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Original Article

Histology and histochemistry of the accessory gland of the female reproductive tract of *Rhodnius neglectus* Lent, 1954 (Hemiptera: Reduviidae)

Histologia e histoquímica das glândulas acessórias do sistema reprodutor feminino de *Rhodnius neglectus* Lent, 1954 (Hemiptera: Reduviidae)

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Abstract

Rhodnius neglectus is a wild triatomine, vector of the protozoan *Trypanosoma cruzi*, which causes Chagas' disease, and feeds on the blood of small mammals, being essential for its growth and reproduction. Accessory glands of the female reproductive tract are important in insect reproduction, but their anatomy and histology in *R. neglectus* are poorly studied. The aim of this work was to describe the histology and histochemistry of the accessory gland of the female reproductive tract of *R. neglectus*. The reproductive tract of five females of *R. neglectus* was dissected and the accessory glands transferred to Zamboni's fixative solution, dehydrated in a crescent series of ethanol, embedded in historesin, sectioned at 2 µm thick, stained with toluidine blue for histological analysis or mercury bromophenol blue for detection of total proteins. The accessory gland *R. neglectus* is tubular, without branches, opening in the dorsal region of the vagina and differing along its length in proximal and distal regions. In the proximal region, the gland is lined by the cuticle with a layer of columnar cells associated with muscle fibers. In the distal region of the gland, the epithelium has spherical secretory cells with terminal apparatus and conducting canaliculi opening in the lumen through pores in the cuticle. Proteins were identified in the gland lumen, terminal apparatus, nucleus and cytoplasm of secretory cells. The histology of the *R. neglectus* gland is similar to that found in other species of this genus, but with variations in the shape and size of its distal region.

Keywords: Chagas' disease, oviposition, secretion, Triatominae.

Resumo

Rhodnius neglectus é um triatomíneo silvestre, vetor do protozoário *Trypanosoma cruzi*, causador da doença de Chagas. Este inseto se alimenta do sangue de pequenos mamíferos, que é essencial para o seu crescimento e reprodução. As glândulas acessórias do sistema reprodutor feminino são importantes na reprodução de insetos, mas sua anatomia e histologia em *R. neglectus* são pouco conhecidas. O objetivo deste trabalho foi descrever a histologia e histoquímica da glândula acessória do aparelho reprodutor feminino de *R. neglectus*. O sistema reprodutor de cinco fêmeas de *R. neglectus* foi dissecado e as glândulas acessórias transferidas para solução fixadora de Zamboni, desidratadas em série crescente de etanol, embebidas em historesina, seccionadas com 2 µm de espessura, coradas com azul de toluidina para análise histológica ou submetidas ao teste de mercúrio-bromofenol para detecção de proteínas totais. O sistema reprodutor de *R. neglectus* tem uma glândula acessória tubular, sem ramificações, abrindo-se na região dorsal da vagina, sendo diferenciada em regiões proximal e distal. Na região proximal, a glândula é revestida internamente pela cutícula com uma camada de células colunares associadas a fibras musculares. Na região distal ocorrem células secretoras esféricas com aparato terminal e canalículos condutores que se abrem no lúmen da glândula através de poros na cutícula. O teste histoquímico revelou a presença de proteínas no lúmen da glândula e no aparato terminal, núcleo e citoplasma das células secretoras. A histologia da glândula de *R. neglectus* é semelhante à das espécies desse gênero, mas com variações na forma e no tamanho de sua região distal.

Palavras-chave: doença de Chagas, oviposição, secreção, Triatominae.

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1. Introduction

Triatomines are wild hematophagous insects, among them, *Rhodnius neglectus* Lent, 1954 (Hemiptera: Reduviidae) vector of the protozoan *Trypanosoma cruzi* Chagas, 1909 (Kinetoplastida: Trypanosomatidae), which causes Chagas' disease, feeds on the blood of small mammals (Gurgel-Gonçalves et al. 2003; Tartarotti et al., 2006). This insect is usually found on palm leaves and bird nests, in addition to reports in human homes (Rodrigues et al., 2009; Carvalho et al. 2014; Ramos et al. 2018). *R. neglectus* is endemic to the Brazilian Cerrado, but it is widely distributed in the Central, Northeast, Southeast and North regions of the country (Gurgel-Gonçalves and Cuba, 2009; Rodrigues et al., 2009; Ramos et al. 2018). The importance of this hematophagous insect for human health (Gurgel-Gonçalves et al., 2012) increases the need to knowledge its reproductive biology to contribute to the management of this Chagas' disease vector.

