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ULTRASTRUCTURE OF THE THORACIC SALIVARY GLANDS OF *Polistes versicolor* (OLIVIER, 1791) (HYMENOPTERA, VESPIDAE)

Thalita Rocha and Flávio Henrique Caetano

Department of Biology, Institute of Biosciences, Paulista State University (UNESP), Rio Claro, SP, Brazil.

ABSTRACT

The salivary system of the Vespidae consists of mandibular, hypopharyngeal and salivary (thoracic) glands. Thoracic salivary glands are related to the foraging activities, adult-adult and adult-larvae trophalaxis, larval feeding and nest construction. The study of these glands is important to understand the mechanisms of secretion and their relation with behavioral habits. In this report we describe the ultrastructure of the thoracic salivary glands of adult *Polistes versicolor*. The glands were excised from anesthetized specimens and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate, pH 7.2, post-fixed in 1% OsO_4 in the same buffer and dehydrated in ethanol series. Transmission electron microscopy showed that the thoracic salivary glands had a secretory portion consisting of pseudoacini. Each pseudoacinose secretory unit included a central cell (TA) surrounded by several parietal cells (TB). From these pseudoacini, canaliculi arose and fused to form the main ducts (ducts 1, 2 and 3 of increasing diameter). The duct system was formed by epithelial type C (TC) cells, but lacked a reservoir. Type D (TD) cells occurred near the base of duct 2. These findings indicate that *P. versicolor* thoracic salivary glands consist of several cell types that have specific roles in secretion biosynthesis (TA), modification (TB, TC, TD) and transport (TC).

Key words: Hymenoptera, salivary system, thoracic salivary glands, ultrastructure, Vespidae

INTRODUCTION

Most winged insects have thoracic salivary glands that consist of a secretory portion and a common duct. In Hymenoptera, the salivary glands consist of several branched secretory tubules, or of secretory units that are acinose in shape. A reservoir may also be present. Tubular secretory units occur in ants [6] whereas in wasps only acinose secretory units are found [9,12,17]. In bees, both secretory units are found, as well as an intermediate structure known as a pseudotubular secretory unit [18].

The salivary system of the Vespidae includes mandibular, hypopharyngeal and salivary glands, with the latter usually occurring in the thorax (thoracic salivary glands). Some bees also show these glands in the head. The glands of the salivary system develop from the ectoderm and undergo major changes in development during metamorphosis. Several studies have suggested a relationship between the salivary system and food digestion, larval feeding, the synthesis of pheromones, and nest construction [5,19,21].

Thoracic salivary glands products provide a source of glue used to hold together the fibers of the nest paper [19,21]. However, the role of the thoracic salivary glands is still a matter of debate. Jeanne [10] suggested that in *Dolichovespula* sp. these glands produced the rubbery material of the petiole of nests, whereas Deleurance [4] reported that in *Polistes* sp. thoracic salivary glands were a source of brood food. Current knowledge about this gland in wasps is limited to observations based on light microscopy [4,9,17,19].

Polistes versicolor (Olivier, 1791) is one of the most widespread Vespidae species in South America, and is very common in São Paulo State, Brazil [8,21]. The genus *Polistes* has been extensively studied because it is believed that this is a key genus for understanding the relationships between the evolution of social insects and wasp societies [19,21].

Considering the importance of the salivary gland system in Vespidae, in this study we used transmission electron microscopy (TEM) to examine the ultrastructure of *P. versicolor* thoracic salivary glands by pointing to the differences between its component cells.

Correspondence to: Dr. Flávio Henrique Caetano
Departamento de Biologia, Instituto de Biociências, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Avenida 24A, nº. 1515, Bela Vista, CEP 13506-900, Rio Claro, SP, Brasil. Tel: (55) (19) 3526-4141, Fax: (55) (19) 3526-4136, E-mail: fcaetano@rc.unesp.br, or tharocha@yahoo.com

MATERIAL AND METHODS

Adult specimens of *P. versicolor* were collected in Rio Claro, São Paulo state, Brazil (22º24'36" S; 47º33'36" W). The specimens were dissected under a stereomicroscope and the thoracic salivary glands transferred to 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 2 h, rinsed twice (15 min each) in 0.1 M sodium cacodylate buffer, and post-fixed in 1% osmium tetroxide in the same buffer (2 h). After a further rinse, the glands were partly dehydrated in 10% ethanol for 15 min.

