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Micron 36 (2005) 449–460

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Ultrastructural studies of the mandibular glands of the minima, media and soldier ants of *Atta sexdens rubropilosa* (Forel 1908) (Hymenoptera: Formicidae)

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Received 5 July 2004; revised 20 December 2004; accepted 31 January 2005

Abstract

The mandibular glands of Hymenoptera are structures associated with the mandibles and constitute part of the salivary glands system. Histological studies in workers of *Atta sexdens rubropilosa* revealed that this gland contains two portions: a secretory and a storage portion or reservoir. Both portions are connected by means of canaliculi. The object of the present work was the study of the ultratructure of the mandibular glands of minima, media and soldier ant of *A. s. rubropilosa* by TEM techniques. The glands, in the three castes studied, possess a reservoir, constituted by a simple pavementous epithelium surrounded by the cuticular intima and the secretory portion is constituted by cells of rounded shape. The secretory cells, mainly of minima and soldier, were rich in smooth endoplasmic reticulum. The media worker and soldier presented a large number of mitochondria, of varying shape. Well-developed Golgi complexes were also present in the soldiers. The secretory cells in minima, media and soldier were provided with collecting intracellular canaliculi, which were linked to the reservoir through the extracellular portion. The cytoplasm of the canaliculi-forming cell was poor in organelles. In the individuals of the three castes of *A. s. rubropilosa*, the presence of lipid secretion granules suggested, beyond the other functions, also a possible pheromonal action. The different roles executed by the different insect castes are directly dependent on the glandular products and, consequently, on the secretory cellular characteristics.

Keywords: Mandibular gland; *Atta sexdens rubropilosa*; Ultrastructure; Pheromone

1. Introduction

The mandibular glands of the Hymenoptera are structures linked to the mandibles. These glands were first described by Meinert (1860) in *Apis*, as paired structures located on each side of the head and which possess an excretory duct located at the internal surface of the joint that links the mandibles to the head. Later, these glands were described again in *Apis* and solitary bees by Schiemenz (1883) and Bordas (1895), who generalized as to their occurrence in all hymenopterans.

The Hymenoptera possess glandular structures that are embryologically joined to the mouthparts, in other words, they evolved from the same embryonic fold as the mouthparts, thus constituting part of the salivary gland system. Four pairs of glands conform this system in ants: labial or salivary of the thorax, mandibular, post-pharyngeal, and maxillary or hypopharyngeal.

The glands joined to the mandibles, known as the mandibular glands, are found in the Apterigota, Blattodea, Mantodea, Isoptera, Coleoptera, and Hymenoptera. They are also present in the larvae of the Lepidoptera, although they are absent in adults of this group (Cruz-Landim, 2002). These glands are common to all social insects, occurring in queens, workers, and males (Billen and Morgan, 1998) and also in the solitary Hymenoptera.

In the Hymenoptera, the mandibular glands are present in the adults of all species and, in general, two types of

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0968-4328/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.micron.2005.01.006

mandibular glands can be found: the ectomandibular glands, or mandibular glands I, and the mesomandibular glands, also known as intramandibular or mandibular glands II (Spradbery, 1991).

The mandibular glands originate from the ectoderm during the process of metamorphosis, so their development is post-embryonic (Gama, 1978). During the differentiation process, the layer of epidermal cells gives rise to the three types of cells that constitute the glands: (1) short epithelial cells that form the reservoir coated by a cuticle at its luminal surface; (2) cylindrical secretory cells surrounding the reservoir; (3) cells of the canaliculi, which join the secretory cells to the reservoir (Cruz-Landim, 2002). The reservoir undergoes a funnelling process, thus forming the main duct that opens at the base of the mandible joint without any associated musculature (Billen and Schoeters, 1994).

Noirot and Quennedey (1974, 1991), while studying the ultrastructure of the exocrine glands of insects in general, proposed a classification for the characterization of the morphology of glandular structures. The classification proposed groups the secretory glandular cells into three different types (I, II, III), depending on the morphogenesis of the secretory cells, on their position in relation to the cuticle, and on the manner of secretion elimination by the gland (Quennedey, 1998). Type III secretory cells, which constitute the mandibular glands of insects, are usually spherical and provided by a duct that joins them to a pore in the cuticle, through which the secretion is eliminated. The plasma membrane of this type of secretory cells creates an infolding, apparently intracellular, into which the cell secretes a cuticle. This space acquires the shape of an intracellular canaliculum that runs through the cytoplasm and has been denominated as intracellular canaliculum, although topologically speaking it is located outside the cells. The plasma membrane that delimitates this canalicular space might present microvilli or tubular infoldings and thus form a pericanalicular labyrinth (Cruz-Landim, 2002).

Histological studies in workers of the ant *Atta sexdens rubropilosa* performed by Pavon and Camargo-Mathias (2001) revealed the presence of two cellular types in the mandibular glands of these individuals: one forming the secretory portion, in which the secretory cells present a rounded morphology and with a pronounced nucleus, and another that composes the reservoir and is formed by a simple squamous epithelium. Both portions are connected through canaliculi that possess intra and extra-cytoplasmic portions. Toledo (1967) previously described the histology of the mandibular glands of this ant and showed the existence of rounded secretory cells with large elliptical nuclei that were rich in granules, with canaliculi of a feathered intracellular portion (rabdome) and an extracellular portion.

The ultrastructure of the mandibular glands of *Apis* was observed by Costa-Leonardo (1981), who showed the presence of rough endoplasmic reticulum, Golgi complex, numerous free ribosomes, and secretion granules in the cytoplasm of the secretory cells. Salles and Cruz-Landim (1996) showed in *Oxytrigona tataira* the presence of poorly developed rough endoplasmic reticulum, a large amount of smooth endoplasmic reticulum, and numerous mitochondria.

Although the mandibular glands are linked to the mouthparts, their product is not primarily used in the ingestion and digestion of food sources. According to some authors, the mandibular glands evolved separately from the digestive function and acquired a role related to behaviour (Gama, 1985). According to Brough (1978) and Delage-Darchen (1976), the mandibular glands of insects, together with the post-pharyngeal glands, produce pheromones that are widely used in establishing the social order of the colony.

It has been established that, in insects, processes such as fecundation, communication, social integration, territory delimitation, etc. are functions that depend directly on the glandular products (Wilson, 1971; Michener, 1974). Birch (1974) observed that the mandibular gland is the site of alarm pheromone synthesis in species of the subfamilies Ponerinae, Dorylinae, Myrmicinae, and Formicinae.

Therefore, in insects, the secretion of the mandibular gland is generally involved in the defense and communication systems (Buschinger and Maschwitz, 1984; Hölldobler and Wilson, 1990; Billen and Morgan, 1998), but it may also act as a fungal-growth inhibitor (Marsaro Junior et al., 2001). In the males of some species, the mandibular gland might even serve as the source of sexual pheromones (Ayasse et al., 2001).

