SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Comparative Analysis of Morphological, Structural and Morphometric Patterns of *Polistes versicolor* (Olivier) (Hymenoptera: Vespidae) Hypopharyngeal Glands

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Análise Comparada do Padrão Morfológico, Estrutural e Morfométrico das Glândulas Hipofaríngeas de *Polistes versicolor* (Olivier) (Hymenoptera: Vespidae)

RESUMO - O presente estudo descreve, por meio de técnicas de histologia e ultramorfologia, os padrões morfológico e estrutural das glândulas hipofaríngeas de *Polistes versicolor* (Olivier), comparando-os aos de outros grupos de Hymenoptera. Além disso, analisa a presença de variações intraespecíficas dessas glândulas por meio de análises morfométricas das células secretoras de vespas em diferentes idades. Na espécie estudada foi constatada a presença de glândulas com características primitivas, possuindo células secretoras que desembocam individualmente na placa hipofaríngea. O padrão morfológico encontrado nas glândulas hipofaríngeas foi basicamente o mesmo para todas as vespas estudadas, entretanto o comprimento das células glandulares apresentou variações significativas entre indivíduos. Aparentemente essas variações não estão relacionadas com a idade.

PALAVRAS-CHAVE: Vespa, sistema salivar, evolução, morfologia, morfometria

ABSTRACT - Using ultramorphological and histochemical techniques, this study describes the structural and morphological patterns of *Polistes versicolor* (Olivier) hypopharyngeal glands, comparing them with patterns of other Hymenopteran groups. Besides, the presence of intra-specific variation was evidenced by morphometric analysis of the secretory cells in wasps with different ages. The studied species presented glands with primitive characteristics, with secretory cells discharging individually in the hypopharyngeal plate. The morphological pattern found in hypopharyngeal glands was basically the same for all studied wasps, however, the length of glandular cells showed significant variations between individuals. Apparently these variations are not related with age.

KEY WORDS: Wasp, salivary system, evolution, morphology, morphometry

The Hymenoptera possess a series of glands connected to the bucal cavity, which together constitute the salivary system, formed by the thoracic salivary glands, the mandible and the hypopharyngeal glands (Bordas 1895). Some of these glands show as simple epidermic modified cells, whereas others present more complex morphology (Cruz-Landim 1967). The hypopharyngeal glands may present morphological patterns that vary from differentiated epidermic cells to well defined organs (Cruz-Landim & Costa 1998).

Most of the studies on hypopharyngeal glands were carried out with *Apis* (Cruz-Landim 1967, Crailsheim & Stolberg 1989, Knecht & Kaatz 1990) and some meliponine (Cruz-Landim 1967, Cruz-Landim *et al.* 1987), whose gland morphology and performance are very well studied. In these insects, the hypopharyngeal glands are more developed in the workers caste, mainly while they are taking care of the brood. During this phase, they are named nurses and they offer the gland secretion as food to all individuals (Szoldertz & Crailsheim 1993). After the nurse phase, the workers begin to forage and their hypopharyngeal glands stop producing food and reduce. However, they remain active, and produce digestive enzymes like invertase (Maurizio 1959, Simpson *et al.* 1968).

In the ants, the hypopharyngeal glands are, among those from the salivary system, the less known functionally (Caetano *et al.* 2002). In *Camponotus (Myrmothrix) rufipes* (Fabr.), the hypopharyngeal glands reach their highest development in the queen, are small in the workers and even smaller in the males. Its function in the ants is not known. However, it is possible that its secretion is ingested as food, helping in the digestion process, or may be regurgitated to feed other individuals of the colony (Gama 1985).

Studies with hypopharyngeal glands of other social

insects considered primitive, like those from the *Polistes* genus, are rare. Differently from the more derived groups, hierarchy occurs in a linear way and there are no morphological differences between castes in *Polistes*. The main factor that determines the social organization occurs by means of confrontations and physical aggression, where the dominant wasp (queen or α female) becomes the main responsible by egg laying, whereas the subordinates (workers) stay in charge of the remaining tasks (Pardi 1948).

