ISSN- 0102-9010

ULTRASTRUCTURE OF THE ECTAL MANDIBULAR GLAND OF THE PAPER-WASP *Polistes versicolor* (HYMENOPTERA: VESPIDAE)

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ABSTRACT

The ultrastructural features of the ectal mandibular glands of the paper wasp *Polistes versicolor* are described. These glands contained a secretory portion and a sac-shaped reservoir. Muscle fibers were observed around the secretory cells close to where slender ducts arose to reach the reservoir sac. These fibers were probably involved in the release of secretion. The early secretory cells contained rough endoplasmic reticulum and Golgi complex with varying features, as well as the mitochondria necessary to sustain the energy requirements for the secretory activity. Smooth endoplasmic reticulum appeared only during the late stages of the secretion cycle. Synthesis and secretion occurred simultaneously and continuously within each cell, with the secretory cycle being essentially asynchronous throughout the gland. The cells of the reservoir wall differed from those in other hymenopterans by their lack of a dense cuticle lining the lumen. These cells also produced lipids that probably will contribute to the ultimate composition of the secretion.

Key words: Ectal mandibular gland, paper wasp, Polistinae, salivary system, ultrastructure

INTRODUCTION

Polistes versicolor (Hymenoptera, Vespidae, Polistinae) is a widely distributed wasp throughout South America, including southeastern Brazil [18]. As in other hymenopterans, *P. versicolor* has numerous exocrine glands that constitute one of the most versatile biosynthetic organ systems in the animal kingdom [12]. Among these glands, those of the salivary system are of particular importance. In wasps, the salivary system includes ectal mandibular glands, hypopharyngeal glands, and the salivary glands of the thorax [13,14]. Jeanne [21] suggested that, primitively, these glands possibly had a hole in feeding, although their currently known functions vary considerably. The ectal mandibular gland of *Polistes* is homologous to the mandibular gland of other hymenopterans. While its function has not been completely elucidated, this gland has morpho-functional traits which suggest that it may be involved in the production of pheromones [15], which are important in wasp communication and social life. Thus, a knowledge of the ultrastructure of the glandular system of the Vespidae may contribute to our understanding of the evolution of sociality and colony organization in these wasps. In this work, we examined the ultrastructural organization of the ectal mandibular gland of *P. versicolor* in order to understand the secretory processes in this gland.

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MATERIAL AND METHODS

The ectal mandibular glands of *P. versicolor* were dissected in fixative (2.5% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2), and stored in this solution for 24 h for complete fixation. The tissues were then washed twice (15 min each) in 0.1 M sodium phosphate buffer prior to post-fixation in 1% osmium tetroxide in water for 2 h. The material was then washed again with sodium phosphate buffer and with 10% ethanol (15 min each). The gland was subsequently contrasted with 2% uranyl acetate in 10% ethanol (1:1) for 12 h, then dehydrated in a graded acetone series and immersed in an acetone:resin solution (1:1) for 12 h, followed by embedding in an Epon-Araldite mixture at 60ºC. Sections 200 nm thick (Sorval MT2-B ultramicrotome) were double-stained with aqueous uranyl acetate (5 µg/ml for 45 min) and lead citrate (4 µg/ml in 10 N NaOH, for 15 min), and then washed in 0.02 M NaOH and in distilled water (1 min each) before examining in a Philips CM 100 transmission electron microscope.

RESULTS

The ectal mandibular gland of *P. versicolor* consisted of round secretory cells which reached the sac-shaped reservoir by a thin duct and were joined together by specialized areas of peripheral cytoplasm. Secretory cells in various stages of activity were seen within a single ectal mandibular gland (Fig. 1A),

which made it possible to establish the complete secretory cycle. All of the cells in full secretory activity showed a nuclear envelope with pores, uncondensed chromatin, rough endoplasmic reticulum (RER) with flat cisternae, numerous mitochondria and a Golgi apparatus consisting of flat, overlain sacs (Fig. 1A,B). Despite the evidence of high synthetic activity, few secretory vesicles were observed at this secretory stage.