In view of all the data mentioned above, the object of the present work was to describe the ultrastructure of the diverse cells that constitute the mandibular glands of workers of the three worker castes of *A. s. rubropilosa*, in an attempt to uncover new morphological information that might be useful to our understanding of the biology, physiology and social behaviour of these individuals.

2. Materials and methods

For the present study, minima, media and soldier worker of the ant *A. s. rubropilosa* were collected from artificial nests kept in the laboratories of the Center for the Study of Social Insects—*Centro de Estudo de Insetos Sociais* (CEIS)—at the UNESP, campus at Rio Claro, SP, Brazil.

2.1. Transmission electron microscopy

Workers of the three castes studied were dissected directly in 0.5% glutaraldehyde fixative solution, and their mandibular glands removed. Next, the glands were fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer for 2 h at 4 °C. Two 15-min washes in cacodylate buffer followed this process. Post-fixation was performed in 1% osmium tetroxide for 1 h at 4 °C followed by another two 15-min washes in the same buffer. For contrast, the material was immersed in a solution of uranyl acetate in acetone for

30 min. After dehydration, the material was embedded in Epon resin diluted in acetone (1:1) and incubated at 4 °C with agitation for 24 h. The material was then transferred to pure Epon resin and incubated at 60 °C for 72 h, until completely polymerized. Semi- and ultrathin sections were obtained with the aid of a Porter Blum ultramicrotome. The semithin sections were stained with Azur II (1%) and Methylene Blue (1%). The ultrathin sections were placed on copper grids and stained with uranyl acetate and lead citrate. The grids were studied and photographed in a transmission electron microscope, PHILIPS CM100.

3. Results

3.1. Reservoir

The reservoir of the mandibular glands for the storage of the secretion has a sac-shaped organization and is joined to the secretory cells by means of canaliculi. No morphological differences can be found among the three castes studied.

The wall of the reservoir of the mandibular glands of the soldier, media and minima worker of *A. s. rubropilosa* is formed by a simple squamous epithelium, internally coated by a thin cuticle (Figs. 1 and 7). The flattened cells of the reservoir possess fusiform nuclei, with fine chromatin granules in the minima worker (Fig. 1B), and sparse heterochromatic bundles in the media worker and soldier (Figs. 1D,F and 7). A nucleolus is always highly evident in the media worker (Fig. 1D). The reservoir, although in intimate contact with the secretory cells, does not establish cellular junctions in any of the individuals of the three castes studied (Fig. 1C and E). Inside the reservoir of the minima worker, the presence of a homogenous secretion can be seen (Fig. 1A). A detail of a nervos laying next to a secretory cell of the media workers was also observed (Fig. 1C).

3.2. Canaliculi

Although the secretory cells and the reservoir are in close contact, the connection between these two regions is only established through canaliculi that possess intra and extra-cytoplasmic portions. The intra- and extra-cytoplasmic portions are formed by different cells. The intracellular portion is produced by the secretory cell itself, while the extracellular portion is produced by duct cells, also known as canaliculi-forming cells. The intracellular canaliculi runs through the interior of the glandular cell and is coated by an interrupted and porous cuticle. The extracellular portions run independently from each other, and after crossing the reservoir wall, they release the secretion directly into its lumen.

3.2.1. Extra-cytoplasmic or extracellular portion

In the mandibular glands of minima, media and soldier of *A. s. rubropilosa*, the extracellular portion of the canaliculi shows a sinuous course, originates in the secretory cells and ending in the reservoir. There is a thick cuticle coating the internal face of the lumen of these canaliculi (Fig. 2). Depending on the region of the canaliculi, it is possible to see the presence of secretion in the lumen (Fig. 2C and D). The extracellular portion is secreted by duct cells, or canaliculi-forming cells, which are formed in the soldier and minima and media worker of this species (Figs. 2B,C,F and 7). The canaliculi-forming cell is unique for each canaliculi; it possess a cytoplasm poor in organelles and a nucleus with sparse chromatin and nucleolus, that is more evident in the soldiers (Fig. 2F). The cells of the canaliculi are connected to it by means of membranous interdigitations (Fig. 2A–C).

3.2.2. Intra-cytoplasmic or intracellular portion

The intracellular portion of the collecting canaliculi has been studied in the secretory cells of the mandibular glands of the minima, media and soldier of *A. s. rubropilosa*. This portion is found to be distributed throughout the cytoplasm of the secretory cell, and present a cuticular coating in its lumen, which is interrupted from space to space (Fig. 3). A pericanalicular space was always observed in the three castes and was characterized by the presence of numerous microvilli (Figs. 3 and 7). In the minima worker (Fig. 3C), it was possible to observe a longitudinal section of a canaliculum. Lipid secretion granules are observed surrounding the canaliculi in the minima worker and soldier (Figs. 3B,D and 7). Specifically in the soldier, mitochondria with varying shapes are found surrounding the canaliculi (Fig. 3D). The accumulation of secretion in the pericanalicular space is so great in the small workers that it pushes the microvilli apart (Fig. 3B), thus indicating the passage of the secretion into the lumen of the canaliculi.

3.3. Secretory portion

3.3.1. Minima worker

In the secretory cells of minima worker we have observed a single type of secretory cell (Fig. 4A) with distinct plasma membrane and evident basal lamina (Fig. 4A). The nuclei of the secretory cells contain heterochromatic bundles (Fig. 5A). The irregular shape of the nuclei should be noted (Figs. 5A and 7), suggesting an increase in the exchange surface between this organelle and the cytoplasm. The secretory cells of this caste exhibit numerous mitochondria of circular shape and with few crests (Fig. 4A), as well as a smooth endoplasmic reticulum, organized in a lamelar manner (Fig. 5F). In the cytoplasm of the secretory cells, we note the presence of myelinic bodies (Fig. 5A and D).

3.3.2. Soldier and media worker

The results obtained for the mandibular glands of these two castes are very similar and, therefore, they will be discussed together.

