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Original article

Comparative morphology of the head glands in species of Protepeolini and Emphorini (Hymenoptera: Apidae)

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Abstract – The tribe Protepeolini is formed by parasitic bees with *Leiopodus* being the only genus of the tribe. Protepeolini bees parasitize the nests of members of the tribe Emphorini. Secretions of the head glands are important to the biology of bees as they may act as chemical signals in parasitism strategies. In this paper, we describe the morphology and histoarchitecture of head glands in three Protepeolini species and compare them with those of their Emphorini hosts. Mandibular and hypopharingeal glands were identified , but no head salivary glands were observed. Only parasitic species displayed sexual dimorphism in the morphology of mandibular glands, with males showing well-developed secretory portions and a characteristic organization of the secretory tissue which was previously unknown in bees. The possible role of mandibular head glands is discussed in relationship to behavioral parameters of *Leiopodus*.

Protepeolini / Emphorini / head glands / immunocytochemistry

1. INTRODUCTION

The tribe Protepeolini comprises the single genus *Leiopodus* (Smith); of the five recognized species, one of them ranges from southwestern United States of America to Guatemala, while the remaining four species are exclusively South American. All the species of Protepeolini tribe are kleptoparasites of members of the tribe Emphorini (Roig-Alsina and Rozen 1994).

The role of the exocrine glandular system is fundamental to the biology of Hymenoptera. In wasps, ants and bees, several glands are present in the antenna, head, thorax, legs and abdomen. Within the members of Apoidea, cephalic salivary, hypopharyngeal and mandibular glands display a diversity of forms (Heselhaus 1922; Nedel 1960; Cruz Landim 1967, 2008). The morphology and function of head glands have been widely studied in social bees (Jarau et al. 2004; Deseyn and Billen 2005; Šobotnik et al. 2008; Reichle et al. 2011). The chemical composition of the mandibular gland secretions of *Melipona quadrifasciata* workers has been determined by a combination of gas cromatography and mass spectrometry. Diverse hydrocarbon molecules were identified in the secretions of newly emerged workers, nurse bees and foragers of this species (Cruz Landim et al. 2012).

In non-parasitic solitary bees, cephalic secretions of females appear to be involved in the attraction of conspecific males, predator deterrence and nest hygiene (Cane and Tengö 1981; Cane et al. 1983; Cane 1986), while in males of certain species, the main function of the glandular

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secretion is probably the marking of territory for sexual competition (Hefetz et al. 1982; Vinson et al. 1982). The nature of the chemical communication by means of the secretions of head glands and the corresponding behavioral responses has been reported in the relationship between solitary bees and the corresponding cleptoparasitic counterparts (Duffield et al. 1990; Hefetz et al. 1982). The existence of a close chemical relationship of bees belonging to the genus *Andrena* with their parasites of the genus *Nomada* suggested that a long-standing coexistence has been established between them and that species of *Nomada* have developed a signaling system to mimic the odors of their hosts (Tengö and Bergstrom 1977).

Morphological studies in the slave-making ant *Polyergus rufescens* have shown specific modifications of the mandible and their associated glands compared to those present in closely-related non-parasitic species. Such a phenomenon suggests morphological and functional adaptations related to the parasitic habits of *P. rufences* (Grasso et al. 2004). However, little information is available regarding this subject in parasitic bees (Cruz Landim 1967, 2008).

Studies on the anatomy of the cephalic exocrine glands as well as characterization of the secretions by means of immunocytochemistry are necessary to understand the role of these organs in behavioral relationships. Here, we describe the histoarchitecture of the head glands in three species of the genus *Leiopodus* by means of light microscopy, scanning electron microscopy and immunocytochemistry, and compare their structures with those of the corresponding hosts of the tribe Emphorini.

2. MATERIALS AND METHODS

2.1. Insects

In order to describe the anatomy of the cephalic glands, we have used insects of the tribe Protepeolini (*Leiopodus lacertinus*, *L. trocantericus*, *L. abnormis*) as well as specimens of the genera *Melitoma* (Lepeletier and Serville) *Ptilothrix* (Smith), and *Diadasina* (Moure) corresponding to the tribe Emphorini, which were captured in the field of the provinces of Buenos Aires and Formosa (Argentina). For the description of the histology and cell morphology of the glandular tissue, we have chosen males and females of *L. lacertinus* and *Melitoma segmentaria*. These bees were captured in the reserve Costanera Sur, Ciudad Autónoma of Buenos Aires.

2.2. Morphology

The head capsule of each bee was opened using fine scissors and the tissues were flushed with cold fixative [4 % paraformaldehyde and 0.4 % picric acid in 0.16 M phosphate buffer (PB), pH 6.9; Settembrini et al. 2008]. The tissues were kept overnight in the same fixative at 4 °C. After that, the samples were rinsed in 0.01 MPB saline (0.13 M NaCl in PB, pH 7.4, PBS) and stained with basic fuchsine, hematoxylin and eosin stain or aniline blue.

The glands were examined under a Zeiss stereomicroscope and the diameters of the acini were measured with an ocular micrometer placed in the eyepiece of the stereomicroscope. In order to standardize the measurements of the glands, each one was divided by the intertegular distance. Statistical analyses were performed according to Niculita et al. (2008).

2.3. Histology

For light-microscopy observations, heads were fixed as stated in 2.2. The glands were dissected out and remained in the fixative overnight at 4 °C. After several washes in PBS, the tissues were dehydrated in graded ethanol series from 50 to 100 %, cleared in xylene, and embedded in paraffin. Serial 5-μm sections were placed onto gelatine-coated slides. After that, the sections were rehydrated and stained with hematoxylin and eosin, dehydrated, and further mounted in Permount (Sigma-Aldrich, MO, USA). Photographs were taken with a Nikon E800 microscope equipped with a Nikon digital sight DS-5Mc camera. Images were modified only to enhance contrast (Adobe Photoshop; Adobe Systems).

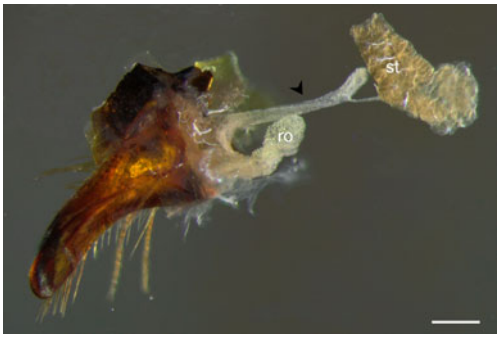

Figure 1. Mandibular gland of *Melitoma segmentaria* female. Dissection of the secretory tissue (*st*) was done to expose the reservoir duct (*black arrowhead*). Reservoir organ (*ro*). *Scale bars* 250 μm.