In more advanced stages of the secretion cycle, the endoplasmic reticulum (ER) cisternae became more numerous, changed into the tubule-vesicular form and only few ribosomes were seen attached to the ER membranes, suggesting that probably the RER is changing to the smooth endoplasmic reticulum (SER) type. At this stage a few electrondense mitochondria and myelinic bodies were seen (Fig. 1C). In a subsequent stage, cells with several secretory vesicles were detected (Fig. 1D); these exhibited a homogenous matrix of average electron-density, as well as highly electron-dense foci of a fibrous nature. The mitochondria became more numerous, varying from small and electron-dense to large and vermiform with an electron-lucent matrix; smooth endoplasmic reticulum (SER) was also seen (Fig. 1E).

With increasing activity the cells became more vacuolated, the myelinic bodies became more numerous, and the vermiform mitochondria started to degenerate. The Golgi complex became dilated saccules and were situated in a depression of the nuclear envelope (Fig. 1F); the SER appeared reduced.

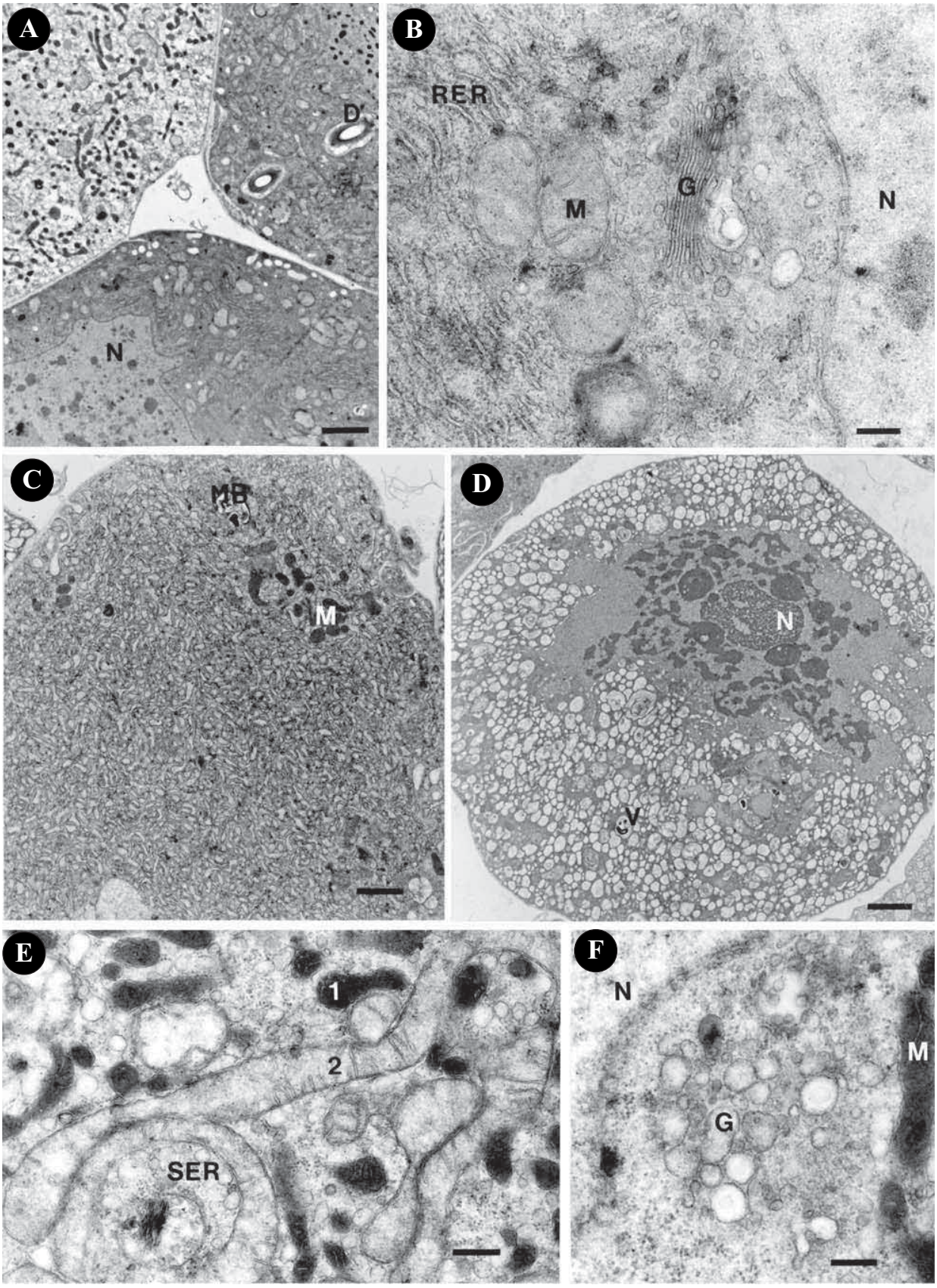
From this stage on, the cells were characterized by a vacuolated cytoplasm containing myelinic bodies (Fig. 1D). Membrane bound-secretory bodies exhibited a highly dense core, irregular in shape, which sometimes appeared connected into the body surface. (Fig. 2A). In addition numerous and large lipid droplets are seen interspersed among mitochondria and free ribosomes in the more advanced stages of secretion (Fig. 2B). During the last stage of secretion the cells had a completely vacuolated cytoplasm, with autophagic bodies and numerous myelinic formations. In some cells the nucleus presented ameboid shape, electrondense nuclear matrix, clumps of heterochromatin, suggesting to be involved in an apoptotic process as depicted in Fig. 1D.

