

Distribution of constitutive heterochromatin in *Triatoma melanocephala* (Hemiptera, Triatominae)

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Genet. Mol. Res. 13 (3): 7899-7903 (2014)

Received January 29, 2014

Accepted August 14, 2014

Published September 29, 2014

DOI <http://dx.doi.org/10.4238/2014.September.29.2>

ABSTRACT. In principle, *Triatoma melanocephala* was included in the Brasiliensis subcomplex on the basis of morphological parameters and geographical layout, since there were no other relevant data available in the literature. On the basis of karyotype, it has been proposed to exclude *T. melanocephala*, as well as of *T. vitticeps* and *T. tibiamaculata*, from the subcomplex, which shows fragmentation of the X sex chromosomes, thereby approaching the species of North America. Therefore, the present study aimed to determine the pattern of constitutive heterochromatin of *T. melanocephala* to provide new data on the cytotaxonomy of this vector of Chagas disease and especially to try to relate this species to some group, complex or subcomplex of triatomine species, aiding in their classification. This species showed no

constitutive heterochromatin in the autosomes or X sex chromosome, but only the Y sex chromosome. The number of chromosomes and heterochromatin pattern of *T. melanocephala* proved to be identical to that described for *Panstrongylus lutzi*. Thus, the present study demonstrated a tentative relationship between *T. melanocephala* and *P. lutzi*. However, we emphasize that other comparative studies should be conducted between these species, such as experimental crosses and molecular, enzymatic, morphological, and morphometric analyses to determine whether these species are actually evolutionarily related or if the number of chromosomes and the heterochromatin pattern emerged as homoplasies in *T. melanocephala* and *P. lutzi*.

Key words: Cytogenetics; Homoplasies; Taxonomy; Triatominae

INTRODUCTION

Triatomines are insects that are taxonomically included in the order Hemiptera and suborder Heteroptera within the family Reduviidae and subfamily Triatominae (Lent and Wygodzinsky, 1979). These organisms are of great medical importance, because all species that belong to the subfamily Triatominae are both bloodsucking and susceptible to infection by the protozoan *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae) and are therefore potential vectors of Chagas disease (Noireau et al., 2009).

Specific identification and systematics of triatomines have been primarily based on morphological observations, mainly by light microscopy (Lent and Wygodzinsky, 1979). However, experimental crosses (Mendonça et al., 2014), molecular (Mendonça et al., 2009; Gardim et al., 2014), enzymatic (Costa et al., 1997), and cytogenetic (Panzera et al., 2010, 2012; Alevi et al., 2012a, 2013a; Succi et al., 2014) techniques have been used as important tools to aid in the classification of species.

Cytogenetic tools were used to differentiate *Triatoma garciabesi* from *T. sordida*, elevating it to the level of species (Panzera et al., 1997). Cytogenetic data, such as the number of chromosomes and the pattern of constitutive heterochromatin, were also used to group the species of the Brasiliensis subcomplex (Panzera et al., 2010; Alevi et al., 2012a,b, 2013a,b,c,d,e, 2014a,b). Thus, cytotaxonomy has turned out to be an important tool in the systematics of triatomines.

T. melanocephala is a species endemic to Brazil, specifically the States of Bahia, Paraíba, Pernambuco, Rio Grande do Norte, and Sergipe (Gurgel-Gonçalves et al., 2012). Recently, this organism was found infected with *T. cruzi* inside homes in the State of Pernambuco (Silva et al., 2012).

In principle, this species was included in the Brasiliensis subcomplex on the basis of morphological parameters and geographical layout (Schofield and Galvão, 2009), since there were no other relevant data available in the literature. Alevi et al. (2012a), according to karyotyping, proposed the exclusion of *T. melanocephala*, as well as *T. vitticeps* and *T. tibiamaculata*, from the subcomplex, which shows fragmentation of X sex chromosomes, thereby approaching the species of North America.

Therefore, the present study aimed to determine the pattern of constitutive heterochromatin of *T. melanocephala* to provide new data on the cytotaxonomy of this vector of Chagas

disease and especially to try to relate this species with some group, complex or subcomplex of triatomine species, aiding in their classification.

MATERIAL AND METHODS

Seminiferous tubules of at least ten adult males of *T. melanocephala*, from the “Insectarium of Triatominae” established in Araraquara, São Paulo, were shredded, crushed and fixed in liquid nitrogen on a glass slide, and chromosomes were stained by C-banding (Sumner, 1972).

