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Spermiotaxonomy of the tribe Rhodniini (Hemiptera, Triatominae)

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ABSTRACT. The tribe Rhodniini is a monophyletic group composed of 22 species, with 19 in the *Rhodnius* genus and three in the *Psammolestes* genus. These insects are morphologically very similar (cryptic species), and new tools are important for investigating the taxonomy of these vectors. Spermiotaxonomy is an important tool in differentiating between related species, and this study analyzed the spermatids of Rhodniini species to elucidate their spermiotaxonomy. All of the Rhodniini species contained two heteropyknotic filaments in the extremities of their cells. Although spermiotaxonomy has been an important tool in differentiating between species of the *Triatoma* genus, all of the species in the *Rhodnius* genus exhibited the same characteristics in their male gametes. However, spermatid analysis made it possible to confirm the monophyly of the Rhodniini tribe, because *Psammolestes tertius* had the same pattern as that described for *Rhodnius*. The results of this study demonstrate that spermiotaxonomy, in addition to being an important tool for differentiating between related species of *Triatoma*, can be used as an optimization tool in phylogenetic analyses.

Key words: *Rhodnius*; *Psammolestes*; Spermiogenesis; Cytotaxonomy

INTRODUCTION

The triatomines are insects that belong to the Hemiptera order, Heteroptera suborder, Reduviidae family, and Triatominae subfamily (Lent and Wygodzinsky, 1979). The Triatominae subfamily is composed of 150 species, grouped in 18 genera and six tribes (Alevi et al., 2015). All of the species of the subfamily are bloodsucking and potential vectors of the *Trypanosoma cruzi* protozoan, which is an etiological agent of Chagas disease (Noireau et al., 2009).

The tribe Rhodniini is a monophyletic group (Monteiro et al., 2000) composed of 22 species, with 19 in the *Rhodnius* genus and three in the *Psammolestes* genus (Rosa et al., 2012; Alevi et al., 2012; Abad-Franch et al., 2013). These insects are morphologically very similar (cryptic species) (Monteiro et al., 2000), and new tools are important for investigating the taxonomy of these vectors.

Recently, spermiotaxonomy was used in the *Triatoma* genus to differentiate between related species (Alevi et al., 2013a, 2014). Cytogenetic analysis of male gametes of *Triatoma melanocephala* and *T. vitticeps*, which are considered sister species (Alevi et al., 2013b; Gardim et al., 2014), allowed the differentiation of these species (Alevi et al., 2014). In addition, the same analysis also made it possible to differentiate *T. lenti* from *T. sherlocki* (Alevi et al., 2013a). Therefore, this study aimed to analyze spermatids of species of the Rhodniini tribe to elucidate the spermiotaxonomy of these vectors.

MATERIAL AND METHODS

Three adult males of each species (Table 1) were cytogenetically analyzed. The insects were donated by “Insetário de Triatominae” of the Biological Sciences Department of the Faculty of Pharmaceutical Sciences, State University of São Paulo, Campus Araraquara, São Paulo, Brazil. Microscope slides with the biological material (seminiferous tubules) were prepared by the crushing technique and stained with lacto-acetic orcein (De Vaio et al., 1985) with modifications 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 by 1000X.

RESULTS

All of the species of the Rhodniini tribe exhibited the same spermatid characteristics, i.e., the presence of two heteropyknotic filaments in the extremities of their cells (Figure 1 and Table 1).

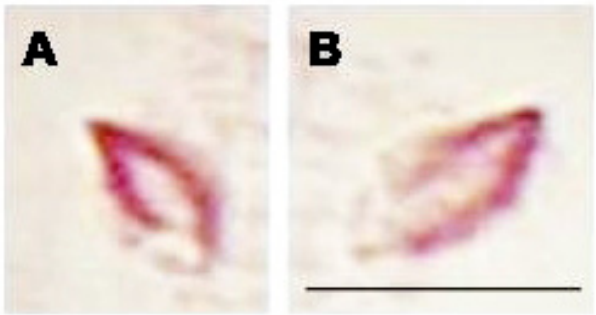

Figure 1. Early spermatids of *Psammolestes tertius* (**A**) and *Rhodnius montenegrensis* (**B**). All of the species analyzed had two heteropyknotic filaments in their haploid cells. Bar: 10 μm.

Table 1. Heteropyknotic patterns in spermatids of species of the tribe Rhodniini.

Species	Heteropyknotic pattern in spermatids	Reference
Rhodnius colombiensis	Two heteropyknotic filaments	This study
Rhodnius domesticus	Two heteropyknotic filaments	Morielle and Azeredo-Oliveira (2004)
Rhodnius montenegrensis	Two heteropyknotic filaments	This study
Rhodnius nasutus	Two heteropyknotic filaments	This study
Rhodnius neglectus	Two heteropyknotic filaments	This study
Rhodnius neivai	Two heteropyknotic filaments	This study
Rhodnius pictipes	Two heteropyknotic filaments	This study
Rhodnius prolixus	Two heteropyknotic filaments	This study
Rhodnius robustus	Two heteropyknotic filaments	This study
Psammolestes tertius	Two heteropyknotic filaments	This study

DISCUSSION

Abad-Franch et al. (2013) suggested that there may be many errors related to the taxonomy of the genus *Rhodnius*, mainly because of cryptic species. The authors propose that *R. milesi* is probably an *R. neglectus* variant from south-eastern Amazonia, *R. zeledoni* closely resembles *R. domesticus*, and *R. montenegrensis* probably represents one of the *R. robustus* lineages (Monteiro et al., 2000).

Although spermiotaxonomy has been an important tool in differentiating between triatomines of the *Triatoma* genus (Alevi et al., 2013a, 2014), all of the species in the *Rhodnius* genus exhibited the same male gamete characteristics. Therefore, the taxonomic issues raised by Abad-Franch et al. (2013) could not be assessed using this tool.

However, spermatid analysis made it possible to confirm the monophyly of the Rhodniini tribe, because *P. tertius* exhibited the same pattern as described for *Rhodnius*. This phylogenetic relationship was proposed by Lent and Wygodzinsky (1979) based on morphological characteristics, and by Monteiro et al. (2000) based on molecular analyses. This parameter was recently used to evaluate relationships between species of the Brasiliensis subcomplex, and it was possible to exclude *T. melanocephala*, *T. vitticeps*, and *T. tibiamaculata*, as well as suggest that *T. lenti* is the sixth subcomplex member (Alevi et al., 2014). The results of this study demonstrate that spermiotaxonomy, in addition to being an important tool for differentiating between related species of *Triatoma*, can be used as an optimization tool in phylogenetic analyses.

Conflicts of interest

The authors declare no conflict of interest.

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