SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

Ultramorphology and Histology of the Larval Salivary Gland of *Pachycondyla villosa* (Fabricius) (Hymenoptera: Formicidae, Ponerinae)

FERNANDO J. ZARA[1] AND FLÁVIO H. CAETANO[2]

[1]Faculdade de Ciências Matemáticas, da Natureza e Técnologia da Informação, Universidade Metodista de Piracicaba - UNIMEP (Campus Taquaral), Rod. do Açúcar, km 156, 13400-911, Piracicaba, SP
[2]Depto. Biologia, Instituto de Biociências, Universidade Estadual Paulista - UNESP, C. postal 199, 13506-900 Rio Claro, SP

Neotropical Entomology 32(1):059-068 (2003)

Utramorfologia e Histologia da Glândula Salivar Larval de *Pachycondyla villosa* (Fabricius) (Hymenoptera: Formicidae, Ponerinae)

RESUMO - A glândula salivar apresenta-se com um duto anterior único, formado por um epitélio colunar, dois dutos laterais curtos, os quais apresentam-se com epitélio cúbico simples e que na sua porção mais proximal torna-se colunar. Posterior a estes, encontram-se os dois reservatórios, os quais possuem o epitélio bastante delgado e é neste reservatório que a região secretora da glândula se abre. Os ramos dorsal e ventral da região secretora da glândula conectam-se por meio de comissuras transversais, sendo que, posteriormente, a região secretora termina em forma de "alça". A região secretora é uniforme, não apresenta tipos celulares distintos e é formada por um epitélio cúbico simples. Neste trabalho é apresentada, também, a revisão sobre a morfologia da glândula salivar larval em insetos, principalmente com relação aos Hymenoptera-Aculeata.

PALAVRAS-CHAVE: Insecta, formiga, MEV, morfologia

ABSTRACT - The salivary gland possesses a single anterior duct with a columnar epithelium, two lateral ducts with simple cubical epithelium, which becomes columnar in the proximal portion, and two thin-walled reservoirs, from which two secretory regions arise. The dorsal and ventral branches of the secretory regions are connected via transverse commissures and ends in a loop. The secretory region is uniform and consists on a simple cubical epithelium. This paper also presents a review on the morphology of the salivary glands in insects, mainly in Hymenoptera-Aculeata.

KEY WORDS: Insect, ant, SEM, morphology

The ant *Pachycondyla villosa* (Fabricius) is restricted to the Neotropical areas, and occurs from central Mexico to Northern Argentina (Kempf 1972). The ants usually construct their nests on trees and in some bromeliads of the species *Aechmea bromeliifolia*.

P. villosa presents four larval instars and has no exclusive components which could act as pheromone, detected by gas chromatography (Zara & Caetano 2001, Zara *et al.* 2002). Previous papers on the morphology of ant larval salivary glands indicate that some species possess reservoir and one or two secretory tubes on each side of the body. (Wheeler 1910, Athias-Henriot 1947, Gama 1985).

According to Petralia & Vinson (1980) and Petralia & Haut (1986), ant larval salivary glands may be involved in silk production and/or digestion. Digestive enzyme activity has been detected in the larval salivary glands of the ant, *Acromyrmex octospinosus* (Reich) (Febvay & Kermarrec 1981). In *Solenopsis invicta* (Buren), the proteolitical activity was detected and this activity can indicate digestive enzymes production (Petralia *et al.* 1980). Apart from this digestive function, Ponerinae ants as well as members of other subfamilies, cease feeding at the end of the last instar and begin to produce silk for their cocoon (Wheeler & Wheeler 1976).

Several papers have been published on the histology of Hymenoptera larvae salivary glands mostly concerning bees (Nelson 1924, Cruz-Landim & Mello 1981) and wasps (Edwards 1980) but only a few dealing with ants (Valentini 1951, Lappano 1958, Ofer 1970). None of those authors, however, worked with any member of the Ponerinae. In those papers a great variation on the Hymenoptera salivary glands morphology was described. However, these variations were not compared one to another (or among each other). In this paper, the ultramorphology and histology of salivary glands at the last larval instar of the *P. villosa* is described and a review on the morphology of the salivary glands of insects, mainly in Hymenoptera-Aculeata, is presented.

Materials and Methods

The Insects and Instars. One bromeliad of the genus *Aechmea*, where the ants had nested, was collected in the gardens of the UNESP (Univesidade Estadual de São Paulo), campus of Rio Claro County, (22°24'36''S; 47°33'36''W, 612 m average altitude). The larvae were separated and anaesthetized by thermal shock (3 to 5 min. at 4°C) for head capsule measurement. The last larval instar was determined according to Zara (1995) and Zara & Caetano (2001), which is based on the head capsule size.

Ultramorphology. For the scanning electron microscopy, salivary glands were removed and fixed in 4% paraformaldehyde and 4% glutaraldehyde, for 24h, dehydrated in an ascending ethanol series (70% to 100%), subjected to two15-min. baths each with 100% acetone and then critically pointed dried (Balzers CPD 030) using 100% acetone. After dehydration the material was placed on aluminium supports using double-sided sticky-tape and sputter coated with gold (Balzers SD 050 sputtering). The salivary glands were examined in Jeol P15 SEM.

Histology. *P. villosa* larvae were fixed directly in 4% paraformaldehyde in a 0.1M phosphate buffer (pH 7.2). The material was then dehydrated in 70%, 80%, 90% and 95% ethanol solutions for 20 min. and transferred to a resin solution (JB4 - Polaron Instruments/Bio Rad) for 72h at 4°C. Finally the material was transferred to resin-filled moulds containing a catalyst. Sections were stained with hematoxiline/eosine, according to routine procedure, or stained using the mercuric-bromophenol blue reaction to detect protein (Lison 1960, Gabe 1976).

