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Original Article

Cytotaxonomy of *Dipetalogaster maxima* Uhler, 1894 (Hemiptera, Reduviidae, Triatominae)

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(With 2 figures)

Abstract

The Triatomini tribe consists of ten genera and is regarded as one of the most important tribes from epidemiological point of view. The genus *Dipetalogaster* Usinger, 1939 is composed only by the species *Dipetalogaster maxima* Uhler, 1894. This triatomine is exclusive of the Mexico and is a potential vector for Chagas disease. Besides the epidemiological importance, the insects of the Triatominae subfamily are important biological models for cytogenetic studies. Therefore, in order to contribute to the knowledge on the reproductive biology and assist in citotaxonomy of *D. maxima*, this study aimed to describe spermatogenesis, as well as confirm the karyotype and heterochromatic patterns of this Mexican triatomine species. The seminiferous tubules were torn, fixed to a cover slip and underwent the cytogenetic technique of Lacto-acetic orcein and C-banding. Through the cytogenetics analysis of testicular material *D. maxima* it was possible to confirm the karyotype (2n = 22), describe the stages of spermatogenesis and characterize the heterochromatic pattern (restricted to sex chromosome Y) of the species. *D. maxima* showed the same arrangement of heterochromatin described for *Triatoma lecticularia* (Stål, 1859) (a species that occur in United States of American and Mexico and is phylogenetically related with *D. maxima*), highlighting the importance of this analysis as an optimization tool to explore phylogenetic correlations.

Keywords: cytogenetics, meiosis, Mexican triatomine.

Citotaxonomia de *Dipetalogaster maxima* Uhler, 1894 (Hemiptera, Reduviidae, Triatominae)

Resumo

A tribo Triatomini consiste em dez gêneros e é considerada uma das tribos mais importantes do ponto de vista epidemiológico. O gênero *Dipetalogaster* Usinger, 1939 é composto apenas pela espécie *Dipetalogaster maxima* Uhler, 1894. Este triatomíneo é exclusivo do México e é um vetor potencial da doença de Chagas. Além da importância epidemiológica, os insetos da subfamília Triatominae são importantes modelos biológicos para estudos citogenéticos. Portanto, a fim de contribuir para o conhecimento da biologia reprodutiva e complementar o conceito específico de *D. maxima*, este trabalho objetivou descrever a espermatogênese, bem como confirmar o padrão cariotípico e heterocromático desta espécie mexicana, com foco citotaxonômico. Os túbulos seminíferos foram dilacerados, fixados em uma lamínula e submetidos à técnica citogenética de Orceína lacto-acética e Bandamento-C. Por meio da análise citogenética do material testicular de *D. maxima* foi possível confirmar o cariótipo (2n = 22), descrever os estágios da espermatogênese e caracterizar o padrão heterocromático (restrito ao cromossomo Y sexual) da espécie. *D. maxima* apresentou o mesmo arranjo de heterocromatina descrito para *Triatoma lecticularia* (Stål, 1859) (espécie que ocorre no México e nos Estados Unidos da América, filogeneticamente relacionada com *D. maxima*), destacando a importância desta técnica como ferramenta para explorar correlações filogenéticas.

Palavras-chave: citogenética, meiose, triatomíneo Mexicano.

1. Introduction

The Triatominae subfamily consists of 153 species distributed in 19 genera and five tribes (Galvão, 2014; Oliveira and Alevi, 2017; Dorn et al., 2018; Oliveira et al., 2018), being all species considered as potential insect vector of Chagas disease. The Triatomini tribe is one of the most important from an epidemiological point of view and brings together ten genera of triatomines (Galvão, 2014).