2.4. Immunohistochemistry of whole mounts

Fixed glands (n=10) were rinsed in PBS and transferred to PBS containing 1 % Triton X100 (PBST) for at least 48 h. After that, they were immersed in PBS containing 5 % normal goat serum for 1 h in order to diminish non-specific binding. The same solution was also used to dilute the primary and secondary antisera. After this, the glands were incubated overnight in a wet chamber with mouse monoclonal anti β-tubulin antibodies (Sigma-Aldrich) diluted 1:1,000. After 3 washes with PBS, preparations were incubated overnight with rabbit anti-mouse IgG coupled to FITC (Molecular Probes, USA) diluted 1:100 in 5 % NGS. Nuclei were counterstained with DAPI (4′, 6-diamidino-2′-phenylindole dihydrochloride; Molecular Probes), at a concentration of 1 mg/mL at RT for 1 h. Glands were mounted with 10 % 1, 4-diazobicyclo-(2,2,2-octane (DABCO™) in glycerol and observed with an Olympus FV300 laser-scanning confocal microscope mounted on an Olympus BX61 microscope and equipped with argon (488 nm, 10 mW), green HeNe (543 nm, 1 mW) and red HeNe (633 nm, 10 mW) lasers and the appropriate filters. Stacks of digitized images were merged and processed using Fluoview 3.2 software (Olympus, Tokyo, Japan) as image acquisition software. If required, images were modified only to enhance contrast (Adobe Photoshop; Adobe Systems)

2.5. Scanning electronic microscopy (SEM)

Fixed glands were rinsed several times with PBS. After this, they were dehydrated in graded ethanol series of 20, 40, 60, and 90 % (v/v) to absolute

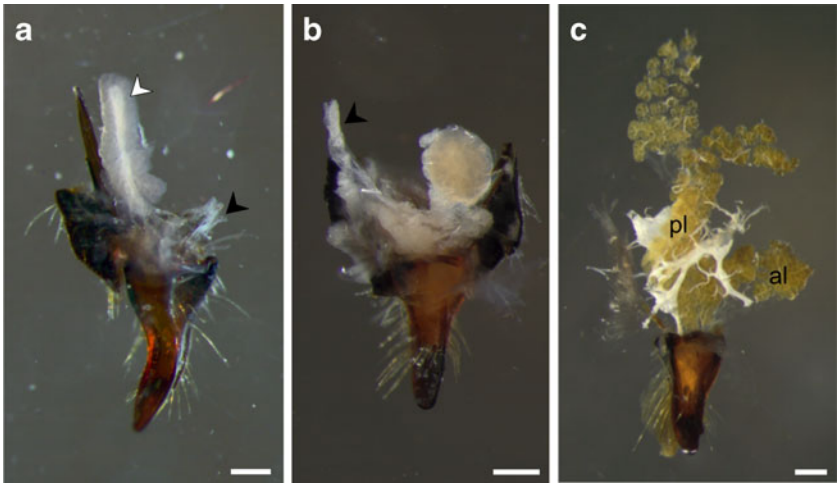

Figure 2. Mandibular glands of *Melitoma segmentaria* male (**a**) and *Leiopodus lacertinus* female (**b**) and male (**c**). *Black arrowheads* point to the reservoir organs and *white arrowheads* mark the reservoir duct. Anterior lobe (*al*), posterior lobe (*pl*). *Scale bars* 250 μm.

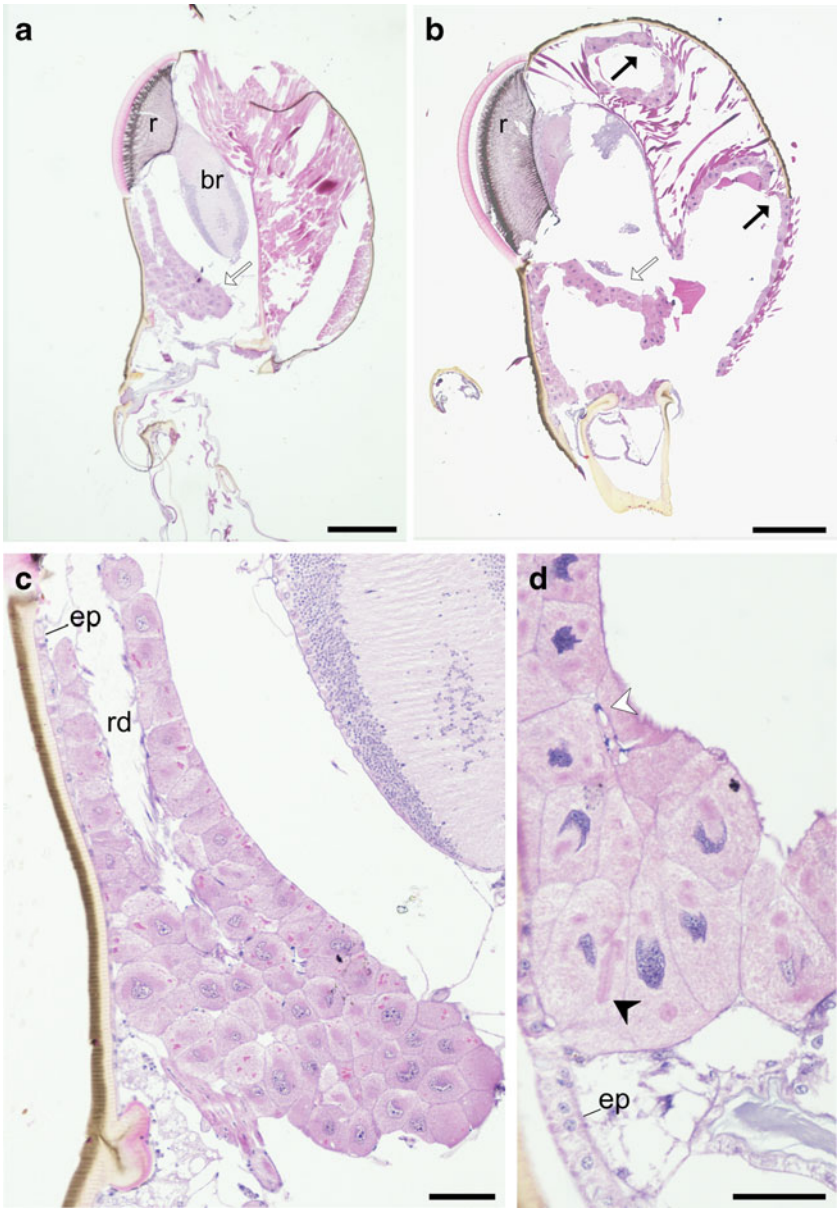

Figure 3. Bright-field micrographs of parasagital sections of male specimens of *Melitoma segmentaria* (**a**, **c**) and *Leiopodus lacertinus* (**b**, **d**). **a**, **b** *White arrows* show the position of the anterior lobes of the mandibular glands, *black arrows* mark the posterior lobes present only in *L. lacertinus.* **c**, **d** Higher magnifications of (**a**, **b**) showing the organization of glandular cells and the origin of reservoir duct (*rd*). *Black arrowhead* marks the end apparatus in a longitudinal section, *white arrowhead* marks a duct cell surrounded by glandular cells. Note the presence of eosinophilic material in many secretory cells. Brain (*br*), epithelial cell (*ep*), retina (*r*). *Scale bars* (**a**, **b**) 200 μm, (**c**) 40 μm, (**d**) 20 μm.

ethanol for 1 h each and either critical-point or air-dried (Settembrini 1984). Glands were further coated with gold palladium and examined in a Philips XL30 SEM microscope.