Thus, ultramorphological and histological analyses of the hypopharyngeal glands of *Polistes versicolor* (Olivier) are here accomplished, and their morphological pattern is determined and compared with those described in the literature. Besides, the occurrence of variations in the gland cells among individual of distinct ages is verified by morphometry. These data may help to infer on the role of these glands in the colonies of primitive eusocial insects.

Material and Methods

The *P. versicolor* specimens were collected in colonies present at the Universidade Estadual Paulista, campus of Rio Claro (22°23'31,5" S; 47°32'63,8" W), and their hypopharyngeal glands were removed and examined by ultramorphological, histological and morphometric techniques.

The wasps were anaesthetized at low temperature and dissected under stereomicroscopy. Dissecting was performed directly in 4% paraphormaldeyde, where the material was maintained for 2h for fixation.

The glands destined to the ultramorphological analyses were dehydrated in a crescent series of acetone (50% to 100%) and taken to the Balzers critical point CPD 030, for complete dehydration. The glands were then placed on a double-faced tape over a metallic support for scanning electron microscope (SEM), and covered with carbon and gold in the Balzers Vaporizer SCD 050. Observation and photography were performed in the SEM Jeol P15.

In order to carry out the histological analyses, the glands were transferred from the paraphormaldeyde to a buffer sodium phosphate solution (pH 7.4), where they were kept for 24h. After this procedure, the glands were dehydrated in ethanol solutions at crescent concentrations (70, 80, 90 and 95%) for about 30 min in each solution, and embedded in Leica resin for 24h at 4°C before inclusion. Resin polymerisation was performed in a chamber at 37°C. The material was longitudinally and transversally sliced, with 5 μm thickness, and the cuts were placed on histological slides and stained with haematoxylin and eosin (H.E.), mounted and, afterwards, analysed and photographed under a Zeiss photomicroscope.

For the morphometric analyses, 30 specimens were used, previously marked with paint for aeromodelism, to determine their ages. The wasps were divided in five age groups named: newly emerged, 10 days old, 20 day old, 30 days old and over 30 days (n = 7 wasps per group). After dissection, the hypopharyngeal glands were transferred to excavated slides, with paraphormaldeyde, and analysed under Axioskop microscope, provided with Axiohome system, measuring the diameter of 10 secretory unities per gland. All the data were plotted and analysed in a computer, using the statistical software Sigma Stat, were the means and standard errors were obtained, and the variance analysis tests were performed to detect the occurrence of significant differences among the gland cells of each wasp and among age groups.

Results

The hypopharyngeal glands in *P. versicolor* are found in the oral cavity (Fig. 1). All studied glands are paired structures composed by a group of individual secretory unities, connected to the pharynx, under a thicker cuticle region, named hypopharyngeal plate (Fig. 2), where associated muscular fibbers are seen (Fig. 2A). Besides, a furrow placed between the secretory portions is clearly seen on the plate (Fig. 2B).

Each secretory unity is formed by a single spherical cell

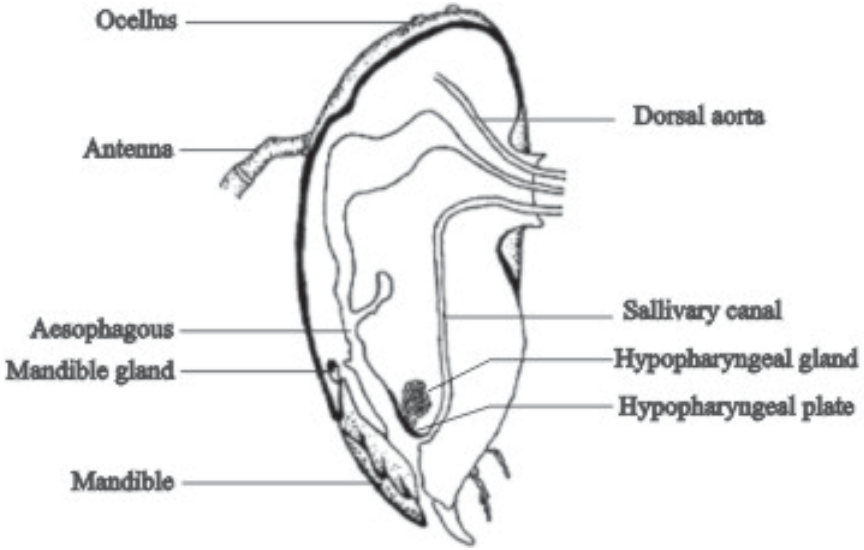

Figure 1. Scheme of a longitudinal cut of *P. versicolor* head, showing the site of the hypopharyngeal glands.

