The spermathecal duct of earwig *Doru luteipes* (Dermaptera: Forficulidae) contributes to spermatozoa survival

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During the mating of insects, the spermatozoa are transferred to the females and stored in the spermatheca (Pascini & Martins 2017). The spermatozoa are stored in this organ to avoid mechanical damage and contact with the hemolymph, remaining in an environment adequate for their maintenance until fertilization (Collins et al. 2004; Al-Lawati et al. 2009; King et al. 2011; Pascini & Martins 2017).

Although the morphology and number vary depending on the insect taxon, the spermatheca consists of a reservoir for spermatozoa storage associated with a muscular duct that transitions into the common oviduct or vagina (Martins & Serrão 2002; Martins et al. 2008; Chapman 2013; Souza et al. 2016; Pascini & Martins 2017).

The shape of the spermatheca may be spherical, tubular, or reniform (Kocorek & Danielczok-Demska 2002; Rodrigues et al. 2008; Souza et al. 2008, 2016; Pascini & Martins 2017). The spermathecal duct may be short, long, with dilations, and may have 1 or 2 flanges at the extremities (Kocorek & Danielczok-Demska 2002; Martins & Serrão 2002; Souza et al. 2016).

In some insects, there is an associated spermathecal gland releasing compounds into the reservoir lumen, contributing to the maintenance of spermatozoid viability (Cruz-Landim & Serrão 2002; Souza et al. 2008; Pascini & Martins 2017). Moreover in insects without spermathecal glands, the epithelial cells of the reservoir seem to assume a glandular function (Chapman 2013; Souza et al. 2016).

In Dermaptera, the spermatheca has been described as an unpaired organ, spherical or tubular in shape, connected to a long, sinuous duct, which may be branched in some species (Hudson 1973; Mariani 1994; Klass 2003; Kamimura 2007; Kamimura & Lee 2014). Although these studies have contributed to understanding the anatomical aspects of the spermatheca in those species, there is no data regarding the histology of the spermatheca in Dermaptera. Our objective is to describe the anatomy, histology, and histochemistry of the spermatheca in the earwig *Doru luteipes* (Scudder) (Dermaptera: Forficulidae), a natural enemy of many pests, such as *Spodoptera frugiperda* Smith & Abbott (Lepdoptera: Noctuidae) (Reis et al. 1988) and *Ascia monuste orseis* (Godart) (Lepidoptera: Pieridae) (Picanço et al. 2003).

Ten *D. luteipes* females were collected from corn crops in the municipality of Rio Paranaíba (19.209722°S, 46.133694°W), Minas Gerais, Brazil. The individuals were transferred to the Laboratório de Biologia Celular e Estrutural of the Universidade Federal de Viçosa – Rio Paranaíba Campus and cryo-anesthetized at −4 °C. The spermathecae were dissected in 125 mM NaCl, and transferred to Zamboni fixative solution (Stefanini et al. 1967) for 12 h at 5 °C.

Three *D. luteipes* spermathecae were washed in phosphate buffer pH 7.2 (PBS), dehydrated in increasing ethanol series (70, 80, 90, 95, and 98%), transferred to hexamethyldisilazane for 10 min, and dried at room temperature (Nation 1983). Subsequently, the samples were mounted on aluminum stubs, coated with gold (20 nm thickness), and analyzed with an LEO VP 1430 (Carl Zeiss, Jena, Germany) scanning electron microscope in the Núcleo de Microscopia e Microanálises of the Universidade Federal de Viçosa.

The 7 remaining spermathecae were dehydrated in a graded ethanol series (70, 80, 90, and 95%), and embedded in historesin (Leica Biosystems, Nussloch, Germany). Sections were cut into 3 µm thicknesses with a microtome (Leica Biosystems RM2255, Nussloch, Germany), stained with toluidine blue, and analyzed with a light microscope. Some sections of the spermathecae were subjected to the following histochemical tests: mercury bromophenol for detection of total proteins, and P.A.S. (Periodic Acid-Schiff) for detection of neutral polysaccharides and glycoconjugates, according to Bancroft and Gamble (2008).