3. RESULTS

3.1. Salivary glands

Cephalic salivary glands were not found in any of the 30 samples from the 6 species. On the other hand, thoracic salivary were identified in all these species.

3.2. Mandibular glands

3.2.1. General morphology

Two main structures were observed in the glands: one of them is the yellow or whitish secretory tissue and the other one is a transparent flattened structure which corresponds to reservoir tissue (Fig. 1, 2a–c). In the mandibular gland, the reservoir tissue is attached to mandible which provides support to the secretory cells and is responsible for the collection of the secretion. The reservoir duct corresponds to the part of the reservoir tissue surrounded by the secretory tissue (Figs. 1, 2a).

In bees, two classes of mandibular glands are present: unilobar (type 1) or bifid (type 2). In both types of glands, one lobe is always present frontolaterally at the level of the optic lobes (Fig. 3a, b). In type 2 glands, a second lobe is located posteriorly to the brain near the base of the mandible. Depending on the species, the posterior lobe of the gland contains secretory tissue or it is devoid of secretory units. In the latter case, the posterior lobe is termed reservoir organ (Fig. 1). In all the bee samples analyzed here, with the exception of *Leiopodus* males, bifid glands having reservoir organs were observed (Figs. 1, 2a, b). In *Leiopodus* males, the reservoir organ was absent. However, a second large glandular lobe was found behind the brain, close to the genal area (Figs. 2c, 3b).

In Emphorini males and females, the secretory tissue is formed by pyramidal masses, with their apices oriented opposite to the base of the mandible (Figs. 2a, 3a, c). Sexual dimorphism was observed in the glands of the Protepeolini, with females showing an oval and compact secretory tissue (Fig. 2c). Instead, in males, the secretory tissue adopted the morphology of a raceme formed by glandular units (Fig, 2c). In all species analyzed here, with the exception of

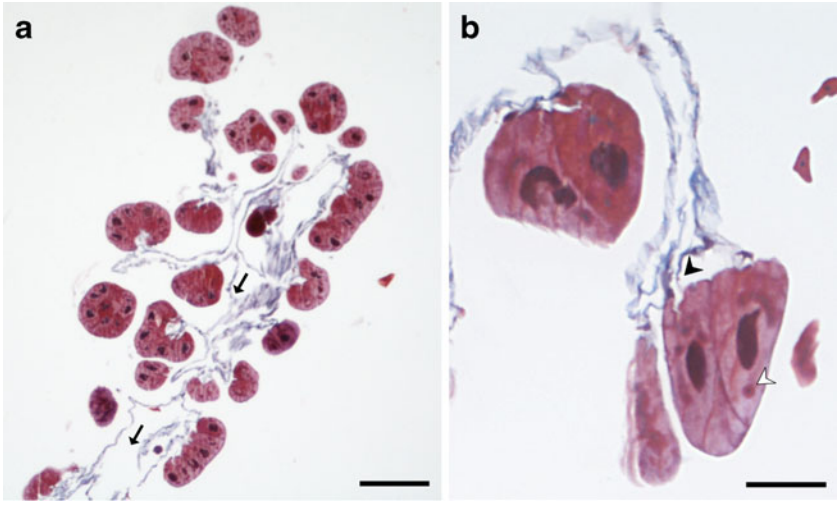

Figure 4. Bright-field micrographs of the posterior lobe of the mandibular gland of *Leiopodus lacertinus*, male. **a** Several acini are joined to the reservoir duct. *Black arrows* mark the course in the reservoir duct of the exocrine secretion from the acini to the base of the mandible. **b** Higher magnification of the micrograph (**a**) showing two acini. The flattened duct cells are above the secretory cells. *Black arrowhead* marks the axial penetration of the duct cell, *white arrowhead* points to the end apparatus. *Scale bars* (**a**) 200 μm, (**b**) 30 μm.

Leiopodus males, the reservoir duct is an elongated white sac running medially to the secretory tissue (Figs. 1, 2a). In *Leiopodus* males, the reservoir duct of each of the lobes was detected only with high resolution microscopy (Figs. 4a, b, 5c).

3.2.2. Histology and immunocytochemistry

In *L. lacertinus* females as well as in both sexes specimens of *M. segmentaria*, the secretory tissue is organized forming a single mass (Figs. 6a–c, 7a–d). At the level of the base of the mandible, males of *L. lacertinus* showed a similar tissue organization to that observed in *Melitoma* (Fig. 5c, d). On the other hand, the distal portion of *L. lacertinus* male gland has a unique feature, showing a raceme of acini; each acinus is composed by 3–7 cells (Figs. 4b, 5a–d, 8). The secretory epithelium is simple cylindrical, with large cells of about 40–60 μm high (Figs. 3d, 4a, b).

The reservoir tissue consists of a flattened epithelium covered by a tiny cuticular luminal

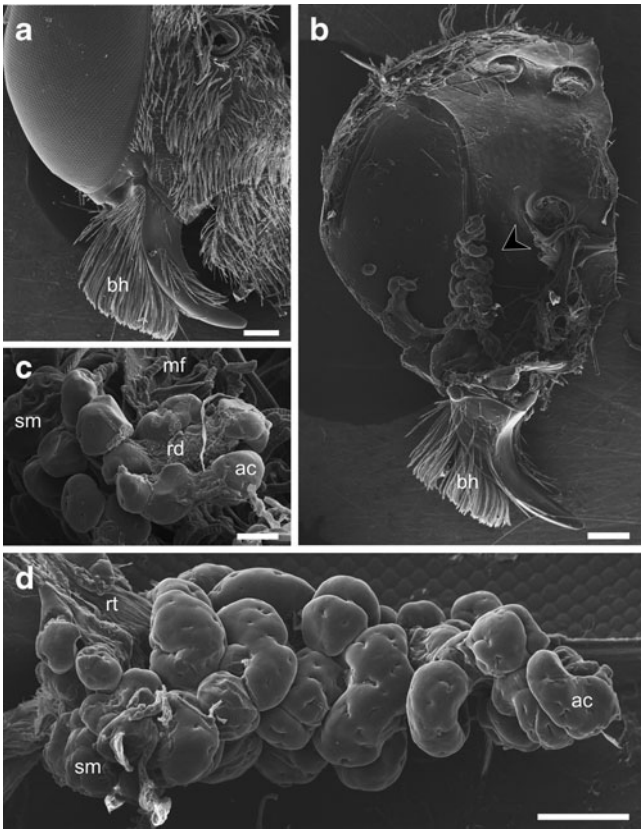

Figure 5. Scanning electron micrographs of the head and glands of a *Leiopodus lacertinus* male. **a** Anterior view of left side of head, showing the high density of brush hairs (*bh*) in the external midline of mandible. **b** Posterior view of the left side of the head, *black arrowhead* marks the secretory tissue of the mandibular gland. **c** Organization in acini (*ac*) of the exocrine tissue with the exception at the *left* of the figure which shows a non-acinar secretory mass (*sm*). Note the presence of reservoir duct (*rd*) connecting the acini. **d** Higher magnification of (**a**) showing several acini and a bigger lobe of non-acinar secretory epithelium near of the base of mandible. Brush hairs of mandible (*br*), muscle fiber (*mf*), reservoir tissue (*rt*), reservoir duct (*rd*). *Scale bars* (**a**, **b**) 200 μm, (**c**) 50 μm, (**d**) 100 μm.

