Spermatogenesis and nucleolar behavior in *Triatoma vandae* and *Triatoma williami* (Hemiptera, Triatominae)

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ABSTRACT. This study describes spermatogenesis in *Triatoma vandae* and the nucleolar behavior of *T. vandae* and *Triatoma williami*, with a cytotaxonomic focus. Analysis of mitotic and meiotic metaphases of *T. vandae* confirms the species karyotype. *T. vandae* presents some characteristics during meiosis that differentiate it from *T. williami*, including the presence of a chromocenter with two sex chromosomes individualized during early prophase, and the presence of a bi- or tripartite corpuscle in polyploid nuclei. It was possible to observe the compaction of chromatin during prophase resulting in holocentric chromosomes. During metaphase, the autosomes presented a ring shape and the sex chromosomes were in the center of the ring. These chromosomes were separated in anaphase. Although it is common, we did not observe the phenomenon of late migration of the sex chromosomes. By means of silver ion impregnation it was possible to describe nucleologenesis in *T. vandae* and *T. williami*. In both species we observed persistence of the nucleolar material during meiosis. In addition to the cells in meiotic division, we also observed the

presence of polyploid nuclei in the seminiferous tubule walls that nourish the cells during cell division. The nucleolar markings reflect their capacity for synthetic activity. *T. vandae* and *T. williami* presented only one nucleolar corpuscle, which reflects low synthetic activity. This study confirms the karyotype of *T. vandae*, describes characteristics that differentiate *T. vandae* and *T. williami* during meiosis, and describes the phenomenon of nucleolar persistence in both species.

INTRODUCTION

Chagas disease is a parasitic illness that causes human health problems with major socioeconomic impacts in Latin America (WHO, 2012). The disease is caused by the protozoan *Trypanosoma cruzi* and the main vectors of transmission are the triatomines (Chagas, 1909).

In 2013, seven to eight million people were estimated to be infected worldwide, mostly in Latin America (WHO, 2013). In Brazil, the number of infected is estimated at two and a half million individuals (Neto, 2009). Due to a lack of anti-trypanosomal drugs, the main method of disease control has been vector control (WHO, 2013), thus demonstrating the importance of studies to aid in the control of Chagas disease.

In this context, cytogenetic studies that can elucidate the taxonomy of the triatomines are important tools for vector control programs because the correct classification of these insects allows vector control programs to focus on species of primary importance in the transmission of Chagas disease (Alevi et al., 2012a).

Triatomine bugs are hematophagous insects classified in the order Hemiptera, suborder Heteroptera, family Reduviidae, and subfamily Triatominae (Lent and Wygodzinsky, 1979). These vectors have some peculiar characteristics, such as holocentric chromosomes with diffuse kinetochores (Panzera et al., 1996), inverted meiosis for sex chromosomes (Gómez-Palacio et al., 2008), and nucleolar persistence during meiosis (Tartarotti and Azeredo-Oliveira, 1999).

Schofield and Galvão (2009) grouped triatomines into complexes and specific subcomplexes, based primarily on geographical distribution and morphological characters. However, by means of new tools these groupings are being reassessed and, in some cases, modified, as can be observed for the species of the brasiliensis subcomplex (Alevi et al., 2012a,b, 2013a,b,c,d, 2014a,b; Gardim et al., 2014; Mendonça et al., 2014).

The matogrossensis subcomplex is the new name of the *T. oliverai* complex proposed by Carcavallo et al. (2000). This subcomplex is composed of nine species that occur in the midwestern region of Brazil: *T. baratai, T. costalimai, T. deaneorum, T. guazu, T. jatai, T. jurbergi, T. matogrossensis, T. vandae,* and *T. williami* (Schofield and Galvão, 2009; Obara et al., 2012; Gonçalves et al., 2013).

Gardim et al. (2013), in their phylogenetic reconstruction based on COI, Cytb, and 16S, did not identify the matogrossensis subcomplex as a monophyletic group, and placed its species into three different clades: 1) *T. jurbergi* + *T. matogrossensis* + *T. vandae*; 2) *T. guasayana* as the sister group of clade 1; and 3) *T. williami* + *T. guazu*. To further clarify the relationships of these species, the present study aims to describe spermatogenesis in *T. vandae* and the nucleolar behavior *T. vandae* and *T. williami*, with a cytotaxonomic focus.