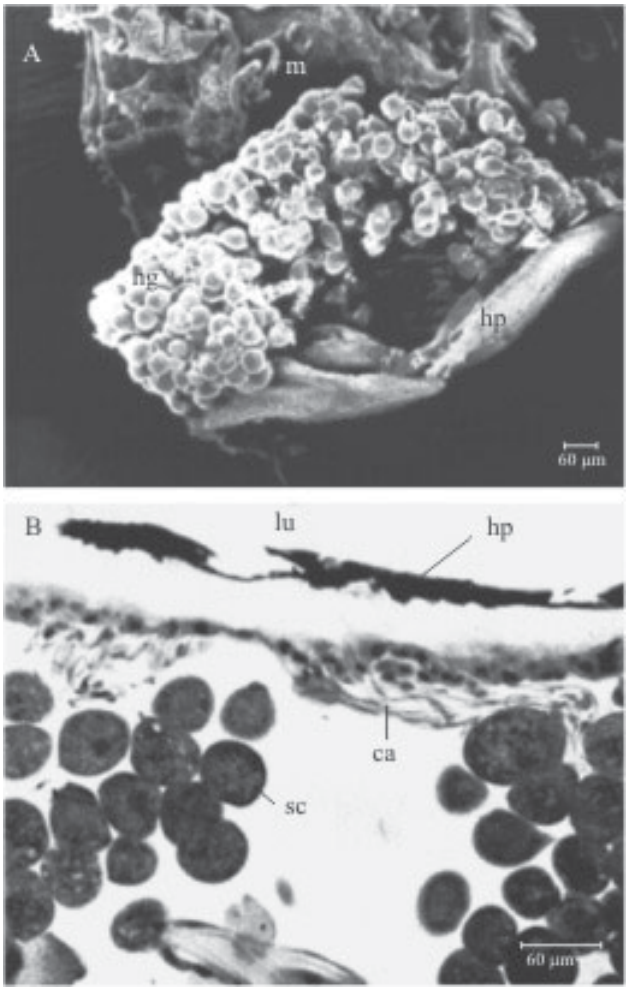

Figure 2. General aspect of the hypopharyngeal glands: (A) total mounting; (B) transversal cut. In B, the two portions of the gland are seen. (ca – canaliculus; sc – secretory cell; hg – hypopharyngeal glands; lu – pharyngeal lumen; m – muscle; hp – hypopharyngeal plate).

