Fine Structure of the Male Accessory Glands of *Triatoma rubrofasciata* (De Geer, 1773) (Hemiptera, Triatominae)

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ABSTRACT Male of *Triatoma rubrofasciata* has four elongated sac-like reproductive mesodermic accessory glands, lined by an inner single layer of secretory cells, with basal plasma membrane infolds and short apical microvilli, and externally enveloped by a thin visceral muscle layer. The secretory cells have a well-developed rough endoplasmic reticulum, Golgi complex, mitochondria, and secretory granules. In one day old adult the gland cells are poorly developed, presenting small, electron-transparent secretory granules scattered among the rough endoplasmatic reticulum, whereas in three days old adult these cells have the cisternae of the rough endoplasmatic reticulum varing size degree, filled with granular electrondense content. In five days old males the secretory granules increase in diameter, being released to the gland lumen. Therefore, there is an increase of the secretory activity according to male maturation. *Microsc. Res. Tech. 70:355–360, 2007.* © 2007 Wiley-Liss, Inc.

INTRODUCTION

The morphology of the insect male reproductive tract has been described in some species, contributing for comprehension of their physiology, evolution, and behavior (Bahadur, 1975; Ferreira et al., 2004; Forbes and Do-Van-Quy, 1965; Hodapp and Jones, 1961; Lemos et al., 2005; Louis and Kumar, 1973; Raabe, 1986; Wheeler and Krutzsch, 1992).

The male accessory glands of the reproductive tract play an important reproductive role among the species, providing the success of protection and transferring of the spermatozoa to the female reproductive apparatus (Leopold, 1976). The male accessory glands contents are transferred to female during mating; these substances inhibit her receptivity for another mating, stimulating egg laying, besides to serve as a source of energy for ejaculated spermatozoa (Adams, 2001; Blum et al., 1962; Friedel and Gillot, 1977; Gillot, 2003; Marchini et al., 2003; Mikheyev, 2004; Paemen et al., 1990).

Triatomines are vectors of *Trypanosoma cruzi*, the ethiologic agent of Chagas disease. Although all the species may be potential vectors of Chagas disease, species living in human homes participate actively of the transmission.

In Triatominae there are four male accessory glands classified in anterior, external, internal, and dorsal according to insect body plan (Barth, 1958). The four glands opens together forming a hilus from which arise a common gland duct that opens into the ejaculatory duct (Barth, 1958).

In *Rhodnius prolixus* Stal, 1859, the spermatophore is produced from contents of three glands with transparent, viscous content, whereas the fourth gland has an opaque, granular aspect, being its secretion responsible for the movements of the spermatozoa inside the female (Davey, 1958). Adult males of *Panstrongylus megistus* (Burmeister, 1835) start to accumulate the accessory gland secretion from two days old, there being not difference of the secretion types between the dorsal and internal glands, whereas those from anterior and external glands present different proteins patterns (Regis et al., 1985, 1987).

Triatoma rubrofasciata (De Geer, 1773) is an endemic species confined to seaport regions, being frequently found in cities of the Brazilian coast (Macario-Rebelo et al., 1999). It is commonly the vector of *Trypanosoma conorhini,* which infects *Rattus rattus,* and this insect is in close association with the rat. Natural infection of *T. rubrofasciata* with *T. cruzi* has been reported in Brazil (Dias and Neves, 1943; Lucena, 1940) although, the biting and defecation habits of this bug make it a relatively inefficient vector (Braga and Lima, 1999).

We describe the ultrastructure of the male accessory glands of *T. rubrofasciata* during the process of sexual maturation, contributing for the knowledge of reproductive aspects of this species.

