Research Article / Artículo de Investigación

Description of the spermathecae and testicles of *Triatoma melanocephala* Neiva & Pinto, 1923 (Hemiptera: Reduviidae: Triatominae)

Descripción de las espermatecas y testículos de *Triatoma melanocephala* Neiva y Pinto, 1923 (Hemiptera: Reduviidae: Triatominae)

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Abstract. *Triatoma melanocephala* is an endemic species of Brazil. Phenotypic (external morphology) and molecular studies were performed to contribute to the taxonomy and phylogeny of triatomines, since few studies on the internal morphology of these were found. In order to contribute to the biological, morphological and taxonomic knowledge of the subfamily Triatominae, this work describes the internal morphology of the reproductive apparatus of adult males and females of *T. melanocephala*. For this work 30 specimens from Jequié, Bahia, Brazil were used. *Triatoma melanocephala's* reproductive system included slim spermathecae insertions, large/medium rounded terminal bodies, and irregular ellipsoid-shaped testicles with a transparent, vitreous appearance. These results contribute to the knowledge of the reproductive biology of this species of vectorial importance and show taxonomic relevance, as well as possible application in systematic studies.

Key words: Chagas disease; gonads; internal morphology; triatomine; vectors.

Resumen. *Triatoma melanocephala* es una especie endémica de Brasil. Se realizaron estudios fenotípicos (morfología externa) y moleculares para contribuir a la taxonomía y filogenia de los triatominos, ya que se encontraron pocos estudios sobre la morfología interna de estos. Con el fin de contribuir al conocimiento biológico, morfológico y taxonómico de la subfamilia Triatominae, este trabajo muestra la morfología interna del aparato reproductor de adultos machos y hembras de *T. melanocephala*. Para este trabajo se utilizaron 30 especímenes recolectados en Jequié, Bahía, Brasil. El sistema reproductivo de *T. melanocephala* incluye inserciones delgadas de espermatecas, cuerpos grandes/medianos con terminaciones redondeadas, y testículos de forma elipsoidal irregular con una apariencia transparente y vitrea. Estos resultados contribuyen al conocimiento de la biología reproductiva de esta especie de importancia vectorial y muestran relevancia taxonómica, así como su posible aplicación en estudios sistemáticos.

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Palabras clave: Enfermedad de Chagas; gónadas; morfología interna; triatomino; vectores.

Introduction

Triatoma melanocephala Neiva & Pinto, 1923 (Hemiptera: Triatominae) is a wild triatomine that occurs in the states of Bahia, Paraíba, Pernambuco, Rio Grande do Norte and Sergipe (Galvão *et al*. 2003; Galvão 2014). Chromosomal (Alevi *et al*. 2012, 2014a), meiotic (Alevi *et al*. 2013), morphological (Sherlock & Guitton 1980) and, mainly, phylogenetic (Justi *et al.* 2014, 2016) data demonstrate that this species, together with *Triatoma vitticeps* (Stål, 1859), form the *T. vitticeps* subcomplex (Alevi *et al*. 2017). It is believed that species from this subcomplex reached the Atlantic coast by dispersal and diversified before the Northern Andean uplift (23–10 Ma) (Justi *et al*. 2016).

The protozoan *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida: Trypanosomatidae) was isolated from a specimen of *T. melanocephala* from Bahia (Ribeiro *et al*. 2014). This information and the reports of capture of this triatomine in human dwellings (Sherlock & Guitton 1980; Galvão 2014) emphasize its epidemiological importance in the vectorial transmission of Chagas disease (CD). In addition to that, Sherlock and Guitton (1980) suggest that *T. melanocephala* is probably an important link between the cycles of wild and domestic transmission, transmitting *T. cruzi* from domestic reservoirs to humans.