Results

Ultramorphology. The salivary gland of the last instar of *P. villosa* consists of a single anterior duct, beginning at the labium, followed by two short lateral ducts, leading into two flattened reservoirs lateral to the digestive system. Reservoirs are followed by two secretory regions with a very convoluted appearance (Figs. 1 and 2). The region of the anterior duct presents a smoother surface in comparison with the lateral ducts, as well as a flatter appearance (Fig. 3). The reservoir region has a distinct morphology: flatter and wider in comparison with the preceding region with a lobular surface (Fig. 4). The differences between the two types of surfaces are apparent in the transition region between the reservoir and lateral ducts: the reservoir consisting of large trapezoidal cells, whist the ducts consist of smaller pentagonal cells (Fig. 4, arrows). When the reservoir is swollen with secretion, its cells resemble those of the lateral ducts, albeit being larger and pentagonal (Figs. 2 and 5).

Each secretory region presents two secretory tubes (dorsal and ventral), distally linked, forming a loop, each previously entering its respective reservoir (Fig. 2). The secretory region has a smooth surface and includes three transverse commissures connecting the dorsal and ventral tubes (Figs. 2 and 6). When the gland was split at several points, fibrous material was found in the lumen (Figs. 7).

Many tracheal tubes were present on the epithelium of the secretory region, reservoirs and anterior duct (Figs. 3, 5 and 6).

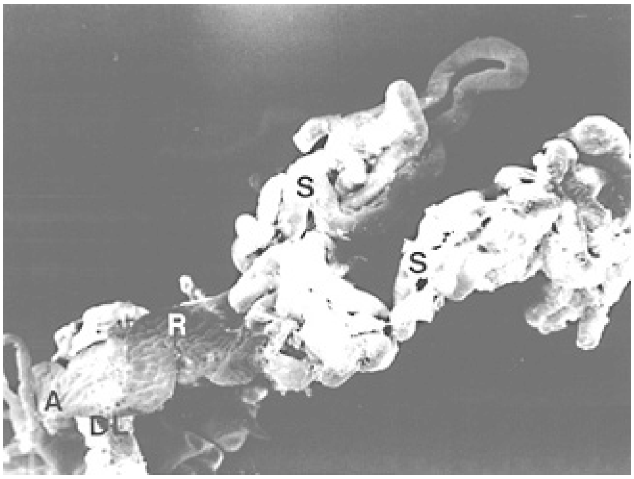

Figure 1. Overall view of *P. villosa* salivary gland, showing: convoluted secretory regions (S), the wider and flatter reservoir (R), lateral duct (DL) and anterior duct (A). Magnification: 72X

Histology. The reservoirs are located in the mid to fore region of the body, while the secretory region is found in the mid to rear part (Fig. 8). The anterior duct under the sub-oesophageal ganglion has a columnar epithelium, the cells of which have large elliptic nuclei and the basal region cells show various vacuoles (Figs. 9 and 10). The lumen is lined by a thin cuticle and gives a positive reaction to the mercuric-bromophenol blue test (Fig. 10). The distal portion of the lateral ducts has simple cubical epithelium, with large rounded nuclei. The cells also contain vacuoles in the basal region and have a very distinct brush border; no cuticle was found (Fig.13). The lateral ducts run along side the oesophagus, only withdrawing from it at the level where the anterior duct is formed, near the sub-oesophageal ganglion (Figs. 11 and 13). In the proximal portion of the lateral duct a mercuric-bromophenol blue positive secretion is present (Figs. 10 to 12), and the duct shows the same characteristics as in the anterior duct, including the presence of cuticle (Fig.12). The reservoirs have a very thin epithelium (Fig.14), with flattened cells, nuclei and cytoplasm containing several vacuoles (Fig. 15). In the lumen there is also evidence of mercuric-bromophenol blue positive material (Fig. 15) The secretory region has a simple cubical epithelium, with rounded or oval nuclei. The cytoplasm contains some vacuole and fibrous material can be observed in the lumen (Fig. 16), which also gives a positive reaction in the mercuric-bromophenol blue test. No histological differences were observed, either between the distal and proximal portions of the secretory region, or between these and the transverse commissures or the dorsal and ventral branches of the secretory region.

