

Sensilla and Secretory Glands in the Antennae of a Primitive Ant: *Dinoponera lucida* (Formicidae: Ponerinae)

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ABSTRACT Morphology of the antennae of the female workers of the ponerine ant *Dinoponera lucida* was examined by light and scanning electron microscopy. In several antennomers, we found secretory gland cells of class I and III. Class III gland cells release their secretion through single pores in the antennal surface, whereas class I secretory cells are seen as tall epidermal cells close to the cuticle. Both gland types have weak reaction for total proteins and neutral polysaccharides. Six distinct sensilla types were observed: trichodea, chaetica, campaniform, basiconica, placodea, and coeloconica. The possible sensory functions of these sensilla and the gland functions are discussed. *Microsc. Res. Tech.* 69:885–890, 2006. © 2006 Wiley-Liss, Inc.

INTRODUCTION

The social behavior of Hymenoptera depends on communication between individuals (Holldöbler and Wilson, 1990; Michener, 1974) and models for ants colonial organization emphasize the importance of interactions between colony members (Cassill and Tschinkel, 1999; Franks and Deneubourg, 1997).

Antennation, the touch of a surface with antennae, is widely distributed during interactions in Hymenoptera (Romani et al., 2005). This occurs because the antennae have receptors for volatile odors, pheromones, water vapor, and carbon dioxide, besides contact chemoreceptors (Kleineidam et al., 2000; McIver, 1975; Mitchell et al., 1999; Ozaki et al., 2005; Stange and Stowe, 1999). Thus, antennation is usually interpreted as stimuli of sensory exploration. However, antennal contacts between individuals of different sexes and fanning in parasitoid and social Hymenoptera have been associated with secretion and dispersion of species-specific substances (Billen, 2000; Bin and Vinson, 1986; Bin et al., 1999; Isidoro et al., 1996; Romani et al., 2002, 2003, 2005; Strohm and Linsenmair, 1995).

Dinoponera lucida ants are about 3 cm long, with predatory habits, without caste polymorphism and restricted to the Atlantic rain forest in the states of Espírito Santo and Bahia, Brazil (Campiolo et al., 2003). This restricted distribution of *D. lucida* and deforestation make this species a threatened one.

The antennae of Ponerinae ants were described in 14 species, but the genus *Dinoponera* was not included in the survey (Hashimoto, 1990).

The aim of this study was to expand the knowledge of the morphology of *D. lucida* antennae, with the first detailed analysis of this appendage for this ant.

MATERIALS AND METHODS

Dinoponera lucida female ants were collected from Ilhéus, state of Bahia, Brazil, and transferred to Zamboni's fixative solution (Stefanini et al., 1967). Antennae

were removed, dehydrated in a graded ethanol series, and embedded in historesin JB4. Sections (5 µm thick) were stained with hematoxylin and eosin. Some of these sections were submitted to mercury–bromophenol and PAS histochemical tests (Pearse, 1980) to detect proteins and neutral carbohydrates, respectively.

Another set of antennae was removed and dehydrated in a graded ethanol series, transferred to hexamethyldizilane (HMDS) for 10 min and air dried. These antennae were then coated with a 30-nm-thick gold layer and analyzed with a scanning electron microscope (LEO VP1430). For viewing internal structures, antennomers were cut in half longitudinally with a razor blade before dehydration, while for viewing internal cuticular structures cut, antennomers were treated with 10% potassium hydroxide for 30 min, and then rinsed with water before being dehydrated, air dried, and gold covered (Bin et al., 1999).

RESULTS

The antennae of *D. lucida* ants have 12 antennomers (Fig. 1) and the histological sections (Figs. 2–11) shows two types of secretory glands in all antennomers, except in the first one that corresponds to the scapus.

Type I secretory gland cells are tall epidermal cells forming a monolayer close to the antennal cuticle (Figs. 2 and 12) contrasting with flattened cells of the ordinary epithelium. The cell surface of the gland cells in contact with the cuticle has many infoldings (Figs. 5 and 6) and a thin subcuticular space (Fig. 2). They have a central nucleus with uncondensed chromatin, an evi-

