



Spermatogenesis in *Nesotriatoma bruneri* (Usinger 1944) (Hemiptera, Triatominae)

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ABSTRACT. The *Nesotriatoma* genus consists of the species *N. flavida*, *N. bruneri* and *N. obscura*, forming the Flavida complex. Variation in the size and morphological differences intraspecific of *N. flavida* led to the description of *N. bruneri*. Two years later, the same author proposed the synonymization of *N. bruneri* with *N. flavida*. Only in 1981 the specific status *N. bruneri* was recovered by means of morphological analysis of the genitalia. However, recently by genetic analysis, it was suggested that *N. bruneri* and *N. flavida* should be again synonymized. As Chagas disease has no cure, the main way to minimize the incidence of this disease is by vector control. Thus, grouping biological data from these hematophagous insects can assist in the development of vector control programs and mainly assist in taxonomic issues of synonymization. Thus, this paper describes

spermatogenesis of *N. bruneri*. Three adult *N. bruneri* males were cytogenetically analyzed. The meiotic behavior observed for *N. bruneri* was very similar to that observed for the triatomine species with 23 chromosomes: during prophase, chromatin compaction was observed, the chromocenter composition was characterized (X_1 , X_2 and Y), and the species karyotype was confirmed as $2n = 23$ ($20A + X_1X_2Y$), as it was observed for *N. flavida*. Moreover, it was possible to observe anaphase and telophase. Thus, this study describes reproductive aspects of *N. bruneri* in order to contribute to the biological knowledge of these insects of epidemiological importance. Furthermore, this corroborates the synonymization of *N. bruneri* with *N. flavida*.

Key words: Cytogenetics; *Nesotriatoma*; Flavida complex

INTRODUCTION

The triatomines are hematophagous insects that belong to the Hemiptera order, Heteroptera suborder, Reduviidae family, and Triatominae subfamily (Lent and Wygodzinsky, 1979). The Heteroptera subfamily is composed of 150 species, grouped into 18 genera and six tribes (Alevi et al., 2015a), with all species being potential vectors of Chagas disease.

The Triatomini tribe is composed of 101 species grouped into nine genera (Alevi et al., 2013). The *Nesotriatoma* genus consists of the species *N. flavida* Neiva, 1911, *N. bruneri* Usinger, 1994, and *N. obscura* Maldonado and Farr, 1962 (Galvão et al., 2003). These species were initially grouped in the *Triatoma* genus (Lent and Wygodzinsky, 1979), and, only in 2002, the *Nesotriatoma* genus was revalidated by molecular analysis (Hypsa et al., 2002) to form the Flavida complex (Schofield and Galvão, 2009).

N. bruneri is a species of triatomine endemic to Cuba (Galvão et al., 2003). Variation in the size and morphological differences intraspecific of *N. flavida* led to the description of *N. bruneri* (Usinger, 1944). However, two years later the same author proposed the synonymization of *N. bruneri* with *N. flavida* (Usinger, 1946). Only in 1981 the specific status *N. bruneri* was recovered by means of morphological analysis of the genitalia (Lent and Jurberg, 1981). However, recently by genetic analysis, Alevi et al. (2016a) suggest that *N. bruneri* and *N. flavida* should be synonymized again.

Although no natural infections have been ascribed to *N. flavida* and *N. bruneri*, Fraga et al. (2011) suggested that these species should be considered in future epidemiological surveillance programs. As Chagas disease has no cure, the main way to minimize the incidence of this disease is by vector control. Thus, grouping biological data from these hematophagous insects can assist in the development of vector control programs and mainly assist in taxonomic issues of synonymization. Thus, this paper describes spermatogenesis of *N. bruneri*.

MATERIAL AND METHODS

Three adult males of *N. bruneri* were cytogenetically analyzed. The insects were donated by Insetário of Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil. Microscope

slides containing biological material (seminiferous tubules) were prepared using the crushing technique and stained with lacto-acetic orcein (De Vaio et al., 1985) with modifications as described by Alevi et al. (2012). The slides were analyzed using a Jenavallight microscope (Zeiss) coupled to a digital camera and an AxioVision LE 4.8 image analyzer (Zeiss). The images were magnified 1000X.

