

Convolutated and venom glands of different species of wasps

Glândulas convolutas e de veneno de diferentes espécies de vespas

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ABSTRACT

The social Hymenoptera increasingly arouse the attention of researchers due to the great economic and ecological importance to the species belonging to this order. The processes of pollination performed by these insects, the biological control, the structures of nests and colonies, the social organization, the accidents arising from their stings and the pharmacological potential of venoms synthesized by them, represent some of the features that make these individuals, targets of important studies. The sting apparatus and venom represent fundamental defense mechanisms of these insects. In order to characterize the convoluted and the venom gland of these species and gain an understanding of the relationship between the components, this study aimed to analyze through morphology and histochemical techniques the venom glands of the wasps, *Polistes versicolor*, *Agelaia palipes palipes* and *Polybia paulista*, and also the convoluted gland - a structure found in the reservoir, establishing the defining characteristics of these structures. In addition to the morphological features described about these structures, our results showed the presence of secretion produced by the convoluted gland cells which indicates that this structure contributes to the final composition of the venom produced and stored in the reservoir.

Keywords: social insects, histochemistry, histology.

RESUMO

Os Hymenoptera sociais despertam cada vez mais a atenção dos pesquisadores devido à grande importância econômica e ecológica das espécies pertencentes a esta ordem. Os processos de polinização realizados por esses insetos, o controle biológico, as estruturas de ninhos e colônias, a organização social, os acidentes decorrentes de suas ferroadas e o potencial farmacológico de venenos sintetizados por eles, representam algumas das características que tornam esses indivíduos alvos de estudos importantes. O aparelho de ferrão e seu veneno representam mecanismos fundamentais de defesa desses insetos. Para caracterizar as glândulas convolutas e de veneno destas espécies e compreender a relação entre seus componentes, este estudo teve como objetivo analisar, por meio de técnicas morfológicas e histoquímicas, as glândulas de veneno das vespas, *Polistes versicolor*, *Agelaia palipes palipes* e *Polybia paulista*, e também a glândula convoluta - uma estrutura encontrada no interior do reservatório de veneno, estabelecendo as características definidoras dessas estruturas. Além das características morfológicas descritas sobre essas estruturas, nossos resultados mostraram a presença de secreção produzida pelas células da glândula convoluta, o que indica que esta estrutura contribui para a composição final do veneno produzido e armazenado no reservatório.

Palavras-chave: insetos sociais, histoquímica, histologia.



INTRODUCTION

Social insects attract considerable attention due to the structure of their colonies and their economic importance (Prezoto, 1999). Social wasps stand out in this scenario because they play an important role as bioindicators of environmental quality (Souza et al., 2010) and act on the balance of ecosystems, such as biological control and pollination processes (Prezoto and Machado 1999; Clemente et al., 2012).

Among the insect groups, bees are prominent in studies on the use of floral resources in the Neotropical region; however, wasps are also very important in this context, representing a considerable portion of foragers (Heithaus, 1979a, b). During foraging occurs the collection of resources such as: water, animal protein - important in the feeding of immature and carbohydrates -for the feeding of adults (Prezoto et al., 2008; Elisei et al., 2010; Barbosa et al., 2014). Often the interaction between social wasps and some flowers is so strong that these plants produce aromatic substances with the aim of attracting these insects (Brodmann et al., 2008). Quirino and Machado (2001) found three species of plants in the genus *Combretum* that were pollinated by wasps of the genera *Polistes*, *Polybia*, *Synoeca*, *Mischocyttarus* and *Protonectarina*.

The architecture of the Vespidae nests is a defensive strategy against predators (Jeanne, 1975). Another type of defense used by wasps is that characteristic of all Aculeata: use of the sting apparatus. Some species present the self-amputation of the stinger and others, do not (Chaud Netto et al., 1994). Social wasps are known to have a peculiar defense behavior, through which they inject their venom with followed stings (Edery et al., 1978; Fitzgerald and Flood, 2006).