The secretory cells were joined to the reservoir by thin cuticular ducts (Fig. 2C-E). These ducts were contained in accessory or duct cells which penetrated the cytoplasm of the secretory cells and branched into increasingly thinner canaliculi, to form a terminal apparatus that collected the secretion. The canaliculi were surrounded by microvilli of the secretory cells. Between the microvilli and canaliculi there was a peri-canalicular space, in which the secretion released by the vesicles accumulated (Fig. 2C). Myofilaments within muscle cells were observed surrounding the secretory cells, mainly in the region where the duct joined the cell (Fig. 2D).

The free portion of the accessory cells was seen in the space between the secretory cells and the reservoir (Fig. 2E). The accessory cells synthesized and organized the cuticular layer surrounding the lumen of the duct and provided a connection between the end apparatus and the reservoir (Fig. 2E). The junctions between the secretory and duct cells were stabilized by cytoplasmic interdigitations and septate junctions (Fig. 2D).

The cells of the reservoir had a nucleus with one or more well-developed nucleoli, a large amount of euchromatin and numerous pores in their envelope (Fig. 3A). They were flat cells connected to each other by interdigitations and septate junctions (Fig. 3B). In *P. versicolor*, the cell lining of the reservoir was absent or reduced to a loose endocuticle, whereas the apices of these cells formed irregular processes resembling flat microvilli (Fig. 2F and 3B). In addition to elongated mitochondria (Fig. 3A), the reservoir cells can have modified mitochondria containing a lipid deposit (Fig. 3C,D). Myelinic bodies were also observed (Fig. 3E).