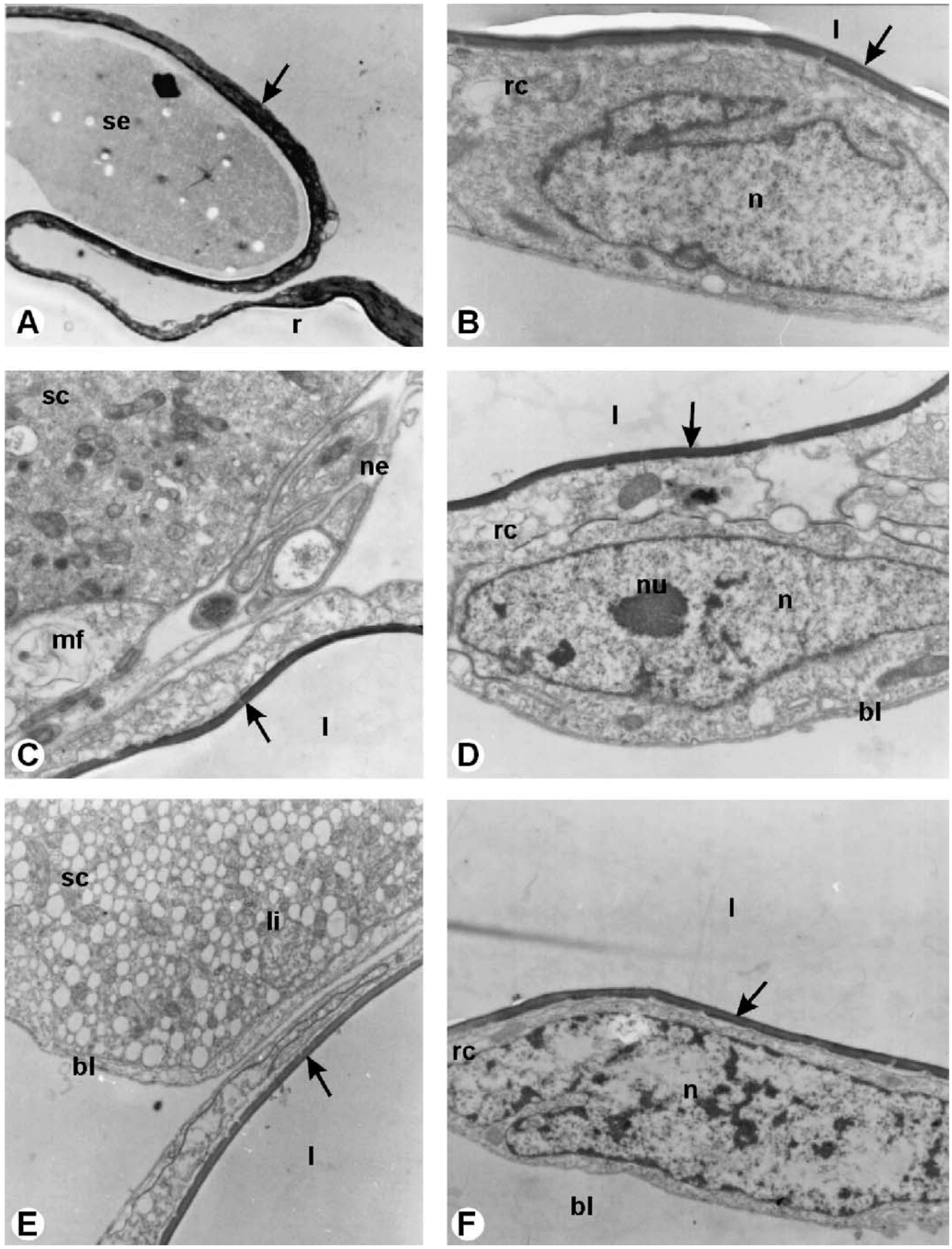

Fig. 1. Mandibular glands of *Atta sexdens rubropilosa*. (A,B) Minima worker. (A) Reservoir (r) with homogeneous secretion (se) in its lumen. Arrow, cuticle. Magnification: 810×. (B) Reservoir cell (rc). Arrow, cuticle; n, nucleus; l, lumen of the reservoir. Magnification: 2900×. (C,D) Media worker. (C) Contact between a secretory cell (sc) and the reservoir. Arrow, cuticle; l, lumen of the reservoir; mf, myelin body; ne, nerve. Magnification: 2050×. (D) Reservoir cell (rc). Arrow, cuticle; n, nucleus; nu, nucleolus; l, lumen of the reservoir; bl, basal lamina. Magnification: 2050×. (E,F) Soldier. (E) Contact between a secretory cell (sc) and the reservoir. Arrow, cuticle; l, lumen of the reservoir; bl, basal lamina; li, lipids. Magnification: 2050×. (F) Cell of the reservoir (rc). Arrow, cuticle; n, nucleus; l, lumen of the reservoir; bl, basal lamina. Magnification: 2050×.

The mandibular glands of soldier and media worker present secretory cells that are either two different types of cells or the same type of secretory cells, but at different stages of the secretory cycle (Fig. 4B–E). The cells of the secretory portion, although they are close to each other, possess different plasma membranes (Fig. 4B, C and E). The point of contact between these cells is represented by the basal lamina, which in some cases appears to be unique and shared by several cells (Fig. 4B, C and E). The elliptical nuclei of the secretory cells contains chromatin with numerous heterochromatic bundles (Figs. 5B,C and 7). Nucleoli (Fig. 5C), in addition to several pores in the nuclear envelope, were found (Fig. 5B and C). The secretory cells exhibit as their main cytoplasmic characteristic