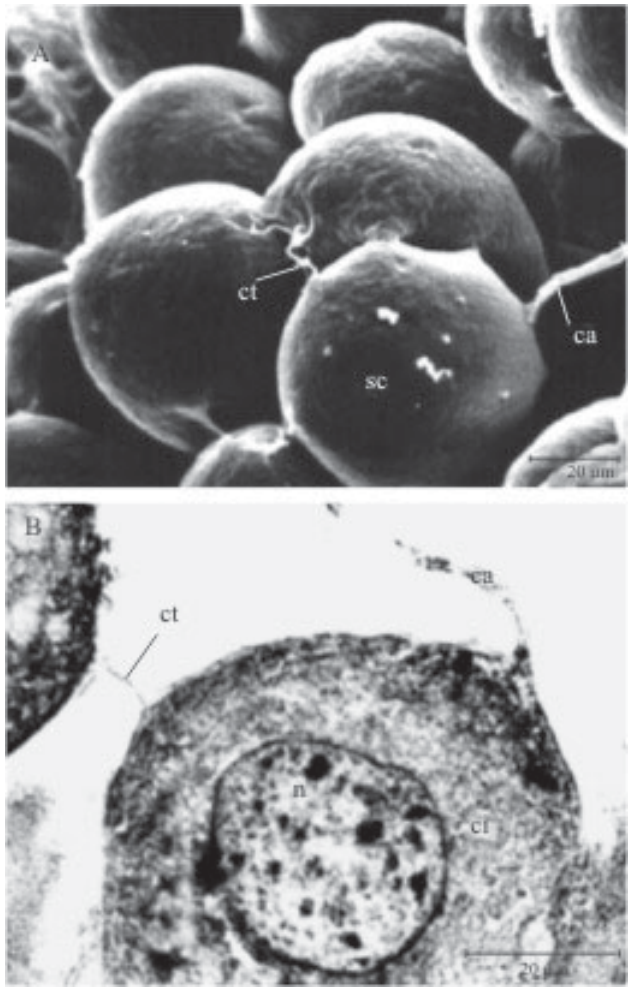

Figure 3. (A and B) Secretory cells of the hypopharyngeal glands. Observe the canaliculi collector of secretion connected to the cells and the conjuntive tissue among them. (ca – canaliculus; ci – citoplasma; sc – secretory cell; n – nucleus; ct – conjuntive tissue).

(Figs. 2 and 3), which contains a well-developed and conspicuous nucleus, with high quantity of nucleoli (Fig. 3B). A thin canaliculus that collects secretion connects each cell to the hypopharyngeal plate (Fig. 3), goes through it and opens on the external surface of the plate, in the oral cavity, by means of pores (Figs. 4A, 4C and 4D).

The group of secretory cells in each side of the hypopharyngeal plate takes the aspect of a bunch surrounded and attached by a conjuntive tissue. The sorrounding tissue presents as filaments between the cells (Fig. 3).

Bellow the hypopharyngeal plate, the epithelium is more developed just where it is crossed by the canaliculi of the secretory cells (Figs. 4A and 4B). Also, in this region, the endocuticle widens, in such a way that the epithelium presents well detached from the exocuticle, highly esclerotized (Figs. 4A and 4B). The epithelium cells, normally flat and with fusiforme nucleus in the rest of the epithelium, change into cubic, with spherical nuclei (Fig. 4B).

In the hypopharyngeal plate, turned to the oral cavity, there are numerous filiforme cuticle projections, disposed before to the pore region where the secretion is released (Figs. 4C and 4D).

Besides the morphological observations, the diameter of 10 secretory unities per wasp was calculated in 30 wasps from different age groups. In each wasp group, the mean diameter of the gland cells significantly varied (Table 1). The differences are considered significant according to the variance analysis, with $P < 0.05$ (newly-emerged $F = 3.15$ g.l. $= 6$ $P = 0.0144$; ten days $F = 4.29$ g.l. $= 6$ $P = 0.0011$; 20 days $F = 5.37$ g.l. $= 6$ $P = 0.0002$; 30 days $F = 6.33$ g.l. $= 6$ $P < 0.0001$; over 30 days $F = 16.8$ g.l. $= 6$ $P < 0.0001$).

The individuals with cells whose mean diameter are significantly different from the others by the Newman-Keuls test, are about 10 µm different, as observed in all age groups (Table 1). Nevertheless, the variance analysis among groups showed different results, and no significant differences were found ($F = 1.8$, g.l. $= 4$, $P = 0.1541$).

