

Revisiting the Chromosomal Diversification of the Genus *Rhodnius* (Stål, 1859) (Hemiptera, Triatominae)

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Abstract. Although all triatomines are considered as potential vectors of the Chagas disease, the *Triatoma*, *Panstrongylus*, and *Rhodnius* genera are the most important from the epidemiological point of view. Based on cytogenetic analyzes carried out so far (C banding and FISH), the species of the genus *Rhodnius* show little interspecific chromosomal variation. Thus, we analyzed the distribution of AT- and CG-rich DNA in the chromatin and chromosomes of the genus *Rhodnius* and discuss the chromosome evolution of these vectors. Except for *Rhodnius domesticus*, *Rhodnius nasutus*, *Rhodnius pictipes*, *Rhodnius colombiensis*, and *Rhodnius pallescens*, all *Rhodnius* species have euchromatic autosomes with the absence of AT- and CG-rich blocks. Curiously, the same species that have heterochromatin blocks in the autosomes also have chromomycin A3 (CMA₃) blocks dispersed in the prophasic nucleus (demonstrating that the heterochromatin of these species is rich in CG). Thus, we characterize the AT- and CG-rich DNA pattern for the genus *Rhodnius*, and we suggest that the pattern of CG-rich heterochromatin in the autosomes of these vectors evolved independently in *pallescens*, *pictipes*, and *prolixus* groups.

Triatomines (Hemiptera, Triatominae) are hematophagous insects of great epidemiological importance because they are vectors of the protozoan *Trypanosoma cruzi* (Chagas, 1909) (Kinetoplastida, Trypanosomatidae), the etiological agent of Chagas disease—a neglected disease that affects about eight million people, resulting in approximately 10,000 deaths per year from clinical manifestations (such as terminal heart failure, thromboembolic complications, refractory ventricular arrhythmias, and sudden death).^{1,2} Although all of triatomine species, of both sexes, are considered as potential vectors of Chagas disease, the *Triatoma* (Laporte, 1832), *Panstrongylus* (Berg, 1879), and *Rhodnius* (Stål, 1859) genera are the most important from the epidemiological point of view.³

The genus *Rhodnius* is a paraphyletic group composed of 23 species grouped into three large groups (*pallescens*, *pictipes*, and *prolixus*) (Table 1). The genus paraphilia is related to the fact that the species of the *prolixus* group present greater phylogenetic proximity with the *Psammolestes* (Bergroth, 1911 genus) than with other groups of *Rhodnius* spp.^{4,5} In an attempt to solve this issue, Hypsa et al.⁶ suggested changing the generic status from *Psammolestes* to *Rhodnius*. However, because of morphological and habitat divergences, the genus *Psammolestes* was maintained by the scientific community.⁷

The species of the genus *Rhodnius* show little interspecific chromosomal variation because all species analyzed so far have karyotype $2n = 22$ (20A + XY) (Table 1) and 45S rDNA clusters restricted to sex chromosomes.⁸ Besides, most species have heterochromatin restricted to the Y sex chromosome (Table 1). However, the knowledge of the genomic composition of heterochromatin base pairs (AT and CG) is limited to *Rhodnius pallescens* (Barber, 1932),⁹ and *Rhodnius prolixus* (Stål, 1859),¹⁰ highlighting the necessity for further studies on the molecular cytogenetics of these vectors. Based on the data presented

earlier, we analyzed the distribution of AT- and CG-rich DNA in the chromatin and chromosomes of the genus *Rhodnius*, and discuss the chromosome evolution of these vectors.

At least five adult males from each species (10 *R. prolixus*, 10 *Rhodnius robustus* (Larrousse, 1927), 10 *Rhodnius neglectus* (Lent, 1954), five *Rhodnius nasutus* (Stål, 1859), five *Rhodnius domesticus* (Neiva and Pinto, 1923), 10 *Rhodnius montenegrensis* (Rosa et al. [2012]), and five *Rhodnius marabaensis* (Souza et al. [2016])) were used. They had been assigned by the Triatominae Insectarium within the Department of Biological Sciences, in the College of Pharmaceutical Sciences, at Sao Paulo State University's Araraquara campus (FCFAR/UNESP), São Paulo, Brazil. The seminiferous tubules of the specimens were squashed and fixed to a coverslip. Then, they underwent the cytogenetic technique of CMA₃/4',6-diamidino-2-phenylindole (DAPI)⁹ and C banding.¹¹ The biological material was analyzed using a Jenaval light microscope (Zeiss) attached to a digital camera, with the AxioVision LE 4.8 image analyzer (Copyright 2006–2009 Carl Zeiss Imaging Solutions GmbH), and using a fluorescence microscope Zeiss-Axioskop and Olympus BX-FLA.

