



Short communication

Distribution of constitutive heterochromatin in two species of triatomines: *Triatoma lenti* Sherlock and Serafim (1967) and *Triatoma sherlocki* Papa, Jurberg, Carcavallo, Cerqueira & Barata (2002)

Kaio Cesar Chaboli Alevi^{a,*}, Priscila Pasqüetto Mendonça^a, Nathália Paiva Pereira^a, Ana Letícia Guerra^a, Camila Helena Facina^a, João Aristeu da Rosa^b, Maria Tercília Vilela de Azeredo Oliveira^a

^a 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

^b Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista – Araraquara, Rod. Araraquara-Jaú km 1, 14801-902 Araraquara, SP, Brazil

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ABSTRACT

Triatoma lenti and *Triatoma sherlocki* are hemipterans that belong to the brasiliensis subcomplex. In triatomines, the constitutive heterochromatin pattern is species-specific and allows, in many cases, for the grouping of species. Thus, we cytogenetically analyzed *T. sherlocki* and *T. lenti* using C-banding, and we compared the results with previous ones obtained in other species of the brasiliensis subcomplex. Both species were found to have a male diploid chromosome number of 22 chromosomes ($2n = 20A + XY$) with heterochromatic blocks at one or both chromosomal ends of all autosomal pairs. During early meiotic prophase, they showed a large heteropycnotic chromocenter constituted by the association of both sex chromosomes plus two autosomal pairs and many heterochromatic blocks dispersed inside the nucleus. All of these cytogenetic characteristics are similar to those observed in other species of brasiliensis subcomplex, results which confirm the grouping of *T. sherlocki* and *T. lenti* within this subcomplex. However, we emphasize the importance of other approaches, such as molecular analysis, to confirm the placement of *T. lenti* within the brasiliensis subcomplex.

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Triatoma lenti is an endemic species of triatomine present only in the Brazilian states of Bahia, Goiás, and Roraima (Gurgel-Gonçalves et al., 2012) and it was found to be infected by the protozoan *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae) (Sherlock and Guitton, 1974). This triatomine is considered to be a potential vector of Chagas disease of secondary importance (Salvatella et al., 1998). *Triatoma sherlocki* is an endemic species of triatomine present only in the Brazilian state of Bahia (Gurgel-Gonçalves et al., 2012). Specimens in all bionomic stages were found in human dwellings and were infected by the protozoan *T. cruzi*, a finding which indicates a process of domestication of this vector (Almeida et al., 2009). This species has some morphological characteristics that are similar to those of *T. lenti*, such as reduced hemelytra, reddish orange-colored rings on the femur, and spots in the connective (Papa et al., 2002). Although *T. sherlocki* lacks the ability to fly, it has developed legs that allow for its dispersion (Almeida et al., 2012).

The brasiliensis subcomplex is present in South America and includes nine species: *Triatoma brasiliensis*, *Triatoma juazeirensis*, *Triatoma melanica*, *Triatoma melanocephala*, *Triatoma petrochiae*, *T. lenti*, *T. sherlocki*, *Triatoma tibiamaculata*, and *Triatoma vitticeps*, which are grouped by molecular and morphometric comparisons (Schofield and Galvão, 2009). The inclusion of *T. lenti* and *T. sherlocki* in this group was based only on morphological characters and geographical distribution, because no genetic analyses were performed on these two species. Recent studies based on mitochondrial sequences suggest that *T. sherlocki* is included in this subcomplex as a sister species of *T. melanica* (Mendonça et al., 2009), and their chromosome number and meiotic processes were described (Panzera et al., 2010). Through the use of cytogenetic data, Alevi et al. (2012) proposed the exclusion of *T. melanocephala*, *T. tibiamaculata*, and *T. vitticeps* from the brasiliensis subcomplex because of their cytogenetic similarities to triatomine species from North America.

Ueshima (1966) proposed that the chromosome number and male meiotic process are useful cytogenetic parameters to clarify evolutionary relationships among different triatomine groups. Furthermore, Pérez et al. (1992) reported the importance of the C-banding technique to reveal several cytogenetic traits that are useful in the characterization and differentiation of triatomine

* 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 (K.C.C. Alevi).

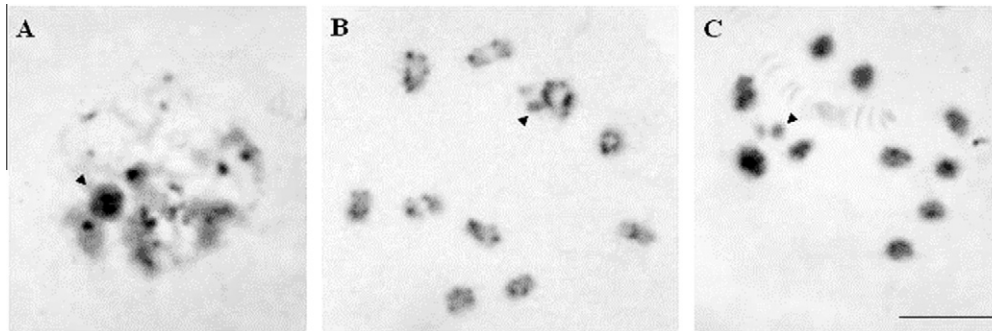


Fig. 1. Male meiotic in *Triatoma sherlocki* $2n = 20A + XY$. (A) Prophase I (diffuse stage). Note a large chromocenter (arrowhead). (B) Final diplotene. Note the C-heterochromatic blocks in one or both chromosomal ends of autosomes and on the Y chromosome (arrowhead). (C) Diakinesis with large heterochromatic blocks in the autosomes. Bar: 10 μ m.