Figure 1. A. Close contact between three secretory cells, each in different stages of the secretory cycle. **D** - duct. **N** - nucleus. Bar = 0.25 μm. **B.** Secretory cell in the initial stage of secretion. Note the nuclear envelope with pores, and the Golgi complex (**G**) in the form of flat, stacked saccules lying next to the nucleus (**N**). The rough endoplasmic reticulum (**RER**) and mitochondria (**M**) are also well developed. Bar = 0.21 μm. **C.** General view of a secretory cell with a large amount of endoplasmic reticulum in tubule-vesicular form, where probably the RER has been changing to SER. **M** - mitochondria. **MB** - myelinic bodies. Bar = 0.7 μm. **D.** Secretory cell displaying characteristics of programmed cell death, possibly at the end of the secretory cycle. Note the nucleus (**N**) with thickly condensed chromatin, the extensively vacuolated cytoplasm, and autophagic vacuoles (**V**). Bar = 1.25 μm. **E.** Ultrastructural changes in mitochondria, during the secretory cycle, from small size and strongly electron-density in its matrix (**1**) to large and vermiform with a weakly electron dense matrix (**2**). The vesicular **SER** can be seen between the mitochondria. Bar = 0.2 μm. **F.** Detail of the Golgi complex (**G**) with dilated sacculi at a depression in the nuclear envelope. This feature only appeared during late stages of the secretory cycle. **M** - mitochondria, **N** - nucleus. Bar = 0.1 μm.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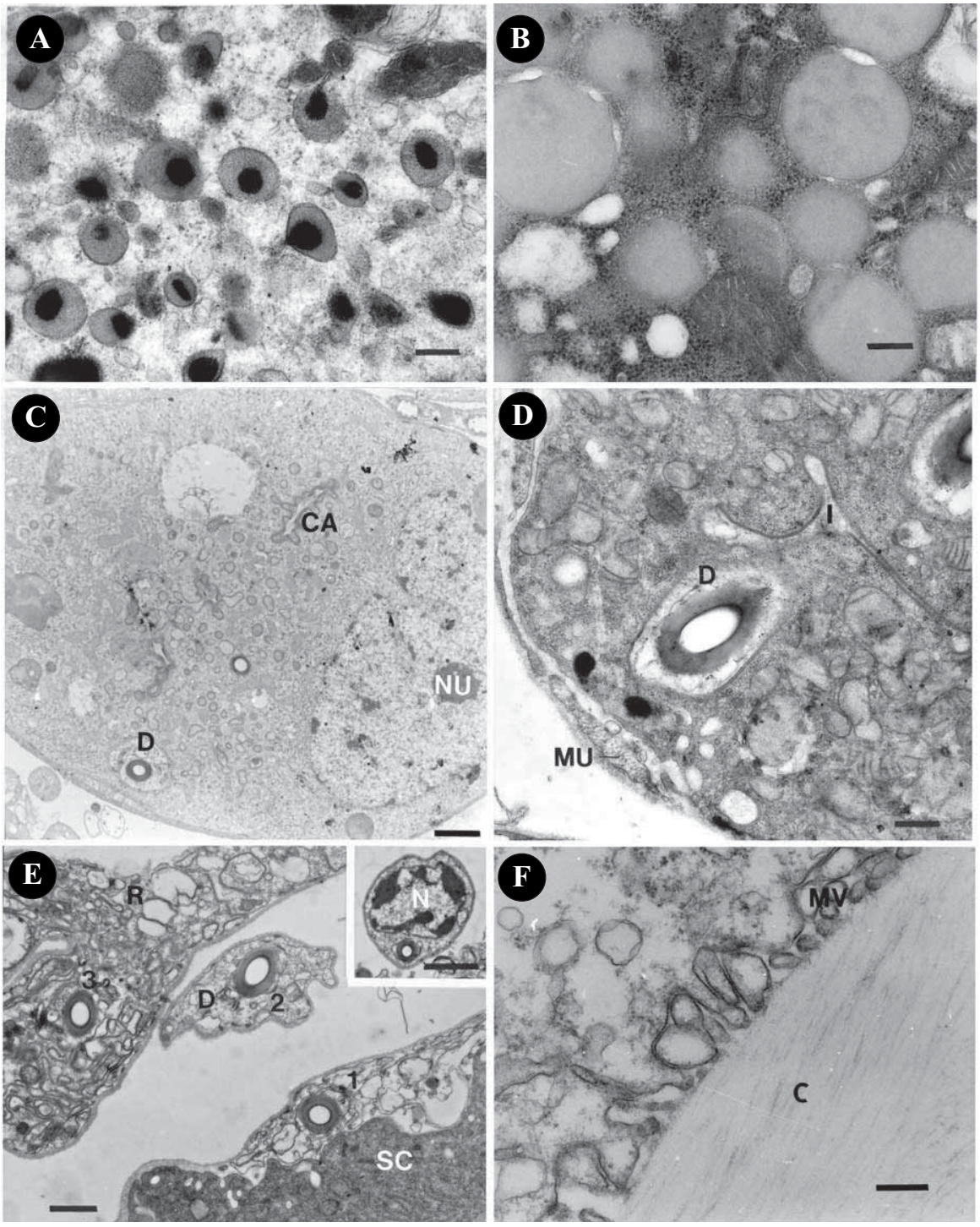

