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# Macroscopic and microscopic characterization of the anatomy of the digestive, excretory and reproductive systems in *Dermestes maculatus* (Coleoptera: Dermestidae), and evaluation of the effect of Roundup Full® II on the anatomy of these systems

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**Caracterización macroscópica y microscópica de la anatomía de los sistemas digestivo, excretor y reproductivo en *Dermestes maculatus* (Coleoptera: Dermestidae), y evaluación del efecto del Roundup Full® II sobre la anatomía de dichos sistemas**

**RESUMEN.** Roundup Full® II (RFII) es actualmente el herbicida más utilizado en Argentina y el mundo. *Dermestes maculatus* De Geer (Coleoptera: Dermestidae) es una especie cosmopolita y se ha observado en cadáveres o restos de animales y humanos. Los envenenamientos con RFII pueden deberse a exposiciones accidentales durante la aplicación del herbicida o a suicidios. La anatomía macroscópica y microscópica de tejidos y órganos de los organismos es parte de la descripción de las especies, lo que permite evaluar el efecto tóxico de xenobióticos sobre esas estructuras. Los objetivos del estudio fueron describir la anatomía macroscópica y microscópica de los sistemas digestivo, excretor y reproductivo de *D. maculatus*, de interés forense, e investigar el efecto del RFII sobre la anatomía de tales sistemas. Se describió la anatomía macroscópica y microscópica de los sistemas mencionados en larvas, adultos y pupas de *D. maculatus*. No se observaron cambios reconocibles en órganos digestivos y excretores de larvas y adultos debido a RFII, ni alteraciones en gónadas de pupas y adultos resultantes del tratamiento de larvas con el herbicida. Los resultados aportan nueva información sobre la anatomía de esta importante especie de insecto y para la entomología forense, contribuyendo a futuros estudios en estas áreas.

**PALABRAS CLAVE.** Anatomía macroscópica de insectos. Anatomía microscópica de insectos. Escarabajos de la piel. Herbicida.

**ABSTRACT.** Roundup Full® II (RFII) is presently the most widely used herbicide in Argentina and in the world. *Dermestes maculatus* De Geer (Coleoptera: Dermestidae) is a cosmopolitan species and has been observed in animal and human cadavers or remains. Poisonings with RFII may be due to accidental exposures during the application of the herbicide or to suicides. The macroscopic and microscopic anatomy of tissues and organs of organisms is part of the species description, which allows evaluating the toxic effect of xenobiotics on these structures. The objectives of this study were the macroscopic and microscopic description of the digestive, excretory and reproductive systems of *D. maculatus*, of forensic interest, and the investigation of the effect of RFII on the anatomy of these systems. The macroscopic and microscopic anatomy of the mentioned systems in larvae, adults and pupae of *D. maculatus* were described. No recognizable changes were observed in digestive and excretory organs of larvae and adults due to RFII, and no alterations were found in gonads of pupae and adults resulting from treating larvae with the herbicide. The results provide new information on the anatomy of this important species of insect and for forensic entomology, contributing to future studies in these areas.

**KEYWORDS.** Herbicide. Hide beetles. Insect macroscopic anatomy. Insect microscopic anatomy.

 

## INTRODUCTION

Glyphosate or N-(phosphonomethyl) glycine, is a broad-spectrum herbicide used to kill annual and perennial grasses, broadleaf grasses, and undesirable woody species. The mechanism of action of this herbicide is to inhibit the enzyme, enol-pyruvyl-shikimate-phosphate-synthetase, resulting in the inhibition of plant growth and survival. This herbicide is usually marketed as a formulation, that is, in addition to the active principle there are other ingredients, such as surfactants, that facilitate handling or increase the effectiveness of the herbicide. Roundup Full II is presently the most widely used herbicide in Argentina and in the world (Mensah et al., 2015). This herbicide is biodegradable and persists in the environment only for a short time. Glyphosate and formulations have been reported to have adverse effects in terrestrial and aquatic environments in vertebrates and invertebrates organisms (Mensah et al., 2015). Some authors have suggested a synergistic effect of the surfactant, as well as, the other ingredients (Comstock et al., 2007). The tolerance or mechanism of toxicity of glyphosate in humans differs from that of other animals (Talbot et al., 1991; Tominack et al., 1991). Until now, it has been postulated that in humans the mechanism of glyphosate could be the uncoupling of mitochondrial oxidative phosphorylation (Talbot et al., 1991). Poisonings may be due to accidental exposures during the application of the herbicide when the handling is not correct and/or the safety and health regulations at work are not complied with (Damalas & Eleftherohorinos, 2011). Cases of lethal poisoning due to the ingestion of glyphosate (acute poisoning) have also been reported, that is, suicides and suicide attempts (Talbot et al., 1991; Tominack et al., 1991; Lee et al., 2015). In addition to death, poisoning can imply variable symptoms, even involving nephrotoxicity, hepatotoxicity, gastrointestinal, etc., as well as, multiorgan toxicity (Tominack et al., 1991; Beswick & Millo, 2011; Hour et al., 2012).