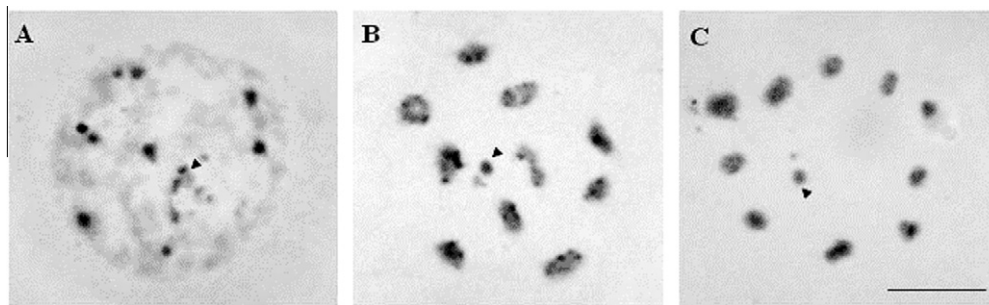


Fig. 2. Male meiotic in *Triatoma lenti* $2n = 20A + XY$. (A) Prophase I (diffuse stage). Note a large chromocenter (arrowhead). (B) Final diplotene. Note the heterochromatic blocks in one or both ends of autosomes and the Y chromosome (arrowhead). (C) Diakinesis with large heterochromatic blocks in the autosomes. Bar: 10 μ m.

species. In triatomines, the C-heterochromatin pattern in autosomes and sex chromosomes is species-specific, and their diagnosis allows for species identification and the grouping of species (Panzera et al., 1998, 2000, 2010). Thus, we have cytogenetically analyzed *T. sherlocki* and *T. lenti* using C-banding, and we have compared them to the other species of the brasiliensis subcomplex.

Seminiferous tubules of ten adult males of *T. lenti* and *T. sherlocki* from the Triatominae Insectarium located in the city of Araquara, Sao Paulo, Brazil were shredded, crushed and fixed in liquid nitrogen on a glass slide. They were then stained using C-banding (Sumner, 1972).

The analysis of the prophase I (diffuse stage) of *T. sherlocki* (Fig. 1A, arrowhead) and *T. lenti* (Fig. 2A, arrowhead) revealed a large chromocenter made up of the association of both sex chromosomes plus two autosomal pairs and many heterochromatic blocks arranged inside the nucleus. Both species were found to have heterochromatic blocks in one or both chromosomal ends of autosomes during final diplotene (Figs. 1 and 2B). Diakinesis in *T. sherlocki* (Fig. 1C) and *T. lenti* (Fig. 2C) revealed large heterochromatic blocks in the autosomes and showed a diploid chromosomal set of $2n = 22$ ($20A + XY$). Note that the Y sex chromosome is greater and more heterochromatic than the X (arrowhead).

All species included in the brasiliensis subcomplex except *T. vitticeps*, *T. melanocephala*, and *T. tibiamaculata* share some cytogenetic characteristics that allow for species distinction between this complex and other triatomine groups (Pérez et al., 1992; Panzera et al., 1998, 2010). The first characteristic is the diploid chromosome number consisting of 20 autosomes plus two sex chromosomes (XY in males and XX in females). The second is the amount of autosomal C-heterochromatin (25–32%). The third is the location of the autosomal C-blocks (at one or both chromosomal ends in all autosomal pairs), and the fourth is the chromosome configuration during early meiotic prophase (a large

chromocenter made up of the association of both sex chromosomes plus two autosomal pairs, and multiple C-dots spread in the nucleus) (Panzera et al., 2000, 2010). These cytogenetic similarities help to group the species within the brasiliensis subcomplex, but it also makes it difficult to differentiate between them (Panzera et al., 2000). However, *T. vitticeps*, *T. melanocephala*, and *T. tibiamaculata* have been found to have multiple sex systems and different patterns of C-heterochromatin (Panzera et al., 2010; Alevi et al., 2012). *T. tibiamaculata* possesses terminal C-dots in some autosomal pairs; *T. vitticeps* does not have autosomal C-heterochromatin (Panzera et al., 2012), while in *T. melanocephala*, the heterochromatic distribution is unknown.

Cytogenetic approaches have been used to clarify the taxonomic status of several triatomine groups, such as those of the infestans (*Triatoma infestans*, *Triatoma platensis*, and *Triatoma delponteii*) and sordida (*Triatoma sordida*, *Triatoma patagonica*, and *Triatoma guasayana*) subcomplexes (Panzera et al., 1995, 1997, respectively). These studies were confirmed using isozymes that demonstrated the efficiency of cytogenetics in taxonomic and evolutionary problem solving (Pereira et al., 1996). However, though it is possible to observe that *T. lenti* possesses cytogenetic characteristics that are similar to those of the brasiliensis subcomplex, more studies should be conducted so that the organism can be properly grouped into this subcomplex. As for *T. petrochiae*, it is considered to be a species that is closely related to the organisms of the complex because it possesses cytogenetic and morphologic characteristics similar to those of the triatomines complex (Panzera et al., 2000); however, isozyme studies confirmed the specific status of *T. petrochiae* and suggested that this organism evolved independently (Monteiro et al., 1998).

Thus, this study confirms the current grouping of *T. sherlocki* and *T. lenti* because both species were found to have the same chromosomal traits observed in the other species of the brasiliensis

subcomplex. However, we emphasize the importance of other approaches, such as molecular analysis, so that the position of *T. lenti* relative to the brasiliensis subcomplex can be better clarified.

Ethical standards

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

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