The spermatheca of the *D. luteipes* female was a single structure with an enlarged reservoir associated with a long (about 5 × larger and three-fourths its diam) and sinuous duct without associated spermathecal glands (Fig. 1A-B). The hemolymph side of the spermathecal hypodermis had muscle layers on both the reservoir and the duct (Fig. 1C-E). In the spermathecal reservoir, and proximal and middle portions of the duct, the muscles were longitudinal (Fig. 1C-D), whereas in the distal portion they were circular (Fig. 1E).

The spermathecal reservoir consisted of a single layer of columnar cells, a cuticular intima, and a lumen. The intima identifies the organ as ectodermal, and the cells as hypodermis (Fig. 2A). The luminal content and the basal region of epithelial cells of the spermathecal reservoir were positive for P.A.S (Periodic Acid-Schiff). (Fig. 2B) and protein (Fig. 2C) in tests.

In the spermathecal duct of *D. luteipes*, the single layered epithelium had different cell types: (i) columnar cells with a large nucleus, and with intracellular canaliculi that opened into the lumen; and (ii) cubic cells, without intracellular canaliculi, closely associated with the cuticle (Fig. 2D-F). The spermathecal duct cells were positive for P.A.S. (Fig. 2E) and proteins (Fig. 2F).

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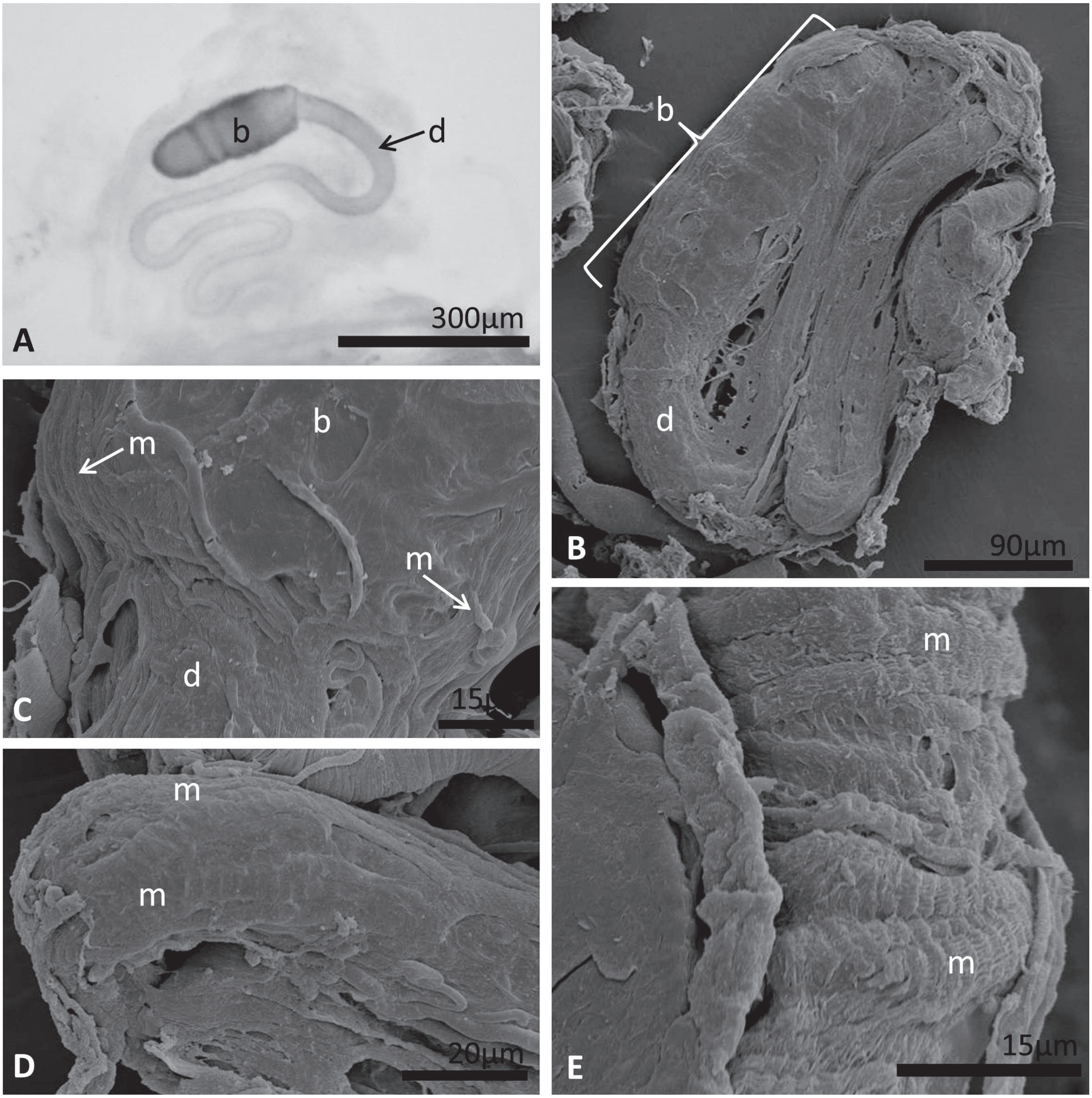