The tissues were then stained in 2% uranyl acetate in 10% ethanol (4 h) and completely dehydrated in an ethanol series. This was followed by washing in acetone: 100% ethanol (1:1, v/v), 100% acetone, and acetone in Epon-Araldite (1:1). The glands was then embedded in Epon-Araldite and sections were cut with a MT2-B ultramicrotome. Ultrathin sections were stained with 2% uranyl acetate for 45 min and with lead citrate [20] for 10 min prior to observation in Zeiss EM9S-2 transmission electron microscope.

RESULTS

The thoracic salivary glands of *P. versicolor* consisted of various types of cells. Each pseudoacinus included a central or type A cell (TA) (Figs.1B-E and 2A,B), surrounded by several parietal or type B cells (TB) (Figs.1B-E and 2B).

The cytoplasm of the central cell contained a large number of secretory vesicles, with products of different electrondensities (Figs.1B-E and 2A,B). The

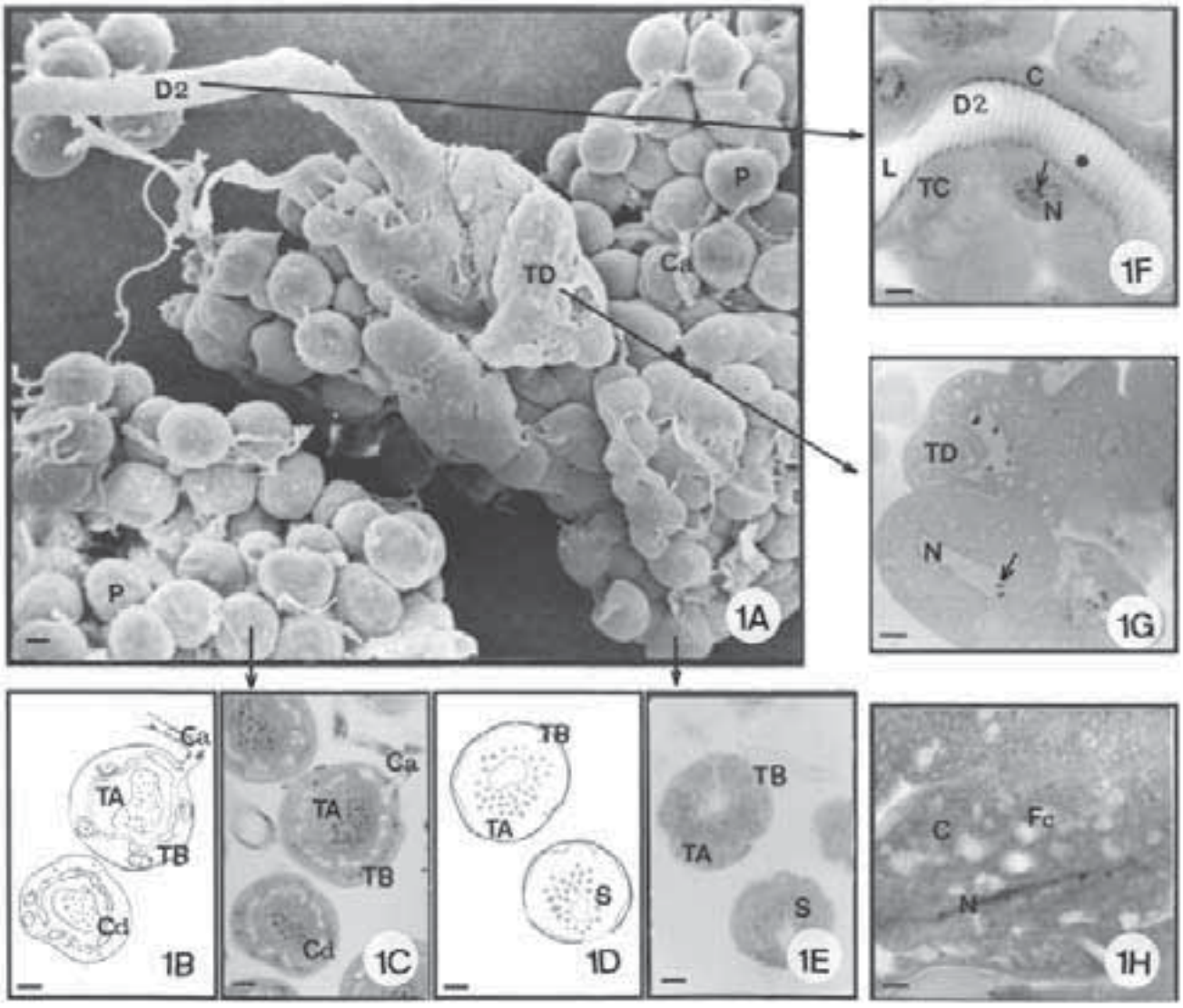

Figure 1. **A)** General aspect of the thoracic salivary glands of *P. versicolor* seen in scanning electron microscopy (SEM). **B, C)** Semi-thin cross-section and diagram of the pseudoacini (**P** in Fig.1A) showing the central cell (**TA**), the parietal cells (**TB**) and the collecting duct (**Cd**) between these cells. **Ca** – canaliculus. **D, E)** Semi-thin cross-section and diagram showing the secretory vesicles (**S**) in the central cell (**TA**) and parietal cells (**TB**). **F)** Semi-thin section showing the epithelial duct cells (**TC**) in duct 2 (**D2**). Note the taenidia–chitin (*****) in the lumen (**L**). **G)** Semi-thin section of the associated duct cell (**TD**) near the base of duct 2 (**D2**). **H)** Semi-thin section of the fat body cells (**Fc**) present near the thoracic salivary glands. **Arrow** – nucleolus, **C** – cytoplasm, **N** – nucleus. Bar = 1.5 μm in all panels.