All species had the same number of chromosomes, $2n = 22$ (20A + XY) (Table 1), confirming the hypothesis that all species of the Rhodniini tribe have 22 chromosomes.²³ Taking into account that the ancestral karyotype is $2n = 22$,^{23,24} Alevi et al.²³ highlight that during the diversification of the *pallescens*, *pictipes*, and *prolixus* groups, there were no evolutionary events (as agmatoploidy and simplploidy) that resulted in changes in the number of chromosomes.

Except for *R. domesticus*, *R. nasutus*, *Rhodnius pictipes* (Stål, 1872), *Rhodnius colombiensis* (Mejia, Galvão and Jurberg, 1999), and *R. pallescens*, all *Rhodnius* species have euchromatic autosomes with the absence of AT- and CG-rich blocks (Figure 1A and B) (Table 1). Curiously, the same species that have heterochromatin blocks in the autosomes also have CMA₃ blocks dispersed in the prophasic nucleus (Figure 1D) (Table 1), emphasizing that the heterochromatin present in the chromosomes of *R. domesticus*, *R. nasutus*, *R. pictipes*, *R. colombiensis*, and *R. pallescens* is rich in CG regions.

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TABLE 1
Cytogenetic characteristics of *Rhodnius* spp.

<i>Rhodnius</i> genus	Karyotype	C-banding			CMA ₃ /DAPI		
		A	X	Y	A	X	Y
<i>prolixus</i> group							
	<i>Rhodnius barretti</i>	–	–	–	–	–	–
	<i>Rhodnius dalessandroi</i>	–	–	–	–	–	–
	<i>Rhodnius domesticus</i>	2n = 22 (20A + XY)*	Yes†	No†	Yes†	CMA ₃ ⁺	CMA ₃ ⁺ DAPI ⁺
	<i>Rhodnius milesi</i>	2n = 22 (20A + XY)‡	No	No	Yes	–	CMA ₃ ⁺ DAPI ⁺
	<i>Rhodnius marabaensis</i>	2n = 22 (20A + XY)	No	No	Yes	–	CMA ₃ ⁺ DAPI ⁺
	<i>Rhodnius montenegrensis</i>	2n = 22 (20A + XY)†	No	No	Yes	–	CMA ₃ ⁺ DAPI ⁺
	<i>Rhodnius nasutus</i>	2n = 22 (20A + XY)§	Yes	No	Yes	CMA ₃ ⁺	CMA ₃ ⁺ DAPI ⁺
	<i>Rhodnius neglectus</i>	2n = 22 (20A + XY)¶	No¶	No¶	Yes¶	–	CMA ₃ ⁺ DAPI ⁺
	<i>Rhodnius neivai</i>	2n = 22 (20A + XY)#	No	No	Yes	–	CMA ₃ ⁺ DAPI ⁺
	<i>Rhodnius prolixus</i>	2n = 22 (20A + XY)**	No	No	Yes	–	CMA ₃ ⁺ DAPI ⁺
	<i>Rhodnius robustus</i>	2n = 22 (20A + XY)††	No	No	Yes	–	CMA ₃ ⁺ DAPI ⁺
<i>pictipes</i> group							
	<i>Rhodnius amazonicus</i>	–	–	–	–	–	–
	<i>Rhodnius brethesi</i>	2n = 22 (20A + XY)	No	No	Yes	–	CMA ₃ ⁺ DAPI ⁺
	<i>Rhodnius paraensis</i>	–	–	–	–	–	–
	<i>Rhodnius pictipes</i>	2n = 22 (20A + XY)††	Yes§	No§	Yes§	CMA ₃ ⁺	CMA ₃ ⁺ DAPI ⁺
	<i>Rhodnius stali</i>	2n = 22 (20A + XY)*	No	No	Yes	–	CMA ₃ ⁺ DAPI ⁺
	<i>Rhodnius zeledoni</i>	–	–	–	–	–	–
<i>pallescens</i> group							
	<i>Rhodnius colombiensis</i>	2n = 22 (20A + XY)*	Yes‡‡	No‡‡	Yes‡‡	CMA ₃ ⁺	CMA ₃ ⁺ DAPI ⁺
	<i>Rhodnius ecuadoriensis</i>	2n = 22 (20A + XY)	No	No	Yes	–	CMA ₃ ⁺ DAPI ⁺
	<i>Rhodnius pallescens</i>	2n = 22 (20A + XY)§§	Yes	No	Yes	CMA ₃ ⁺	CMA ₃ ⁺ DAPI ⁺

A: autosome, X: X sex chromosome, Y: Y sex chromosome.

