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New record and cytogenetic analysis of *Psammolestes tertius* Lent & Jurberg, 1965 (Hemiptera, Reduviidae, Triatominae) from Bahia State, Brazil

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ABSTRACT. This paper reports on the first occurrence of *Psammolestes tertius* in the Chapada Diamantina region, located in the city of Seabra, Bahia State, in northeastern Brazil. Following an active search, 24 *P. tertius* specimens were collected from *Phacellodomus rufifrons* (rufous-fronted thornbird) nests. The insects did not present any symptoms of infection by *Trypanosoma cruzi*. *P. tertius* males were cytogenetically analyzed, and the results were compared with those of other specimens from the Brazilian State of Ceará. Triatomines from both locations

presented the same cytogenetic characteristics: 22 chromosomes, little variation in the size of the autosomes, Y chromosomes that were larger than the X chromosomes, a chromocenter formed only by the sex chromosomes during prophase, and autosomes lacking constitutive heterochromatin. However, it is important to note that this species shows intraspecific chromosomal variation. In light of the results obtained, it is recommended that more studies be performed to characterize *P. tertius*. These studies will be particularly helpful in understanding this species in ecological, biological, biogeographical, and phylogenetic terms.

Key words: Triatominae subfamily; Chapada Diamantina; Chromosomal variation

INTRODUCTION

The Triatominae subfamily is composed of 151 species grouped into 18 genera (Alevi et al., 2015a; Mendonça et al., 2016). All triatomine species are bloodsucking bugs and are potential vectors of the protozoan *Trypanosoma cruzi* Chagas, 1909, an etiological agent of Chagas disease.

In Brazil, 65 triatomine species have been reported. They are distributed into ten genera: *Alberprosenia* Martínez and Carcavallo, 1977; *Belminus* Stål, 1859; *Cavernicola* Barber, 1937; *Eratyrus* Stål, 1859; *Microtriatoma* (Lent, 1951); *Panstrongylus* Berg, 1879; *Parabelminus* Lent, 1943; *Psammolestes* Bergroth, 1911; *Rhodnius* Stål, 1859; and *Triatoma* Laporte, 1832. With 23 species, the Brazilian State of Bahia has the greatest species diversity to date. It is believed that one of the reasons for this richness in registered species is the variety of biomes and ecoregions in the state, which include the Cerrado, the Caatinga scrubland, and the Atlantic Forest (Galvão, 2014).

Chapada Diamantina is located in the center of Bahia State. This region has been the focus of many studies (Juncá et al., 2008; Funch et al., 2009) because it is considered a refuge for many species (Rodrigues-Filho et al., 2002). Sherlock and Serafim (1972) reported the presence of nine triatomine species in the Chapada Diamantina region: *Panstrongylus diasi*, *P. geniculatus*, *P. lutzi*, *P. megistus*, *Triatoma bahiensis*, *T. maculata*, *T. melanocephala*, *T. sordida*, and *T. tibiamaculata*. Forty years later, Souza et al. (2012) reported the occurrence of ten triatomine species in the Chapada Diamantina: *P. diasi*, *P. geniculatus*, *P. lutzi*, *P. megistus*, *T. brasiliensis*, *T. infestans*, *T. melanocephala*, *T. lenti*, *T. pseudomaculata*, and *T. sordida*. In other words, in the last forty years, only *T. brasiliensis* was newly reported in the Chapada Diamantina region. It is important to note that Sherlock and Serafim (1972) misidentified *T. pseudomaculata* as *T. maculata*, since *T. maculata* is a species restricted to the State of Roraima in Brazil (Galvão et al., 2003).

