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Use of *Alphitobius diaperinus* as an alternative method of osteotechnics

Uso do *Alphitobius diaperinus* como método alternativo de osteotécnica

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Abstract
This study aimed to develop an alternative biological osteotechnical method using lesser mealworms (*Alphitobius diaperinus*), a scavenging insect found in poultry production systems. Ten small to medium-sized animal cadavers of different species and two isolated bone pieces were used. After the soft tissues were removed, the bones were accommodated with the insects until they were thoroughly cleaned. Next, the pieces were evaluated, bleached, dried, and the skeletons assembled. The use of lesser mealworms in the biological preparation of skeletons has shown very promising results since this insect is easy to acquire, maintain, and handle; it does not emit unpleasant odors into the environment; it provides complete cleaning of the skeletons, reaching places that are difficult to access. In addition, they present great agility in cleaning the carcasses, dispensing the previous fixation of the soft tissues to prevent putrefaction or the appearance of microorganisms, such as fungal colonies.
Keywords: maceration; beetles; skeletons

Resumo
Buscou-se com o presente estudo desenvolver um método alternativo de osteotécnica biológica baseado no uso de cascudinhos (*Alphitobius diaperinus*), um inseto de comportamento necrófago encontrado nos sistemas de produção avícola. Foram utilizados dez cadáveres de animais de pequeno a médio porte e de diferentes espécies, e duas peças ósseas isoladas. Após a retirada dos tecidos moles, os ossos foram acomodados junto aos insetos até a sua limpeza completa. Na sequência, as peças foram avaliadas, clareadas, secas e os esqueletos montados. A utilização dos cascudinhos na preparação biológica de esqueletos demonstrou resultados bastantes promissores, visto que este inseto é de fácil aquisição, manutenção e manipulação, não emite odores desagradáveis ao ambiente, proporciona uma limpeza completa dos esqueletos, atingindo lugares de difícil acesso. Além disso, apresentam grande agilidade na limpeza das carcaças, sendo dispensável a fixação prévia dos tecidos moles para prevenir a putrefação ou o aparecimento de microrganismos, como colônias de fungos.
Palavras-chave: maceração; besouros; esqueletos

1. Introduction

The use of osteological models contributes to educational and scientific activities as they allow the identification of some aspects of the animal's life such as locomotion and posture(1). During the preparation of these osteological specimens, mechanical, biological, or chemical processes can be applied separately or in combination(2).

In the biological preparation, crabs, clothes moths, woodworms, tapeworms, isopods, and *Dermestes* have been reported to be used, the latter being the most common(3). *Dermestes maculatus* is a widespread beetle that feeds on dead animal carcasses(4). This species has been reproduced in laboratories for decades for preparing biological materials(5) because it produces less unpleasant odors when compared to other maceration processes and keeps the specimens fully articulated during the cleaning process(6). However, when applying this method to the preparation of bat skulls, Oliveira(7) observed that *Dermestes* caused structural damage to some pieces due to the consumption of bone extract, even in the presence of soft tissue.

With similar characteristics, *Alphitobius diaperinus* is a cosmopolitan insect found in abundance in the substrate of poultry farms. This African-origin species is naturally associated with bat caves and bird nests, feeding on fragments of fur, feathers, and, occasionally, carcasses(8). In poultry houses, the beetle and its larvae feed on excrement and feed and engage in necrophagy on carcasses or dying birds(9). Its biological cycle varies considerably but tends to be completed in approximately 55 days under favorable temperature and humidity conditions, contributing to its rapid development in facilities(10).

Given the diversity of possible methods for the preparation of osteological specimens and the importance

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that these specimens have in the scientific and educational environment, we aimed to develop an alternative method for the biological preparation of skeletons using *A. diaperinus*.

