Morphology and Histochemistry of the Intramandibular Glands in Attini and Ponerini (Hymenoptera, Formicidae) Species

LUIZA CARLA BARBOSA MARTINS[1] AND JOSÉ EDUARDO SERRÃO[2]
[1]*Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa-36570-000, Minas Gerais, Brasil*
[2]*Departamento de Biologia Geral, Universidade Federal de Viçosa, Viçosa-36570-000, Minas Gerais, Brasil*

KEY WORDS ants; histology; exocrine gland; mandibles; phylogeny

ABSTRACT An understanding of the internal morphology of the ant mandible is important in explaining the relationship between the exocrine system and the behavioral and phylogenetic characteristics of different subfamilies of Formicidae. In this study, we investigated the occurrence of intramandibular glands in ants from the Ponerini (Ponerinae) and Attini (Myrmicinae). These ants possess glands from classes I and III, and secretory epithelial cells with a reservoir. The intramandibular glands show a distinct histology in the studied species, varying in their location, degree of development, and chemical content. Using this information, it is possible to hypothesize that the glands from different tribes produce different substances, which may indicate a variety of functions, depending on the chemical nature of the cellular constituents. A cladistic analysis using the characters of the intramandibular glands separated both tribes, suggesting that structural differences in the intramandibular glands may contribute to future phylogenetic studies of the Formicidae. *Microsc. Res. Tech. 74:763–771, 2011.*

INTRODUCTION

A common characteristic of social insects is the variety of exocrine glands on the various parts of their bodies. The high number and diversity of these glands relate to the many important functions that secretions have in the lives of social insects (Billen, 2008; Billen and Morgan, 1998; Hölldobler and Wilson, 1990; Noirot and Quennedey, 1991). At this time, a total of 105 different exocrine glands have been recognized in the various groups of social insects (Billen, 2008).

Noirot and Quennedey (1991) classify the epidermal glands of insects into three classes. Class I comprises epidermal cells that take on a secretory function, and release compounds to the exterior of the body by diffusion through the cuticle. The class II glands comprise cells which also release secretions to the body surface through the cuticle, but, in this case, since the cells are not in contact with the cuticle, the secretion is first passed through an epidermal cell. In the class III glands, the secretory cells usually originate from the epidermis. The secretory cells are usually spherical and are linked by a canal cell to a pore in the cuticle, where the secretion is released.

In the Hymenoptera, there are usually two types of mandibular glands: (i) the ectomandibular or mandibular glands and (ii) the mesomandibular or intramandibular glands (Cruz-Landim and Abdalla, 2002). The ectomandibular glands are the best known and studied, and form the basis our knowledge of the "mandibular glands" in general, in contrast to the less well understood intramandibular glands (Cruz-Landim and Abdalla, 2002).

The intramandibular glands are classified as tegumental glands and differentiated with the mandible epidermis in the pupa (Cruz-Landim and Abdalla, 2002). They were described for the first time in *Atta sexdens rubropilosa* ants by Toledo (1967).

Study of the morphology of the intramandibular glands in ants can help us to understand the relationship between the exocrine gland system and the behavioral and phylogenetic characteristics of the different ant species. This study investigated the occurrence of intramandibular glands in ants from the tribes Ponerini (Ponerinae) and Attini (Myrmicinae).

MATERIALS AND METHODS

Ants

Worker castes of the species of Formicidae listed in Table 1 were obtained by field collection in the States of Minas Gerais and Bahia (Brazil), and transferred to Zamboni's fixative solution (Stefanini et al., 1967). Voucher specimens were deposited in the Entomological Regional Museum from Federal University of Viçosa (UFVB).

Histology and Histochemistry

The mandibles of three specimens each species annotated in the Table 1 were removed from the fixed specimens, dehydrated in a graded ethanol series and embedded in historesin (Leica). The mandibles were sectioned longitudinally in 3-μm slices and stained with

*Correspondence to: José Eduardo Serrão, Departamento de Biologia Geral, Universidade Federal de Viçosa, Avenida Peter Henry Rolfs, s/n, CEP. 36570-000-Viçosa, Minas Gerais, Brasil. E-mail: jeserrao@ufv.br

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TABLE 1. List of taxa analyzed and locality

Species	Collect site
Myrmicinae	
Attini	
Acromyrmex subterraneus brunneus (Forel, 1911)	Paraopeba, Minas Gerais
Acromyrmex niger (Fr. Smith, 1858)	Viçosa, Minas Gerais
Acromyrmex subterraneus molestans (Santschi, 1925)	Teixeiras, Minas Gerais
Atta bisphaerica (Forel, 1908)	Teixeiras, Minas Gerais
Atta laevigata (Fr. Smith, 1858)	Teixeiras, Minas Gerais
Atta sexdens rubropilosa (Forel, 1908)	Teixeiras, Minas Gerais
Ponerinae	
Ponerini	
Hypoponera sp1.	Viçosa, Minas Gerais
Leptogenys arcuata (Roger, 1861)	Ilhéus, Bahia
Leptogenys sp1.	Ilhéus, Bahia
Odontomachus haematodus (Linnaeus, 1758)	Itajuípe, Bahia
Pachycondyla sp1.	Ibicuí, Bahia
Pachycondyla crassinoda (Latreille, 1802)	Porto Seguro, Bahia
Pachycondyla harpax (Fabricius, 1804)	Itajuípe, Bahia
Pachycondyla impressa Roger, 1861	Itajuípe, Bahia
Pachycondyla stigma (Fabricius, 1804)	Boa Nova, Bahia
Pachycondyla veranae (Forel, 1922)	Guaratinga, Bahia
Pachycondyla villosa (Fabricius, 1804)	Itajuípe, Bahia
Formicinae	
Camponotus rufipes (Fabricius, 1775)	Itajuípe, Bahia