Many species of social wasps are also important in the biological control of pests, mainly lepidoptera of crops such as eucalyptus, vegetables, coffee, corn and tomatoes (Bach, 1951; Marques and Carvalho, 1993; Prezoto and Machado, 1999; Richter, 2000).

Another important characteristic of social insects is the variety of exocrine glands found in bees, wasps, ants and termites. The Hymenoptera, among invertebrate groups, represent the only species possessing inoculator's

venom devices, which is produced by a gland - the venom gland (Nakajima, 1986).

This gland has ectodermal origin and is located on the posterior portion of the abdomen, between the rectum and ovaries, being constituted by a tubular secretory thin, forked at the end. This tubule ends into a reservoir which through an excretory duct, binds to the stinger (Snodgrass, 1956). The venom gland of wasps is similar to that of ants because it is composed of two free secretory tubules and a reservoir (Maschwitz and Kloft, 1971). This reservoir contains a suspended, tubular structure - the convoluted gland (Schoeters and Billen, 1995), which is absent in bees. Many studies related to the mechanisms of action and allergenicity of venoms, as well as the biology and morphology of aculeate animals (bees, ants and wasps) have been focused on a small number of species (Oliveira et al., 1999; Bilo et al., 2005; King et al., 1984; King and Spangfort, 2000; Brand et al., 1972; De Graaf et al., 2009).

On bees, the epithelium of this gland is mainly composed of two layers of cells. The external is composed of Class III cells (Noirot and Quennedey, 1974) which form a pseudo-epithelial organization. The internal layer consists of low cells which coat the face overlying the lumen of the gland with a cuticular layer (Cruz-Landim, 2009). In this cuticle layer are found the canaliculi, structures responsible for transporting the secretion produced by the glands into the lumen to be stored in the reservoir. These canaliculi end in a region called the terminal apparatus, which possesses many microvilli (Cruz-Landim and Kitajima, 1966; Bridges and Owen, 1984).

The production and interaction among all the compounds of these venoms must be understood to be able to use it in pharmacology, to minimize the damage in case of accidents and to use them as new drugs. Based on previous studies, the current study aims to establish the morphological and histochemical features present in the venom glands of the wasps, *Polistes Versicolor* (Olivier, 1791), *Agelaia palipes palipes* (Olivier, 1791) and *Polybia paulista* (Ihering, 1896) characterizing the defining aspects of each species to further facilitate the understanding of the production and contents of their venom.

MATERIAL AND METHODS

Biological Material

Wasps colonies of the species *P. versicolor*, *A. p. palipes* and *P. paulista* were collected in the Department of Biology in the Institute of Biosciences at Rio Claro, Brazil, campus of the Universidade Estadual Paulista “Júlio de Mesquita Filho”, UNESP.

Methods

Obtaining the animals

Two different colonies (nests) of each wasp specie were selected and collected with the aid of an entomological network. No chemical was used in this process.

Histology

For each colony, 5 individuals were collected and 12 slides were made for each individual.

After collecting and anesthetizing the animals for 5 minutes in the freezer, the sting were pulled and the venom glands were dissected in insect saline solution (7.5 g/L NaCl, 2.38 g/L Na₂HPO₄, 2.72 g/L KH₂PO₄) and fixed in 4% paraformaldehyde, except for the materials that were stained with PAS and Sudan Black, which were fixed in aqueous Bouin's solution and formaldehyde-calcium, respectively. After 24 hours of fixation, all the materials were placed in sodium phosphate buffer for 30 minutes, dehydrated for 30 minutes in solutions with increasing concentrations of alcohol and then incubated in embedding resin for three days. After this procedure, the materials were embedded in Historesin (Leica Microsystems - Germany). Sections of 4 µm were made using Leica Microtome (Germany) and mounted on glass slides. After that, the venom glands were processed for different treatments, as described below and examined and photographed on an Olympus BX51 photomicroscope. Images were obtained with a digital camera (Olympus DP-71) and image acquisition was conducted with DP Controller software. All the dyes products were obtained from Sigma Aldrich (Brazil).

- Morphological analysis: Hematoxylin/Eosin (Junqueira and Junqueira, 1983).