The female reproductive tract of insects is formed by a pair of ovaries and lateral oviducts, a common oviduct, spermathecae, and accessory glands (Chapman, 2013). The accessory gland is usually unique in hematophagous Hemiptera, as reported for *Triatoma lecticularia* (Stal, 1859), *Rhodnius brethesi* Matta, 1919, *Rhodnius nasutus* Stal, 1859, and *Rhodnius pictipes* Stal, 1872 (Chiang et al., 2012; Monteiro et al., 2019). However, in other non-hematophagous hemipterans, including *Cosmoclopius nigroannulatus* (Stal, 1860) (Reduviidae) (Jahnke et al., 2006), *Diaphorina citri* Kuwayama, 1908 (Liviidae) (Dossi and Cônsoli., 2014), and *Acyrthosiphon pisum* (Harris, 1776) (Aphididae) (Wieczorek et al. al., 2019) the female reproductive tract has two accessory glands). In crickets, the accessory gland is characterized by a complex of tubules with numerous secretory branches, as reported for *Acheta domesticus* (Linnaeus, 1758), *Gryllus bimaculatus* De Geer, 1773, *Gryllus assimilalis* (Fabricius, 1775) and *Teleogryllus commodus* (Walker, 1869) (Orthoptera: Gryllidae) (Sturm, 2008; 2012).

The accessory gland of the female reproductive in insects has been associated with production of compounds the play role as a lubricant, improving the transport of fertilized eggs, protecting them against desiccation and predators, in addition to the attachment of eggs to the substrate at the time of oviposition (Chapman 2013). The antibacterial function of the secretion produced by the female accessory gland has been also reported in Diptera (Belardinelli et al., 2005). Proteins, lipids and carbohydrates are the main components found in the secretion released by the accessory glands (Lococo and Huebner, 1980b; Sturm 2002).

The accessory gland of the female reproductive tract of triatomines that show oviposition behavior without egg attachement to the substrate, such as *Triatoma dimidiata* (Latreille 1811) and *Triatoma sordida* (Stal 1859) (Hemiptera: Reduviidae), is reduced or absent (Chiang et al., 2012). On the other hand, this gland, in *Rhodnius prolixus* Stal, 1859 (Hemiptera: Reduviidae), is well-developed with a tubular shape, numerous spherical secretory cells and an excretory duct surrounded by muscle tissue (Lococo and Huebner, 1980b). This gland is known as cementum because it releases rich-protein secretion that spreads on the egg surface, strongly attaching it to the substrate (Lococo and Huebner, 1980b; Davey, 2007).

The accessory gland is highly specialized and fundamental in the reproductive process and success of insects, although it is not fully known in some species. The aim of this work was to describe the histology and histochemistry of the accessory gland of the female reproductive tract of *R. neglectus*.

2. Materials and Methods

2.1. Insects

Females of *R. neglectus* were obtained from the Department of Parasitology, Universidade Federal do Triângulo Mineiro (UFTM, 19°45' S, 47°55' W), Uberaba, state of Minas Gerais, Brazil. The colony was stablished from insects collected in Tocantinópolis, state of Tocantins, Brazil in 2014 and maintained in the laboratory at room temperature and natural illumination cycle. The females were transferred to the Cellular and Structural Biology Laboratory of the Universidade Federal de Viçosa Campus Rio Paranaíba in Rio Paranaíba, state of Minas Gerais, Brazil. The experiments carried out with animals were in accordance with the license 256, approved by the Animal Use Ethics Committee (CEUA) from UFTM (Carvalho-Costa et al., 2015). This insect was reared in cylindrical acrylic containers with pieces of folded cardboard to increase the surface area of refuge. The containers were sealed with a thin cotton fabric allowing the blood meal in chicken (Mendes, 2014).