MATERIAL AND METHODS

Five specimens of *T. vandae* and *T. williami* were taken from a colony maintained at the "Triatominae Insectarium" of Universidade Estadual Paulista (UNESP, Araraquara, SP, Brazil). The seminiferous tubules of adult triatomine males were analyzed because spermatogenesis in Hemiptera continues into adulthood and so the different stages of meiosis can be observed. After dissection, the seminiferous tubules were placed in methanol acetic acid (3:1) and then stored in a freezer at -20°C. For slide preparation, each tubule received two baths in distilled water for 5 minutes and then was placed in a 45% acetic acid solution for 10 minutes. The tubule was squashed and the material was stained with lacto-acetic orcein (De Vaio et al., 1985, with modifications according to Alevi et al., 2012a) and impregnated by silver ions (Howell and Black, 1980). The slides were examined under a Zeiss-Jenaval light microscope (magnified 1000X) and the images were captured using the Zeiss AxioVision LE 4.8 software (Carl Zeiss Microscopy GmbH, Jena, Germany).

RESULTS

Lacto-acetic orcein

The conventional cytogenetic technique of lacto-acetic orcein staining was used to examine spermatogenesis in *T. vandae* (Figure 1). The polyploid nuclei of *T. vandae* showed a bi- or tripartite corpuscle (Figure 1a, arrow). Through examination of the cell in mitotic metaphase it was possible to confirm the number of chromosomes in the species as 2n = 22 (20A + XY) (Figure 1b). During meiosis, we observed compaction of chromatin and a chromocenter formed by the sex chromosomes in cells in prophase (Figure 1c-e, arrows). In the initial diffuse stage it was possible to distinguish the sex chromosomes (Figure 1c, arrow) that were later compacted during the intermediate diffuse stage (Figure 1d, arrow) and the final stage (Figure 1e, arrow). Cells in metaphase I (Figure 1f) and metaphase II (Figure 1g) also confirmed the karyotype as 2n = 22. During anaphase, we observed migration of the chromosomes to the poles of the cells (Figure 1h-i).
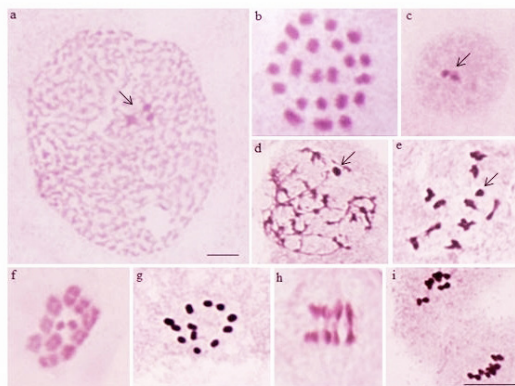

Figure 1. Spermatogenesis of *Triatoma vandae*. **a.** Note the polyploidy nuclei of *T. vandae* showed a bi- or tripartite corpuscle (arrow). **b.** Mitotic metaphase. **c.-e.** Note the compaction of chromatin and a chromocenter formed by the sex chromosomes in prophase (arrows). Note that in the initial diffuse stage (c) it was possible to distinguish the sex chromosomes (arrow), which then compacted during the intermediate diffuse stage (d, arrow) and the final stage (e, arrow). **f.** Metaphase I. **g.** Metaphase II. **h.-i.** Anaphase. Note the migration of the chromosomes to the poles of the cells. Bar: 10 μm.

Impregnation by silver ions

By means of silver ion impregnation it was possible to describe the nucleolar behavior of *T. vandae* and *T. williami* during meiosis. As *T. vandae* and *T. williami* showed the same characteristics, we illustrate only the process in *T. vandae* (Figure 2). Polyploid nuclei exhibited only one nucleolar corpuscle (Figure 2a). During prophase, we observed the presence of nucleolar material (Figure 2b-d, arrows). Note the presence of nucleolus fragments during the stages of prophase (Figure 2b-c, arrows), and the persistence of these fragments during metaphase (Figure 2e, arrow) and anaphase (Figure 2f, arrow).
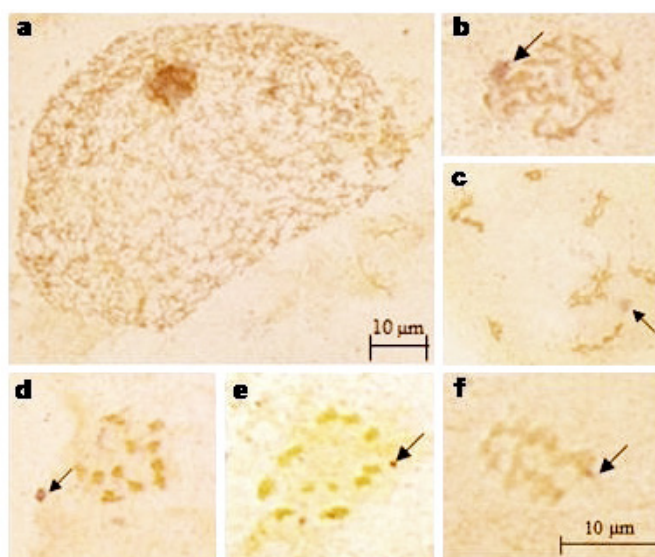

