Received: 9 October 2018 | Revised: 17 January 2019 | Accepted: 2 February 2019

DOI: 10.1002/jemt.23242

MICROSCOPY RESEARCH & TECHNIQUE WILEY

RESEARCH ARTICLE

Morphology of the mandibular gland of the ant *Paraponera clavata* (Hymenoptera: Paraponerinae)

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Review Editor: George Perry

Funding information
Programa de Pós-Graduação em Biodiversidade, Ambiente e Saúde (PPGBAS); Conselho Nacional de Pesquisa (CNPq); Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão (FAPEMA); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

Abstract

The ant *Paraponera clavata* (Fabricius, 1775) is the only extant species of Paraponerinae and is widely distributed in Brazilian forests. Aspects of its biology are documented extensively in the literature; however, knowledge of *P. clavata* internal morphology, specifically of exocrine glands, is restricted to the venom apparatus. The objective of this study was to describe the mandibular gland morphology of *P. clavata* workers. The mandibular gland is composed of a reservoir connected to a cluster of Type III secretory cells with cytoplasm rich in mitochondria and lipid droplets, similar to that of other ants. Notably, the glandular secretion is rich in protein and has a solid aspect. This is the first morphological description of the mandibular gland of *P. clavata*.

Research Highlights

This study presents the morphological description of the mandibular gland of *Paraponera clavata* (Hymenoptera: Paraponerinae). Singular characteristics of the gland are described: the glandular secretion is rich in protein and has a solid aspect.

KEYWORDS

ant, histochemistry, mandibular glands, ultrastructure

1 | INTRODUCTION

Paraponera clavata (Fabricius) is the only living species of ant subfamily Paraponerinae. It is found exclusively in the Neotropical region and is widely distributed in Brazilian tropical forests (Arias-Penna, 2007; Baccaro et al., 2015).

Some aspects of the biology of *P. clavata*, such as nesting and feeding habits, are well documented (Breed & Bennett, 1985; Jandt, Larson, Tellez, & McGlynn, 2013; Janzen & Carroll, 1983; Lattke, 2003; Longino & Hanson, 1995; McGee & Eaton, 2013). However, data on the internal morphology are restricted to the venom apparatus (Aili et al., 2017; Hermann et al., 1984; Hermann & Blum, 1966; Piek et al., 1991; Torres, Quinet, Hant, & Martins, 2013; Touchard et al., 2016).

Eusocial Hymenoptera (ants, wasps, and bees) are rich in exocrine glands, which are essential for chemical communication (Billen, 2008; Guerino & Cruz-Landin, 2003). In ants, at least 78 types of exocrine glands with important functions in marking and mating, among others, have been described (Billen, 2008; Billen, Stroobants, Wenseleers, Hashim, & Ito, 2013; Caetano, Jaffé, & Zara, 2002; Hölldobler, Obermayer, Plowes, & Fisher, 2014).

The salivary system of ants is composed of the mandibular, intramandibular, hypopharyngeal, postpharyngeal, and thoracic salivary glands (Caetano et al., 2002; Gama, 1978; Schoeters & Billen, 1994). Some of these glands perform digestive functions, such as the hypopharyngeal (Amaral & Caetano, 2005) and the thoracic salivary glands (Spradbery, 1973). However, the mandibular glands are associated with the production of pheromones for nestmate recognition (Caetano et al., 2002; Gama, 1985) and, mainly, with alarm communication (Cammaerts, Evershed, & Morgan, 1983; Fales, Blum, Crewe, & Brand, 1972; Martins, Delabie, & Serrão, 2016).