* Dujardin et al.¹²

† Alevi et al.¹³

‡ Panzera et al.¹⁴

§ Pérez et al.¹⁵

|| Panzera et al.¹⁶

¶ Barth.¹⁷

Koshy.¹⁸

** Schreiber and Pellegrino.¹⁹

†† Koshy.²⁰

‡‡ Panzera et al.²¹

§§ Panzera et al.²²

Besides, all species had the euchromatic X sex chromosome and CMA₃⁺ (Figure 1B and D), and the heterochromatic Y chromosome and DAPI⁺ (Figure 1A and C) (Table 1).

Taking into account that *Rhodnius* species whose autosomes present heterochromatic blocks and CMA₃⁺ belong to different groups (*prolixus* group: *R. domesticus* and *R. nasutus*; *pictipes* group: *R. pictipes*; and *pallescens* group: *R. colombiensis* and *R. pallescens*), we suggest that the gain/loss of CG-rich heterochromatin was an event that took place independently in the three groups of *Rhodnius* and can be related to different evolutionary factors. Alevi et al.²⁵ for example, suggest that the different patterns of heterochromatin losses observed in the *pallescens* group can be associated with adaptation to different environments occupied by species.

The divergences in the composition of the autosomes can also be used as taxonomic tools to differentiate some species of *Rhodnius* because events of phenotypic plasticity and cryptic speciation have been reported for this genus of medical importance.²⁶ Within the *prolixus* group, for example, *R. domesticus* and *R. nasutus* can be distinguished from all other species with euchromatic autosomes; within the *pictipes* group, *R. pictipes* can be distinguished from all other species by cytotaxonomy; besides, *R. colombiensis* and *R. pallescens* can be differentiated from *R. ecuadoriensis* (Lent and León, 1958) (euchromatic autosomes). Still, *R. colombiensis* and *R. pallescens* can also be differentiated from each other by the amount of heterochromatin in the autosomes.²⁵

Recently, the heterochromatin pattern and the composition of AT and CG was one of the tools used to describe

R. taquarussuensis Rosa et al.²⁷ However, molecular analyzes and experimental crosses showed that *R. taquarussuensis* was *R. neglectus* with chromosomal polymorphisms.²⁸ Based on this, it is evident that although cytogenetic analyses are of great taxonomic importance,^{29,30} the confirmation that different cytotypes represent valid taxa must be performed through integrative taxonomy.³¹

Thus, we characterize the AT- and CG-rich DNA pattern for the genus *Rhodnius*, and we suggest that the pattern of CG-rich heterochromatin in the autosomes of these vectors evolved independently in *pallescens*, *pictipes*, and *prolixus* groups.

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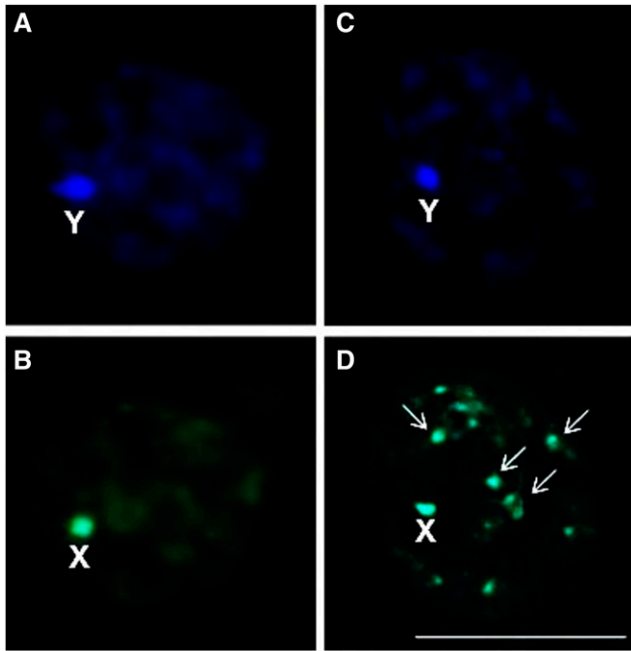


FIGURE 1. Composition of the heterochromatin base pairs in pro-phases of *Rhodnius marabaensis* (A and B) and *Rhodnius domesticus* (C and D). (A and C) Y sex chromosome rich in AT. (B) X sex chromosome rich in CG. (D) X sex chromosome and autosomes rich in GC. Bar: 10 μ m. This figure appears in color at www.ajtmh.org.

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