MATERIALS AND METHODS

Colonies of *T. rubrofasciata* from urban area of São Luís, State of Maranhão, Brazil, were maintained in the Department of Entomology, Oswaldo Cruz Institute, FIOCRUZ. Fifth instar male nymphs sexed according to Lent and Jurberg (1969), were maintained in a dark glass flask (30 × 15 cm^2) closed with nylon screen at 29 ± 1°C and 80 ± 5% rh with 12 h photoperiod. Inside the flask, a
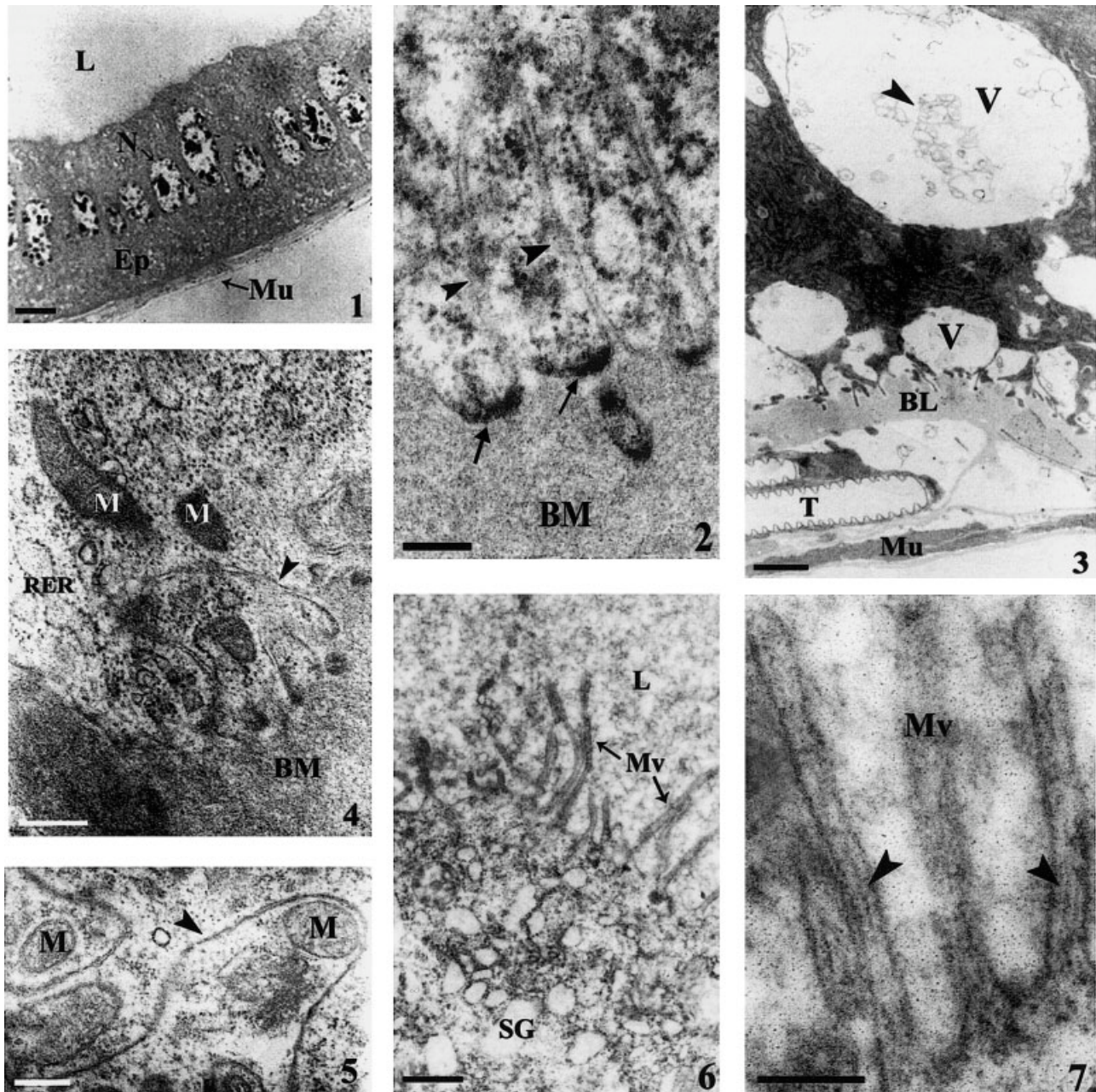
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folded filter paper was placed to increase the contact surface and refuge. The insects were fed weekly with blood of Swiss mouse (Protocol CEUA-FIOCRUZ P0100-01).