Some sections were reacted with hematoxylin (10 minutes) and washed in running water for 10 minutes. They were subsequently subjected to an aqueous solution of eosin for 5 minutes and washed with distilled water.

- Detection of nucleic acids with Toluidine Blue (Mello and Vidal, 1980).

For detection of the nucleic acids, the sections were stained with 1% aqueous toluidine blue (pH 9.0) for 10 minutes and washed in distilled water.

- Detection of proteins with Mercury-Bromophenol Blue (Pearse, 1960).

Slides containing sections of the venom glands were placed in a solution of mercury-bromophenol blue for 1 hour at room temperature, washed for 5 minutes in 0.5% aqueous acetic acid and then washed in distilled water.

- Detection of DNA by the Feulgen reaction (Feulgen and Rossenbeck, 1924) and counterstaining with 1% fast green.

The slides were placed in HCl for 45 minutes and then in distilled water for 5 minutes. Then they were placed in Schiff reagent for 1 hour in the dark and in sulphurous water for 1 minute. The slides were washed in running water for 15 minutes and then the sections were counterstained with 1% fast green for 30 seconds and washed in distilled water.

- Detection of polysaccharides by PAS reaction (periodic-acid Schiff stain) (McManus, 1946) and counterstaining with Methyl Green.

To stain polysaccharides, the slides were fixed in aqueous Bouin's solution, washed in 1% periodic acid for 5 minutes and then washed in distilled water. They were then subjected to Schiff reagent (basic fuchsin bleached by the loss of aldehyde) for 1 hour and washed in tap water for 30 minutes. Next, the sections were counterstained for 20 seconds with methyl green and washed. In all procedures described above, after drying, the slides were mounted in Canada balsam.

- Detection of lipids with Sudan Black B (Junqueira and Junqueira, 1983).

The slides containing sections of the venom glands were fixed in formaldehyde-calcium. The slides were dehydrated and included as a routine technique for inclusion in Leica Historesin, described above. For lipid detection, the slides were washed in 70% alcohol and stained with Sudan Black for 15 minutes and with

Neutral Red for 2 to 4 minutes. All slides were mounted in glycerin gelatin after complete drying.

RESULTS

Based on the analysis of the venom glands of the species *P. versicolor*, *A. p. palipes* and *P. paulista* we could show that the cellular organization of this structure is basically the same. They consist of two thin secretory tubules that end in a reservoir. Two types of cells were found in the epithelium of the gland: secretory cells, which had larger nuclei and were found in the external portion of the gland and the inner layer cells which have smaller nuclei and line the gland lumen (Figures 1A, 2A and 3A).

In the venom gland of the three species of the analyzed wasps, the cuticle layer covering the luminal face could be visualized as well as the canaliculi – structures responsible for transporting the venom into the lumen of the gland (Figures 1E, 2B and 3E). These canaliculi stood out more in the venom glands of *P. versicolor*, appearing more prominent and in a great number (Figures 1B, 1C and 1E). Under light microscope, showed the presence of secretory vesicles in the epithelium of the gland (Figures 1F, 3D and 3F).

Inside the reservoir of wasps it was visualized a tubular structure called convoluted gland (Figures 3G and 3K). Analyzing this structure in *P. versicolor*, *A. p. palipes*