Microscopic anatomy allows evaluating the toxic effect of xenobiotics on organs and tissues of organisms (Latif et al., 2013), because it is a technique that consists of visualizing the structure, as well as, the changes to which the organs/tissues have been subjected. So, it is used in scientific studies, forensic investigation, etc. (Gurina & Simms, 2023). The same concept can be applied for macroscopic anatomy. Forensic entomotoxicology is an area of forensic entomology that involves, among other objectives, the qualitative and/or quantitative determination of xenobiotics in scavenging insects (Goff et al., 1991; Gagliano & Aventaggiato, 2001). Toxicological analysis of insects found at the site of death or on a carcass can help determine the cause of death or the circumstances surrounding it, can contribute to the estimation of the postmortem interval (PMI) (Bourel et al., 1999; O'Brien & Turner, 2004) and/or with the geographical area where the body rests or its origin (Nuorteva, 1977).

In arthropods there is little literature on histology effects of glyphosate or its formulations. In crustaceans were examined alterations in somatic and ovarian growth (Avigliano et al., 2018), in digestive tubules, connective tissues and haemocytes infiltration (Séguin et al., 2017), and in main organs (Binhua et al., 2023). In insects a previous study evaluated the effects of Roundup Full II on the development of *Dermestes maculatus* De Geer (Coleoptera: Dermestidae) and *Lucilia sericata* Meigen (Diptera: Calliphoridae), but there are no reports on this herbicide or any formulation-based glyphosate effects in hide beetles macroscopic and microscopic anatomy. *Dermestes maculatus* is a cosmopolitan species that has been observed in animal and human cadavers or remains; they are pests of stored animal products and they can also damage constructions (Zanetti et al., 2020). Besides of beetle adaptations for feeding on dry materials, they are able to colonize, feed and reproduce on fresh tissues soon after death (Gunn, 2018; Zanetti et al., 2019).

This study aimed to provide descriptions of the macroscopic and microscopic anatomy of digestive, excretory and reproductive systems in a species of forensic interest such as *D. maculatus*, and to investigate the effect of Roundup Full II (RFII) on the anatomy of these systems.

## MATERIAL AND METHODS

### Rearing of *Dermestes maculatus* under treatment with RFII.

Colonies of hide beetles were maintained in plastic containers with cat litter and pieces of distilled water-soaked cotton at 20±5 ºC, 40 % relative humidity, and 12:12 h Light/Dark photoperiod in the vivarium of Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur. To provide another pupation site or refuge for beetles, pieces of soft wood were also added. Hide beetles were supplied with pig trotters. For the analysis of RFII, this herbicide was provided to beetles in a diet as was described in Zanetti et al. (2021). In brief, the preparation of diet consisted in cutting pork muscle into small pieces and homogenized them with distilled water. Then, 7.69 ml/kg RFII (potassium salt of N-phosphonomethyl glycine 66.2 % p/v, Monsanto Argentina S.A.I.C) was added to the mixture, which was homogenized again. Finally, powdered agar (Laboratorios Britania S.A., Argentina) was dissolved in boiling distilled water and thoroughly mixed into the pork preparation. The dose represented a potential lethal acute overdose in humans (Zanetti & Centeno, 2024). The control was a homogenate of pork muscle (which was obtained as was described above) without herbicide. For more details on the protocol, see Zanetti et al. (2016, 2021). Twenty adults of *D. maculatus* from the colony were placed inside a plastic container with 3 cm of cat litter, pieces of cotton, and pieces of soft wood. The hide beetles were provided with the diet as food. The containers were maintained at 30±0.1 ºC, 42 ± 3 % relative humidity and 12:12 h Light/Dark photoperiod inside an incubator (Ingelab, Argentina). From the beginning to the end of the trial daily observations were made to verify the development of hide beetles (from egg to adult emergence). Twenty recently moulted specimens of larval instar 5, 6 or 7, pupa of 72 h old and emerged adult females were randomly extracted, killed in 5 % ethanol and preserved in formaldehyde 10 %.

