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# Cytogenetic characterisation of *Triatoma rubrofasciata* (De Geer) (Hemiptera, Triatominae) spermatocytes and its cytotaxonomic application

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*Triatoma rubrofasciata* (De Geer) (Hemiptera, Triatominae) was the first Triatominae species formally described, as *Cimex rubrofasciatus* De Geer, 1773. This insect presents anthropogenic habits (Galvão 2014) and is considered of global epidemiological importance, since it has a pantropical distribution and was found infected with the protozoan *Trypanosoma cruzi* (Chagas, 1909) (Sherlock & Serafim 1974; Brazil & Silva 1983), the etiological agent of Chagas disease.

This vector is found in approximately 45 countries (Galvão *et al.* 2003) and the Old World populations reached the New World probably by maritime transport (Patterson *et al.* 2001; Hypsa *et al.* 2002; Galvão 2014). In addition, this species had its dispersion favoured by the interaction between residential settlement and human activities (Silveira & Rezende 1994).

*Triatoma rubrofasciata* is the only species of triatomine present on the African continent and has been reported from Angola, Democratic Republic of Congo, Guinea-Conakry, Sierra Leone, South Africa and Tanzania (Dujardin *et al.* 2015). Related studies regarding entomological aspects of *T. cruzi* in Africa are scarce in the literature. However some exemplars of *T. cruzi* were identified from African mammals (Hamilton *et al.* 2009), which highlights the need for new epidemiological studies, including ones on insect vector species.

Biological studies of *T. rubrofasciata* are restricted to starvation resistance (Cortéz & Gonçalves 1998), interspecific morphometric variability (Patterson *et al.* 2001), morphometry of testis follicles (Freitas *et al.* 2007a), colouration of the testicular peritoneal sheath (Alevi *et al.* 2014a), male accessory gland ultrastructure (Freitas *et al.* 2007b), phylogeny (Hypsa *et al.* 2002; Justi *et al.* 2014) and karyotypic studies (Manna 1950; Alevi *et al.* 2015a).

The application of different cytogenetic markers has contributed substantially to triatomine taxonomy (Ueshima 1966; Pérez *et al.* 1992; Panzera *et al.* 1997, 2010, 2012; Alevi *et al.* 2012a, 2013a, 2014b, 2015a). Thus, this study aims to cytogenetically characterise spermatocytes in the early prophase of *T. rubrofasciata* and compare it with all other species in the genus *Triatoma* that have been analysed cytotaxonomically.

Four adult males from the National and International Reference Laboratory in Taxonomy of Triatominae, FIOCRUZ, Rio de Janeiro, Brazil, were analysed cytogenetically. Seminiferous tubules were first shredded and squashed 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 analysis of the initial prophase of *T. rubrofasciata* allowed us to observe that this species has a chromocentre formed by the sex chromosomes (Fig. 1, arrow) and many heteropycnotic blocks dispersed in the nucleus. This result was compared with that described for 33 species of the genus *Triatoma* (Table 1).

The comparative analysis of spermatocytes in early prophase were important taxonomic tools to assist in the differentiation of species in the *T. maculata* complex (Santos *et al.* 2007), to assist in the grouping of the *T. brasiliensis* subcomplex (Panzera *et al.* 2000, Alevi *et al.* 2012a, 2013a, 2014b) and to demonstrate intraspecific variation in *T. sordida* (Panzera *et al.* 1997) and *T. dimidiata* (Panzera *et al.* 2006).

The comparative analysis of *T. rubrofasciata* with the species of the genus *Triatoma*, whether by chromocentre composition, or by arrangement of heteropycnotic/heterochromatic blocks in the nucleus allowed the differentiation of this vec-

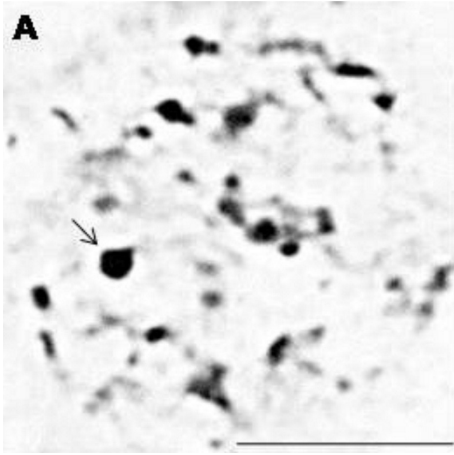
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**Table 1.** Cytogenetic characteristics in spermatocytes of *Triatoma*.