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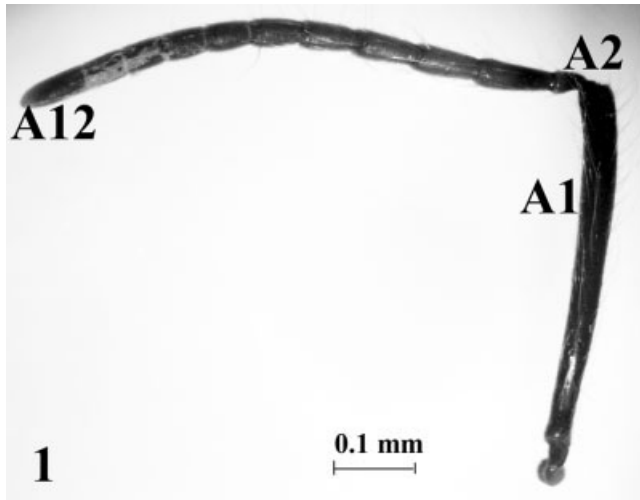


Fig. 1. The right antenna of *Dinoponera lucida* female showing the antennomer 1 or scapus (A1), antennomer 2 or pedicel (A2) and the antennal flagellum totaling 12 antennomers (A12).

dent nucleolus (Figs. 5 and 6), and cytoplasm with unstained granules, which negatively reacted with mercury bromophenol and PAS (Figs. 6–8). However, the basal part of the cells showed positive PAS reaction (Figs. 9–11).

Type III secretory gland cells are isolated cells located between the antennal nerve and the body wall (Figs. 2, 5, and 11). These cells are globular with developed nucleus and several nucleoli (Figs. 2–4, and 12). They open to the exterior through a conducting canal formed by a cell with flattened nucleus (Figs. 4 and 12) that originates from an infolding of the gland cell. The canals appear as intracellular canaliculi in the histological sections (Figs. 3 and 10). The conducting canal opens in the antennal cuticle by isolated pores (Figs. 13 and 18). The histochemical tests were weakly positive in these gland cells (Figs. 7 and 11) showing glycogen in the conducting canals (Fig. 10).

Scanning electron micrographs of antennae of *D. lucida* female ant are shown in Figures 14–19. The antennae of *D. lucida* have sensilla of the trichodea, basiconica, campaniform, placodea, and coeloconica types (Fig. 14).

Trichodea sensilla are either long (140–160 μm in length), located in the scapus (antennomer I) and in the antennomers apex, except in the last antennomer (Fig. 15) or short (20–30 μm in length) with enlarged base and distributed in all antennomers (Fig. 15).

Sensilla basiconica (Fig. 16) are found in the middle–apical portion of antennomers. Sensilla campaniform

(Fig. 17) appear in low numbers (2–3) in the antennomers apex. All antennomers have about 7–9 sensilla coeloconica (Fig. 18) and groups of 3–6 sensilla placodea (Fig. 19).

DISCUSSION

The secretory gland cells at the antennae of *D. lucida* ants are class I when close to the body cuticle and else they belong to class III, with conducting canals that open in pores in the cuticle (Noirot and Quennedey, 1974, 1991). The occurrence of gland cells of these two classes in a same region of the body was also reported for *Apis mellifera* and Meliponini (Cruz-Landim and Abdalla, 2002), wasps (Romani et al., 2005), and *Bombus panscorum* (Romani et al., 2002, 2003). The gland status of the epidermal cells close to the antennal cuticle is supported by their thickness associated with the infoldings in the cell surface near to cuticle and the subcuticular space (Cruz-Landim and Abdalla, 2002). These gland cells release their secretion to the subcuticular space following diffusion across the cuticle (Cruz-Landim, 1993; Noirot and Quennedey, 1991). On the other hand, class III glands always open in pores onto the body surface (Cruz-Landim and Abdalla, 2002; Guerino and Cruz-Landim, 2003; Noirot and Quennedey, 1974, 1991).

