

Ultramorphology and Histology of the Ectal Mandibular Gland in *Polistes versicolor* (Olivier) (Hymenoptera: Vespidae)

Thiago Augusto Ortega Pietrobon¹ and Flávio Henrique Caetano^{2,*}

¹ FAM, R. Belem, 233, Jd. N. S. de Fátima, 13478–620, Americana, sp-Brazil

² UNESP, Depto. de Biologia, Campus de Rio Claro, 13506–900, CP. 199, Rio Claro, SP-Brazil

Received October 10, 2002; accepted January 30, 2003

Summary The ectal mandibular gland (EMG) of wasps is homologous to the mandibular gland of ants and bees. This gland belongs to salivary system and its function still unknown. The EMG of *Polistes versicolor* showed histological and ultramorphological features similar to that found in ants and other wasps. This gland is constituted by a secretory region and a reservoir. The secretory region contains individual secretory cells that showed several nucleoli. The reservoir has a club shape and is connected to each mandible, by a duct that opens on its external side, which there are cuticular projections. The EMG of males is smaller than those of females. Our results suggested that the EMG secrete volatile compounds that are liberated when the mandibles still open.

Key words Salivary system, Ectal mandibular gland, Histology, Ultramorphology, Wasps, *Polistes*.

The wasp *Polistes versicolor*, as all hymenopterans, possess numerous exocrine glands that play important roles in the behavior of these insects (Downing 1991). Among these glands is the salivary system, which, in wasps, is composed by the ectal mandibular, hypopharyngeal, and salivary glands of the thorax (Chapman 1975). According to Jeanne (1996), these glands were once strictly related to feeding, but nowadays it is believed that they possess numerous functions. Some of these functions have not been completely elucidated, nevertheless, in some species, they seem to be related to the social organization of the colony and they vary according to caste, sex and age of the individual (Cruz-Landim and Saenz 1972).

Morphologically, the ectal mandibular gland is described as a pair of glands internally localized at each side of the head. They are composed by a group of secretory cells joined by cuticular canaliculi to a sac-shaped reservoir, which opens at the base of the mandible (Cruz-Landim and Saenz 1972, Landolt and Akre 1979, Downing and Jeanne 1982, Conte and Cruz-Landim 1997). The secretory cells appear isolated and spherical in shape, showing a low affinity to dyes. The reservoir resembles a thin membrane but with cellular organization (Cruz-Landim and Saenz 1972). In general, the ectal mandibular gland is relatively small when compared to the mandibular glands of bees and ants (Downing 1991).

This work aims at describing the ultramorphology and histology of the ectal mandibular gland of *P. versicolor* while trying to correlate its shape and function to the various aspects of the biology of this species.

Materials and methods

Wasps of the species *Polistes versicolor* were collected and dissected in physiological solution for insects. The extracted ectal mandibular glands were prepared for histology by being fixed in 4% paraformaldehyde, dehydrated in a standard ethanol series from 70–90%, and finally embedded in

* Corresponding author, e-mail: fcaetano@rc.unesp.br

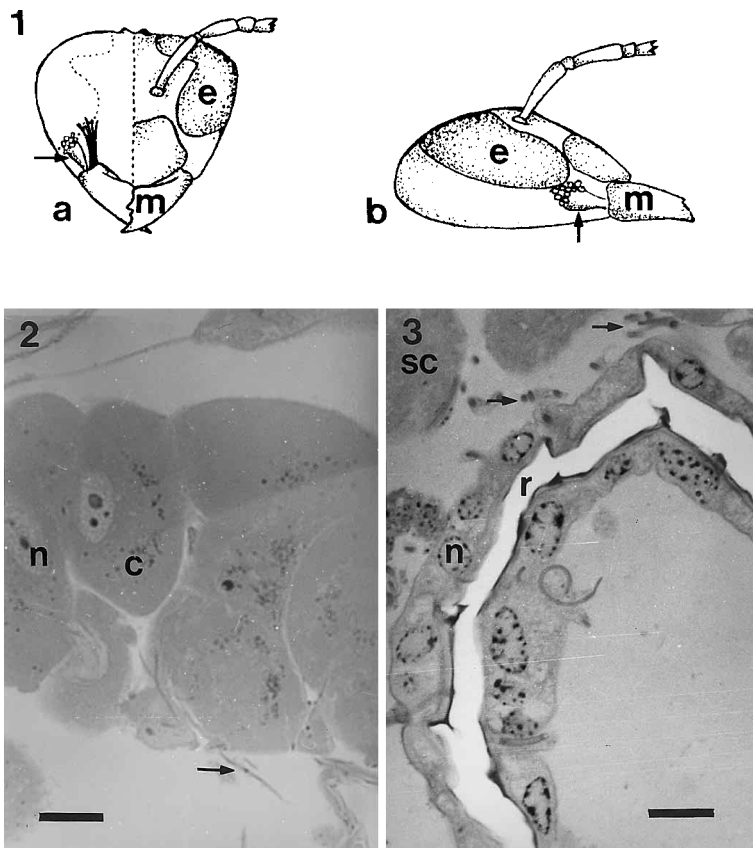
Leica historesin. The material was then cut under a microtome to a thickness between 1.0 and 4.0 μm . The sections were stained with Hematoxiline Harris and aqueous Eosin. The slides were viewed and photographed under a Zeiss photomicroscope.