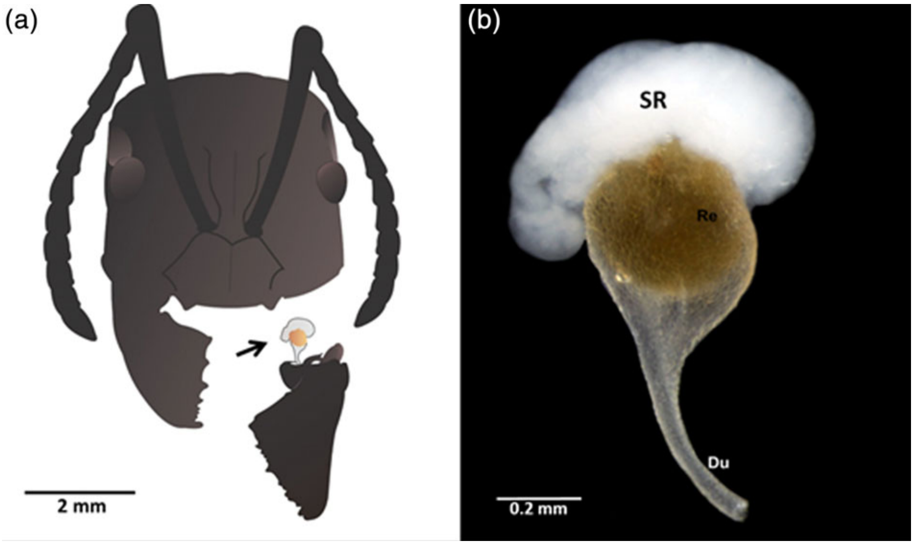

FIGURE 1 (a) Schematic drawing of the head of *Paraponera clavata*, showing the location of the mandibular gland (arrow). (b) External anatomy of the mandibular gland showing the secretory region (SR), reservoir (Re), and excretory duct (Du) [Color figure can be viewed at wileyonlinelibrary.com]

Mandibular glands generally comprise a cluster of secretory cells connected to a reservoir from which an individual duct opens to the base of the mandible (Caetano et al., 2002; Hermann, Hunt, & Buren, 1971). However, the morphology of the mandibular gland can vary according to caste and species (Grasso et al., 2004; Serrão, Martins, Santos, & Gonçalves, 2015).

The purpose of this study was to describe the morphology of the mandibular gland in *P. clavata* workers.

2 | MATERIALS AND METHODS

2.1 | Biological material

Adult *P. clavata* workers were manually collected from three nests located in the Inhamum Environmental Protection Area (04°53′S 43°24′W), Caxias, Maranhão, Brazil, and transferred to the Myrmecology Laboratory (LAMIR) of the State University of Maranhão (UEMA), Brazil. Workers were maintained in plastic boxes lined with soil and substrate collected from the original nests. Artificial nests were kept at 27 ± 2°C under a photoperiod of 12 h light and 12 h darkness, and workers were fed a diet of apples, honey, and locust nymphs.

2.2 | External morphology

Five Adult *P. clavata* workers were cryoanesthetized at −4°C, and their mandibular glands were dissected in 125 mM NaCl. Immediately after extraction, mandibular glands were photographed using a Zeiss® Discovery V12 stereomicroscope equipped with a digital camera (AxioCam ICc 1, 1.4 megapixels) and Zen® 2012 software, which was also used to take measurements of the external structures.

