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Cleaning Osteological Specimens with Beetles of the genus *Dermestes* Linnaeus, 1758 (Coleoptera: Dermestidae)

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Abstract

Within many biological collections around the world, it is known that *Dermestes* beetles are used in the preparation of osteological material, as part of the collection management protocols. Despite the fact that the use of these beetles is common, management so that the yield of a colony is optimal is not entirely clarified. In this study, we present the conditions and procedures in the management of a colony of dermestids in order to provide a standard system within the collections. The colony must be kept in an isolation infrastructure, under controlled conditions of temperature (23.26 to 28.54 °C), relative humidity (52.43%) and darkness. The material intended for osteological cleaning should be introduced into the colony without viscera, eyes, tongue, brain, skin, hair or feathers, and large areas of muscle. If the material is dehydrated or has been preserved with chemicals, these should be treated beforehand with distilled or deionized water (to rehydrate or wash, respectively). After the beetles remove all soft tissues, the material should be cleaned with ammonia to remove fat and beetle eggs. Subsequently, the skeleton or bones are dried at 40 °C. The dermestarium should be cleaned every two to three months to prevent the colony from decreasing due to contamination.

Keywords: Biological collections; beetles of the genus *Dermestes*; cleaning bones.

Introduction

The identification of many vertebrate species, especially mammals and in some cases fish, amphibians, birds and reptiles, requires the examination and comparison of osteological characters (presence of canaliculi, grooves) that can only be studied with clean skeletons. Cleaning is one of the phases of curatorship in many scientific disciplines, including archeology and anthropology (Leeper, 2015). Even so, specimen cleaning is not always appropriate, since information is lost from bones, cartilage, muscles, tendons, veins, arteries. The decision to clean a skeleton should be based

on the need to obtain information at the osteological level.

When any cleaning process is carried out, damage and instability to the osteological specimens may result, either by the reactions that occur between the reagents or water with the bones, proteins or minerals. Two principles should be followed-the "principle of least", that is, the process must be carried out in the shortest time possible and with the least amount of reagents; and the "principle of durability", which seeks to enhance the useful life of collections (Simmons and Muñoz-Saba, 2005a).

Due to the lack of protocols that clarifies the need to clean osteological specimens and specify the steps to follow and elements to be cleaned, specimen preparators, and taxonomists proceed according to their criteria or traditionally process, but not necessarily in the most suitable manner. An innovative way to clean bones is through the use of beetles of the genus *Dermestes* (Meeuse, 1965). However, there is little available data on their biology and the optimal environmental conditions for the maintenance of beetle colonies (Franco, *et al*., 2001).

Simmons and Muñoz-Saba (2005a), and Leeper (2015) present the following considerations that must be taken into account to make a decision about the cleaning bones: (1) why is bone cleaning necessary?; (2) when a specimen should be cleaned?; (3) how a skeleton should be cleaned?; (4) how long will the cleaning process take?; (5) what information will be lost in the of cleaning bones?; (6) can the specimen support the procedure?; (7) what criteria allows choosing the best cleaning process?; (8) will the specimen remain stable after cleaning?; (9) how will the procedure affect the useful life of the specimen?; (10) what is the purpose of the specimen in the collection?; (11) are articulation or other elements required?; (12) how much fat is acceptable?; and (13) specimen bleaching?.

As curators, it is a great responsibility to maintain a dermestarium in optimal conditions to prepare the samples in the best way. This article establishes a protocol for cleaning osteological specimens with *Dermestes* beetles, essential in the curation processes of zoological collections, based on literature review and the authors' experience. The purpose of this publication is to clarify why the cleaning process must be carried out and the procedures to be performed, and thus avoid irremediable damage and loss of its information.

Existing methods of cleaning bone material

Some traditional methods for cleaning bone material (Simmons and Muñoz-Saba, 2015b; Brito de Oliveira, 2018 and references cited therein) include:

1. Bacterial maceration—placing the specimens in cold water, with or without the addition of enzymatic detergents.
2. Bacterial maceration—placing the specimens in hot water, without the addition of enzymatic detergents.
3. Chemical cleaning with sodium hydroxide or potassium hydroxide, followed by immersion in a solution of ammonia or sodium perborate solution to neutralize the reaction.
4. Manual cleaning.
5. Cleaning with proteolytic enzymes (specific proteins: papain, pepsin, trypsin) (Offele, *et al*., 2007; Leeper, 2015).
6. Cleaning with organisms—larvae of the genus *Dermestes* (Dermestidae: Coleoptera) (Hall and Russell, 1932; Borell, 1938; Tiemeier, 1939; Russell, 1947; Meeuse, 1965; Sommer and Anderson, 1974; Hefti, *et al*., 1980; Valcarcel and Johnson, 1981; Williams and Rogers, 1989; Jannett and Davies, 1993; Franco, *et al*., 2001; Simmons and Muñoz-Saba, 2005b; McDonald, 2006; Offele, *et al*., 2007; Leeper, 2015); isopods (Isopoda: Malacostraca) (Maiorana and Van Valen 1985; Warburg, 1993); mealworms (Allen and Neill, 1950); clothes moth larvae (Banta, 1961); and crabs (Sealander and Leonard, 1954).
7. Composting and burials (Leeper, 2015).