For the ultramorphological analysis, the material was fixed in Karnovsky solution, dehydrated in a standard ethanol series and critical point dried (Balzers CPD 030). The dehydrated tissues were mounted in a metallic support and sputtered with a thin layer of carbon and gold (Balzers SCD 050). The samples were analyzed and photographed under a scanning electron microscope JEOL (JSM P15).

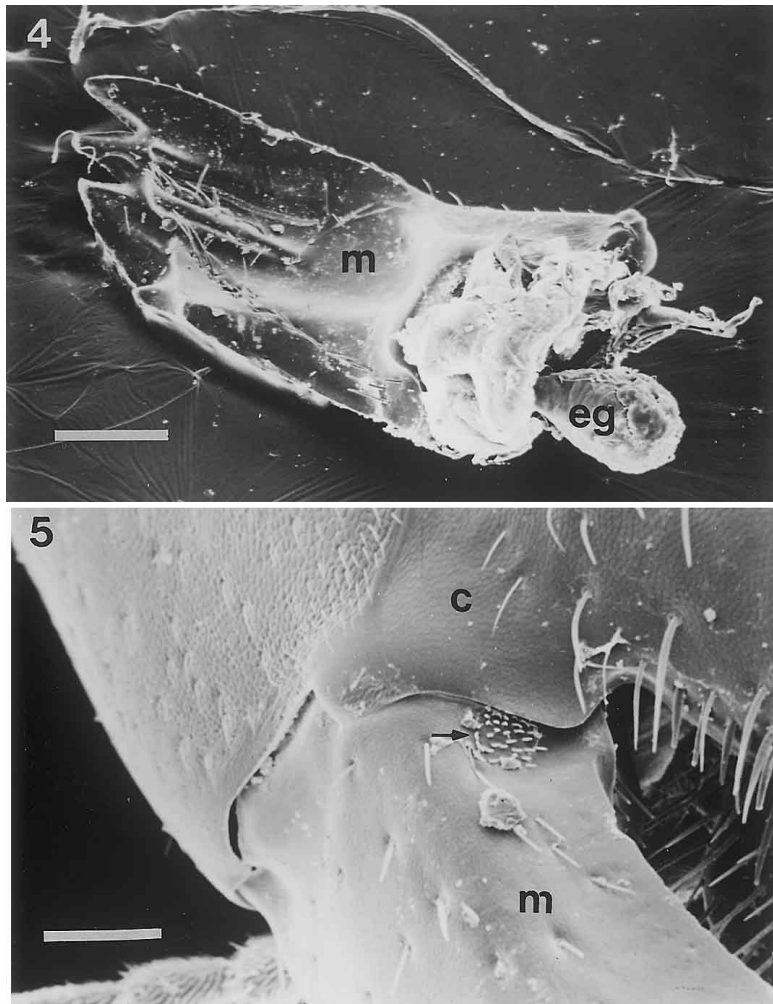
Results and discussion

The ectal mandibular gland of *P. versicolor* is situated laterally in the cephalic cavity, extending from the base of the mandible until the eye's margin. It is found between the internal face of the gena and the muscular fibers attached to the mandible (Fig. 1).

The gland can be divided into 2 portions: the secretory portion and the reservoir (Figs. 6, 7). The secretory portion is composed by isolated cells, with low affinity to dyes, with nuclei showing



Figs. 1–3. 1) Schematic representation of the wasp's head, indicating the position of the ectal mandibular gland (arrow). Its lateral position inside the head and its extension from the base of the mandible (m) until the base of the eye (e) can be noted. In (a) we show the frontal view and (b) is a lateral view. (c) Clypeus. 2) Section of the EMG of *P. versicolor*. The secretory cells can be observed, showing the cytoplasm (c) and nucleus (n) with nucleoli. (arrow) Canaliculi. (Scale bar 10 μm). 3) Detail of the walls of the reservoir (r) showing the flat cells and their nuclei (n) with nucleoli. (sc) Secretory cell. (arrow) Canaliculi. (Scale bar 10 μm).