Fig. 1. Anatomy of the spermatheca in *Doru luteipes*. (A) Light micrograph showing the spermathecal reservoir (b) and duct (d). (B) Scanning electron micrograph of the spermathecal reservoir (b) and duct (d). (C) Transitional region between the reservoir (b) and the duct (d) showing longitudinal muscles (m). (D) Median portion of the spermathecal duct with longitudinal muscles (m). (E) Distal region of the spermathecal duct with circular muscles (m).

Two different, family-level spermathecal configurations are found in Dermaptera (Klass 2003; Kamimura 2007): single and multiple. Families with single spermatheca include Apachyidae, Labiduridae, Anisolabididae, Chelisochidae, Spongiphoridae, and Forficulidae. Branched spermatheca are found in the families Karschiellidae, Pygidicranidae, and Diplatyidae. This configuration is considered primitive, whereas the single spermatheca is considered derived.

Doru luteipes has the spermatheca with an enlarged reservoir and sinuous duct, such as reported in other Forficulidae (Hudson 1973; Mariani 1994; Schneider & Klass 2013), whereas in Chelisochidae and Spongiphoridae the reservoir is tubular, and of the same diam as the duct; that is, there is no abrupt demarcation in diam between the 2 (Hudson 1973; Schneider & Klass 2013).

The epithelial cells of the *D. luteipes* spermathecal reservoir are columnar, as are the secretory cells in the spermathecae of some Hymenoptera species (Dallai 1975; Martins & Serrão 2002; Souza et al. 2008). These cells in *D. luteipes* are rich in proteins and glycoconjugates, suggesting that they may contribute to spermatozoa maintenance. In addition, epithelial cells of the spermathecal duct in *D. luteipes* have an end apparatus from which emerge a duct cell