Maceration in hot water or cleaning skeletons with enzymatic detergents or chemical products (such as hydrogen peroxide or carbon tetrachloride) are common practices, but they are not recommended because they may damage the bone, causing porosity and deterioration over time. It is possible that these techniques affect not only the soft tissue but also the morphology and molecular integrity of the bone tissue, therefore, information loss may result. Preferred cleaning methods include maceration in cold water (without detergents), and cleaning with biological organisms (Simmons and Muñoz-Saba, 2005b; Offele, *et al*., 2007; Leeper, 2015).

Beetles of the genus *Dermestes* are the main organism used for cleaning bones in biological collections. The advantages include: (1) less monitoring time required (Russell, 1947, Hooper; 1950; Brito de Oliveira, 2018); (2) the articulation of the skeleton can be maintained if the specimen is removed before the cartilage is ingested or of the ligaments

joining the phalanges and some small bones (e.g., sesamoid) are not consumed in their entirety (Leeper, 2015); and (3) the deterioration of the bone tissue is minimal, which allows research at the molecular level. Other processes cause the DNA to be degraded by hydrolysis and oxidation (Arismendi, *et al*., 2004; Offele, *et al*., 2007 Leeper, 2015).

Problems with cleaning with Dermestes

Cleaning with beetles of the genus *Dermestes* is complex, and when the colony is not at its peak of activity it may be considered to be an inefficient method as it takes days or months to complete the process (Leeper, 2015), while other methods require only hours or days (Thompson and Robel, 1968). The colony must be maintained in a location away from the biological collections in order to prevent the beetles from escaping and infesting the collection.

The maintenance of the colony can also be a common problem, as the population may suddenly decrease in numbers, probably due to variations in environmental conditions; therefore, the dermestarium, must provide appropriate conditions of temperature, humidity and light (Sealander and Leonard, 1954; Leeper, 2015; Mori, 1979).

Unlike other methods of osteological preparation, the process carried out with beetles does not end when the specimens are removed from the colony- the bones must still be degreased, especially in animals with large bones that remain yellow and produce a strong odour (Hamon, 1964), for example, species of orders Artiodactyla, Carnivora, Cetacea, Cingulata, Perissodactyla, Pilosa, Primates, Rodentia (Hystricomorpha), Sirenia. Controlling the amount of time that the material is in the beetle colony is fundamental, because the bones may be damaged by dermatosis (the ingestion of bony tissue by the beetles). Special care must be taken with thin bones, because the beetles tend to make small holes in bones in order to reach the marrow (Leeper, 2015). The beetles may cause the roots of teeth to become translucent as they remove the pigment from dental plaque (Offele, *et al*., 2007).

Results

Dermestes Linnaeus, 1758 (Coleoptera: Dermestidae)

The beetles of the genus *Dermestes* measure between 2 and 12 mm as adults and 7 mm in the larval stage. The coloration is dark with yellow or white patterns. The adults are photophobic and prefer warm, humid, and dark environments (Russell, 1947; Valcarcel and Johnson, 1981; Muñoz-Saba and Simmons, 2005; McDonald, 2006). Dermestids are characterized by four stages of development: egg, larva, pupa, and adult. They have a development time of about 45 days. The larval stage is the longest (30 days), characterized by rapid growth and high consumption of food. Larvae ingest more soft tissue than adults (Leeper, 2015). The pupa has a duration of one week. Five days after the adult's emergence, the females begin laying eggs, reaching an average of 426 in 100 days (Russell, 1947; Valcarcel and Johnson, 1981).

The species of beetle used for the cleaning of bone material in the Zoological Collections of the nstitute of Natural Sciences (ICN) of the National University of Colombia is *Dermestes carnivorus* Fabricius, 1775, which reaches 7 to 8 mm in adult stage, has elytra with fine yellow hairs, albino pubescence in the abdominal sternites, and two marked areas of black hairiness in the fourth abdominal sternite. Sexual dimorphism is manifested by the presence in males of a pubescent tuft in the middle of the abdominal sternite room (Delobel and Tran, 1993) (Figure 1).

