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Morphology of the ovary and spermatheca of the leafcutter ant *Acromyrmex rugosus* queens (Hymenoptera: Formicidae)

Cliver Fernandes Farder-Gomes[1,], Marco Antonio Oliveira[2], Terezinha Maria Castro Della Lucia[1,3], and José Eduardo Serrão[1,4]*

Abstract

The leafcutter ant *Acromyrmex rugosus* Smith (Hymenoptera: Formicidae) is considered a pest of several crops. In this study we investigated the morphology of the ovary and spermatheca of *A. rugosus* queens. The ovary is meroistic polytrophic with 12 ovarioles per ovary. Each ovariole has a short terminal filament, a germarium, and a long vitellarium with growth follicles. The nurse chamber near the germarium is larger than the egg chamber. The follicular cells surrounding the egg chamber are cuboidal, with a well-developed nucleus, whereas those surrounding the nurse chamber are flattened. The oocyte increases in volume along the ovariole toward the lateral oviduct. Oocytes have multiple accessory nuclei. Mature oocytes have cytoplasm rich in yolk granules. The reservoir epithelium of the spermatheca shows morphological differences, with both columnar and flattened cells. The spermathecal gland has elongated and acinus-like cells.

Key Words: egg chamber; nurse chamber; ovariole; pest; spermathecal gland

Resumo

A formiga cortadeira *Acromyrmex rugosus* Smith (Hymenoptera: Formicidae) é considerada uma praga de várias culturas. Este estudo investigou a morfologia do ovário e espermateca de rainhas de *A. rugosus*. O ovário é meroístico politrófico com 12 ovaríolos por ovário. Cada ováriolo tem um filamento terminal curto, um germário e um longo vitelário com folículos em crescimento. A câmara nutridora perto do germário é maior que a câmara ovocítica. As células foliculares que circundam a câmara ovocítica são cuboidais com núcleo bem desenvolvido, enquanto as que circundam a câmara nutridora são achatadas. O ovócito aumenta em volume ao longo do ovaríolo em direção ao oviduto lateral. Os ovócitos têm múltiplos núcleos acessórios. O ovócito maduro apresentou citoplasma rico em grânulos de vitelo. O epitélio do reservatório da espermateca apresenta diferenças morfológicas, com células colunares e achatadas. A glândula espermatecal tem células alongadas e semelhantes a ácino.

Palavras Chave: câmara ovocítica; câmara nutridora; peste; glândula espermatecal

Leafcutter ants are eusocial insects that occur exclusively in the Americas and have a wide Neotropical distribution (Della Lucia et al. 2014). Species of the genus *Atta* and *Acromyrmex* (both Hymenoptera: Formicidae) cause significant economic damage to agriculture and forestry (Mundim et al. 2012). However, they also are useful, because colonies of these insects play a role in nutrient turnover, increasing soil nutrient concentration (Sousa-Souto et al. 2007; Sternberg et al. 2007; Pinto-Tomás et al. 2009).

The leafcutter ant *Acromyrmex rugosus* Smith (Hymenoptera: Formicidae) is found in the Cerrado and Caatinga biomes from Brazil, and it is considered a pest of cotton, beans, cassava, corn, orange, and eucalyptus plantations (Gonçalves 1961). Their colonies are monogynous, with a single reproductive queen, and the number of workers may range from 112 to 2,029 individuals (Soares et al. 2006).

In insects, the female reproductive tract consists of a pair of ovaries, lateral oviducts that open into a common oviduct, a spermatheca that usually contains an associated gland, and a vagina (Bünning 1994; Tsai & Perrier 1996). Each ovary is formed by elongated structures, termed ovarioles, that consist of a terminal filament, a germarium, and a vitellarium (Tsai & Perrier 1996; Belles & Piulachs 2015). In ants, the ovaries are classified as meroistic polytrophic in which the nurse cells accompany the oocytes, forming the ovarian follicles. These follicles consist of an oocytic chamber and a nurse chamber, surrounded by a layer of follicular cells (Antunes et al. 2002; Cruz-Landim 2009; Mao et al. 2016).

