SYSTEMATICS, MORPHOLOGY AND PHYSIOLOGY

The Metasternal and Brindley's Glands of *Triatoma brasiliensis* Neiva (Hemiptera: Reduviidae)

Ana Cristina R Vitta, José E Serrão, Eraldo R Lima, Evaldo F Vilela

Depto. de Biologia Geral, Univ. Federal de Viçosa (UFV), Viçosa, MG; jeserrao@ufv.br, mothman@insecta.ufv.br, evilela@ufv.br

Edited by Takumasa Kondo – CORPOICA, Colombia

Neotropical Entomology 38(2):231-236 (2009)

Glândulas de Brindley e Metasternal de *Triatoma brasiliensis* Neiva (Hemiptera: Reduviidae)

RESUMO - *Triatoma brasiliensis* Neiva é o principal inseto vetor da doença de Chagas no Nordeste do Brasil. Os adultos da espécie apresentam dois pares de glândulas exócrinas, as glândulas metasternais e as glândulas de Brindley que liberam compostos voláteis, possivelmente com função de defesa, alarme e/ou acasalamento. O conhecimento anatômico e histológico das glândulas de Brindley e metasternal no gênero *Triatoma* é escasso e, considerando a relevância dessas glândulas na sua biologia, o objetivo do presente trabalho foi estudar e conhecer a sua morfologia. As glândulas de Brindley e metasternal de *T. brasiliensis* possuem uma unidade glandular similar àquela descrita para *Rhodnius prolixus* Stål e *Panstrongylus megistus* Burmeister, formada principalmente por um aparato secretor, sáculo e ducto coletor.

PALAVRAS-CHAVE: Doença de Chagas, triatomíneo e morfologia

ABSTRACT - *Triatoma brasiliensis* Neiva is the most important vector of the Chagas' disease in the semiarid zones of North-eastern Brazil. Adult bugs have two main pairs of exocrine glands, the metasternal and the Brindley's glands, which release volatiles possibly with defense, alarm and/or mating functions. To date, anatomical and histological studies of the metasternal and the Brindley's glands in the genus *Triatoma* are scarce and, considering the relevance of these exocrine glands, the present work aimed at studying their morphology in *T. brasiliensis*. The metasternal and the Brindley's glands of *T. brasiliensis* consist of glandular units similar to those described for *Rhodnius prolixus* Stål and *Panstrongylus megistus* Burmeister, comprising a secretory apparatus, saccule and collector duct.

KEY WORDS: Chagas' disease, triatomine, morphology

Triatoma brasiliensis Neiva is the most important vector of the Chagas' disease in the semiarid zones of North-eastern Brazil. Adult bugs have two main pairs of exocrine glands, the metasternal and the Brindley's glands, which release volatiles possibly with defense, alarm and/or mating functions (Manrique *et al* 2006, Scholfield 1979, Rossiter & Staddon 1983).

The Brindley's glands are simple sac-like structures located dorsally, extending into the lateral part of the second abdominal segment of Reduvioidae (Kälin & Barrett 1975, Staddon 1983, Weirauch 2006). Disturbed adult triatomine bugs release a secretion from these glands, whose main component is isobutyric acid with putative alarm and defense functions (Games *et al* 1974, Ward 1981, Manrique *et al* 2006). The Brindley's glands in *Rodnius prolixus* Stål are formed by saccules, secretory cells and ducts (Kalin & Barrett 1975) that resemble the dermal gland type "B" consisting of four cell types (Wigglesworth 1933). Subsequently, Barrett *et al* (1979) reported the presence of another type of glandular unity named type "A". In *Panstrongylus megistus* Burmeister though, the Brindley's glands consist of two cell types (Schofield & Upton 1978).

The metasternal glands are widespread among Heteroptera (Weirauch 2006) and lie ventrally on the metathorax, consisting of a small pear-shaped reservoir and an unbranched secretory tubule opening laterally to the sternal apophyseal pit. The single known compound in Triatominae, 3-methyl-2-hexanone, is released by the metasternal glands of *Dipetalogaster maximus* Uhler (Rossiter & Staddon 1983). Alcohols and ketones are the main compounds from these glands in *Triatoma infestans* Klug, which may play a role in the sexual and alarm communication (Manrique *et al*. 2006).

The present work aimed at studing the morphology of these glands in *T. brasiliensis*. The anatomical and histological studies of the metasternal and the Brindley's glands in the genus *Triatoma* will put this species in a systematic context considering that no species in this genus have been hitherto studied in detail.

