SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Ultramorphology and Histology of the *Polistes versicolor* (Oliver) (Vespidae) Thorax Salivary Gland Compared with Other Hymenoptera

THALITA ROCHA AND FLÁVIO H. CAETANO

Depto. Biologia,Universidade Estadual Paulista "Júlio de Mesquita Filho", Instituto de Biociências Av. 24A, 1515, Bela Vista, 13506-900, Rio Claro, SP

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Ultramorfologia e Histologia da Glândula Salivar do Tórax de *Polistes versicolor* (Olivier) (Vespidae), Comparada com a de Outros Hymenoptera

RESUMO - O sistema salivar dos Hymenoptera é constituído pelas glândulas mandibulares, hipofaríngeas e salivares do tórax. É de grande importância por estar relacionado a diversos aspectos da vida destes insetos, como produção de feromônios, alimentação da cria, digestão dos alimentos e construção do ninho. Indivíduos adultos de *Polistes versicolor* (Olivier) foram dissecados, as glândulas salivares do tórax retiradas e processadas para exames com microscopia eletrônica de varredura e histológico. A glândula salivar do tórax de *P. versicolor* apresenta as unidades secretoras alveolares, sendo constituída por pseudoácinos e não apresenta reservatório. Quatro tipos de células estão presentes na glândula. As células T1 e T2, constituem os pseudoácinos e, diferem entre si, principalmente, pelo grande número de vesículas secretoras em T2. Na base dos dutos coletores da glândula há um agrupamento de células T3, também com características secretoras. A secreção produzida nos pseudoácinos é conduzida por canalículos e dutos ao exterior, sendo os últimos constituídos por células T4. A comparação dessas características com as de diferentes espécies de Hymenoptera, já estudadas, demonstrou que as glândulas salivares do tórax não podem ser utilizadas como único fator de comparação em estudos evolutivos.

PALAVRAS-CHAVE: Vespa, sistema salivar, morfologia, tipo de células

ABSTRACT - The salivary system of the Hymenoptera consists of the mandible, hypopharynx and thoracic salivary glands. It is very important because it is related to various aspects of the life of the insects, such as pheromone production, feeding the young, food digestion and nest building. Adult *Polistes versicolor* (Olivier) individuals were dissected, the thoracic salivary glands removed and processed for scanning electronic microscopy and histological examination. The *P. versicolor* thoracic salivary gland presents alveolar secretory units, consists of pseudoacines and does not have a reservoir. Four types of cells are present in the gland. The T1 and T2 cells make up the pseudoacines and differ mainly by the many secretory vessels in T2. There is a cluster of T3 cells at the base of the gland duct collectors, also with secretory characteristics. The secretion produced in the pseudoacines is conducted by canals and ducts to the outside, and the latter are made of T4 cells. The comparison of these characteristics with those of different Hymenoptera species, already studied, showed that the thoracic salivary gland cannot be used as a single comparison factor in evolutionary studies.

KEY WORDS: Wasp, salivary system, morphology, cell type

The Vespidae family is divided into 11 subfamilies, including the Polistinae subfamily (Spradbery 1973). Among the 29 genera of this subfamily the *Polistes* genus is the most studied. It has been considered a 'key genus' for the understanding of the evolution of social insects and of wasp societies (Evans 1958) because of the small differentiation among the casts and its wide distribution throughout the tropical region of the old and new worlds (Richards 1978). *Polistes versicolor* (Olivier) is one of the most widespread species in South America and is common in several Brazilian states, especially São Paulo (Gobbi & Zucchi 1980).

Similar to other insects, the Hymenoptera present glandular structures embryonically linked to the mouth parts that form the salivary system (Chapman 1975). The components of this system are the mandible, hypopharynx and thoracic salivary glands. In ants in addition to these glands there is the post pharynx gland (Gama 1985, Caetano *et al.* 2001).

The thoracic salivary gland in Hymenoptera is also called

the labial gland or simply the salivary gland and is associated with the dilution and lubrication of food and nest building (Spradber 1973). According to Landolt & Akre (1979) the secretion eliminated by this gland in wasps is related to production of the 'glue' used to keep the cell walls together that form the nest and also for the production of the material that forms the peciole that sustains it. However Edwards (1980) believed that, because this gland is well developed in old dominant females and males, which do not act directly in nest building, the thoracic salivary gland is not related to this function alone.