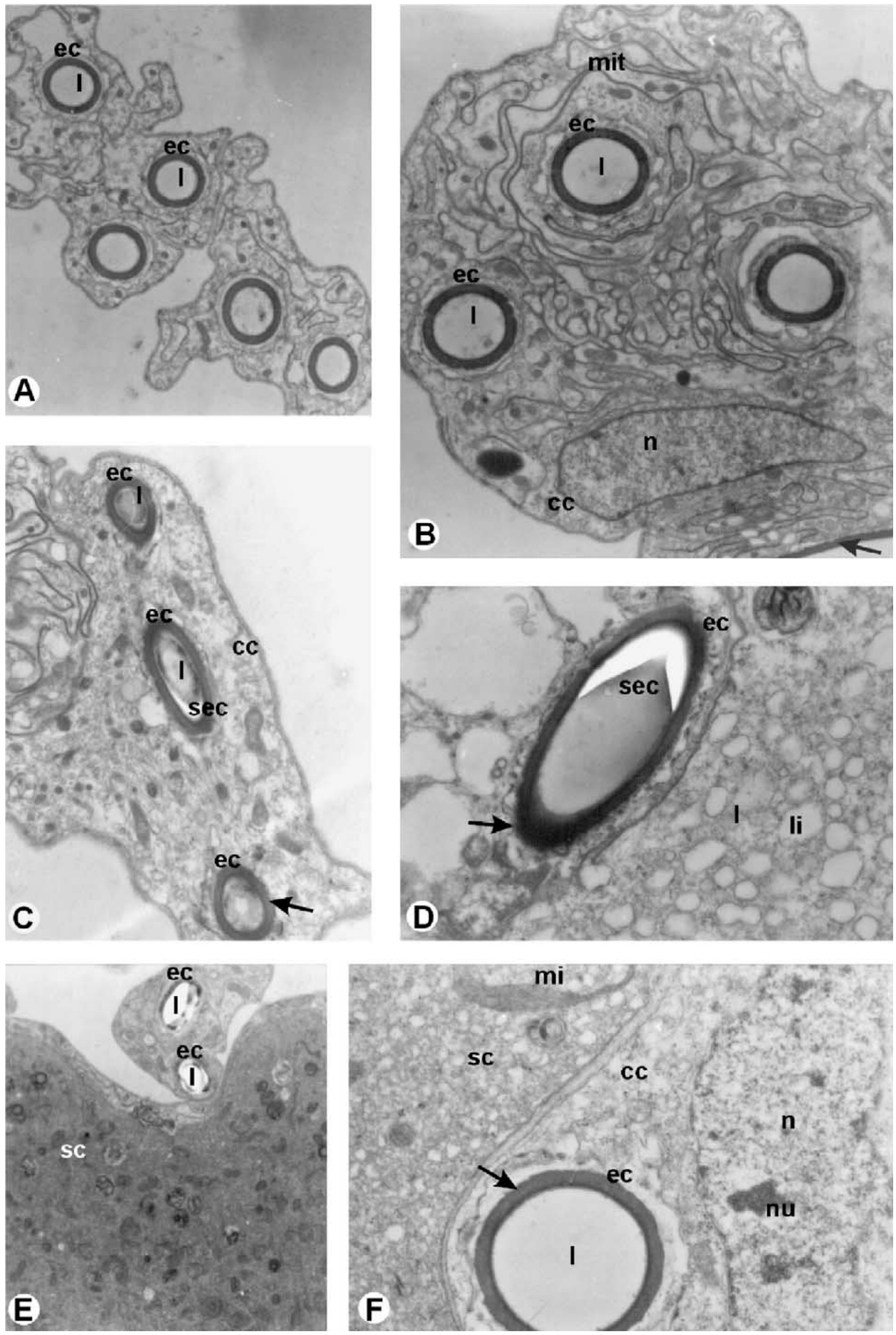

Fig. 2. Mandibular glands of *Atta sexdens rubropilosa*. (A,B) Minima worker. (A) Extra-cytoplasmic canaliculi (ec). l, lumen of the canaliculi. Magnification: 2050×. (B) Canaliculi-forming cell (cc) and extra-cytoplasmic canaliculi (ec). Arrow, cuticle; l, lumen of the canaliculi; n, nucleus; mit, interdigitation joint. Magnification: 2050×. (C,D) Media worker. (C) Canaliculi-forming cell (cc) and extra-cytoplasmic canaliculi (ec). Arrow, cuticle; l, lumen of the canaliculi; sec, secretion. Magnification: 2050×. (D) extra-cytoplasmic canaliculi (ec). Arrow, cuticle; sec, secretion; l, lumen of the reservoir; li, lipids. Magnification: 2900×. (E,F) Soldier. (E) Extra-cytoplasmic canaliculi (ec). l, lumen of the canaliculi; sc, secretory cell. Magnification: 810×. (F) Canaliculi-forming cell (cc) and extra-cytoplasmic canaliculi (ec). Arrow, cuticle; sc, secretory cell; l, lumen of the canaliculi; n, nucleus; nu, nucleoli; mi, mitochondria. Magnification: 4200×.

the presence of numerous mitochondria with few crests and with varying morphologies, from small and electron-dense (Figs. 4E, 5C,G, 6E and 7) to large and elongated (Figs. 4E, 6C and 7). The mitochondria frequently appear to be associated to lipid droplets (Figs. 3D, 4E, 5G, 6D and 7). A large amount of smooth endoplasmic reticulum was found in the cytoplasm of the secretory cells (Figs. 4C,E, 6B,C and 7). Golgi complexes with slightly dilated cisterns and several lysosomes are found only in the secretory cells of soldier (Figs. 6A and 7). Electron-lucid vesicles containing