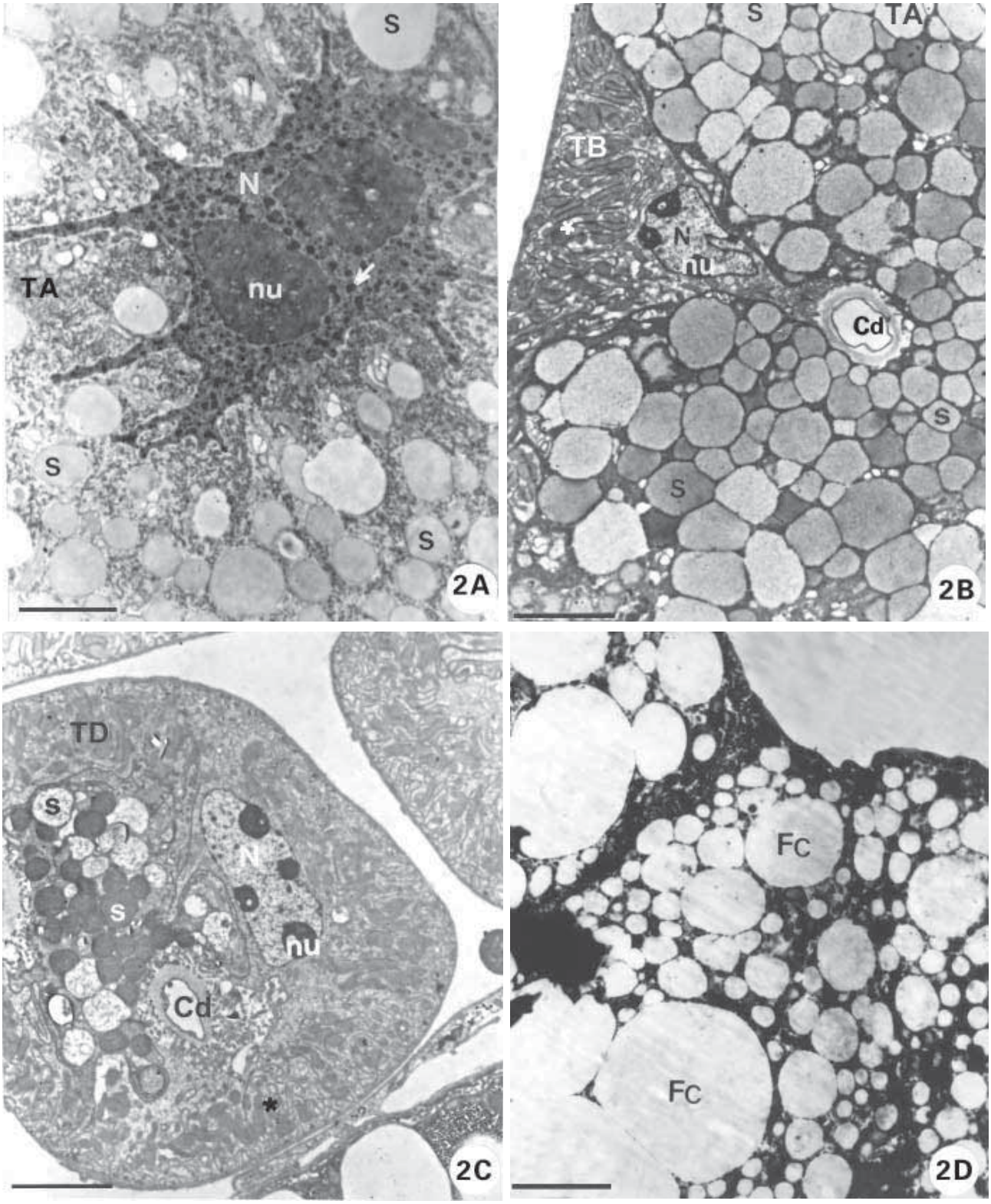

Figure 2. **A)** A central cell (**TA**) of a pseudoacinus of the thoracic salivary gland of *P. versicolor* showing an irregular nucleus (**N**) with a large nucleolus (**nu**) and numerous clumps of heterochromatin (**arrows**). Note the variety of secretory vesicles (**S**). **B)** Detail of a pseudoacinus showing the peripheral region with a parietal cell (**TB**) and the central cell (**TA**) cytoplasm. Note the collecting duct (**Cd**) between the central cell (**TA**) and parietal cell (**TB**). The basal plasma membrane of the parietal cell (**TB**) is involved in labyrinthine invaginations that form areas of cytoplasm with mitochondria (*). **N** - nucleus, **nu** - nucleolus, **S** - secretory vesicles. **C)** Main features of the associated duct cell (**TD**). The basal plasma membrane is involved in labyrinthine invaginations associated with mitochondria (*) similar to those seen in parietal cells (**TB** in Fig. **2B**). Note the secretory vesicles (**S**) similar to those of the central cells (**TA** in Fig. **2B**), and the regular nucleus (**N**). **Arrowhead** – microvilli, **Cd** - collecting duct, **nu** - nucleolus. **D)** Detail of a fat body cell showing the lipid droplets (**Fc**). Bar = 5 μm in all cases.

nucleus was always irregular in shape, with large nucleoli and randomly distributed clumps of heterochromatin (Fig. 2A). In contrast, the cytoplasm of the parietal cells contained no secretory vesicles (Figs. 1B-E and 2B). These cells penetrated the central cell, together with the collecting duct, and were encircled by microvilli (Figs. 1B,E and 2B). The basal plasma membrane of the parietal cells formed a labyrinth of invaginations that contained cytoplasm with mitochondria (Fig. 2B). The nucleus of parietal cells was regular in shape and contained two or more nucleoli that were smaller than those of the central cell. The collecting duct in the pseudoacini was located between the central cell and the parietal cells (Figs. 1B,E and 2B).