Starved adults of *T. rubrofasciata* with one, three, and five days old were dissected in saline solution for insect (0.1 M NaCl, 0.1 M KCl). The male accessory glands were isolated and fixed in 2.5% glutaraldehyde in sodium cacodilate buffer 0.1 M, pH 7.2 for 1 h and postfixed in 1% osmium tetroxide in the same buffer for 1h. The samples were dehydrated in an acetone series (50 to 100%) and embedded in resin Epon-Araldite.

Thin sections were stained with toluidine blue and analyzed in a light microscope Axiolab Zeiss. Ultrathin sections were contrasted with 5% uranyl acetate and 1% lead citrate and analyzed in a transmission electron microscope Zeiss EM 109.
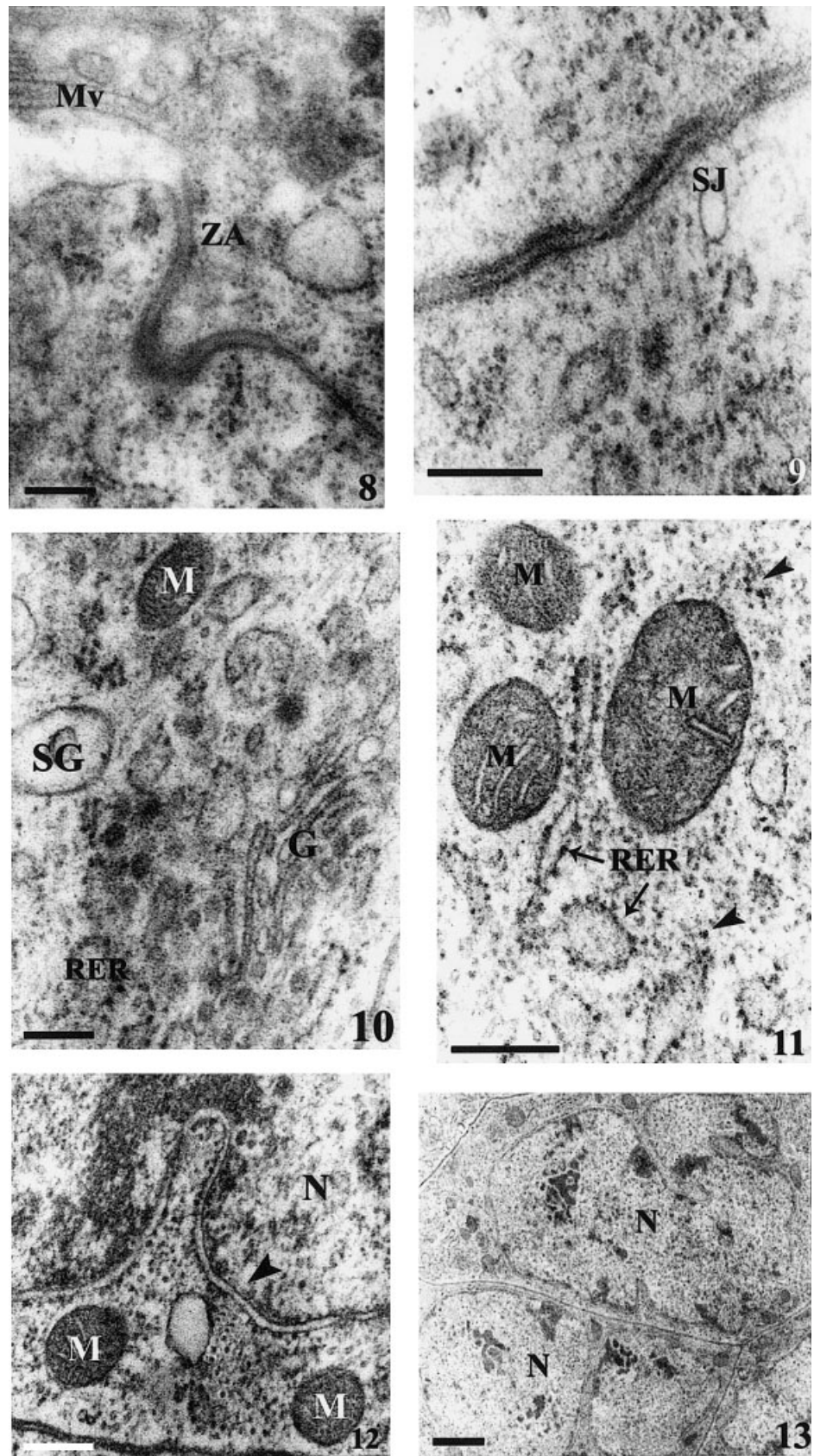