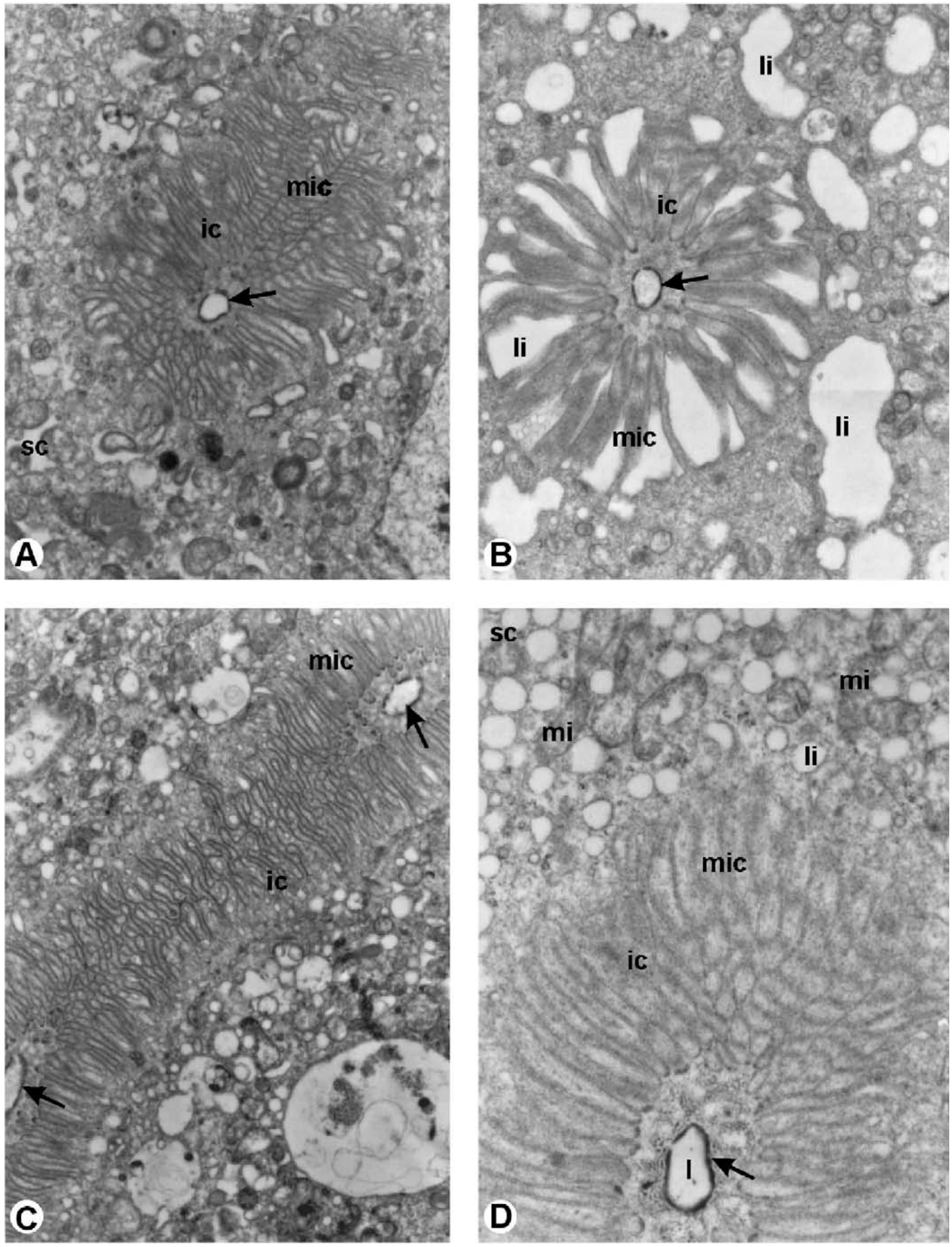

Fig. 3. Mandibular glands of *Atta sexdens rubropilosa*. (A) Media worker. Secretory cell (sc) with intra-cytoplasmic canaliculi (ic). Arrow, cuticle; mic, microvilli. Magnification: 2050×. (B,C) Minima worker. (B) Intra-cytoplasmic canaliculi (ic) with their microvilli (mic) pushed apart due to accumulation of a lipidic secretion (li). Arrow, cuticle. Magnification: 2900×. (C) Longitudinal section of intra-cytoplasmic canaliculi (ic). Arrow, cuticle; mic, microvilli. Magnification: 2050×. (D) Secretory cell (sc) with intra-cytoplasmic canaliculi (ic). Arrow, cuticle; mic, microvilli; l, lumen of the canaliculi; li, lipids; mi, mitochondria. Magnification: 1250×.

lipids (Figs. 4C,E, 6D and 7), microtubules (Figs. 6E and 7), and myelinic bodies (Figs. 5H,I and 7) are also present.

4. Discussion

The initial study of the mandibular glands of minima, media and soldier of the *ant A. s. rubropilosa* (Pavon and Camargo-Mathias, 2001) showed that these structures are paired, one located on each side of the head, and associated to the mandibles. The morphological study demonstrated a sac-shaped organization, in which a thin-walled reservoir is associated with the secretory cells. These two regions are connected by means of canaliculi that possess intra- and extra-cytoplasmic portions. An excretory duct that opens on the surface of the mandible plays the role

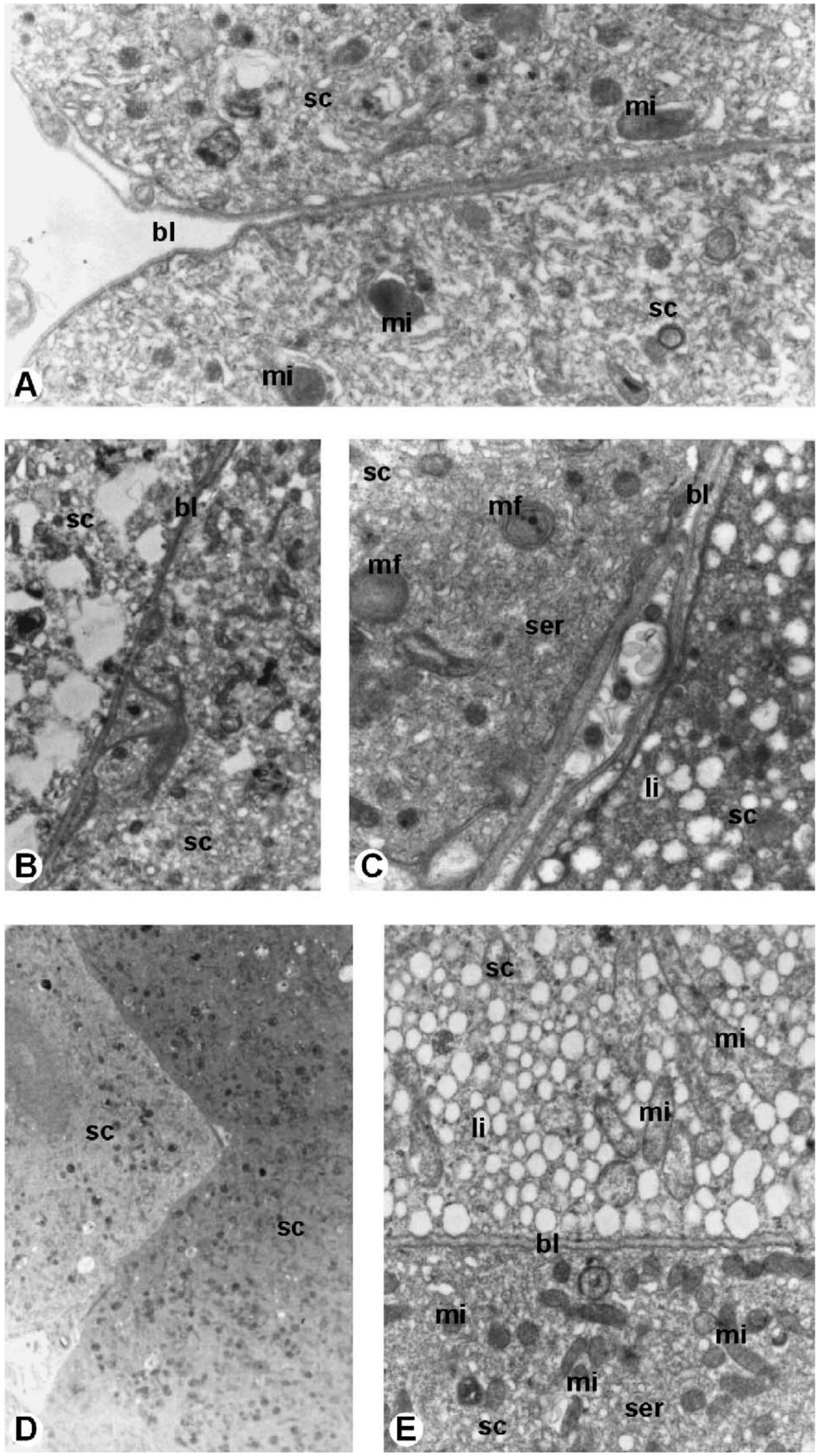

Fig. 4. Mandibular glands of *Atta sexdens rubropilosa*. (A) Minima worker. Evident secretory cell (sc) and basal lamina (bl). mi, mitochondria. Magnification: 2050×. (B,C) Media worker. (B) sc, secretory cell; bl, basal lamina. Magnification: 2050×. (C) sc, secretory cell; bl, basal lamina; mf, myelin body; li, lipids; ser, smooth endoplasmic reticulum. Magnification: 2050×. (D,E) Soldier. (D) cs, secretory cell. Magnification: 420×. (E) sc, secretory cell; bl, basal lamina; mi, mitochondria; li, lipids; ser, smooth endoplasmic reticulum. Magnification: 2900×.