The anterior part of the ovariole, called a terminal filament, consists of a filiform aggregate of undifferentiated cells that joins the ovarioles to each other and to the body wall (Amaral & Machado-Santelli 2009; Cruz-Landim 2009). Oogenesis begins in the germarium with the division of germ cells (Pearson et al. 2016). Each cystoblast undergoes successive mitoses to produce a cyst with many cells. The oocyte originates from 1 of these cells, whereas the nurse cells originate from others (Buning 1994; Patrício & Cruz-Landim 2006). The main portion of the ovariole is the vitellarium, in which oocytes grow and store yolk

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(Cruz-Landim 2009; Farder-Gomes et al. 2019). As the oocytes mature, they move towards the ovariole, so that oocytes in early stages of development are at the germarium end and the mature ones are at the oviduct end, ready for fertilization (Farder-Gomes et al. 2019).

After mating, many female insects store spermatozoa in the spermatheca, which protects and supplies essential compounds for their survival and nutrition (Stacconi & Romani 2011; Pascini & Martins 2016). The spermatheca is an important structure for queen ants, as they mate only once and the amount of spermatozoa stored during the single nuptial flight determines their reproductive success and colony longevity (Tschinkel & Porter 1988; Baer et al. 2006).

The female reproductive tract of leafcutter ants has been studied in few species and exhibit a great variability in the number of ovaries and spermatheca (Tschinkel 1987; Antunes et al. 2002; Dijkstra et al. 2005; Ortiz & Camargo-Mathias 2006, 2007; Cardoso et al. 2008). Although these studies provide data on the morphology, more detailed information on anatomy and histology of the reproductive tract of *A. rugosus* are scarce, and additional studies are important for understanding the basic organization of this ant species, and allowing comparative studies with other ants. The objective of this study was to investigate the morphology of the ovary and spermatheca of *A. rugosus* queens.

Materials and Methods

Four *A. rugosus* queens were collected by nest excavation and 3 fertilized winged foraging females were collected from a trail in Florestal (19.871200°S, 44.423900°W), Minas Gerais State, Brazil. All queens were known to be fertilized because they had spermatozoa stored in the spermatheca. The specimens were dissected in 125 mM NaCl and the reproductive tract transferred to Zamboni's fixative solution (Stefanini et al. 1967) for 24 h. The samples were dehydrated in a graded ethanol series (70, 80, 90, and 95% for 15 min each). Next, the samples were embedded in historesin Leica, and the 3 µm thickness histological sections were stained with hematoxylin and eosin, analyzed with an Olympus BX-60 light microscope (Olympus Corporation, Shinjuku, Tokyo, Japan), and photographed with an Olympus QColor 3 camera (Olympus Corporation, Shinjuku, Tokyo, Japan).

Results

The reproductive tract of *A. rugosus* had a pair of ovaries with 12 ovarioles each, lateral oviducts connecting the ovaries to a common oviduct, a spermatheca, and an extensive network of trachea associated with the ovaries (Fig. 1).

Each ovariole had a short terminal filament in the apical portion, followed by a dilated apical region, the germarium, in which somatic and germinative cells were indistinguishable (Fig. 2A). After the germarium, the vitellarium was the longest ovariole region, with many follicles formed by egg, and nurse chambers with 11 nurse cells, surrounded by a layer of follicular cells, characterizing a meroistic polytrophic ovary (Fig. 2B).

The nurse chamber was larger than the egg chamber near the germarium, with large nurse cells with well-developed nucleus (Fig. 2B, C). In this region of the ovariole, oocytes in the initial stages of development showed basophilic and homogeneous cytoplasm (Fig. 2C). The follicular cells that surrounded the egg chamber were cuboidal and had a well-developed nucleus, whereas those surrounding the nurse chamber were flattened (Fig. 2C, E). The follicular epithelium was disrupted by a connection between the nurse and egg chambers, and the oocytes had multiple accessory nuclei (Fig. 2E).