2.2. Histology and histochemistry

Five *R. neglectus* females were cold anesthetized at 0 °C for five minutes and their reproductive tract dissected in 125 mM NaCl. The accessory gland was isolated and transferred to Zamboni's fixative solution (Stefanini et al., 1967) for 24 hours at 4°C and then dehydrated in a graded ethanol series (70%, 80%, 90%, and 95%) for 15 minutes each. The accessory glands were embedded in Leica® historesin, sectioned at 2 µm thick in a Leica® rotatory microtome and stained in toluidine blue-borax. Some sections were submitted to the mercury bromophenol blue test to evidence total proteins (Bancroft and Gamble, 2008). These samples were analyzed in an Olympus® CX41 light microscope coupled with a Nikon D3100 camera.

3. Results

The female reproductive tract of *R. neglectus* consisted of a pair of ovaries and lateral oviducts opening in a common oviduct, spermathecae, and an accessory gland (Figure 1). The accessory gland of *R. neglectus* was connected to the dorsal region of the vagina, and was well-developed with an elongated, tubular-shaped, unbranched and coiled shape in the terminal region (Figure 1).

The accessory gland, along its length, differs between the proximal and distal regions (Figure 1). In the proximal

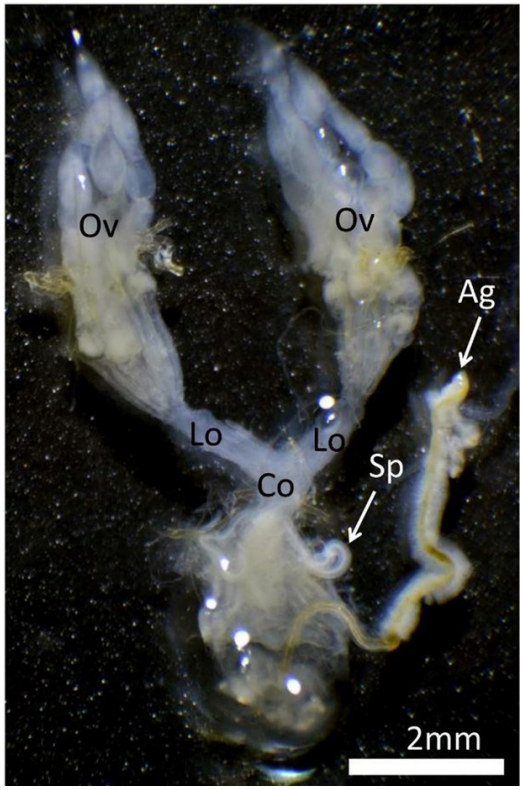

Figure 1. Female reproductive system of *Rhodnius neglectus* (Hemiptera: Reduviidae), consisting of two ovaries (Ov) with lateral oviducts (Lo) connected to the common oviduct (Co), spermathcae (Sp) and accessory glands (Ag).

region, the epithelium was formed by a single layer of cubic cells with the lumen lined by a folded cuticular intima and the basal surface associated with thick longitudinal muscles (Figures 2A, D). The coiled distal region had the epithelium with spherical secretory cells with terminal apparatus containing a canaliculus that open in the lumen through pores in the cuticular intima (Figures 2B, C, D). In the terminal apparatus of the cells occurred the presence of secretion (Figure 2B). The secretory cells of the accessory gland of *R. neglectus* were spherical with a well-developed nucleus rich in decondensed chromatin and evident nucleolus (Figure 2C). Positive staining to protein was found in the region of secretory cells, terminal apparatus, canaliculi and in the lumen of the accessory gland of *R. neglectus* (Figures 3A, B).

4. Discussion

The anatomical features of the *R. neglectus* female reproductive tract, with a pair of lateral oviduct, common oviduct, spermathecae and an accessory gland, are common in insects, including Hemiptera representatives, such as Reduviidae *Rhodnius montenegrensis* da Rosa et al., 2012, *Triatoma infestans* (Klug, 1834) (Nascimento et al., 2017; 2019) and Liviidae *D. citri* (Dossi and Cônsoli., 2014). However, in Pentatomidae the accessory gland is absent (Lemos et al. 2005).

The female accessory gland of *R. neglectus* is well-developed due to the oviposition behavior of this insect, producing a large amount of secretion to attach the eggs to the substrate, as other *Rhodnius* spp. (Rabinovich et al., 2010; Chiang et al., 2012).