RESULTS AND DISCUSSION

The initial prophase showed a large chromocenter (Figure 1A, arrowhead) consisting of the sex chromosomes and some heterochromatic blocks dispersed in the nucleus (Figure 1A, arrows). According to metaphase I analysis (Figure 1B and C), we confirmed that males of *T. melanocephala* have the karyotype $2n = 24 (20A + X_1X_2X_3Y)$. Moreover, by analyzing the metaphase I in polar view (Figure 1B and C) and side view (Figure 1D), we observed that this species shows no constitutive heterochromatin in autosomes and X sex chromosome (arrows), but only the Y sex chromosome (arrowheads).

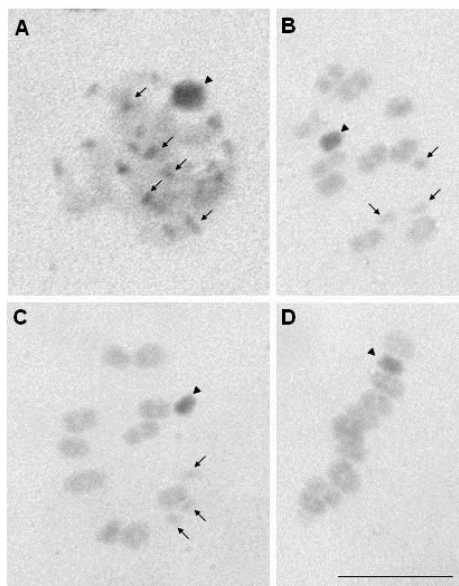


Figure 1. Seminiferous tubule of *Triatoma melanocephala* stained by C-banding. **A.** Initial prophase. Note a large chromocenter (arrowhead) and heterochromatic blocks (arrows) in the nucleus. **B. C. D.** Metaphase I. Note that the Y sex chromosomes (arrowheads) and that the X sex chromosomes (arrows) show no constitutive heterochromatin. Bar: 10 μm .

The fragmentation of the sex chromosome X is a derived characteristic, since according to Ueshima (1966), the sex determination system in the ancestral subfamily Triatominae is XY. This peculiarity has been described in the literature in only four species, namely *T.*

melanocephala (Alevi et al., 2012a), *T. vitticeps* (Schreiber and Pellegrino, 1950), *T. eratyru-siformis* (Panzeria et al., 2010), and *Panstrongylus lutzi* (Santos, 2010). Of these species, only *T. vitticeps* and *P. lutzi* show the heterochromatin pattern described. Both, as observed in *T. melanocephala*, show no constitutive heterochromatin in autosomes. However, *T. vitticeps* shows constitutive heterochromatin in the X sex chromosome (Severi-Aguiar et al., 2006).

T. melanocephala and *T. vitticeps* species were considered synonymous by Lent and Wygodzinsky (1979). The only morphological characteristics that differentiate these species are the absence of testacea range in the head and the presence of two brown spots on the thorax (Sherlock and Guitton, 1980). Alevi et al. (2013b), by analyzing the heteropycnotic pattern, observed a direct correlation between the species, since *T. melanocephala* showed the same characteristics described by Severi-Aguiar et al. (2006) for *T. vitticeps*. Thus, although the number of chromosomes and the heteropycnotic pattern are identical, the presence of constitutive heterochromatin in the X sex chromosome can be considered a diagnostic characteristic of *T. vitticeps*, since *T. melanocephala* has no heterochromatic blocks in the X sex chromosome.

The heterochromatin banding pattern of *T. melanocephala* proved to be identical to that described for *P. lutzi*. Both showed heterochromatic blocks in prophase, the same number of chromosomes and the same arrangement of constitutive heterochromatin in the chromosomes, i.e., only the Y sex chromosome showed heterochromatin. Santos (2010) believe that possibly the heterochromatic bands observed in prophase are not seen during metaphase due to the high degree of condensation of chromosomes. However, we believe that the blocks do not really exist during metaphase, since the C-banding technique is specific for constitutive heterochromatin, and since it was totally clear that only chromosome heterochromatin was in the Y sex chromosome.

CONCLUSION

Thus, we demonstrated here a tentative relationship between *T. melanocephala* and *P. lutzi*. However, we emphasize that other comparative studies should be conducted between these species, such as experimental crosses and molecular, enzymatic, morphological, and morphometric analyses to determine whether these species are actually evolutionarily related or if the number of chromosomes and the heterochromatin pattern emerged as homoplasies in *T. melanocephala* and *P. lutzi*.

ACKNOWLEDGMENTS

Research supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; Process #2012/22361-2) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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