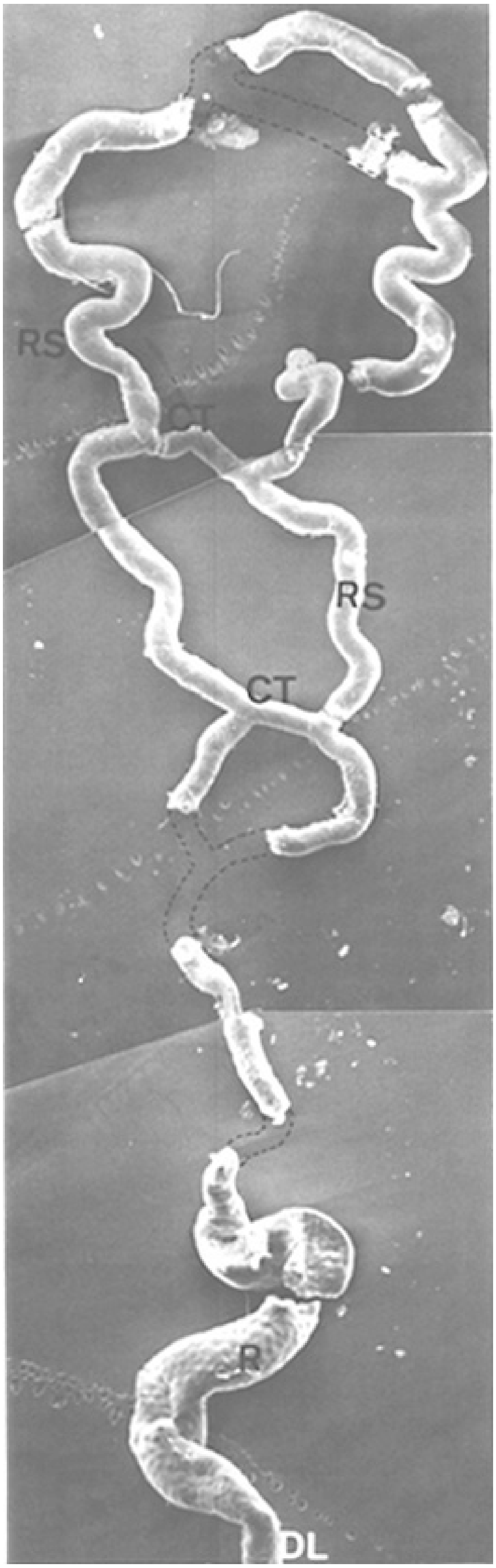

Figure 2. Assembled micrographs showing sequence of a gland of *P. villosa*, with swollen reservoir, secretory tubes (RS) with convolutions and transverse commissures (CT). Magnification: 50X. R = reservoir; DL = Lateral duct

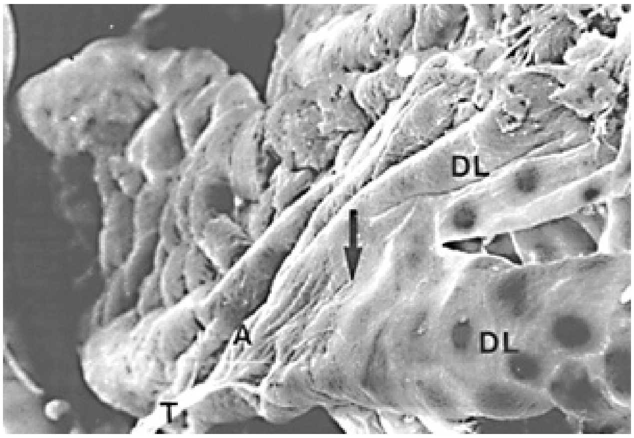

Figure 3. Electron micrograph showing the joining of the lateral ducts (DL), forming the anterior duct (A) of *P. villosa*. Arrow indicates the smooth surface of anterior duct. Magnification: 480X. T = Trachea

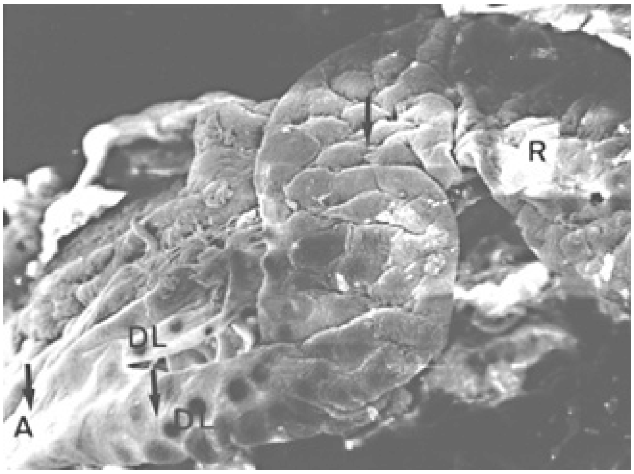

Figure 4. Reservoir (R) of the salivary gland of *P. villosa*, showing its flattened form opening on the lateral duct (DL), with the region of joining of these ducts forming the anterior duct (A). The difference between the surfaces of the three regions can be noticed (arrows). Magnification: 240X

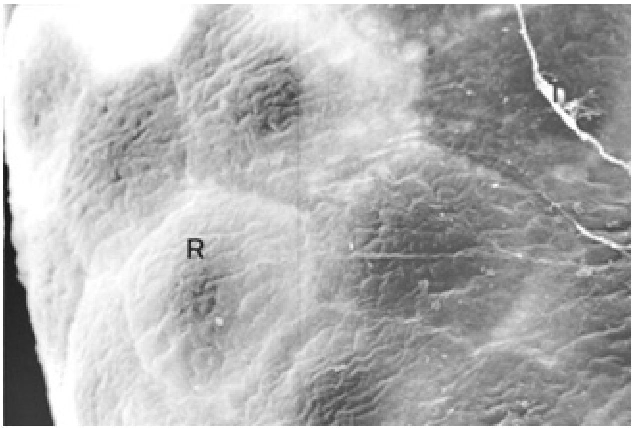

Figure 5. Detail of the basal surface of the reservoir (R) of *P. villosa*, full of secretion. This micrograph shows the pentagon-shape assumed by the cells. Magnification: 1500X. T = Trachea

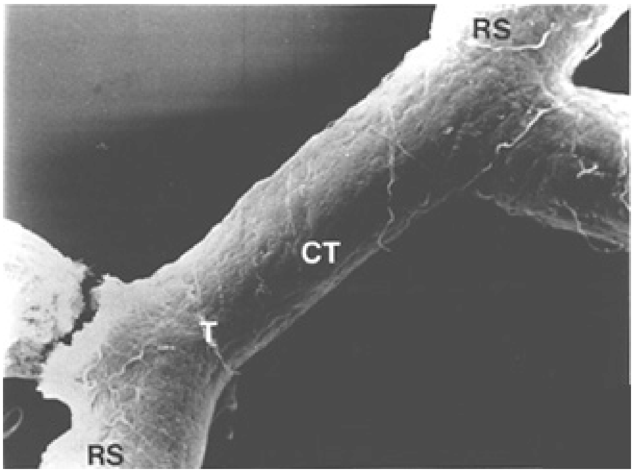

Figure 6. Detail of the transversal commissures (CT) of *P. villosa* linking one secretory tube (RS) to the other, showing its smooth surface and the presence of trachea (T). Magnification: 600X