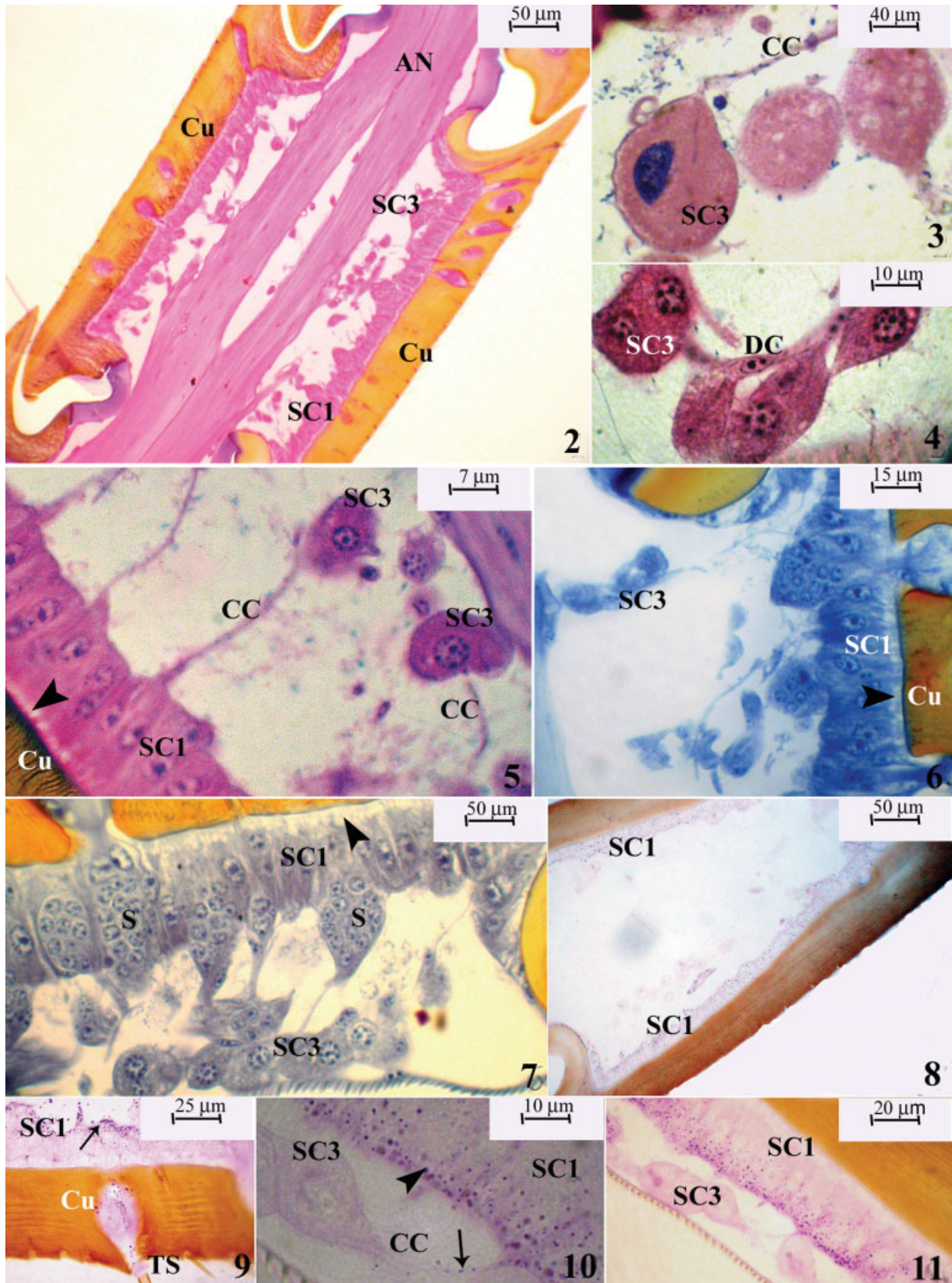
The histochemical tests showed that class I and class III glands have secretions without protein and carbohydrate because mercury–bromophenol blue and PAS tests presented a weakly positive, while cytoplasm granules were never positive. In bees, similar glands found in the abdomen release hydrocarbons, alcohols and fatty acids used in chemical communication in these insects (Smith et al., 1993; Wossler and Crewe, 1999; Wossler et al., 2000). In ants, cuticular hydrocarbons are used for nestmate and non-nestmate discrimination (Holldöbler and Wilson, 1990). So we suggest that both antennal glands of *D. lucida* may play a role in the production, at least, some of these cuticular hydrocarbons.

Opening of secretory gland cells of different classes in a common reservoir may result in release of different substances in the secretion (Staddon, 1979), but when different classes of gland cells do not have a common reservoir and release secretions at the same locale, their secretions may have differences in the volatility and/or release time (Romani et al., 2005). The gland cells of classes I and III of *D. lucida* lack reservoirs or accumulation of secretory granules. This suggests a constitutive secretory mechanism, which corroborates the hypothesis of differences on volatility of their secretions other than on release in different times.