hematoxyline and eosin. Some slices of mandible were also tested for histochemistry as follows: Mercury-bromophenol blue for protein staining; PAS (Periodic acid-Schiff) for polysaccharide and glyco-conjugate; and Nile blue for lipid identification, according to Pearse (1985).

Morphometry

Morphometric data on gland cells were obtained from 10 sections/ant with aid of the software Image-Pro Plus version 4.5 (Media cybernetics). The total area of the cell and the nucleus were obtained and used to determine the nucleus/cytoplasm ratio with the formula: NCR = $N/C - N$, were N is the area of the nucleus and C the area of the cell.

Scanning Electronic Microscope

Ant mandibles were removed, dehydrated in a graded ethanol series, transferred to hexamethyldisilazane (HMDS) for 5 min and air dried. They were then glued into aluminum supports, covered with gold (20 nm) and observed in a scanning electron microscope, LEO VP1430, in the Microscopy and Microanalysis Nucleus of the Federal University of Viçosa (UFV), Minas Gerais, Brazil.

Phylogenetic Analysis

The comparison of the intramandibular glands between the various ant species resulted in a seven-character matrix, which was evaluated for phylogenetic comparison. The characters were polarized based on a comparison with an out-group (Maddison et al., 1984; Nixon and Carpenter, 1993; Watrous and Wheeler, 1981) using *Camponotus rufipes* (Fabricius, 1775) (Formicinae) as the out-group.

The characters present in the out-group were considered as plesiomorphic and coded as (0), and the apomorphic characters as (1) and (2) in the case of non-ordered multi-state characters. The cladistic analysis was conducted using the PAUP computer program, version 4.0b10 (Swofford, 1998) with a heuristic search and TBR algorithm. The results were analyzed using the TreeView program, version 16.6.

The evaluated characters of the intramandibular glands (Fig. 1) were:

1. Type of epithelium: flattened (0), cuboidal (1), and columnar (2).
2. Nucleus size of the epithelial cells: large when nucleus/cytoplasm ratio >0.20 (0) and small when nucleus/cytoplasm ratio ≤ 0.20 (1).
3. Type III gland: without a cytoplasm vacuole (0), highly vacuolated (1), and weakly vacuolated (2).
4. Size of the nucleus of the type III gland cell: small when nucleus/cytoplasm ratio ≤ 0.14 (0), large when nucleus/cytoplasm ratio >0.14 (1).
5. Glandular reservoir: absent (0) and present (1).
6. Other types of cells: absent (0) and present (1).
7. Cytoplasm granules: absent (0) and present (1).