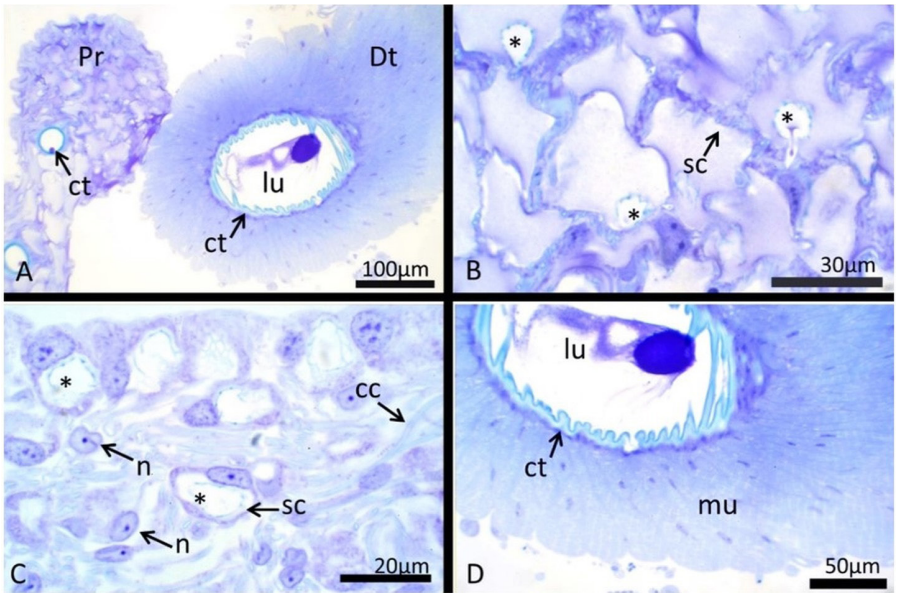

Figure 2. Light micrographs of the accessory gland of the female reproductive tract of *Rhodnius neglectus* (Hemiptera: Reduviidae). (A) proximal (Pr) and distal regions (Dt) showing lumen (lu) covered by cuticle (ct); (B) Distal region showing secretory cells (sc) with secretion in the terminal apparatus (asterisk); (C) Secretory cells (sc) of the distal region with a nucleus (n) rich in decondensed chromatin and evident nucleolus (arrowheads) and the canaliculi (cc); (D) Proximal region showing cubic epithelium with thick cuticle (ct) and well-developed muscles (mu).

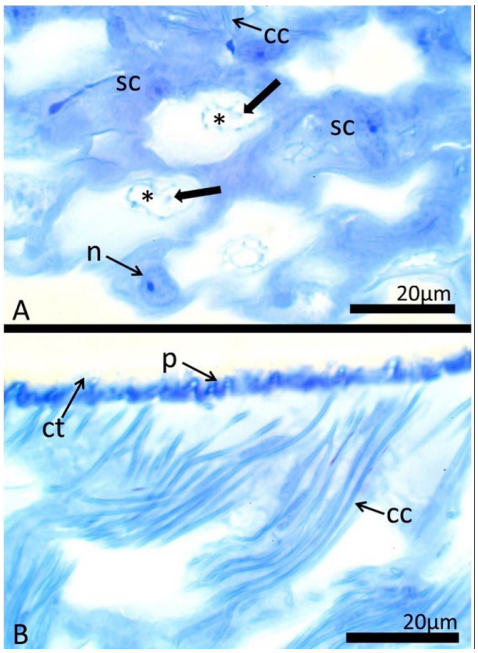

Figure 3. Light micrographs of the accessory gland of the female reproductive tract of *Rhodnius neglectus* (Hemiptera: Reduviidae) submitted to the mercury-bromophenol test. (A) Secretory cells (SC) showing positive reaction for proteins (black arrows) in the terminal apparatus (asterisks), nucleus (n), and canaliculi (cc); (B) Positive reaction for protein in the cuticle (ct) of the reservoir and in the canaliculi (cc).