Environmental requirements for *Dermestes carnivorus* are shown in Table 1. At higher temperatures, the beetles become dehydrated (Valcarcel and Johnson, 1981), and at lower temperatures the population size decreases (Hefti, *et al*., 1980); If the beetle colony is maintained at high temperatures, individuals will disperse to cooler microclimates (McDonald, 2006), generally under the specimens to be cleaning and in the deepest of the dermestarium.

The dermestarium must be able to maintain the correct level of relative humidity (Valcarcel and Johnson, 1981), this will allow the proper development of the colony. A Very high relative humidity causes the growth of mold and bacteria, which are harmful to both pupae and larvae (Meeuse, 1965; Williams and Rogers, 1989). Providing a dark environment is another important factor because the beetles are phototropically negative (Valcarcel and Johnson, 1981; Muñoz-Saba and Simmons, 2005; Leeper, 2015), therefore, the activity is affected by this.

The environmental conditions of the room where the colony is located are affected by the temperature and relative humidity of the external environment (Leeper, 2015), therefore, it is recommended follow the proposal of Simmons and Muñoz-Saba (2005c), referred to as the theory of enclosures, based on the fact that it is easier to control the environmental conditions of a small enclosure (a microenvironment) than in a large room. The proliferation of beetle frass and the

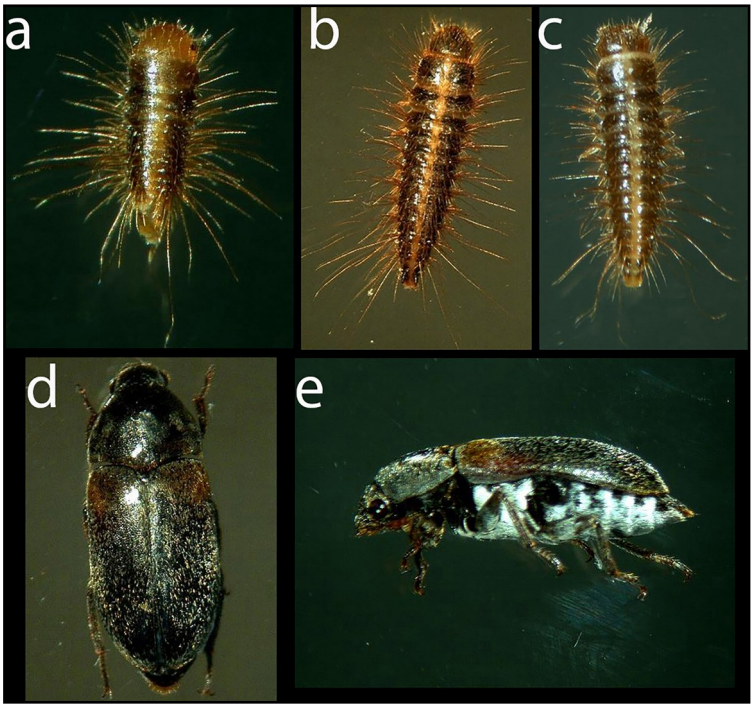

Figure 1. Dermestes carnivorus Fabricius, 1775. The different stages of larvae development: (a) stage I; (b) stage II; (c) stage III; and adults in (d) dorsal view, and (e) lateral view. The diagnostic characters are highlighted. Image: Yaneth Muñoz-Saba, 2019

presence of deteriorated inorganic material should be avoided. In general, conditions that are ideal for pests result in a considerable reduction of the beetle colony.

Diet

Dermestids consume primarily carrion and vegetable and animal fibers such as skin, meat, fish, hair, horn, and wool (Russell, 1947; Valcarcel and Johnson, 1981). The beetles prefer muscle tissue (because it is a source of protein) over tissues that are denser in collagen, which they tend to ingest only after the muscle tissue has been consumed (Leeper, 2015). Larvae eat bones, wool, silk, skin, feathers, leather, glue, and cellulose-based textiles; adults also consume nectar, and pollen, particularly from white flowers (Muñoz-Saba and Simmons, 2005).

Reproduction

In order to promote egg production, the ambient humidity and the amount of fat available to the colony must be carefully regulated. Some fat must remain in the carcass to be cleaned as about 15% fat is necessary for a balanced diet (Valcarcel and Johnson, 1981), without which the beetles are smaller and lay fewer eggs (Russell, 1947). Most dermestid species thrive in relative humidity of about 52.43% (Table 1).