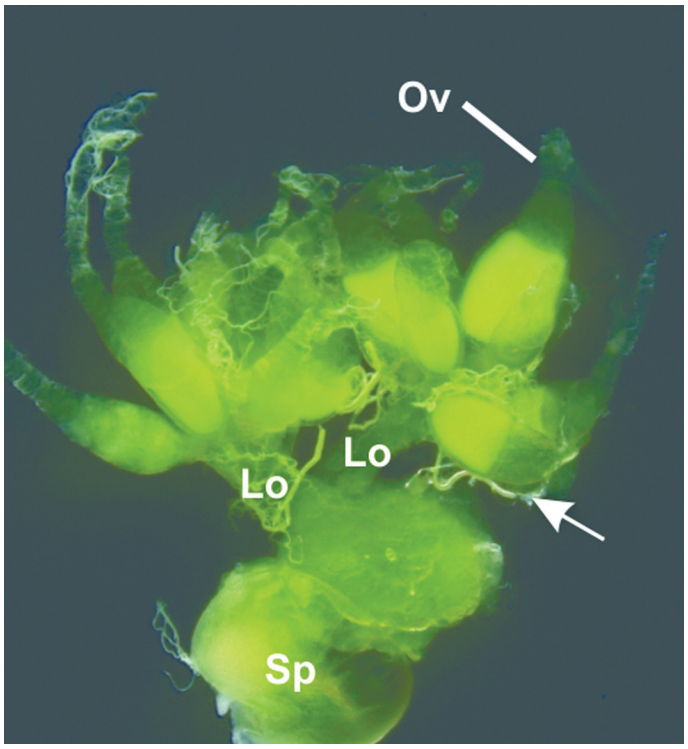


Fig. 1. General appearance of the *Acromyrmex rugosus* reproductive system. Ovariole (Ov); lateral oviduct (Lo); spermatheca (Sp); trachea associated with the ovarioles (white arrow). Scale bar: 500 µm.

The oocytes increased in volume as they moved toward the lateral oviduct, and the egg chambers gradually became larger than the nurse chambers. During the oocyte maturation, yolk granules began to accumulate in the ooplasm (Fig. 2F, G). The nurse cells were restricted to a small region of the nurse chamber (Fig. 2H) and showed degenerative features, such as cytoplasmic reduction and nuclear chromatin condensation. The mature egg chamber showed flattened follicular cells and oocyte with cytoplasm rich in yolk granules (Fig. 2H).

Acromyrmex rugosus had 1 lobate spermatheca connected to the common oviduct via a narrow duct (Fig. 1). The epithelium of the spermathecal reservoir showed morphological differences, with the upper 2/3 formed by columnar cells and the remainder with flattened cells (Fig. 3A). The transition between the columnar and flattened epithelium occurred gradually (Fig. 3B). The spermatheca showed an external secretory portion with elongated, acinus-like cells forming the spermathecal gland (Fig. 3A, C). These cells had a well-developed nucleus and granule-rich cytoplasm (Fig. 3A, B). Muscle layers surrounded the entire spermatheca below the gland (Fig. 3A) and in the insertion region of the spermathecal duct. Many muscle formed the spermathecal pump (Fig. 3D).

Discussion

The number of ovarioles found in *A. rugosus* was different from that found in *Acromyrmex subterraneus subterraneus* (Forel) (Hymenoptera: Formicidae), with 28 ovarioles per ovary (Antunes et al. 2002); *Acromyrmex ameliae* De Souza, Soares & Della Lucia (Hymenoptera: Formicidae) with 13 to 15 ovarioles per ovary (Soares et al. 2010); *Acromyrmex octospinosus* Reich (Hymenoptera: Formicidae) and *Acromyrmex echinatior* (Forel) (Hymenoptera: Formicidae), with 5 to 6 ovarioles per ovary (Dijkstra et al. 2005); and queens of the genus *Atta* with more than 300 ovarioles per ovary (Tschinkel 1987). The number of ovarioles per ovary in ants vary