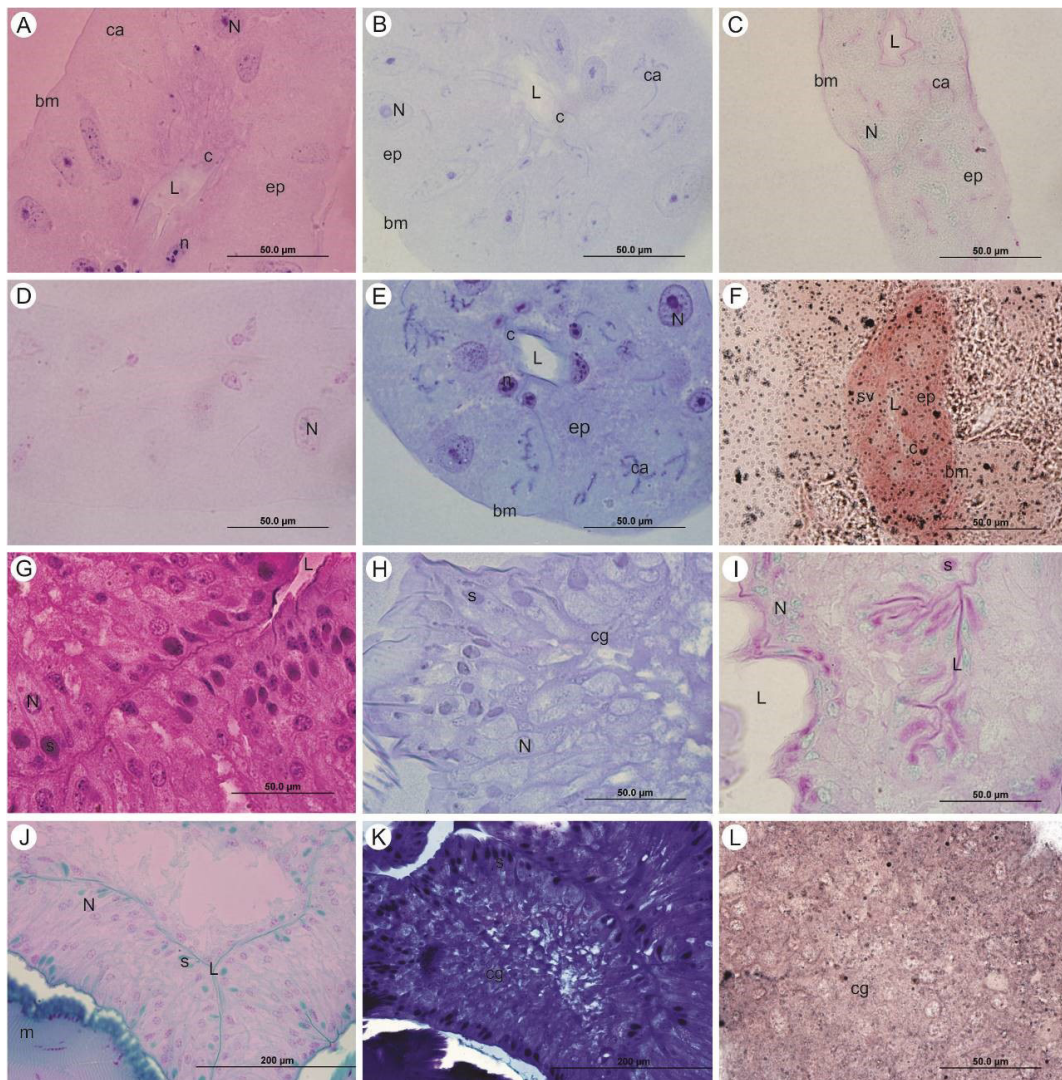


Figure 1. Histological sections of the venom gland of *Polistes versicolor* reacted with Hematoxylin and Eosin (A), Bromophenol Blue (B), PAS reaction (C), Feulgen reaction (D), Toluidine Blue (E) and Sudan Black (F). Histological sections of the convoluted gland of *Polistes versicolor* reacted with Hematoxylin and Eosin (G), Bromophenol Blue (H), PAS reaction (I), Feulgen reaction (J), Toluidine Blue (K) and Sudan Black (L). N - secretory cell nucleus, n - inner layer nucleus, c - cuticle, ca - canaliculus, L - lumen, ep - epithelium, bm - basal membrane, sv - secretion vesicle, s - secretion, cg - convoluted gland, m - muscle layer of the reservoir.