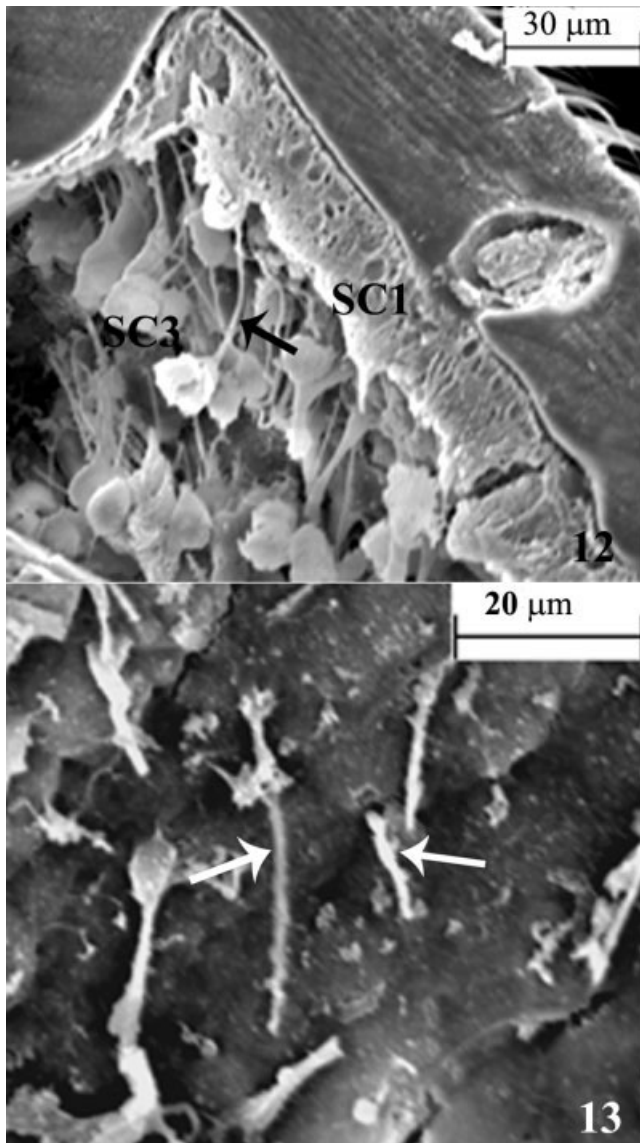
Antennal glands of male insects play a role in the courtship behavior during reproduction (Feliccioli et al.,

Figs. 2–11. Histological sections of antennae of *Dinoponera lucida* female ants. **Fig. 2:** Longitudinal section at the 9th antennomer showing class I gland cells (SC1) close to the cuticle (Cu), as well as class III gland cells (SC3). AN, antennal nerve. **Fig. 3:** Gland cell of class III and the conducting canal (CC) at the 7th antennomer. **Fig. 4:** Gland cells of class III and duct cell (direct current (DC)) that forms the conducting canal at the 4th antennomer. **Fig. 5:** Two class III gland cells with their conducting canals crossing the epithelium of class I cells (SC1) at the 7th antennomer. Arrowhead, subcuticular space; Cu, antennal cuticle. **Fig. 6:** Gland cells of classes I (SC1) and III (SC3) at the 5th antennomer. Note the infoldings (arrowhead) in

the cell surface. Cu, antennal cuticle (mercury–bromophenol). **Fig. 7:** Gland cells of classes I and III and neurons somata (S) showing the subcuticular space (arrowhead) at the 6th antennomer (mercury–bromophenol). **Fig. 8:** Longitudinal section of the 8th antennomer showing PAS positive class I gland cells. **Fig. 9:** Detail of positive PAS granules (arrow) in the class I glands at the 5th antennomer. Cu, antennal cuticle; Tr, sensillum trichodea. **Fig. 10:** Detail of PAS positive granules (arrow) in the conducting canal of a class III gland and in the class I gland (arrowhead) at the 4th antennomer. **Fig. 11:** Gland cells of classes I (SC1) and III (SC3) showing positive PAS reactions at the 10th antennomer. Note weak reaction in class III glands.



Figs. 2-11



Figs. 12–13. Scanning electron micrographs of antennae of *Dinoponera lucida* female ant. **Fig. 12:** Internal view of 4th antennomer with glands of class I (SC1) and of class III (SC3) with its conducting canal (arrow). **Fig. 13:** Internal view of 6th antennomer showing the conducting canals (arrows) entering in the antennal cuticle (antennae treated with potassium hydroxide).