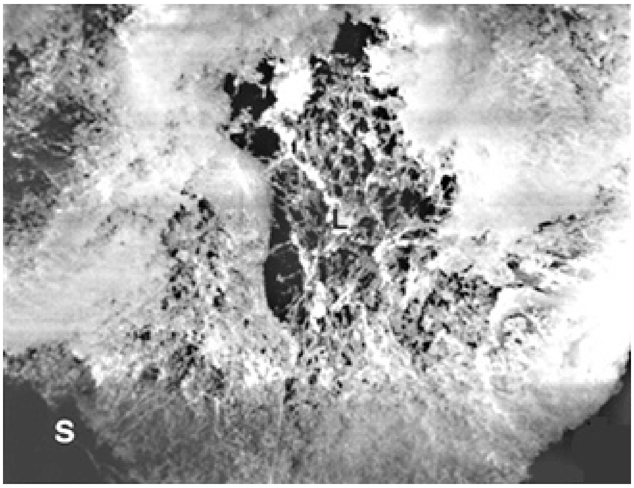

Figure 7. Detail of the lumen (L) in the secretory region (S) of *P. villosa* salivary gland, showing the presence of fibrous material. Magnification: 3000X

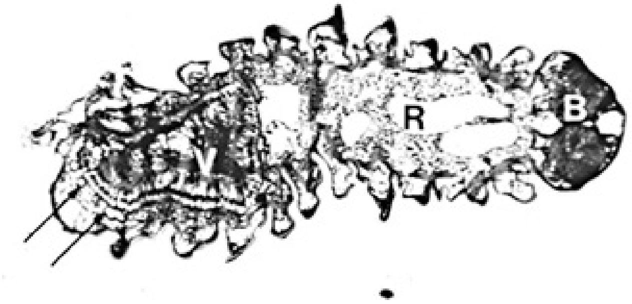

Figure 8. Longitudinal section of *P. villosa* larva, showing the arrangement of the salivary gland. The reservoirs (R), quite ample, are found in the forepart of the body, whilst the secretory tubes (arrows) are sited mainly in the posterior part of the body, by the ventricle (V). Hematoxilin-eosin. Magnification: 55X. B = brain

Figure 9. Overall view of the elliptical anterior duct (A) of *P. villosa* larva, with secretion in the lumen. The presence of the sub-oesophageal ganglion (SE) can be observed between the single duct and the oesophagus (E). Mercuric-bromophenol blue test. Magnification: 215X

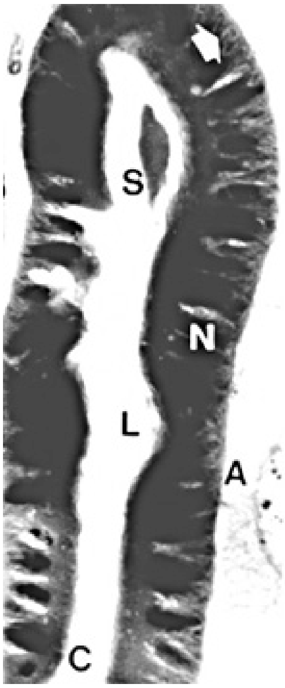

Figure10. Detail of the anterior duct (A) of *P. villosa* showing the basal portion slightly vacuolised (arrow), the stretched nuclei (N) and the presence of thin cuticle lining the lumen (C). Protein positive secretion (S) can be seen in the lumen (L). Mercuric-bromophenol blue test. Magnification: 550X

Figure 11. Transversal section of lateral ducts (DL) of *P. villosa* salivary gland near the formation of the anterior duct. This histological section shows the union of lateral ducts close to sub-oesophagigeal ganglion (SE). Mercuric-bromophenol blue test. Magnification: 215X

Figure 12. Detail of the previous figure showing the resemblance between the end portion epithelium of the lateral ducts (DL) and the anterior duct. Mercuric-bromophenol blue test. Magnification: 550X. C = cuticle, S = secretion

Discussion

Scanning electron microscopy allowed a more accurate observation of larval salivary gland morphology, in comparison with that obtained by the photo-stereomicroscopic system. The larval salivary glands of *P. villosa* have been found to differ from those described for Diptera larvae (Phillips & Swift 1965, Harrod & Kastritsis 1972, Riparbelli *et al*. 1993, Riparbelli *et al*. 1994), Lepidoptera (Cruz-Landim 1973, Wiley & Lai-Fook 1974, Akai 1983, Sorour *et al*. 1990, Victoriano 1998) and for those of other Hymenoptera - Aculeata, such as bees of the *Apis* genera (Nelson 1924, Cruz-Landim & Mello 1969), *Melipona* (Silva de Moraes 1972, Silva de Moraes & Cruz-Landim 1979) and *Scaptotrigona* (Cruz-Landim & Mello 1981) in that they possess a reservoir and have two secretory tubes, ending in a “blunt bottom”, at each side of the body, therefore not ending in a “loop”. Compared to the wasps *Vespula germanica* L. (Edwards 1980), *Pseudopolybia vespiceps* (de Saussure), *Mischocyttarus atramentarius* Zikan, *Polistes versicolor* Oliver and *Occipitalia traili* (Cameron.) (Caetano, unpublished data), the gland differs in that it has a reservoir, short lateral ducts, joints and by not ending in a blunt bottom. In the ant larvae, this gland differs by having two secretory tubes at each side of the body instead of a single one, as in *Plagiolepis crosi* (Sant.), *Camponotus compressus* (Fabr.), (Athia-Henriot 1947) *Tapinoma sirothi* Krausse, *Pheidole pallidula* (Nyl.) (Valentini 1951) and ending in “blunt bottom “as described for *Camponotus rufipes* Fabr. (Gama 1985), *C. pennsylvanicus* (Degeer.), *Formica pallidefulva* Smith. (Petrallia & Haut 1986), *Camponotus silvaticus* (Oliver)