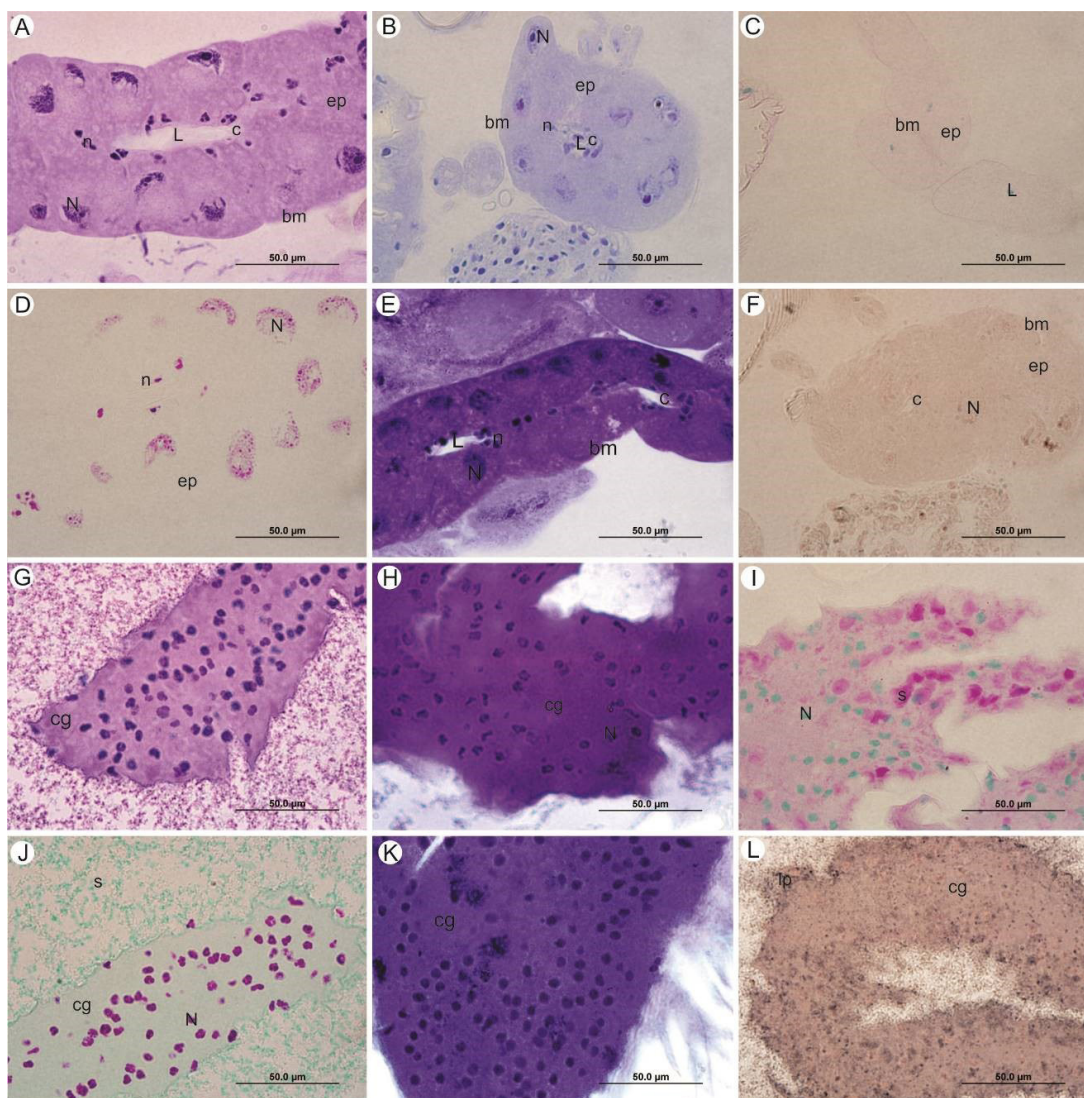


Figure 2. Histological sections of the venom gland of *Agelaia palipes palipes* reacted with Hematoxylin and Eosin (A), Bromophenol Blue (B), PAS reaction (C), Feulgen reaction (D), Toluidine Blue (E) and Sudan Black (F). Histological sections of the convoluted gland of *Agelaia palipes palipes* reacted with Hematoxylin and Eosin (G), Bromophenol Blue (H), PAS reaction (I), Feulgen reaction (J), Toluidine Blue (K) and Sudan Black (L). N - secretory cell nucleus, n - inner layer nucleus, c - cuticle, L - lumen, ep - epithelium, bm - basal membrane, s - secretion, cg - convoluted gland, lp - lipideous.