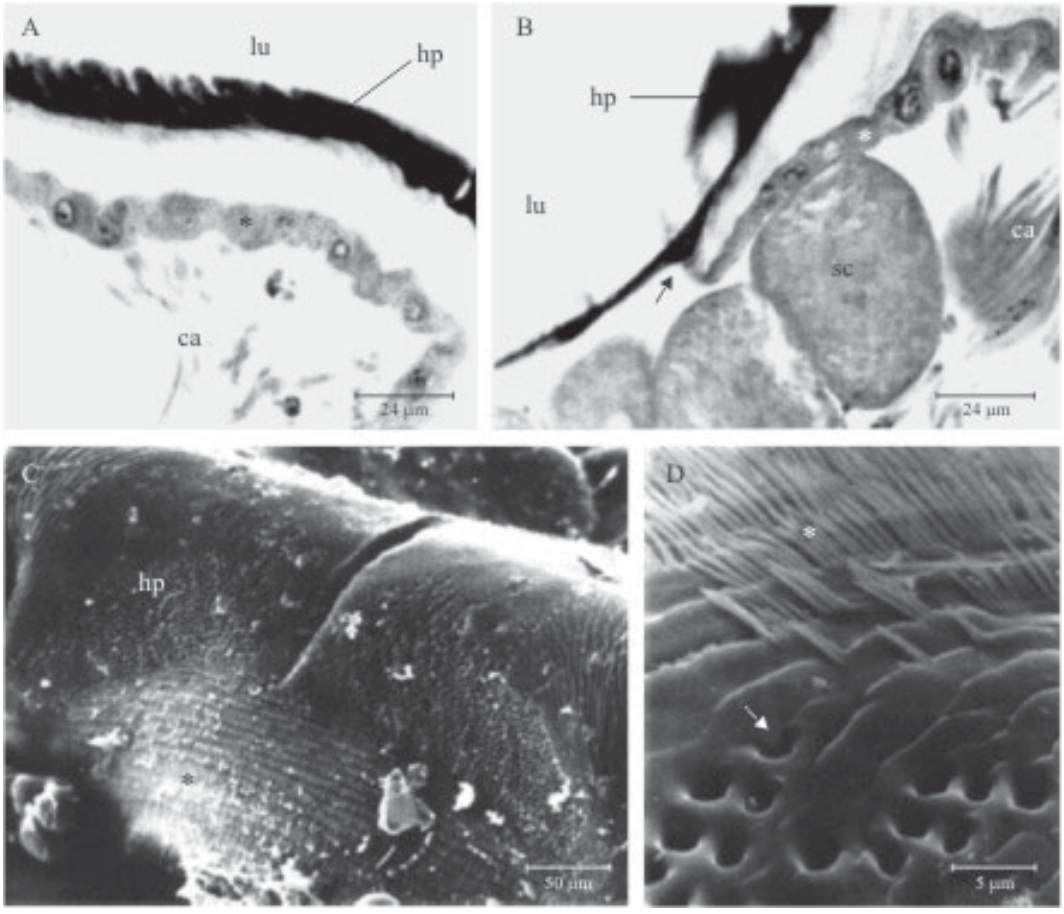

Figure 4. (A) Junction of the canaliculi into the hypopharyngeal plate. Note the canaliculi crossing the plate and the cubic epithelium under (*). (B) Transition area from plane to cubic epithelium – arrow. Note the epithelium (*) coming off the hypopharyngeal plate. (C) External face of the hypopharyngeal plate, turned towards the bucal cavity, with pores and lined with a series of cuticular filiform projections (*). (D) Detail showing the pores where the secretion is released (arrow) and the cuticular filiform projections (*). (ca – canaliculus; sc – secretory cell; lu – pharyngeal lumen; hp – hypopharyngeal plate).

Discussion

The ultramorphological analysis of the hypopharyngeal glands of *P. versicolor* revealed that they are composed by secretory unities directly and individually linked to the hypopharyngeal plate by mean of their canaliculi. These morphological aspects were considered primitive by Cruz-Landim & Costa (1998). According to these authors, hypopharyngeal glands with these characteristics are named type 1 glands; other three types (2, 3 e 4) are described and related to an evolution sequence. After the type 1, the hypopharyngeal plate invaginated in the area where the collector canaliculi are connected, so forming the type 2. In a sequence, the invagination prolonged and originated an axial

Table 1. Mean diameter (µm) of the secretory cells of the hypopharyngeal glands of wasps in different age groups. (n = 10)

Newly emerged		10 days old		20 days old		30 days old		Over 30 days	
Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
49.6	1.37	55.8	1.35	53.0	1.47	51.7	2.01	49.6	1.37
53.2	1.39	57.9	1.15	54.5	2.03	52.0	2.38	52.3	1.16
53.3	2.90	58.7	2.22	55.5	1.32	54.3	1.98	59.2	2.34
54.1	1.35	60.0	1.07	57.9	2.05	56.3	1.35	60.0	1.07
54.5	2.03	61.3	2.14	60.2	1.41	59.8	2.25	62.3	1.68
57.9	2.05	65.8	2.85	60.3	1.40	61.2	1.37	65.0	1.99
58.7	2.22	68.6	2.88	65.0	1.99	66.1	2.27	70.9	1.81

canal, where the canaliculi are linked (type 3) and, at last, the axial canal extended even more, becoming 1 cm long (type 4).