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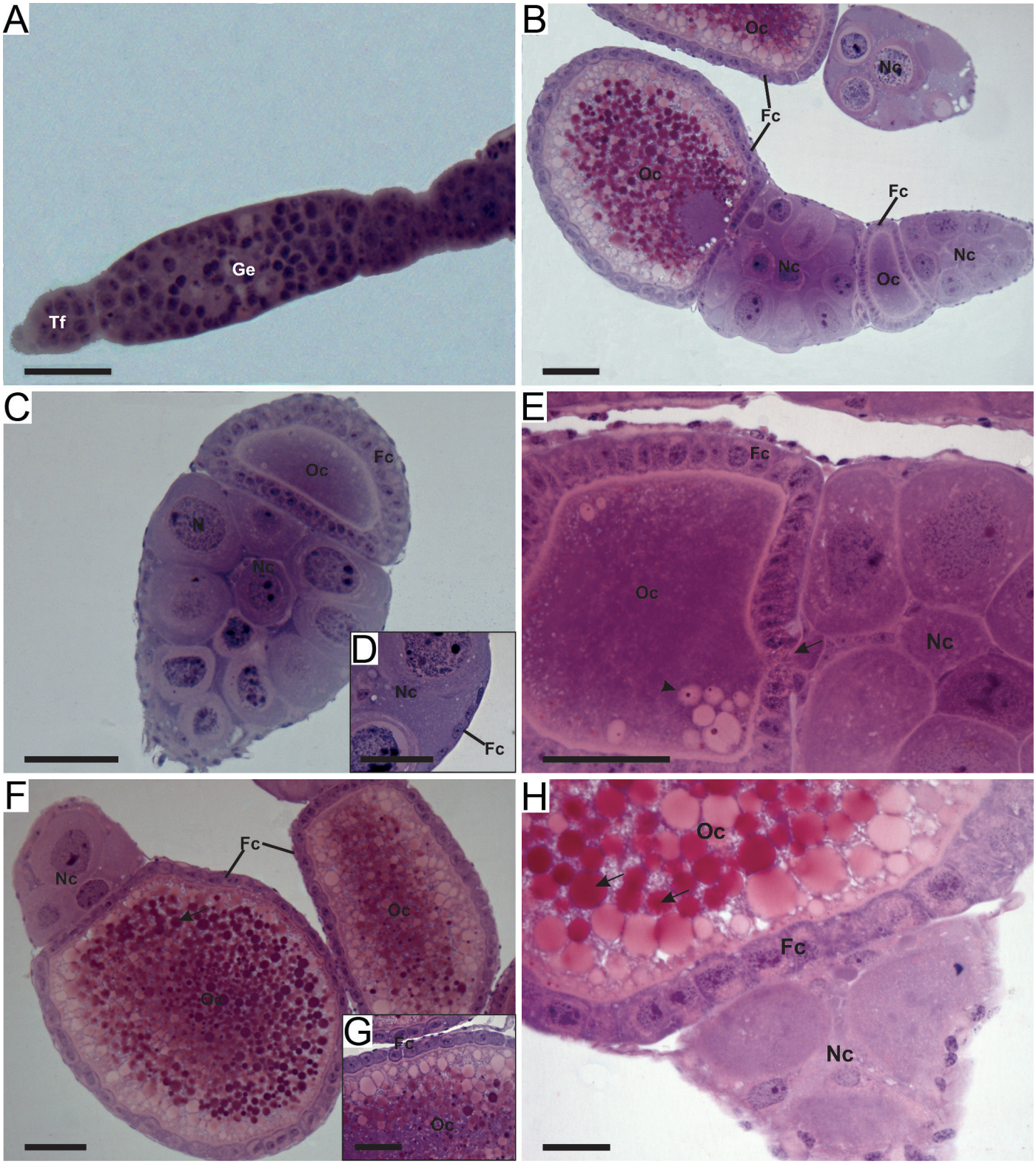


Fig. 2. Light micrographs of an *Acromyrmex rugosus* ovariole. (A) Terminal filament (Tf) and germarium (Ge). Scale bar: 30 µm. (B) The vitellarium region with the egg chamber (Oc) and nurse chamber (Nc) at various stages of development, covered by follicular cells (Fc). Scale bar: 30 µm. (C) A follicle at the early stage of development with a small egg chamber (Oc) enveloped by cuboidal follicular cells (Fc) and a well-developed nurse chamber (Nc). N, nurse cell nucleus. Scale bar: 20 µm. (D) Flat follicular cells (Fc) covering the nurse chamber (Nc). Scale bar: 20 µm. (E) A follicle with an oocyte (Oc) with multiple accessory nuclei (black arrowhead). A disruption in the follicular epithelium that allows communication between the egg and nurse chambers (black arrow). Scale bar: 20 µm. (F) Oocytes (Oc) in the late maturation stages with a large number of yolk granules in the cytoplasm (black arrow), enveloped by cuboidal follicular (Fc) cells. The nurse chamber (Nc) is smaller than the egg chamber (Oc). Scale bar: 30 µm. (G) Cuboidal follicular epithelium (Fc) covering the oocyte (Oc). Scale bar: 20 µm. (H) A follicle at the final stage of development with degenerating nurse cells (Nc). Yolk granules in the ooplasm (black arrow). Oc, oocyte; Fc, follicular cells. Scale bar: 10 µm.