All of the pseudoacini had a canaliculus that arose from the collecting duct (Fig. 1). Two or more of these canaliculi merged to form ducts of different diameters and a larger lumen. The first ducts (D1) (Figs. 3A,B)

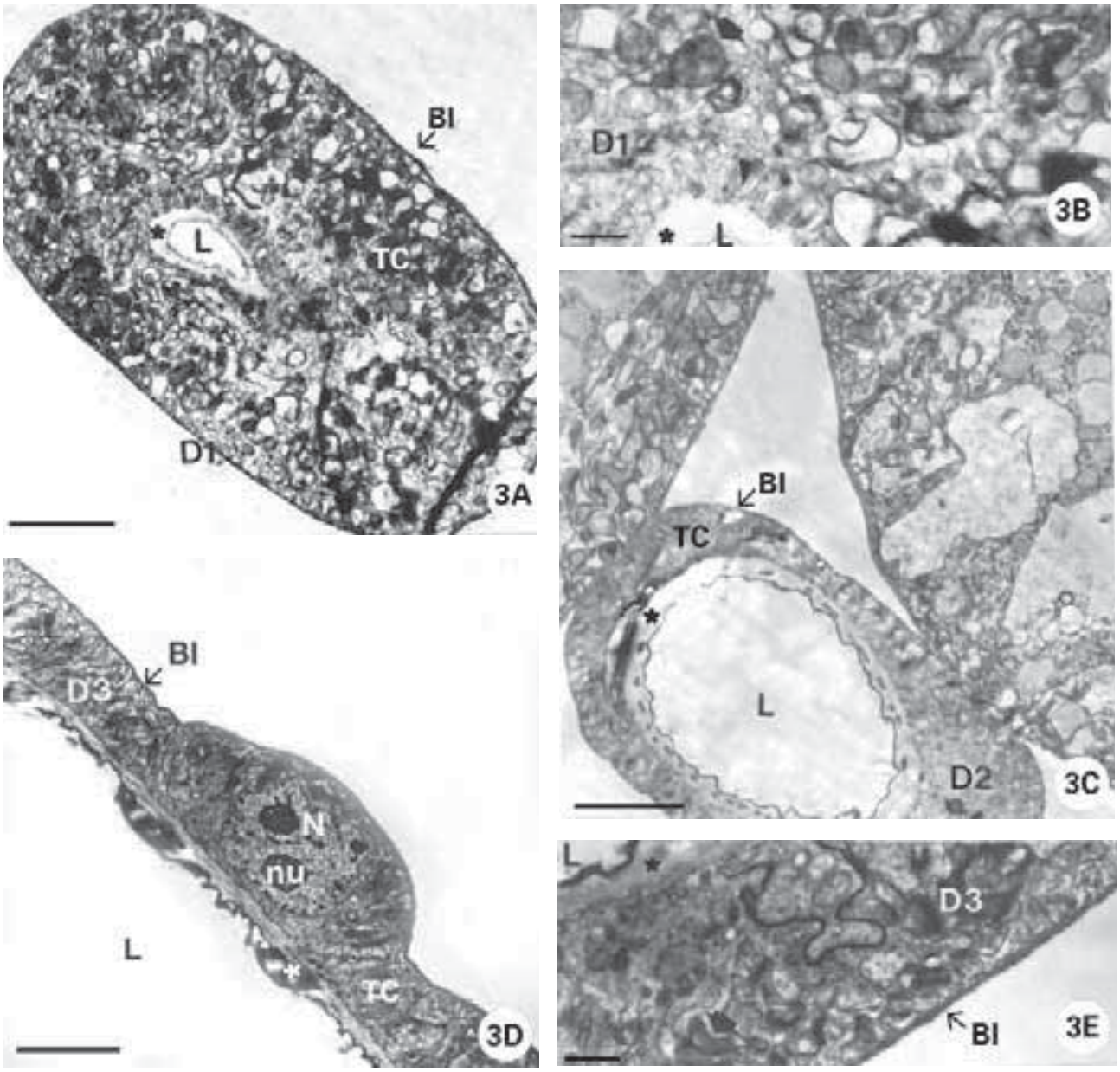

Figure 3. **A)** General view of duct 1 (**D1**) showing an epithelial duct cell (**TC**), with a large number of mitochondria and the basal lamina (**Bl**). The lumen (**L**) is lined by taenidia-chitin (*). **B)** Detail of a duct 1 epithelial duct cell (**TC**) showing the mitochondria (**thick arrow**) and the microvilli (**arrowhead**). **L -** lumen, * - chitin. **C)** General aspect of duct 2 showing the lumen (**L**) lined by taenidia-chitin (*). **Bl** - basal lamina, **TC -** epithelial duct cell. **D)** Detail of duct 3 (**D3**) showing an epithelial duct cell (**TC**), the nucleus (**N**) and nucleolus (**nu**). The duct lumen (**L**) is lined by taenidia–chitin (*) similar to ducts 1 and 2. **Bl** - basal lamina. **E)** Detail of an epithelial cell (**TC**) in duct 3 (**D3**) showing the basal plasma membrane involved in a labyrinthine pathway with associated mitochondria (**thick arrow**) similar to duct 1 but lower in number. **Bl** - basal lamina, **L** - lumen, * - chitin. Bar = 5 μm in all cases.

fused to form ducts 2 (D2) (Figs. 1A,F and 3C) that in turn fused to form ducts 3 (D3) (Fig. 3D,E). Type C cells (TC) formed epithelium of the ducts 1, 2 and 3 (Figs. 1F and 3A,C,D). The morphology of these epithelial duct cells varied according to the duct formed.

The epithelial duct cells in D1 contained numerous mitochondria (Fig. 3A,B) that decreased in number in D2 and D3 (Fig. 3C,E). The basal plasma membrane of theses cells in D1 was similar to that of parietal cells and type D cells (TD) (Fig. 2B,C and 3A). The apical plasma membrane of the epithelial duct