The genus *Dipetalogaster* Usinger, 1939 is composed only by the species *Dipetalogaster maxima* (Uhler, 1894). This triatomine is exclusive to Mexico (Galvão et al., 2003) and is a potential vector of Chagas disease, because has been found infected by the protozoan *Trypanosoma cruzi* (Chagas, 1909), in natural environments (Jimenez et al., 2003) and occasionally in rural households and peripheral areas (Lent and Wygodzinsky, 1979; Estrada et al., 1995). Costa et al. (1986, 1987, 1992) performed bionomic studies and analyzed the population dynamics and the influence of diet on several factors such as rhythm and posture, egg viability, fertility curve, female mortality, resistance to fasting and biological cycle.

Besides the epidemiological importance, the triatomines are important biological models for cytogenetic studies, because they have some peculiarities, as holocentric chromosomes, diffuse kinetochores and inverted meiosis to the sex chromosomes (Ueshima, 1966). Ueshima (1966) first proposed the use of cytogenetic data as a tool in taxonomy (cytotaxonomy) of triatomines. Recently, cytogenetic analyzes have proved to be important tools for studying the taxonomy of these vectors, emphasizing karyosystematic (Alevi et al., 2012, 2015a, 2018), meiotic (Panzera et al., 1998; Mendonça et al., 2014; Alevi et al., 2014, 2016a), of the pattern of constitutive heterochromatin (Perez et al., 1992; Panzera et al., 2000, 2010; Alevi et al., 2015b), heterochromatin base pair composition (Bardella et al., 2016; Alevi et al., 2017) and of the distribution of Nucleolus Organizer Regions (NOR) studies (Panzera et al., 2012; Pita et al., 2013, 2016).

Therefore, in order to contribute to the knowledge on the reproductive biology and assist in cytotaxonomic of *D. maxima*, this study aimed to describe spermatogenesis, as well as confirm the karyotype and heterochromatic pattern of this Mexican triatomine species.

2. Material and Methods

In this study, two adult males of *D. maxima* (Figure 1A) were used [the specimens were classified as *D. maxima* according to Lent and Wygodzinsky (1979)]. They had been assigned by insectarium of the Laboratory of Triatomines and Chagas Disease Epidemiology at the René Rachou Research Center (CPqRR/FICRUZ), Minas Gerais, Brazil. The testicles (Figure 1B1) of adult males were removed and seminiferous tubules (Figure 1B2) were torn and fixed to a cover slip. They then underwent the cytogenetic technique of Lacto-acetic orcein (for analysis of karyotype and spermatogenesis) [De Vaio et al. (1985), with modifications according to Alevi et al. (2012)] and C-banding for analysis of heterochromatin pattern (Sumner, 1972) and 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). The images obtained were magnified by a factor of 1000x.

3. Results and Discussion

Through the analysis of stained testicular material of *D. maxima* with Lacto-acetic orcein, it was possible to confirm the karyotype (Figure 1C) and describe spermatogenesis of the species (Figure 2). Analysis of meiotic metaphase *D. maxima* allowed possible to confirm the karyotype 2n = 22 chromosomes (20A + XY) (Figure 1C), originally described by Ueshima (1966).

Although the diverse in number of chromosomes (2n = 21, 22, 23, 24 and 25) in the Triatomini (Ueshima, 1966; Alevi et al., 2018), the karyotype 2n = 22 is the most frequently described for that tribe (Ueshima, 1966; Alevi et al., 2015a). It is believed that this number of