Figure 2. Nucleolar behavior of *Triatoma vandae*. **a.** Polyploid nuclei showed only one nucleolar corpuscle. **b.-d.** Prophase. Note the presence of nucleolar material (arrows). **e.** Metaphase. Note the nucleolar persistence (arrow). **f.** Anaphase. Note the nucleolar persistence (arrow). Bar: 10 μm.

DISCUSSION

T. vandae and *T. williami* are potential vectors of Chagas disease in the midwestern region of Brazil, where *T. vandae* has been collected in the states of Mato Grosso and Mato Grosso do Sul and *T. williami* in Goiás, Mato Grosso, and Mato Grosso do Sul (Gurgel-Gonçalves et al., 2012). It is known that *T. williami* is an invasive species of residential areas (peri and intradomiciliary) and, in 2011, it was found infected by *T. cruzi* (Arrais-Silva et al., 2011). Much about the biology, ecology, and epidemiology of *T. vandae* is still unknown, which emphasizes the importance of this study.

Analysis of the mitotic and meiotic metaphase of *T. vandae* allowed us to confirm the species karyotype which was previously described by Panzera et al. (2010). This number of chromosomes is observed in all insects in the matogrossensis subcomplex (Panzera et al., 2010; Succi et al., 2013; Alevi et al., 2014c).

Recently, the spermatogenesis of *T. williami* has been described (Succi et al., 2013). *T. vandae* presents some characteristics during meiosis that differentiate it from *T. williami*, including

the presence of a chromocenter with two sex chromosomes individualized during early prophase, and the presence of a bi- or tripartite corpuscle in polyploid nuclei. These results, in combination with morphological analyses of eggs and nymphs that also identified species differences (Silva et al., 2005), are important taxonomic tools.

The chromosomes of *T. vandae* behaved in a way similar to that described for the Triatominae subfamily (Ueshima, 1966). It was possible to observe the compaction of chromatin during prophase resulting in holocentric chromosomes. During metaphase, the autosomes exhibited a ring shape and the sex chromosomes were in the center of the ring. These chromosomes were separated in anaphase. Although it is common, we did not observe the phenomenon of late migration of the sex chromosomes.

By means of silver ion impregnation it was possible to observe nucleologenesis in *T. vandae* and *T. williami*. The nucleolus is a nuclear structure common to all eukaryotic cells and is responsible for ribosome biogenesis, which occurs by means of a series of events involving rRNA gene transcription, pre-processing of ribosomal RNAs, and the meeting of pre-ribosomal particles (Scherr et al., 1997). Variations in the pattern of transcriptional activity, expressed by differences in the size or number of nucleoli, may result in a reduction or an absence of rDNA, and may be involved in the process of gene regulation (Galletti-Júnior et al., 1985).

In both species we observed persistence of the nucleolar material during meiosis. The phenomenon of nucleolar persistence during spermatogenesis is characterized by the presence of the nucleolus or nucleolar corpuscles during meiotic metaphase, since, generally, in eukaryotes the nucleolus is fragmented in late prophase and is only reorganized at the end of prophase. This phenomenon has been described in 21 species of triatomines (Tartarotti and Azeredo-Oliveira, 1999; Morielle-Souza and Azeredo-Oliveira, 2004, 2007; Severi-Aguiar and Azeredo-Oliveira, 2005; Severi-Aguiar et al., 2006; Costa et al., 2008; Alevi et al., 2013e, 2014d), and Alevi et al. (2014d) have proposed that it is a synapomorphy of the subfamily Triatominae.

In addition to the cells in meiotic division, we also observed the presence of polyploid nuclei in the seminiferous tubule walls that nourish the cells during cell division. Thus, the nucleolar markings reflect the capacity of synthetic activity. *T. vandae* and *T. williami* presented only one nucleolar corpuscle, which reflects low synthetic activity. Alevi et al. (2013e) analyzed polyploid nuclei of *T. melanocephala* and *T. lenti* and suggested that *T. lenti* exhibited greater synthetic activity because of its large number of nucleolar corpuscles.

Thus, this study confirms the karyotype of *T. vandae*, presents characteristics that differentiate *T. vandae* and *T. williami* during meiosis, and describes the phenomenon of nucleolar persistence in both species. These data are important for evolutionary studies of these vectors of Chagas disease.

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