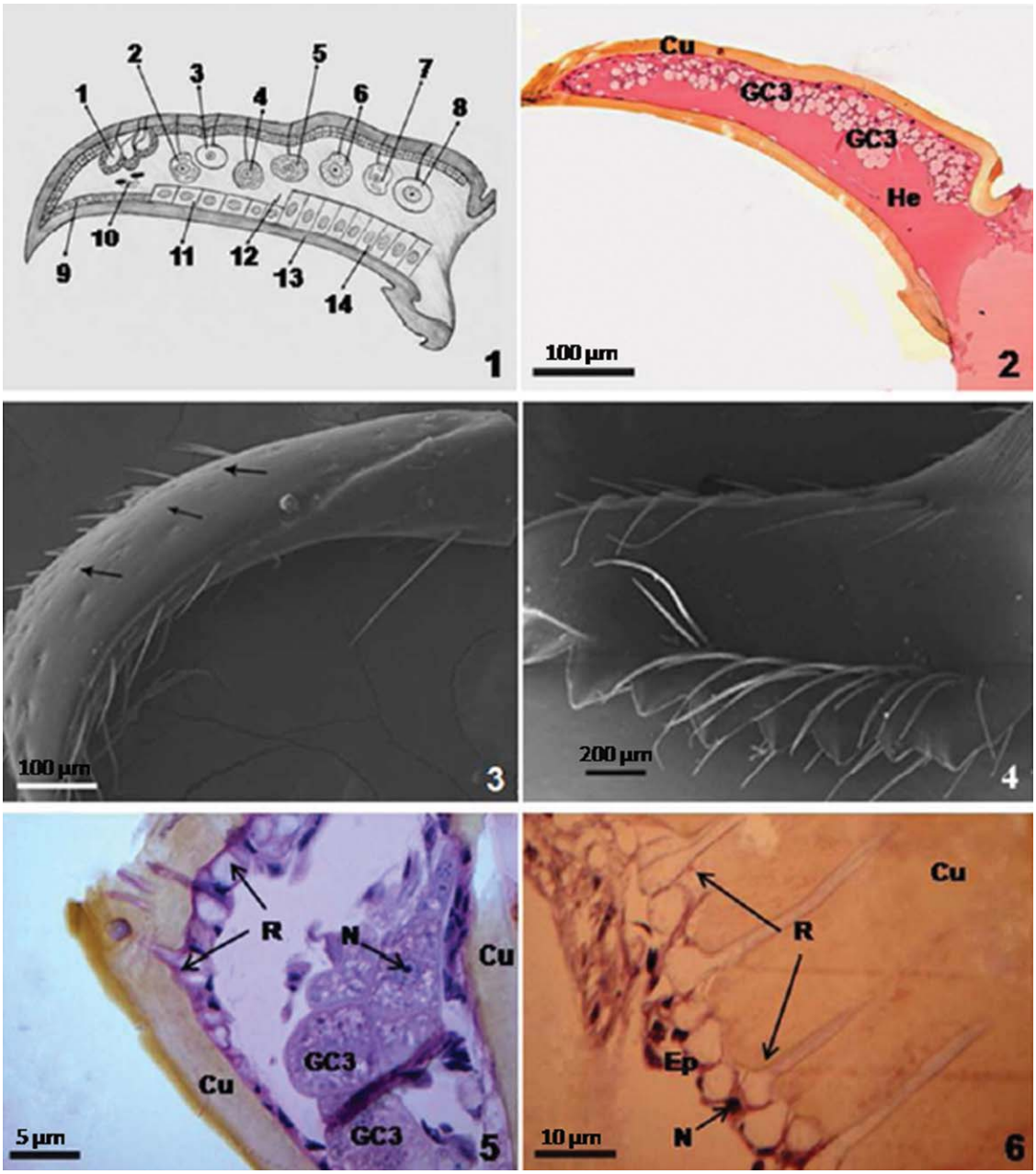
RESULTS

The intramandibular glands in the ants studied can be divided into three categories: (i) class I glands, characterized by cuboidal or columnar epidermal cells; (ii) class III unicellular glands, isolated cells in the internal cavity of the mandible characterized by the presence of canaliculi that open to pores on the surface of the mandible; and (iii) secretory epithelial cells with a reservoir, formed by hypertrophy of the epidermal cells in specific areas of the mandible and containing a wide reservoir (Fig. 1). Characteristically, the pores of the class III glands occur on the superior surface of the mandible (Figs. 2–4). However, the occurrence and structure of these three types of gland, varies in the different ant species, as described below.

In the Attini, all of the *Acromyrmex* and *Atta* species studied the intermandibular epidermis has flattened cells with epithelial glands with a reservoir (Figs. 5 and 6) and unicellular glands of class III.

In *Acromyrmex subterraneus brunneus* and *Acromyrmex niger*, the flattened epithelium of the intramandibular epidermis has cells with a smaller nucleus compared with those of *A. subterraneus molestans*. In all of the Attini species, some regions of the intramandibular epidermis contain cells that have become hypertrophied, forming a reservoir (Fig. 5).