The way to minimize the incidence of CD is the control of vectors, which requires the knowledge of the different biological aspects of these hematophagous insects. The first biological information described for *T. melanocephala* was morphological characteristics (Sherlock & Guitton 1980). Currently, several other analyses such as chromosome (Alevi *et al.* 2012, 2014a; Pita *et al*. 2021), meiotic (Alevi *et al*. 2013), cell (Alevi *et al*. 2016a, b), spermiotaxonomy (Alevi *et al*. 2014b), biological (Oliveira *et al*. 2015), molecular (Gardim *et al*. 2014), epidemiological (Ribeiro *et al*. 2014), reproductive (Alevi *et al.* 2014c), evolutionary (Justi *et al*. 2014, 2016; Alevi *et al*. 2017), morphological and morphometric studies (Oliveira *et al*. 2015) were performed in *T. melanocephala*.

Studies on the internal anatomy of triatomines are related to the works of Snodgrass (1935), Davey (1958), Lacombe (1965) and Barth (1980). Snodgrass (1935) describes the reproductive tract of females of species of the class Insecta as consisting of two ovaries, formed by smaller cylindrical groups called ovarioles, two lateral oviducts leading to the common oviduct, where the pairs of spermathecae and the accessory gland are inserted (the common oviduct already has an opening for the coupling bag). For its part, the male reproductive system consists of a pair of testicles, lateral ducts, vas deferens, the seminal vesicle, accessory glands, and the ejaculatory duct, which leads to the phallus, the copulatory organ of arthropods.

The main way to mitigate the incidence of CD is through the control of vectors and, for this, it is necessary to know the different biological aspects of these hematophagous insects. Thus, the present study sought to provide an anatomical and morphological characterization of the male and female reproductive systems of *T. melanocephala*, with the purpose of knowing the reproductive biology of this species.

Material and Methods

The specimens of *T. melanocephala* used are maintained in the Insectarium of Triatominae of the School of Pharmaceutical Sciences/UNESP since October 8, 2009 (CTA 219). The specimens of *T. melanocephala* that originated the colony were collected in the peridomicile of the municipality of Jequié, state of Bahia, by a team of the Secretary of Health of the State of Bahia (Sesab) and sent by Eliane Góes Nascimento and Renato Freitas de Araújo.

Morphological study by optical microscopy (MO)

For the observation of the reproductive apparatus by means of optical microscopy, the procedures established by Lacombe (1965) and Nascimento *et al*. (2017) were adopted. The images were captured under a stereoscopic microscope Leica MZ APO and Motic Advanced 3.2 plus an image analysis system. The number of insects used was 15 male and 15 female.

Morphological study by scanning electron microscopy (SEM)

For SEM observations, the spermathecae and testis of *T. melanocephala* were previously prepared following the methods described by Nascimento *et al*. (2017). The images were subsequently examined and recorded following Rosa *et al*. (1992) and Oliveira *et al*. (2015).

Results

Optical microscopy

The complete female reproductive system of *T. melanocephala* has: two ovaries, two lateral oviducts, a common oviduct, two spermathecae, an accessory gland, and a genital chamber. The study of ovaries of adult females of *T. melanocephala* showed that they are double and consist of several ovarioles, as it can be seen in Fig. 1A. The ovaries are divided into three parts: terminal filament, ovarian or ovary tubes, and peduncle or chalice. The terminal filament ends on the thoracic adipose body, promoting the positioning of the ovary. The ovary is covered by a tunic of its own, which is lined externally by the peritoneal membrane that goes from the lateral oviduct to the terminal filament, having little contact with the ovaries.

The spermathecae of *T. melanocephala* presented a variation in the positioning (Fig. 1) and differences in size and shape depending on whether they are full or empty of sperm (Figs. 1B, C). Slim insertions were found in the common oviduct, and there were large and medium bodies with rounded terminal portions (Figs. 1B, C).