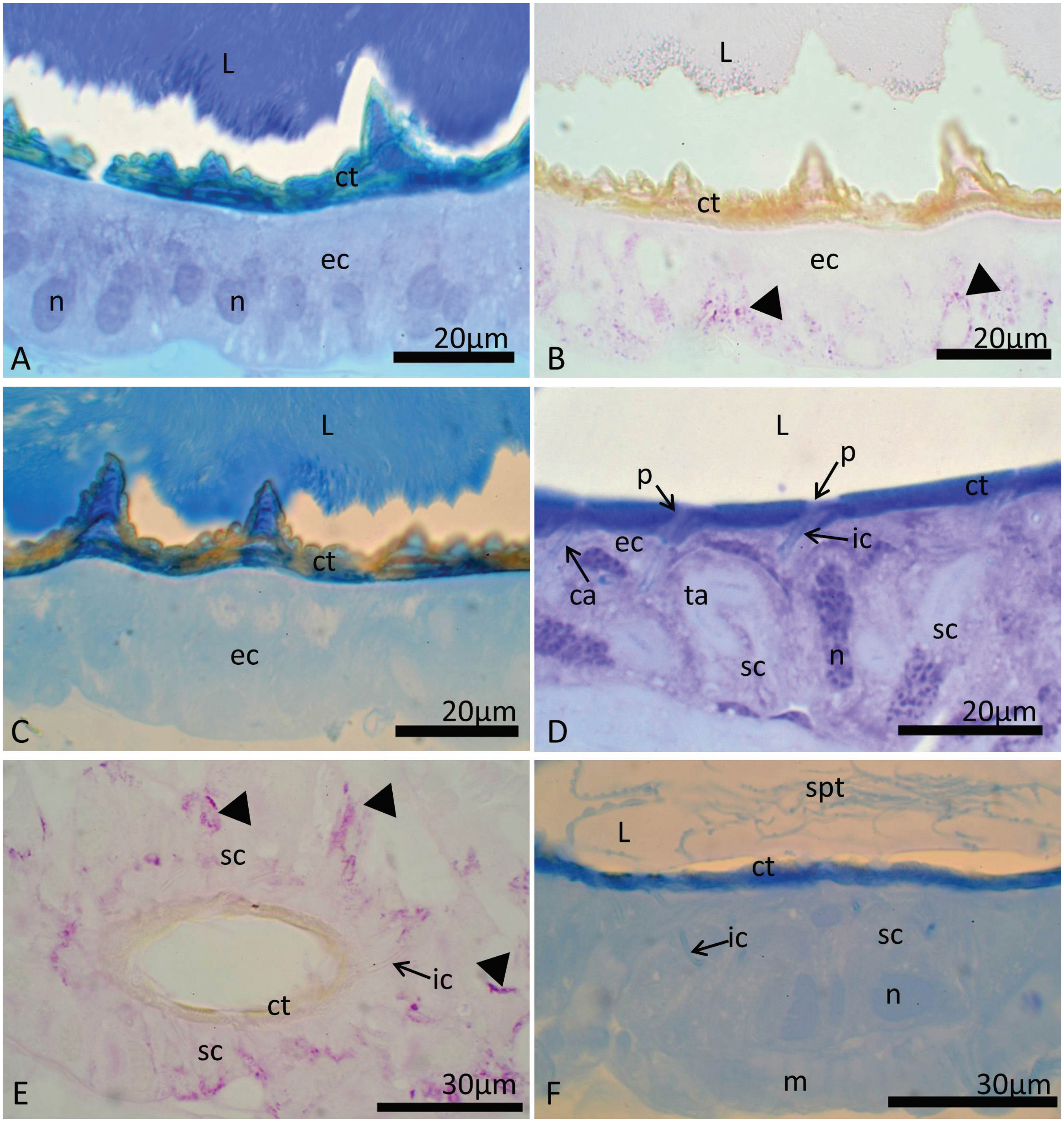

Fig. 2. Light micrographs of the spermatheca of *Doru luteipes*. (A) Section of the spermathecal reservoir containing epithelial cells (ec). (B) Section of the spermathecal reservoir with epithelial cells (ec) positive for P.A.S. (arrow head). (C) Spermathecal reservoir showing epithelial cells (ec) and luminal content (lu) positive for proteins. (D) Section of the spermathecal duct showing epithelial cells (ec) and class III secretory cells (sc) with intracellular canaliculi (ic), which open in pores (p) through the cuticle (ct). (E) Secretory cells (sc) of the duct with P.A.S.-positive regions (arrow heads). (F) Cells of the spermathecal duct positive for proteins. lu: lumen; ea: end apparatus; spt: spermatozoa; m: muscular fiber.