Material and Methods

Insects. *Triatoma brasiliensis* adults were maintained at 27° ± 1°C, 64 ± 10% RH, subjected to an illumination regime of 12:12L/D and fed on rabbit blood. Experiments were conducted with both 30-50 day-old males and females.

Scanning electron microscope (SEM). The metasternal (n = 8) and the Brindley's glands (n = 8) of adult males and females were dissected in saline solution and transferred to Zamboni´s solution (Stefanini *et al* 1967) for 24h at 4°C. Subsequently, samples were washed in sodium phosphate buffer 0.1 M, pH 7.4 for 5 min, dehydrated in a graded ethanol series, transferred to hexamethyldizilasane (HMDS) for 10 min and air dried. Glands were then coated with a 30-nm-thick gold layer and analyzed with a scanning electron microscope JEOL VP1430. Some metasternal glands were transferred to distilled water for 5 min and to 1% triton 100-X during 15 min to rupture cells and expose their ducts following fixation and the standard procedure for SEM describe above.

Histology. After dissection, the metasternal (n = 6) and the Brindley's glands (n = 6) were transferred to Zamboni's fixative solution (Stefanini *et al* 1967). Glands were dehydrated in a graded ethanol series and embedded in historesin JB4. Sections 5 μm thick were stained with hematoxyline and eosin Phloxine stain. Ultrathin sections were contrasted with 5% uranyl acetate and 1% lead citrate (Bancroft & Stevens 1996).

Results

Brindley's glands. The Brindley's glands of *T. brasiliensis* are located between the abdomen and the dorsolateral part of the metathorax. The glands possess a secretory portion in a dilated sac-like structure opening into a collector duct (Figs 1, 7). Attached onto the receiving duct wall, there is a well-developed tendon connecting the duct wall to a muscular bundle, named here retractor muscle (Figs 2, 7).

The secretory portion of the wall of the Brindley's gland of *T. brasiliensis* comprises a layer of secretory cells, over which there is a layer of flattened cells covered by cuticle (Figs 7, 8). The flattened cells have elongated nuclei with condensed chromatin. A thickened cuticle over the epithelium coats the gland lumen. The epithelium may be folded according to the amount of secretion accumulated in the gland (Fig 8).

The secretory unit consists of gland cells formed by a secretory apparatus, a saccule and a collecting canal surrounded by cuticle. The gland cells are columnar and the well-developed nucleus has predominantly uncondensed chromatin with an evident nucleolus (Fig 8). The cytoplasm is characterized by the presence of granules that were negative for either acid or alkaline staining.

The secretory apparatus is represented by a wide extracellular cavity in which a surface folding may be seen. A cuticular canal, the collecting canal, opens onto the secretory apparatus and into the lumen of the gland by means of a canalicle (Fig 8).

We found both cell types "A" and "B". There are few type "A" cells, which are characterized by a U-shaped narrowed saccule, whereas the type "B" cells are abundant and have a spherical widened saccule (Fig 10).