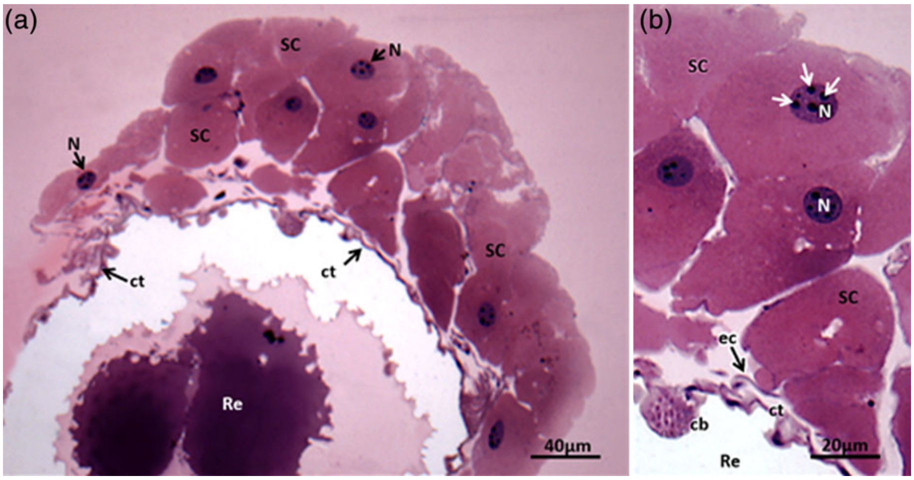

FIGURE 2 Histological sections (stained with hematoxylin and eosin). (a) Prominent secretory layer and reservoir of the mandibular gland. (b) Basal portion of the secretory layer and collecting canaliculi intersecting the reservoir (white arrows: nucleoli). N, nucleus; SC, secretory cells; ct, reservoir cuticle; Re, reservoir; ec, extracellular canaliculi; cb, bundle of canaliculi [Color figure can be viewed at wileyonlinelibrary.com]

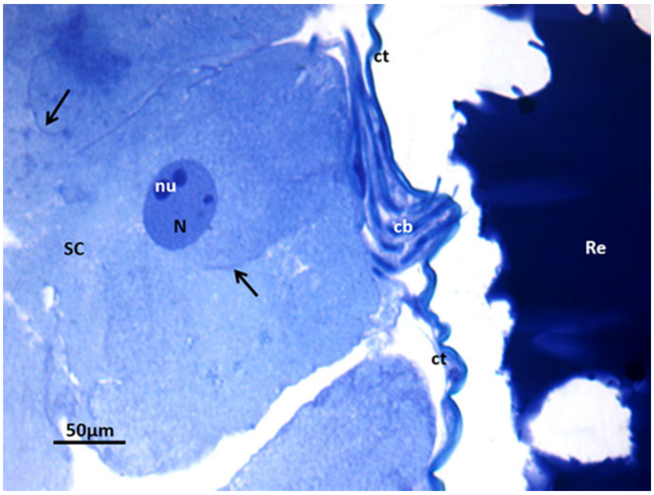

FIGURE 3 Histological section (stained with toluidine blue). Secretory cells, with collecting canaliculi at the intersection with the reservoir. Intracellular canaliculi (arrows) within secretory cells. SC, secretory cells; N, nucleus; nu, nucleolus; ct, reservoir cuticle; Re, reservoir; cb, bundle of canaliculi [Color figure can be viewed at wileyonlinelibrary.com]

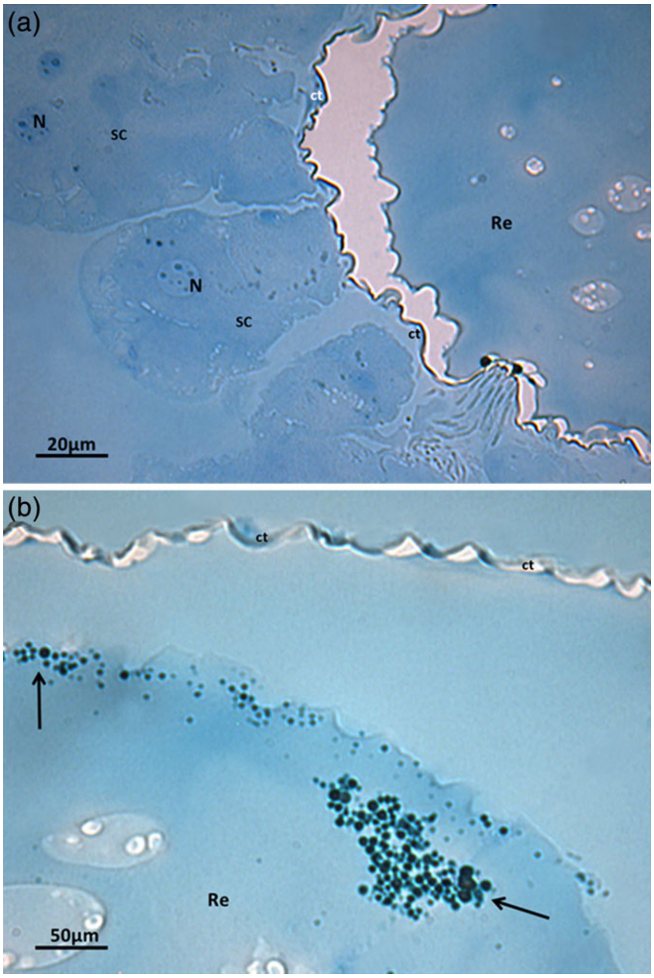

FIGURE 4 Histological sections stained with Nile blue for lipid detection. (a) Cytoplasm and nucleus of glandular cells, which were negative for lipids. (b) Lipid-positive granules in the reservoir (arrows). SC, secretory cells; N, nucleus; Re, reservoir; ct, reservoir cuticle [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Histochemical analysis of the mandibular gland of *Paraponera clavata*