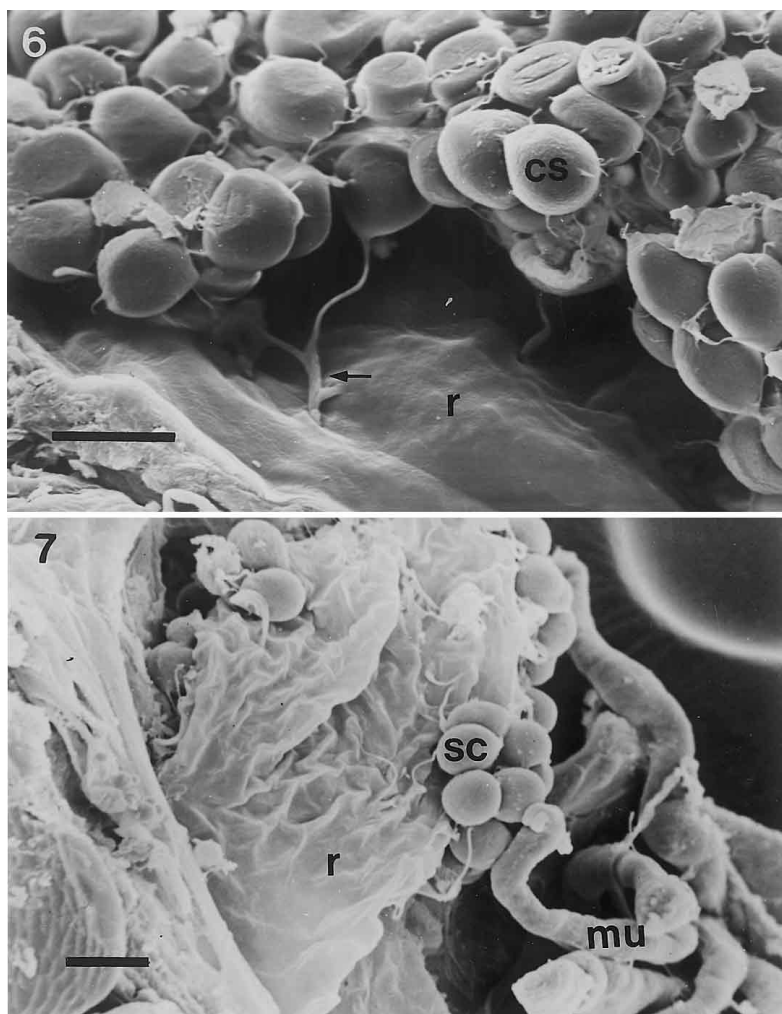


Figs. 4, 5. 4) General view of the ectal mandibular gland (eg) of *P. versicolor*, connected to the mandible (m). (Scale bar 250 μ m). 5) Detail of the head, showing part of the right mandible (m) opened, leaving the opening region of the EMG exposed (arrow). (c) Clypeus. (Scale bar 100 μ m).

1 or more nucleoli (Fig. 2), indicating a high rate of synthesis. Plasma membrane processes occur among these cells, connecting one to other, which do not constitute true cytoplasmic bridges although they might allow the exchange of substances between neighboring cells (Fig. 6). Conte and Cruz-Landim (1997) observed filiform projections of unknown nature that covered the whole surface of the secretory cells. However, even though we used the same species and techniques, we did not observe such projections. In similar studies in other species of the same genus, no author has mentioned these kind of projections, leading us to believe that the projections observed by Conte and Cruz-Landim (1997) were artifacts of the technique.

Each secretory cell is joined to the reservoir by a canaliculi that release the secretion to be stored (Figs. 2, 3, 6). The secretory cells are characterized as type III of Noirot and Quennedey (1974); therefore, the canaliculi is nothing more than a continuation of the collector cell. This arrangement is known in several exocrine glands of insects, being characterized by an isolated cell that communicates to the reservoir or directly to the exterior (Chapman 1975).