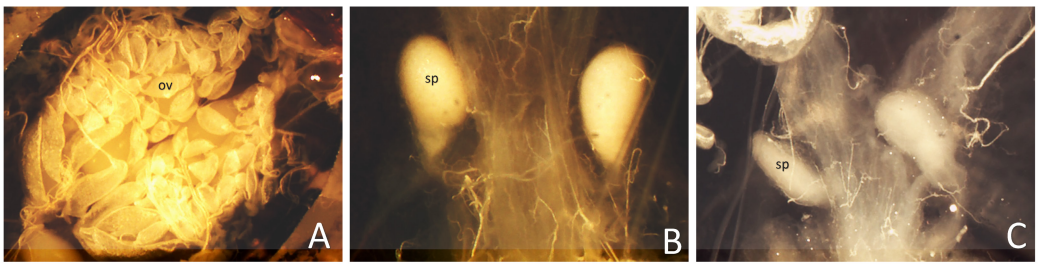

Figure 1. Female reproductive system of adult *T. melanocephala* by optical microscopy. **A.** Overview of the (ov) ovarioles magnification 10X. **B.** Full spermathecae. **C.** Nearly empty spermathecae. (sp) spermathecae Magnification 40x. / Sistema reproductivo femenino del adulto de *T. melanocephala* visto por microscopia óptica. **A.** Visión general de los (ov) ovariolos con aumento de 10X. **B.** Espermatecas llenas. **C.** Espermatecas casi vacías. (sp) espermatecas. Aumento de 40x.

The complete male reproductive system has: two testicles, two vas deferens, seminiferous vesicle, two accessory glands and ejaculatory duct. The testicles of *T. melanocephala* have seven testicular follicles that are encased by a peritoneal sheath (Figs. 2A, B, C). They presented an irregular contour, approximately ellipsoid shaped, and a transparent coloration with slight vitreous luster (Fig. 2A).

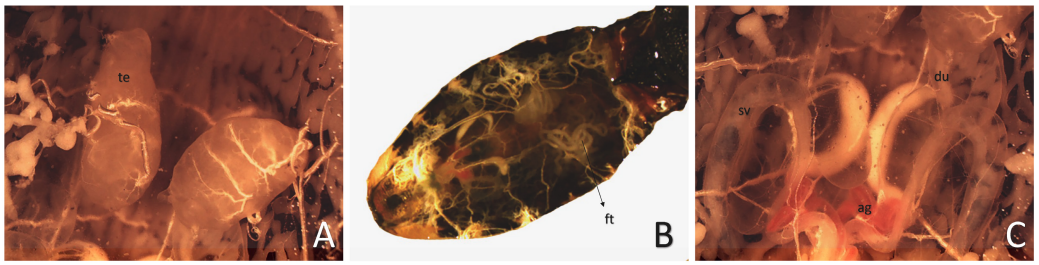

Figure 2. Male reproductive system of adult male *T. melanocephala* by optical microscopy. **A.** Overview of the (Te) testicles; magnification 20X. **B.** Overview of the (Ft) follicles testicular; magnification 20x. **C.** Overview of the (du) ductus deferens 20x; (ag) accessory glands 20x and (sv) seminiferous vesicle; magnification 20x. / Sistema reproductivo masculino del adulto de *T. melanocephala* visto por microscopía óptica. **A.** Visión general de los (Te) testículos; aumento de 20x. **B.** Visión general de los (Ft) folículos testiculares; aumento de 20x. **C.** Visión general del (du) conducto deferente; 20x; (ag) glándulas accesorias 20x y (sv) vesícula seminal; aumento de 20x.

Scanning Electron Microscopy

The spermathecae of *T. melanocephala* showed a rounded shape with variations when they are full of sperm. When empty, they presented the same format in all samples analyzed (Fig. 3).