Although a high number of adult beetles are required to maintain a functioning colony for breeding purposes, the growing larvae consume the greatest amount of food and are the most important bone cleaners (Hall and Russell, 1932).

References	Temperature (°C)	
	Minimum	Maximum
Hall and Russell, 1932		28.88
Hefti, et al., 1980	22.00	28.00
Leeper, 2015	20.80	30.20
Meeuse, 1965	28.00	30.00
Muñoz-Saba, obs. pers., 2003	20.01	26.31
Russell, 1947	21.11	29.44
Sommer and Anderson, 1974	27.00	29.00
Valcarcel and Johnson, 1981	23.89	26.67
Average	23.26	28.54

Table 1. Environmental conditions required by Dermestes.

Colony Infrastructure

Building

To avoid inadvertent contamination of to the collections, the beetle colony should not be kept in the same building as the collections. The space where the colony is located should be equipped with an air extractor (to reduce the odor from the colony), an oven, several terraria, and a flat bench for the curation of the material (stainless steel is preferred), with a stainless-steel pot for washing (Figure 2).

Top

The lid of the dermestarium or cabinetry must seal completely so that no beetles can escape (Valcarcel and Johnson, 1981), and be equipped with a pair of external aluminum handles on the upper surface (about 7 cm from the widest part and 11 cm from the mesh) to facilitate removal (Figure 2d). For example, in a dermestarium that measures 52.30 cm long, 36.20 cm wide, and 25.50 cm high, the lid should be 51.00 cm x 34.80 cm with a hole 13.00 cm in diameter in the center, covered with 1.0 mm stainless steel mesh and attached with silicone (Figure 2f).

Substrate

Each dermestarium should contain a small dish of water about 100 mm tall, located in one corner, covered with gauze that is attached to the dish with an elastic band to prevent the beetles from falling in and drowning (Valcarcel and Johnson, 1981). The water in the dish provides the necessary humidity for the maintenance of the colony (Figure 2e). If a water dish is not included inside the dermestarium, use an atomizer to spray water on the specimens to keep the tissues soft (Leeper, 2015). The walls of the dermestarium should not be sprayed, as this is ineffectual (because the water evaporates quickly), does not moisten the cotton, and the glass sides will remain humid and attract fungi (Sommer and Anderson, 1974).

The substrate inside the dermestarium should be composed of sheets of acid free cotton fiber (Valcarcel and Johnson, 1981). The use of loose fabric is recommended to allow aeration and to permit the frass to fall to the bottom of the dermestarium and thus avoid staining the specimen that is being cleaned. The use of gauze (100% cotton) is not recommended, because its mesh allows the larvae and pupae to pass through, and emerging