Figure 2A. Detail of secretory vesicles with the electron-dense core material, which probably represents immature secretory products. Bar = 0.1 µm. In **B,** the secretory vesicles are in a most advanced stage of maturation, resembling lipid droplets and contained only vestiges of the immature secretion. Bar = 0.1 µm. **C.** General view of a secretory cell showing the duct (**D**) and branched canaliculi (**CA**) that permeate the whole cytoplasm. **NU** - nucleolus. Bar = 0.6 µm. **D.** A duct (**D**) is present at the junction between the secretory cell and the accessory cell; this zone is reinforced by interdigitations (**I**). Note the myofilaments (**MU**) at the periphery of the secretory cell. Bar = 0.2 µm. **E.** General view of the space between the secretory cells (**SC**) and the reservoir (**R**), showing the duct of accessory cells (**D**) at various levels (**1**), (**2**), (**3**). Note the path followed by the end apparatus-duct complex from the uptake to the release of the secretory product. Bar = 0.5 µm. The region of the nucleus (**N**) of the duct cell is shown in more detail in the inset. Bar = 0.1 µm. **F.** Detail of the reservoir showing the apical region of its cells with numerous microvilli (**MV**) and the loose endocuticle (**C**). Bar = 0.25 µm.

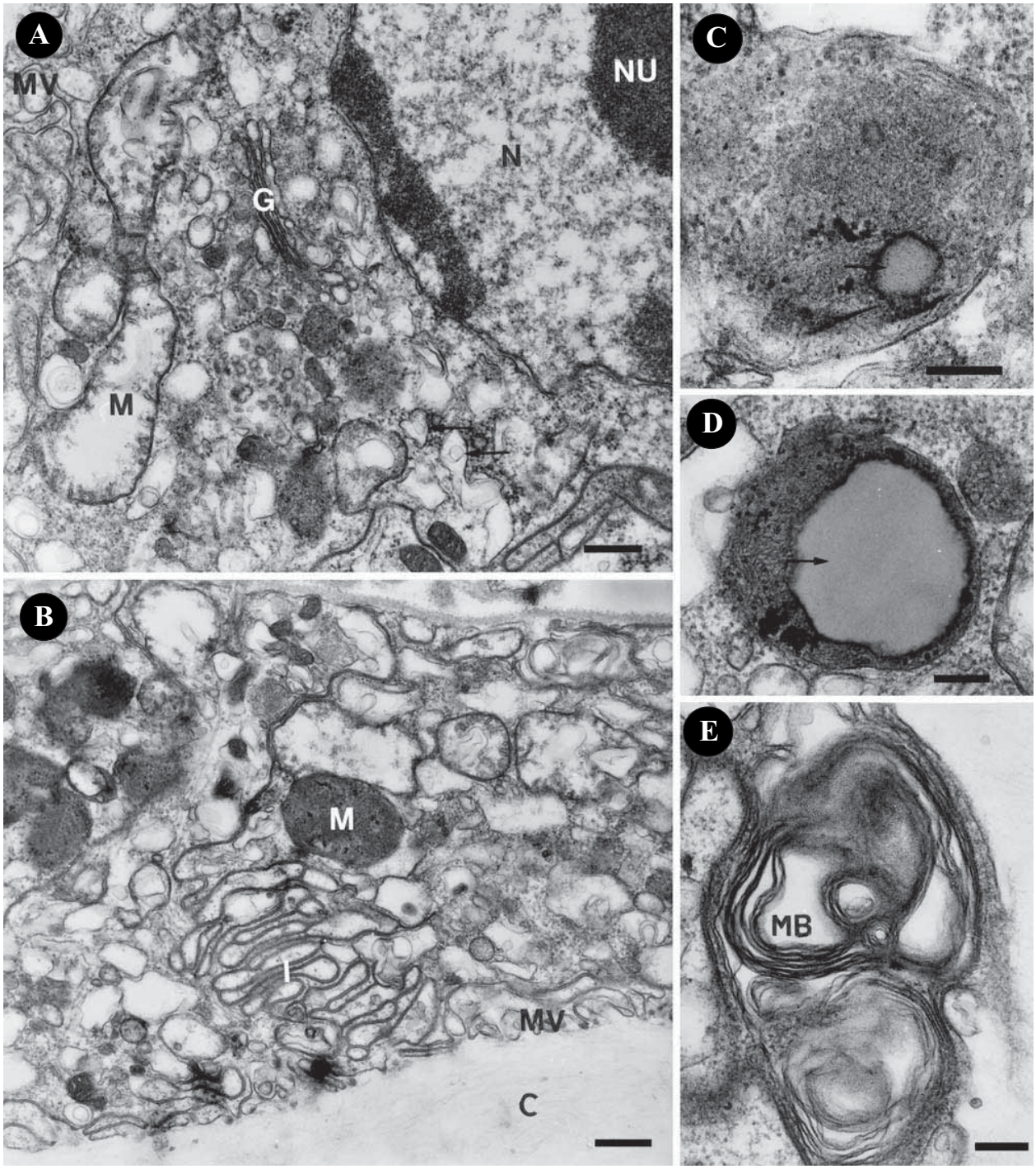

Figure 3A. Electron micrograph of a reservoir cell, showing its secretory features. Note the nucleus (**N**) with a well-developed nucleolus (**NU**). In the cytoplasm, large mitochondria (**M**), and Golgi complexes (**G**) can be seen. The apical portion of the cell contains numerous irregular cytoplasmic processes, resembling microvilli (**MV**). Bar = 0.2 µm. **B.