cells in D1 also formed microvilli, as in parietal cells (Figs. 2B and 3A,B). In contrast, microvilli were not seen in D2 and D3 and the number of mitochondria is higher in D2 than in D1 and D3 (Fig. 3A,C,E). In D1, the appearance of the epithelial duct cells resembled that of cells involved in the active transport of molecules and differed from D2 and D3.

Type D (TD) or associated duct cells were found near the base of the D2 (Figs. 1G and 2C), with no other intervening cells. The cytoplasm of these cells was similar to that of the central cell and contained vesicles with contents of different electrondensities, but fewer in number (Figs. 2A,B,C). In contrast, the nucleus and basal plasma membrane of theses cells resembled that of parietal cells.

The lumen of D1, D2 and D3 ducts was lined by taenidia (chitin) (Figs. 1F and 3A,C,D) produced by the epithelial duct cells.

Fat body cells were observed between the secretory pseudoacini (Fig. 1H). The fat body is the principal lipid store in insects and is not part of the thoracic salivary glands. Fat body cells provide other cells with a nutrient reservoir. These cells occur in all cavities of the head, thorax and abdomen and may be parietal (adhered to the cuticle) or visceral (located among the organs) [11].

DISCUSSION

The thoracic salivary glands of *P. versicolor* are similar to those of *Xylocopa frontalis*, *X. suspecta*, *Centris fuscata*, *Hemisiella tarsata* [2], *Polistes canadensis, P. actaeon* [3], *Vespula pensylvanica* [12] and some ant species of the subfamilies Ponerinae and Dolichoderinae [7] in that they contain a single central cell per acinus. The central cell of pseudoacini in *P. versicolor* thoracic salivary glands is surrounded by several parietal cells [17]. This arrangement is seen in all hymenopteran species that have acinose thoracic salivary glands.