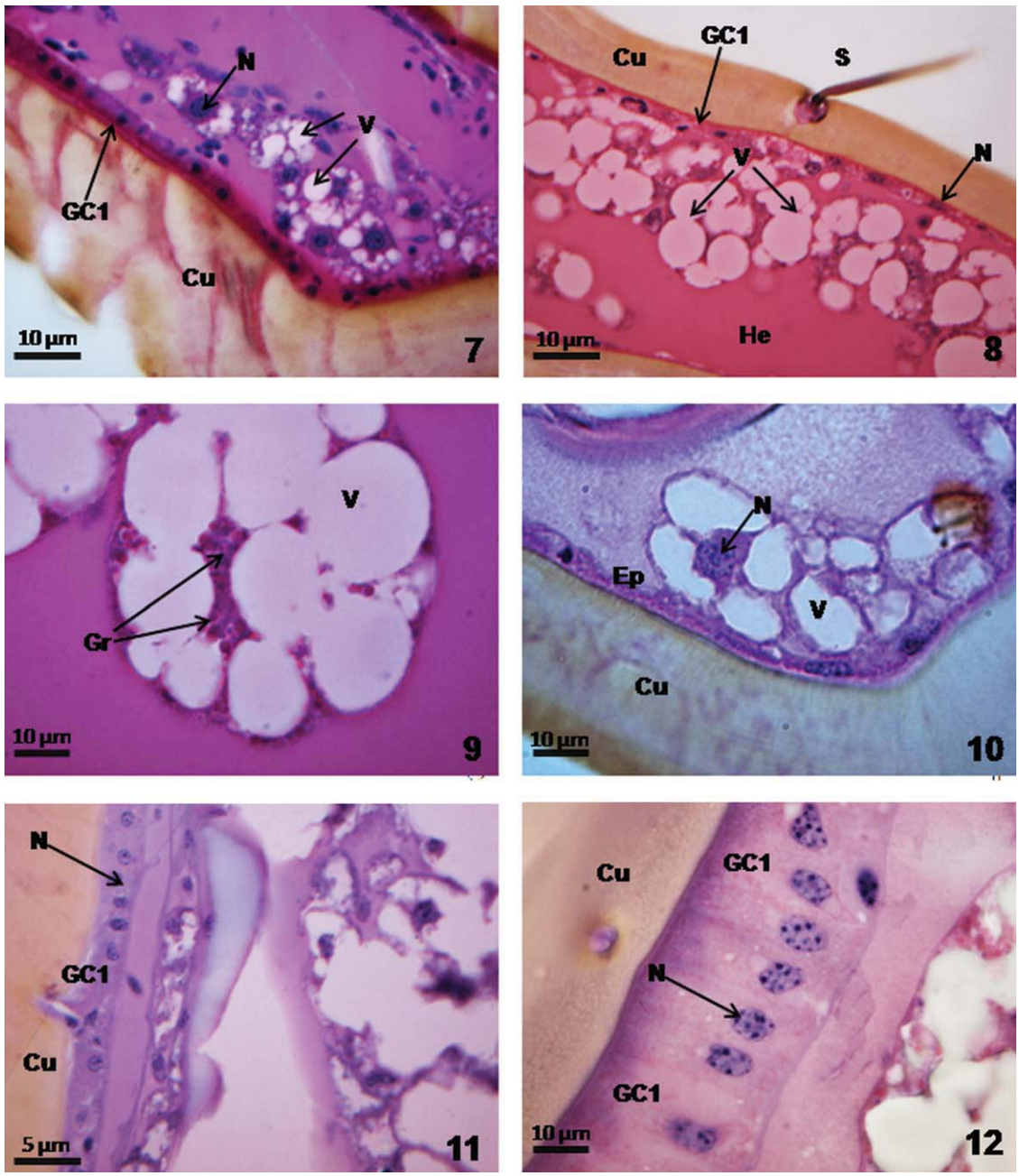
In *A. subterraneus molestans*, *A. subterraneus brunneus*, and the *Atta* species, the nucleus of the class III secretory cells was well developed and contained decondensed chromatin (Fig. 5). In *A. niger* and *Atta bisphaerica*, the class III secretory cells were highly vacuolated. In *A. niger* and *A. subterraneus molestans*, the cytoplasm of the class III secretory cells contained an accumulation of secretory granules, which were lacking in *A. subterraneus brunneus* and the *Atta* species.



Figs. 1–6. **Fig. 1:** Mandibles of ants. Schematic drawn showing the different cells found in the mandible of ants [(1) gland with reservoir; (2) gland class III with few vacuoles and developed nucleus; (3) gland class III without vacuole; (4) gland calss III with many vacuoles and developed nucleus; (5) gland class III vacuolated and with small nucleus; (6) gland class III with granules; (7) gland class III with few vacuoles and small nucleus; (8) gland class III without vacuole and developed nucleus; (9) epidermis with flattened cells; (10) other cell types; (11) gland class I with cuboidal cells; (12) hemocoel; (13) cuticle; and (14) gland class I with columnar cells. (draw without scale)]. **Fig. 2:** Histological section of the mandible of *Leptogenys* sp1 showing gland class 3 (GC3). **Fig. 3:** Scanning electron micrograph of superior surface of the mandible of *Leptogenys* sp1 showing pores (arrows). **Fig. 4:** Scanning electron micrograph of the inferior surface of the mandible of *Pachycondyla crassinoda*, without pores. **Fig. 5:** Histological section of the mandible of *Acromyrmex subterraneus molestans* showing flattened epidermis (Ep), glands with reservoir (R), and gland cells class III (GC3). **Fig. 6:** Histological section of the mandible of *Atta laevigata* showing glands with reservoir (R). Cu, cuticle; He, hemocoel; and N, nucleus. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Among the Ponerini, *Hypoponera* sp1 had an epidermis containing cuboidal cells characteristic of class I epidermal cells (Fig. 7) and glands of class III with highly vacuolated secretory cells (Fig. 7). The nuclei of class I and class III secretory cells were well developed, containing decondensed chromatin (Fig. 7) and secretory granules were widespread in the cytoplasm of the class III secretory cells.