** Detail of the relationship between two cells in the reservoir. Note the interdigitations (**I**) in the anterior region, next to the lumen of the reservoir. In other regions (central and basal), the junction is simple. The basal lamina is moderately electron-dense and the cuticle is limited to an inner layer (**C**). **M** - mitochondria. **MV** - microvilli. Bar = 0.2 µm. **C-D.** Mitochondria modified for the production and storage of lipids (**arrow**). The beginning of the accumulation is shown in **C**, while in **D** the lipids occupy most of the organelle, compressing the matrix and the crests towards the periphery. Bar = 0.1 µm. **E.** Myelinic bodies (**MB**) between the apical microvilli of reservoir cells. Bar = 0.1 µm.

DISCUSSION

The morphological organization of the ectal mandibular gland of *P. versicolor* agreed with the description by Conte and Cruz-Landim [8], and was similar to that reported for wasps and ants in general [4,5,13,17,20,24,29,31,32,34].

The muscle fibers surrounding the secretory cells have not been described for other species. These fibers may be involved in cell movements which could facilitate the flow of secretion towards the reservoir or the uptake of substances from the hemolymph.

The basal portion of the secretory cells of the ectal mandibular gland of *P. versicolor* differed from that of *Apis mellifera* since the former lacked the peculiar invaginations observed in the honeybee [9]. The secretory cells of *A. mellifera* form an epithelium, in which only the basal portion of the cells is in contact with the hemolymph. Thus, the invaginations serve to increase the surface area of these cells. This same organization occurs in the post-pharyngeal gland of *Dinoponera australis*, in which the secretory cells also display wide basal surfaces designed to increase the area of absorption [3]. Since large surface areas of the secretory cells of *P. versicolor* are exposed to hemolymph,very few or none invaginations are necessary.