The salivary gland system, including the thoracic salivary gland, has anatomical and histological variations through which the evolutionary lines followed by these organs can be established within the group (Cruz-Landim 1967), and they can in certain cases be correlated with cast or species adaptations (Cruz-Landim & Saenz 1972).

Thus studies of the thoracic salivary gland not only in *P. versicolor* but also in other Hymenoptera species are important because the characterization of the gland and its structures simplifies recognition of the group to which it belongs and also supplies information on understanding its function. The comparison of these glandular structures among the different Hymenoptera groups can help in the evolutionary understanding of the species and their possible co-evolutionary relationships.

The objective of the present study was to investigate the ultramorphology and histology of the *P. versicolor* thoracic salivary gland based on scanning electronic microscopy and histology techniques in an attempt to determine the possible cell types that form it and compare it with different Hymenoptera species.

Material and Methods

Adult *P. versicolor* individuals were collected in Rio Claro – SP – Brazil (22°24'36"S; 47°33'36"W) anesthetized at low temperature (4°C) and processed according to the Scanning Electronic Microscopy (SEM) and Histology techniques for examination under light microscope (LM).

Ultramorphology. The thoracic salivary glands obtained were kept in modified Karnovisky fixing solution (84 ml/L glutaraldeyde 25%, 500 ml/L paraphormaldeyde 8%, buffered with sodium phosphate buffer 0.2M – pH 7.3 q.s. to 1 L) for 2h, dehydrated in ethanol and then taken to the critical point (Balzer CPD 030) for complete dehydration. The material was fixed on metal supports and covered with gold with a spray Sputtering Balzer SCD 050 for observation under SEM, JEOL JMS P15.

Histology. The thoracic salivary glands obtained were kept in 4% paraphormaldeyde fixing solution for 2h, submitted to sodium phosphate buffer (0.1M - pH 7.4) for 24h and taken for dehydration in ethanol. The material was then blocked and cut 5 µm thickness and the histological slides stained with Harris Hematoxiline and Aqueous Eosin.

Results

The *P. versicolor* thoracic salivary gland is located anther-lateral-dorsally in the thorax. It is morphologically divided into four lobes, one pair located in the prothorax and the other in the mesothorax. Each lobe consists of four globular secretory units, called pseudoacines (Figs. 1 and 2), joined by conjunctive tissue (Figs. 1a, 1b and 2b) considered an alveolar type gland.