RESULTS

By analyzing slides stained by the orcein technique, it was possible to describe the spermatogenesis of *N. bruneri* (Figure 1). During prophase (Figure 1A-C), it was possible to observe the compaction of chromatin and to characterize the chromocenter composition (X_1 , X_2 , and Y), with the Y sex chromosome being the largest and most heteropycnotic. At the end of prophase, specifically in the diplotene phase, chiasmus was observed between the autosomes (Figure 1C) and the species karyotype $2n = 23$ ($20A + X_1X_2Y$) was confirmed. Moreover, it was possible to observe anaphase, with the separation of chromosomes (Figure 1D), and telophase with decondensed chromatin (Figure 1E).

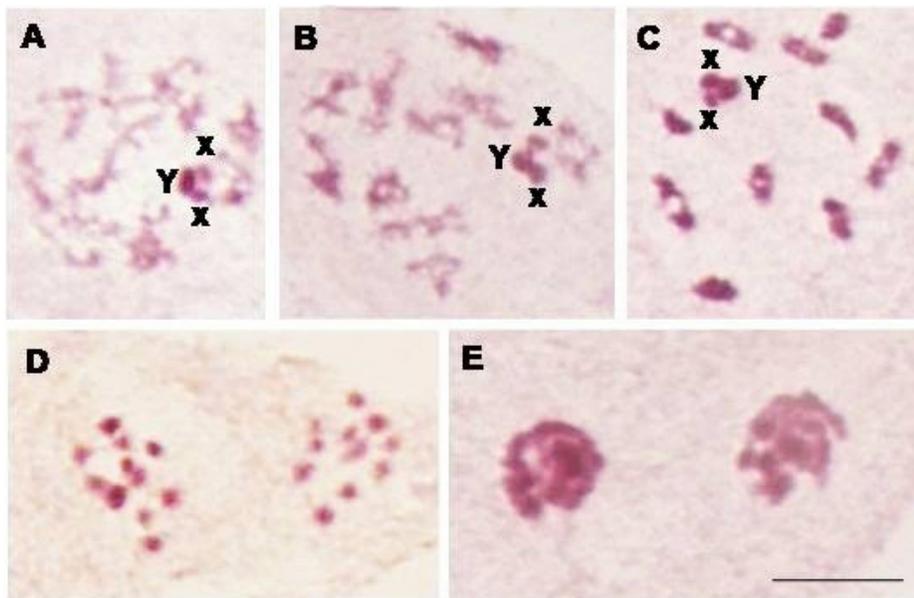


Figure 1. Spermatogenesis of *Nesotriatoma bruneri*. A.-C. Prophase. Note the compaction of chromatin and chromocenter (X_1 , X_2 , and Y) (A-C) and chiasmus (C). D. anaphase. E. telophase. Bar: 10 μ m.

DISCUSSION

The meiotic behavior observed for *N. bruneri* was very similar to that observed for the triatomine species with 23 chromosomes, with approximately 34% of species having the described karyotype (Alevi et al., 2015b). Moreover, this species presented the same number of chromosomes as previously described for *N. flavida* (Dujardin et al., 2002).

Characterization of the chromocenter during meiotic prophase is an extremely important taxonomic tool for triatomines (Alevi et al., 2016b), and has already been applied to differentiate species of *Maculata* (Dos Santos et al., 2007) and *Sordida* subcomplexes (Panzera et al., 1997), and to assist in the grouping of species of the *Infestans* (Panzera et al., 1995), *Matogrossensis* (Alevi et al., 2015c), *Rubrovaria* (Alevi et al., 2015c), and *Brasiliensis* subcomplexes (Alevi et al., 2014). In the case of the *Flavida* complex, this tool is important to corroborate the synonymization of *N. bruneri* with *N. flavida*, since *N. flavida* also features the chromocenter formed by sex chromosomes X_1 , X_2 , and Y.

The only triatomine species present in Cuba are *N. bruneri*, *N. flavida*, *Triatoma rubrofasciata* (De Geer, 1773), and *Bolboderia scabrosa* Valdés, 1919 (González and Broche, 2006). With the synonymization of *N. bruneri* and *N. flavida*, *N. flavida* can be distinguished from *T. rubrofasciata* by karyotype, which features 25 chromosomes (Alevi et al., 2015b). *B. scabrosa* has never been analyzed cytogenetically.

Thus, this study describes the reproductive aspects of *N. bruneri*, in order to contribute to the biological knowledge of these insects of epidemiological importance. Furthermore, corroborates the synonymization of *N. bruneri* with *N. flavida*.

Conflicts of interest

The authors declare no conflict of interest.

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