Genetics and Molecular Research

Differentiation between *Triatoma arthurneivai* and *Triatoma wygodzinskyi* (Hemiptera: Reduviidae: Triatominae) using cytotaxonomy

K.C.C. Alevi[1], C.H.L. Imperador[1], F.F.F. Moreira[2], J. Jurberg[2] and M.T.V. Azeredo-Oliveira[1]

[1]Laboratório de Biologia Celular, Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista "Júlio de Mesquita Filho", São José do Rio Preto, SP, Brasil
[2]Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brasil

Corresponding author: K.C.C. Alevi
E-mail: kaiochaboli@hotmail.com

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ABSTRACT. Using classic morphometric techniques to examine the head and thorax of *Triatoma* specimens, researchers identified a possible taxonomic problem involving *T. arthurneivai* (Lent & Martins) and *T. wygodzinskyi* (Lent). A recent geometric morphometric study indicated that the insects captured outside the Serra do Cipó region, State of Minas Gerais, Brazil, were *T. wygodzinskyi*. The misidentification of *T. arthurneivai* as *T. wygodzinskyi* could result in several problems associated with entoepidemiological lifting, the biological characterization of the species, and phylogenetic reconstruction. For the first time, we describe the use of cytogenetic analysis as a tool for differentiation between *T. arthurneivai* and *T. wygodzinskyi*. The results indicated that both species had the same number of chromosomes 2n = 22 (20A + XY). However, analyses of spermatocytes during early

prophase indicated that it was possible to differentiate *T. arthurneivai* and *T. wygodzinskyi*, because only *T. arthurneivai* exhibited heteropycnotic blocks distributed in the chromatin. Therefore, we highlight the analysis of spermatocytes as a taxonomic tool for the characterization of *T. arthurneivai* and *T. wygodzinskyi*, and suggest that the technique can be used for entoepidemiological lifting in vector control programs. Thus, the results presented here, in conjunction with morphometric analyses, are of utmost taxonomic and epidemiological importance for the identification of *T. arthurneivai* and *T. wygodzinskyi* specimens.

Key words: Cytogenetics; Taxonomy; Maculata subcomplex

INTRODUCTION

The description of *Triatoma arthurneivai* (Lent & Martins) was based on a single female specimen collected in Serra do Cipó, State of Minas Gerais, Brazil (Lent and Martins, 1940). After approximately 10 years, Pellegrino (1951) collected specimens of this species in Santa Rita de Caldas, in southern Minas Gerais. Using the specimens collected by Pellegrino (1951), Lent (1951) described *T. wygodzinskyi* (Lent). In addition to those collected in Minas Gerais, specimens first identified as *T. arthurneivai* were also collected in the States of São Paulo [Sorocaba (Corrêa et al., 1962) and Itupararanga/Votorantim (Forattini et al., 1968)] and Paraná (Stumpf et al., 1981).

Using classic morphometric techniques to examine the head and thorax, Dos Santos et al. (2007) suggested a possible taxonomic problem involving *T. arthurneivai* and *T. wygodzinskyi*. Indeed, a recent geometric morphometric study showed that the insects captured outside the Serra do Cipó region were *T. wygodzinskyi* specimens (Carbajal de la Fuente et al., 2011). These authors reported that the populations of *T. arthurneivai* from São Paulo, which were studied for more than 40 years by several authors (Corrêa et al., 1962, 1965; Pinto Alves and Noda, 1964; Juarez, 1970; Forattini et al., 1968, 1972; Barretto and Ribeiro, 1981; Hypsa et al., 2002; de Paula et al., 2005; Rosa et al., 2005; Dos Santos et al., 2007; Bargues et al., 2008), were actually *T. wygodzinskyi* specimens.

The misidentification of *T. arthurneivai* as *T. wygodzinskyi* could result in several problems associated with entoepidemiological lifting (Pinto Alves and Noda, 1964), the biological characterization of the species (Forattini et al., 1968; Juarez, 1970), and phylogenetic reconstruction (Hypsa et al., 2002; de Paula et al., 2005; Bargues et al., 2008). The identification of new inexpensive tools that aid the correct identification of the species is of great epidemiological importance. Therefore, we describe the use of cytogenetic analysis as a tool for differentiation of *T. arthurneivai* and *T. wygodzinskyi*.

MATERIAL AND METHODS

At least two adult males from each species (*T. arthurneivai* and *T. wygodzinskyi*) were used, and the specimens were assigned by the insectariums of the National and International Laboratory of Reference for Triatominae Taxonomy, Oswaldo Cruz Institute (FIOCRUZ), Rio de Janeiro, Brazil (*T. arthurneivai*) and the Laboratory of Triatomines and Chagas Disease Epidemiology at the René Rachou Research Center (CPqRR/FICRUZ), Minas Gerais, Brazil (*T. wygodzinskyi*).