Figs. 8–13. Male accessory glands of *Triatoma rubrofasciata*. **Fig. 8:** *Zonula adherens* (ZA) in apical portion of the cell. Microvilli (Mv) (bar = 0.5 μm). **Fig. 9:** Septate junction (SJ) with narrow intercellular space (bar = 0.5 μm). **Figs. 10–11:** Middle region. Rough endoplasmic reticulum (RER); Golgi complex (G); Secretory granule (SG); Mitochondria (M); free polyribosome (arrowhead) (bar = 0.5 μm). **Fig. 12:** Nucleus (N) with nuclear envelope presenting folds (arrowhead) (bar = 0.5 μm). **Fig. 13:** Nucleus under low resolution. Notice deep folds in the nuclear membrane (bar = 2 μm).
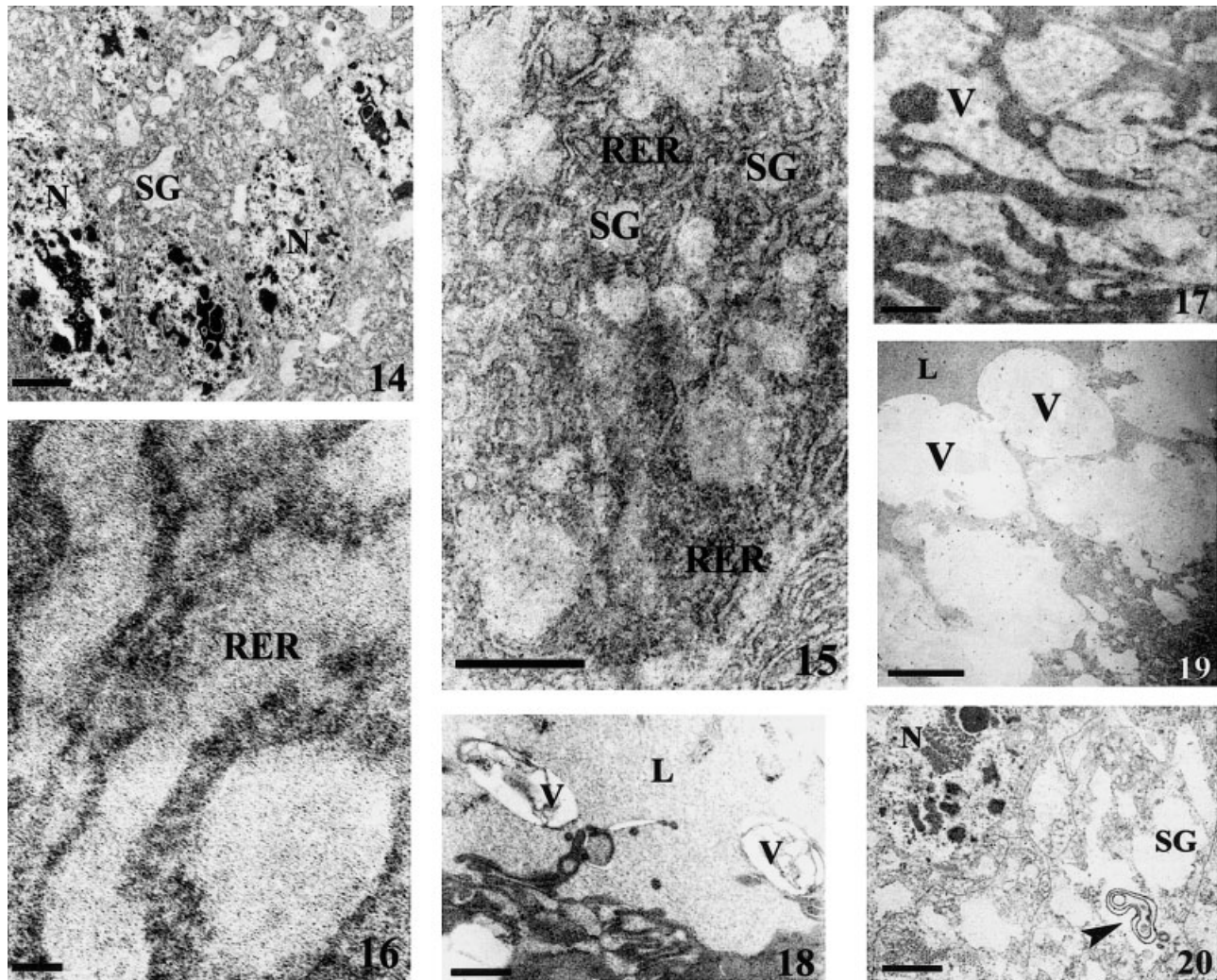
Figs. 14–20. Male accessory glands of *Triatoma rubrofasciata*. **Fig. 14:** Nucleus (N) present chromatin decondensed. Secretory granule (SG) (bar = 5 μm). **Fig. 15:** Glandular epithelium in adult with one day old presents small secretory granules (SG) among the rough endoplasmatic reticulum (RER) (bar = 1 μm). **Fig. 16:** Rough endoplasmic reticulum (RER) cisternae dilated in the glandular cells of three day old adult (bar = 0.1 μm). **Fig. 17:** Vesicles (V) with granular secretion (bar = 1 μm). **Fig. 18:** Vesicles (V) with membranous content casted of to the gland lumen (L) (bar = 1 μm). **Fig. 19:** Vesicles (V) eliminated to the gland lumen (L) (bar = 10 μm). **Fig. 20:** Myelin figure (arrowhead) and secretory granules (SG) in the cytoplasm. Nucleus (N) (bar = 5 μm).