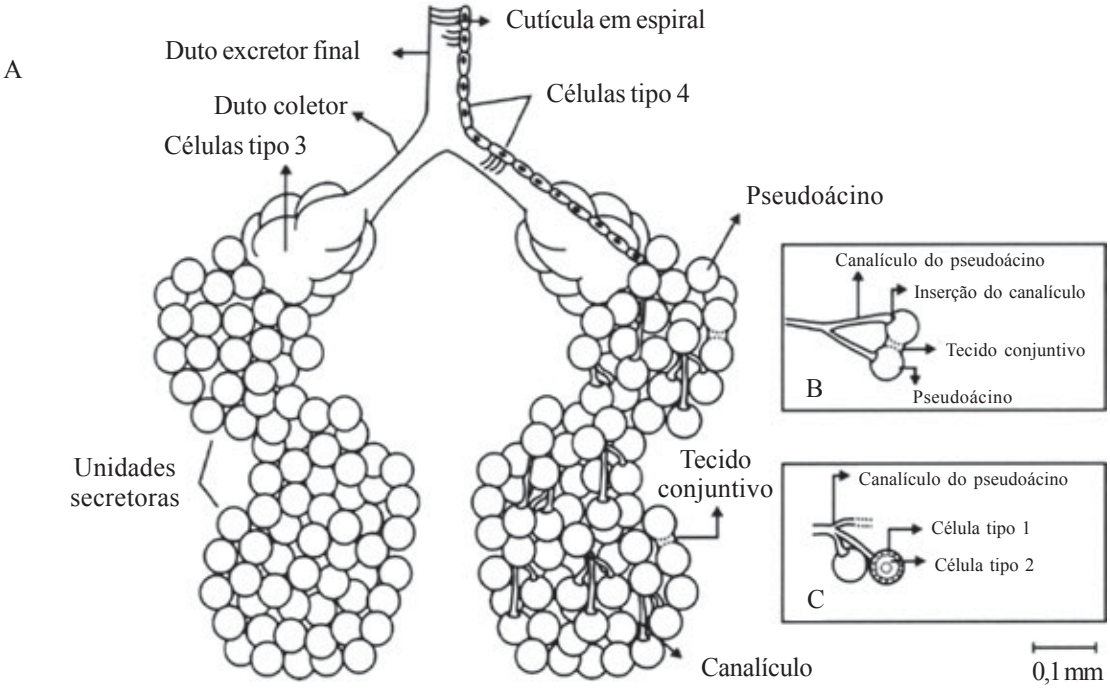

Figure 1. General scheme of the *P. versicolor* thoracic salivary gland.

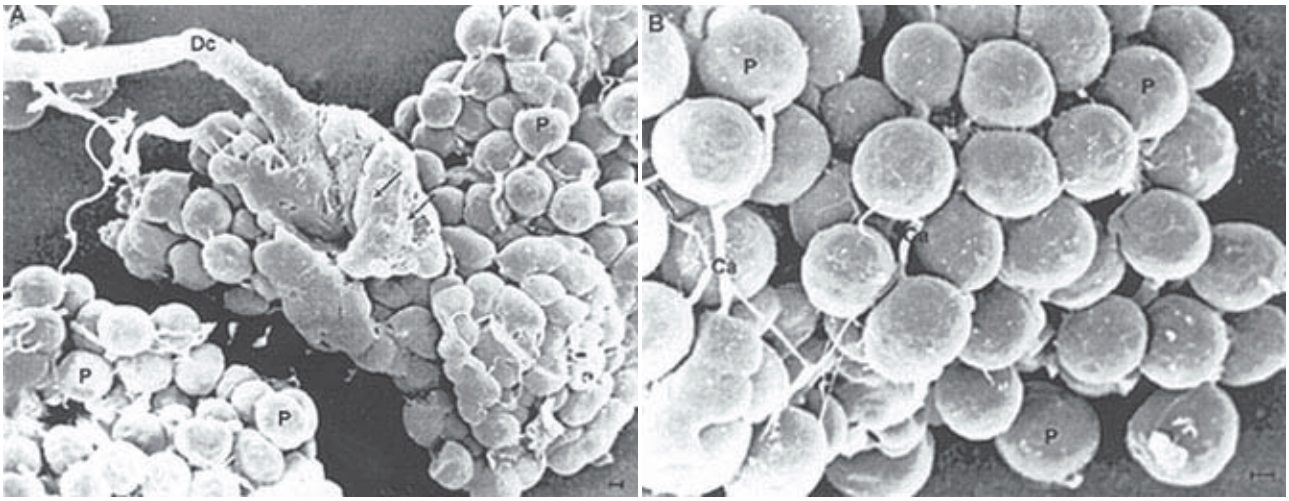

Figure 2. A) Differentiated cell clustering (arrows) to the gland collector duct (Dc). B) Detail of the secretory units showing the pseudoacines (P), and the canals (Ca). Scale: 1cm = 920X.

The pseudoacines are formed by a large central cell surrounded by an average of 11 peripheral cells (Figs. 1c and 3). The pseudoacine central cell, demoninated here as type 2 cell (T2) (Fig. 3) presents a rounded nucleus with many nucleoles. These cells, when in secretory activity, present irregular nucleus morphologically, due to the many secretory vessels (Fig. 3b).

The peripheral cells, type 1 cells (T1) are small, oval and stain weakly with H-E. Their nucleus is central and round and does not present secretory vessels in its cytoplasm (Figs. 3a and 4c).

The *P. versicolor* thoracic salivary gland does not have a reservoir. A cluster of cells was observed in the region close to the gland collector ducts, which presented different morphology from that observed in the secretory units (Figs. 1a, 2a and 4a). These cells, called type 3 (T3) (Fig. 4b) are large, rounded in shape and have an elongated nucleus, their cytoplasm presents secretory vessels (Fig. 4b) but in a smaller number compared to the T2 cells, when in secretory activity (Fig. 3b).