(canaliculi) that is interpreted to transport secretions to the lumen. The presence of canaliculi indicates that cells belong to gland cell Class III, following Noirot and Quennedey (1974, 1991). Class III secretory cells are found in the spermatheca of insects without spermathecal glands, such as *Murgantia histrionica* (Hahn) (Hemiptera: Pentatomidae) (Stacconi & Romani 2011) and *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae) (Souza et al. 2016). The histochemical tests show that these Class III duct cells of *D. luteipes* are rich in proteins and glycoconjugates, suggesting that they may release these compounds into duct lumen. In the bumble bee (Hymenoptera), the cells of the spermathecal duct produce polysaccharides for the spermatozoa (Schoeters & Billen 2000).

The lumen of the spermathecal reservoir and duct of *D. luteipes* are lined with a thick cuticle, as expected due to the ectodermal origin of this organ in the insects (Chapman 2013; Pascini & Martins 2017). The reservoir is important for the maintenance of the spermatozoa, isolating them from the external environment, and preventing contact with the hemolymph molecules (Pascini & Martins 2017).

Generally, spermathecal muscles occur close to the hilum, forming a spermathecal pump that controls release of spermatozoa during oocytes fecundation (Kocorek & Danielczok-Demska 2002; Martins & Serrão 2002; Souza et al. 2016). In some Hymenoptera, the spermathecal duct is short and associated with muscle, and in some Heteroptera, the region containing muscles are delimited by a flange (Kocorek & Danielczok-Demska 2002; Martins & Serrão 2002; Souza et al. 2008). In *D. luteipes*, the muscles have different shapes according to the spermathecal region, with longitudinal ones in the reservoir, proximal, and middle portions of the duct, and circular muscles in the distal portion of the duct. These muscles seem to contribute to control of spermatozoa release, as well as in maintaining the sinuous shape of the duct.

Overall, *D. luteipes* has spermatheca with anatomy similar to that of other Forficulidae. However, this is the first histological and histochemical description, showing the secretory function of the cells in the spermathecal reservoir and duct of a species of Dermaptera.

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Summary

The spermatheca of female insects is responsible for storing spermatozoa until fertilization. In Dermaptera, there are anatomical data for the spermatheca, but the histology is still unknown. This study describes the anatomy, histology, and hystochemistry of the *Doru luteipes* (Scudder) (Dermaptera: Forficulidae) spermatheca. The *D. luteipes* spermatheca is a single structure with an enlargement (reservoir) opening through a sinuous duct. The epithelial cells of the reservoir, and those of the duct, are different throughout the organ. These cells are rich in neutral polysaccharides, glycoconjugates, and proteins, indicating that the secretions function in the maintenance of spermatozoa viability. Muscles occur both in the reservoir and the duct, an uncommon feature in the spermatheca of other insects. This is the first histological description of the *D. luteipes* spermatheca, showing that both the duct and reservoir epithelial cells contribute compounds to maintain the spermatozoa.

Key Words: secretion; spermatozoa; viability; mating

Sumário

Nas fêmeas dos insetos, as espermatecas são órgãos responsáveis pelo estoque dos espermatozoides até a fertilização. Em Dermaptera, há dados anatômicas das espermatecas, mas a histologia ainda é desconhecida. O objetivo deste trabalho foi descrever a anatomia, histologia e histoquímica da espermateca de *Doru luteipes*. A espermateca de *D. luteipes* é uma estrutura única formada por um bulbo dilatado associado a um ducto sinuoso. As células do bulbo e àquelas do ducto são diferentes ao longo do órgão. As células do bulbo e do ducto são ricas em polissacarídeos neutros, glicoconjugados e proteínas indicando que as secreções produzidas participam da manutenção da viabilidade dos espermatozoides. Fibras musculares ocorrem tanto no bulbo quanto no ducto, característica incomum em espermatecas de outros insetos. Esta é a primeira descrição histológica da espermateca de *D. luteipes*, mostrando que as células do bulbo e ducto contribuem com secreções para manutenção dos espermatozoides sendo que as células do ducto tem maior participação neste processo e as fibras musculares estão distribuídas de forma diferente daquelas encontradas em espermatecas de outros insetos.

Palavras Chave: secreção; espermatozoides; viabilidade; cópula

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