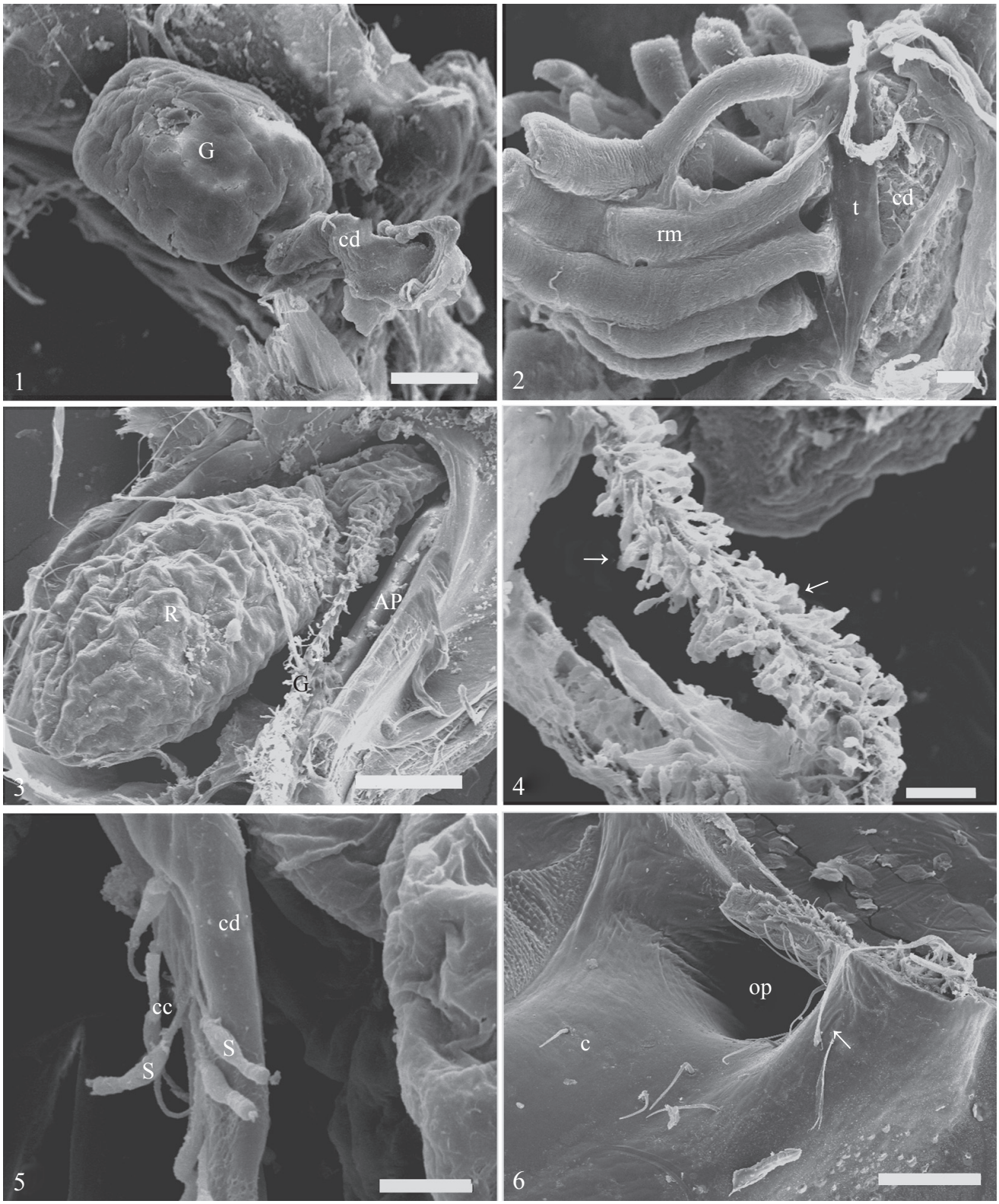
Metasternal glands. The metasternal glands are located in the metathoracic region, laterally to each metacoxal cavity (Fig 3). The metasternal gland of *T. brasiliensis* consists of an elongated secretory tubule that opens into a reservoir in an elongated sac-like shape (Fig 9). Each gland, together with its reservoir, is closely attached to an apophysis by a retractor muscle (Fig 3). The gland opens to the exterior by means of a small opening near to the apophysis (Fig 6). In each secretory cell, the secretion product is collected by the collecting canal, which dilates in a sac-like form and protrudes in the secretory cell invagination, so that the set of collecting canals opens independently onto the collector duct that reaches the gland reservoir (Figs 4, 5).

The secretory portion of the metasternal glands consists of a monolayer of columnar gland cells with spherical nuclei (Fig 10). The cytoplasm of the gland cells has some acidophil granules. Each gland cell has a secretory apparatus and a receiving duct opening into the gland lumen, which is narrowed and covered by a thick cuticle (Figs 10, 11). The metasternal gland reservoir is covered by a thin cuticle and surrounded by a monolayer of epithelial cells, which are flattened as well as their nuclei (Figs 9, 10).

Discussion

The Brindley's and the mestasternal glands of *T. brasiliensis* reveal glandular units that are similar to those described for both *R. prolixus* (Kalin & Barrett 1975) and *P. megistus* (Schofield & Upton 1978, Santos-Mallet & Souza 1990), which under light microscope are mainly comprised by a secretory apparatus, a saccule and a collecting canal. Such gland unit resembles the dermal gland type "B" reported by Wigglesworth (1933) and Lai-Fook (1970) for *R. prolixus*. Subsequently, Barrett *et al*. (1979) reported the presence of another type of glandular unit named type "A" structurally different from type "B". Our observations have confirmed that the Brindley's glands of *T. brasiliensis* are formed by a set of the two glandular unit types "A" and "B", randomly distributed in the gland. As reported by Barret *et al* (1979) the type "B" cells seem to be more numerous than type "A".