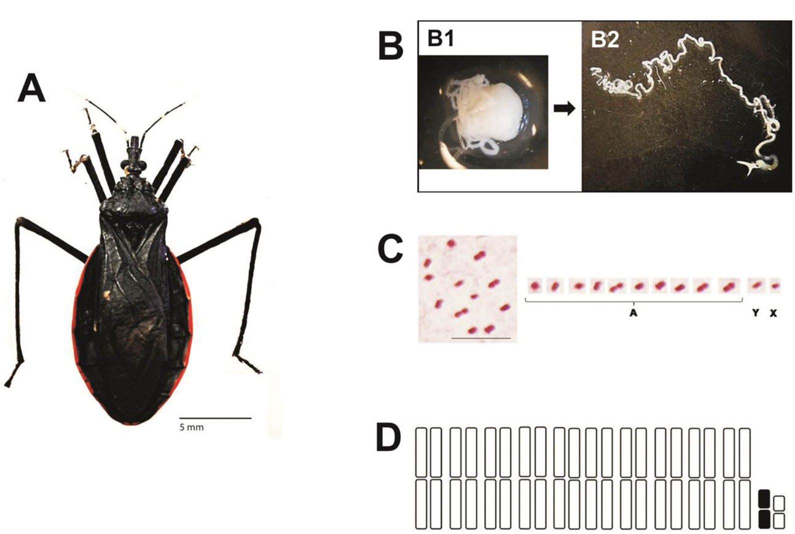

Figure 1. (A) *Dipetalogaster maxima* male; (B) Testicle (B1) and seminiferous tubule (B2) of *D. maxima*; (C) Karyotype of *D. maxima* [2n = 22 (20A +XY)]. Bar: 10 µm. and (D) Ideogram representing the disposition of constitutive heterochromatin in *D. maxima*. Note the presence of heterochromatin restricted to sex chromosome Y.