**Macroscopic anatomy**

Control and RFII-treated specimens were removed from formaldehyde 10 % and dissected under stereomicroscope (NXZ, China). Photographs were taken using a digital camera (Optika, Italy) which was mounted to a stereomicroscope (Zeiss, Germany) to observe the digestive, excretory and reproductive structures (anterior, median, and posterior gut; Malpighian tubule; ovary, bursa copulatrix, spermatheca, spermathecal gland, ducts, and vagina). Possible alterations in the form and number of these structures in response to RFII were qualitatively examined. Five replicates of each treatment were performed.

**Microscopic anatomy**

Before fixing the specimens with 10 % formaldehyde for 24 h, control, as well as, treated specimens with RFII were removed from ethanol 5 % and manipulated under stereomicroscope (NXZ, China) to make punctures with an entomological pin and to remove legs from larva and adult specimens to allow fixative to enter the body, in adults were removed elytra as well. After fixation, specimens were dehydrated in ascending grades of alcohol followed by infiltration and inclusion in paraffin (Ross et al., 1997). Five µm microtome sections were cut into a rolling ribbon. The ribbon was placed on the glass slide and stained with haematoxylin and eosin (Ross et al., 1997). Finally, slides were mounted, cover slip and examined under optic microscope (Olympus BX51). Photographs were taken using an Olympus C-7070 digital camera which was mounted to the microscope to observe any changes in nucleus, cytoplasm, membrane, microvilli, intima, and cell form, of the digestive, excretory and reproductive structures mentioned above. This allowed a qualitative comparison of glyphosate treatment with control. An appropriate magnification was used (this was indicated in the figure captions). Five replicates of each treatment were performed.

## RESULTS AND DISCUSSION

**Adults**

The digestive structure besides mouthparts is a gut which can be divided in foregut, midgut and hindgut. The foregut is made up of a pharynx, an oesophagus, a proventriculus and a cardiac valve (Fig. 1a). The pharynx consists of a simple columnar epithelium and muscles. The oesophagus is surrounded by longitudinal and transverse muscles and columnar epithelial cells with microvilli (Fig. 1b-c). The proventriculus is the widest part of the foregut and has a simple columnar epithelium with an oval nucleus and microvilli. Surrounding the epithelium there are layers of circular and longitudinal muscles. This could be seen in Fig. 1d. A well-developed intima with spicules or spines facing lumen is present in both oesophagus and proventriculus. The division of the foregut surrounded by muscle is also described in *Meligethes* (Odonthogethes) *chinensis* Kirejtshuk (Coleoptera: Nitidulidae) (Chen et al., 2023). The foregut is the shortest region of the intestine and this characteristic is also observed in other insect species, suggesting that this characteristic is due to the fact that they do not need to store large amounts of food (Terra, 1990), which is further supported by the absence of crop. In *Oxelytrum discicolle* Brullé (Coleoptera: Silphidae) and *Eusomus ovulum* Germar (Coleoptera: Curculionidae), developed musculature is also found associated with the epithelium and spicules present in the cuticle that covers the lumen of the foregut (Özyurt Koçakoğlu et al., 2020; Toni et al., 2022). Such structures would be associated with the movement and crushing of food (Snodgrass, 1935). At the union of proventriculus with ventriculus a ring of six spherical gastric caeca is observed as is shown in Fig. 1a, b. The caecum consisted of a monolayer columnar epithelium with microvilli and a central rounded nucleus with a nucleolus and parches of heterochromatin (Fig. 1e); also, nidi of regenerative cells are located in some places of the caecum (Fig. 1f). The midgut is represented by the ventriculus (Fig. 2a). From the exterior to the interior (lumen) it has outer longitudinal muscles and inner circular muscles, followed by a basal membrane where is placed a simple columnar epithelium with an oval nucleus in the center (with patches of heterochromatin) and a brush border in the apical surface bordering with the gut lumen, often forming folds. Outside the muscle layers are found several tracheoles and tracheae. All these structures can be seen in Fig. 2b. Microvilli secrete a thin membrane, the peritrophic membrane. A similar microscopic anatomy is observed in the proventriculus and midgut of *E. ovulum* (Özyurt Koçakoğlu et al., 2020) and of *Epicauta waterhousei* Haag-Rutenberg (Coleoptera: Meloidae), but this latter species differs in the simple cuboidal epithelium (Senarat et al., 2014). In *M.* (O.) *chinensis* females the macroscopic anatomy of midgut is also similar (Chen et al., 2023) to that of hide beetles. Throughout the midgut there are small blunt-fingered diverticula or evaginations arranged in the form of rings (Fig. 2a, c, d). The diverticula consisted of a columnar epithelium with an oval nucleus with heterochromatin, microvilli and a very narrow lumen (Fig. 2b). Both gastric caeca and diverticula are consistent with that of Cleridae species (Ekis & Gupta, 1971), and also in other species of Coleoptera such as: *Heteronychus* *arator* Fabricius (Coleoptera: Scarabaeidae) (Sheehan et al., 1982), *O. discicolle* (Caldeira et al., 2007), *Nicrophorus vespillodes* Herbst (Coleoptera: Silphidae) (Vogel et al., 2017), and *M.* (O.) *chinensis* (Chen et al., 2023). Such coincidence could be explained because the species belong to the same order and suborder (Vanin & Ide, 2002). The presence of crypts with regenerative cells indicates that the epithelium of the midgut can be constantly renewed, mainly in necrophagous beetles that feed on food surrounded by the peritrophic matrix (Erlandson et al., 2019; Hegedus et al., 2019).