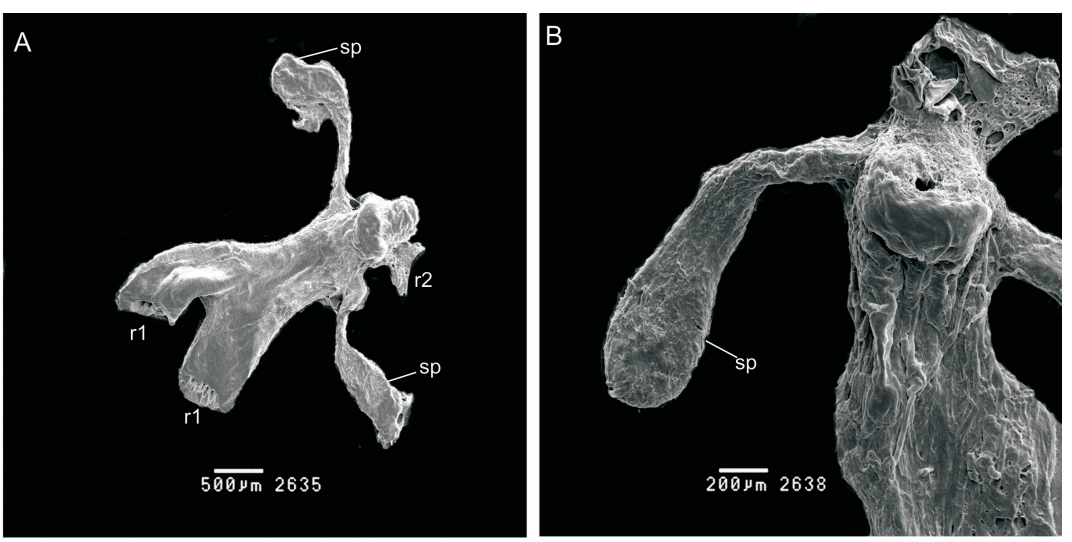

Figure 3. Female reproductive system by scanning electron microscopy (SEM). **A.** Spermathecae adult female *T. melanocephala*: (sp) spermathecae; (R^1 and R^2) region where the cuts for assembling the parts were made where two oviducts side and genital second camera (Ramírez Pérez 1969). **B.** Detail of spermathecae. / Sistema reproductivo femenino observado por microscopía electrónica de barrido (MEB). **A.** Espermatecas de la hembra adulta de *T. melanocephala*: (sp) espermatecas; (R^1 y R^2) región donde se realizaron los cortes para el ensamblaje de las partes donde se encuentran los dos oviductos laterales y la segunda cámara genital (Ramírez Pérez 1969). **B.** Detalle de las espermatecas.

The testicles of *T. melanocephala* presented a rounded shape, having a differentiation in the regions of connection with anterior vas deferens and terminal filament (Fig. 4).

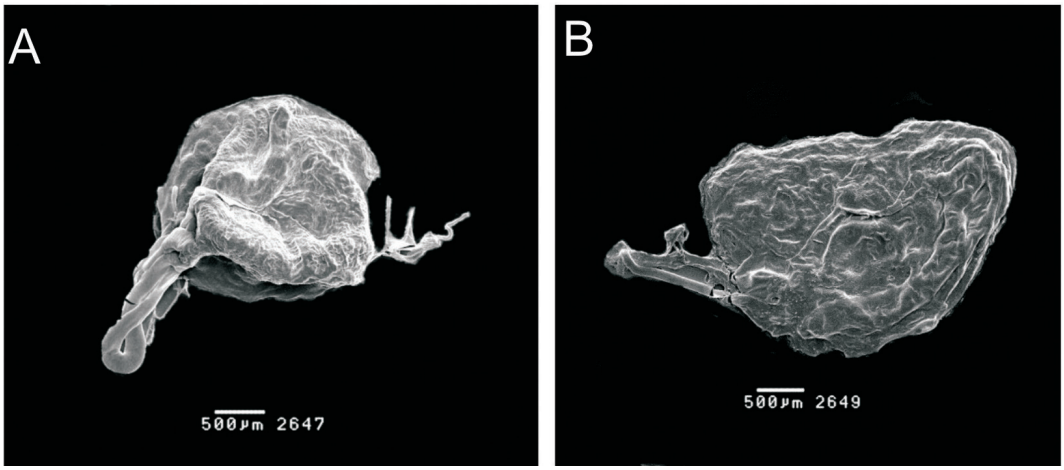

Figure 4. Testicles of adult male *T. melanocephala* by **(A)** Testicle dorsal view **(B)** Testicle ventral view by scanning electron microscopy. / Testículos del adulto de *T. melanocephala* observados por **(A)** vista dorsal del testículo **(B)** vista ventral del testículo mediante microscopía electrónica de barrido.

