



## Short communication

Karyotype of *Triatoma melanocephala* Neiva and Pinto (1923). Does this species fit in the Brasiliensis subcomplex?Kaio Cesar Chaboli Alevi<sup>a,\*</sup>, Priscila Pasqüetto Mendonça<sup>a</sup>, Nathália Paiva Pereira<sup>a</sup>, João Aristeu da Rosa<sup>b</sup>, Maria Tercília Vilela de Azeredo Oliveira<sup>a</sup><sup>a</sup> Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista – São José do Rio Preto, Rua Cristovão Colombo 2265, 15054-000 São José do Rio Preto, SP, Brazil<sup>b</sup> Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista – Araraquara, Rod. Araraquara-Jaú km 1, 14801-902 Araraquara, SP, Brazil

## ARTICLE INFO

## Article history:

Received 25 March 2012

Received in revised form 19 June 2012

Accepted 21 June 2012

Available online 1 July 2012

## Keywords:

Cytogenetics

Taxonomy

Triatominae

Brasiliensis subcomplex

## 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  $2n = 24$  ( $20A + X_1X_2X_3Y$ ). 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.

© 2012 Elsevier B.V. All rights reserved.

## Introduction

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 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. brasiliensis*, *T. juazeirensis*, *T. melanica*, *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).

\* Corresponding author. Address: Instituto de Biociências, Letras e Ciências Exatas, IBILCE – UNESP, Rua Cristovão Colombo, 2265, Jardim Nazareth, 15054-000 São José do Rio Preto, SP, Brazil. Tel.: +55 (17) 32212380x2378.

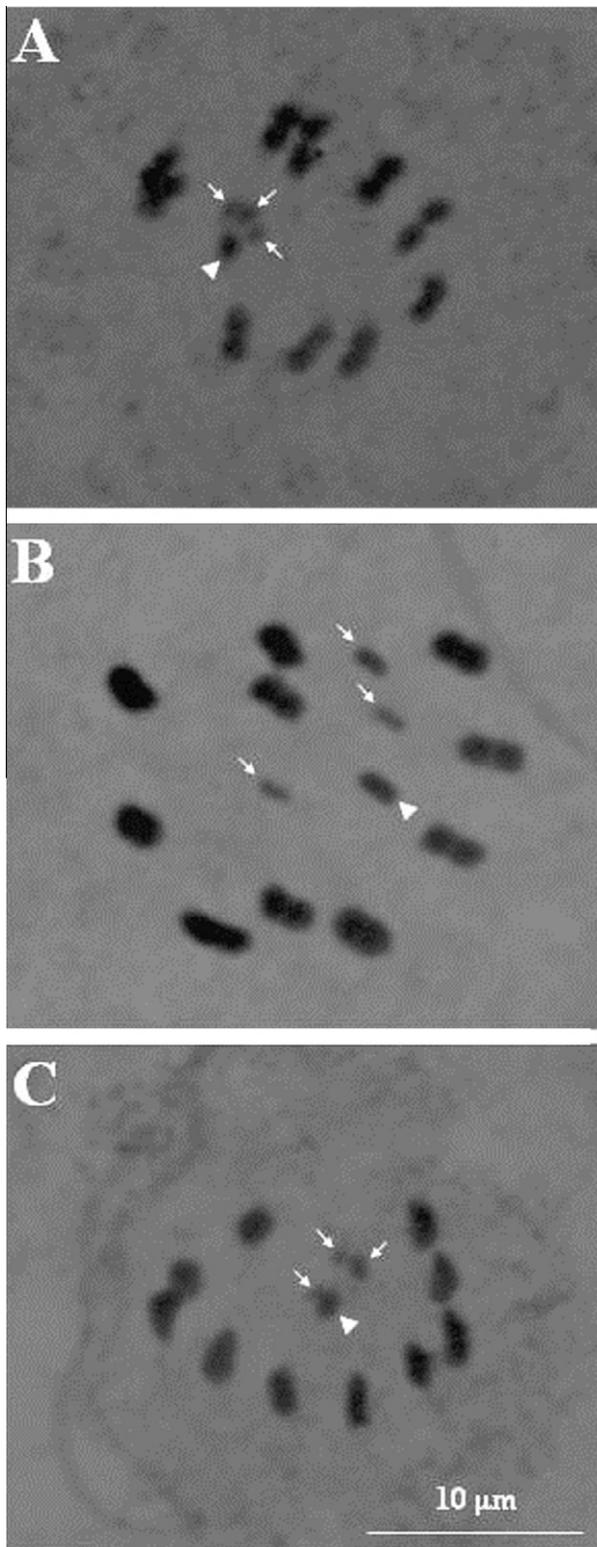
E-mail address: [kaiochaboli@hotmail.com](mailto:kaiochaboli@hotmail.com) (K.C.C. Alevi).