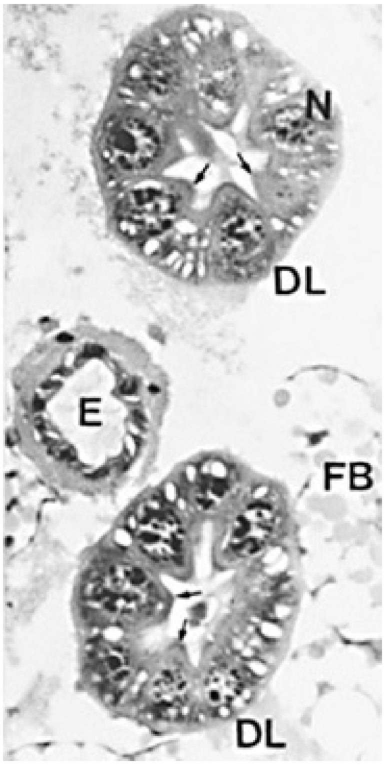

Figure 13. Aspect of the lateral ducts near the reservoir of *P. villosa* larva. In this region, the epithelium is cubical with large, rounded nuclei (N), the cytoplasm presents several vacuole and a brush border can been seen at the apical region (arrows). This figure further shows the closeness of these ducts to the oesophagus (E). Hematoxilin-eosin Magnification: 340X. FB = Fat body

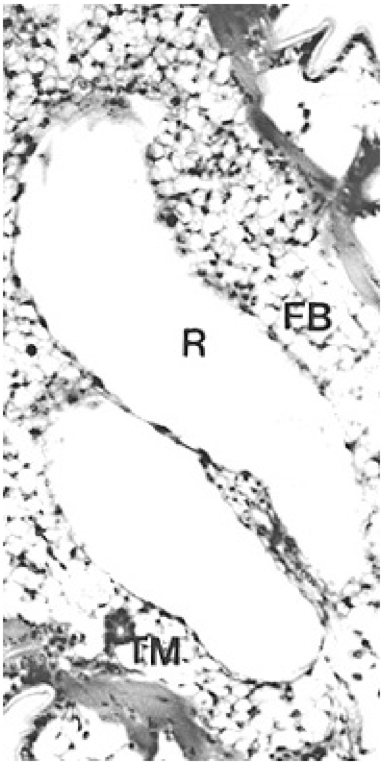

Figure 14. Overall view of the ample reservoir (R) of the salivary gland of *P. villosa* formed by a rather thin epithelium, immersed in the fat body (FB). Magnification: 215X. Hematoxilin-eosin. TM = Malpighian tubes

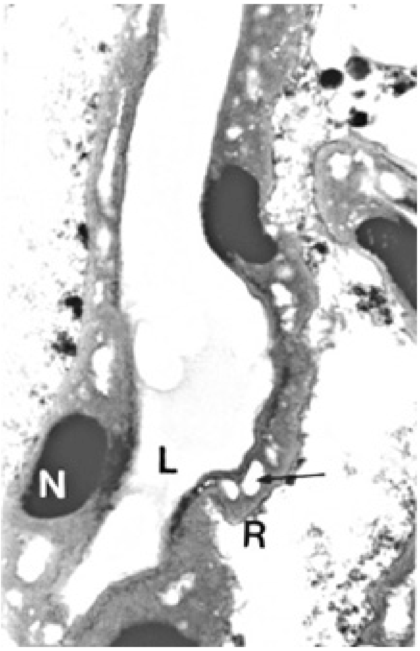

Figure 15. Detail of the epithelium of the reservoir (R) of *P. villosa* showing the flattened cells with several vacuoles (arrow) in the cytoplasm. The presence of protein positive reacting material can be noted in the lumen (L). Mercuric-bromophenol blue test. Magnification: 550X. N = nucleus

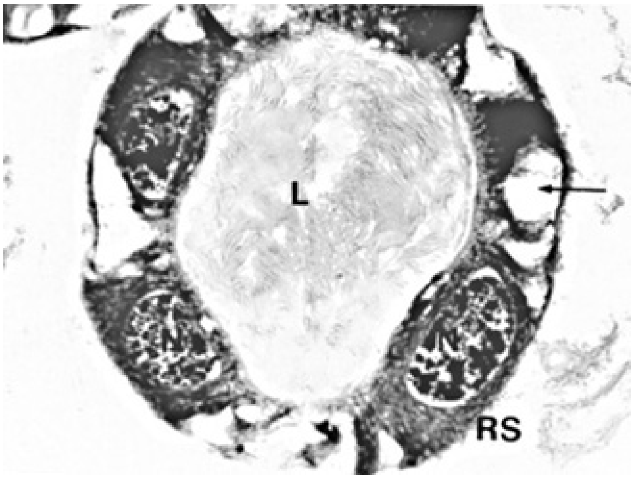

Figure 16. Transversal section of a secretory tube (RS) of the salivary gland of *P. villosa* showing the simple cubical epithelium, with very large nuclei (N) containing heterochromatine blocks and cytoplasm with large vacuoles (arrow). In the wider lumen (L), the presence of fibrous secretion can be noted. Hematoxilin-eosin. Magnification: 550X

Monomorium ajier Bernard, and many other species described by Valentini (1951), *Messor rufo-testaceus* (Forster) and *M. aegyptiacus* (Em.) (Athias-Henriot 1947), *Eciton burchelli* Westw. (Lappano 1958), *Neivamyrmex nigriscens* (Cresson) (Wang & Happ 1974) and *Formica pratensis* males Retz (Emmert 1968). Morphological data of larval salivary gland for different subfamilies of ants are presented in Table 1.

Apart from these differences, *P. villosa* salivary glands have transverse commissures in the secretory region, not seen in any of the aforementioned species. Larvae of *Bombus* spp and *B. attratus* Franklin do have many secretory tubes on either side of the body, with short transverse commissures so that they anastomose across the middle, but in these species, they do not end in a "loop" (Flower & Kenchington 1967, Mello & Vidal 1979).