RESULTS

The four accessory glands of the male reproductive tract of *T. rubrofasciata* are elongated sac-like. This anatomical aspect changes according to the aging. In the first day of adult life the glands are wither, without secretion storage. At the three days old, the glands have a shining aspect, with enlarged volume. The glands volume increases at the five days old males because of intracellular secretion storage.

The four accessory glands have the same structure. The gland lumen is lined by a single layer of secretory cells with basal plasma membrane infolds (Fig. 1) that have granular, homogeneous aspect. In the region of the basal infolds of the plasma membrane focal contact in association with the basal lamina can be seen, (Fig. 2). All glands are surrounded by thin visceral muscle layer and trachea, whose tracheolas are in closely association with the gland epithelium (Fig. 3).

The basal portion of gland cells has polyribosomes, rough endoplasmic reticulum, and mitochondria closely related to the deep folds of the plasma membrane (Figs. 4, 5). The cell apex presents microvilli supported by microfilaments ending in the apical cytoplasm (Figs. 6, 7) where rough endoplasmic reticulum and polyribosomes are found. The contact between the cells is maintained by *zonula adherens* (Fig. 8) and septate junctions with narrow intercellular space (Fig. 9).

The middle portion of the cell has Golgi complex, rough endoplasmic reticulum with dilated cisterns (Fig. 10), polyribosomes, and numerous mitochondria in the whole cytoplasm, many of them large and polymorphic (Figs. 4, 5, 11). The nuclear envelope is sinuous (Figs. 12, 13). The nucleus has predominance of descondensed chromatin and one or two nucleoli (Fig. 14).

Some ultrastructural features of the male accessory glands change according to the aging. The glands in one

day old adult are poorly developed with gland cells likely described above, with small secretory granules (0.22–0.40 μm) filled with electron-transparent content, scattered among the rough endoplasmatic reticulum (Fig. 15). In three days old adult, male accessory glands are well-developed, showing the rough endoplasmatic reticulum with dilated cisternae varying size (Fig. 16) containing granular secretion of low electron-density inside (Fig. 17). Some secretory granules with membranous content are placed both in the basal and apical regions (Fig. 2), which are released to the gland lumen (Fig. 18). At the five days old, the secretory granules increase in diameter (1.12–1.50 μm) and are released to the lumen (Fig. 19). Besides the secretory granules, the cytoplasm of the gland cell presents myelin figures (Fig. 20).

DISCUSSION

The four accessory glands of *T. rubrofasciata* male are mesodermic origin since they have not a cuticle lining the gland lumen (Chapman, 1998; Leopold, 1976) as in *Triatoma infestans* (Barth, 1958; Klug, 1834).

The gland cells of the accessory glands of the male reproductive tract of *T. rubrofasciata* have a single nucleus, differently of the data of Barth (1958) that related two nuclei in accessory glands of *T. infestans*. The founding of two nuclei in the cells of male accessory glands might be an artifact, because we find that the nuclear envelope has many folds, which under low resolution may be interpreted as two nuclei.

In the fly *Drosophila funebris* the secretory activity in the male accessory glands occurrs in the first week of the adult life, when the reproductive activity of the males is higher (Federer and Chen, 1982). In triatomines, the first mating in *T. infestans* males occur often after the second blood meal, whereas in *P. megistus,* secretion storage in male accessory glands may be found in two days old males, independent of blood meal (Regis et al., 1985). The *T. rubrofasciata* specimens herein analyzed remained starved during adult stage and we find secretory granules in cell apical region and released to gland lumen in three days old male, suggesting that reproductive activity in *T. rubrofasciata* starts from the third day of adult life, independent of the nutritional state of the insect.

The secretory release in some case can be merocrine, although the presence of membranes inside the gland lumen suggest apocrine secretion. The occurrence of both apocrine and merocrine mechanisms for release of secretory products were found in accessory glands of males of *Leptinotarsa decemlineata* (De Loof and Lagasse, 1972), *Periplaneta americana* (Adiyodi and Adiyodi, 1974), and *Tenebrio molitor* (Gadzama et al., 1977). Moreover, the great number of mitochondria and the depth basal plasma membrane infolds in the basal portion of the cells of male accessory glands of *T. rubrofasciata* suggest an absorptive activity of substances from hemolymph, likely found in *P. megistus* (Regis et al., 1987).

Cells with higher secretory activity have many secretory granules, rough endoplasmatic reticulum, Golgi complex, and mitochondria. However, because of the presence of secretory granules with different sizes, shape, and electron density in the gland cells of accessory glands and mainly because of the two different mechanisms of cell secretion releasing of *T. rubrofasciata* males, cythochemical analyses are necessary to make, in order to determine with more accuracy the secretion nature of these glands.

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