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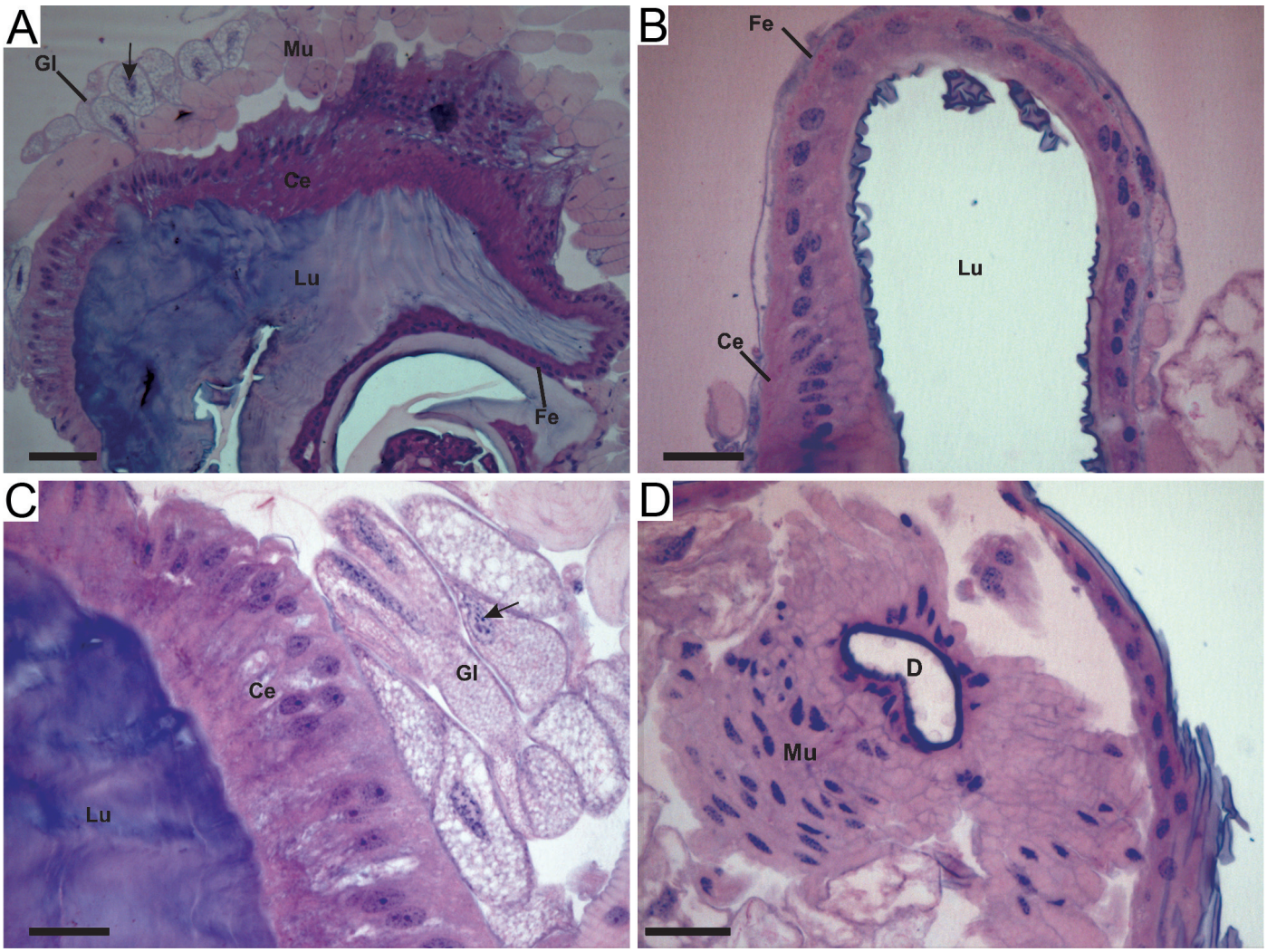