and *P. paulista* it was possible to note a predominance of columnar cells, and some nuclei with an elongated shape similar to the cell shape. Analyzing the convoluted gland in portions close to the lumen of the reservoir, we observed a secretion that, based on its position and appearance, appeared to be released into the lumen and thus contributed to the production of venom that is stored in the reservoir (Figures 1G, 1H, 1I, 2I, 3I and 3L). By Hematoxylin/Eosin, nuclei of cells in the venom glands appeared purple and practically no secretion was identified in the lumen (Figures 1A, 2A and 3A). In the convoluted gland of this species of wasps, the secretion produced appears darker, suggesting that it is

more acidic than the secretion found in the venom gland lumen (Figures 1G, 2G and 3G). We also can say that the basement membrane appears more clearly defined in the photomicrographs of *P. paulista* (Figure 3A).

With the technique of Bromophenol Blue, specific for proteins, we can see that the canaliculi, responsible for dump the secretion produced into the lumen, appears in the epithelium of the gland, showing a positive reaction (Figures 1B and 3B). The convoluted glands of *A. p. palipes* and *P. paulista* were more strongly stained by Bromophenol Blue, appearing darker than the venom gland (Figures 2H and 3H). In the convoluted gland of *P. versicolor*, the apical portions of the cells were

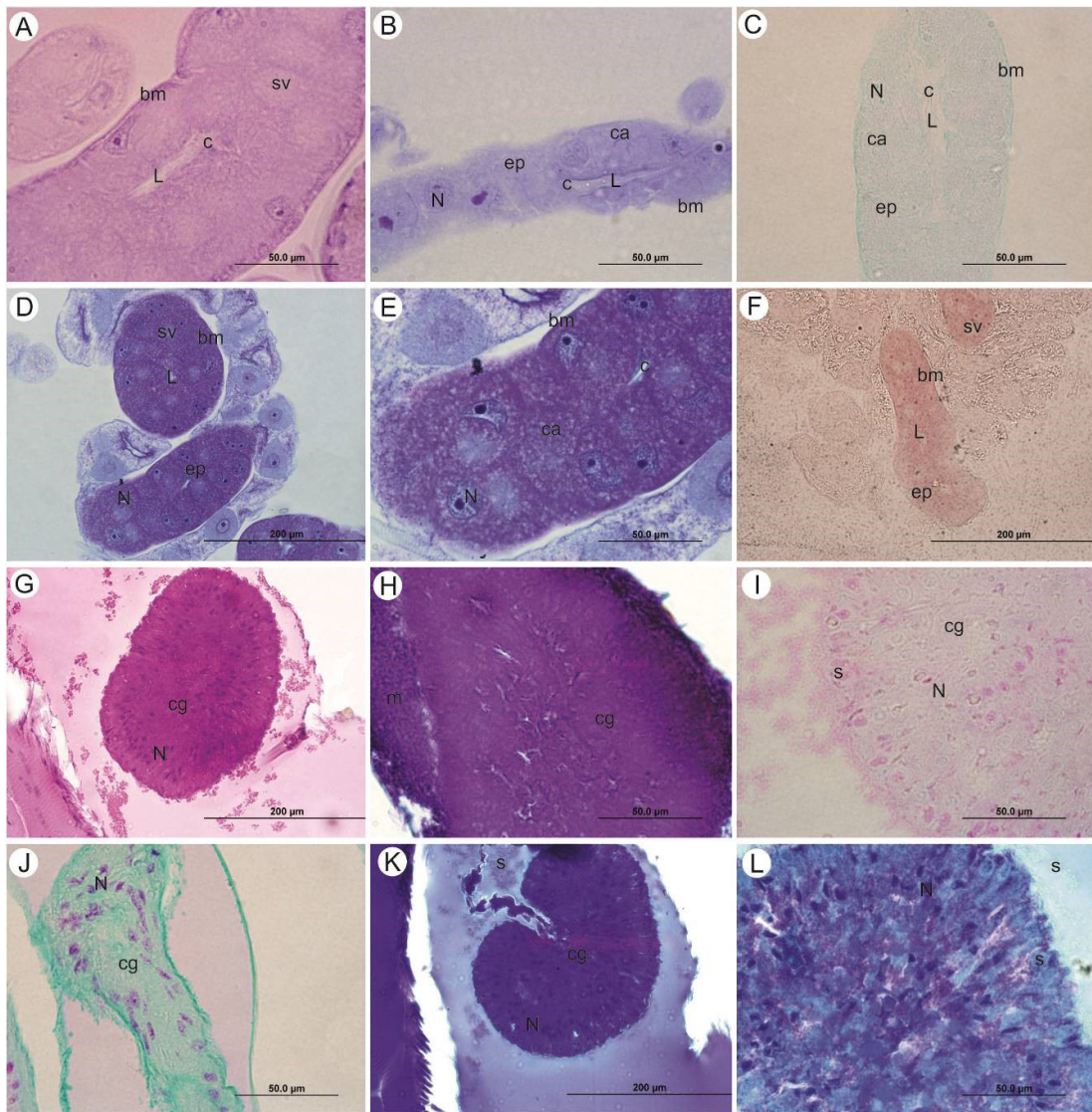


Figure 3. Histological sections of the venom gland of *Polybia paulista* reacted with Hematoxylin and Eosin (A), Bromophenol Blue (B), PAS reaction (C), Toluidine Blue (D and E) and Sudan Black (F). Histological sections of the convoluted gland of *Polybia paulista* reacted with Hematoxylin and Eosin (G), Bromophenol Blue (H), PAS reaction (I), Feulgen reaction (J), Toluidine Blue (K and L). N - secretory cell nucleus, c - cuticle, ca - canaliculus, L - lumen, ep - epithelium, bm - basal membrane, sv - secretion vesicle, s - secretion, cg - convoluted gland, m - muscle layer of the reservoir.

stained, clearly indicating the presence of secretion in these areas (Figure 1H).