The various stages of activity observed in the secretory cells suggested asynchronous secretory cycles among these cells. An asynchronous secretory cells cycle has also been observed in glands of other hymenopterans, such as the salivary gland of larvae of *Pachycondyla (=Neoponera) villosa* [35]. Costa-Leonardo [9] reported that it was not possible to identify the stages of the biosynthesis and accumulation of secretion or cyclic excretion in the mandibular glands. Once initiated the secretory process is continuous as long as the active phase lasts. Costa-Leonardo [9] also proposed a hormonal control for synthesis and secretion in *A. mellifera*, and this may be true of *P. (=N.) villosa*. An asynchronous activation of the membrane receptors for hormones in secretory cells could explain the "uncoordinated" secretory cycle of these glands.

Analysis of the secretory cells of the ectal mandibular gland of *P. versicolor* during the different stages of the secretory cycle revealed similarities with *A. mellifera*. The first stages of the cycle in *P. versicolor* resembled those of the mandibular glands of virgin honeybee queens. In more advanced stages of the secretory cycle, *A. mellifera* had RER with a vesicular appearance (Fig. 1C), as also observed in *P. versicolor*, but here the concomitant marked decrease of ribosomes strongly suggests that the RER type is being replaced by the SER type. Finally, queens of *A. mellifera* (one year old or more) had secretory cells with a more vacuolated cytoplasm and myelinic bodies [11] which resembled the pattern seen in the most advanced stages of the secretory cycle of the ectal mandibular gland of *P. versicolor*.

Alike in bees (*A. mellifera*) [9-11], ants (*Pachycondyla striata*) [23], and wasps (*Polistes dominulus*) [16], lipid droplets were detected in the cytoplasm of the secretory cells of *P. versicolor*, agreeing with, histochemical tests which demonstrated the presence of lipids in the secretion stored in the reservoir [27]. Meirelles *et al.* [25] observed that the lipids stored in the silk gland of *A. mellifera* larvae were exogenous in origin and were possibly taken up from the hemolymph. The secretory cells of the ectal mandibular gland of *P. versicolor* may be able to acquire lipids directly from the hemolymph, in addition to have ability for storaging lipid droplets. Such lipids may be rapidly processed by the SER that appears late in the secretory cycle of these cells. We suggest that the the reservoir cells may be capable of synthesizing the lipid fraction of the secretion.

The secretory products contained in the vesicles of *P. versicolor*, which crowded around the end apparatus, differed from those of *P. striata* since they showed an electron-dense core against a transparent background; this core was vestigial in more advanced stages. Structures like that are typically of protein nature. In *P. striata*, the secretion contained only liquid material [23], whereas in *A. mellifera* highly electron-dense granules were also observed [9]; no such granules were seen in *P. versicolor*. Costa-Leonardo [9], Mathias *et al*. [23], and Fortunato *et al.* [16] suggested that the variation in the electron density of the secretory products contained within the vesicles could be related to maturational processes.

Changes in the structure and organization of the mitochondria, Golgi apparatus and RER, were observed throughout the secretory cycle. Similar alterations were also detected in larval salivary glands of *P. (=N.) villosa* [35]. According to these authors, coordinated variations in the RER and Golgi apparatus could be related to different physiological stages, with the secretory products being able to vary in the different stages or cell types. In *P. versicolor* the variation seen in secretory cell structure reflected the

different stages of the secretory cycle rather different cell types. Although different compounds were assumed to be produced at each stage of the secretory cycle, it was not possible to differentiate with certainty the type of secretion at the ultrastructural level. The secretion was transparent to moderately electron-dense in all of the sections examined.