In general, the function of the hypopharyngeal glands can only be inferred indirectly, by relating their morphology and degree of development to the activities of the individuals and their behavioural characteristics, and also by analogy with *Apis mellifera* (L.), where the function of these glands is relatively well established (Simpson 1960). In this species, they are only present in the worker and they are related to the production of royal jelly (Knecht & Kaatz 1990). From the known hypopharyngeal glands, those belonging to *Apis* are among the most derived, the type 4 glands (Snodgrass 1956, Cruz-Landim 1977, Costa & Cruz-Landim 1977).

It seems that the evolution of the glands type 1 towards the type 4 may have happened independently, within the different groups. Also, the development of the hypopharyngeal glands do not keep a strict correlation with the phylogeny of the Hymenoptera, but do keep with the degree of sociability of the species and the function of the individual in the colony (Cruz-Landim & Costa 1998).

Even among the bee species, the lower the degree of sociability, the more the morphology of the hypopharyngeal glands tends to type 1. For instance, this kind of gland was found in several solitary bees (Cruz-Landim 1967).

In other Hymenoptera variations related to the species biology are also encountered. Among the Sphecidae, Sphecinae possess type 1 glands and in several aspects resemble to wasps, while Nissoninae present glands type 3 and 4 and behave more like social bees (Saenz & Cruz-Landim 1972). Among the ants, *Dinoponera australis* (Emery), considered primitive, presents hypopharyngeal glands type 1 (Caetano *et al.* 2002). In *C.* (*Myrmothrix*) *rufipes*, considered derived and possessing a more biologically complex social organization, the glands are composed by secretory cells linked to a single excretory duct. Besides, in this species, the development of the glands is differentiated among castes and between sexes (Gama 1985). However, in some species of Polistinae wasps considered highly derived, as in *Polybia* species, the hypopharyngeal glands possess the same pattern encountered in *P. versicolor* (Cruz-Landim & Saenz 1972), although the studies in this genus were held only with workers.

In relation to the secretory unities, *P. versicolor* presents the same morphological pattern described in the literature for other insects (Cruz-Landim 1967, Cruz-Landim & Saenz 1972, Gama 1985). Its hypopharyngeal glands are constituted by spherical cells with large and central nuclei, with high quantity of nucleoli, evidencing high activity of synthesis. These cells were well developed in all analysed individuals, what indicates that they must have a relatively important role for these wasps.

The diameter of the gland cells of *P. versicolor* varied from about 50 μm to 70 μm within the individual, with significant differences among wasps with the same age. However, the variation in the gland length showed no relation with age, as shown by the statistical analyses.

Morphometric studies held with *Scaptotrigona postica* (Latreille) showed a relation between age and the development of the hypopharyngeal glands (Costa & Cruz-Landim 2000). However, this relation may occur due to the synchrony on the development of the workers' activities during their lives (Michener 1974), i.e., workers of the same age perform the same tasks, therefore, they possess hypopharyngeal glands in similar stages. So, the activities performed by the bees are those related to the stimuli for the gland development and not the age. This fact can be proved when bee glands that are under degenerative process restart secretion production when the bees are induced to return to take care of the brood (Gracioli *et al.* 1999). In *P. versicolor* there is not a defined relation between age and the repertoire of activities of the colony (Zara & Balestieri 2000). In this case, individuals of the same age can carry out different activities and, so, they can have glands in different stages of development.

The cellular length of newly emerged wasps also varied, what may result from events held before adult phase. West-Eberhard (1969) observed that the larvae of *Polistes* might receive food in different manners, in order that those well fed showed bigger after emergence. Perhaps the same factor could interfere on the development of the hypopharyngeal glands of *P. versicolor* during the larval phase.

As it seems, the hypopharyngeal glands are very important in the biology of social insects, because they possess a plasticity that follows the evolution of social life in different groups. So, the more complex the type of social organization is, the higher is the morphological differentiation of the hypopharyngeal glands. The morphological pattern of the hypopharyngeal glands of *P. versicolor*, which is considered a key species to understand the evolution of the social insects (Evans 1958), is in a primitive stage and, at least externally, shows identical among individuals. The variation encountered in the cellular length of the glands showed no relation with age, but may be involved with the single activities performed by the wasps.

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