Structure	Lipids	Carbohydrates	Proteins
Secretory cell nucleus	–	–	++
Secretory cell cytoplasm	–	–	+++
Reservoir	+++[a]	+	+++

Staining intensity: negative (–), weakly positive (+), positive (++), and strongly positive (+++).
[a]Observed only in small granules.

2.3 | Light microscopy

For the light microscopy, samples were five adult *P. clavata* workers were cryoanesthetized, and the dissected mandibular glands were fixed with Zamboni's fixative (Stefanini, De Martino, & Zamboni, 1967) for 24 h. The glands were then dehydrated in a graded ethanol series (70%, 80%, 90%, and 95%) and embedded in LR White resin. Three-micrometer-thick sections were obtained using a Leica microtome, stained with hematoxylin and eosin or toluidine blue, and analyzed under a light microscope (Olympus BX-60) equipped with a digital camera and QCapture software.

2.4 | Histochemistry

Histological sections of the mandibular glands were stained with mercuric bromophenol blue for protein detection, periodic acid–Schiff (PAS) for carbohydrate detection, and Nile blue for lipid detection, as proposed by Pearse (1985).

2.5 | Transmission electron microscopy

For transmission electron microscopy, samples were five adult *P. clavata* workers were cryoanesthetized, and their mandibular glands were dissected in 0.1 M sodium cacodylate buffer solution, pH 7.2, with 0.2 M sucrose and fixed with 2.5% glutaraldehyde for 12 h. Subsequently, glands were washed in buffer and postfixed in 1% osmium tetroxide for 2 h. Samples were washed twice with buffer, dehydrated in a graded ethanol series (70%, 80%, 90%, and 95%), and soaked in LR White resin. Ultrathin

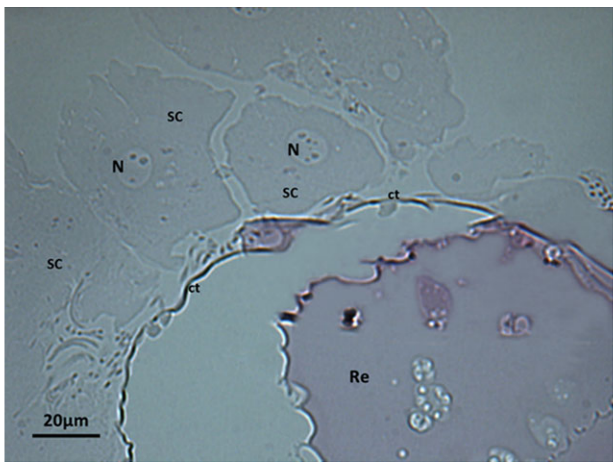

FIGURE 5 Secretory cells show negative staining for carbohydrates (periodic acid–Schiff, PAS) in the nucleus and cytoplasm, and the reservoir shows weakly positive staining. SC, secretory cells; N, nucleus; Re, reservoir; ct, reservoir cuticle [Color figure can be viewed at wileyonlinelibrary.com]