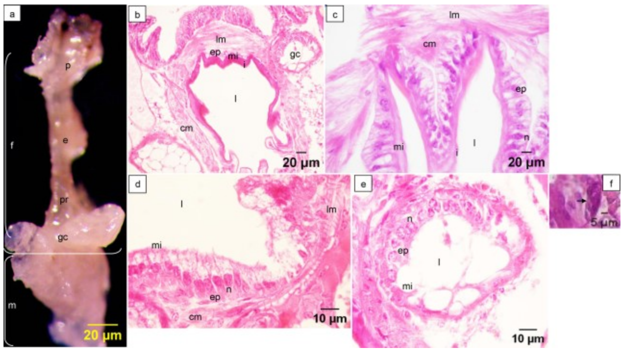

**Fig. 1. Digestive system of *Dermestes maculatus* adult. a. Macroscopic anatomy of foregut and first section of midgut (5x). b. Longitudinal section of foregut (40x). c. Longitudinal section of oesophagus (40x). d. Longitudinal section of proventriculus (100x). e. Longitudinal section of gastric caeca (100x). f. Longitudinal section of nidi of regenerative cells of gastric caecum (20x).** References= cm: circular muscle, e: oesophagus, ep: epithelium, f: foregut, gc: gastric caeca, i: intima, l: lumen, lm: longitudinal muscle, m: midgut, mi: microvilli, n: nucleus, p: pharynx, pr: proventriculus. Black arrow shows nidi of regenerative cells.

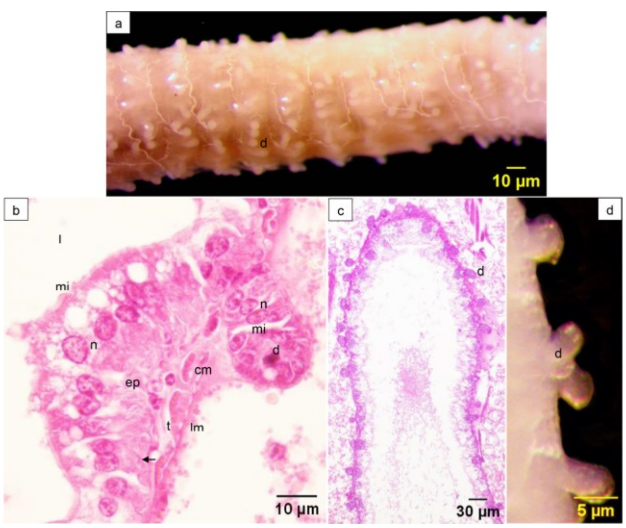

**Fig. 2. Midgut of *Dermestes maculatus* adult. a. Ventriculus macroscopic anatomy (5x). b. Longitudinal section of ventriculus (100x). c. Idem b (20x). d. Macroscopic anatomy detail of diverticula (5x).** References= cm: circular muscle, d: diverticula, ep: epithelium, l: lumen, lm: longitudinal muscle, mi: microvilli, n: nucleus, t: tracheole. Black arrow shows basal membrane.