The comparative morphology of salivary glands for Hymenoptera - Aculeata is summarized in the Table 2. When compared to Hymenoptera - Symphyta salivary glands, the differences become more pronounced. In these insects, the salivary glands present isolated or bunched secretory units, linked to the reservoir epithelium by a secretory channel. The tubular reservoir has a single ramification at each side of the body in *Neurotoma* and *Cladius*. However, in *Celphus cinctus* Norton the tubular reservoirs are compound by two tubes, one shorter than the other (Kenchington 1972). The lumen of the *P. villosa* salivary glands secretory region presents fibrous material scattered along its entire length. In this respect it differs from the silkworm *Bombyx mori* L. (Akai 1983, Akai *et al.*1987) in which the silk protein is found in the lumen in spherical bodies, and only assumes a fibber shape when passing through the spinneret. The histology of anterior duct epithelium in *P. villosa* resembles that described for other species of ants, consisting of a columnar epithelium lined by cuticle (Valentini 1951, Lappano 1958, Emmert 1968). The

Table 1. Morphological data of salivary gland in different Formicidae subfamilies.

Subfamily	Species with 2 secretory tubes	Species with 4 secretory tubes	Secretory tubes ending in "loop"	Secretory tubes ending in "blunt bottom"	Transverse commissures
Ponerinae	0	1	+	-	+
Ecitoninae	3	0	-	+	-
Dolichoderinae	1	1	-	+	-
Formicinae	2	11	-	+	-
Myrmicinae	1	9	-	+	-

+ presence of the character, - absence of the character

denomination given to this salivary gland region varies widely, being described as a common duct (Lappano 1958, Emmert 1968), "canal impair" (Valentini 1951), "tube impair" (Athias-Henriot 1947) and final excretory duct (Gama 1985). The lateral ducts, also called "labial ducts" (Lappano 1958; Wang & Happ 1974), "canal d'écoulement lateral" (Athias-Henriot 1947) and "paired duct" (Emmert 1968, Petralia & Vinson 1980), possess a varied morphology, with the distal portion consisting of rounded nuclei cubical cells and the apex presenting a brush border without the presence of cuticle. The proximal region of the lateral ducts shows cells resembling those in the single anterior duct, the presence of cuticle also being observed. Within the larvae, the salivary glands are found near the oesophagus, the joining of the lateral ducts occurring between the sub-oesophageal ganglion and the oesophagus. This arrangement differs from that described for *S. invicta* (Petralia & Vinson 1980), which occurs between the sub-oesophageal ganglion and the protothoraxic ganglion, and for *C. rufipes* (Gama 1985), occurring at the last protothoraxic ganglion.

The pair of reservoirs strongly resemble the description of *C. pennsylvanicus* (Petralia & Haut 1986), *C. rufipes* (Gama 1985), *S. invicta* (Petralia & Vinson 1980, Petralia *et al*. 1980) *Pheidole pallidula* (Valentini 1951), *Paedaulgus termitoletes* (Forel) (Wheeler 1910), in which the epithelium is thin, consisting of flat cells with oval nuclei, containing many heterochromatine blocks. The reservoirs are quite ample and Wheeler (1918) speculates that they could function as food stores, to be provided to adults. This function, however, cannot be assigned to *P. villosa* reservoirs, needing experimental confirmation for Ponerinae (Hölldobler 1985, Hölldobler & Wilson 1990). The secretory region presents a simple cubical epithelium, its diameter consisting of four to five cells. The basal regions of the cells are very vacuolated, resembling that observed in *C. rufipes*, and this could indicate a capture of substances from the hemolymph to form glandular secretions (Terra & Bianchi 1975, Gama 1985). This type of epithelium seems to be the most common in Hymenoptera larval salivary glands, as observed in *Melipona quadrifasciata* (Lep.) (Mello & Vidal 1971), *Scaptotrigona postica* Latreille (Cruz-Landim & Mello 1981), *E. burchelli* (Lappano 1958) *N. nigriscens* (Wang & Happ 1974), *C. rufipes* (Gama 1985), *S. invicta* (Petralia & Vinson 1980), *C. pennsylvanicus* (Petralia & Haut 1986).

The presence of fibrous secretion can be observed in the secretory region, thus secretion related to the silk produced for building the cocoon, and the occurrence of this kind of secretion is also reported for *C. rufipes* (Gama 1985), *Polirhachis simplex* (Forel) (Ofer 1970). Besides the silk, the salivary system can also produce digestive enzymes, as suggested for *E. burchelli* (Lappano 1958) or for the Myrmicinae *Acromyrmex octospinosus* (Febvay & Kermarrec 1981) and *S. invicta* (Petralia *et al*. 1980). The production of digestive enzymes in this region of the gland may also be occurring in *P. villosa*, where the presence of small vesicles (microapocrine secretion) in the lumen was observed in larvae at the beginning of the last instar by transmission electron microscopy (Zara, unpublish data). Only a single cell type was identified in the secretory region, which agrees with the observations on *Apis* (Nelson 1924), *M. quadrifasciata* (Mello & Vidal 1971, Silva de Moraes & Cruz-Landim 1979), *S. postica* (Cruz-Landim & Mello 1981) and *S. invicta* (Petralia *et al.* 1980). The presence of protein identified by the mercuric-bromophenol blue test was observed in all regions of the gland, and a similar reaction was observed in *S. invicta* (Petralia *et al*. 1980). In summary, the salivary glands of the last larval instar of *P. villosa* differs from that observed in the majority of Hymenoptera currently studied, mainly because of their loop shape, three transverse commissures, and presence of mercuric-bromophenol blue positive fibrous material, probably silk, in the lumen.

Acknowledgments

This research work was supported by FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) proc. 98/00576-8.

Literature Cited

Athias-Henriot, C. 1947. Recherches sur les larves de quelques fourmis d'Algérie. Bull. Biol. Fr. Belg. 81: 247-272.

Akai, H. 1983. The structure and ultrastructure of the silk gland. Experientia 39: 443-449.

