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Short Communication

Spermatogenesis in *Triatoma melanocephala* (Hemiptera: Triatominae)

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ABSTRACT. *Triatoma melanocephala* is a rare species of Hemiptera. It belonged to the Brasiliensis subcomplex and presents morphological characteristics very close to those of *Triatoma vitticeps*. We investigated spermatogenesis of *T. melanocephala* and compared it with that of *T. vitticeps* in order to determine whether these organisms have similar cytogenetic characteristics. Lacto-acetic orcein staining was used to observe all stages of meiosis. These two species were found to have the same karyotype ($2n = 20A + X_1X_2X_3Y$), heteropycnotic corpuscles in the polyploid spermatogonial cells, interfasic and profasic nucleus, and chromocenter with four sex chromosomes during prophase. Thus, we conclude that besides the morphologic similarity of *T. melanocephala* with *T. vitticeps*, they also have similar spermatogenesis and cytogenetics.

Key words: Triatomine; Cytogenetics; Meiosis; Lacto-acetic orcein; Brasiliensis subcomplex

The species *Triatoma melanocephala*, described by Neiva and Pinto (1923), is a rare species of Hemiptera that is found exclusively in the Brazilian States of Bahia, Pernambuco, Paraíba, Rio Grande do Norte, and Sergipe (Gurgel-Gonçalves et al., 2012). It is known to be infected by *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae), the causative agent of Chagas disease. The species is likely an important link of sylvatic and domestic transmission cycles, which transmits the parasite of mice and marsupials to humans (Sherlock and Guitton, 1980).

Sherlock and Guitton (1980), from their morphological description of *T. melanocephala*, proposed that the hemipteran has morphologic characteristics very close to those of *Triatoma vitticeps*, where the only distinguishing features are the absence of range testacea in the head and the presence of two brown spots on the thorax.

The species belonged to the Brasiliensis subcomplex (Schofield and Galvão, 2009). This subcomplex is present in South America and consisted of 9 species: *T. brasiliensis*, *T. juazeirensis*, *T. melanica*, *T. melanocephala*, *T. petrochiae*, *T. lenti*, *T. sherlocki*, *T. tibiamaculata*, and *T. vitticeps*. However, recently, by means of karyotype analysis, it was proposed to exclude *T. melanocephala*, *T. vitticeps*, and *T. tibiamaculata* from the Brasiliensis subcomplex (Alevi et al., 2012a). Thus, cytogenetic studies can be considered an important tool for the classification of this subcomplex (Alevi et al., 2012a,b; 2013a,b).

Most of the cytogenetic results on triatomines have been obtained on the basis of chromosome comparisons relying on conventional staining. In these studies, the comparison criteria were often the number, morphology, and arrangement of chromosomes in the metaphase plate. The data obtained from these investigations gave the first indications of the processes that occurred during chromosomal evolution in these insects (Pérez et al., 1992; Panzera et al., 1996; Tavares and Azeredo-Oliveira, 1997).

This paper describes the spermatogenesis of *T. melanocephala* and compares it with that of *T. vitticeps* to find out whether these organisms have convergent cytogenetic characteristics.

Seminiferous tubules of 15 adult males of *T. menalocephala* were first shredded and smashed on a slide, which was then placed in liquid nitrogen. The preparation was stained using the lacto-acetic orcein cytogenetic technique of De Vaio et al. (1985), with modifications according to Alevi et al. (2012a). The slides were analyzed using a light microscope (Jenaval-Zeiss), which was coupled to a digital camera and an AxioVision LE 4.8 image analyzer (Copyright© 2006-2009 Carl Zeiss Imaging Solutions Gmb H). The images were magnified 1000X.