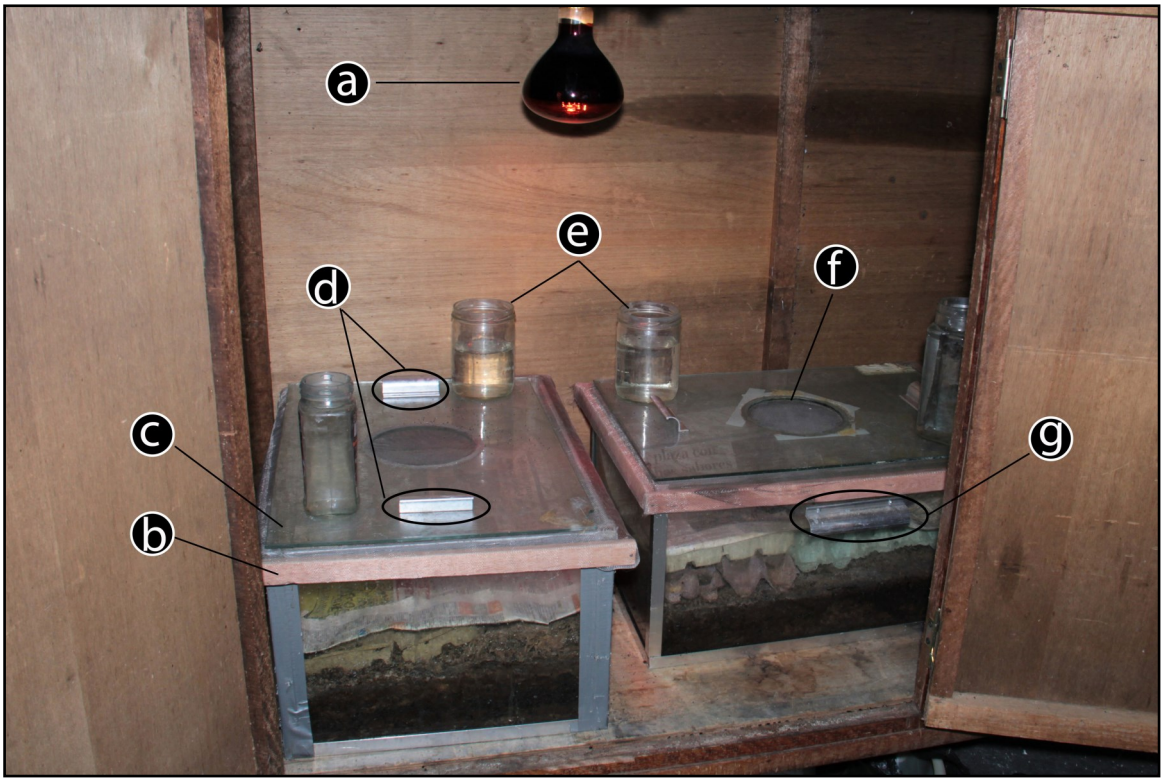

Figure 2. Area with dermestarium where the Dermestes colony is located. (a) UV lamp; (b) safety frame lined with metal mesh; (c) glass lid; (d) glass lid handles; (e) jar with water; (f) ventilation hole in glass cover; (g) dermestarium handles. Image: Juan Carlos Sánchez-Nivicela, 2019.

adults will become trapped in it. The cotton layer should have a thickness equivalent to one quarter of the height of the dermestarium to provide a suitable place to house eggs, larvae, and pupae (Sommer and Anderson, 1974) (Figure 3a).

Container where the material is located

Each specimen to be cleaned should be placed in a corrugated cardboard container in which the larvae can pupate (Tiemeuer, 1939) (Figure 3c, d). The use of metal or Plexiglas containers (PMMA, Polymethyl methacrilate) has also been proposed (Valcarcel and Johnson, 1981), but these materials do not provide the correct conditions for the pupae. Containers of synthetic material are not advisable because they cannot harbor pupae and may be consumed by the beetles, causing probable intoxication and subseqent death. The containers should not be removed from the dermestarium because if so, the pupae will be lost. When the containers deteriorate, they should be disposed of after being carefully inspected with a magnifying glass for pupae; when pupae are found, they should be placed in another container, not in the cotton, so as not to vary their environmental conditions excessively.

Cleaning the dermestarium

1. Remove the cotton substrate from the dermestarium and dispose of it in a bag labeled as biological waste. The fragments of cotton should be examined meticulously for pupae and eggs with a magnifying glass. Transfer the adults, eggs, larvae and pupae to another clean dermestarium to avoid reducing the population size (Valcarcel and Johnson, 1981).
2. Wipe the inside of the dermestarium with a clean, dry cloth to remove organic matter and dust.
3. Wash the inside of the glass with warm water (23-25 °C), applied evenly with a clean cloth.
4. Use a non-foaming alkaline detergent that does not contain chlorine, applied evenly with a clean cloth.
5. Wait five minutes.
6. Scrub the inside of the dermestarium with a clean cloth to better incorporate the detergent and act on organic matter and biofilms.
7. Wait five minutes.
8. Rinse away the detergent with warm water (23-25 °C) until no residue remains.
9. Allow the dermestarium to dry completely at room temperature.