In *P. versicolor*, as in *X. frontalis*, *X. suspecta*, *C. fuscata* and *H. tarsata*, the cytoplasm of the central cell contains a large number of vesicles of varying sizes with secretory products of different electrondensities [1]. The presence of a large number of vesicles in the cytoplasm and the irregular nuclear morphology of the central cell suggested that these cells are the secretory cells of the pseudoacini in *P. versicolor* thoracic salivary glands. The positive periodic acid-Schiff test for carbohydrates suggested that the central cell produced proteinaceous and carbohydrate material [16].

Acinar glands are typically formed by a small duct surrounded by a variable number of cells. The thoracic salivary glands of *P. versicolor* differed from this arrangement in that the central cell was surrounded by parietal cells with a single collecting duct between these cells. Based on these differences, we suggest that the term pseudoacinus is more appropriated for this gland in *P. versicolor* [17].

The parietal cells in *P. versicolor* are similar to those of *X. frontalis*, *X. suspecta* and *C. fuscata* since they are located in the outer part of the acini and their apices penetrate towards the central cell [1].

Type 1 ducts differed from D2 and D3 and their peculiar epithelial duct cells have not been seen in other insects. The microvilli of these cells may be involved in concentrating the secretion whereas the mitochondria associated with the basal plasma membrane suggest that these cells may also modify the ionic concentration of the secretory fluid in the duct, in a manner similar to the salivary gland cells of *Calliphora vomitoria* [14]. The epithelial duct cells of D2 and D3 had the appearance of conducting cells.

The cytoplasm of the associated duct cells was similar to that of the central cell, but had mitochondria associated with the basal plasma membrane, in a manner similar to the epithelial duct cells in D1. The nucleus and basal plasma membrane of the associated duct cells resembled those of parietal cells.

According to Landolt and Akre [12], parietal cells and epithelial duct cells may regulate the water and ion content of the secretion. The highly folded apical plasma membranes, with the formation of microvilli, and the presence of numerous mitochondria associated with the basal plasma membrane, provides a greatly increased surface area and energy for active transport, respectively. This arrangement suggests that there is active uptake of material from the hemolymph, with subsequent transfer to the ducts [12], as seen in

parietal cells, associated duct cells, and epithelial duct cells in D1. The movement of water and ions from the hemolymph to the duct network by these cells could produce a large volume of fluid. Since the thoracic salivary glands secretion is partly proteinaceous [16], it could serve as brood food, as suggested for *Polistes* salivary glands [4] or as a mucoproteinaceus glue [16] used in paper making [19].

The secretion is apparently not accumulated or stored in *P. versicolor* thoracic salivary glands since there is no reservoir, as also seen in other wasp species. However, a reservoir has been described in some ants and bees [1,2,6,7,13,15].

The lumen of the ducts in *P. versicolor* thoracic salivary glands is lined with twisted taenidia, as also described in *V. pensylvanica* [12] and bees (*Bombus* sp. and *Tricholletes* sp.) [2]. The chitin present in these taenidia is produced by the epithelial duct cells.

The variety of cell types in *P. versicolor* thoracic salivary glands suggests that each cell type has a specific role in the biosynthesis (central cell), modification (parietal cells, associated duct cells and epithelial duct cells in D1) and transport (epithelial duct cells in D2 and D3) of secretion.

