

First cytogenetic study of *Cavernicola pilosa* Barber, 1937 (Hemiptera, Triatominae)

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ABSTRACT. *Cavernicola pilosa* is a triatomine species that lives in caves and feeds on bat blood. This vector has a wide geographical distribution, and is found in Brazil, Colombia, Panama, Peru, and Venezuela. Little is known about the reproductive biology of this species, because most previous studies have only characterized its morphology, morphometry, ecology, and epidemiology. Therefore, this study aimed to obtain preliminary data related to spermatogenesis in *C. pilosa* by conducting cytogenetic analysis. Analysis of the heterochromatic pattern of *C. pilosa* during the initial prophase revealed that heterochromatic blocks are only present in the sex chromosomes. Based on the analyses of the meiotic metaphase and prophase, we found that the sex determination system of *C. pilosa* is XY and the chromosomes are holocentric. *C. pilosa* spermatids are filamentous and have long flagella. It was not possible to detect corpuscle

or filament heteropycnosis in spermatids of this species. The initial cytogenetic data presented in this study are important in characterizing the spermatogenesis and heterochromatic patterns of *C. pilosa*. Our results suggest that adaptation to troglodytism did not result in differences in spermatogenesis in this vector.

Key words: Tribe Cavernicolini; Spermatogenesis; Heterochromatin

INTRODUCTION

Triatomines are hematophagous vectors of the protozoan *Trypanosoma cruzi*, 1909 (Kinetoplastida, Trypanosomatidae), which is the etiological agent of Chagas disease. The Triatominae subfamily includes 148 species (Rosa et al., 2012; Abad-Franch et al., 2013; Alevi et al., 2013a; Gonçalves et al., 2013; Jurberg et al., 2013; Poinar Jr., 2013) that are distributed in 18 genera and six tribes (Galvão et al., 2003; Tartarotti et al., 2006; Alevi et al., 2013a).

The tribe Cavernicolini Usinger, 1944, is composed of only two species of the genus *Cavernicola* Barber, 1937, namely *Cavernicola lenti* Barrett & Arias, 1985 and *Cavernicola pilosa* Barber, 1937. Both members of the Cavernicolini tribe (as the *Cavernicola* genus) were initially described from *C. pilosa*. Oliveira et al. (2007) redescribed the Cavernicolini tribe as the *Cavernicola* genus based on the morphological and morphometric characteristics of *C. lenti* and *C. pilosa*.

C. pilosa mainly inhabits caves, feeds on bat (*Desmodus rotundus*) blood (Lent and Wygodzinsky, 1979) and disperse passively in bat hair (Oliveira et al., 2008). This vector has a wide geographical distribution, and is found in Brazil (Bahia, Espírito Santo, Goiás, Minas Gerais, Mato Grosso do Sul, Mato Grosso, Pará, Paraná, and Tocantins), Colombia (Cundinamarca, Meta, Tolima, and Valle), Panama, Peru (Loreto), and Venezuela (Cojedes, Portuguesa, Táchira, Lara, and Amazonas) (Galvão et al., 2003; Gurguel-Gonçalves et al., 2012).

Little is known about the reproductive biology of this vector, because previous studies have only characterized its morphology and morphometry (Lent and Jurberg, 1969; Oliveira et al., 2007), ecology (Oliveira et al., 2008), and epidemiology (Dias et al., 1942; Baker et al., 1978; Silva et al., 1992). Therefore, this study aimed to obtain preliminary data concerning spermatogenesis in this species.

MATERIAL AND METHODS

Because of the difficulty in collecting and maintaining *C. pilosa* in insectariums, we only used one fifth-instar nymph that was collected in a cave in Marabá, PA, Brazil. This insect was dissected and its seminiferous tubules were placed in methanol:acetic acid (3:1). For slide preparation, each tubule was bathed twice in distilled water for 5 min. The tubules were then placed in a 45% acetic acid solution for 10 min, squashed, stained with lacto-aceto-orcein (De Vaio et al., 1985, with modifications by Alevi et al., 2012), and C-banding (Sumner, 1972). The slides were examined (total increase of 1000X) under a Jenaval light microscope (Zeiss, Jena, Germany), and images were captured using AxioVision LE 4.8 (Zeiss).