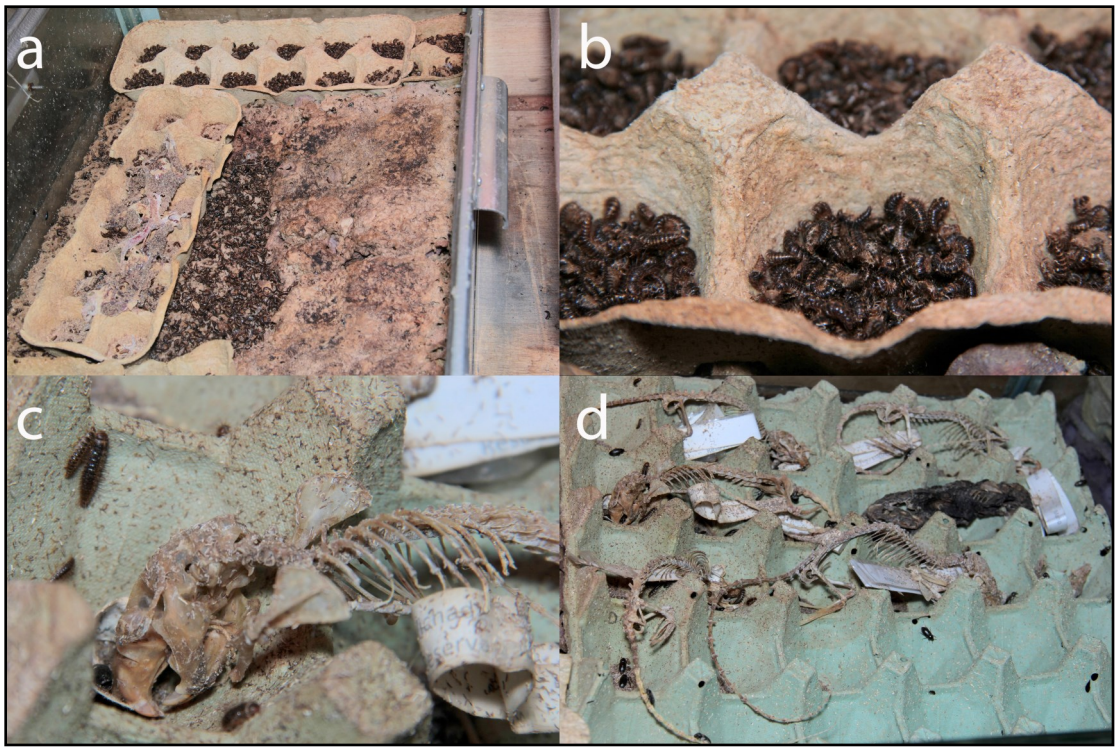

Figure 3. Inside view of the dermestarium: (a) Substrate and corrugated cardboard containers; (b) dermestid larvae in the substrate; (c, d) larvae and adults of dermestid beetles feeding on the remains. Image: Juan Carlos Sánchez-Nivicela, 2019.

10. Apply a disinfectant that does not contain chlorine, iodine, or alcohol. A disinfectant based on quaternary ammonium, acids, or hydrogen peroxide should be used.
11. Allow the dermestarium to dry for 48 hours at room temperature.
12. Carry out a final wash with distilled or deionized water if a strong odor remains.
13. Allow to dry for 48 hours at room temperature.

Curation of specimens while undergoing cleaning

Protect specimen tags and labels by covering them with transparent tape (e.g., Tesa® magic tape, Bezt®, Scotch®) or by enclosing them in a Mylar envelope that is taped shut around the string of the tag. Tags and labels are generally made of materials that the beetles will consume so they may be damaged (resulting in information loss). To the specimens that undergo the process of the solution of concentrated such as chicken broth, beef broth, or fish broth, must be protect yours the labels by covering them with transparent tape and a resealable polyethylene or polypropylene bag; at the end of this process the bag is removed (the broth dilutes the adhesive of the tape). Original labels should not be discarded no matter how badly damaged they are, but rather protected with a covering of inert plastic (e.g., polyethylene or polyester). The information from damaged labels should be transcribed on a new, acid-free paper label (Simmons and Muñoz-Saba, 2005a). All specimens being skeletonized should be labeled (McDonald, 2006). If a specimen does not have any information associated with it prepare a label using good quality acid-free paper and a technical pen with black carbon ink. The label should include the letters NN and the specimen should be assigned a number. Attach labels to the specimen before beginning the cleaning process, using a soft thread.

Determine how the specimen is preserved: e.g., fresh, dehydrated, or in alcohol or formaldehyde

It is necessary to know if the samples were stored in a reagent, to define the procedure prior to the entry of the specimens into the dermestarium. If they are not carried out, the population of *Dermestes* will decrease considerably.