2. Material and methods

The lesser mealworms (*A. diaperinus*) were collected using a mug and a pot with a lid from the aviary of the Instituto Federal Catarinense (IFC), Concórdia Campus, located in Santa Catarina, Brazil. They were housed in plastic containers with a smooth inner surface and covered on top with a small-grain wire mesh, allowing the passage of air and blocking the entrance of other animals. The plastic boxes were placed in an outdoor environment, built with wooden slats and black canvas, which protected them from rain, sunlight, and wind. The enclosure's internal temperature was maintained within the range of 25 to 30 °C with an automatic heating system based on incandescent lamps equipped with a display to mark the enclosure's temperature. At the bottom of each box, a layer of approximately four centimeters of shavings was placed as substrate, and small empty plastic pots were filled with moist absorbent cotton. Small portions of chicken feed were added to maintain the beetles, and the absorbent cotton was moistened weekly. After collecting and accommodating the beetles, the carcasses of the experimental animals were readily placed in the boxes without requiring time for the development of the *A. diaperinus* population.

Ten whole animal cadavers were used, namely, one fancy rat (*Rattus norvegicus*), two rufous-bellied thrushes (*Turdus rufiventris*), one black-tufted marmoset (*Callithrix penicillata*), one suindara (*Tyto furcata*), one coral snake (*Micrurus altirostris*), one domestic dog puppy (*Canis lupus familiaris*), one nine-banded armadillo (*Dasypus novemcinctus*), one nutria (*Myocastor coypus*), and one jacunda fish (*Crenicichla lacustris*). Three isolated bone parts were used, namely, one goat (*Capra aegagrus hircus*) head, one Japanese quail (*Coturnix japonica*) head, and one red-breasted toucan (*Ramphastos dicolorus*) head. To test the action of these insects on already prepared anatomical parts, two bird taxidermy specimens were used, namely, a common ground dove (*Columbia* sp.) and a surucua trogon (*Trogon surrucura*). All the specimens used were already in the animal anatomy laboratory of IFC, Concordia campus, which consisted of donations, animals found deceased within the campus, or deceased from being run over by cars on highways.

The parts underwent cleaning and removal of the skin, viscera, and excess musculature. They were then weighed and placed on a plastic base with low edges to avoid the loss of bone structures due to the movement caused by the action of the insects, and subsequently placed in the container where the lesser mealworms were kept. Daily inspections were conducted to monitor the process and collect information about the odors generated, fungal growth, and the consumption of the carcass tissues.

After the beetles thoroughly cleaned the soft tissues, the pieces were weighed and evaluated for the insects' consumption of the bone matrix. Afterward, they were submerged in 70% alcohol for 30 min, placed in a 10% hydrogen peroxide solution until they reached the desired light color, and dried in the sun, thus being ready to assemble the skeletons. Photographic records were collected during the processing of each specimen, and the results were tabulated for ease of interpretation. The produced pieces are on display in the animal anatomy laboratory of the IFC, Concordia, and are available for visiting and use in classes.

3. Results and discussion

The results obtained from the lesser mealworms were compared with the reports in the literature using dermestids, as this is the most employed methodology involving living organisms and shows promising results in cleaning osteological specimens[2]. The lesser mealworm (*A. diaperinus*) is considered a pest of the poultry production system because of the favorable environmental factors for its rapid proliferation[11]. When entering the facility to collect them, large quantities of these beetles, both in their larval and adult stages, were observed just below the carcasses of dead birds in the aviary, especially in the period after the delivery of the flock, which facilitated their capture in large quantities. It is worth noting that each batch of chickens takes an average of 45 days to prepare[12], which allows for the implementation or replacement of the beetles quickly and throughout the year.

Dermestes can be obtained through donations from institutions that have these insects or captured from nature[14] since they are often found on decomposing carcasses[15]; however, after collection, these beetles must be reproduced until a suitable amount is reached for the cleaning work. Unlike *Dermestes*, lesser mealworms can be sent directly to the cleaning process due to the ease and abundance of collection, speeding up the processing of the pieces.