The retractor muscle of the Brindley's glands of *T. brasiliensis* is comprised of a well-developed tendon directly connected to the collector duct enabling contraction for secretion release. In *R. prolixus*, the retractor muscle is connected to the collector duct through three ramifications from the tendon (Barrett *et al* 1979). Although some glands, such as those secreting terpene in *Anisomorpha buprestoides* Stoll, are surrounded by a set of intrinsic muscles that enables contraction for secretion release (Happ *et al* 1966), in both *R. prolixus* and *T. brasiliensis* a single muscle is associated with the Brindley's glands. Furthermore, no muscle was found surrounding either the glandular lumen or the epithelium.



Figs 1-6 Scanning electron micrographs of the Brindley's (1-2) and metasternal (3-6) glands of *Triatoma brasiliensis*. 1) Secretory portion (G) and collector duct (cd). Bar = 100 μm. 2) The tendon (t) and associated retractor muscles (rm) of the collecting duct. Bar = 20 μm. 3) Reservoir (R) and secretory portion (G) of the metasternal gland. AP – metasternal apophysis. Bar = 100 μm. 4) Detailed view of secretory portion of the metasternal gland showing the sclerotized units (arrows). Bar = 20 μm. 5) The collector duct (cd) with associated saccule (s) and collecting canal (cc) of the metasternal gland. Bar = 10 μm. 6) opening (op) of the apophysis and of the metasternal gland (arrowhead). c – body cuticle. Bar = 100 μm.