The hindgut is divided in ileum, colon, and rectum (Fig. 3a). The junction of the midgut and ileum coincides with the opening of the Malpighian tubules, and the junction of the ileum and colon coincides with the distal Malpighian tubules (Fig. 3b). The folded ileum is composed of sclerotized intima in contact with lumen, a columnar epithelium, and muscle layers (inner circular and external longitudinal) (Fig. 3c). The nucleus of the epithelial cells of ileum is oval and located in the middle of the cell. The colon and the rectum have the same tissues as ileum (Fig. 3d-e). All divisions are surrounded by the tracheae and tracheoles. The presence of circular and longitudinal muscles, as well as, tracheae and tracheoles, surrounding the three sections, is also described for clerid species (Ekis & Gupta, 1971), and other species of Coleoptera such as: *E. ovulum* (Özyurt Koçakoğlu et al., 2020), *O. discicolle* (Toni et al., 2022), and *M.* (O.) *chinensis* (Chen et al., 2023). *Aegorhinus superciliosus* Guérin-Méneville (Coleoptera: Curculionidae) shows a different arrangement of muscle layers than *D. maculatus,* as it has an outer wall of circular muscles and an inner layer of longitudinal muscles (Medel et al., 2013). *Epiphaneus malachiticus* Boheman (Coleoptera: Curculionidae) presents a different epithelium from that in hide beetles because is of cuboidal cells (Candan et al., 2019).

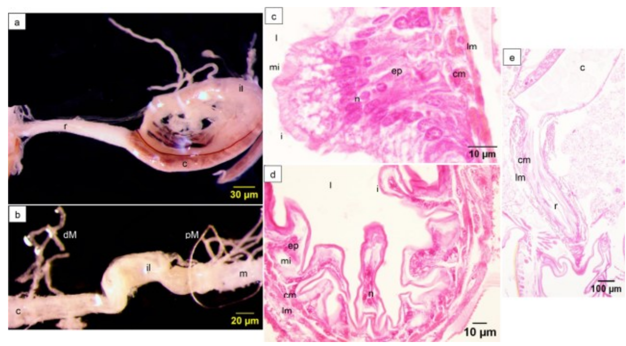

**Fig. 3. Hindgut of *Dermestes maculatus* adult. a. Macroscopic anatomy (3.2x). b. Macroscopic anatomy insertion of Malpighian tubules (4x). c. Longitudinal section of ileum (100x). d. Longitudinal section of colon (100x). e. Longitudinal section of the colon and rectum (4x).** References= c: colon, cm: circular muscle, dM: distal Malpighian tubules, ep: epithelium, i: intima, il: ileum, l: lumen, lm: longitudinal muscle, m: midgut, mi: microvilli, n: nucleus, pM: proximal Malpighian tubules, r: rectum.

The excretory system consists of six Malpighian tubules that are characterized by large cuboidal cells with microvilli which form the brush border (Fig. 4a-c). A large oval or spherical nucleus is found in the central cytoplasm of epithelium containing a nucleolus and heterochromatin. The tubular epithelium is covered by an extremely thick basal lamina. Outside the basal lamina are found several tracheoles and a series of circular and longitudinal muscles (Fig. 4b-c). The proximal tubules insert at the junction of the ventricle and ileum, and the distal tubules insert at the junction of the ileum and colon. The macroscopic and microscopic anatomy described in hide beetles is also observed in other beetles (Aldigail et al., 2013; Candan et al., 2019; Özyurt Koçakoğlu et al., 2020). The union of the tubules to the colon forms a cryptonephridial system. Similarities can be found in species of Cleridae, and other species of Coleoptera such as: *M.* (O.) *chinensis*, *E. ovulum* and *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) (Ekis & Gupta, 1971; Bell et al., 2012; Özyurt Koçakoğlu et al., 2020; Chen et al., 2023). The cryptonephridial system is considered an adaptation to minimize water loss. These cells present an apical edge that increases the nutrient absorption surface (Serrão & Cruz-Landim, 1996).

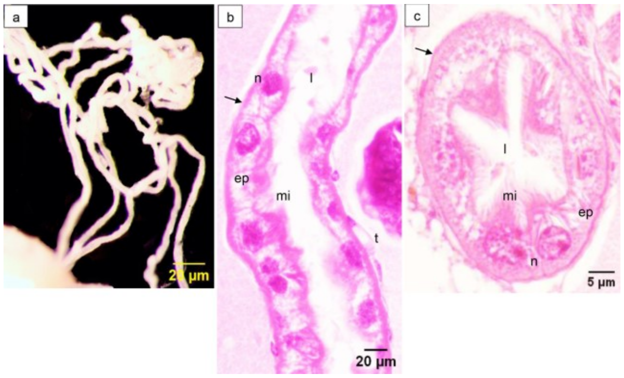

**Fig. 4. Malpighian tubules of *Dermestes maculatus*. a. Macroscopic anatomy (5x). b and c. Longitudinal section of Malpighian tubules (100x).** References= ep: epithelium, l: lumen, mi: microvilli, n: nucleus, t: tracheole. Black arrows show basal membrane.