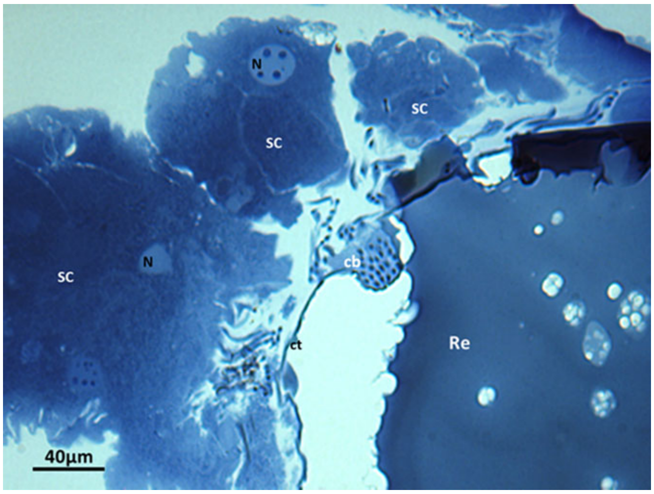

FIGURE 6 Cytoplasm of glandular cell and reservoir with positive staining for proteins (mercuric bromophenol blue) and nucleus of glandular cell with weakly positive staining. SC, secretory cells; N, nucleus; Re, reservoir; cb, bundle of canaliculi; ct, reservoir cuticle [Color figure can be viewed at wileyonlinelibrary.com]

sections (50–90 nm) obtained using an Sorvall MT2-BMT2-B ultramicrotome (Sorvall Instruments, Wilmington, DE) were stained with 1% aqueous uranyl acetate and lead citrate (Reynolds, 1963) for 20 min each and analyzed using a Zeiss EM109 transmission electron microscope.

3 | RESULTS

Paraponera clavata mandibular glands were located in the head, easily located in the proximal part of the mandible of the mandibles (Figure 1a), and contained a secretory region, a reservoir, and an excretory duct (Figure 1b).

As shown in Figure 1b, the secretory region consisted of a cluster of cells (colored white) situated above the reservoir (colored brown), which was 0.45 mm in diameter and had a solid consistency. The basal portion of the reservoir was elongated, forming the excretory duct (0.40 mm in length and 50 μm in diameter), which opened into the mandible (Figure 1a).

The secretory region of the mandibular gland had well-developed globular cells with acidophilic cytoplasm and nucleus with predominance of decondensed chromatin and some nucleoli (Figure 2a,b). Cells were individually connected to the reservoir through a narrow canaliculus (Figure 3). Canaliculi were arranged in bundles (Figures 2b, 3, and 6) of 20–30, which crossed the wall of the gland reservoir.

The reservoir was lined by a simple epithelium of flattened cells, and the inner surface was covered by a thin cuticle (Figures 2 and 3). It was filled with heterogeneous content with core region strongly acidophilic, whereas the peripheral content was weakly acidophilic (Figure 2a).