The reservoir is a membranous sac, club-shaped, with walls composed by flat cells whose nu-



Figs. 6, 7. 6) Detail showing the secretory portion and the reservoir clearly separated. Note a canaliculi (arrow) parting from the secretory cell (sc) and fusing to the wall of the reservoir (r). (Scale bar 20 μ m). 7) Detail of the EMG of the male, from which part of the secretory cells (sc) were removed in order to expose the reservoir (r). (mu) Muscle. (Scale bar 25 μ m).

clei possess several nucleoli (Figs. 3, 4). Once again, the description of this portion of the gland coincides with the description made by several other authors (Cruz-Landim and Saenz 1972, Landolt and Akre 1979, Conte and Cruz-Landim 1997). When present, the secretion was homogeneously stained by Eosin, which bonds electrostatically to positively charged elements such as proteins with NH_3^+ radicals (Taboga and Vilamaior 2001).

The male's ectal mandibular gland shows the same ultramorphological aspects than those founded in females, with the exception of being smaller, especially with regards to the reservoir (Fig. 7). The reservoir in males is almost completely covered by the secretory cells, contradicting with the results found by Cruz-Landim and Saenz (1972), who suggested the absence of reservoirs in these individuals. Wenzel (1987) reported that in males of *P. major* the ectal mandibular gland is more highly developed than in females. Perhaps this variation is related to the different stages of the secretory cycle. Only an accomplish study with accompanied to the development of this gland related to age will allow us to reach a conclusion regarding this matter.

Landolt and Akre (1979) and Downing and Jeanne (1982) observed in several species of wasps a long and sclerotized duct that goes across the mandible, liberating the secretion close to its base. Such duct was not observed in *P. versicolor*, probably due to the site of sectioning of the material. Nevertheless, the opening of the gland is found just beside the trulleum region, where a group of filiform cuticular processes can be found, which could help disperse the secretion (Fig. 5). This arrangement and function was proposed for *P. fuscatus* by Downing and Jeanne (1982); for the ant *Acromyrmex subterraneus* by Mayhé-Nunes and Caetano (1994); and for *Atta laevigata* by Hernández and Caetano (1995). These projections are also found in other species of wasps, such as the species studied by Landolt and Akre (1979). When the mandibles are closed, the dispersion area is protected under the clypeus, being exposed only when the mandibles are open. When the mandibles are being opened, the muscle fibers traction exerts certain pressure over the reservoir, forcing the secretion release. Therefore, we believe that the degree at which the mandibles are opened must play an important role in the process of secretion of the ectal mandibular gland. According to Downing and Jeanne (1982), the dominant females of *P. fuscatus* keep their mandibles opened during their dominance interactions, thus exposing the opening of the ectal mandibular gland, which probably produce important chemical signals for the preservation of status. Fortunato *et al.* (2000) compared the glandular activity and the development of the colony in *P. dominulus* and suggested that the secretion of the ectal mandibular gland might be involved in the chemical defense of the colony. In this same species, Fortunato *et al.* (2001) observed that this secretion is liberated only when the wasps are disturbed. Due to these observations and assays performed with the secretion, these authors concluded that it might be involved in warning signaling vertebrates.

Since each caste and/or age can show different behaviors in which the mandibles are used (for reviews see Zara and Balestieri 2000), it is probable that the secretion of the ectal mandibular gland has different functions in each group. This was suggested by Cruz-Landim and Saenz (1972) when they observed differences in the volumes of the secretory portions among individuals of different castes and sex. According this fact, Fortunato *et al.* (2001) also reported individual variations in the compounds concentration in the secretion of the ectal mandibular gland of the *P. dominulus*.

The ectal mandibular gland of *P. versicolor* is similar to the ones of other wasps and to the mandibular gland of ants regards to their histology and ultramorphology. However, they differ widely from the mandibular glands of bees. In *Apis* this gland consists of a simple sac with a cuticular wall, covered by a layer of large secretory cells and opening into the membrane that connects the mandible to the head (Simpson 1960, Cruz-Landim 1961). On the other hand, in ants and wasps this gland is composed by a sac-shaped reservoir, with secretory cells disposed over the distal portion joined to the reservoir by means of canaliculi. This arrangement has been reported for *Atta sexdens rubropilosa* (Toledo 1967), *Pachycondyla striata* (Tomotake *et al.* 1992), *Camponotus rufipes* (Gama 1985), *Acromyrmex* sp. (Mayhé-Nunes and Caetano 1994), *Atta laevigata* (Hernández and Caetano 1995) and members of the subfamily Polistinae (Cruz-Landim and Saenz 1972).

Judging by the position of the opening region of the gland, by the mechanism of exposition of this region, by the group of filiform processes present in this region, and by its histological aspect, we can conclude that the secretion is constituted by volatile substances, possibly pheromones. These observations relate wasps to other Hymenoptera, such as bees and ants, in which the mechanism of action of this gland's secretion has been more widely studied.