Some triatomine species are associated with bird nests (Lent and Wygodzinsky, 1979). *Psammolestes tertius* is one of the species with which triatomines have been associated. In most cases, these insects are found living in furnariid nests, particularly in those of the species *Phacellodomus rufifrons* Wied, 1821; *P. ruber* Vieillot, 1817 (Gurgel-Gonçalves and Cuba, 2011); and *Anumbius annumbi* Vieillot, 1817 (Turienzo and Di Iorio, 2008). Thus, in order to determine whether this species is present in the Chapada Diamantina region of Brazil, active searches of *P. rufifrons* nests were performed in the city of Seabra, Bahia State, Brazil (12°32.449'S and 41°32.896'W), and *P. tertius* specimens were captured.

MATERIAL AND METHODS

The triatomines were collected from the city of Seabra, Bahia (Figure 1), with help from the Central Laboratory for Public Health (LACEN-BA), and the Endemic Disease Control Center. On active searches of *P. rufifrons* nests made of thorny twigs, and hanging 1064 m high, 24 *P. tertius* specimens were collected, five of which were adult females, two of which were adult males, and 17 of which were nymphs. Beetles from the families Cerambycidae, Chrysomelidae, and Curculionidae were also identified around the nests, as were phytophagous hemipterans from the family Lygaeidae.

The *P. tertius* specimens were identified in the Parasitology Laboratory of the Department of Biological Science, School of Pharmaceutical Sciences at São Paulo State University, Araraquara (UNESP/FCFAR), São Paulo, Brazil. Identification was based on the criteria described previously by Lent and Wygodzinsky (1979). Feces from the specimens were examined. For this examination, the feces samples were diluted in saline solution and observed under a Leica MZ APO stereomicroscope. No Trypanosomatid forms were found. After identification, these insects were cytogenetically analyzed using lacto-aceto orcein techniques (De Vaio et al., 1985) with modifications according to Alevi et al. (2012), and also using C-banding (Sumner, 1972). The results were compared with those obtained by Panzera et al. (1998) who studied *P. tertius* from the Brazilian State of Ceará, in order to determine whether *P. tertius* exhibits any intraspecific chromosomal variation.

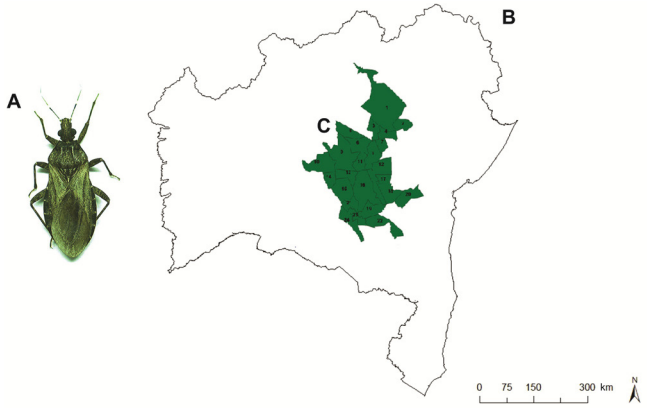

Figure 1. A. *Psammolestes tertius* male. **B.** Map of the Brazilian State of Bahia. **C.** Cities within the Chapada Diamantina region: 1 - Morro do Chapéu, 2 - Tapiramuta, 3 - Bonito, 4 - Utinga, 5 - Souto Soares, 6 - Iraquara, 7 - Wagner, 8 - Lençóis, 9 - Seabra, 10 - Ibitiara, 11 - Palmeira, 12 - Andarai, 13 - Boninal, 14 - Novo Horizonte, 15 - Piatã, 16 - Mucugê, 17 - Nova Redenção, 18 - Itaetê, 19 - Ibicoara, 20 - Marcíonilio Souza, 21 - Abaira, 22 - Barra da Estiva, 23 - Jussiape, and 24 - Rio de Contas.

RESULTS AND DISCUSSION

The genus *Psammolestes* Bergroth, 1911, includes three species grouped into the Rhodniini tribe: *P. arthuri* (Pinto, 1926); *P. coreodes* Bergroth, 1911; and *P. tertius* Lent and Jurberg, 1965. *P. arthuri* distribution was found to be restricted to Colombia and Venezuela, while *P. coreodes* was reported in Argentina, Bolivia, Brazil, and Paraguay. *P. tertius* distribution is restricted to Brazil (Galvão, 2014).