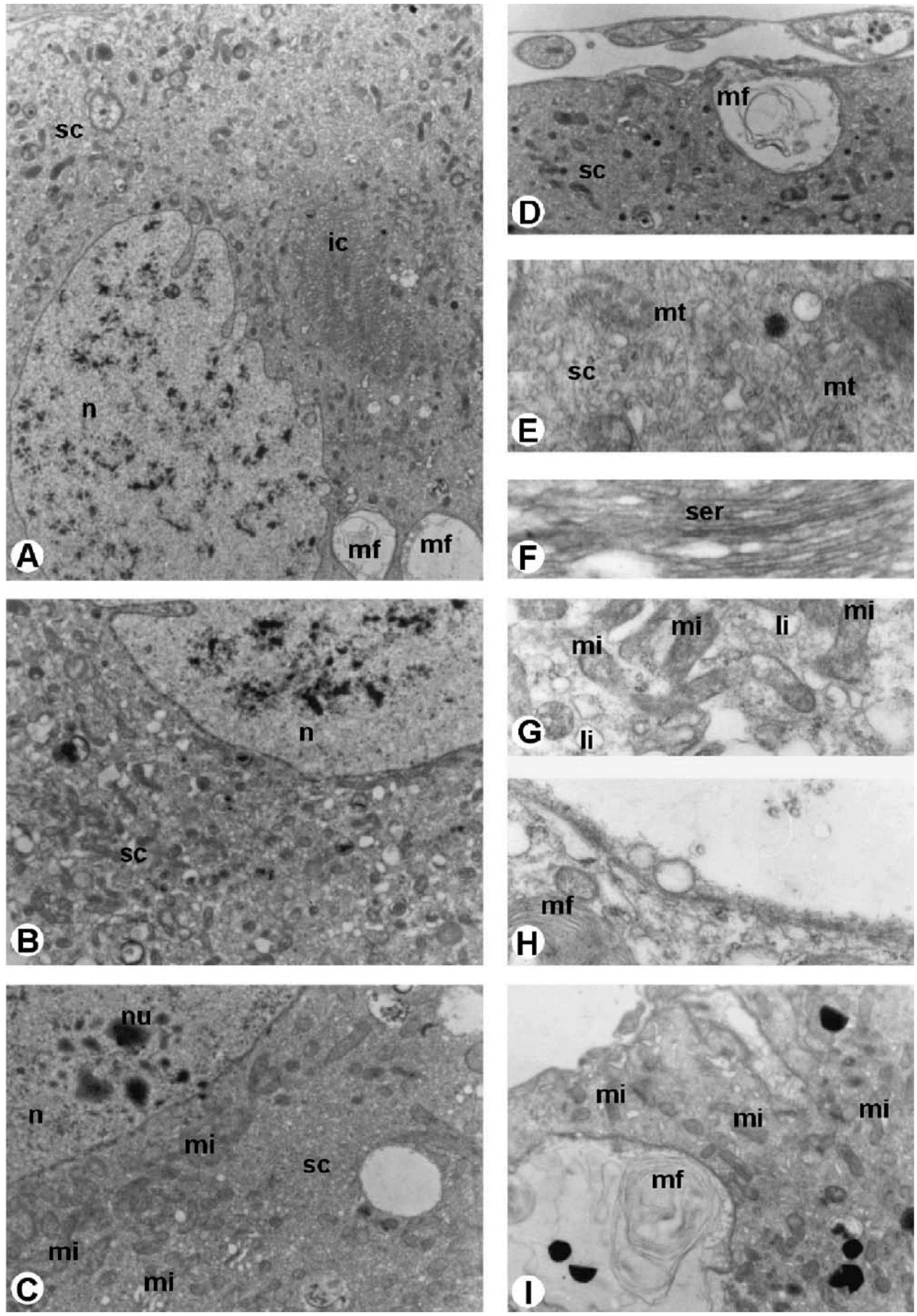

Fig. 5. Mandibular glands of *Atta sexdens rubropilosa*. (A,D–F) Minima worker. (A) sc, secretory cell; n, nucleus; ic, intra-cytoplasmic canaliculi; mf, myelin body. Magnification: 810×. (D) sc, secretory cell; mf, myelin body. Magnification: 2050×. (E) sc, secretory cell; mt, microtubules. Magnification: 2050×. (F) ser, smooth endoplasmic reticulum. Magnification: 1350×. (B,G,H) Media worker. (B) sc, secretory cell; n, nucleus. Magnification: 4200×. (G) mi, mitochondria; li, lipids. Magnification: 4200×. (H) mf, myelin body. Magnification: 2050×. (C,I) Soldier. (C) sc, secretory cell; n, nucleus; nu, nucleolus; mi, mitochondria. Magnification: 2050×. (I) mf, myelin body; mi, mitochondria. Magnification: 1450×.

of passing the product synthesized by the secretory cells towards the exterior.

The secretory cells of the mandibular glands of *A. s. rubropilosa* belong to the class III according to the classification of Noirot and Quennedey (1974).

Our ultrastructural study reveals in the canaliculi of the mandibular glands of *A. s. rubropilosa* the presence of two different cellular types. The intracellular portion is formed by the secretory cell itself, while the extracellular portion is produced by specialized cells known as canaliculi-forming cells (Costa-Leonardo and Cruz-Landim, 1985). The intra-cellular portion runs inside the glandular cell and is internally coated by an interrupted and porous cuticle, which gives a discontinuous appearance to this cuticle and causes