Freshly preserved specimens: fresh material

1. Extract the internal organs from the specimen, including the tongue, eyes, brain, viscera, skin, hair, or feathers (Borell, 1938; Tiemeuer, 1939; McDonald, 2006) with care to avoid damage to the bony cavity. These tissues should be removed to avoid contamination from decomposition and subsequent degradation (Williams and Rogers, 1989) and to speed up the cleaning process. The carcass need to be skinned.
2. Make incisions in the muscle masses to provide easy access for the larvae and adult beetles.
3. For large vertebrates separate the skull at the second cervical vertebra (C2-axis) to avoid breaking the occipital. This allows the beetles to easily enter the vertebral column and cranial cavity.
4. Place the specimen in a corrugated cardboard container and set it in the dermestarium. Each specimen should be placed in a separate container to avoid mixing of skeletal elements. In some cases, small parts or small bones may become detached from the specimen and may be moved about by beetles. If the specimen is located directly on the cotton, the small bones that become loose or break off (or even small skulls) may be lost in the cotton (Figure 3d).
5. Check the colony 24 hours after introducing the new material to be cleaned, and again after that at least once every 48 hours. The length of the cleaning process depends on the size of the specimen to be prepared and the activity level in the colony. Small specimens (e.g., skulls of birds, bats, shrews, mice) should be examined daily in an active colony to avoid damage to small bones such as the hyoid or the nasal cartilages, which are important characters for the identification of species (Carleton, 1980; Griffiths, 1982; Weissengruber, 2002; McDonald, 2006). Larger specimens may be checked less frequently.
6. Take the small and medium skulls by hand, approach them to the light bulb, between 5 to 10 seconds, with the purpose of skull heating, the larvae migrate from the narrow structures. With fine-tipped forceps, the larvae are collected and locate in the aquarium.
7. Extract the specimens from the beetle colony when the bones are clean but before they are disarticulated (McDonald, 2006) (Figure 4a, b).
8. Place the cleaned specimens individually in heat-resistant glass jars. Using a plastic funnel, add to each jar a solution of one part 40% ammonia and four parts of distilled or deionized water (Russell, 1947). If necessary, turn the skulls using fine-tipped forceps to allow the ammonia to enter through the foramen magnum so that the skull will submerge. The specimens should be left in the ammonia for 3 to 6 hours to eliminate eggs, larvae, and pupae of beetles that are inside the specimens. The fat present in the specimens,

especially in long bones, is also reduced or eliminated with this procedure (Tiemeuer, 1939; McDonald, 2006).

9. Extract the bones from the ammonia and filter the contents of the jar through a plastic funnel containing four overlapping layers of clean gauze to collect small, loose bones. This procedure must be performed very carefully; search for small bones with the help of a magnifying glass (Figure 4a).
10. To eliminate the ammonia and its odor, wash the jar, fill it with distilled or deionized water, and place the bone material in the jar for 12 hours. Then and filter it again to locate any small bones that have become loose.
11. Change the water again and repeat after 12 hours.
12. Check the specimen for remnants of muscle or tendons—if any remain, remove them carefully using fine-tipped forceps.
13. Gently wash the bones with a soft bristle brush using slight circular movements. Use particular care with small bones (e.g., the hyoid). Rinse the bones with distilled or deionized water (Meeuse, 1965). This procedure is carried out to remove adherent grease and dirt that can lodge in cracks and rough surfaces, especially on large specimens (Sommer and Anderson, 1974; Leeper, 2015). The fat could later attract collection pests.
14. Verify that there are no larvae or adult beetles among the bones. If any are found, extract them using fine-tipped forceps, being careful not to damage the specimen (Borell, 1938). The larvae may lodge in the cranial cavity, the neuronal channels of articulated skeletons, or any other small cavity or crevice. It is important to make sure that the beetles, in all their stages, are eliminated from the bones. Any remaining live beetles will continue to feed on the bones (McDonald, 2006), and dead beetles will become food for other pests. Only those remnants of *Dermestes* located in completely inaccessible places (e.g., deep in the nasal turbinates, inside the tympanic bullae) may not be eliminated because the preservation of the specimen is prioritized and trying to remove them would cause too much damage to the bones.
15. Place the cleaned specimen in a heat-resistant glass jar. Dry it for 24 to 48 hours, depending on the size of the animal, in an oven at a temperature of 40 °C (Sommer and Anderson, 1974).
16. Remove the specimen from the oven, allow it to cool, and then remove it from the jar.
17. Remove and discard the string attached to the labels. Labels usually become separated from specimens during the cleaning process, and

Figure 4. Osteological specimens: (a) Individuals recently removed from the dermestarium (left), bone collection (center) and storage process (right); (b) Individuals medial size (left) and small size (right) after the cleaning process; (c) Skulls totally clean (include degreasing process) in medial size (left) and small size (right). Image: Juan Carlos Sánchez-Nivicela, 2019.

the remaining string may become food or a niche for collection pests (Muñoz-Saba and Simmons, 2005).

18. Store the specimen with its tags and labels in a resealable polyethylene or polypropylene bag the size of appropriate size, or in a rigid polystyrene or acid-free paper box (Figure 4a).

Dehydrated specimens

Dehydrated specimens may be placed individually in heat-resistant glass jars that are filled with distilled or deionized water and left for 24 hours to rehydrate, then processed following steps 3 to 19 above.