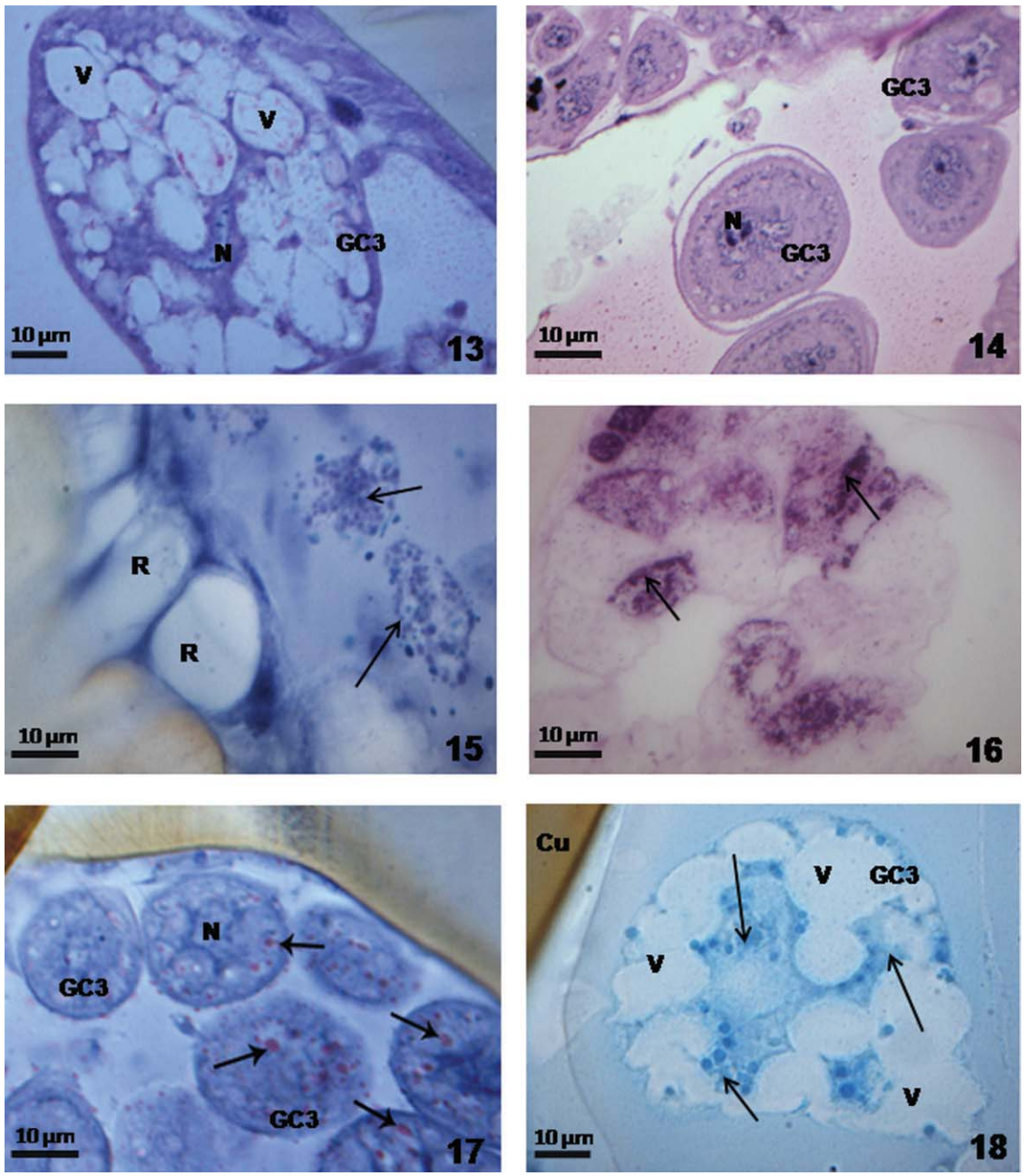
The two species of *Leptogenys* showed a flattened epidermis, and the class III secretory cells were vacuo-



Figs. 7–12. **Fig. 7:** Histological sections of the mandibles of ants. *Hypoponera* sp1 showing cuboidal cells of gland class I (GC1); gland class III with many vacuoles (V) and nucleus (N). **Fig. 8:** Leptogenys arcuata showing cuboidal cells of gland class I (GC1) and gland class III with vacuoles (V) and secretory granules (Gr). **Fig. 9:** Detailed view of gland class III of *Leptogenys arcuata* showing vacuoles (V) and secretory granules (Gr). **Fig. 10:** *Pachycondyla* sp1 showing flattened epidermis (Ep) and gland class III with vacuoles (V) and well developed nucleus (N). **Fig. 11:** *Pachycondyla veranae* showing cuboidal cells of gland class I (GC1) with well developed nucleus (N). **Fig. 12:** *Pachycondyla crassinoda* showing columnar cells of gland class I (GC1) with well developed nucleus (N). Cu, cuticle; He, hemocoel; and S, sensillum.

lated with large nuclei (Fig. 8). In *Leptogenys* species granules were found in the cytoplasm of class III secretory cells (Fig. 9) and other cell types, probably hemocytes and trophocytes, occurred in the interior of the mandible.

In the *Pachycondyla* species, including *P. stigma*, the epidermis is formed by flattened cells (Fig. 10). Class I glandular cells varied among species, being cuboidal in *P. veranae* and *P. villosa* (Fig. 11) and columnar in *P. crassinoda, P. harpax*, and *P. impressa* (Fig. 12). The class III secretory cells were vacuolated in *Pachycondyla* sp1, *P. crassinoda, P. harpax, P. veranae*, and *P. villosa* (Figs. 10 and 13), whereas in *P. impressa* and *P. stigma* vacuoles were absent (Fig. 14). The nuclei were smaller in the class III glandular cells of *P. crassinoda, P. harpax, P. stigma*, and *P. villosa* compared