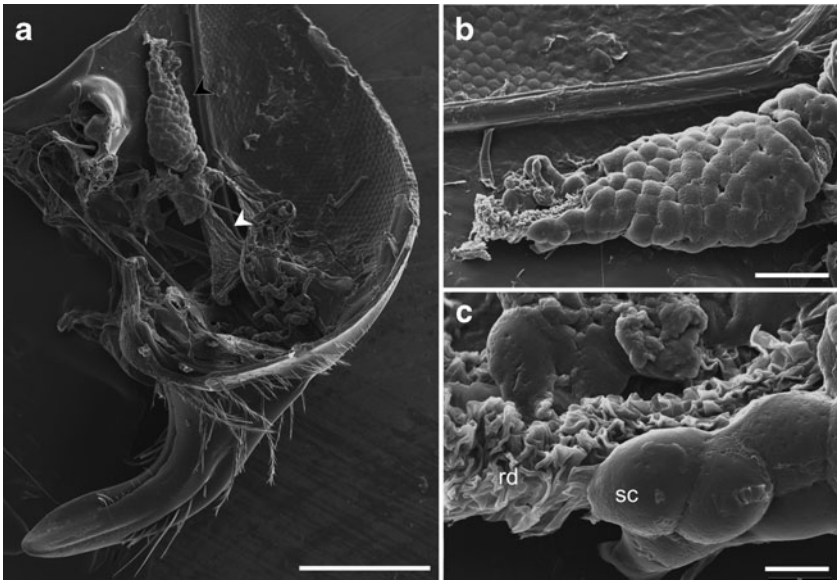

Figure 6. Scanning electron micrographs of the mandibular gland of *Melitoma segmentaria* male. **a** Posterior view of the right side of the head, *black arrowhead* marks the secretory tissue of the mandibular gland, *white arrowhead* marks a zone near the base without secretory tissue. **b** The distal part of the gland is dissected out to expose the inner part of the reservoir duct (*rd*). **c** Note the convoluted surface of the reservoir tissue. secretory cell (*sc*). *Scale bars* (**a**) 500 μm, (**b**) 100 μm, (**c**) 20 μm.

layer (Fig. 9). Projections of the apical surface of the cells into the lumen of the reservoir were observed by SEM and light microscopy (Figs. 6c, 9). The reservoir tissue delivers the secretion to the gland opening which is located medially in the upper surface of the mandible (Fig. 10a, b). In all the males of *Leiopodus*, brush hairs were observed in the mandible (Fig. 5a, b).

In samples from *L. lacertinus* and *M. segmentaria*, the secretory unit consists of two cell types. The duct cell forms the wall of a ductule, which penetrates the glandular cell in an axial orientation to drain the secretion to the reservoir duct (Figs. 4b, 8). Septate junctions between duct and glandular cells were observed at higher magnification (Fig. 7c). The intracellular part of the duct system (end apparatus) consists of a ductule located inside of the gland cell and surrounded by cytoplasm, a fact which was also evidenced in SEM and light microscope observations of histological sections (Figs. 3d, 4b, 8). The morphology of the end apparatus forming a ring or annulus close to the secretory cell nucleus was reconstructed by immunofluorescence using anti β-tubulin antibodies (Fig. 11a–d). External sections of duct cells and reservoir tissue also presented high intensity in β-tubulin-like immunoreactivity (Fig. 11b).

3.3. Hypopharyngeal glands

3.3.1. General morphology

The hypopharyngeal gland, located in the middle of the head at the level of the clypeus, is formed by a pair of lobes connected to the hypopharyngeal plate (Fig. 12a). In the genus *Leiopodus* and in *Ptilotrhix relata*, the hypopharyngeal glands are sacular with a main axis parallel to the hypopharyngeal plate (Fig. 12a, b). However, in *Melitoma* and *Diadasina* they are rounded (Figs. 12d, 13c).

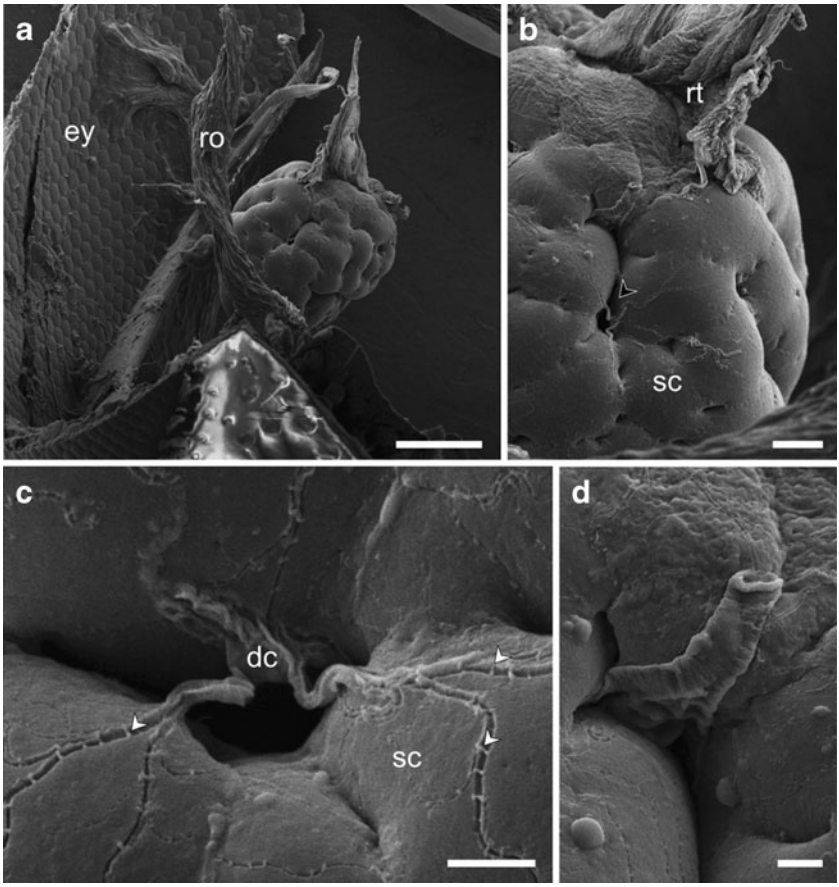

Figure 7. Scanning electron micrographs of *Leiopodus lacertinus* female. **a** Lateral view of the head showing the spherical mandibular gland. Note the posterior position of the reservoir organ (*ro*) with respect to the gland; eye (*e*). **b** Higher magnification of *boxed area* in (**a**). Note the reservoir tissue (*rt*), *arrowhead* points to a deep groove between 2 secretory cells. **c** Duct cell (*dc*) and external collector duct, *white arrowheads* mark the intercellular bridges between ducts and secretory cells. **d** A trachea sending branches to secretory cells. *Scale bars* (**a**) 100 μm, (**b**) 20 μm, (**c**, **c**) 5 μm.