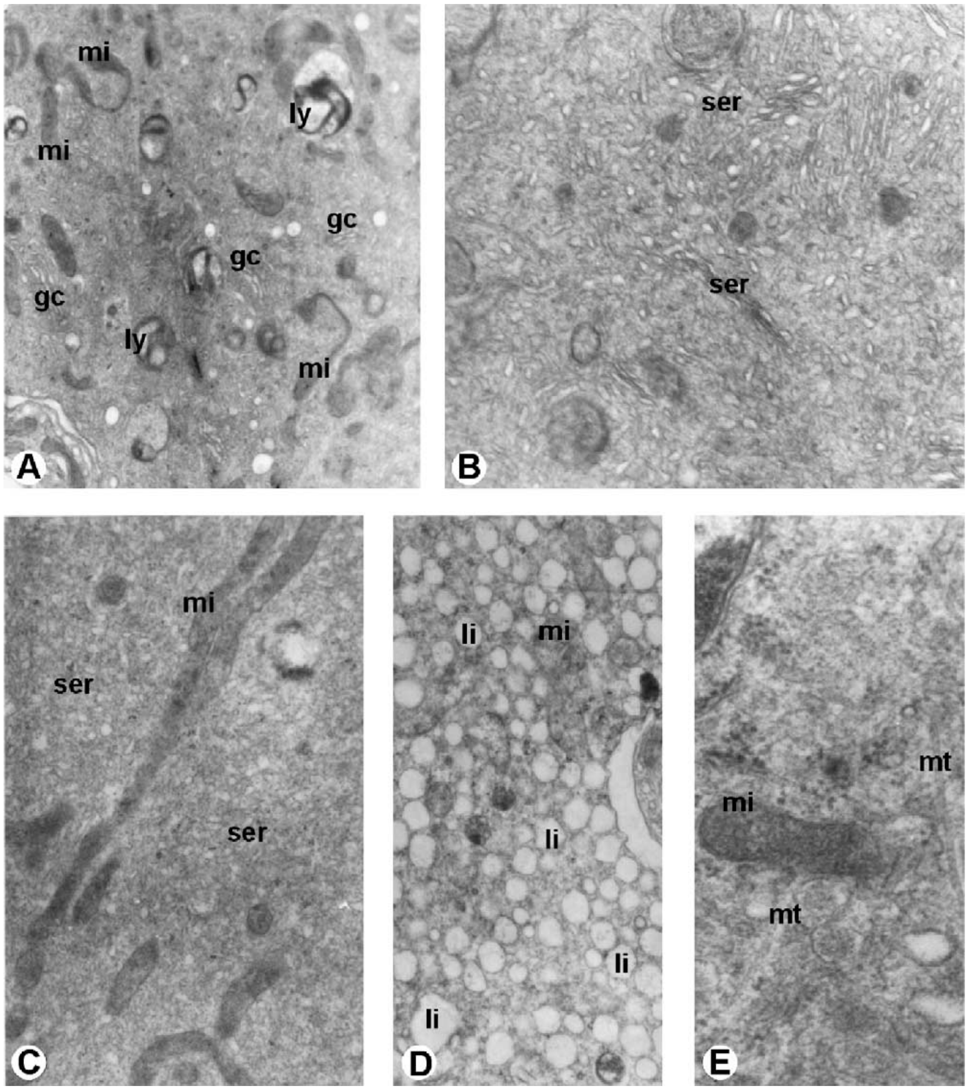

Fig. 6. Mandibular glands of soldier of *Atta sexdens rubropilosa*. (A) mi, mitochondria; gc, Golgi complex; ly, lysosomes. Magnification: 2900×. (B) ser, smooth endoplasmic reticulum. Magnification: 4200×. (C) ser, smooth endoplasmic reticulum; mi, mitochondria. Magnification: 4200×. (D) mi, mitochondria; li, lipids. Magnification: 4200×. (E) mi, mitochondria; mt, microtubules. Magnification: 1050×.

a widening of the cavity, which allows the formation of a space, known as pericanalicular space (Costa-Leonardo, 1981). Knecht and Kaatz (1990), in studies concerning the hypopharyngeal glands of *Apis mellifera*, termed this pericanalicular space as 'the intracellular reservoir', a term that would not be correct according to other authors, since the secretion at this particular point is already outside of the cell. The plasma membrane that delimitates this secretory space is differentiated into numerous microvilli, which would have the function of increasing the exchange surface. Beams and Anderson (1961) described the intracellular portion of the canaliculi as being in fact extracellular, because it is delimitated by a cuticle and thus separated from the cell. Cruz-Landim and Hadek (1969) reported that the intracellular canaliculi are produced by an infolding of the plasma membrane and, therefore, the intracellular canaliculi would be, topologically speaking, extracellular.

The mandibular glands of media worker and soldier of *A. s. rubropilosa* present secretory cells with two different ultrastructural aspects, which could be a single type at different stages of the secretory cycle or may be really a two different cells. The asynchrony of the secretory cycle has already been described in other hymenopterans, such as in the salivary gland of the ant *Pachycondyla (=Neoponera) villosa* (Zara and Caetano, 2002) and for the ectomandibular gland of the wasp *Polistes versicolor* (Pietrobon and Caetano, 2004). According to Pietrobon and Caetano (2004), the asynchronous activation of the pheromone membrane receptors in the secretory cells of the ectomandibular gland of the wasp *P. versicolor* makes the cells go through secretory cycles. Cruz-Landim (2002), while studying the mandibular glands of several Hymenoptera, confirmed that these glands exhibit growth stages, either of activity or regression, which gives them a different cytoplasmic appearance.

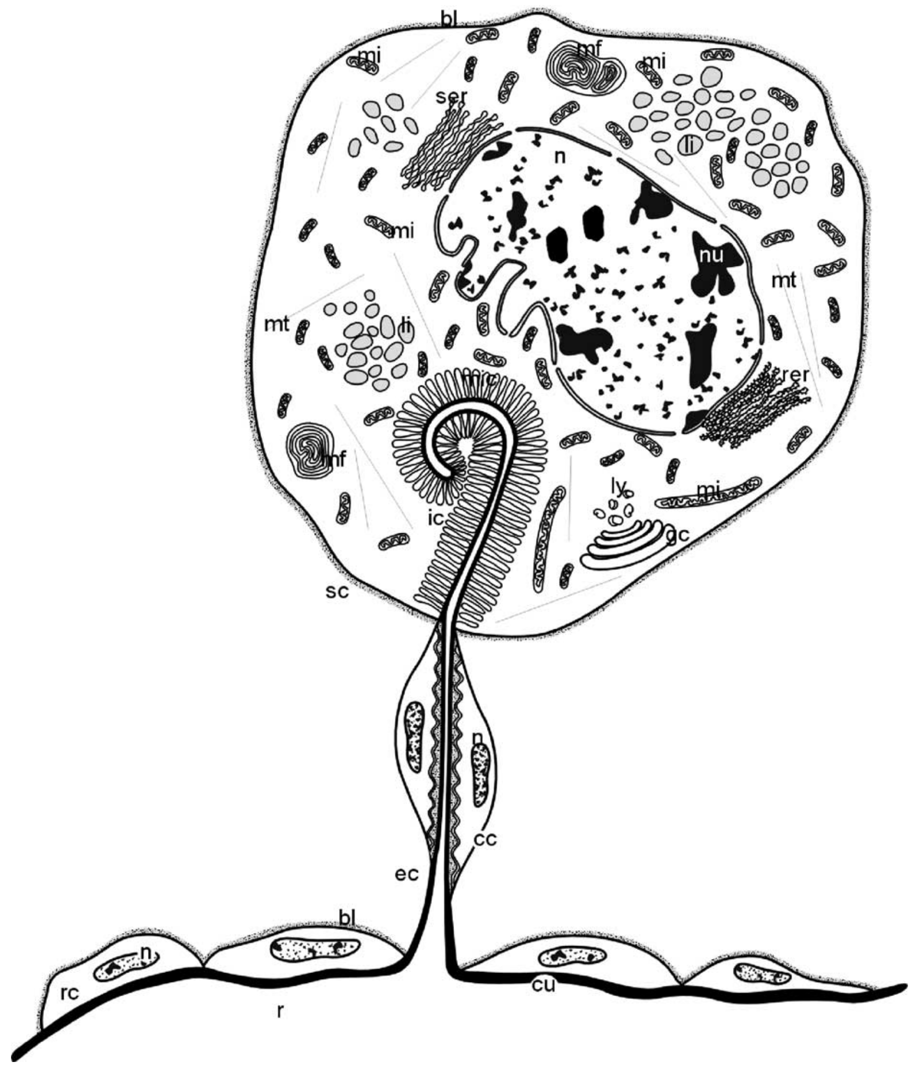

Fig. 7. Schematic representation of the mandibular glands of *Atta sexdens rubropilosa*. sc, secretory cell; r, reservoir; n, nucleus; nu, nucleolus; ic, intra-cytoplasmic canaliculi; ec, extra-cytoplasmic canaliculi; mf, myelin body; mi, mitochondria; gc, Golgi complex; ly, lysosomes; mt, microtubules; ser, smooth endoplasmic reticulum; rer, rough endoplasmic reticulum; mic, microvilli; li, lipids; cc, canaliculi-forming cell; rc, reservoir cell; cu, cuticle; bl, basal lamima.