Figs. 13–18. **Fig. 13:** Histological sections of the mandibles of ants. *Pachycondyla harpax* showing gland class III (GC3) with vacuoles (V) and small nucleus (N). **Fig. 14:** *Pachycondyla impressa* showing gland class III (GC3) with amoeboid nucleus (N). **Fig. 15:** *Atta laevigata* showing positive reaction for proteins (arrows) in gland class III. Bromophenol blue test. **Fig. 16:** *Acromyrmex subterraneus brunneus* showing positive regions for carbohydrates (arrows). PAS test. **Fig. 17:** *Pachycondyla crassinoda* showing positive reaction for lipids (arrows) in gland class III (GC3). Nile blue. **Fig. 18:** *Leptogenys* sp1 showing positive reaction for proteins (arrows) in gland class III (GC3). Bromophenol blue. Cu, cuticle; Ep, epidermis; G, gland; He, hemocoel; R, reservoir; and S, sensillum. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

with those of *P. impressa* and *P. veranae*. Granules were found in the cytoplasm of the class III secretory cells in *P. crassinoda, P. harpax*, and *P. stigma.* Other types of cell, probably hemocytes and trophocytes, were seen in *P. crassinoda, P. impressa, P. stigma*, and *P. veranae.* In all species of *Pachycondyla*, the nuclei of class I and III secretory cells showed decondensed chromatin (Figs. 10 and 12).

Glandular epithelial cells with a reservoir were not observed in any of the Ponerini species.

Histochemistry

The results of the three histochemical tests are summarized in Table 2.

In the Attini, the histochemical tests for proteins with mercury-bromophenol and for carboydtrates with

TABLE 2. Histochemical tests in the intramandibular glans of Attini and Ponerini ants

Tribe/Species	Gland class I			Gland class III		
	Lipid	Carbohydrate	Protein	Lipid	Carbohydrate	Protein
Attini						
Acromyrmex subterraneus brunneus	ga	ga	ga	+	+++	+++
Acromyrmex niger	ga	ga	ga	+	++	+
Acromyrmex subterraneus molestans	ga	ga	ga	++	+++	+
Atta bisphaerica	ga	ga	ga	++	+	+
Atta laevigata	ga	ga	ga	+	+++	++
Atta sexdens rubropilosa	ga	ga	ga	−	+	+
Ponerini						
Hypoponera sp1.	+	−	+	+	−	+
Leptogenys arcuata	ga	ga	ga	+	nd	+
Leptogenys sp1.	ga	ga	ga	+	nd	++
Odontomachus haematodus	−	−	ga	+	−	+
Pachycondyla sp1.	ga	ga	ga	+	−	nd
Pachycondyla crassinoda	++	−		+	−	nd
Pachycondyla harpax	−	−	+	−	−	+
Pachycondyla impressa	−	−	+++	−	−	+++
Pachycondyla stigma	ga	ga	−	+	+	−
Pachycondyla veranae	++	+	−	+	+	−
Pachycondyla villosa	++	−	+	++	−	++

−, negative; +, weakly positive reaction; ++, positive reaction; +++, strong positive reaction; ga, gland absent; and nd, not determined.

PAS were positive for class III secretory cells in all species (Figs. 15 and 16). The Nile blue test did not detected lipids, except in the class III glandular cells of *Atta sexdens rubropilosa*.

For the six species of Ponerini, the histochemical results with Nile blue, PAS, and mercury-bromophenol were similar for both class I and III secretory cells, showing the presence of lipid in *Hypoponera* sp1, *Pachycondyla crassinoda, P. veranae*, *and P. villosa* (Fig. 17) and carbohydrate in *P. veranae*.

In the species of Ponerini that had only class III glandular cells, the Nile blue test was positive in *Pachycondyla stigma, Leptogenys arcuata*, and *Odontomachus hematodus*, the PAS was positive in *P. stigma*, and the mercury-bromophenol test showed the presence of proteins in *Leptogenys* sp1, *L. arcuata* and *O. hematodus* (Fig. 18).

Phylogenetic Analysis

The distribution of the evaluated characters of the intramandibular glands between the studied species is shown in Table 3. The parsimony analysis resolved 75 trees and the consensus tree is shown in Figure 19.

According to the retrieved topology, polytomy was found in all the species tested, except for *Camponotus rufipes* which was used as the out-group. Despite the polytomy, there were three resolved clades. One contained the Attini, which is supported by the presence of intramandibular glands with a reservoir (character 5). Two others contained Ponerini species, one being the clade *Leptogenys, Pachycondyla crassinola, P. harpax* and *P. stigma,* supported by the presence of cytoplasmatic granules (character 7).

In the Attini clade, *Atta sexdens rubropilosa* was a sister group of *Acromyrmex subterraneus brunneus* and *Atta laevigata*. In Ponerini, two clades were distinguished, each containing new polytomies. The two studied species of *Leptogenys* were in different clades as were the *Pachycondyla* species. Within *Pachycondyla* there were two clades: one for *P. impressa* and *P. veranae,* and for *P. crassinola* and *P. harpax* and another for all species studied of *Hypoponera*, *Pachycondyla*, *Leptogenys* and *Odontomachus* in the other clade of Ponerini.