References

- Chapman, R. F. 1975. The Insects-Structure and Function. American Elsevier, New York.
- Conte, H. and Cruz-Landim, C. 1997. Scanning electron microscopy of the mandibular gland of *Polistes versicolor* (Hymenoptera, Vespidae). Rev. Brasil. Biol. 57: 147-150.
- Cruz-Landim, C. 1961. Istologia e anatomia comparata delle ghiandole salivari, mandibolari e ipofaringee delle Api (Hymenoptera, Apoidea). Simposia Genetica et Biologica Italica 12: 288-307.

- and Saenz, M. H. P. 1972. Estudo comparativo de algumas glândulas dos Vespoide (Hymenoptera). *Papeis Avulsos Zool.* **25**: 251–263.
- Downing, H. A. 1991. The Function and Evolution of Exocrine Glands. In: Ross, K. G. and Matthews, R. W. (eds.). *The Social Biology of Wasps*. Cornell University Press, London. pp. 540–569.
- and Jeanne, R. L. 1982. A description of the ectal mandibular gland in the paper wasp *Polistes fuscatus* (Hymenoptera, Vespidae). *Psyche* **89**: 317–320.
- Fortunato, A., Maile, R., Turillazzi, S., Morgan, E. D., Moneti, G., Jones, G. R. and Pieraccini, G. 2001. Defensive of secretion of ectal mandibular glands of *Polistes dominulus*. *J. Chem. Ecol.* **27**: 569–579.
- , Turillazzi, S. and Delfino, G. 2000. Ectal mandibular gland in *Polistes dominulus* (Christ) (Hymenoptera, Vespidae): Ultrastructural modifications over the secretory cycle. *J. Morphol.* **244**: 45–55.
- Gama, V. 1985. Sistema salivar de *Camponotus (Myrmothix) rufipes* (Fabricius, 1775) (Hymenoptera: Formicidae). *Rev. Brasil. Biol.* **45**: 317–359.
- Hernández, J. U. and Caetano, F. H. 1995. Characterization of the mandible and mandibular glands in different castes of the leaf-cutting ant *Atta laevigata* (F. Smith) (Hymenoptera: Formicidae) using scanning electron microscopy. *Bol. Entomol. Venez. N.S.* **10**: 51–56.
- Jeanne, R. L. 1996. The Evolution of Exocrine Gland Function in Wasps. In: Turillazzi, S. and West-Eberhard, M. J. (eds.). *Natural History and Evolution of Paper Wasps*. Oxford Univ. Press, New York. pp. 145–160.
- Landolt, P. J. and Akre, R. D. 1979. Occurrence and location of exocrine glands in some social Vespidae (Hymenoptera). *Annals of the Entomological Society of America* **72**: 141–148.
- Mayhé-Nunes, A. J. and Caetano, F. H. 1994. Ultramorphology of, and comparison between, the mandibular gland and mandibles of two species of *Acromyrmex* (Hymenoptera, Formicidae). *Naturalia* **19**: 17–27.
- Noirot, C. and Quennedey, A. 1974. Fine structure of insect epidermal glands. *Ann. Rev. Entomol.* **19**: 61–80.
- Simpson, J. 1960. The functions of the salivary glands of *Apis mellifera*. *J. Insect Physiol.* **4**: 107–121.
- Taboga, S. R. and Vilamaior, P. S. L. 2001. Métodos de Estudo da Célula. 2. Citoquímica. In: Carvalho, H. F. and Recco-Pimentel, S. M. (eds.). *A Célula 2001*. Malone, Barueri. pp. 15–38.
- Toledo, L. F. A. 1967. Histo-anatomia de glândulas de *Atta sexdens rubropilosa* Forel (Hymenoptera). *Arq. Inst. Biol.* **34**: 321–329.
- Tomotake, M. E. M., Mathias, M. I. C., Yabuki, A. T. and Caetano, F. H. 1992. Scanning electron microscopy of mandibular glands of workers and queens of the ants *Pahycondila striata* (Hymenoptera: Ponerinae). *J. Adv. Zool.* **13**: 1–6.
- Wenzel, J. W. 1987. Male reproductive behavior and mandibular glands in *Polistes major* (Hymenoptera: Vespidae). *Insectes Sociaux* **34**: 44–57.
- Zara, J. F. and Balestieri, J. B. P. 2000. Behavioral catalogue of *Polistes versicolor* Oliver (Vespidae: Polistinae) Post-emergent colonies. *Naturalia* **25**: 301–319.
-