The mandibular glands of minima, media and soldier of *A. s. rubropilosa* may undergo through the different stages.

The secretory cells of the mandibular glands of the three castes of *A. s. rubropilosa*, although topologically lying close to each other, possess different plasma membranes. The secretory cells establish contact between one another through the basal lamina, which is shared frequently; cellular junctions of any sort were not detected.

The total absence of infoldings of the plasma membrane of the secretory cells of the three castes studied was evident, thus suggesting that this fact is due to the large area of contact of the secretory portion with the haemolymph. This was also observed in the ectomandibular glands of the wasp *P. versicolor* (Pietrobon and Caetano, 2004).

The presence of numerous microtubules was noted in the cytoplasm of the secretory cells of the media worker and soldier of *A. s. rubropilosa*. These microtubules might be related to the occurrence of a process of cellular transport, or to the processes of shape change of the cells caused by the distension of the lumen of the reservoir due to the presence of secretion.

The nuclei of the secretory cells of the mandibular glands minima, media and soldier of *A. s. rubropilosa* show chromatin with numerous heterochromatic bundles, in addition to many pores in the nuclear envelope. This same nuclear characteristic occurs in the tegumental abdominal glands of *Oxae flavescens* (Guerino and Cruz-Landim, 2002).

Among the cytoplasmic organelles of the secretory cells of the mandibular glands of minima, media and soldier of *A. s. rubropilosa*, the most numerous were the mitochondria with few crests and with varying shapes, from small and electron-dense to large and elongated and frequently associated to lipid droplets. Gonçalves (2004), while studying the mandibular glands of reproductive individuals of the termite *Coptotermes gestroi*, showed the presence of mitochondria associated to lipid droplets. Similarly, Caetano et al. (2002) reported for the post-pharyngeal glands of *Dinoponera australis* the presence of mitochondria with various shapes. These same authors suggested that, in addition to the production of energy for the vital processes, another role of mitochondria in insects would be the production of lipids. According to Billen (1991), insect glands that have the function of producing pheromones are rich in elongated mitochondria, which agrees with our results. Caetano (1998) reported that the presence of mitochondria surrounding the nuclei of the cells of the post-pharyngeal gland of *D. australis* rarely occurred. In our study, mainly in the mandibular glands of soldiers of *A. s. rubropilosa*, the data obtained show the opposite situation.

Large amounts of smooth endoplasmic reticulum were found in the cytoplasm of the secretory cells of the mandibular glands of the three castes of *A. s. rubropilosa*. It has also been reported in pheromone-secreting glands of the Isoptera (Quennedey, 1971) and Hymenoptera (Ribeiro, 1999). Other organelles like Golgi complexes, lysosomes and myelin bodies were only found in the secretory cells of soldiers of *A. s. rubropilosa*. The presence of lysosomes in the cells of workers of *A. mellifera* was observed by Cruz-Landim and Silva de Moraes (1977) and led the authors to suggest a degenerative process for the gland as well as the formation of myelin bodies or cytolysosomes as products of the autodigestion of segregated cytoplasmic portions (reticulum and mitochondria) into vacuoles. Therefore, the presence of lysosomes and myelin bodies in the secretory cells of *A. s. rubropilosa* suggests that a process of autodigestion of segregated cytoplasmic portions is occurring in these glands and consequently indicates the occurrence of several stages of the same secretory cycle, as occurs in *A. s. rubropilosa*.

Camargo-Mathias et al. (1991) also reported the presence of myelin bodies concentrically arranged in the mandibular glands of worker ants of *Neoponera villosa*. These structures might be considered as bodies originated from myeloid or lamellar vesicles after the elimination of the secretion, or of part of the secretion, contained within (Gracioli and Silva de Moraes, 2002).

The present study with minima, media and soldier workers of *A. s. rubropilosa* showed that the soldier has the most elaborate secretion apparatus, thus suggesting that in addition to the secretory cellular activity, the secretion produced must be of a predominant lipidic nature. Corroborating the high secretory activity in the soldier, the nuclear envelope presented a large amount of pores. These data suggest a high rate for the production of ribosomal RNA, thus confirming the histochemical results previously obtained by Pavon and Camargo-Mathias (2004). These characteristics suggest a correlation between the defense and social communication roles that the soldier caste of *A. s. rubropilosa* executes.

According to the present results, the mechanism for the production and release of the secretion in the mandibular glands of *A. s. rubropilosa* seems to occur in several stages: (1) the secretion is synthesized by the secretory cells, (2) the secretion is stored in cytoplasmic vesicles, (3) the secretion is released into the pericanalicular space and collected by the intracellular portion of the collecting canaliculi, (4) the extracellular portion of the excretory canaliculi release it into the reservoir in which the secretion will be stored, and (5) afterwards, the secretion will be released via the excretory duct (see Fig. 7).

The wall of the reservoir of the mandibular glands of *A. s. rubropilosa* is formed by a simple squamous epithelium. Guerino and Cruz-Landim (2002), while studying the tegumental abdominal glands of *O. flavescens*, showed that the reservoir is also formed by a layer of short cells, which are continuous to the epidermis and coated by a cuticle on its luminal face.

In the individuals of the three castes of *A. s. rubropilosa*, the presence of lipid secretion granules suggested, beyond the other functions, also a possible pheromonal action.

The different roles executed by the different insect castes are directly dependent on the glandular products and, consequently, on the secretory cellular characteristics. It is known that in ants the soldiers are the individuals that leave the nest to forage, in addition to being involved with nest defense. Such a situation leaves the animal highly vulnerable to infection and predation by other animals. Therefore, it would be expected that the mandibular gland of individuals of this caste would have a more developed secretory apparatus. In the case of the media worker, and especially in the minima worker, which are specialized in caring for the delicate portions of the fungus that feeds the colony, the secretory characteristics of the mandibular glands are different specialization.

Acknowledgements

This work was financed by the Brazilian Research Agency FAPESP (*Fundação de Amparo à Pesquisa do Estado de São Paulo*), Process No. 03/00553-8. We are grateful to Antonio T. Yabuki, Monika Iamonte and Cristiane Márcia Mileo for the technical support.

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