There are two ovaries of the telotrophic meroistic type with twenty ovarioles each one. Each ovariole is elongated in shape with broad base and with a terminal filament in the apical region (Fig. 5a-b). Next to this filament is a germarium, a set of developing oocytes and nurse cells contained within follicles, and a pedicel or posterior connection to an oviduct (Fig. 5c-d). The developing oocyte with spherical nucleus, and nurse cells, is enclosed by cuboidal epithelium with large nucleus (follicular cells) (Fig. 5d-e). The nurse cells are generally hexagonal shaped cells containing a spherical nucleus (Fig. 5f). Between the oocyte and the pedicel there is a pre-follicular plug. In Fig. 6a the other reproductive structures can be seen. The lateral oviduct is formed by an epithelium of cells with oval nuclei. The middle oviduct is formed by an epithelium of large and irregular cells, with oval and basally positioned nuclei (Fig. 6b). The bursa copulatrix lumen is surrounded by a cuticular intima, a single layer of columnar epithelium, and a thin muscle layer (Fig. 6c). The spermatheca is made up of a thin muscular envelope and a simple epithelium of irregular flat cells. This can be seen in Fig. 6d and also with spermatozoa inside. The spermathecal gland has an epithelium consisting of large glandular cells with an irregular shape, which present abundant cytoplasmic secretions (Fig. 6e). The spermathecal duct is made up of a thick muscular envelope and an epithelium made up of compact/columnar cells with rounded or oval nuclei in a basal position (Fig. 6d). The vagina is covered by a thick muscular structure, followed by an epithelial layer that supports a cuticular intima (Fig. 6f). The macroscopic and microscopic anatomy of the female reproductive system of hide beetles are very similar to that of clerids (Opitz, 2014), *Odontotaenius striatopunctatus* Percheron (Coleoptera: Passalidae) (Cruz Rosales & Castillo, 2008), *Rhynchophorus ferrugineus* Olivier (Coleoptera: Curculionidae) (Wan Nurul 'Ain et al., 2018), *Trypophloeus klimeschi* Eggers (Coleoptera: Curculionidae) (Gao et al., 2021), and *Capnodis tenebrionis* L. (Coleoptera: Buprestidae) (Özyurt Koçakoğlu et al., 2022), differentiating *D. maculatus* from these species in the number of ovarioles. This condition in insects can differ depending on the insect reproductive strategy (Özyurt Koçakoğlu et al., 2022).

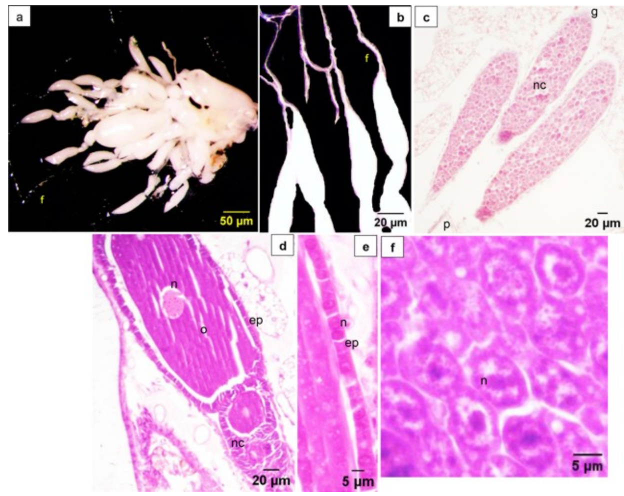

**Fig. 5. Female reproductive system of *Dermestes maculatus*. a. Macroscopic anatomy of ovary (2x). b. Detail of macroscopic anatomy of the ovarioles (3.2x). c. Longitudinal section of ovarioles (20x). d. Longitudinal section of ovarioles with oocytes in different stages of development (40x). e. Detail of follicular cells (100x). f. Detail of nurse cells (100x).** References= ep: epithelium, f: filament, g: germarium, n: nucleus, nc: nurse cell, o: oocyte, p: pedicel.