It was possible to observe all stages of meiosis during spermatogenesis (Figure 1). The polyploid nucleus, which is a cell of the wall of the seminiferous tubule responsible for nutrition of the cells during the meiotic division, showed a heteropycnotic body (Figure 1A, arrow). We observed the interphase nucleus (Figure 1B), the prophase nucleus (Figure 1C) and the spermatogonial metaphase nucleus (Figure 1D). Interphase and mitotic prophase also displayed one heteropycnotic body (arrow). The initial meiotic prophase demonstrated the four individual sex chromosomes (Figure 1E, arrows). This chromocenter formed by sex chromosomes remained throughout prophase I (Figure 1F and G, arrows). The nucleus in metaphase I (Figure 1H) allowed us to observe the diploid chromosome set of the species ($2n = 20A + X_1X_2X_3Y$). Note that the Y chromosome is larger and more heteropycnotic than the X chromosomes. Furthermore, it was possible to observe two larger and more heteropycnotic autosomes (arrows). It was also possible to observe metaphases in the lateral view (Figure 1I), late anaphase (Figure 1J) and telophase (Figure 1K).

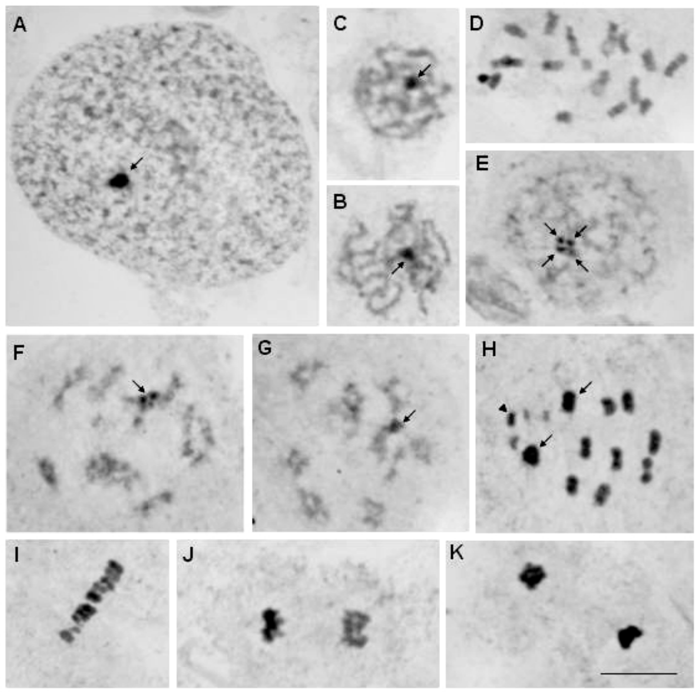

Figure 1. Seminiferous tubule of *Triatoma melanocephala* stained by lacto-acetic orcein. **A.** Polyploid nucleus. Note the heteropycnotic corpuscle (arrow). **B.** Interfasic nucleus. **C.** Spermatogonial prophase. **D.** Spermatogonial metaphase. **E.** Initial prophase demonstrated the four individualized sex chromosomes (arrows). **F.** and **G.** Prophase I. Note the persistence of chromocenter. **H.** Metaphase I (2n = 20 A + $X_1X_2X_3Y$). **I.** Metaphases in lateral view. **J.** Anaphase. **K.** Telophase.

The basic number of chromosomes for triatomines is 2n = 22, with 10 pairs of autosomes and 1 pair of sex chromosomes (Ueshima, 1966). However, there are species with multiple sex chromosomes (XY, X_1X_2Y, $X_1X_2X_3Y$), caused by fragmentation of the original X (Manna, 1950; Ueshima, 1966), as in *T. tibiamaculata* (X_1X_2Y) (Panzera et al., 1996) and *T. vitticeps* ($X_1X_2X_3Y$) (Schreiber and Pellegrino, 1950). The karyotype of *T. melanocephala* has been recently described (Alevi et al., 2012a) and approaches that of *T. vitticeps* and *T. eratyrusiformis*, because among the 140 species of triatomines described, only the males of these species have a diploid chromosome number 2n = 24 (20A + $X_1X_2X_3Y$) (Panzera et al., 2010).

In addition to karyotype, *T. melanocephala* has some characteristics during spermatogenesis that resembles those of *T. vitticeps*, such as heteropycnotic bodies present in the polyploid, interphase and prophase nucleus (Severi-Aguiar et al., 2006). Moreover, Severi-Aguiar et al. (2006) also observed a chromocenter with four individual sex chromosomes during prophase in *T. vitticeps*.

Thus, on the basis of comparative analysis of spermatogenesis of *T. melanocephala*, we propose that beyond morphologic characteristics, this species has similar cytogenetic characteristics as *T. vitticeps*.

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