

Karyotype of *Rhodnius montenegrensis* (Hemiptera, Triatominae)

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ABSTRACT. The Triatominae subfamily comprises 6 tribes. The tribe Rhodniini comprises 2 genera and 22 nominal species. *Rhodnius montenegrensis* (Hemiptera, Triatominae) was recently described as evolutionarily related to *R. robustus*. Therefore, in order to contribute to karyosystematic study of the tribe Rhodniini, this report describes the number of chromosomes and compares the karyotype of *R. montenegrensis* to that of all other species in the tribe, in order to determine the karyotypic evolution of the tribe Rhodniini. The seminiferous tubules of adult males, after being removed and fixated on a cover slip, were processed with lacto-aceto-orcein for cytogenetic analysis. *R. montenegrensis*, as well as all other species of the tribe Rhodniini showed 22 chromosomes (20 autosomes + XY). Thus, we hereby describe the karyotype of the species *R. montenegrensis* and mainly highlight that the

tribe Rhodniini displays karyotypic homogeneity, demonstrating itself as a derived group to a lesser extent when compared to the number of chromosomes of the common ancestors of the subfamily Triatominae.

Key words: Tribe Rhodniini; Cytotaxonomy; Karyosystematic

INTRODUCTION

Triatomines are insects taxonomically included in the Order Hemiptera and Suborder Heteroptera, within the Family Reduviidae, and subfamily Triatominae (Lent and Wygodzinsky, 1979). The subfamily Triatominae consists of 148 species (Abad-Franch et al., 2013; Alevi et al., 2013d; Jurberg et al., 2013; Poinar, 2013). All triatomine species are susceptible to infection by the protozoan *Trypanosoma cruzi* (Kinetoplastida, Trypanosomatidae) and therefore potential vectors of Chagas disease. Infection occurs through food with infected blood and all instars that are likely to ingest the parasite, since hematophagy is mandatory at all stages of the insect's lifecycle (Noireau et al., 2009).

The subfamily Triatominae comprises 6 tribes, namely, Alberproseniini, Bolboderini, Cavernicolini, Linshcosteusini, Rhodniini, and Triatomini (Galvão et al., 2003; Tartarotti et al., 2006; Alevi et al., 2013d). The tribe Rhodniini comprises of 2 genera and 22 nominal species (Table 1). Recently, da Rosa et al. (2012, 2014) described the species *Rhodnius montenegrensis* as related to *R. robustus* that belongs to *R. prolixus* complex. This species was found to be infected by the protozoan *Trypanosoma rangeli* (Meneguetti et al., 2014).

Table 1. Species of triatomines that constitute the tribe Rhodniini with their respective karyotypes.

Tribe Rhodniini	Karyotype	Described by
<i>P. arthuri</i>	Not described	
<i>P. coreodes</i>	2n = 20A + XY	Schreiber and Pellegrino (1950)
<i>P. tertius</i>	2n = 20A + XY	Panzer et al. (1998)
<i>R. amazonicus</i>	Not described	
<i>R. barretti</i>	Not described	
<i>R. brethesi</i>	2n = 20A + XY	Panzer et al. (1998)
<i>R. colombiensis</i>	2n = 20A + XY	Dujardin et al. (2002)
<i>R. dalessandroi</i>	Not described	
<i>R. domesticus</i>	2n = 20A + XY	Dujardin et al. (2002)
<i>R. ecuadoriensis</i>	2n = 20A + XY	Panzer et al. (1996)
<i>R. milesi</i>	2n = 20A + XY	Panzer et al. (2010)
<i>R. montenegrensis</i>	2n = 20A + XY	First described
<i>R. nasutus</i>	2n = 20A + XY	Pérez et al. (1992)
<i>R. neglectus</i>	2n = 20A + XY	Barth (1956)
<i>R. neivai</i>	2n = 20A + XY	Koshy (1979)
<i>R. pallescens</i>	2n = 20A + XY	Panzer et al. (1996)
<i>R. paraensis</i>	Not described	
<i>R. pictipes</i>	2n = 20A + XY	Koshy (1979)
<i>R. prolixus</i>	2n = 20A + XY	Schreiber and Pellegrino (1950)
<i>R. robustus</i>	2n = 20A + XY	Koshy (1979)
<i>R. stali</i>	2n = 20A + XY	Dujardin et al. (2002)
<i>R. zeledoni</i>	Not described	

P = genus *Psammolestes*, R = genus *Rhodnius*.

Therefore, in order to contribute to karyosystematic study of the tribe Rhodniini, this study describes the number of chromosomes and compares the karyotype of the species *R.*

montenegrensis with all other species of the tribe, in order to determine karyotypic evolution in the tribe Rhodniini.