TABLE 3. Matrix of intramandibular gland characters

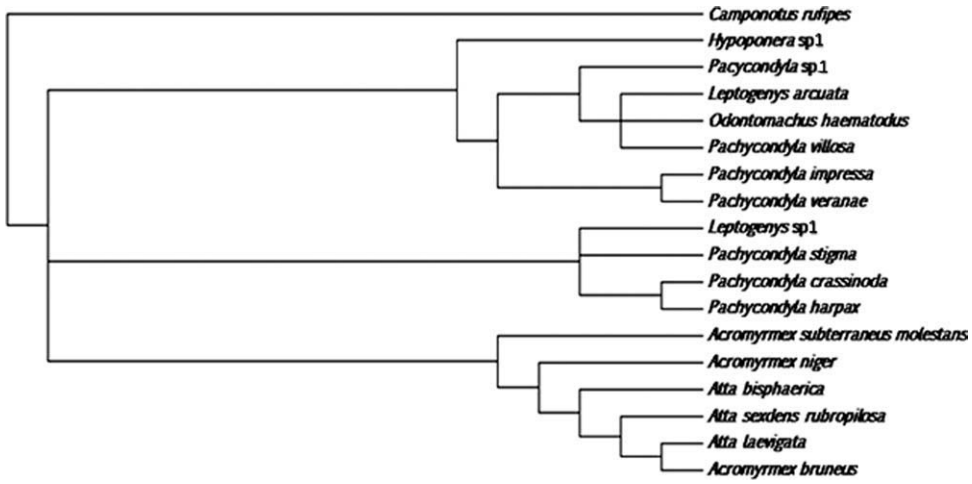
Species	Characters						
	1	2	3	4	5	6	7
Acromyrmex subterraneus brunneus	0	1	2	0	1	0	0
Acromyrmex niger	0	1	1	0	1	1	1
Acromyrmex subterraneus molestans	0	0	2	1	1	1	1
Atta bisphaerica	0	1	1	1	1	1	0
Atta laevigata	0	1	2	0	1	1	0
Atta sexdens rubropilosa	0	1	0	1	1	1	0
Hypoponera sp1.	1	0	1	1	0	1	1
Leptogenys arcuata	0	0	1	0	0	0	0
Leptogenys sp1.	0	0	1	1	0	1	1
Odontomachus haematodus	0	1	0	0	0	0	0
Pachycondyla sp1.	0	0	1	1	0	0	0
Pachycondyla crassinoda	2	0	1	0	0	1	1
Pachycondyla harpax	2	0	1	0	0	0	1
Pachycondyla impressa	2	0	0	1	0	1	0
Pachycondyla stigma	0	0	0	1	0	1	1
Pachycondyla veranae	1	0	1	1	0	1	0
Pachycondyla villosa	1	0	1	0	0	0	0
Camponotus rufipes	0	0	1	1	0	1	1

For explanation see material and methods section.

DISCUSSION

The results of this work show that the intramandibular glands contained classes I and III secretory cells according to the classification of Noirot and Quennedey (1974, 1991) and that their secretions are released through cuticular pores (intrinsic pores of the cuticle resulting from its deposition during molt) and conducting canals that open to pores on the body surface, respectively. Secretory epithelial cells with a reservoir, similar to the ones reported by Amaral and Caetano (2006) and Billen (2008) also occurred.

The occurrence of class I and III secretory cells in the same region of the body corroborates data reported for



Figs. 19. Phylogenetic relationships based on intramandibular gland characters. Notice three clades: one for all Attini and another two for Ponerini.

bees (Costa-Leonardo, 1978; Cruz-Landim and Abdalla, 2002; Romani et al., 2002, 2003; Smith et al., 1993; Wossler and Crewe, 1999; Wossler et al., 2000), wasps (Romani et al., 2005) and ants (Marques-Silva et al., 2006). In contrast, Schoeters and Billen (1994) have suggested that in 11 subfamilies of ants the intramandibular glands comprise only class III secretory cells. Billen and Espadaler (2002) reported the occurrence of class I intramandibular secretory cells in *Pyramica membranifera* (Myrmicinae: Dacetini) for the first time. In this study, we showed that this class of glandular cell is present in the Ponerini *Hypoponera* sp1., *Pachycondyla crassinoda, P. harpax, P. impressa, P. veranae*, and *P. villosa*. The class I gland may be related to secretions used in feeding. *Pyramica membranifera* and the species of Ponerini studied here are all predators (Bolton, 1998), while the fungus-feeding Attini species studied do not have class I glandular cells. Billen and Espadaler (2002) point out that the intramandibular secretory cells of class I are associated with the predatory habit in Dacetini, and also suggest that the class I glandular epithelial cells may be a source of alomones [chemical of one species that when in contact with a different species affect the behavior or physiology of the receiver to the benefit the originator but not the receiver (Dicke and Sabelis, 1992)].

Attini species had secretory epithelial cells with a reservoir, in addition to class III secretory cells, as found in *Atta sexdens rubropilosa* (Amaral and Caetano, 2006). The opening of a number of different secretory glandular cells into a common reservoir results in the release of a mixture of substances (Staddon, 1979). In contrast, when the different classes of glandular cells do not share a common reservoir their secretions may show differences in volatility and/or timing of release (Marques-Silva et al., 2006; Romani et al., 2005).

In Attini, the class III glandular cells had some vacuoles and cytoplasm containing stored proteins, lipids, and carbohydrates, suggesting high secretory activity of these cells. The presence of all these chemical substances reflects the complexity and size of the colonies in which Attini species live, and the degree to which the division of labor and colony architecture is regulated by chemical signaling (Jaffé, 1984).