3.3.2. Histology

In *M. segmentaria,* the organization of the glandular tissue is similar to that of *L. lacertinus*. Thus, a group of spheroid secretory cells of about 30–50 μm in diameter are gathered to form a single unit (Fig. 13b, c). The duct cells and the end apparatus were stained with anti β-tubulin antibodies (Figs. 13b, c, 14). Unlike what was observed in mandibular glands, every duct cell drains the secretion independently, at the base of the hypopharyngeal plate (Fig. 12b–d).

3.4. Morphometric studies

The mandibular glands of *Leiopodus* males were the largest among all the glands (Table 1). Relative measures of these glands showed that in *L. lacertinus* males they were significantly larger than in females (t=6.93; P<0.0001). In the other species of *Leiopodus*, it was found that males also tended to have larger mandibular glands than females (Table 1). On the other hand, *M. segmentaria, Diadasina distincta* and *Ptilotrix relata* did not show sexual dimorphism.

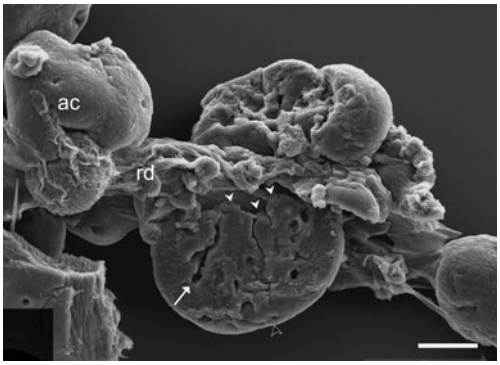

Figure 8. Scanning electron micrograph of mandibular gland of *Leiopodus lacertinus* male. Parasagittal section of one acinus (*ac*), *white arrowheads* mark the apical zones of glandular cells facing the reservoir, *black arrowhead* marks the border between two glandular cells. *White arrow* marks an intracellular canaliculus inside a gland cell. Reservoir duct (*rd*). *Scale bar* 25 μm.

No significant difference in the size of the hypopharyngeal glands was found in all species analyzed here. However, in some specimens of *P. relata*, hypopharyngeal glands of females appeared larger than those of males (Table 1).

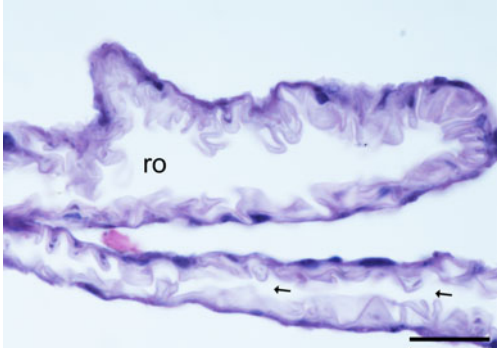

Figure 9. Bright-field micrograph of reservoir organ *Leiopodus lacertinus* female. **a** Longitudinal section of the reservoir organ (*ro*) formed by squamous epithelial cells projecting to the lumen. Note the thin cuticular layer in the luminal surface of the cells. *Black arrows* mark the pathway of the glandular secretion. *Scale bar* 10 μm.

4. DISCUSSION

4.1. Head glands in solitary bees

In this paper, we describe the morphology and perform a morphometrical analysis of head glands in species of Protepeolini and Emphorini. In all the species analyzed here, we did not detect cephalic salivary glands, a fact which is in agreement with the report by Cruz Landim (1967). In solitary bees of the subfamily Apinae, only species of the genus *Centris* possess head salivary glands (Cruz Landim 1967, 2008); however, in Emphorini and Protepeolini, we confirm the presence of these organs only in the thoracic region (personal observations)

4.2. Mandibular gland

In Hymenoptera, several reports have described the cell components of the exocrine glands which showed a similar organization in wasps, ants and bees (Vallet et al. 1991; Fortunato et al. 2000; do Amaral and Machado-Santelli 2008; Cruz Landim et al. 2011). Our observations about the presence of β-tubulin-like immunoreactivity in secretory cells and in the reservoir duct are in agreement with previous reports in ants (do Amaral and Machado-Santelli 2008). Regarding the reservoir tissue, ultrastructural studies in *Apis mellifera* have shown microtubules in the intima layer (Vallet et al. 1991). By means of immunofluorescence and light and scanning electron microscopy, we were able to classify the cellular units of mandibular and hypopharyngeal exocrine glands as belonging to Class III according to the report from Noirot and Quennedey (1974). We confirm that in the solitary bees studied here, the terminal duct of the gland ends in the articulation membrane of the anterior side of the mandibule as has been described in *A. mellifera* and *Melipona bicolor* (Simpson 1960; Gracioli et al. 2004).

In social bees, the secretory tissue of the mandibular gland varied in size and cell morphology along the adult cycle and among the different castes of individuals of a social colony (Nedel 1960; Lensky et al. 1985; Vallet

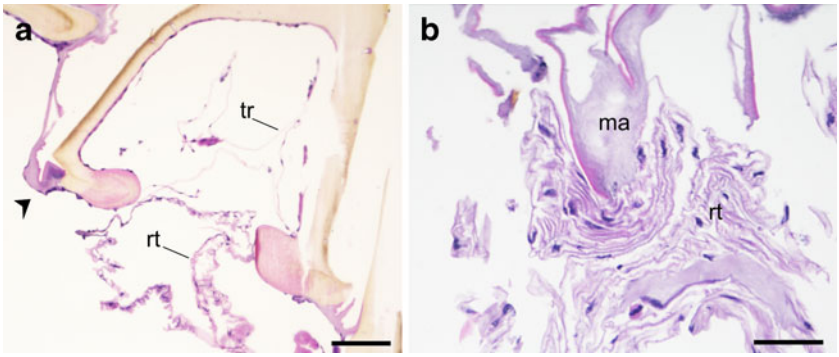

Figure 10. Bright-field micrographs of the base of the mandible. *Melitoma segmentaria* male. **a** Reservoir tissue (*rt*) near of articulation of the mandible. *Black arrowhead* marks the joint of the mandible and articulation membrane. Note tracheal tissue inside of mandible (*tr*). **b** Reservoir tissue penetrates the articulation membrane (*ma*). *Scale bars* (**a**) 40 μm, (**b**) 20 μm.

et al. 1991; Gracioli-Vitti et al. 2004; Cruz Landim et al. 2011). Regarding solitary bees, morphological changes in the mandibular gland have been reported in *Centris* males during the

