized by checkered beetles after the amount of days mentioned above, meaning that the cadaver was in advanced stages of decomposition. We thought that the relation time-marks/artifacts would not be exactly a minimum PMI, but could be useful when there is no other indicator or could contribute with other data to the estimation of PMI. Medugu and Kabir[19] observed when they studied the infestation on different fish species, that *D. maculatus* caused much higher losses than *N. rufipes*, but general susceptibility depended on fish species. These authors suggested that these results may be were due to the relatively shorter development period of *N. rufipes* compared to *D. maculatus*. We obtained some preliminary results of the life cycle of *N. rufipes*[20] which showed that 13 days after the adults were place together, the first larval stage appeared. The larvae were found between the muscle fibers (beef). It took 13 days for pupal chambers to be built (a total of four larval stages were observed). The emergence of the imago occurred approximately 7 days after pupation. A total 32 days elapsed from the moment that adults were placed together to the emergence of the imago. Also, the food quality and the dietary needs of *N. rufipes* may be are other factors that contributed to the differences found between the two species. Hasan and Phillips[21] suggested in their study of mass-rearing clerids that diets could be a factor for the differences found with respect to other studies.

Other results evidenced in this work showed that these cadaveric beetles frequently used the spaces between and under the phalanges of the pig trotters. *D. maculatus* also used these sites.[5] The fecal pellets and shavings were soon seen accumulating over the trotters, soil substrate and cotton. Their identification is important because they could be used as a forensic indicator of beetle presence, as suggested by Schroeder et al.[22] and Zanetti et al.[5] with *D. maculatus*.

Necrobia rufipes can build chambers of different materials.[20] In our study, the sandy soil and cotton were used by the larvae to pupate. This is important to consider at a crime or cadaver finding scene when collecting entomological evidence. May be these beetles did not pupate in their food to prevent cannibalism as suggested Keyenberg[23] for *Dermestes lardarius* L. and *Dermestes vulpinus* Fabricius. This explanation was also suggested by Zanetti et al.[5] when they studied the taphonomic marks caused by *D. maculatus*.

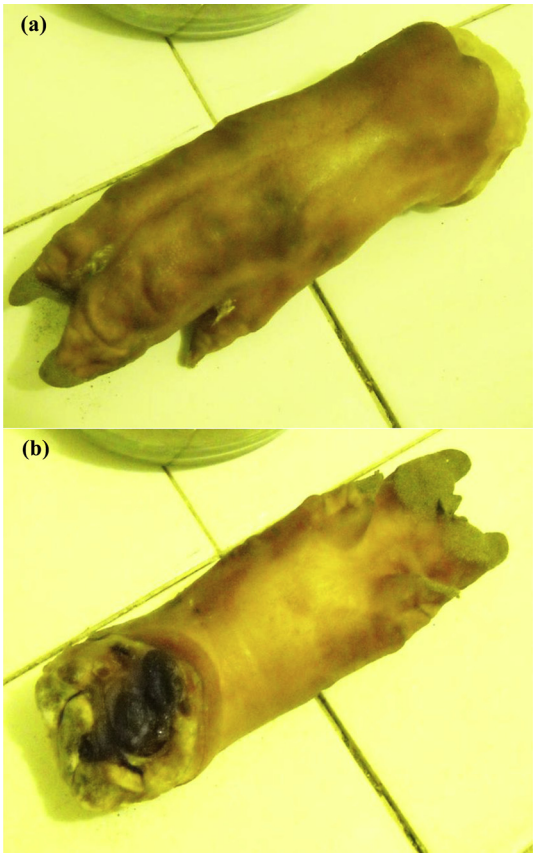

Fig. 6. Control (without insect activity). (a) Dorsal view. (b) Ventral view.

Previously we provided information on the taphonomic effects of *D. maculatus* and now with this study we do it with another species of forensic interest. It is important to take care during the examination of a cadaver to determine if insect artifacts have taken place on other superficial ante mortem injuries,[24,25] resulting in the modification of wounds and/or loss of identifying features.[10,26–29] Also, insect activity can simulate vital lesions.[10,27] Moreover,

Fig. 7. Muscular tissue (beef) with holes and tunnels caused by the scavenger action, particularly of the larvae. The horizontal marker equals 1 cm.

insect injuries can be confused with postmortem injuries caused by aggressors.[10]

Although the replicates have not been compared quantitatively, the aim of this work was to initially corroborate if checkered beetles produce marks and to provide qualitatively data about them. Because several conditions were controlled, the differences found in one of the replicates could be consequence of the size progeny of the beetles or some physical/pathological condition of the trotter that we could not detect.

In conclusion, *N. rufipes* is a scavenger capable of producing artifacts in different tissues of pig extremities, as observed under controlled conditions. Although further research is needed on the life cycle of cadaveric species and their behavior, this study contributes data of significant application in biology, ecology, anthropology and forensics.

Conflict of interests

The authors declare that they have no competing interests.

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Ethical approval

None.

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