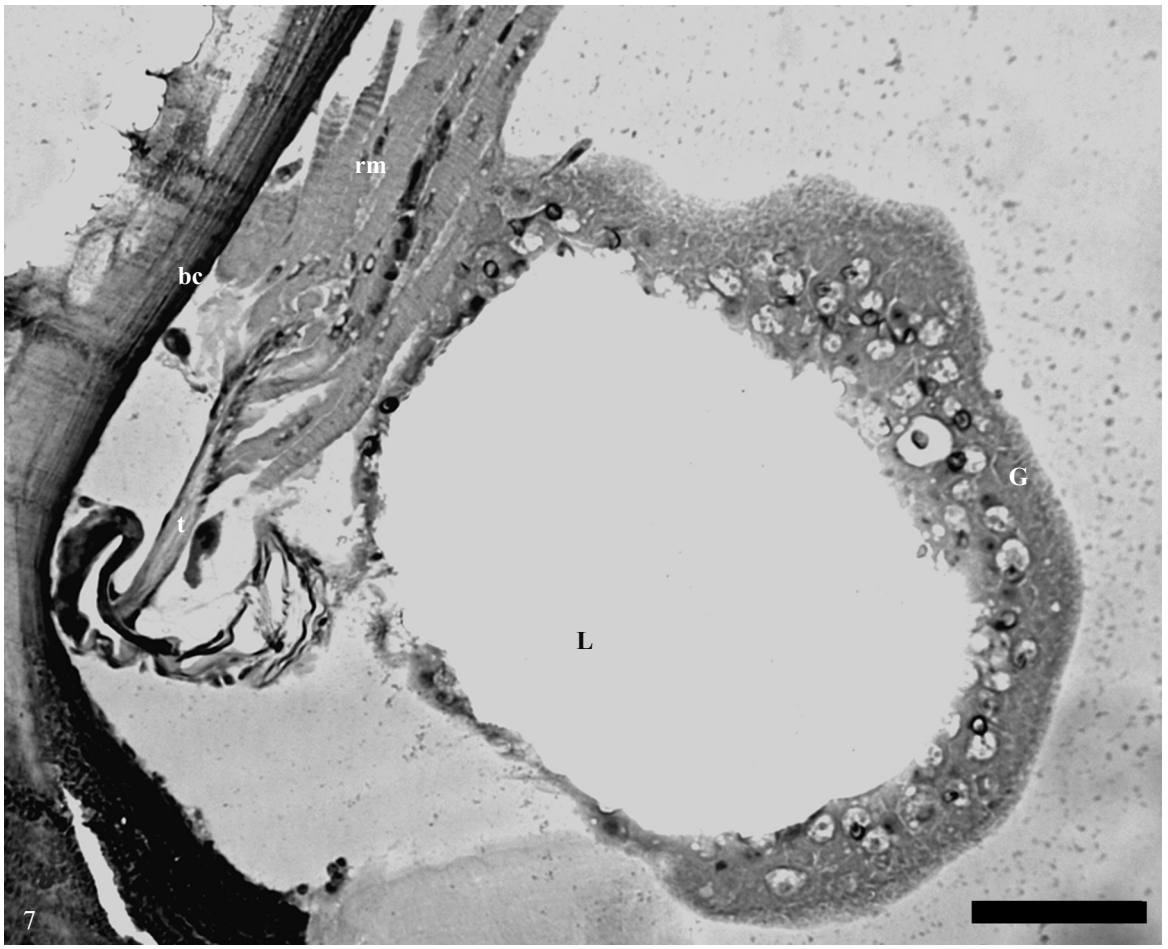


Fig 7 Light micrography of the section of the Bindley's gland of *Triatoma brasiliensis* showing the secretory portion (G) and the gland lumen (L). Note the collector duct (cd) associated with retractor muscles (rm) through the tendon (t). bc – body cuticle. Bar = 20 μm.

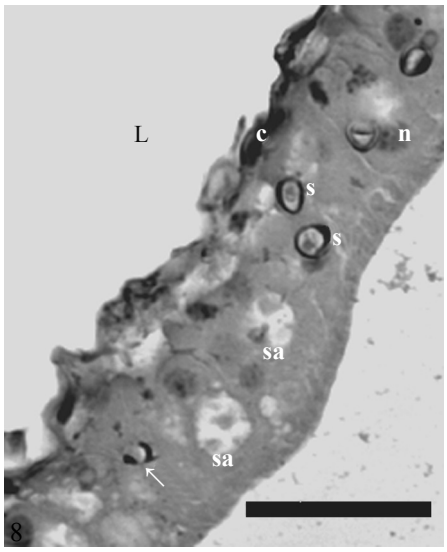
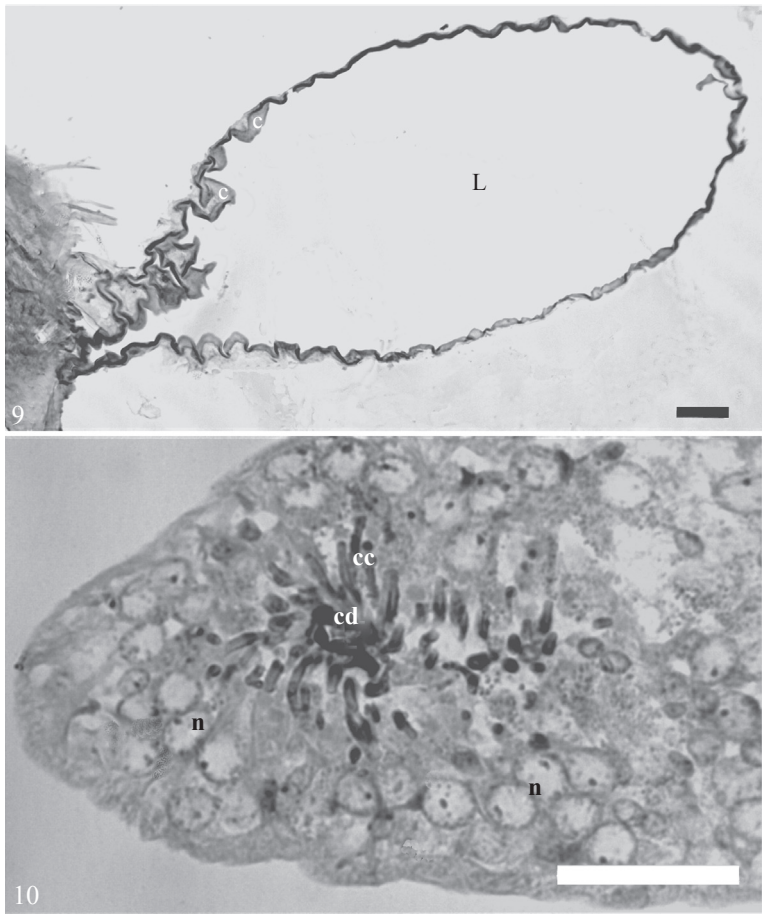


Fig 8 The Brindley's gland of *Triatoma brasiliensis* showing the gland epithelium. Note the secretory apparatus (sa) and the saccule (s) of gland cells type "B" and the U-shape saccule (arrowhead) of the gland cell type "A". L – gland lumen; n – nucleus; c – cuticle. Bar = 20 μm.

