Short communication

Karyotype of *Triatoma melanocephala* Neiva and Pinto (1923). Does this species fit in the Brasiliensis subcomplex?

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**Abstract**

*Triatoma melanocephala* is a rare species of Hemiptera that belongs to the Brasiliensis subcomplex. This subcomplex is composed of cryptic species, and the basic number of chromosomes for triatomines of this subcomplex is 2n = 22; however, *T. melanocephala* showed a karyotype of 2n = 24 (20A + X1X2X3Y). Thus, this study allowed us to describe the karyotype of the species and, more specifically, to propose the exclusion of *T. melanocephala*, as well as *T. vitticeps* and *T. tibiamaculata*, which also has fragmentation of the X chromosome, from the Brasiliensis subcomplex.

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**Introduction**

The species *Triatoma melanocephala*, described by Neiva and Pinto (1923), is a rare species of Hemiptera that was found exclusively in the Brazilian states of Bahia, Pernambuco, Paraíba, Rio Grande do Norte and Sergipe (Gurgel-Gonçalves et al., 2012). It was found 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 that transmits the parasite of mice and marsupials to humans (Sherlock and Guitton, 1980).

The species belongs to the Brasiliensis subcomplex (Schofield and Galvão, 2009). This subcomplex is present in South America and consists of nine species: *T. brasiensis*, *T. juazeirensis*, *T. melania*, *T. melanocephala*, *T. petrochiae*, *T. lenti*, *T. sherlocki*, *T. tibiamaculata* and *T. vitticeps* (Schofield and Galvão, 2009).

According to Monteiro et al. (2001), it is difficult to explain the Brasiliensis subcomplex classification by morphological parameters alone, because these species are cryptic; i.e., morphologically similar. Thus, cytogenetic studies can be considered an important tool for the differentiation of these species (Pérez et al., 1992).

Most of the cytogenetic results on triatomines were obtained based on chromosome comparisons that relied on conventional staining. In these studies, the comparison criteria were often the number, the morphology, and the disposition 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).

The basic number of chromosomes for triatomines is 2n = 22, with 10 pairs of autosomes and one pair of sex chromosomes (Ueshima, 1966). However, there are species with multiple sex chromosomes (XY, X1X2Y, X1X2X3Y), caused by fragmentation of the original X (Manna, 1950; Ueshima, 1966).

Seminiferous tubules of fifteen adult males of *T. melanocephala* were first shredded, smashed, and set the slide in liquid nitrogen. Then were stained with the lacto-acetic orcein cytogenetic technique of De Vaio et al. (1985), with modifications to the biological material, which is no longer the whole testicle, but instead only a seminiferous tubule, and to the duration in acetic acid, which is now only 10 min. Based on the analysis of meiotic metaphases (Fig. 1), we observed that males of *T. melanocephala* have the karyotype 2n = 24 (20A + X1X2X3Y), demonstrating that this organism does not have the modal chromosome set found in triatomines (2n = 22) (Ueshima, 1966).

The species *T. tibiamaculata* and *T. vitticeps* share some morphological similarities with the species of the Brasiliensis subcomplex,
but when analyzed cytogenetically and molecularly, they are related more closely to the Triatominae of North America, which have multiple sex chromosomes (Panzera et al., 2010). The karyotype of *T. tibiamaculata* is 2n = 20A + X1X2Y (Panzera et al., 1996) and the karotype of *T. vitticeps* is 2n = 20A + X1X2X3Y (Schreiber and Pellegrino, 1950). Mitochondrial DNA sequences also confirm the close phylogenetic relationship between these Hemiptera and the ones present in North America (Schofield and Galvão, 2009).

Only *T. eratyrusformis* and *T. vitticeps* karyotypes have been described previously as 20A + X1X2Y (Panzera et al., 2010). This similarity highlights the importance of karyotype studies in combination with other techniques for understanding the classification of insects in a subcomplex, because until this study was developed, the inclusion of only *T. vitticeps* and *T. tibiamaculata* in this subcomplex was questioned (Schofield and Galvão, 2009).

The present study showed a direct karyotypic relationship between *T. melanocephala*, *T. vitticeps* and *T. tibiamaculata*. All species presented fragmentation of the X chromosome and, thus, are similar to North American species. Therefore, we propose the exclusion of *T. melanocephala*, *T. vitticeps*, and *T. tibiamaculata* from the Brasiliensis subcomplex. This study underscores the importance of cytogenetics in the correct classification of triatomines, vectors of Chagas disease across Brazil.

The correct classification of the triatomines enables differentiate species of primary importance for species of minor importance, since, currently, some species in the process of domiciliation; i.e., leaving the natural habitat and began living in urban environments such as indoors and peridomicate (Dias and Schofeld, 1998). Thus, through the correct classification is possible to distinguish the main species vectors of Chagas disease and thereby allow greater attention of vector control programs for these species.

### Ethical standards

The experiments comply with the current laws of the country in which they were performed.

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### References


*Fig. 1.* Seminiferous tubule of *Triatoma melanocephala* stained by lacto-acetic orcin. (A and B) Metaphase I with ten bivalent autosomes and three sex chromosomes. (C) Metaphase II. Arrows: X chromosome fragmentation (X1X2X3). Arrowhead: Y chromosome.