Specimens preserved in fluid, with chemicals, or otherwise contaminated

Specimens that are in a fluid preservative (e.g., formaldehyde or alcohol), that have been contaminated with chemicals such as borax or phenol, or are contaminated with fungi or other organisms must be cleaned before processing. Place such specimens in individual heat resistant glass jars and immerse then in distilled or deionized water for 24 hours (Meeuse, 1965). After processing as described in step 3 above, the specimen may need to be coated with animal fat or vegetable oil to make it palatable to the beetles (Laurie and Hill, 1951; Hooper, 1956). Alternatively, the specimen may be treated with a concentrated solution made with cubes of instant broth that has been allowed to cool, this reduce the cleaning time of chemically preserved osteological material, and also facilitated dermestid cleaning of a maggot-contaminated specimen (Nicholson and Smith, 2010). Submerge the specimen in the cooled broth for 12 hours, then dry in an oven for 6 hours at a temperature of 40 °C, then process through steps 4 to 19 above.

Considerations to the process

To minimize the dehydration of tissues, it is recommended that specimens to be cleaned should not be dried prior to placing them in the dermestid colony (Valcarcel and Johnson, 1981), with the exception of specimens previously preserved in fluid or otherwise chemically dehydrated. Specimens that are not completely cleaned despite being exposed to the dermestids for a long time should be isolated to force the larvae to eliminate the remaining traces of tissue (Borell, 1938). Fresh specimens should not be added to a dermestarium once the processing of other specimens has begun as the beetles tend to prefer fresh material.

Should the beetle colony decrease considerably, a piece of meat with fat should be added to encourage the beetles to pupate (Borell, 1938; Russell, 1947; Meeuse, 1965). When the colony is not checked frequently (e.g., on weekends or holidays) a piece of meat with fat wrapped in moist cotton may be added to the dermestarium to provide an adequate nest for the eggs and pupae; this will ensure that the colony has enough soft tissue for the larvae, but keep in mind that the beetles will abandon older osteological material for fresh meat (Leeper, 2015).

If treating the cleaned bones with ammonia (steps 9 to 19) cannot be carried out immediately, the specimens should be placed in a resealable polyethylene or polypropylene bag and frozen to a temperature of -18 to -20 °C (McDonald, 2006).

Specimen history

All procedures and processes carried out during the preparation of osteological material by *Dermestes* beetles (hydration, elimination of chemicals, cleaning, degreasing, drying) should be recorded as part of the permanent specimen record (e.g., in catalogs and databases). This information is important because how specimens are prepared often affects their use in subsequent research (e.g., DNA sequencing).

Cleaning time

Under ideal conditions, an active dermestid colony can be expected to clean fresh small skulls and skeletons in three to ten days (Tiemeuer, 1939; Meeuse, 1965; Hefti, *et al*., 1980; Leeper, 2015). Large specimens and those that have been dehydrated or subjected to some chemical treatment will require more time (Meeuse, 1965), as much as 20 to 30 days. The length of time required in the dermestid colony depends on (1) the condition of the colony (2); how the specimen is preserved (fresh, dehydrated, in fluid); (3) the size of the specimen; and (4) the amount of tissue to be removed (Meeuse, 1965).

The useful life of a colony is two to three months before the accumulation of larvae and frass reduce its efficiency to a very low rate (Meeuse, 1965), this depend of use and of care. Because of this, it is necessary to clean the terraria and transfer the adults, larvae, pupae, and eggs to two other terraria every two or three months.

Risks of working with beetles of the genus *Dermestes*

The following considerations must be taken into account when working with colonies of beetles of

the genus *Dermestes* and when cleaning osteological material:

Diseases

Direct contact with the larvae should be avoided because shed hairs and frass may cause skin allergies (e.g., contact dermatitis). The frass may also cause irritation of the respiratory tract (Tiemeier, 1939; Meeuse, 1965; Simmons and Muñoz-Saba, 2005).

Pests in Collection

Dermestes beetles are a common pest in biological collections, where they feed on a wide variety of materials, especially skins, feathers, hair, wood, paper, wool, silk, and dried fruits (Muñoz-Saba and Simmons, 2005). Therefore, care must be taken to avoid dermestid infestations in the collection (McDonald, 2006).

Biosecurity

Due to the biological risk incurred in cleaning osteological material with beetles of the genus *Dermestes*, the following precautions should be taken:

Personal protective equipment

Industrial coveralls should be worn to prevent clothing from becoming impregnated with the odors that are produced in the process as well as contamination from insect frass. Use of a long-sleeved lab coat (preferably disposable) is recommended, as well as the use of a nylon head covering, safety glasses or goggles, a well-fitting dust mask, and nitrile gloves.

Biological and chemical residues

The Biosecurity Protocols for Biological and Chemical Residues established by each institution must be followed.

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