In an experiment conducted by Gomes and Oliveira[14] that evaluated the assembly and maintenance of *Dermestes* colonies, exhaust fans were required due to the uncomfortable odors released into the processing environment. The same authors recommended that the place used to house *Dermestes* be separate from biological materials. All this care is important to avoid infestations because these beetles pose a risk as they can consume fur, paper, hair, feathers, and tissues[16]. In this experiment, the simple housing, made with a tarpaulin and frame, met all the basic requirements for managing the *A. diaperinus* colony and the maceration activities. No abnormal odors besides those belonging to the poultry litter brought along at harvest time were detected. No insects escaped into the environment, and the enclosures were not damaged. No large investments were necessary to introduce the insects into the laboratory routine and their rearing in places close to the laboratory, facilitating the follow-up of the skeleton preparations.

Seeking to provide conditions like those found in poultry houses, such as temperature, humidity, and food, which are responsible for the perpetuation of the beetles in the production environment[17], it was observed that controlling the temperature, treating the insects with chicken feed, and humidifying the substrate with a sprayer were sufficient to maintain the strong performance of the insects throughout the period. The temperature factor, especially the temperature reduction, seems to influence the processing speed; however, even exposed to temperatures of 4 °C inside the hatchery for a week at night due to problems in the heating equipment, it was observed that after the stabilization of the ideal temperature, the activity of the lesser mealworms was re-established. In *Dermestes* breeding, Leeper[18] reports that environmental variations can cause the population to drop suddenly, as can the accumulation of organic matter in the cotton substrate, requiring frequent cleaning. To maintain dermestids, the breeding grounds should be supplied with more organic material, such as meat or animal bones (previously fixed with alcohol), or even dog food can be used[14].

According to Gomes and Oliveira[14], before going to the dermestid colony, the material to be processed should be preserved in 70% alcohol or 10% formaldehyde. By the end, it should be completely dry to avoid contamination by fungus or other parasites, thus avoiding the foul odor and the consequent reduction in the *Dermestes* population. In the present experiment, no previous conservation measures were applied to the pieces worked on; yet no colonies of fungi or other insects were observed on the pieces, nor were there unpleasant odors. These facts may relate to the rapid consumption of the soft tissues by the lesser mealworms, which provides practicality, economy, and safety in the processing developed in this work.

During the experiment, 11 small to medium-sized osteological specimens of different species were produced. Lesser mealworms have been found to have a great capacity to consume soft tissue in a short period of time (Table 1) and have an aptitude for performing skeletal cleaning in the preparation of osteological models.

Table 1. Amount of tissue consumed by the lesser mealworms and time spent to finish cleaning each prepared piece.

Piece	Initial weight (g)	Final weight (g)	Time (days)
Fancy rat	86	14	7
Japanese quail (head)	11	3	3
Thrush bird	21	3	1
Black-tufted marmoset	124	34	8
Suindara	100	28	2
Coral snake	17	3	1
Dog puppy	170	48	7
Armadillo (head and torso)	324	80	6
Armadillo (limbs)	174	79	6
Nutria	491	234	10
Goat (head)	746	297	30
Jacunda fish	29	4	3

According to Hefti and collaborators[13], it is impossible to predetermine the processing time because many variables directly influence the result, such as the size and composition of the beetle population, available food, temperature, and light incidence on the breeding sites. In the study by Rodrigues et al.[6], where *Dermestes* were used to clean whole skeletons of some animals, a processing time of four months was obtained for an iguana, one month for a pig and a ferret, 20 days for the common ground dove, and 10 days for a bat and an owl. Although many variables can interfere with the final result, it is noted that compared to *Dermestes*, the lesser mealworms showed an 80% reduction in the time required to clean an owl skeleton, taking only two days for completion. In this study, to clean the complete skeleton of a puppy (260 mm in length), the process took seven days. This was half the time found in a work with *Dermestes* by Oliveira[7], who reported that it took 14 to 28 days to clean the heads of animals measuring 17.7 to 31.3 mm in length.