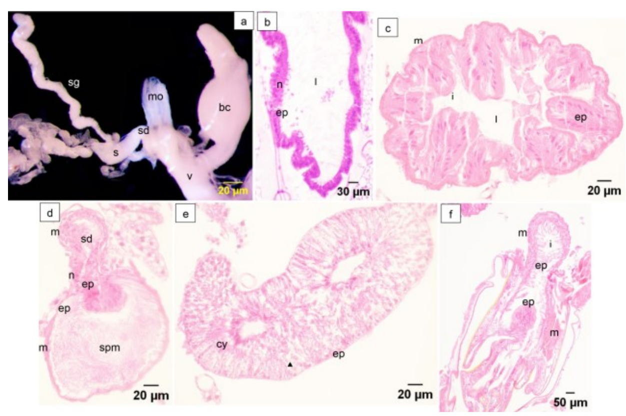

**Fig. 6. Female reproductive system of *Dermestes maculatus*. a. Macroscopic anatomy of common oviduct, bursa copulatrix, spermatheca with spermathecal duct, spermathecal gland, and vagina (4x). b. Longitudinal section of median oviduct (40x). c. Longitudinal section of bursa copulatrix (40x). d. Longitudinal section of spermatheca with spermathecal duct (40x). e. Longitudinal section of spermathecal gland (40x). f. Longitudinal section of vagina (20x).** References= bc: bursa copulatrix, cy: cytoplasm, ep: epithelium, i: intima, l: lumen, m: muscle, mo: middle oviduct, n: nucleus, s: spermatheca, sd: spermathecal duct, sg: spermatechal gland, spm: sperm, v: vagina. Arrowhead shows cytoplasmic secretions.

**Larva**

As in adults, following the mouthparts a gut with three regions is distinguished: foregut, midgut and hindgut. The foregut has the same divisions as in adults. Fig. 7a shows the oesophagus which has the same histology as in adults. Also, at the union of proventriculus with ventriculus is located a ring of six ball shaped gastric caeca. Their size increases from the ventral to the dorsal area. The microscopic anatomy of the caeca is the same as in adults (Fig. 7b). The midgut is similar to that of the adult but there are no diverticula, regenerative cells can be found within the epithelium forming basal nests, with an oval nucleus with patches of heterochromatin (Fig. 7c). Outside the muscle layers are found several tracheoles and tracheae. The findings of the present work match with those of Caldeira et al. (2007) and similarities are also observed with other species such as *H. arator* (Sheehan et al., 1982), *Trogoderma granarium* Everts (Coleoptera: Dermestidae) (Szczepanik & Ignatowicz, 1999), and *O. discicolle,* although this latter lacks gastric caeca (Toni et al., 2022). The cells of midgut may be multifunctional to produce digestive enzymes and peritrophic matrix and absorbing nutrients, since there is no anatomical or histological regionalization in the midgut of *D. maculatus* larvae (Toni et al., 2022). The hindgut showed the same macroscopic and microscopic anatomy as in adults. All divisions are surrounded by the tracheae and tracheoles and the epithelium is columnar (Fig. 7d-f). Some differences present *H. arator* because the hindgut has small, long, finger-like papillae on the inner surface of the anterior colon (Sheehan et al., 1982), and *O. discicolle* has a cuboidal epithelium along the hindgut (Toni et al., 2022).

The excretory system consists of Malpighian tubules and these have the same microscopic anatomy as in adults (Fig. 7g). In *H. arator* there are four Malpighian tubules (Sheehan et al., 1982).

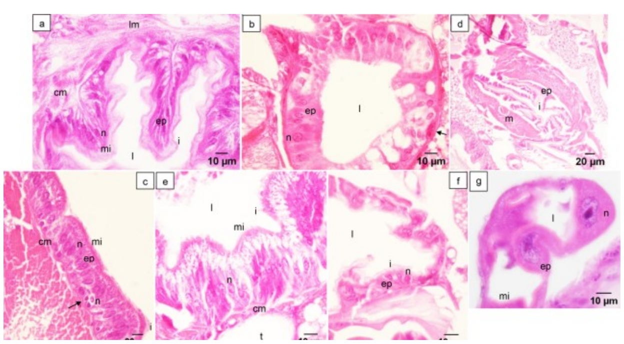


**Fig. 7. Microscopic anatomy of alimentary and excretory system of *Dermestes maculatus* larvae. a. Longitudinal section of oesophagus (100x). b. Longitudinal section of gastric caecum (100x). c. Longitudinal section of midgut (100x). d. Longitudinal section of ileum (40x). e. Longitudinal section of colon (100x). f. Longitudinal section of rectum (100x). g. Longitudinal section of Malpighian tubule (100x).** References= ep: epithelium, cm: circular muscle, i: intima, l: lumen, lm: longitudinal muscle, m: muscle, mi: microvilli, n: nucleus, t:trachea. Black arrows show nidi of regenerative cells.