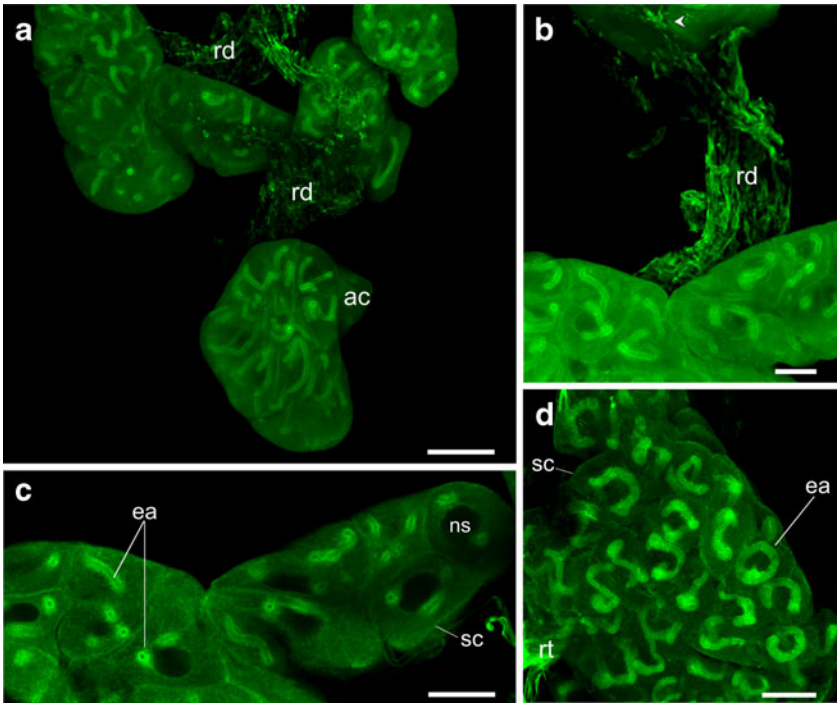

Figure 11. Confocal immunofluorescence of the mandibular gland of *Leiopodus lacertinus* male (**a**–**c**) and female (**d**). Anti β-tubulin monoclonal antibodies. **a**, **b** Note the connection of acini (*ac*) by reservoir duct (*rd*). **c** Higher magnification of area in (**a**), showing a immunostained end apparatus (*ea*) in different planes of section. The nuclei of secretory cells are not counterstained (*ns*). **d** Note the annular configuration of the end apparatus around the nuclei of the secretory cells (*sc*). *Scale bars* (**a**) 70 μm, (**b**) 30 μm, (**c**) 40 μm, (**d**) 60 μm.

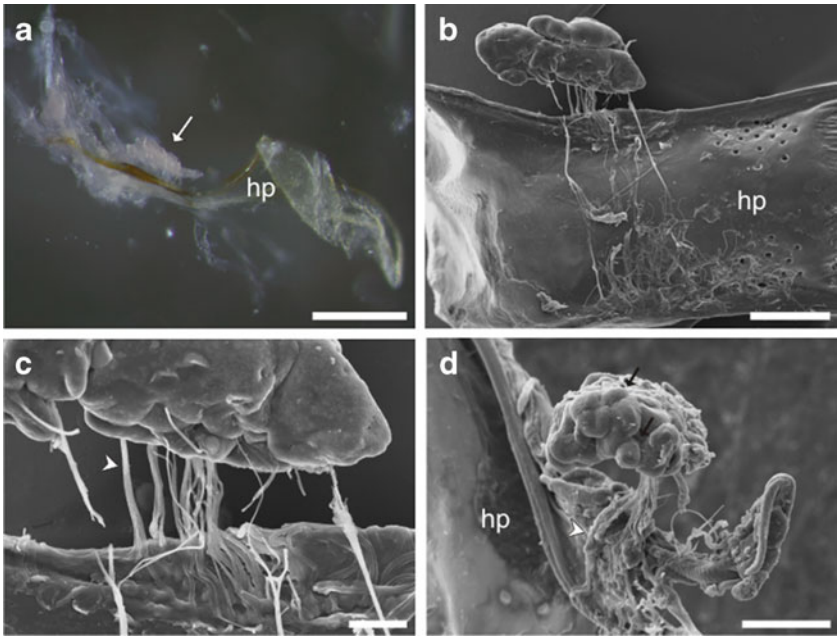

Figure 12. Hypopharyngeal glands of *Leiopodus lacertinus* and *Melitoma segmentaria*. **a** Stereomicroscopy of *L. lacertinus* female, *white arrow* points to the gland, hypopharyngeal plate (*hp*). **b**, **c** Scanning electron micrographs of *L. lacertinus* female hypopharyngeal gland. *White arrowhead* marks a series of independent ducts that communicate each secretory cell with the hypopharyngeal plate. **d** Scanning electron micrographs of hypopharyngeal gland of *Melitoma segmentaria* male, *black arrows* point to secretory cells while a *white arrowhead* marks a duct. *Scale bars* (**a**) 500 μm, (**b**) 150 μm, (**c**) 20 μm.

act of territorial marking (Vinson et al. 1982). In our studies, we have found a variety in the coloration patterns of fresh glands from intense yellow to white or transparent. We have also found that many specimens showed either right or left glands lacking a secretory mass. No intermediate stages of development were found. The bifid structure of the mandibular glands (type 2 glands), with one secretory lobe and a reservoir, has been also observed in other species of Apinae (Cruz Landim 1967, 2008; Vinson et al. 1982). It has been hypothesized that type 1 mandibular glands having only a small mass of secretory tissue represent a primitive condition (Cruz Landim 1967). A later evolutive step, referred to as the *melipona glandular type,* might be represented by the appearance of a non-secretory tissue which might function as a reservoir. The development of a secondary lobe for storage, or "the reservoir organ", might be a further step of this evolutionary process. However, some Meliponini possess a well-developed mass of secretory tissue together with a reduced reservoir (Cruz Landim 1967). The mandibular glands of *Leiopodus* males also display the same organization of the latter meliponini glands, making the reservoir duct the only possible site for storage of the product.

Sexual dimorphisms in mandibular glands have been documented in social bees (Nedel 1960; Cruz Landim 1967; Lensky et al. 1985; Gracioli et al. 2004). Instead, 4 of the 24 species of the studied solitary bees showed differences between males and females (Cruz Landim 1967). It is noteworthy that the distinctive organization in acinar units of the mandibular glands of *Leiopodus* males has not been previously reported in bees. We have also observed dense mandibular brush hairs in this

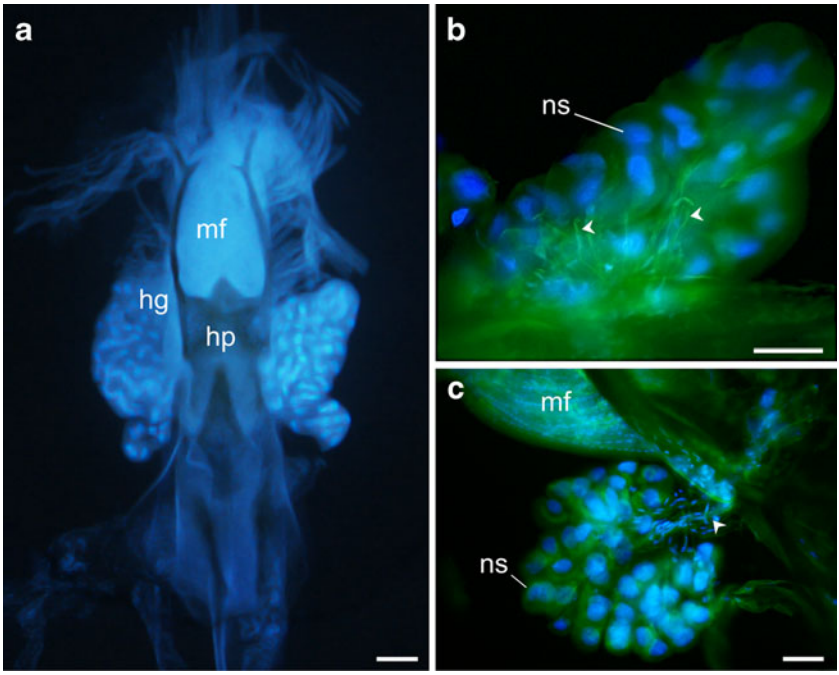

Figure 13. Immunofluorescence of hypopharyngeal glands of *Leiopodus lacertinus* male (**a**) *L. lacertinus* female (**b**) and *Melitoma segmentaria* female (**c**). **a** DAPI staining of the nuclei (*blue*) showing the position of the glands in relationship to the mandible. **b**, **c** Immunostaining with anti β-tubulin antibodies (*green*) and nuclei counterstained with DAPI (*blue*). In (**c**) numerous flattened nuclei indicate the course of duct cells to the hypopharyngeal plate (*hp*). Hypopharyngeal glands (*hg*), nuclei of secretory cells (*ns*), muscle fiber (*mf*). *Scale bars* (**a**)=60 μm; (**b**, **c**) 30 μm.