Interpreting the data obtained through the PAS technique, used to highlight the presence of polysaccharides, the secretion in the convoluted gland of all of analyzed species was also positive. We can show the presence of secretion in this structure, especially in the portions near the lumen of the reservoir a result that allows us to infer that the convoluted gland of wasp contributes to the composition of the secretion produced by the venom gland (Figures 1I, 2I and 3I).

When subject to Feulgen reaction we found that the nuclei of cells of *P. versicolor* and *A. p. palipes* had a more defined shapes while in *P. paulista*, the nuclei exhibited a more elongated shape (Figures 1J, 2J and 3J). Based on the material stained with Toluidine Blue, which aims to identify acid structures, particularly nucleic acids, the secretions had a dark tone due to the acidity of this substance (Figures 1K, 2K and 3L). Furthermore, secretory vesicles could be visualized in the venom glands of *P. paulista* (Figure 3D). By Sudan Black technique, specific for lipids, we observed the presence

of secretory vesicles in the venom gland of *P. versicolor* and *P. paulista* (Figures 1F and 3F). Based on the analysis of the convoluted glands of the wasps, darker portions are viewed compared to the secretion present around this structure (Figures 1L and 2L). Thus, we conclude that there are granules of lipids in the epithelium of this gland. In *A. p. palipes* is evident the presence of these lipids in the apical portions of cells, near the lumen, also indicating that they are part of the constitution of the venom.

DISCUSSION

In this work, the results have shown that despite some morphological variations found in the venom glands of the wasps, in general, the cellular structure remains the same. As described by Snodgrass (1956), this structure consists basically of a thin tubular secretory that flows into a reservoir, which connects to sting through a duct excretory.

For bees, many studies have been conducted to describe morphologically the venom gland (Owen and Bridges, 1976; Bridges and Owen, 1984). The epithelial organization in two layers of cells, the cuticle covering the lumen of the gland and the canaliculi could be seen in the venom gland of the three species of wasps studied in this work, demonstrating in this way, the close similarity between this structure in these three species of wasps and also the resemblance in relation to bees.