Discussion

Analyzing the female reproductive system of *Rhodnius* Stål, 1859, Ramírez Pérez (1969) reports that the female *R. prolixus* Stål, 1859 presents in its adult phase a pair of ovaries located on either side of the digestive tract and a common oviduct that bifurcates anteriorly forming the lateral oviducts, the same being observed in *T. melanocephala*.

When compared to *T. infestans* (Klug, 1834) (Nascimento *et al*. 2017), *T. melanocephala* presented a difference in the number of ovaries and their size. Although new species should be studied, we suggest that this structure of the female reproductive system may be a taxonomic tool to differentiate species, as well as it was observed that the size of the testicular follicles represents an important tool to differentiate the genera of the subfamily Triatominae (Alevi *et al*. 2015).

Snodgrass (1935) mentions that the formation of the spermatic tubes has a similar origin to the formation of the eggs in the oviducts of the females, thus they are denominated testicular follicles. The accessory glands produce an opaque granular secretion responsible for the movement of the spermatozoa in the female, which together give rise to the spermatophore.

All triatomine species have seven testicular follicles (Gonçalves *et al*. 1987). This synapomorphy is not shared with other Hemiptera that present variation in the number of follicles (Pereira *et al*. 2015). The testicular follicles consist of a thin translucent membrane that allows the visualization of the internal cells (Gonçalves *et al*. 1987). Testicular follicle lining cells are known as cystic cells and are responsible for nourishing cells in cell division during spermatogenesis (Schmidt & Dorn 2004). In triatomines, spermatogenesis is cystic, that is, the cell divisions happen synchronized within the spermatogonial cysts (Silistino-Souza *et al*. 2012; Alevi *et al*. 2015).

The coloring of the peritoneal sheath of the testes was characterized as a synapomorphy of Triatominae (Alevi *et al*. 2014c). The transparent coloration observed for all the species analyzed by the authors is extremely different from other Hemiptera that have multicolored sheaths (reddish, orange, yellowish or transparent) (Gomes *et al*. 2013). The testicles of *T. melanocephala* presented an irregular outline, with an approximately ellipsoid shape and a transparent sheath, which corroborates the description proposed by Alevi *et al*. (2014c).

The spermathecae is a compartment responsible for storing the semen released at the time of copulation, until the oocyte is developed. Later the oocyte descends through the lateral oviduct, which has several folds naturally to allow a good dilation at the time of ovulation, when the egg passes through the genital chamber and the spermathecae injects the stored sperm, thus fertilizing the egg (Barth 1973; Ramírez Pérez 1969).

Comparing *T. melanocephala* with the species studied by Nascimento *et al*. (2017), it is possible to observe a similarity in the morphology of the spermathecae of this species with *T. infestans* and differences with *T. brasiliensis* Neiva, 1911, *T. juazeirensis* Costa & Felix, 2007, *T. sherlocki* Papa *et al*., 2002 and *P. tibiamaculatus* (Pinto, 1926). These results are important because *T. melanocephala* was initially grouped with *T. brasiliensis, T. juazeirensis, T. sherlocki* and *P. tibiamaculatus* in the *T. brasiliensis* subcomplex (Schofield & Galvão 2009). However, it is currently known that these species are not phylogenetically related (Alevi *et al*. 2012; Gardim *et al*. 2014; Justi *et al*. 2014, 2016; Oliveira *et al*. 2017), which indicates that the analysis of the spermatheca in SEM has taxonomic value.

Therefore, the parameters of the female and male reproductive systems analyzed in adults of *T. melanocephala* have contributed to the knowledge of the reproductive biology of this species of vectorial importance and have showed taxonomic relevance, as well as possible application in systematic studies.

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