It was also observed that the bones were thoroughly cleaned, even in complex and hard-to-reach places, and that the articulated bone components were preserved, thus avoiding the loss of small bones in the beetle enclosure (Figure 1A). The bones did not require glue or wire for exposure. Other structures, such as costal and xiphoid cartilage, remained intact and adhered, and no damage to the bone matrix was perceived when the skeletons were removed immediately after the cleaning was completed (Figure 1B, C).

In the works with *Dermestes*, attention was given to the time the specimens remained under the beetles' action due to the risk of losing cartilaginous parts or very delicate bones. This was fundamental to monitoring the cleaning of the material daily and its removal when sufficiently clean. Skulls of small, delicate animals can be completely ingested if left too long in the dermestid colony(14). In this study, the specimens were observed daily to evaluate the possibility of injury; however, to test the processing limits of *A. diaperinus* without damaging the worked material, a thrush bird skeleton (total time of two weeks) and a red-breasted toucan head (total time of 24 weeks) were left in the beetles' enclosure. It was found that a few days after cleaning, the beetles acted on the ligaments, partially disarticulating the skeleton of the thrush and causing the degradation of the slender interorbital septum. Disarticulation of the mandible did not occur in the toucan head, but loss of bone matrix of the interorbital septum was observed (Figure 2).

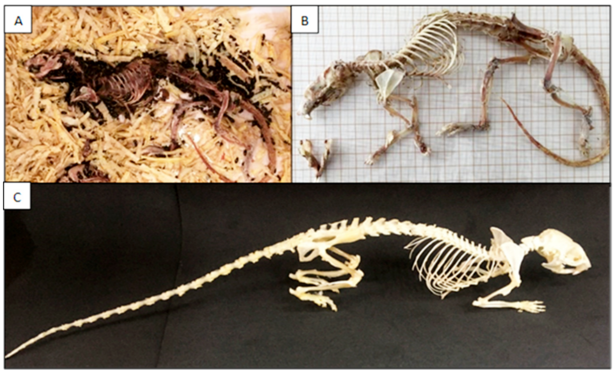

Figure 1. Photographic images of the processing steps of the fancy rat skeleton. In A, the lesser mealworms clean the skeleton; B shows the immediate post-cleaning result of the soft tissues; C displays the assembled skeleton.

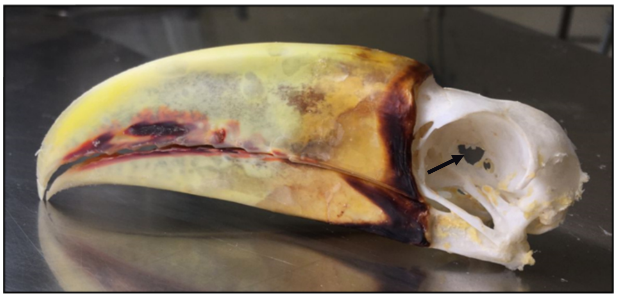

Figure 2. Photographic image of the lateral view of the red-breasted toucan skull showing the consumption of the bone matrix of the interorbital septum (→).

4. Conclusion

The use of beetles of the species *A. diaperinus* in the biological preparation of osteological specimens is a viable alternative because the insects are easily and abundantly found, require simplified management, do not generate unpleasant odors in the environment, have a great capacity for consuming soft organic tissues, and are quick and efficient in cleaning even in delicate specimens with difficult access. Besides the laboratory routine, these insects can be used in research that requires the cleaning of skeletons since, macroscopically, no consumption of the bone matrix was observed in the specimens used in the process when handled according to the methodology of this work.

Conflicting interests

The authors declare that there is no conflict of interest.

Author contributions

Conceptualization: A. D. Dahmer, C. D. da Silva and C. Pietczak. *Investigation*: A. D. Dahmer and C. D. da Silva. *Project management*: A. C. G. dos Reis. *Writing (original draft):* A. D. Dahmer. *Writing (review and editing)*: C. Pietczak and A. C. G. dos Reis

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