1998; Romani et al., 2002, 2003, 2005). Secretions of these glands in *Philanthus triangulum* (Sphecidae) females are used to mark the brood chambers (Strohm and Linsenmair, 1995). The antennal glands of females seem to be common in ants such as reported for *Eciton burchelli* (Billen, 2000) and *Solenopsis invicta* (Isidoro et al., 2000), but their functions are unknown. The specimens of *D. lucida* ants here analyzed were female workers, without developed ovaries. However, the hypothesis that the antennal glands play a role in the reproductive behavior cannot be discarded, because this species and many Ponerinae lack a morphologically specialized queen caste (Peters, 1997). Workers of these ants may mate to reproduce and, for this reason, they are named gamergates. Morphologically, they

belong the worker caste, but they present morphological differences from queens of ant species with caste polymorphism (Peters and Crewe, 1984). Other worker can mate and occupy the position of the old gamergate when this is removed from the colony (Monnin and Peters, 1999). All females of a colony are potentially gamergates and, therefore, with antennal glandular apparatus. Furthermore, there is no morphological distinction between castes, which make all female members of a colony apt to assume reproductive behavior.

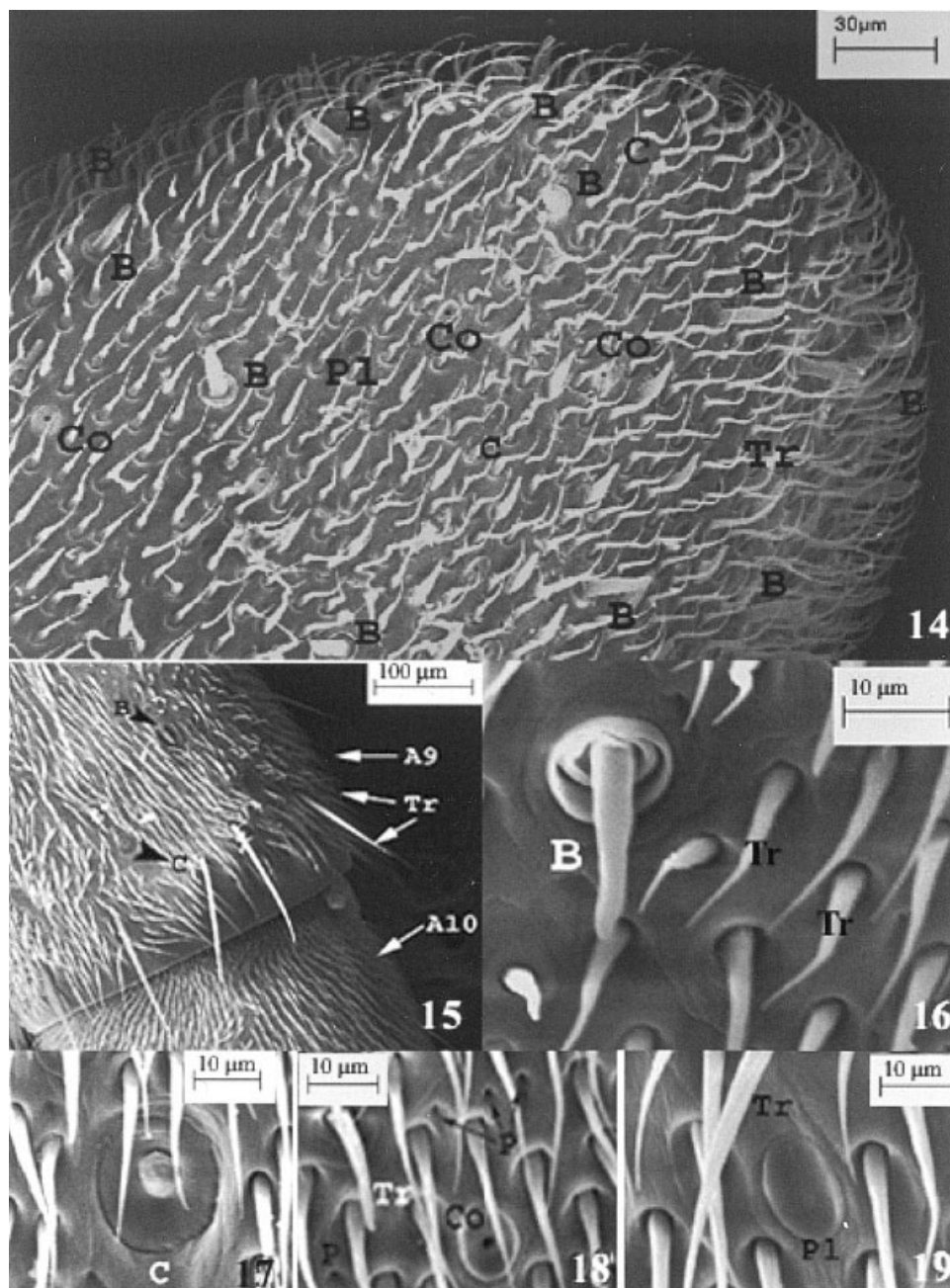
The five types of sensilla in the antennae of *D. lucida* ants were also found in other Hymenoptera (Stort and Moraes-Alves, 1998, 1999). However, these sensilla have variations, and the placodea type was not found on *S. invicta* (Renthal et al., 2003) and on males of the wasps *Polistes dominulus* and *Vespa cabro* (Romani et al., 2005). Furthermore, males and females of the parasitoid *Plutella xylostella* (Roux et al., 2005) have no sensilla basiconica.

