New Evidence of the Monophyletic Relationship of the Genus *Psammolestes* Bergroth, 1911 (Hemiptera, Reduviidae, Triatominae)

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Abstract. The genus *Psammolestes* within the subfamily Triatominae and tribe Rhodniini comprises the species *Psammolestes arthuri*, *Psammolestes coreodes*, and *Psammolestes tertius*, all potential vectors of Chagas disease. A feature of *Psammolestes* is their close association with birds, which makes them an interesting model for evolutionary studies. We analyzed cytogenetically *Psammolestes* spp., with the aim of contributing to the genetic and evolutionary knowledge of these vectors. All species of the *Psammolestes* showed the same chromosomal characteristics: chromocenter formed only by sex chromosomes X and Y, karyotype $2n = 22$ and constitutive heterochromatin, and AT base pairs restricted to the sex chromosome Y. These results corroborate the monophyly of the genus and lead to the hypothesis that during the derivation of *P. tertius*, *P. coreodes*, and *P. arthuri* from their common ancestor, there was no reorganization in the number or structure of chromosomes.

Chagas disease is caused by the protozoan *Trypanosoma cruzi* (Chagas, 1909) and transmitted mainly by triatomines.¹ Presently, the subfamily Triatominae (Hemiptera, Reduviidae) consists of 152 species (150 living species and two fossil ones) distributed in 18 genera and five tribes (Alberproseniini, Bolboderini, Cavernicolini, Rhodniini, and Triatomini),² all the species being potential vectors of *T. cruzi*.

The tribe Rhodniini is a monophyletic group³ consisting of two genera with different phenotypes: one with long thin legs and a long head, living mainly in palm trees (genus *Rhodnius* Stål, 1859), and the other having a short head, strong legs, wide femora, and a very wide rostrum (the widest in all the subfamily), living in nests of birds of the family Furnariidae (genus *Psammolestes* Bergroth, 1911).⁴ The genera include 21 species of *Rhodnius* (divided into the groups *pallascens*, *pictipes*, and *prolixus*) and three species of *Psammolestes*.^{2,5}

Psammolestes coreodes Bergroth, 1911 is distributed in Argentina (Catamarca, Corrientes, Chaco, Entre Ríos, Formosa, Santa Fe, Santiago del Estero, Salta, Jujuy, and Tucumán), Bolivia (Santa Cruz), Brazil (Mato Grosso), and Paraguay (Central); *Psammolestes tertius* Lent and Jurberg, 1965 is distributed in Brazil (Bahia, Ceará, Goiás, Mato Grosso, Maranhão, Minas Gerais, Pará, Paraíba, Pernambuco, and São Paulo) and Peru (San Martín); and *Psammolestes arthuri* (Pinto, 1926) is distributed in Colombia (Meta) and Venezuela (Aragua, Cojedes, Miranda, Guárico, Portuguesa, Yaracuy, Anzoátegui, Apure, Lara, Táchira, Barinas, and Monagas).^{6,7}

Phylogenetic analyses of *P. tertius* and *P. coreodes* suggest that this genus is monophyletic³ (there are no phylogenetic studies of *P. arthuri* in the literature) and presents a phylogenetic relationship with the species of the *prolixus* group,³ which led to suggest the inclusion of the genus *Psammolestes* in the genus *Rhodnius*.⁸ Monteiro et al.³ suggest that perhaps *Psammolestes* should be regarded as a specialized lineage from the *prolixus* group of *Rhodnius* because the genus

Psammolestes and species of the *prolixus* group share a common ancestral origin, which highlights the paraphyly of the genus *Rhodnius*.⁵

Based on mitochondrial DNA data presented by Monteiro et al.,³ Soares et al.⁹ suggest that *Psammolestes* has derived from a form similar to *Rhodnius robustus* Larrousse, 1927. In addition, the authors suggest that these triatomines spread from the Amazon region northward into the llanos of Venezuela, where *P. arthuri* is now abundant in furnariidae nests, and southeastward into the caatinga–cerrado path of Central Brazil. Furthermore, as predicted by Schofield and Dujardin,¹⁰ the authors suggest subsequent differentiation of *P. tertius* along a north–south cline, from the larger specimens of the northeastern caatinga region to the smaller individuals of the central cerrado. According to them, the third species of the genus, *P. coreodes*, from the Chaco region of Argentina and Paraguay, may represent the southernmost differentiation of these descending populations.

Cytogenetic studies on the genus *Psammolestes* started in 1950 with the description of the karyotype of *P. coreodes*.¹¹ After 48 years, the karyotype of *P. tertius* was described,¹² and in 2012, the constitutive heterochromatin pattern of the species was characterized by Panzera et al.¹³ (Table 1). In addition, a more recent cytogenetic study comparing *P. tertius* of different Brazilian states (Bahia and Ceará) was performed and showed the absence of intraspecific chromosome variation.¹⁴ The present work seeks to characterize the karyotype evolution and the chromatin composition of the species of the genus *Psammolestes*, with the aim of contributing to the genetic and evolutionary knowledge of these potential vectors.