During the last stages of the secretory cycle, several dramatic changes were seen in the cytoplasm and nucleus. According to Häcker [19], condensed peripheral chromatin, dilatation of the ER, vacuolation of the cytoplasm together with intact morphology of other cell organelles (in the early stages of the cell death process), and the continuous synthesis of messenger RNA and proteins, are characteristic traits of apoptotic cells. Vacuolization of the cytoplasm occurs in cells undergoing type 3b (cytoplasmic degeneration) cell death [7], and was seen in the present work. In the hypo-pharyngeal glands of *A. mellifera*, Moraes and Bowen [30] described processes of cell death with features similar to necrosis, which complicated identification of the type of programmed cell death. Considering that programmed cell death has been studied mainly in vertebrates, with few reports for adult insects, we can only suggest that the appearance of apoptosis in the secretory cells of the ectal mandibular gland of *P. versicolor* marks the end of the secretory cycle.

The thin cuticular ducts that join the secretory cells to the reservoir appear to be a characteristic of the mandibular glands of the Hymenoptera [9-11,16,23] These cells belong to Class III of Noirot and Quennedey terminology [26], and the terminal apparatus, with its ramified pattern, is exclusive to the exocrine secretory cells of wasps [2]. Costa-Leonardo [9] showed that the junctions between the secretory cells and duct in *A. mellifera*, are stabilized by cytoplasm interdigitations and septate junctions, as also observed in *P. versicolor*. Both of these mechanical type specializations and the distal localization of the secretory cells, suggest that the ducts may anchor the latter to the reservoir, in addition to transporting their products.

The cells of the ectal mandibular gland reservoir in *P. versicolor* were, morphologically very similar to those of other Hymenoptera. As in *Apis mellifera* these cells were flat and joined to one another by interdigitations and septate junctions [9,10]. They also had distinctive secretory traits, in strong contrast to the corresponding cells in *A. mellifera*, which lack the cytoplasmic organelles involved in secretory processes [9]. In *A. mellifera*, the cells of the reservoir only secrete the cuticle that surrounds the reservoir [11], whereas in *P. versicolor* this lining was absent or, more commonly, reduced to a loose endocuticle. The absence of a thick cuticular layer surrounding the reservoir has not been described in the mandibular and ectal mandibular glands of other hymenopterans.

Mitochondria can capture fatty acids from the cytoplasm and break them down into acetyl-CoA to produce energy when the carbohydrate supply is scarce [22]. Modified mitochondria, like those detected in *P. versicolor*, have also been found in the post-pharyngeal gland of the ant *Dinoponera australis*, where they may indicate: 1) an excessive production of lipids, consistent with abundant SER and 2) deactivation of the enzymatic complex involved in the degradation of fatty acids [6]. The limited amount of SER in the ectal mandibular gland of *P. versicolor* appear to be conformed with hypothesis 2. The modified mitochondria in the post-pharyngeal gland of *D. australis* may nevertheless be related to the synthesis of lipids [6].

Ratcliffe and King [28] reported dramatic mitochondrial changes related to autophagic processes involving the formation of lipid droplets and myelinic bodies in the wasp *Nasonia vitripennis*. These myelinic structures were also observed in the cells and between cell apices in the reservoir of the ectal mandibular gland of *P. versicolor*, but without autophagic activity. These structures could be related to the regular processes of degradation and cycling of membranes and lipids which are common in secretory cells. However, Billen and Morgan [2] regarded these lamellar bodies as secretory lipids since they were frequently found in the peri-canalicular space of the terminal apparatus of insect secretory cells. The ultrastructural traits observed in the reservoir cells of the ectal mandibular gland of *P. versicolor* suggested a high level of synthetic activity [1] and could contribute to the secretory product contained inside the reservoir. A similar pattern was observed in venom glands of *Philanthus triangulum* [33], in which the ultimate toxicity of the secretory product is achieved in the reservoir.

ACKNOWLEDGMENTS

This work was supported by FAPESP (Proc. 99/02154-6). We thank Dr. Giovanni Delfino for their suggestions and criticism which greatly improved the manuscript quality.

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Received: March 13, 2003
Accepted: July 16, 2003