The histochemistry shows similar results for gland cells of the class I and III in all studied Ponerini species (Table 2). In this way, no protein and carbohydrates occurs together in the same cell. However, protein and lipid were both abundant in these cells, suggesting that in Ponerini glands of class I and III release substances that are of a protein and/or lipoprotein nature.

Although these results show the occurrence of the different types of intramandibular glands in the different species of ants, the function of these glands remains open to interpretation. According to Quennedey (1998), the class III glandular cells produce attractive and repulsive pheromones. Amaral and Caetano (2006) and Quennedey (1998) have suggested that because class III secretory cells contain carbohydrates and protein, the secretions from them are related to the production of enzymes and glyco-conjugates. Schoeters and Billen (1994) pointed out that the class III glandular cells are directly associated with the release of all the secretions of the intramandibular gland. This is contrary to Amaral and Caetano (2006) who proposed that the class I secretory cells present in the mandibular epidermis are the main source of secretions in the intramandibular glands. Class I and III epidermal glands are commonly found in the abdomen of bees and ants, and are involved in the release of hydrocarbons, alcohol, and fatty acids used for communication by these insects (Grasso et al., 2004; Hora et al., 2010; Serrão et al., 2009; Smith et al., 1993; Wossler and Crewe, 1999; Wossler et al., 2000). In ants, cuticular hydrocarbons are used for recognizing conspecifics (Hölldobler and Wilson, 1990). Marques-Silva et al. (2006) therefore suggested that the class I and III antennal glands of *Dinoponera lucida* might play a role in the production of at least some of these cuticular hydrocarbons, but to date the function of the intramandibular glands remains unknown. Hora et al. (2010) showed that class I glands are responsible by a fertility signal in queens of *Ectatomma tuberculatum.* Grasso et al. (2004) take their universal occurrence in ants to

confirm the importance of glandular secretions to the biology of ants in general.

Because all the Attini and Ponerini studied here had class III glandular cells, we suggest that this gland is probably associated with biological functions (feeding, grooming, and hydration) and/or behavioral traits (communication) common to all the Formicidae. There are two distinct types of trail marking behavior in the two tribes studied: exploratory trails and recruitment trails (Wilson, 1963). The first is found in army ants Ponerini and Dorylini, and released scents are deposited almost continuously by scout workers. In recruitment trails, found in Myrmicinae, Dolichoderinae, and Formicinae, secretions are deposited only by workers returning to the nest after finding a food source (Wilson, 1971). The released scents may have different concentrations and chemical compositions.

Attini and Ponerini are considered derived and basal ants, respectively (Kusnezov, 1955; Peeters, 1991; Taylor, 1978; Wheeler, 1910; Wilson and Hölldobler, 2005), although this is a misinterpretation of cladistic theory, because the terms "derived" and "basal" have not support in the current systematic (Krell and Cranston, 2004). In this way, is more reliable affirm that Ponerini is a sister-group of Attini. Some studies have used specific structures to suggest new hypotheses in evolution and phylogeny (Gotwald, 1969; Hashimoto, 1991a,b, 1996; Hermann, 1969; Perrault, 1999). The diversity in intramandibular gland distribution and morphology shown here suggests that structural differences in the intramandibular glands can contribute to future phylogenetic studies of Formicidae. Despite the occurrence of polytomies in the phylogenetic analyses, indicating the nonresolution of the groups in Attini and Ponerini, both tribes were supported by the characters of the intramandibular glands. Further studies are necessary to resolve the in-groups within both tribes, using a greater number of intramandibular morphological characters, with standardization of physiological state, age, and feeding habits, such as in the studies of Bethylidae wasps (Goulbault et al., 2008).

The morphology, ultrastructure, and physiology of intramandibular glands have previously been studied in several species of Hymenoptera (Amaral and Caetano, 2006; Billen and Espadaler, 2002; Costa-Leonardo, 1978; Cruz-Landim and Abdalla, 2002; Grasso et al., 2004; Nedel, 1960; Ribeiro and Caetano, 2000; Schoeters and Billen, 1994; Toledo, 1967), but none of these studies compared the intramandibular structures in distinct subfamilies using cladistic tools. Our results from the Attini species suggest the presence of two types of secretory intramandibular cells (with a reservoir and class III), and the positive results of the three histochemical tests probably reflects specialization among the various castes. These varying histochemical characteristics could contribute to the division of labor within a colony, mass recruitment and group foraging (Wilson, 1971), producing the substances necessary for the release of key chemical signals for communication.

The occurrence of the class III gland in Ponerini and Attini and the findings from other ant species (Schoeters and Billen, 1994) suggests that this gland is widely distributed among ants. The uniformity of the gland's histology and the lack of evidence of changes in its form suggest a similar function of this gland in all the ants in which it occurs. Any differences in the function of exocrine glands in different ants would be explained by class III glands working together with class I glands, as in Ponerini, and together with secretory glands with a reservoir, as in Attini.

In conclusion, the morphological and histochemical features of the different intramandibular glands suggest that the Attini and Ponerini intramandibular glands produce substances of a distinct nature and that the occurrence of such glands vary according to tribe, and can be used as phylogenetic characters.

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