species. It should be noted that both morphological features have been described in the males of the cabronid wasp *Philanthus triangulum* (Goettler and Strohm 2008). These wasps display an active pattern of territorial marking for attracting females to a territory that do not contain resources that might be attractive to them (Kroiss et al. 2010). In field studies performed at the start of the nesting season of *M. segmentaria*, males of *L. lacertinus* were observed flying in aggregates for several days, at the neighborhood of the host nests. In this period, they did not interact with their sex mates (unpublished observations). Other possible role of the mandibular secretion of the male during parasitism is to mimic the chemical signal that will allow the coespecific females to visit the corresponding host nests (Tengö and Bergstrom 1977).

4.3. Hypopharyngeal gland

Hypopharyngeal glands are well developed in social bees (Cruz Landim 1967). They are supplied with multiple secretory alveoli and an axial collector duct which receives all the secretion. These glands end further in the hypopharyngeal plate. The hypopharyngeal gland is less developed in solitary than in social bees (Cruz Landim 1967, 2008). Moreover, the axial duct is either rudimentary or completely absent in solitary bees. As already mentioned for mandibular glands, the cell units of the hypopharyngeal glands correspond to class III (Noirot and Quennedey 1974). *Melitoma segmentaria* hypopharyngeal glands were included in a group of glands with a short or invisible collector duct (Cruz Landim 1967). We did not observe an axial collector duct in our specimens

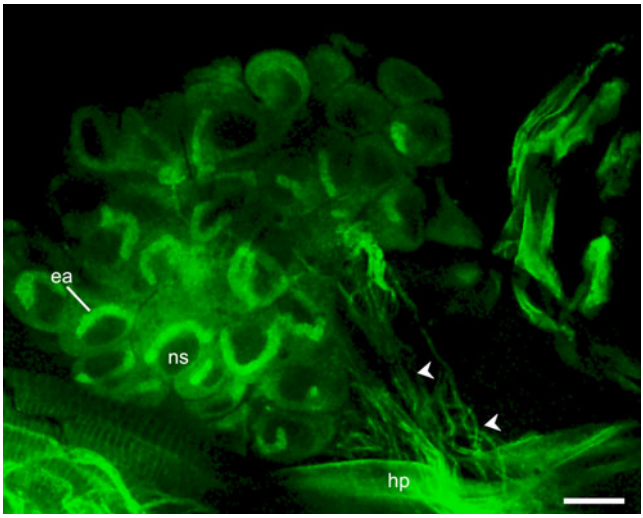

Figure 14. Confocal immunofluorescence of hypopharyngeal glands of *Melitoma segmentaria* male. Anti β-tubulin antibodies. Note the end apparatus (*ea*) around the nuclei of the secretory cells (*ns*). *White arrowheads* mark the multiple canaliculi that end in the hypopharyngeal plate (*hp*). *Scale bars* 20 μm.

of *M. segmentaria*. However, multiple single secretory cells each one with its own duct were seen ending in the hypopharyngeal plate.

No significant differences in the morphology of the glands of host and parasite as well as any pattern of sexual dimorphism within species have been observed. These results suggest that bees of the tribes Protepeolini and Emphorini retain the primitive condition of the hypopharyngeal glands.

Table I. Diameter of mandibular and hypopharyngeal glands (*MGd*; *HPHd*).

Species	Gender	MGd	MGd/Id	*n*	HPGd	HPGd/Id	*n*
Leiopodus lacertinus	♀	0.68±0.07*	0.28±0.03	13	0.47±0.03	0.2±0.03	10
	♂	1.74±0.13	0.68±0.04	11	0.4±0.04	0.17±0.01	6
Leiopodus trocantericus	♀	0.47±0.02	0.25±0.01	2	0.2±0.05	0.1±0.02	2
	♂	1.37±0.12	0.77±0.08	2	0.25±0.05	0.12±0.05	2
Leiopodus abnormis	♀	0.42±0.02	0.21±0.01	2	0.03±0.05	0.1±0.02	2
	♂	1.5	0.85	1	–	–	1
Melitoma segmentaria	♀	0.85±0.05	0.27±0.02	13	0.35±0.02	0.11±0.01	6
	♂	0.83±0.04	0.30±0.02	8	0.34±0.03	0.12±0.01	6
Diadasina distincta	♀	0.75	0.31	1	0.5	0.2	1
	♂	0.62±0.025	0.30±0.01	2	0.37±0.02	0.18±0.02	2
Ptilothrix relata	♀	1.4±0.05	0.39±0.04	4	0.52±0.06	0.18±0.02	4
	♂	0.81±0.08	0.30±0.02	4	0.31±0.07	0.12±0.03	4

The relative gland size (*MGd/Id*, *HP/Id*) was calculated by dividing the gland diameter by the intertegular distance. The values correspond to mean ± SEM

*$P<0.0001$

5. CONCLUSIONS

This study provides new morphological descriptions of the glandular systems so far poorly studied in wild bees. In addition, a comparative analysis between sexes yields valuable elements for understanding the still unknown biology of Protepeolini males. The overt sexual dimorphism of mandibular glands has been demonstrated in *Leiopodus*. The evidence presented here suggests an evolutionary process of specialization of the mandibular glands in males of *Leiopodus*. Ethological studies in the field as well as chemical analysis of head gland secretions are needed to understand their role in the parasitic activities of Proteopeolini.