However, this accessory gland is absent in triatomines without eggs that are attached to the substrate, including *Nesotriato Nesotriatoma bruneri* [= *Nesotriatoma flavida* (Neiva, 1911)] (Chiang et al., 2012; Tellez-Garcia et al., 2019). The tubular shape without ramifications of the accessory gland of the female reproductive tract of *R. neglectus* is similar to that reported in *R. prolixus*, called cement gland and common in this genus (Lococo and Huebner, 1980a; Chiang et al., 2012). However, with variations in the shape of the gland such as that in *R. nasutus* with accessory gland with an enlarged tube that bends over itself, and *R. pictipes*, in which the distal region of the gland is folded forming a globular structure (Chiang et al., 2012; Chiang and Chiang, 2017). The opening of the accessory gland, e.g, in the dorsal region of the vagina in *R. neglectus* varies among insects, as it is a structure involved in the reproduction process, as in *D. citri* opening in the common oviduct (Dossi and Cônsoli, 2014; Alba-Alejandre et al., 2020).

Differentiation along the length of the accessory gland in proximal and distal regions of *R. neglectus* with the proximal shorter and associated with muscles, may indicates a characteristic of transport of the secretion produced in the longer distal region. This gland type with different regions is common in the genus *Rhodnius*, but the distal region may be more dilated in a circular shape as in *R. nasutus* (Chiang et al. 2012; Lococo and Huebner, 1980a).

The gland duct lined by a folded cuticular intima, with cubic epithelium, and muscle fibers in the proximal region, indicates contractile activity of muscles, probably with pumping function to drive and control the release of secretion in the vaginal chamber, as suggested to occur in *R. prolixus* (Lococo and Huebner, 1980a). The release of secretion, by muscle contraction, has been suggested to coat eggs with secretion for oviposition (Chiang and O'Donnell, 2009; Sedra and Lange, 2014).

The presence of secretory cells with terminal apparatus and conducting canaliculus indicates that these cells are of class III, according to the classification of Noirot and Quennedey (1991). The terminal apparatus is a structure in the cytoplasm of the class III secretory cell and is usually located in the apical or basal region of the cell, function to collect the secretion produced in the cell (Stacconi and Romani, 2011; Souza et al., 2016; Pascini and Martins, 2017), confirming the secretion activity of the terminal apparatus by the presence of proteins rich secretion, observed after the histochemical test. Thus, the secretion produced is conducted through a conducting canaliculus associated with the terminal apparatus and released into the lumen through pores present in the cuticle that lines the central duct, such as in other Hemiptera (Lococo and Huebner, 1980a).

Secretory cells in the distal region of the accessory gland of *R. neglectus* female show the nucleus rich in decondensed chromatin and an evident nucleolus, indicating high metabolic activity. This suggests that their cytoplasm may be rich in endoplasmic reticulum, Golgi apparatus, mitochondria and secretory vesicles, that are related to the production of proteins present in the secretion (Stacconi and Romani, 2011; Sturm, 2012; Laghezza Masci et al., 2015).

Positive staining for protein in the secretory cells of the female accessory gland here observed, may be due to the cytoplasm and cell nucleus. Positive protein staining in the conducting canaliculi and lumen of the accessory gland of *R. neglectus* suggest that the secretion produced by the class III secretory cells is rich in protein, such as reported for *R. prolixus*, in which the released rich-protein secretion has adhesive function and acts on oviposition (Lococo and Huebner, 1980a). In addition to *Rhodnius* spp., the presence of proteins has been reported in the accessory gland of the female reproductive tract in other Hemiptera species as *Acyrthosiphon pisum* (Harris, 1776) and *Acyrthosiphon svalbardicum* Heikenheimo, 1968 (Hemiptera: Aphididae) (Wieczorek et al., 2019; 2020), and other insects such as *T. commodus* (Sturm, 2008) and *Phlebotomus papatasi* (Scopoli, 1786) (Diptera: Psychodidae) (Rosetto et al., 2003).

The accessory gland of the female reproductive tract of *R. neglectus* is similar to that of other species of this genus, with tubular morphology, without branches and with numerous secretory cells. The presence of muscle fibers, secretory cells with terminal apparatus, conducting canaliculi, and protein suggest the specialization in the synthesis, release and transport of rich-protein secretion.

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