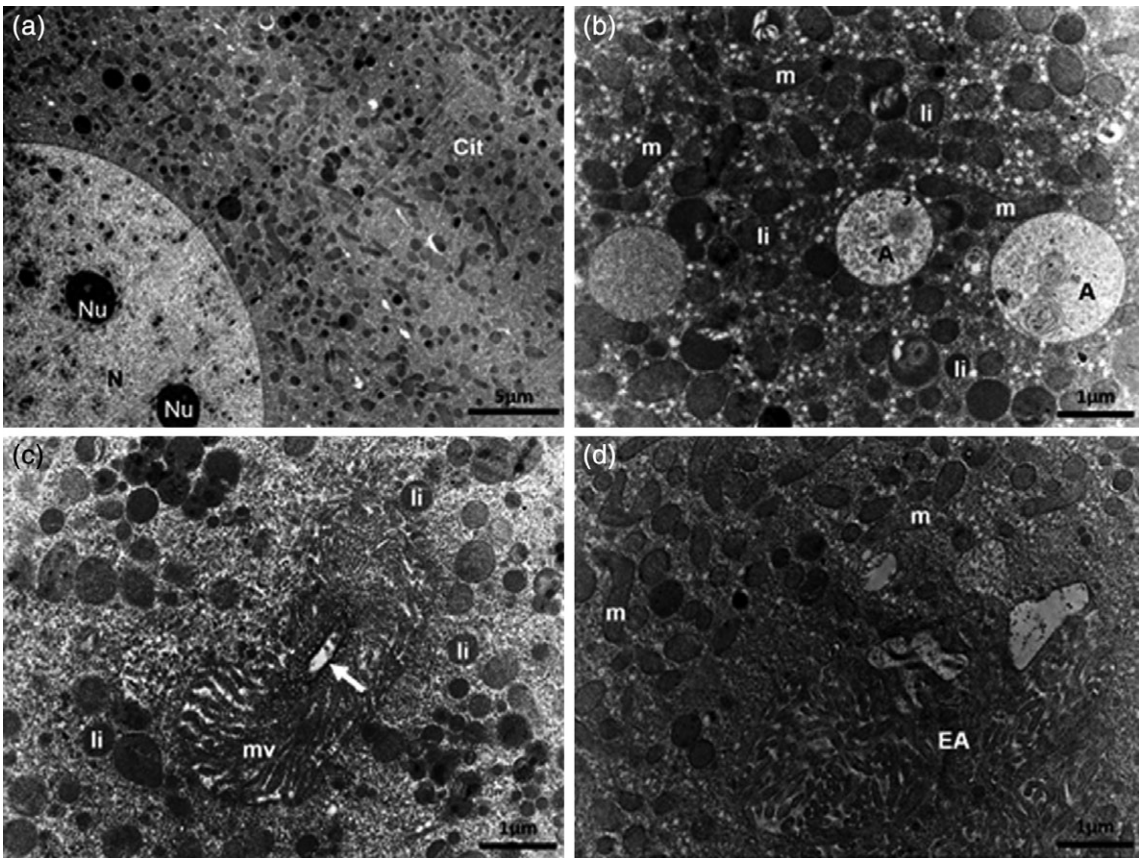

FIGURE 7 Ultrastructure of the mandibular gland of *Paraponera clavata*. (a) Nucleus and cytoplasmic organelles of glandular cell. (b) Cytoplasmic organelles, in particular, lipid droplets, autophagosomes, and mitochondria. (c) Intracellular canaliculus (arrow) and its structures. (d) Cytoplasmic organelles, prominent mitochondria, and end apparatus. N, nucleus; Nu, nucleolus; Cyt, cytoplasm of secretory cell; m, mitochondria; li, lipid droplets; mv, microvilli of the intracellular canaliculus; EA, end apparatus