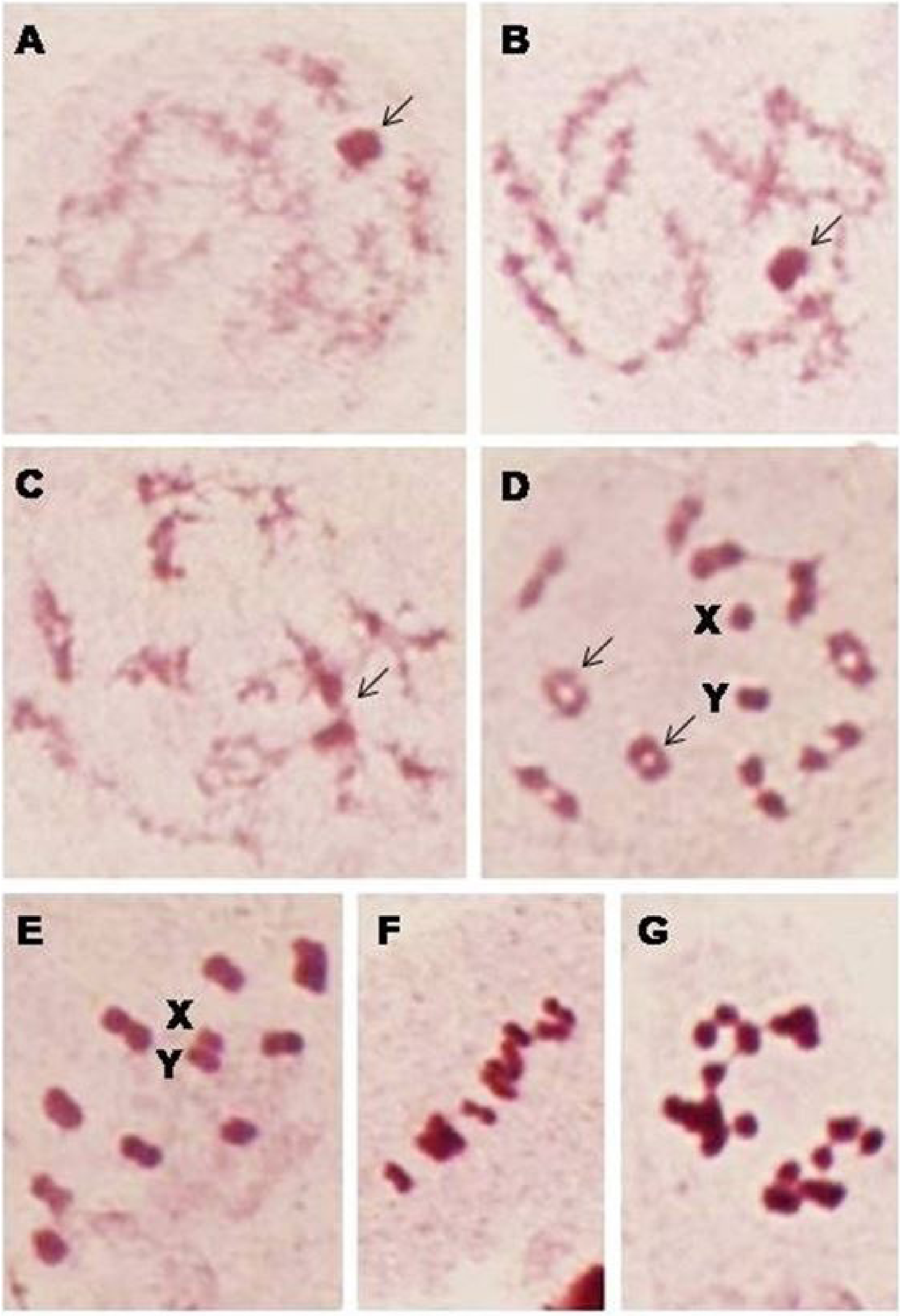

Figure 2. Spermatogenesis of *D. maxima*. (A-D) prophase. Note the compaction of chromatin (A-D). Presence of a heteropycnotic chromocenter (A-C, arrows), that differed in sex chromosomes X and Y. Also note the presence of chiasmus in diplotene (D-E, arrows); (E) metaphase in polar view; (F) metaphase in side view; (G) anaphase; (H-J) spermiogenesis. Bar: 10 µm.

chromosomes is the same presented by the common ancestor of these blood-sucking insects (Ueshima, 1966).

During spermatogenesis (Figure 2), it was possible to describe the meiosis' stages (Figure 2A-G). The spermatogenesis consists of three main stages, namely spermatocytogenesis (mitosis), meiosis and spermiogenesis (Johnson et al., 1997). In triatomine, the spermatogenesis is considered cystic (Alevi et al., 2015c). The prophases' analysis allow to observe compaction of chromatin (Figure 2A-D), the presence of a heteropycnotic chromocenter (Figure 2A-C, arrows) that differed in sex chromosomes X and Y and the presence of chiasma in cell's autosomes in diplotene (Figure 2D, arrows). Recently, the characterization of prophase of these vectors was considered an important cytotaxonomic tool for characterization of the *Triatoma* Laporte, 1832 genus of triatomines (Alevi et al., 2016b). The metaphases were observed in lateral (Figure 2E) and polar view (Figure 2F), confirming the karyotype and holocentric nature of chromosomes. Furthermore, it was also possible to observe the anaphase (Figure 2G) and characterize the elongation phases of spermatid during spermiogenesis (Figure 2H-J).

Through the analysis of stained slides by C-banding technique, was observed that *D. maxima* has constitutive heterochromatin restricted only to the Y sex chromosome, as illustrated by the ideogram (Figure 1D). Our results confirm the ones of Pita et al. (2014) which presents the Y sex chromosome of *D. maxima* as heterochromatic and marked by GUISH probes to repetitive sequences in the genome. *D. maxima* proved to be phylogenetically close to *T. lecticularia* (Stål, 1859) and *Paratriatoma hirsuta* Barber, 1938 (Justi et al., 2014). Although the heterochromatic pattern of *P. hirsuta* has not been characterized, *T. lecticularia* also presents karyotype 2n = 22 (Ueshima, 1966) and heterochromatin restricted to the Y sex chromosome (Panzera et al., 1998), demonstrating that the cytogenetic analyzes can be a tool to explore phylogenetic correlations.

Therefore, this paper describes spermatogenesis of *D. maxima*, confirmed the species' karyotype (2n = 22) and corroborated the phylogenetic relationship of *D.maxima* and *T. lecticularia*.

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