Canals can be observed surrounding the peripheral cells and the center of the pseudoacines (Figs. 3c and 3d) that form a single canal. These canals are projected from the pseudoacines (Figs. 1c, 2b, 3c and 3d) and merge forming the gland collector ducts (Figs. 1a and 2a) and these form the final single excretory duct (Fig. 1a).

The gland collector ducts and the gland excretory duct are formed by a single epithelium consisting of type 4 cells (T4) and are internally lined with chitin, with spirally placed reinforcements. This covering can also be observed in the canals that come from the pseudoacines (Fig. 4c).

Discussion

The location and aspect observed for the *P. versicolor* thoracic salivary gland is the same as reported for *Sphex johannis* Dalla Torre (Sphecidae), *Bicyrtes variegata* (Olivier) (Bembicini), *P. canadensis* L., *Polistes actaeon* (Haliday) (Polistinae) and *Polybia nigra* (Olivier), *Polybia occidentalis scutellaris* (Olivier), *Polybia sericea* (Olivier), *Protopolybia minutissima sedula* (Saussure), *Apoica pallida* (Olivier) (Epiponini) (Cruz-Landim 1967). However, eusocial bees (Cruz-Landim 1967) and some ants of the Myrmecinae and Formicinae subfamilies present a tubular secretory portion. Other bee and ant species (the Ponerinae, Dorylinae and Pseudomyrmicinae subfamilies) present a pseudotubular secretory portion, consisting of short pseudotubes (Cruz-Landim 1973, Gama & Cruz-Landim 1982) therefore, there is variation in the disposition and cell types that form the secretory portion of these glands in the Hymenoptera.

In studies carried out with bees, the most primitive thoracic salivary gland was described as a pseudotubular gland presenting short pseudotubes. Probably this type of gland would have given rise to the alveolar glands that are observed in the Vespidae and Sphecidae species studied to date and the tubular and pseudotubular form consisting of long pseudotubes, presented in the most derived Hymenoptera (Cruz-Landim 1973). Thus the shape of the secretory units in the thoracic salivary gland can be used as a trait for evolutionary inferences in the Hymenoptera group.

In wasps with alveolar thoracic salivary gland the secretory portion consists of acines formed by a large central cell, surrounded by a border of small flattened cells. Such morphology in the acines is conserved in all the wasp species in the Polistinae subfamily studied to date and is maintained, with little variation, in bees, in which the acines can present one or two central cells (Cruz-Landim 1967).

The *P. versicolor* thoracic salivary gland pseudoacines present canals, which collect the excretion produced by the central cell (T2). These canals give rise to the canal that comes from each pseudoacine.

The *P. versicolor* thoracic salivary gland ducts consist of a layer of flattened epithelium cells (T4) and the duct lumens is covered by a thin layer of chitin. This aspect of the duct is similar to that described for other wasps such as *Vespula pensylvanica* (Saussure) (Landolt & Akre 1979); *Tricolletes* bees (Cruz-Landim 1967) and ants of the Ponerinae, Dorylinae, Myrmecinae, Dolichoderinae and Formicinae subfamilies (Gama