P. tertius is an exclusively sylvatic species that has not been associated with the transmission of Chagas disease, particularly because of its feeding habits, which are restricted to Chagas-resistant birds. Although the results were negative for infection by *T. cruzi*, this species has been found to be infected by protozoans (Lent and Wygodzinsky, 1979). The authors report that these flagellates were likely acquired from opossums found in abandoned bird nests that were still inhabited by triatomines.

The cytogenetic analyses performed on *P. tertius* revealed that this species does not present intraspecific chromosomal variation, since the specimens from Bahia presented the same characteristics described for the samples from Ceará (Panzera et al., 1998): 22 chromosomes (20 A + XY) (Figure 2A); little variation in autosome size (Figure 2A); Y chromosomes that were larger than the X chromosomes (Figure 2A), a chromocenter formed only by the X and Y chromosomes during initial prophase (Figure 2B; arrow); and autosomes lacking constitutive heterochromatin, which was restricted to the Y chromosomes (Figure 2C; arrow).

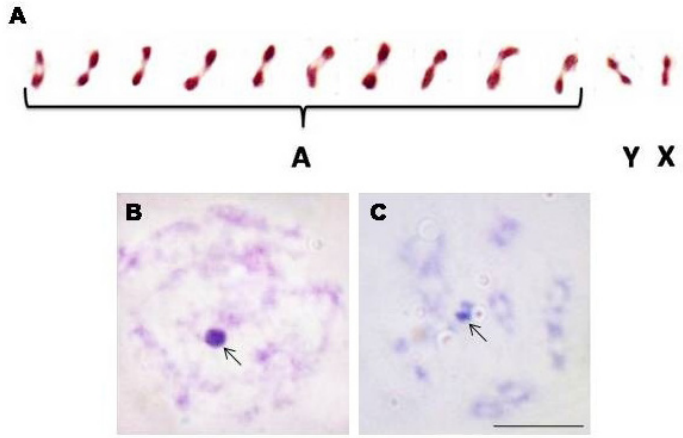

Figure 2. Karyotype (A) and constitutive heterochromatin disposition (B and C) in *Psammolestes tertius*. **A.** Metaphase I: Note that the species has 22 chromosomes, little variation in autosome size, and a Y chromosome that is larger than the X chromosome. **B.** Initial prophase. Note the heterochromatic chromocenter formed by the sex chromosomes (arrow). **C.** Final prophase. Note that only the Y chromosome is heterochromatic (arrow). Bar scale: 10 μm.

Intraspecific chromosomal variation in the Rhodniini tribe was observed in the case of *R. ecuadoriensis* (Pita et al., 2013) and *R. pallescens* (Gómez-Palacio et al., 2008). Both *P. tertius* and *Rhodnius neglectus* have been found to exhibit chromosomal homogeneity among specimens from different Brazilian states (Alevi et al., 2015b). Soares et al. (2001) analyzed two *P. tertius* populations by using morphometry, isoenzymes, and genetic analyses; they were able to distinguish between specimens from Ceará and specimens from the Brazilian State of Minas Gerais. These authors suggest that there is no gene flow between these populations, largely because they are geographically isolated. Although the results in our study did not reveal intraspecific variation, it is important to note that genetic evolution and chromosomal evolution are each guided by very different factors. The factors that cause variations in DNA sequences are more common than those that lead to chromosomal changes (Alevi et al., 2015c).

In conclusion, the presence of *P. tertius* in the Chapada Diamantina region of Brazil is described here for the first time, keeping in mind that this species presents intraspecific

chromosomal homogeneity. Further studies are necessary to characterize this species. These studies will be particularly helpful in understanding this species in ecological, biological, biogeographical, and phylogenetic terms. In addition, studies are also necessary to determine triatomine fauna and their distribution in Brazil, particularly for species belonging to the genus *Psammolestes*.

Conflicts of interest

The authors declare no conflict of interest.

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