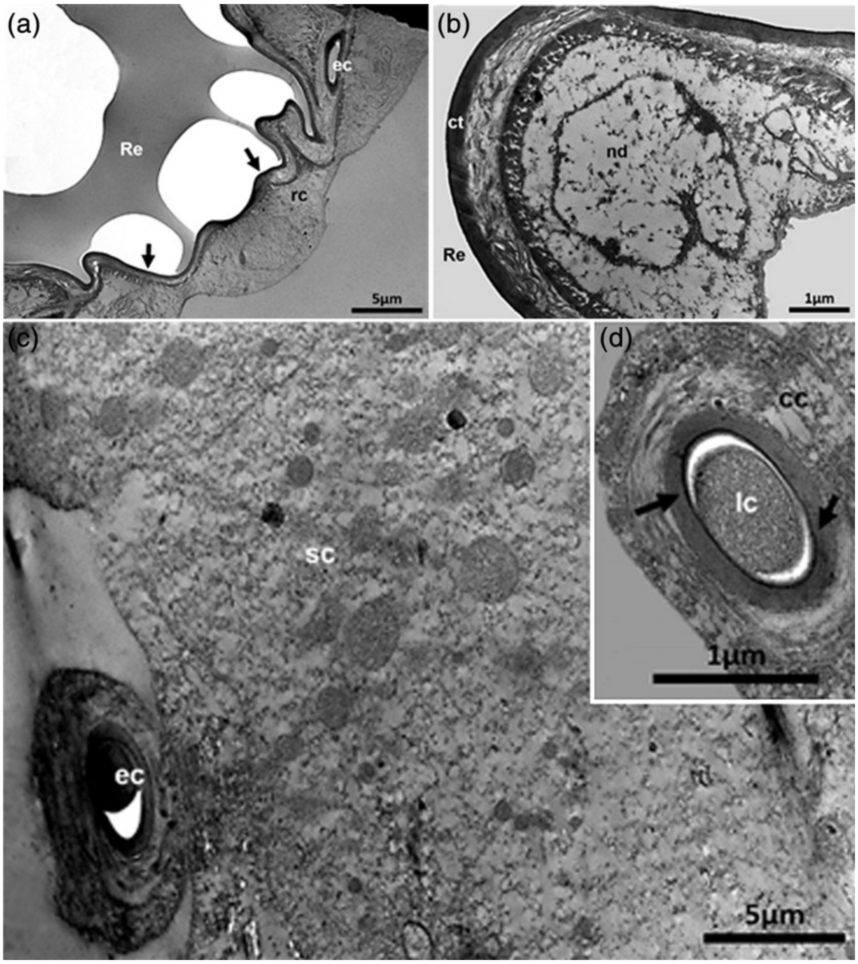

FIGURE 8 (a) Edge of the reservoir (arrows pointing to the cuticle). (b) Cuticle and reservoir cell. (c) Extracellular canaliculus. (d) Cuticle (arrows) and extracellular canaliculus cells. sc, secretory cell; nd, nucleus of reservoir cell; ec, extracellular canaliculus; lc, extracellular canaliculus lumen; cc, cell from which the extracellular canaliculus originates; rc, reservoir cells; Re, reservoir

The histochemical test for lipids showed a strong positive reaction in dispersed granules in the glandular secretion but negative reaction in secretory cells (Figure 4a,b and Table 1).

PAS test showed low carbohydrate content in the glandular secretion (Figure 5 and Table 1).

The cytoplasm of glandular cells and the glandular secretion showed strongly positive staining for proteins (Figure 6 and Table 1).

Transmission electron microscopy images of the secretory cells showed that their cytoplasm was rich in mitochondria and lipid droplets and contained some autophagosomes (Figure 7a,b). The lumen of the apparatus was surrounded by many microvilli (Figure 7c,d).

Canaliculi originated from the end apparatus of secretory cells (Figure 7d). The extracellular portion of canaliculi was formed by flat cells with little cytoplasm covered by a thick cuticle (0.2 μm) (Figure 8c,d).

Cells of the reservoir wall were flattened, and the apical surface was covered by a cuticle of ~0.8 μm thickness (Figure 8a,b).

4 | DISCUSSION

The morphology of the mandibular gland of *P. clavata* is similar to that of another species, the Ponerinae *Dinoponera grandis* (Perty), as to the location and shape of the reservoir (Hermann et al., 1984). However, the clustered arrangement of globular glandular cells of *P. clavata* differs from the ovoid cell clusters of the Formicinae *Polyergus rufescens* (Latreille) (Grasso et al., 2004) and species of *Calomyrmex* (Emery) (Brough, 1977); the Myrmicinae *Atta sexdens* (Forel) (Amaral & Machado-Santelli, 2008) and *Monomorium pharaonis* (Linnaeus) (Boonen, Eelen, Børgesen, & Billen, 2013); and the Ponerinae *Dinoponera gigantea* (Perty) (Caetano et al., 2002), *Pachycondyla striata* (Smith) (Serrão et al., 2015), and *Brachyponera sennaarensis* (Mayr) (Billen & Al-Khalifa, 2018).

An interesting feature of the mandibular gland in *P. clavata* is that the secretion stored in the reservoir is solid, unlike the fluid glandular secretion of this gland in *D. grandis* (Hermann et al., 1984), *D. gigantea* (Caetano et al., 2002), and *P. striata* (Serrão et al., 2015). It is hard to explain how a solid glandular secretion can be discharges. Perhaps, the solid aspect of the secretion results from protein crystallization due to its high content as reveals our histochemical test, which might be break with activation of proteases in the gland content, but this needs further functional and biochemical studies.

The content of the reservoir is heterogeneous, more acidophilic in the central region than at the periphery, which is probably due to a high concentration of proteins in the secretion, as suggested by the strong positive reaction in the histochemical test for proteins. Similar findings were observed in the mandibular gland of *A. sexdens* (Pavon & Camargo-Mathias, 2004, 2006) and of the wasp *Polistes versicolor* (Pietrobon & Caetano, 2003).