Strongly stained and well-distributed granules were found in the cytoplasm of secretory cells of mestasternal glands, suggesting that these glands produce substances different from those secreted by the Brindley's glands, which show non-stained granules. Alcohols and ketones were the main compounds produced by the metasternal glands of *Triatoma infestans* (Manrique *et al* 2006), whereas isobutyric acid is the major component of the Brindley's gland (Games *et al* 1974). Therefore, the presence of acidophil granules in the metasternal glands suggest that other components might be present in this gland, yet alcohols and ketones may be transported to the secretory apparatus together with substances of alkaline nature.

In both metasternal and the Brindley's glands, the description of the glandular cells herein presented agree with that reported by Noirot & Quennedey (1991), showing that they are comprised of type III cells. Hence, we suggest the use of cellular classification into cells type III, already proposed by Noirot & Quennedey (1991), since glandular cells from both glands release their secretions through a collecting canal formed by one or more cells and that such units may be either isolated or associated to other glandular units or accessory structures (extracellular reservoir). That general classification into type III glands does not invalidate the



Figs 9-10 The metasternal gland of *Triatoma brasiliensis*. 9) The reservoir showing the enlarged lumen (L) lined by a thin epithelium with cuticle (c). Bar = 20 μm. 10) The secretory portion showing the gland cells with well-developed nucleus (n) and collecting canal (cc) opening in the collector duct (cd). Bar = 20 μm.

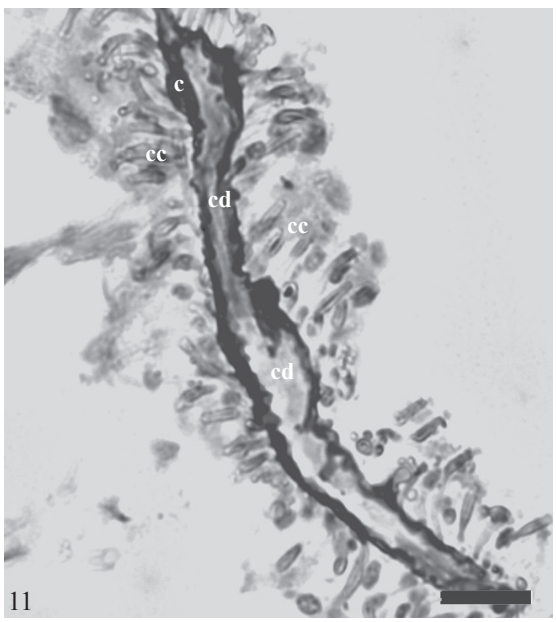


Fig 11 The metasternal gland of *Triatoma brasiliensis* showing the collecting canal (cc) opening in the conducting duct lined by a thin cuticle (c). Bar = 20 μm.

subdivisions of other gland types such as types "A" and "B", which result from particular features in the structure of the cells forming the secretory unity. In this sense, class III glands seem to be ubiquitous among insects, since they have been reported in Isoptera (Noirot & Quennedey 1974), Coleoptera (Delachambre 1975), Dictyoptera (Mercer & Brunet 1959), Phasmatodea (Happ *et al* 1966), Mecoptera (Crossley & Waterhouse 1969) and Hymenoptera (Cruz-Landim 1967, Marques-Silva *et al* 2006, Azevedo *et al* 2007).

The present work is the first structural description of the Brindley's and mestasternal glands of *T. brasiliensis*, which may be used as a reference for further studies on behavior and reproduction patterns of this vector of Chagas' disease.

Acknowledgments

The authors are grateful to Núcleo de Microscopia e Microanálises from Universidade Federal de Viçosa for technical assistance and an anonymous reviewer for providing many insightful comments and suggestions on drafts of this paper. To the Brazilian research agencies CNPq and FAPEMIG for financial support.

References

Azevedo D O, Teixeira E W, Alves M L T, Moreti A C C, Blochtein B, Zanuncio J C, Serrão J E (2007) Comparative analyses of the abdominal tergal glands in *Apis mellifera* (Hymenoptera: Apidae) queens. Anim Biol 57: 329-338.