Species	Prophase		References
	Chromocentre	Chromatin	
<i>Triatoma baratai</i>	X and Y	–	Alevi <i>et al.</i> (2015a)
<i>Triatoma brasiliensis</i>	X, Y and two autosomes	Dispersed blocks	Panzer <i>et al.</i> (2000)
<i>Triatoma carcavalloii</i>	X and Y	–	Alevi <i>et al.</i> (2015a)
<i>Triatoma circummaculata</i>	X and Y	–	Panzer <i>et al.</i> (1998)
<i>Triatoma costalimai</i>	X and Y	–	Alevi <i>et al.</i> (2015a)
<i>Triatoma delpontei</i>	X and Y plus autosomes	–	Panzer <i>et al.</i> (1995)
<i>Triatoma dimidiata</i>	X <sub>1</sub> , X <sub>2</sub> and Y	Dispersed blocks	Panzer <i>et al.</i> (2006)
<i>Triatoma dimidiata</i>	X <sub>1</sub> , X <sub>2</sub> and Y	–	Panzer <i>et al.</i> (2006)
<i>Triatoma garciabesi</i>	X and Y	–	Jurberg <i>et al.</i> (1998)
<i>Triatoma gerstaeckeri</i>	X <sub>1</sub> , X <sub>2</sub> and Y	–	Ueshima (1966)
<i>Triatoma guasayana</i>	X and Y	–	Panzer <i>et al.</i> (1997)
<i>Triatoma guazu</i>	X and Y	–	Alevi <i>et al.</i> (2015a)
<i>Triatoma infestans</i>	X and Y plus autosomes	–	Panzer <i>et al.</i> (1995)
<i>Triatoma juazeirensis</i>	X, Y and two autosomes	Dispersed blocks	Panzer <i>et al.</i> (2000)
<i>Triatoma jurbergi</i>	X and Y	–	Alevi <i>et al.</i> (2015a)
<i>Triatoma klugi</i>	X and Y	–	Costa <i>et al.</i> (2008)
<i>Triatoma lenti</i>	X, Y and two autosomes	Dispersed blocks	Alevi <i>et al.</i> (2012b)
<i>Triatoma lecticularia</i>	X and Y	–	Tavares & Azeredo-Oliveira (2007)
<i>Triatoma maculata</i>	X and Y	Dispersed blocks	Santos <i>et al.</i> (2007)
<i>Triatoma matogrossensis</i>	X and Y	–	Pérez <i>et al.</i> (1992)
<i>Triatoma melanica</i>	X, Y and two autosomes	Dispersed blocks	Alevi <i>et al.</i> (2014c)
<i>Triatoma melanocephala</i>	X <sub>1</sub> , X <sub>2</sub> , X <sub>3</sub> and Y	–	Alevi <i>et al.</i> (2013b)
<i>Triatoma petrochiae</i>	X, Y and two autosomes	Dispersed blocks	Panzer <i>et al.</i> (2000)
<i>Triatoma petrochiae</i>	X and Y	–	Alevi <i>et al.</i> (2014b)
<i>Triatoma pintodiasi</i>	X and Y	–	Alevi <i>et al.</i> (2015c)
<i>Triatoma platensis</i>	X and Y plus autosomes	Chromocentre (several autosomal)	Panzer <i>et al.</i> (1995)
<i>Triatoma protracta</i>	X <sub>1</sub> , X <sub>2</sub> and Y	Dispersed blocks	Ueshima (1966)
<i>Triatoma pseudomaculata</i>	X and Y plus autosomes	Dispersed blocks	Santos <i>et al.</i> (2007)
<i>Triatoma rubrofasciata</i>	X <sub>1</sub> , X <sub>2</sub> and Y	Dispersed blocks	This paper
<i>Triatoma rubrovaria</i>	X and Y	–	Panzer <i>et al.</i> (1995)
<i>Triatoma sherlocki</i>	X, Y and two autosomes	Dispersed blocks	Panzer <i>et al.</i> (2010)
<i>Triatoma sordida</i>	X and Y	–	Panzer <i>et al.</i> (1997)
<i>Triatoma sordida</i>	X and Y plus autosomes	Dispersed blocks	Panzer <i>et al.</i> (1997)
<i>Triatoma tibiamaculata</i>	X <sub>1</sub> , X <sub>2</sub> and Y	Dispersed blocks	Panzer <i>et al.</i> (1998)
<i>Triatoma vandae</i>	X and Y	–	Panzer <i>et al.</i> (2010)
<i>Triatoma vitticeps</i>	X <sub>1</sub> , X <sub>2</sub> , X <sub>3</sub> and Y	–	Panzer <i>et al.</i> (1998)
<i>Triatoma williami</i>	X and Y	–	Succi <i>et al.</i> (2014)

tor from 30 other species (Table 1). Only one of the *T. dimidiata* population, *T. protracta* and *T. tibiamaculata* showed the same characteristics observed for *T. rubrofasciata*. However, none of these species has evolutionary relationship with *T. rubrofasciata* (Justi *et al.* 2014) and by means of karyosystematics can be easily differentiated (Alevi *et al.* 2015b).

Therefore, the analysis of spermatocytes of *T. rubrofasciata* was shown to be an extremely important taxonomic tool, since it allows this species to be distinguished from 30 other species of triatomines of the genus *Triatoma*. The correct classification *T. rubrofasciata* is of global importance and can help vector control programmes.



**Fig. 1.** Initial prophase of *Triatoma rubrofasciata*. Note the presence of a chromocentre formed by the sex chromosomes (arrow) and heteropycnotic blocks dispersed in the nucleus. Scale bar = 10  $\mu$ m.

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