Akai, H., T. Imai & K. Tsubouchi. 1987. Fine-structural changes of liquid silk in silk gland during the spinning stage of *Bombyx* larvae. J. Seric. Sci. Jpn. 56: 131-137.

Cruz-Landim, C. 1973. Ultraestrutura da glândula salivar larval de *Amalo helops megapyrha* (Lepidoptera -

Table 2. Morphology of larval salivary gland for different Hymenoptera-Aculeata families.

Hymenoptera - Aculeata family and species	Reservoir	Number of secretory tubes	Transverse commissures	Ending in a "blunt bottom"	Ending in a "loop"	Reference
APIDAE						
Bombus sp.	-	Many	+	+	-	Flower & Kenchington, 1967
Bombus atratus Franklin	-	Many	+	+	-	Mello & Vidal 1979
Apis mellifera L.	-	2	-	+	-	Nelson 1924; Cruz-Landim & Mello 1969
Melipona quadrifasciata (Lepidoptera)	-	2	-	+	-	Silva de Moraes & Cruz-Landim 1979
Scaptotrigona Postica Latreille	-	2	-	+	-	Cruz-Landim & Mello 1981
VESPIDAE						
Vespula germanica L.	-	4	-	+	-	Edwards 1980
Pseudopolybia vespiceps (de Saussure)	-	4	-	+	-	Caetano, unpublished data
Occiptalia traili (Cameron.)	-	4	-	+	-	Caetano, unpublished data
Mischocyttarus atramentarius Zikan	-	4	-	+	-	Caetano, unpublished data
Polistes versicolor Oliver	-	4	-	+	-	Caetano, unpublished data
FORMICIDAE						
Pachycondyla villosa (Fabr.)	+	4	+	-	+	The present study
Plagiolepis crosi (Sant.)	+	2	-	+	-	Athias-Henriot 1947
Tapinoma simrothi (Krausse)	+	2	-	+	-	Valentini 1951
Pheidole pallidula (Nyl.)	+	2	-	+	-	Valentini 1951
Eciton burchelli Westw.	+	2	-	+	-	Lappano 1958
E. hamatum (Fabr.)	+	2	-	+	-	Tafuri 1951 cited in Lappano 1958
Neivamyrmex nigriscens (Cresson)	+	2	-	+	-	Wang & Happ 1974
Solenopsis invicta Buren	+	4	-	+	-	Petralia *et al.* 1980
Camponotus rufipes Fabr.	+	4	-	+	-	Gama 1985
C. pennsylvanicus (Degeer.)	+	4	-	+	-	Petralia & Haut 1986
C. silvaticus (Oliver)	+	4	-	+	-	Valentini 1951
C. senex (Smith)	+	4	-	+	-	Zara *et al.* in prep.
C. atlanticis Forel	+	4	-	+	-	Valentini 1951
Formica pallidefulva Smith.	+	4	-	+	-	Petralia & Haut 1986
F. pratensis Retz	+	4	-	+	-	Emmert 1968
Monomorium ajier Bernard	+	4	-	+	-	Valentini 1951
M. salomonis (L.)	+	4	-	+	-	Valentini 1951
Atopula hortensi Bernard	+	4	-	+	-	Valentini 1951
Acantholepis frauenfeldi Mayr	+	4	-	+	-	Valentini 1951
Messor minor (Forel)	+	4	-	+	-	Valentini 1951
Tetramorium punicum (Smith)	+	4	-	+	-	Valentini 1951
Cataglyphis viatica (Rog.)	+	4	-	+	-	Valentini 1951
C. bombycina (Rog.)	+	4	-	+	-	Valentini 1951
Aphaenogaster testaceo-pilosa (Lucas)	+	4	-	+	-	Athias-Henriot 1947
Messor rufo-testaceus (Forster)	+	4	-	+	-	Athias-Henriot 1947
Messor aegyptiacus (Emery)	+	4	-	+	-	Athias-Henriot 1947
Bothriomyrmex decaptans Sant.	+	4	-	+	-	Athias-Henriot 1947
Cataglyphis bicolor (Fabr.)	+	4	-	+	-	Athias-Henriot 1947

+ presence of the character, - absence of the character

Arctiidae). An. Acad. Brasil. Ciênc. 45: 565-84.

Cruz-Landim, C. & M.L.S. Mello. 1969. The post-embryonic changes in *Melipona quadrifasciata anthidioides* Lep. (Hym., Apoidea). II. Development of the salivary glands system. J. Morph. 123: 481-502.

Cruz-Landim, C. & R.A. Mello. 1981. Desenvolvimento e envelhecimento de larvas e adultos de Scaptotrigona postica (Latreille) (Hymenoptera: Apidae): aspectos histológicos e histoquímicos. São Paulo, Academia de Ciências do Estado de São Paulo (31), 118p.

Edwards, R. 1980. Social wasps: their biology and control Felcourt, Rentokil Library, 398p.

Emmert, W. 1968. Die postembryonalentwicklung sekretorischer kopfdrüsen von *Formica pratensis* Retz. und *Apis mellifica* L. (Ins., Hym.). Z. Morphol. Tiere 63: 1-62.

Febvay, G. & A. Kermarrec. 1981. Activites enzymatiques des glandes salivaires et de l'intestin moyen d'une fourmi attini (adultes et larves): (*Acromyrmex octospinosus* (Reich) (Formicidae, Attini). Arch. Biol. 92: 299-316.

Flower, N. E. & W. Kenchington. 1967. Studies on insect fibrous proteins: The larval silk of *Apis*, *Bombus* and *Vespa*. J. Royal Microbiol. Soc. 86: 297-310.

Gabe, M. 1976. Histological techniques**.** New York, Springer Verlag, 1106p.

Gama, V. 1985. O sistema salivar de *Camponotus* (Myrmothrix) *rufipes* (Fabricius, 1775) (Hymenoptera: Formicidae). Rev. Bras. Biol. 45: 317-359

Harrod, M.J.E. & C.D. Kastritsis. 1972. Developmental studies in *Drosophila:* VI. Ultrastructural analysis of the salivary glands of *Drosophila pseudoobscura* during the late larval period. J. Ultrastruc. Res. 40: 239-312.