MATERIAL AND METHODS

In this study, we used 10 males of the species *R. montenegrensis*, assigned by the “Triatominae Insectarium” at the Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas, Araraquara campus. The seminiferous tubules of adult males, after being removed and fixated on a cover slip, were processed for cytogenetic analysis using lacto-acetic orcein technique (De Vaio et al., 1985, with modifications described by Alevi et al., 2012a). The biological material was analyzed using Jenaval light microscope (Zeiss) coupled to a digital camera and an image analyzer Axio Vision LE 4.8 (Copyright© 2006-2009 Carl Zeiss Imaging Solutions GmbH). The images were magnified by a factor of 1000.

RESULTS

Analysis of metaphase I (Figure 1A) and metaphase II (Figure 1B) revealed that the species *R. montenegrensis* possesses a diploid chromosome set $2n = 22$ (20A + XY). This chromosome number is shared across all members of the tribe Rhodniini (Table 1).

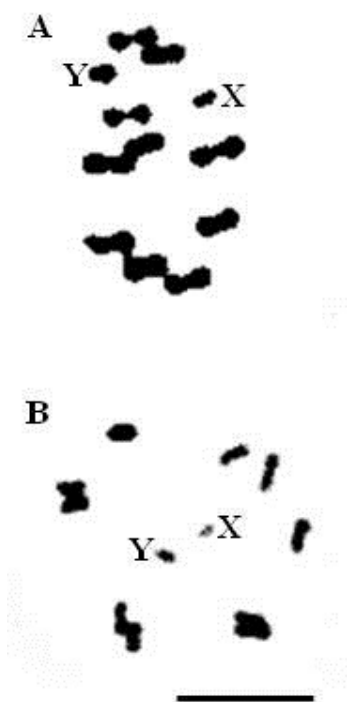


Figure 1. Seminiferous tubule of *Rhodnius montenegrensis* stained by lacto-aceto-orcein. **A.** Metaphase I with 10 bivalent autosomes and sex chromosomes. **B.** Metaphase II with 10 autosomes and sex chromosomes. X = X sex chromosome; Y = Y sex chromosome. Bar: 10 μ m.

DISCUSSION

The similarity between *Rhodnius* species is so great that one species is frequently mistaken for another, due to morphologic similarity. The classification of Rhodniini, as a monophyletic tribe, takes into account characteristics of the genus *Rhodnius* not shared with other triatomines, such as, apical antenna insertion, body forms, post-ocular callosities, male genital characteristics, egg-surface architecture, and presence of nitrophenol in the salivary glands. Besides these characteristics, the genera *Rhodnius* and *Psammolestes* are primarily arboreal species in contrast to the terrestrial habits of most of the other triatomines (Schofield and Dujardin, 1999).

Using DNA sequence analysis of mitochondrial ribosomal RNA, mitochondrial cytochrome b, and nuclear RNA, Monteiro et al. (2000) indicated a paraphyletic nature of the genus *Rhodnius*, supporting the monophyly of the Rhodniini tribe. The Rhodniini, Cavernicolini, Bolboderini, Linshcosteini, and Alberproseniini tribes constitute monophyletic groups, while the Triatomini tribe is considered polyphyletic (Tartarotti et al., 2006). No reports are currently available regarding the number of chromosomes of representatives from Alberproseniini, Cavernicolini, Bolboderini, or Linshcosteini tribes.

Cytogenetic studies have focused mainly on Rhodniini and Triatomini tribes, where 86 karyotypes have been described to date (Alevi et al., 2013d). Cytogenetic data are important tools that assist with taxonomic and evolutionary knowledge of the triatomine bugs (Ueshima, 1966; Pérez et al., 1992; Alevi et al., 2012a,b, 2013a,b,c,d, 2014).

All species of the tribe Rhodniini with the karyotype described, including *R. montenegrensis*, had the same number of chromosomes, namely 22 (20 autosomes + XY). Besides the number of chromosomes, insects of the tribe Rhodniini share the location of the probe 45S of nucleolar organizing region (NOR) which is restricted to sex chromosomes (Pita et al., 2013). Ueshima (1966) indicated that the common ancestor of all organisms of the Triatominae subfamily presents the karyotype $2n = 20A + XY$, as observed in the tribe Rhodniini. The tribe Triatomini is the most derivative, since all mechanisms of sex determination, derived from fragmentation of the sex chromosome X, are found in this group (XY, X_1X_2Y , $X_1X_2X_3Y$) (Alevi et al., 2013d).

Thus, we describe the karyotype of the species *R. montenegrensis* and highlight that the tribe possesses karyotypic homogeneity, demonstrating itself as a derived group to a lesser extent when compared to the number of chromosomes of the common ancestors of the subfamily Triatominae.

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