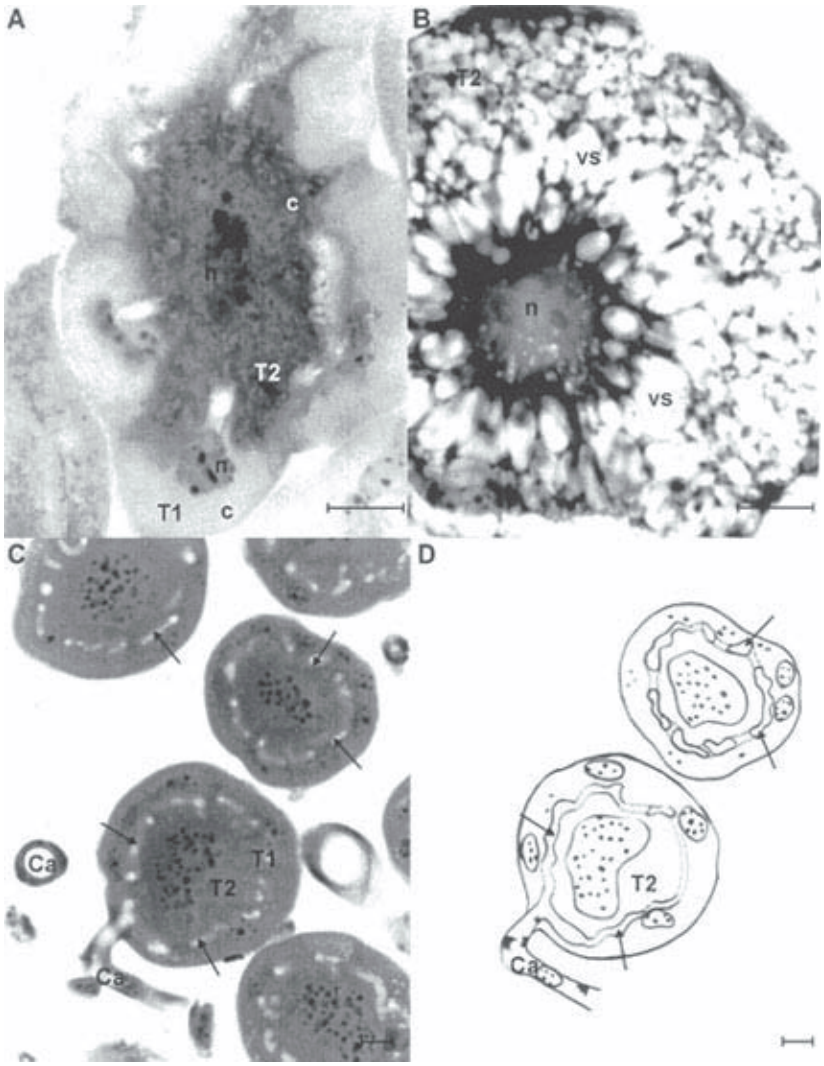

Figure 3. A) Pseudoacine. Detail of type 1 (T1) and type 2 (T2) cells. B) Detail of type 2 (T2) cell in secretory activity. (n) nucleus, (c) cytoplasm, (vs) secretory vessels. C) Detail of the intracytoplasmatic canal (arrows) present in the pseudoacines. (Ca) canal. D) Scheme of the previous figure. Scale: 1cm = 920X.

& Cruz-Landim 1982). Such data leads us to state that the morphology and histology of the ducts is conserved in the Hymenoptera group, considering the species studied up to now.

In *P. versicolor* as well as in the other Polistinae and Epiponini, the union of the gland duct collectors occurs in the thorax, giving rise to the final single excretory duct that leaves to the glossa (Cruz-Landim and Saenz 1972) and this same aspect is observed in *Dinoponera australis* (Emery) (Caetano *et al.* 2002). However, in *Bicyrtes* sp. and *Bombus* sp. the union of the duct collectors of the thorax salivary gland occurs in the head (Saenz & Cruz-Landim 1972).

The union of the duct collectors in the thorax to form the final single excretory duct is a trait present in *P. versicolor* and in more derived Hymenoptera species.

The T cell is responsible for producing glandular secretion, concentrated in the cytoplasm in the form of vesicles. Secretory function cannot be attributed to T1 cells bearing in mind their histological aspects.

The *P. versicolor* thoracic salivary gland presented a different cell clustering (T3) in the portion close to the gland collector duct and this structure has not been observed in wasps studied to date. Possibly these cells are responsible for the production of some type of secretion, mixed to that produced by the T2 cells at the moment of their exit to the final destination.

P. versicolor does not present a reservoir, as observed in other Polistinae (Cruz-Landim & Saenz 1972) and for most of the species of the Ponerinae and Myrmecinae subfamilies (Gam & Cruz-Landim 1982, Caetano *et al.* 2002). The *Rubrica surinamensis* (DeGeer) and *B. variegata* wasps present a dilation in the initial portion of the excretory duct of the gland (Saenz and Cruz-Landim 1972) similar to that found in *Bombus* sp. and *Apis mellifera* L. In *S. johannis* the reservoir is present in the portion close to the excretory duct (Saenz & Cruz-Landim 1972) similarly to ants in the Formicinae subfamily (Gama 1985).