Our results also showed that the convoluted gland secretion produced is apparently more acidic than the secretion in the lumen of the venom gland, being this, another indication of the contribution of the convoluted gland for the final composition of the venom. In bees it was also shown: according to Abreu (2000), the secretion contained in the reservoir is basic, but is altered at the time of the sting so that it appears acidic. Based on this work, this change in the secretion is due to the contribution of a structure present in the dorsal wall of the reservoir that contains the venom. Based on this hypothesis, studies have suggested that the venom can be activated by enzymes produced inside the reservoir (Ratcliffe and King, 1969).

Secretory cells of the venom gland of bees can stock the secretion produced in form of vesicles/hyaline spheres or dense granules. Thus, this secretion would be

responsible for determining the basic characteristic of the cytoplasm. Furthermore, the large amount of RNA present in this structure contributes to the basophilic character of the cell (Cruz-Landim et al., 1967).

Our results showed that these secretory vesicles, as well as in bees, are also observed in the venom gland epithelium of wasps, especially in *P. versicolor* and *P. paulista*. In addition, the venom gland of these species after subjected to the techniques described in this work also showed a basic characteristic when compared to the convoluted gland.

In the present work, by Bromophenol Blue, we observed proteins in the venom gland and in the canaliculi of *P. versicolor*, *A. p. palipes* and *P. paulista*. Analyzing the photomicrographs reacted with PAS, we can note that the convoluted glands of these three species were strongly stained, being very noticeable the presence of polysaccharides in the secretion synthesized and stored in the reservoir, corroborating, in this way the results obtained by Britto and Caetano (2005), that also visualized in the venom gland of *P. versicolor* positive reaction for proteins, that were found in the cytoplasm in the form of vesicles homogeneously distributed; and positive reaction for PAS in the convoluted gland. We showed in this work that the canaliculi, structures that transport the secretion, stained positive for polysaccharides.

Our results confirm those of these same authors with respect to the shape of the convoluted gland cells, which have a columnar appearance with more elongated nuclei, consistent with the shape of the cell. According to them, the faces of these apical cells are directed into the lumen of the reservoir, an area where there is an accumulation of secretion.

Furthermore, we identified portions of the lipids in the convoluted gland of the wasps, mainly in the apical cells and in the secretion founding the lumen of the reservoir, which enhances the results by Britto and Caetano (2005). Oliveira et al. (1999) and Abreu (2000) proposed that the lipids may function to activate the proteins. Thus, when the venom is injected into the tissue, these lipids interact with phospholipids on the plasma membrane, thus facilitating the transport of toxins through the intracellular environment. Based on a work by Kabara and Fischer (1969) apud Mohamed et al. (1983) the main function of the lipids present in the venom of

animals is to facilitate the distribution of toxins in the body and the penetration of toxic peptides into cells. Other researchers have shown that the presence of lipids enhances the lethality of the venoms of some animals (Marie and Ibrahim, 1976).

As proposed by Britto and Caetano (2005) to *P. versicolor*, in *A. p. palipes* and *P. paulista* the secretion synthesized by the tubular portion of the venom gland can be modified through the components produced by the convoluted gland, which are found in the reservoir. The composition of the secretions suggests that they are being eliminated from the convoluted gland toward the lumen of the reservoir. Thus, based on the results obtained and in pre-existing works we conclude that this gland contributes to the production of the venom.

CONCLUSIONS

The results obtained from this study allow us to conclude that despite some variations, the venom gland and the convoluted gland of the wasps *P. versicolor*, *A. p. palipes* and *P. paulista* show great similarity related to cellular organization. In addition, the present study shows that components produced by the convoluted gland modify the secretion synthesized by the tubular portion of the venom gland. These results suggest the importance of establishing the morphological and histochemical characteristics of these structures mainly due to the lack of information about them on wasps. In addition, the data obtained in this work may assist in a future pharmacological application of venom and/or bioprospection of venom of social wasps.

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