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 ( $XY, X_1X_2Y, X_1X_2X_3Y$ ), 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  $2n = 24$  ( $20A + X_1X_2X_3Y$ ), 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,



**Fig. 1.** Seminiferous tubule of *Triatoma melanocephala* stained by lacto-acetic orcein. (A and B) Metaphase I with ten bivalent autosomes and three sex chromosomes. (C) Metaphase II. Arrows: X chromosome fragmentation ( $X_1X_2X_3$ ). Arrowhead: Y chromosome.

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  $2n = 20A + X_1X_2Y$  (Panzera et al., 1996) and the karyotype of *T. vitticeps* is  $2n = 20A + X_1X_2X_3Y$  (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. eratyrusiformis* and *T. vitticeps* karyotypes have been described previously as  $20A + X_1X_2X_3Y$  (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 peridomicile (Dias and Schofield, 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.

#### Acknowledgments

This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

#### References

- De Vaio, E.S., Grucci, B., Castagnino, A.M., Franca, M.E., Martinez, M.E., 1985. Meiotic differences between three triatomine species (Hemiptera:Reduviidae). *Genetica* 67, 185–191.
- Dias, J.C.P., Schofield, C.J., 1998. Controle da transmissão transfusional da doença de Chagas na Iniciativa do Cone Sul. *Rev. Soc. Bras. Med. Trop.* 31, 373–383.
- Gurgel-Gonçalves, R., Galvão, C., Costa, J., Peterson, A.T., 2012. Geographic distribution of chagas disease vectors in Brazil based on ecological niche modeling. *J. Trop. Med.* 2012, 15.
- Manna, G.K., 1950. Multiple sex chromosome mechanism in a reduviid bug, *Conorhinus rubrofasciata* (De Geer). *Proc. Zool. Soc. Bengal* 3, 155–161.
- Monteiro, F.A., Escalante, A.A., Beard, C.B., 2001. Molecular tools and triatomine systematics: a public health perspective. *Trends Parasitol.* 17, 344–347.
- Neiva, A., Pinto, C., 1923. Dos Hemípteros hematofagos do Norte do Brasil com descrição de duas novas espécies. *Brasil Médico* 37, 73–76.
- Panzera, F., Pérez, R., Hornos, S., Panzera, Y., Cestau, R., Delgado, V., Nicolini, P., 1996. Chromosome numbers in the Triatominae (Hemiptera-Reduviidae): a Review. *Mem. Inst. Oswaldo Cruz.* 91, 515–518.
- Panzera, F., Pérez, R., Panzera, Y., Ferrandis, I., Ferreiro, M.J., Calleros, L., 2010. Cytogenetics and genome evolution in the subfamily Triatominae (Hemiptera, Reduviidae). *Cytogenet. Genome Res.* 128, 77–87.
- Pérez, R., Panzera, Y., Scafiezzo, S., Mazzella, M.C., Panzera, F., Dujardin, J.P., Svortzoff, E., 1992. Cytogenetics as a tool for Triatominae species distinction (Hemiptera-Reduviidae). *Mem. Inst. Oswaldo Cruz.* 87, 353–361.
- Schofield, C.J., Galvão, C., 2009. Classification, evolution, and species groups within the Triatominae. *Acta Trop.* 110, 88–100.
- Schreiber, G., Pellegrino, J., 1950. Eteropnicosi di autosomi come possibile meccanismo di speciazione. *Sci. Genet.* 3, 215–226.
- Sherlock, Í.A., Guitton, N., 1980. Fauna triatominae do estado da Bahia, Brasil: IV - *Triatoma Melanocephala* Neiva & Pinto, 1923. *Mem. Inst. Oswaldo Cruz.* 75, 23–31.
- Tavares, M.G., Azeredo-Oliveira, M.T.V., 1997. Cytogenetics study on holocentric chromosomes of five species of triatomines (Heteroptera: Reduviidae). *Cytobios* 89, 51–61.
- Ueshima, N., 1966. Cytotaxonomy of the Triatominae (Reduviidae, Hemiptera). *Chromosoma* 18, 97–122.