The secretion of the mandibular gland of *P. clavata* is rich in protein and poor in lipids and carbohydrates, likely reported for *A. sexdens* (Pavon & Camargo-Mathias, 2006). Nevertheless, this is an intriguing finding because secretory cells are rich in mitochondria and lipid droplets and with few rough endoplasmic reticulum, an expected cytoplasm organelle for cells producing proteins (Alberts et al., 2014). Otherwise, mitochondria and lipid droplets have been reported in the secretory cells of the mandibular gland of ants (Davidson, Kamariah, & Billen, 2011; Billen, Hashim, & Ito, 2016), associated with high metabolic rate (Dailey & Crang, 1978; Gracioli-Vitti & Abdalla, 2006; Santos, Souza, Vieira, Zanuncio, & Serrão, 2015), as lipids are energy reserves used by mitochondria for ATP production.

The secretory cells of the mandibular gland of *P. clavata* have an end apparatus with an intracellular canaliculus characterizing the type III secretory cell according to the classification of Noirot and Quennedey (1974). However, in *P. clavata*, canaliculi of secretory cells are arranged into bundles that open into the reservoir, forming a structure similar to the "sieve plate" found in metapleural (Bot, Obermayer, Hölldobler, & Boomsma, 2001; Lacerda et al., 2010; Schoeters & Billen, 1993; Souza, Soares, Cyrino, & Serrão, 2006), intramandibular glands of other ants (Billen & Delsinne, 2013). In the mandibular glands, canaliculi bundles occur only in the Ponerinae *Dinoponera australis* (Emery) (Caetano et al., 2002), but the function of this bundle arrangement is unknown.

At the ultrastructural level, the organization of the secretory cells is similar to that of the mandibular gland in the majority of other social insects, with a well-developed smooth endoplasmic reticulum, that is indicative for the elaboration of a nonproteinaceous secretion (Boonen et al., 2013; Noirot & Quennedey, 1974). The secretory products are drained from the secretory cells into the duct cells through the end apparatus. This can either appear with tightly packed or loosely arranged microvilli that surround the central cuticular canal, of which the loosely arranged form has been interpreted as a temporary storage space at the cellular level (Billen & Schoeters, 1994; Billen, Ito, Maile, & Morgan, 1998, Boonen et al., 2013). Whereas the duct cells of mandibular glands in other species usually are mainly straight, they appear remarkably folded in queens and workers of *Brachyponera sennaarensis* (Billen & Al-Khalifa, 2018).

Several studies with mandible glands on ants have shown that they perform defensive alarm functions (Billen et al., 1998; Fales et al., 1972), although other functions have already been attributed to these glands as a source of sex pheromones (Gracioli, Moraes, & Cruz-Landim, 2004), repellency of other insects (Brough, 1978), and inhibition of fungi (Akino, Turushima, & Yamaoka, 1995; Marsaro Jr, Della-Lucia, Barbosa, Maffia, & Morandi, 2001) and bacteria (Brough, 1983). For the species *P. clavata*, the probable functions of the mandibular gland remain unproven. The lack of this information, added to the morphological variations presented in this study, support the need for behavioral tests with the extracts of this gland so that the actual functionalities of the mandibular gland in *P. clavata* are known.

This is the first morphological description of the mandibular gland of *P. clavata*, the only extant species of Paraponerinae. The mandibular gland has some characteristics in common with those of ants of other subfamilies, although the high protein content and solid aspect of the glandular secretion is unprecedented.

ACKNOWLEDGMENTS

This study was supported by the following Brazilian institutions: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa e ao Desenvolvimento Científico e Tecnológico do Maranhão (FAPEMA), Conselho Nacional de Pesquisa (CNPq), and Programa de Pós-Graduação em Biodiversidade, Ambiente e Saúde (PPGBAS). The authors thank the Center for Microscopy and Microanalysis of the Federal University of Viçosa and the Maranhão Zoological Collection (CZMA) for the technical support.

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How to cite this article: Andrade TT, Gonçalves WG, Serrão JE, Martins LCB. Morphology of the mandibular gland of the ant *Paraponera clavata* (Hymenoptera: Paraponerinae). *Microsc Res Tech*. 2019;1–8. https://doi.org/10.1002/jemt.23242