Function of the different sensilla types can be deduced from the quantity of their pores. Unfortunately, we were not able to discriminate pores in sensilla of *D. lucida* ants by scanning electron microscopy and we did not obtain indirect evidence of pores by silver staining procedures. This is due to the fact that antennae of this ant are great and strongly sclerotized and, for this reason, the cleaning procedures (Shanbhag et al., 1999; Vilela et al., 2003), fluorescence microscopy (Renthal et al., 2003), and the crystal violet technique (Dey, 1999) failed. However, it is possible to speculate about the functions of the sensilla based on their morphological patterns.

Hymenoptera parasitoids (Roux et al., 2005) and bees (Stort and Moraes-Alves, 1998, 1999) have sensilla placodea with chemoreceptor function. Sensilla basiconica have been reported as contact and volatile chemoreceptors (Ozaki et al., 2005; Renthal et al., 2003), although they may be CO₂-sensitive (Shanbhag et al., 1999). Sensilla coeloconica are considered as hygrothermoreceptors (Roux et al., 2005) or contact chemoreceptors (Fresnau, 1979).

Sensilla campaniform, found near the antennae articulations, can perceive cuticular stresses during mechanical deformations (McIver, 1975), but the function of those present in the antennae and maxillary palps is unknown (Merivee et al., 2002; Spiegel et al., 2005). The presence of groups of sensilla campaniform in the antennomer apex of *D. lucida* antennae suggests that they have mechanoreceptor function to perceive cuticular stresses.

Nonporous sensilla trichodea are mechanoreceptors (Fernandes and Linardi, 2002; Merivee et al., 2002; Renthal et al., 2003; Roux et al., 2005; Spiegel et al., 2005), while those with pores are contact and volatile chemoreceptors (Chapman, 1998). We did not find pores in the sensilla of *D. lucida*, but the sensilla trichodea with enlarged bases (the shorter ones) are usually volatile chemoreceptors (Shanbhag et al., 1999). The long sensilla trichodea have well-developed sockets and are similar to sensilla chaetica of *Drosophila melanogaster* (Shanbhag et al., 1999) and *Antheraea assama* (Lepidoptera: Saturniidae), which present typical innervation of mechanoreceptor sensilla (Singh and Nayak, 1985). The predominance of these long sensilla in the scapus (antennomer I) and in the apex of



Figs. 14–19. Scanning electron micrographs of antennae of *Dinoponera lucida* female ant. **Fig. 14:** Antennomer 12 showing different types of sensilla. B, sensilla basiconica; C, sensilla campaniform; Co, sensilla coeloconica; Pl, sensilla placodea; Tr, sensilla trichodea. **Fig. 15:** Antennomers 9 (A9) and 10 (A10) showing long and short sensilla trichodea (Tr), sensilla basiconica (B), and sensilla campaniform (C). **Fig. 16:** Detailed view of sensilla basiconica (B) and chemoreceptor sensilla trichodea (Tr) on the 7th antennomer. **Fig. 17:** Detailed view of sensilla campaniform (C) on the 9th antennomer. **Fig. 18:** Antennomer 8 showing sensilla coeloconica (CO), exocrine gland pores (P), and sensilla trichodea (Tr). **Fig. 19:** Detailed view of a sensilla placodea (Pl) on 8th antennomer; Tr, sensilla trichodea.

other antennomers, except in the last, suggests that they have a mechanoreceptor function in *D. lucida*.

Our results show five sensilla and two exocrine glands types on the antennae of *D. lucida* females.

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