Fig. 3. Light micrographs of an *Acromyrmex rugosus* spermatheca: (A) General appearance of the spermatheca with regions of columnar (Ce) and flat (Fe) epithelia in the reservoir and spermathecal gland (Gl) containing cells with a well-developed nucleus (black arrow). Scale bar: 30 µm. (B) The reservoir epithelium and transition between columnar (Ce) and flat epithelia (Fe). Scale bar: 10 µm. (C) The spermathecal gland (Gl) containing cells with a well-developed nucleus (black arrow) and cytoplasm with granules. Scale bar: 10 µm. (D) The spermathecal pump with muscles (Mu) associated with the spermathecal duct (D). Scale bar: 10 µm. Lu, lumen; Mu, muscles.

from 2 to 1,300 (Hölldobler & Wilson 1990), and may be related to the queen's reproductive capacity (Antunes et al. 2002). In addition, the number of ovarioles may be influenced by environmental and genetic factors, and food availability during larval development (Bergland et al 2008; Jervis et al. 2008; Green & Extavour 2012).

Nurse cells are important for oocyte development, producing RNA and proteins, which are transported to the oocyte through disruptions in the follicular epithelium (De Loof et al. 1990; Cruz-Landim 2009). The nurse cells of *A. rugosus* degenerated after transfer of their cytoplasm into the oocyte (nurse cell dumping), similar to that reported for other Hymenoptera (Amaral & Machado-Santelli 2009; Dong et al. 2010; Okada et al. 2010). The presence of accessory nuclei in *A. rugosus* oocytes has been reported in other hymenopterans (Martins & Serrão 2004), with a possible role in ribonucleoprotein production for special regions of the ooplasm (Bilinski 1991a, b).

The lobate spermatheca of *A. rugosus* is similar to that reported for other species in the genus *Acromyrmex*, as well as in other myrmicine ants (Wheeler & Krutzsch 1994; Ortiz & Camargo-Mathias 2006, 2007; Cardoso et al. 2008), suggesting that this spermathecal shape is common to this subfamily. The presence of columnar epithelium in the spermathecal reservoir wall of *A. rugosus* also has been reported in *A. subterraneus subterraneus*, *Acromyrmex balzani* (Emery) (Hymenoptera: Formicidae), *Acromyrmex landolti* (Forel) (Hymenoptera: Formicidae), and *Acromyrmex landolti balzani* Emery (Hymenoptera: Formicidae); however, only *A. rugosus*, *A. subterraneus*, and *A. balzani* have spermathecal glands (Ortiz & Camargo-Mathias 2007; Cardoso et al. 2008). The gland secretions act as nutrients for the spermatozoa, contributing to the maintenance of their viability for long periods (den Boer et al. 2009; Wolfner 2011). Columnar epithelium in the spermathecal reservoir has been claimed to play some role in the release of content to the lumen (Ortiz & Camargo-Mathias 2007). Further investigation is needed to determine whether or not secretion from reservoir epithelium and spermathecal gland have similar compounds and functions.

Attini queens mate only at the beginning of their reproductive life, but with many males (polyandry), and store all the spermatozoa in the spermatheca (Pabalan et al. 1996; Gotoh et al. 2009). Therefore, these queens should control the amount of spermatozoa used during oocyte fertilization. The spermathecal pump muscles found in *A. rugosus* may be responsible for opening and closing the spermathecal duct, and control the transport of spermatozoa to the reservoir during mating

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and from the reservoir to the common oviduct during egg fertilization (Cardoso et al. 2008).

Our results show that *A. rugosus* queens have a pair of meroistic polytrophic ovaries with 12 ovarioles each and only 1 lobate spermatheca with a differentiated reservoir epithelium. Data on the organization of the reproductive tract of *Acromyrmex* queens contributes to understanding the details of the reproductive biology of leafcutter ants, and allows comparative studies with other ants.

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