**Pupae**

In pupae of the age studied, reduced or few structures that belong to the digestive, excretory and reproductive systems can be seen. Those structures have the same microscopic anatomy described for the other stages (Fig. 8a-c). The fact that the structures were few or reduced may be consistent with the remodelling involved in the transition from larva to adult (metamorphosis). For example, in species of the family Calliphoridae, Davies & Harvey (2013) observed that four-day pupae comprise mainly fat body (trophocytes) and oenocytes, which contain lipid, and as the pupa develops, neuron and muscle density increases. Helm et al. (2018) indicated that the gut of *Megachile rotundata* Fabricius (Hymenoptera: Megachilidae) was discernible in pupal samples and that an early pupa has gut in the early stages of development. They also pointed out that the organ systems are at different stages of organogenesis throughout metamorphosis.

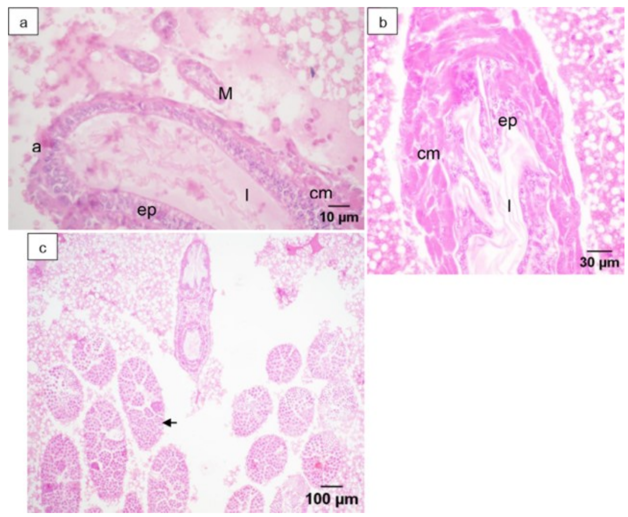


**Fig. 8. Microscopic anatomy of alimentary, excretory and reproductive systems of *Dermestes maculatus* pupa. a. Longitudinal section of alimentary canal and Malpighian tubules (100x). b. Longitudinal section of alimentary canal (40x). c. Longitudinal section of ovarioles (10x).** References= a: alimentary canal, cm: circular muscle, ep: epithelium, l: lumen, M: Malpighian tubule. Black arrow shows nurse cells.

**Toxicicity of RFII**

No recognizable changes (for example, loss of the brush border, damage to epithelial cell nuclei, etc.) were observed in anterior, median, posterior gut and Malpighian tubules of larvae and adults due to RFII. This matched with Mottier et al. (2015) who revealed the absence of atrophies of the wall of the digestive tubules, destructuration of the connective tissue, and haemocyte infiltration in *Crassostrea gigas* Thunberg (Ostreoida: Ostreidae) after a subchronic exposure to glyphosate acid. Likewise, Séguin et al. (2017) showed the same results after exposure of the Pacific oyster to glyphosate Roundup Express® (REX) and POEA. Also, no macroscopic and microscopic alterations were found in gonads of pupae and adults resulting from treating larvae with the herbicide. Likewise, the bursa copulatrix, spermatheca, spermathecal gland, oviducts, spermathecal duct and vagina in adults were not affected by the herbicide. Mottier et al. (2015) did not detect gamete degenerations or gonad necrosis in *C. gigas*. Avigliano et al. (2018) found a higher proportion of reabsorbed vitellogenic oocytes in the ovaries of *Neohelice granulata* Dana (Decapoda: Varunidae) exposed to glyphosate together with an increased area of previtellogenic oocytes. Also, some previtellogenic oocytes were of bigger size compared to control.

Different factors, such as species, xenobiotic type, xenobiotic concentration, dose, stage of development, variability among individuals, pharmacokinetics, among others, could cause different responses to a xenobiotic, therefore the rates of development could be increased, delayed or unchanged (Zanetti et al., 2021; Zanetti & Centeno, 2024).

In conclusion, the results provide new information on the anatomy of this important species of insects valuable for forensic entomology.

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