Five adult males of each species were used for cytogenetic analysis. The wild species considered herein were *P. tertius* (Castro Alves, Bahia, Brazil), *P. coreodes* (Corumbá, Mato Grosso do Sul, Brazil), and *P. arthuri* (Maracay, Aragua, Venezuela), all of them being from the field. The seminiferous tubules were torn apart, crushed, and fixed on slides in liquid nitrogen. The cytogenetic techniques Lacto-Aceto Orcein^{15,16} and C-banding¹⁷ were applied for the description of karyotype, characterization of meiosis, and description of heterochromatin pattern, respectively. Then the cytogenomic technique of CMA₃/DAPI banding was applied according to

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TABLE 1
Cytogenetic characteristics of species of the genus *Psammolestes*

Species	Karyotype	Meiosis	C-banding			CMA ₃ /DAPI		
		Chromocenter	A	X	Y	A	X	Y
<i>Psammolestes arthuri</i>	$2n = 22 (20A + XY)$	XY	–	–	+	–	CMA ⁺	DAPI [–]
<i>Psammolestes coreodes</i>	$2n = 22 (20A + XY)^{11}$	XY	–	–	+	–	CMA ⁺	DAPI [–]
<i>Psammolestes tertius</i>	$2n = 22 (20A + XY)^{12,14}$	XY ¹³	–	–	+ ^{13,14}	–	CMA ⁺	DAPI [–]

FIGURE 1. Cytogenetic analyses of *Psammolestes* spp. Note the X and Y sex chromosomes forming the chromocenter of *Psammolestes tertius* (A, arrow), *Psammolestes coreodes* (B, arrow), and *Psammolestes arthuri* (C, arrow). Note that the autosomes and X chromosome are euchromatic and Y is heterochromatic in *P. tertius* (D), *P. coreodes* (E), and *P. arthuri* (F). Note that the X chromosome is rich in CG (G–I) and the Y chromosome is rich in AT (J–L) and the karyotype is $2n = 22 (20A + XY)$ (M–O) for *P. tertius* (G, J, and M), *P. coreodes* (H, K, and N), and *P. arthuri* (I, L, and O). X: X sex chromosome, Y: Y sex chromosome. Scale bar: 10 μ m. This figure appears in color at www.ajtmh.org.

Schimid¹⁸ with the modifications provided by Severi-Aguiar et al.¹⁹ to differentiate the heterochromatin regions rich in AT and CG. The biological material was analyzed using a Jenaval light microscope (Zeiss, Jena, Germany) and Olympus BX-FLA fluorescence microscope.

All species of the genus *Psammolestes* presented the same chromosomal characteristics, namely, chromocenter formed only by sex chromosomes X and Y during the prophase (Figure 1A–C), euchromatic autosomes and sex chromosome X and heterochromatic sex chromosome Y (Figure 1D–F), sex chromosomes X rich in CG (Figure 1G–I) and Y rich in CG (Figure 1J–L), and karyotype $2n = 22$ (Figure 1M–O). These characteristics confirm the data already described in the literature (Table 1) and corroborate the monophyly of this genus.

The karyotype $2n = 22$ is present in all species of the tribe Rhodniini.^{20,21} This karyotype is the same number of chromosomes as the ancestor of Triatominae,¹⁷ which indicates that the genomic reorganization events that occurred during the evolution of the tribe Rhodniini did not lead to numerical alterations in the chromosomes, unlike what happened to the tribe Triatomini, which presents karyotypes ranging from $2n = 21$ to 25 .^{22,23}

The chromocenter formed only by sex chromosomes X and Y is also shared with the species of *Rhodnius*,^{13,24} which demonstrates that this meiotic behavior is present in all species of the tribe Rhodniini. In the tribe Triatomini, there are species with the pattern described for *Psammolestes* and species that present union of autosomes with sexual chromosomes in the formation of the chromocenter.²⁵ This meiotic behavior can be used as a taxonomic tool to group related species. For example, all species of the *Triatoma brasiliensis* complex present a chromocenter formed by the sex chromosomes plus a pair of autosomes (characteristics that make it possible to differentiate seven species in this complex from all other triatomine complexes).²⁶

With the exception of *Rhodnius colombiensis* Mejia, Galvão and Jurberg, 1999; *Rhodnius nasutus* Stål, 1859; *Rhodnius pallescens* Barber, 1932; *R. pictipes* Stål, 1872; and *R. taquarussuensis* Rosa et al. 2017,^{21,27} all *Rhodnius* species also have constitutive heterochromatin restricted to the sex chromosome Y, as well as observed in *Psammolestes* spp. The distribution pattern of constitutive heterochromatin is extremely important for the taxonomy of Triatominae, being one of the main tools used in the description of the last species of the tribe Rhodniini, namely, *R. taquarussuensis*.²¹ In addition, the three species of the genus *Psammolestes* showed the same DNA composition rich in AT and CG.

Heterochromatin loss/reorganization in AT and CG composition could have occurred during the speciation of *Psammolestes* spp, as observed for the species of the group *pallescens*.²⁸ However, considering that the ancestor of *P. tertius*, *P. coreodes*, and *P. arthuri* was similar to *R. robustus* (which does not present heterochromatin in the autosomes either, only in the sexual chromosome Y²⁷) and especially the degree of specialization of these species during their evolution (they inhabit only bird nests), it can be stated that the species maintained the genetic material without chromosomal changes.

The cytogenetic characteristics analyzed indicate chromosomal homogeneity in the genus *Psammolestes*, which corroborates the monophyletic feature of the genus and suggests that during the derivation of *P. tertius*, *P. coreodes*,

and *P. arthuri* from the common ancestor, there was no reorganization in the number or structure of chromosomes.

Received February 6, 2018. Accepted for publication April 7, 2018.

Published online October 15, 2018.

Acknowledgments: We appreciate Wilma Savini and Jose Manuel Ayala for their support in the Venezuela and Central Laboratory of Public Health and Professor Gonçalves Moniz (LACEN - BA) for field support in Bahia.

Financial support: The study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (process numbers 2013/19764-0 and 2015/11372-1) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil).

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