The biological material used to characterize cells was spermatocytes, which were easily obtained from testicular material by tearing the seminiferous tubules of adult males prior to fixation to a cover slip. The samples were then subjected to a cytogenetic technique that utilized lacto-acetic orcein (De Vaio et al., 1985, with modifications based on Alevi et al., 2012). At least 50 cells from each species were analyzed using a Jenaval light microscope (Zeiss) attached to a digital camera and an Axio Vision LE 4.8 image analyzer (Copyright 2006-2009 Carl Zeiss Imaging Solutions Gmb H), and the obtained images were magnified by a factor of 1000X.

RESULTS

The results of the analyses indicated that both species had the same number of chromosomes 2n = 22 (20A + XY). However, analyses of spermatocytes during early prophase indicated that it was possible to differentiate *T. arthurneivai* and *T. wygodzinskyi*, because several heteropycnotic blocks distributed in the chromatin were present in *T. arthurneivai* (Figure 1A) but not in *T. wygodzinskyi* (Figure 1B). In addition, both species have a chromocenter formed by the X and Y sex chromosomes (Figure 1A and B, arrows).

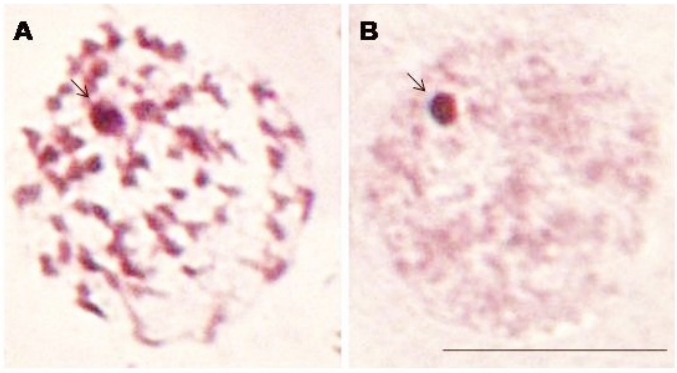

Figure 1. Early prophase of *Triatoma arthurneivai* (**A**) and *T. wygodzinskyi* (**B**). Note that *T. arthurneivai* exhibits several heteropycnotic blocks distributed in the chromatin, but these are not present in *T. wygodzinskyi*. Arrow: chromocenter formed by the X and Y sex chromosomes. Scale bar: 10 µm.

DISCUSSION

All species belonging to the Maculata subcomplex (*T. maculata*, *T. pseudomaculata*, *T. arthurneivai*, and *T. wygodzinskyi*) have the same number of chromosomes (2n = 22; 20A + XY) (Dos Santos et al., 2007). Dos Santos et al. (2007) also examined the meiotic prophase stages of *T. arthurneivai*, *T. maculata*, and *T. pseudomaculata*, and the authors found that the *T. arthurneivai* spermatocyte exhibited a heteropycnotic chromocenter formed by the sex chromosomes. However, our results confirmed that the insects analyzed by Dos Santos et al. (2007) were *T. wygodzinskyi* specimens, which corroborated the conclusion proposed by Carbajal de la Fuente et al. (2010).

The cytogenetic characterization of triatomine spermatocytes was also important for the differentiation between *T. melanocephala* and other members of the Brasiliensis

subcomplex (Alevi et al., 2013, 2014), between *T. maculata* and *T. pseudomaculata* (Dos Santos et al., 2007), between *T. guasayana* and *T. sordida* (Panzera et al., 1997), and between *T. rubrofasciata* and other 30 species of the triatomines (Alevi et al., 2016). The cellular analyses of spermatocytes allowed the characterization of many species-specific patterns, thereby ensuring that morphologically related species were differentiated, so as observed for *T. arthurneivai* and *T. wygodzinskyi*.

The cytogenetic technique that utilized lacto-acetic orcein was cheaper and faster than molecular methods. This colorant has an affinity for basic structures, so histone and non-histone proteins involved in compaction of the genetic material (heteropycnotic blocks) are stained, and this characteristic was of great importance for the cytogenetic and cytotaxonomic studies of triatomines (Ueshima, 1966; De Vaio et al., 1985). The analysis of spermatocytes using orcein as a taxonomic tool for the characterization of *T. arthurneivai* and *T. wygodzinskyi* specimens was highlighted here, and we suggest that it may be used for entoepidemiological lifting in vector control programs.

Therefore, the combination of cytotaxonomic and morphometric analyses (Carbajal de la Fuente et al., 2011) were proven to be of utmost taxonomic and epidemiological importance for the identification of *T. arthurneivai* and *T. wygodzinskyi* specimens.

Conflicts of interest

The authors declare no conflict of interest.

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