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Morphologie comparative des glandes céphaliques chez des espèces de Protepeolini et d'Emphorini (Hymenoptera: Apidae)

Immunochimie / glandes céphaliques / étude comparative / *Leiopodus*

Vergleichende Morphologie der Kopfdruesen von Arten der Protepeolini und Emphorini (Hymenoptera: Apidae)

Protepeolini / Emphorini / Kopfdruesen / Immuncytochemie

REFERENCES

Cane, J.H. (1986) Predator deterrence by mandibular gland secretions of bees (Hymenoptera: Apoidea). J. Chem. Ecol. **12**, 1295–1309

Cane, J.H., Tengö, J.O. (1981) Pheromonal cues direct mate-seeking behaviors of male *Colletes cunicularius* (Hymenoptera: Colletidae). J. Chem. Ecol. **7**, 427–436

Cane, J.H., Gerdin, S., Wife, G. (1983) Mandibular gland secretions of solitary bees (Hymenoptera: Apoidea): potential for nest cell disinfection. J. Kansas Entomol. Soc. **56**, 199–204

Cruz Landim, C. (1967) Estudo comparativo de algumas glándulas das abelhas (Hymenoptera, Apoidea) e respectivas implicações evolutivas. Arq. Zool. Sao Paulo **15**, 177–290

Cruz Landim, C. (2008) Glãndulas mandibulares p315-324. In: Abelhas-Morfologia e Função de Sistemas. UNESP

Cruz Landim, C., Gracioli-Vitti, L.F., Abdalla, F.C. (2011) Ultrastuctural studies of the mandibular gland of *Melipona quadrifasciata* Lepeletier, 1836 (Apidae, Meliponini): a comparison between workers and queens. J. Apic. Res. **50**, 306–315

Cruz Landim, C., Ferreira Caliman, M.J., Gracioli-Vitti, L.F., Zucchi, R. (2012) Correlation between mandibular gland secretion and cuticular hydrocarbons in the stingless bee *Melipona quadrifasciata*. Genet. Mol. Res. **11**, 966–977

Deseyn, J., Billen, J. (2005) Age-dependent morphology and ultrastructure of the hypopharyngeal gland of *Apis mellifera* workers (Hymenoptera, Apidae). Apidologie **36**, 49–57

do Amaral, J.B., Machado-Santelli, G.M. (2008) Salivary system in leaf-cutting ants (*Atta sexdens rubropilosa* Forel, 1908) castes: a confocal study. Micron **39**, 535–540

Duffield, R.M., Simon-Jordan, C., Riddick, E.W., Wheeler, J.W. (1990) Exocrine secretions of bees X. 3,7-Dimethyldeca-2,6-dien-1,10-diol: a sex-specific compound from *Nomada annulata* (Hymenoptera: Anthophoridae). J. Chem. Ecol. **16**, 1069–1076

Fortunato, A., Turillazzi, S., Delfino, G. (2000) Ectal mandibular gland in *Polistes dominulus* (Christ) (Hymenoptera, Vespidae): ultrastructural modifications over the secretory cycle. J. Morphol. **244**, 45–55

Goettler, W., Strohm, E. (2008) Mandibular glands of European beewolves, *Philanthus triangulum* (Hymenoptera, Crabronidae). Arthropod Struct. Dev. **37**, 363–371

Gracioli, L.F., Silva de Moraes, R.L.M., Cruz-Landim, C. (2004) Ultrastructural aspects of the mandibular gland of *Melipona bicolor* Lepeletier, 1836 (Hymenoptera: Apidae, Meliponini) in the castes. Micron **35**, 331–336

Gracioli-Vitti, L., Abdalla, F., Cruz-Landim, C. (2004) Caracterização das glândulas mandibulares nas diferentes classes de adultos de *Scaptotrigona postica* Latreille (Hymenoptera: Apidae). Neotrop. Entomol. **33**, 703–708

Grasso, D.A., Romani, R., Castracani, C., Visicchio, R., Mori, A., Isidoro, N., Le Moli, F. (2004) Mandible associated glands in queens of the slave-making ant *Polyergus rufescens* (Hymenoptera, Formicidae). Insect. Soc. **51**, 74–80

Hefetz, A., Eickwort, G.C., Blum, M.S., Cane, J., Bohart, G.E. (1982) A comparative study of the exocrine products of cleptoparasitic bees (*Holcopasites*) and their hosts (*Calliopsis*) (Hymenoptera: Anthophoridae, Andrenidae). J. Chem. Ecol. **8**, 1389–1397

Heselhaus, F. (1922) Die Hautdrüsen der Apiden und verwandter formen. Zool. Jahrb. Jena Abt. f. Anat. **49**, 369–464

Jarau, S., Hrncir, M., Zucchi, R., Barth, F.G. (2004) A stingless bee uses labial gland secretions for scent trail communication (*Trigona recursa* Smith 1863). J. Comp. Physiol. A **190**, 233–239

Kroiss, J., Lechner, K., Strohm, E. (2010) Male territoriality and mating system in the European beewolf *Philanthus triangulum* F. (Hymenoptera: Crabronidae): evidence for a "hotspot" lek polygyny. J. Ethol. **28**, 295–304

Lensky, Y.P., Cassier, C., Notkin, M., Delorme-Joulie, C., Levlnsohn, M. (1985) Pheromonal activity and fine structure of the mandibular glands of honeybee drones (*Apis mellifera* L.). J. Insect Physiol. **31**, 265–276

Nedel, J.D. (1960) Morphologie und physiologie der mandibeldrüse. Z. Morphol. Ökol. Tiere **49**, 139–183

Niculita, H., Billen, J., Keller, L. (2008) Comparative morphology of cephalic exocrine glands among castes of the black ant *Lasius niger*. Arthropod Struct. Dev. **36**, 135–141

Noirot, C., Quennedey, A. (1974) Fine structure of insect epidermal glands. Annu. Rev. Entomol. **19**, 61–80

Reichle, C., Aguilar, I., Ayasse, M., Jarau, S. (2011) Stingless bees (*Scaptotrigona pectoralis*) learn foreign trail pheromones and use them to find food. J. Comp. Physiol. A. Neuroethol. Sens. Neural B. **147**, 243–249

Roig-Alsina, A., Rozen Jr., J.G. (1994) Revision of the cleptoparasitic bee tribe Protepeolini, including biologies and immature stages. Am. Mus. Novit. **3099**, 1–27

Settembrini, B.P. (1984) The compound eyes of *Triatoma infestans* and *Rhodnius prolixus* (Hemiptera: Reduviidae). J. Med. Entomol. **21**, 477–479

Settembrini, B.P., Galvani, G.L., Villar, M.J. (2008) Localization of cholecystokinin-like immunoreactivity in the central nervous system of *Triatoma infestans* (Insecta: Heteroptera). Tissue Cell **40**, 51–60

Simpson, J. (1960) The functions of the salivary glands of *Apis mellifera*. J. Insect Physiol. **4**, 107–121

Šobotnik, J., Kalinková, B., Cahlíková, L., Weyda, F., Ptáček, V., Valterová, I. (2008) Age-dependent changes in structure and function of the male labial glands in *Bombus terrestris*. J. Insect Physiol. **54**, 204–214

Tengö, J., Bergstrom, G. (1977) Cleptoparasitism and odor mimetism in bees: do *Nomada* males imitate the odor of *Andrena* females? Science **196**, 1117–1119

Vallet, A., Cassier, P., Lensky, Y. (1991) Ontogeny of the fine structure of the mandibular glands of the honeybee (*Apis mellifera* L.) workers and the pheromonal activity of 2-heptanone. J. Insect Physiol **37**, 789–804

Vinson, S.B., Williams, H.J., Frankie, G.W., Wheeler, J.W., Blum, M.S., Coville, R.E. (1982) Mandibular glands of male *Centris adani* (Hymenoptera; Anthophoridae): their morphology, chemical constituents and function in scent marking and territorial behavior. J. Chem. Ecol. **8**, 319–327