The absence of reservoir, as observed in this study and

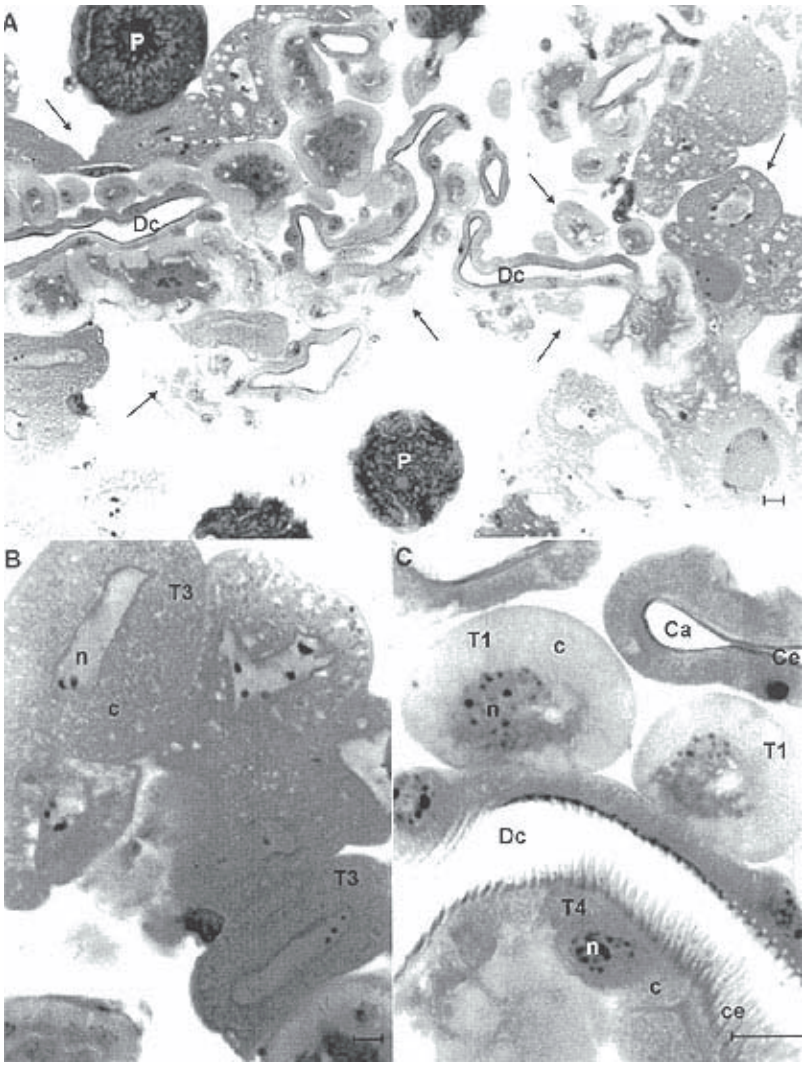

Figure 4. A) General view of the differentiated cell clustering (arrows) under the optic microscope. B) Detail of type 3 (T3) cell. C) Detail of the type 4 (T4) cells that form the gland collector duct (Dc). Observe the internal spiral lining of chitin in the collector duct and the canal. (n) nucleus ,(c) cytoplasm, (Ca) canal. Scale: 1cm = 920X

already described in the literature, can be considered a trait present in the primitive groups of Hymenoptera (Cruz-Landim 1967).

It could be concluded that the alveolar morphology and absence of reservoir are characteristics attributed to less derived groups of Hymenoptera and found in *P. versicolor*. The acine morphology described for *P. versicolor* is maintained in the Hymenoptera species that present alveolar thoracic salivary glands as well as the duct constitution. The presence of canals in the acines was also observed for other wasp species (Saenz 1972). However, the way in which these canals surround the pseudoacines was observed in the present study and in some Apoidea (Cavasin-Oliveira & Cruz-Landim 1998). Such characteristics enabled us to denominate the acines in the *P. versicolor* thoracic salivary gland as pseudoacines.

In spite of belonging to a base group among the Vespidae, the *P. versicolor* thoracic salivary gland also presents characteristics peculiar to derived Hymenoptera species. Thus it is hard to use the total gland structure in comparison among Hymenoptera species.

Acknowledgments

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