Bancroft J D, Stevens A (1996). Theory and practice of histological techniques. 4th edition, Churchill Livingstone, 765p.

Barrett F M, Millen B F, Lai-Fook J (1979) The Brindley's glands of *Rhodnius prolixus*. I. Structure of the mature gland. Can J Zool 57: 1109-1119.

Crossley A C, Waterhouse D F (1969) The ultrastructure of a pheromone-secreting gland in the male scorpion-fly, *Harpobbaticus australis* (Bittacidae: Mecoptera). Tiss Cell 1: 273-294.

Cruz-Landim C (1967) Estudo comparativo de algumas glândulas das abelhas (Hymenoptera, Apoidea) e respectivas implicações evolutivas. Arq Zool 3: 177-290.

Delachambre J (1975) L'ultrastructure es glandes dermiques de *Tenebrio monitor* L. (Insecta: Coeloptera). Tiss Cell 5: 243-257.

Games D E, Schofield C J, Staddon B W (1974) The secretion from Brindley's scent gland in Triatominae. Ann Entomol Soc Am 67: 820-829.

Happ G M, Stradberg J D, Happ C N (1966) The terpene-producing glands of a phasmid insect: cell morphology and histology. J Morphol 119: 143-159.

Kälin M, Barrett F M (1975) Observations on the anatomy, histology, release-site, and function of Brindley's gland in the blood-sucking bug *Rhodnius prolixus*. Ann Entomol Soc Am 68: 126-134.

Lai-Fook J (1970) The fine structure of developing type B dermal glands in *Rhodnius prolixus*. Tiss Cell 2: 119-138.

Manrique G, Vitta A C R, Ferreira R A, Zani C L, Unelius C R, Lazzari C R, Diotaiuti L G Lorenzo M G (2006) Chemical communication in Chagas disease vectors. Source, identity, and potential function of volatiles released by the metasternal and Brindley's glands of *Triatoma infestans* adults. J Chem Ecol 7: 9106-9116.

Marques-Silva S, Matiello-Guss C P, Delabie J H C, Mariano C S F, Zanuncio J C, Serrão J E (2006) Sensilla and secretory glands in the antennae of a primitive ant: *Dinoponera lucida* (Formicidae: Ponerinae). Microsc Res Tech 69: 885-890.

Mercer Eh, Brunet P C J (1959) The electron microscopy of the left collaterial gland of the cockroach. J Biophys Biochem Cytol 5: 257-262.

Noirot C, Quennedey A (1974) Fine structure of insect epidermal glands. Annu Rev Entomol 19: 61-81.

Noirot C, Quennedey A (1991) Glands, gland cells, glandular units; some comments on terminology and classification. Ann Soc Entomol Fr 27:123-128.

Rossiter M, Staddon B W (1983) 3-Methyl-2-hexanone from the bug *Dipetalogaster maximus* (Uhler) (Heteroptera; Reduviidae). Experientia 39: 380-381.

Santos-Mallet J R, Souza W (1990) Histological and ultrastructural aspects of the Brindley´s glands of *Panstrongylus megistus* (Burmeister, 1835) (Hemiptera, Reduviidae). Mem Inst Oswaldo Cruz 85: 141-152.

Schofield C J, Upton C P (1978) Brindley´s scent-glands the metasternal scent glands of *Panstrongylus megistus* (Hemiptera, Reduviidae, Triatominae). Rev Bras Biol 38: 665-678.

Staddon B W (1983) The scent glands of Heteroptera. Adv Insect Physiol 14: 131-157.

Stefanini M, Martino C De, Zamboni L (1967) Fixation of ejaculated spermatozoa for electron microscopy. Nature 216: 173-174.

Ward J P (1981) A comparison of the behavioural responses of the haematophagous bug, *Triatoma infestans* to synthetic homologues of two naturally occurring chemicals (n- and isobutyric acid). Physio. Entomol 6: 325-329.

Weirauch C (2006) Metathoracic glands and associated evaporatory structures in Reduvioidea (Heteroptera: Cimicomorpha), with observation on the mode of function of the metacoxal comb. Eur J Entomol 103: 97-108.

Wigglesworth V B (1933) The physiology of the cuticle and of ecdysis in *Rhodnius prolixus* (Triatomidae, Hemiptera) with special reference to the function of the oenocytes and of the dermal glands. Q J Microsc Sci 76: 269-319.

Received 17/XII/07. Accepted 09/II/09.