Hölldobler, B. 1985. Liquid food transmission and antennation signals in ponerine ants. Israel J. Entomol. 19: 89-99.

Hölldobler, B. & E.O. Wilson. 1990. The ants. London, Springer-Verlag, 732p.

Kempf, W.W. 1972. Catálogo abreviado das formigas da região neotropical (Hym. Formicidae). Petrópolis, Ed. Vozes, 334p.

Kenchington, W. 1972. Variations in silk gland morphology among sawfly larvae (Hymenoptera: Symphyta). J. Entomol. 46: 111-116.

Lappano, E.R. 1958. A morphological study of larval development in polymorphic all-worker broods of the army ants *Eciton burchelli.* Insectes Sociaux 5: 31-66.

Lison, L. 1960. Hitochimie et cytochimie animales: principes et méthodes. Paris, Gauthier-Villars, 842p.

Mello, M.L.S. & B.C. Vidal. 1971. Histochemical and histophysical aspects of silk secretion in *Melipona quadrifasciata* (Hym: Apoidea)**.** Z. Zellforsch. 18: 555-569.

Mello, M.L.S. & B.C Vidal. 1979. The salivary gland secretions of a Neotropical bumblebee. Protoplasma 100: 251-265.

Nelson, J.A. 1924. Anatomy of honey bee. J. Agric. Res. 28: 1167-1229.

Ofer, J. 1970. *Polyrhachis simplex* the weaver ant of Israel. Insectes Sociaux 17: 49-82.

Petralia, R.S. & S.B. Vinson. 1980. Internal anatomy of fourth instar larva of imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). Int. J. Insect Morphol. Embriol. 8: 89-106.

Petralia, R.S., A.A. Sorensen & S.B. Vinson. 1980. The labial gland system of larvae of the imported fire ant, *Solenopsis invicta* Buren. Cell Tissue Res. 206: 145-156.

Petralia, R.S. & C.F. Haut. 1986. Morphology of the labial gland system of the mature larva of black carpenter ant, *Camponotus pennsylvanicus*. Iowa Acad. Sci. 93: 16-20.

Phillips, D.M. & H. Swift. 1965. Cytoplasmic fine structure of *Sciara* salivary glands. J. Cell Biol. 27: 398-409.

Riparbelli, M.G., G. Callaini & R. Dallai. 1993. Spatial organization of microtubules and microfilaments in larval and adult salivary glands of *Drosophila melanogaster*. Tissue Cell 25: 751-762.

Riparbelli, M.G., G. Callaini & R. Dallai. 1994. Cytoskeleton of larval and adult salivary glands of dipteran *Ceratitis capitata*. Implication on microfilaments and microtubules in saliva discharge. Boll. Zool. 61: 6-17.

Silva de Moraes, R.L.M. & C. Cruz-Landim. 1979. Estudos ultraestruturais da glândula salivar de *Melipona quadrifasciata anthidioides* Lep. durante o desenvolvimento larval. Rev. Brasil. Biol. 39: 103-116.

Sorour, J., O. Larink, A. Ramadan, A. Shalaby & S. Osman. 1990. Anatomical, histological, and electron microscopical studies on the salivary glands of larvae of *Spodoptera littoralis* Boisd. (Lep., Noctuidae). Zool. J. Anat. 120: 251-271.

Terra, W.R. & A.G. Bianchi. 1975. Rate of production by the larva of the fly, *Rhynchosciara americana* and storage sites of precursor. J. Insect Physiol. 21: 1547-1550.

Valentini, S. 1951. Sur L'adaptacion des larves de Formicoidea. Ann. Des Sc. Nat. Zool. 11: 249-76.

Victoriano, E. 1998. Morfologia ultra-estrutural da glândula da seda de larva de *Diatraea saccharalis* (Lepidoptera: Pyralidae). Dissertação de mestrado, Instituto de Biociências, Universidade Estadual Paulista, Botucatu, 101p.

Wang, Y.J. & G.M. Happ. 1974. Larval development during the nomadic phase of neartic army ant, *Neivamyrmex nigriscens* (Cresson)(Hymenoptera: Formicidae). Int. J. insect. Morphol. Embryol. 3: 73-86.

Wheeler, C.G. & J. Wheeler. 1976. Ant larvae: review and synthesis. Proc. Entomol. Soc. Wash., D.C. 108p.

Wheeler, W.N. 1918. A study of some ant larvae, with a consideration of the origin and meaning of the social habits among insects. Proc. Amer. Phil. Soc. 57: 293-343.

Wheeler, W.N. 1910. Ants: their structure, development and behavior. New York, Columbia University Press, 663p.

Wiley, M.J. & J. Lai-Fook. 1974. Studies on the silk gland of *Calpodes ethlius* Stoll,(Lepidoptera, Hesperiidae). J. Morphol. 144: 297-310.

Zara, F.J. 1998. Caracterização morfo-histologica, histoquímica e ultra-estrutural da porção secretora da glândula salivary do quarto estágio larval de *Neoponera villosa* (Formicidae: Ponerinae). Dissertação de Mestrado. Instituto de Biociências, Universidade Estadual Paulista, Rio Claro, 126p.

Zara, F.J. & F.H. Caetano. 2001. Number of larval instars of the ant *Pachycondyla (=Neoponera) villosa* (Formicidae: Ponerinae) determinated by the rule of Dyar. Sociobiology 38: 679-686.

Zara, F.J., A. C. Cabrera, K. Jaffé & F.H. Caetano. 2001. Chemical analysis of different larval instars and tissues of *Pachycondyla (=Neoponera) villosa* (Formicidae: Formicidae, Ponerinae). Sociobiology 39: 89-101.

Received 10/01/02. Accepted 25/09/02.