RESULTS

Polyploid nuclei were observed during all stages of spermatogenesis (Figure 1A and B). These cells exhibited one heterochromatic (Figure 1A) and one heteropycnotic corpuscle (Figure

1B). By analyzing the meiotic prophases, it was possible to study the sex chromosomes, which were completely heterochromatic (Figure 2A) and heteropycnotic (Figure 2B). The sex determination system is probably XY. In addition, by analyzing the prophase, we discovered that there were no heterochromatic blocks in the autosomes (Figure 2A). Although it was not possible to ascertain the species karyotype by studying metaphase I (Figure 2C and D), we did find that the chromosomes are holocentric. We characterized the spermatids during spermiogenesis, which were completely filamentous with long flagella (Figure 2E).

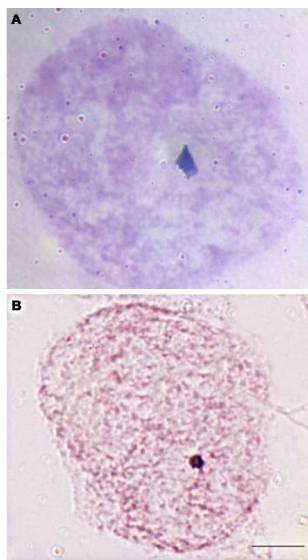


Figure 1. Polyploid nuclei of *Cavernicola pilosa*. Note the presence of a heterochromatic (A) and heteropycnotic corpuscle (B). Bar, 10 μ m.

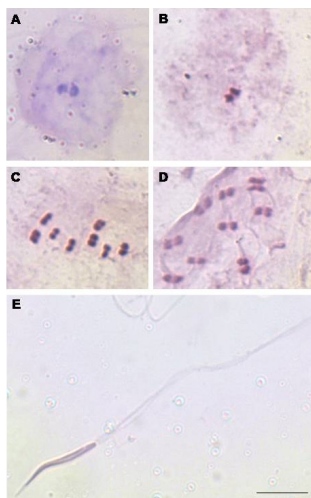


Figure 2. Spermatogenesis in *Cavernicola pilosa*. A. B. Initial prophase; the sex chromosomes were heterochromatic (A) and heteropycnotic (B). C. D. Metaphase I; note the holocentric nature of the chromosomes. E. Spermatid; note the filamentous form and the presence of a flagellum. Bar, 10 μ m.

DISCUSSION

Cytogenetic analyses of the Triatominae subfamily have been conducted for over 100 years (e.g., Payne, 1909), and is of utmost importance to detailed knowledge of cell biology, taxonomy, systematics, and evolution of the triatomines. The cytogenetic characterization of spermatogenesis in *C. pilosa* is important in understanding the reproductive biology and cytogenetics of this vector.

Polyploid nuclei are cells in seminiferous tubule walls that are responsible for cell nutrition during meiotic division. They exhibit high or low transcriptional activity, according to the requirement for reproduction (Alevi et al., 2013b). The absence of heterochromatic blocks in the PN suggests that there is no heterochromatin in *C. pilosa* autosomes, only in its sex chromosomes.

Analyzing the initial prophase to characterize this species is extremely important, because this phase of meiosis has been used as a cytotaxonomic tool for the study of the *Triatoma maculata* (Santos et al., 2007) and *T. brasiliensis* complexes (Alevi et al., 2014). Analyzing the heterochromatic pattern of *C. pilosa* during the initial prophases revealed that heterochromatic blocks are only present in the sex chromosomes.

The commonest sex determination system in the Triatominae is XY. However, there are variations in the fragmentation of X, which results in X_1X_2Y and $X_1X_2X_3Y$ (Ueshima, 1966; Alevi et al., 2013a). The sex determination system in *C. pilosa* is probably XY, based on the meiotic metaphase and prophases.

The holocentric nature of triatomine chromosomes is characterized by the absence of a centromere, i.e., the kinetochore is dispersed throughout the chromosome (Hughes-Schrader and Schrader, 1961). By analyzing the metaphases, it was possible to characterize the chromosomes of *C. pilosa* as holocentric. However, it was not possible to describe the species karyotype.

Analysis of triatomine spermiogenesis has recently been used as a taxonomic tool to differentiate morphologically related species (Alevi et al., 2013c,d, 2014). *C. pilosa* spermatids were completely filamentous with long flagella. It was not possible to detect corpuscle or filament heteropycnosis in this species.

Although further cytogenetic studies should be conducted in *C. pilosa*, these initial cytogenetic data are important in characterizing the spermatogenesis and heterochromatic patterns of *C. pilosa*. Our results suggest that adaptation to troglodytism did not result in differences in spermatogenesis in this vector.

Conflicts of interest

The authors declare no conflict of interest.

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