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REFERENCES

1. Cavasin-Oliveira GM, Cruz-Landim C (1998) Ultrastructure of Apoidea (Hymenoptera, Anthophorinae) salivary glands. I. Alveolar glands. *Rev. Bras. Entomol*. **42**, 1-6.
2. Cruz-Landim C (1967) Estudo comparativo de algumas glândulas de abelhas (Hymenoptera, Apoidea) e respectivas implicações evolutivas. *Arq. Zool. São Paulo* **15**, 177-290.
3. Cruz-Landim C, Saenz MHP (1972) Estudo comparativo de algumas glândulas dos Vespoidea (Hymenoptera). *Pap. Avulsos Zool*. **25**, 251-263.
4. Deleurance EP (1955) Contribution à l´étude biologique des *Polistes* (Hymenoptères, Vespides) II, - Le cycle évolutif du couvain. *Insect. Soc*. **11**, 285-302.
5. Downing HA (1991) The function and evolution of exocrine glands. In: *The Social Biology of Wasps* (Ross KG, Matthews RW, eds). pp. 540-547. Cornell University Press: London.
6. Gama V (1985) O sistema salivar de *Camponotus (Myrmothrix) rufipes* (Fabricius, 1775), (Hymenoptera, Formicinae). *Rev. Bras. Biol*. **45**, 317-359.
7. Gama V, Cruz-Landim C (1982) Estudo comparativo das glândulas do sistema salivar de formigas (Hymenoptera – Formicidae). *Naturalia* **7**, 145-165.
8. Gobbi N, Zucchi R (1980) On the ecology of *Polistes versicolor versicolor* (Olivier) in southern Brazil (Hymenoptera, Vespidae, Polistini). I-Phenological account. *Naturalia* **5**, 97-104.
9. Heselhaus F (1922) Die hautdüsen der Apiden und verwandter formen. *Zool. Jahrb. Abt. Anat. Ontog. Tiere* **43**, 369-464.
10. Jeanne RL (1977) A specialization in nest petiole construction by queens of *Vespula* sp. (Hymenoptera: Vespidae). *N.Y. Entomol. Soc*. **85**, 127-129.
11. Keeney LL (1978) Endocrine regulation of fat body development and function. *Annu. Rev. Entomol*. **23**, 329-352.
12. Landolt PJ, Akre RD (1979) Ultrastructure of the thoracic gland of queens of the western yellow jacket *Vespula pensylvanica* (Hymenoptera: Vespidae). *Ann. Entomol. Soc. Am*. **72**, 586-590.
13. Oliveira GMC, Cruz-Landim C (1996) Ultrastructural study of salivary glands of *Trigona spinipes* (Hymenoptera, Apidae). *Braz. J. morphol. Sci*. **13**, 120.
14. Oschman JL, Berridge MJ (1970) Structural and functional aspects of salivary fluid secretion in *Calliphora*. *Tissue Cell* **2**, 281-310.
15. Reginato RD, Cruz-Landim C (1996) Secretory cycle of glands from salivary system in *Schwarziana quadripunctata* (Apidae, Meliponinae). *Braz. J. morphol. Sci*. **13**, 122-123.
16. Rocha T, Caetano FH (2000) Histoquímica da glândula salivar do tórax de *Polistes versicolor* (Olivier) (Hym.: Vespidae). *Braz. J. morphol. Sci*. (Suppl.) **17**, 157.
17. Rocha T, Caetano FH (2003) Ultramorfologia e histologia da glândula salivar do tórax de *Polistes versicolor* (Olivier, 1791) (Hymenoptera, Vespidae). *Neotrop. Entomol*. **32**, 585-590.
18. Schönitzer K, Seifert P (1990) Anatomy and ultrastructure of the salivary gland in the thorax of the honeybee worker, *Apis mellifera* (Insecta, Hymenoptera). *Zoomorphology* **109**, 211-222.
19. Spradbery JP (1973) Form and function. In: *Wasps*: *an Account of the Biology and Natural History of Solitary and Social Wasps* (Spradbery JP, ed). pp. 33-37. University of Washington Press: Seattle.
20. Venable JH, Coggeshall R (1965) A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol*. **25**, 407.
21. Zara FJ, Balestieri JBP (2000) Behavioural catalogue of *Polistes versicolor* Olivier (Vespidae: Polistinae) post-emergent colonies. *Naturalia* **25**, 301-319.

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