



Chromosomal evolution in the pallescens group (Hemiptera, Triatominae)

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ABSTRACT. *Rhodnius colombiensis*, in conjunction with *R. pallescens* and *R. ecuadoriensis*, forms the monophyletic pallescens group. Cytogenetic analyses of these closely related species would further our understanding of the taxonomy and evolution of this group. In this study, *R. colombiensis* was cytogenetically analyzed, and the results were compared with cytogenetic data from other species of the pallescens group, particularly their chromosomal evolution. We found that this triatomine has heteropycnotic blocks in five autosomal bivalents at both metaphase I and II. The derivation of *R. colombiensis* from *R. pallescens* led to significant loss of heteropycnotic and heterochromatic regions (approximately 50%). *R. ecuadoriensis* is the most differentiated of the group because it has lost all heterochromatin and heteropycnotic blocks in the autosomes. Based on the heteropycnotic and heterochromatic pattern of *R. colombiensis* and the chromosomal evolution analysis of the pallescens group, we

suggest that the karyotype of *R. colombiensis* and *R. ecuadoriensis* lost its heteropycnotic and heterochromatic blocks during speciation. Furthermore, this loss could be related to adaptation to different environments.

Key words: *Rhodnius colombiensis*; Tribe Rhodniini; Cytogenetic

INTRODUCTION

Triatomines are insects that are 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 distributed in 18 genera and 6 tribes (Abad-Franch et al., 2013; Alevi et al., 2013; Jurberg et al., 2013; Poinar Jr, 2013).

The tribe Rhodniini consists of 22 species, 19 of the genus *Rhodnius* and 3 of the genus *Psammolestes* (Abad-Franch et al., 2013; Alevi et al., 2013). Hemipterans of the genus *Rhodnius* are divided into two lineages, Pictipes and Robustus (Table 1), and three species groups, namely, *pallescens*, *prolixus*, and *pictipes* (Abad-Franch et al., 2009).

Rhodnius colombiensis, in conjunction with *R. pallescens* and *R. ecuadoriensis*, form a monophyletic group known as pallescens (Mejia et al., 1999; Schofield and Dujardin, 1999; Abad-Franch et al., 2009; Díaz et al., 2014). Abad-Franch and Monteiro (2007) stated that the cytogenetic analysis of these closely related species would further our understanding of the taxonomy and evolution of this group.

Therefore, specimens of *R. colombiensis* were studied and the results were compared with cytogenetic data from the other species of the pallescens group, particularly their chromosomal evolution.

Table 1. Division of the genus *Rhodnius* into two lineages.

Lineages of the genus <i>Rhodnius</i>	
Robustus lineage	Pictipes lineage
<i>R. barretti</i>	<i>R. amazonicus</i>
<i>R. dalessandroi</i>	<i>R. brethesi</i>
<i>R. domesticus</i>	<i>R. colombiensis</i>
<i>R. milesi</i>	<i>R. ecuadoriensis</i>
<i>R. montenegrensis</i>	<i>R. pallescens</i>
<i>R. nasutus</i>	<i>R. paraensis</i>
<i>R. neivai</i>	<i>R. pictipes</i>
<i>R. neglectus</i>	<i>R. stali</i>
<i>R. prolixus</i>	<i>R. zeledoni</i>
<i>R. robustus</i>	

MATERIAL AND METHODS

We used five *R. colombiensis* males obtained from the Triatominae Insectarium, Department of Biological Sciences, Faculty of Pharmaceutical Sciences, Araraquara campus, Universidade Estadual Paulista, Brazil. The seminiferous tubules of adult males, after being removed and fixed onto a cover slip, were processed for cytogenetic analysis using the lacto-acetic orcein technique (De Vaio et al., 1985, with modifications described by Alevi et al., 2012). The biological material was analyzed using a Jenaval light microscope (Zeiss) coupled to a digital camera and an image analyzer (Axio Vision LE 4.8, Zeiss). The images were magnified by a factor of 1000.

RESULTS

R. colombiensis contains heteropyknotic blocks dispersed in the nucleus of the initial prophase (Figure 1A), as well as at one or both ends of four or five autosomes in metaphase I (Figure 1B) and II (Figure 1C). This is presented in an ideogram (Figure 2B), which can be compared with the arrangement of heteropyknotic/heterochromatic blocks in *R. palleescens* (Figure 2A) and *R. ecuadoriensis* (Figure 2C).

The results were then compared with those obtained by classical and molecular cytogenetic analyses performed in the palleescens group (Panzera et al., 1998, 2012; Dujardin et al., 2002; Morielle-Souza and Azeredo-Oliveira, 2007; Gómez-Palacio et al., 2008; Pita et al., 2013) (Table 2).

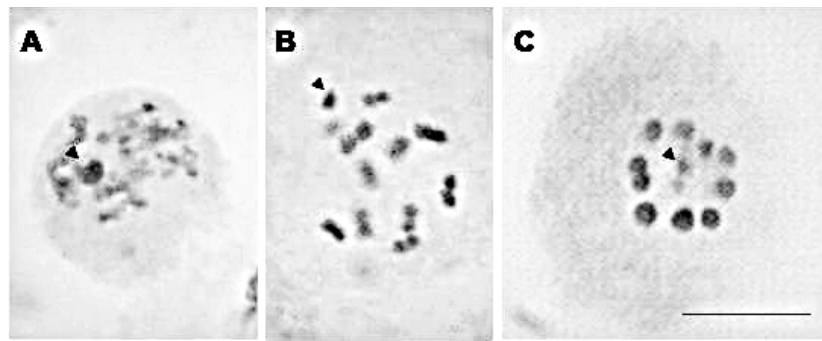


Figure 1. Semiferous tubule of *Rhodnius colombiensis* stained by lacto-acetic orcein. **A.** Prophase I. Note the chromocenter (arrowed) and the heteropyknotic blocks in chromatin. **B.** Metaphase I. Note the heteropyknotic blocks in four or five autosomes and in the Y sex chromosome (arrowed). **C.** Metaphase II. Note the heteropyknotic blocks in four or five autosomes and in the Y sex chromosome (arrowed). Bar = 10 μ m.

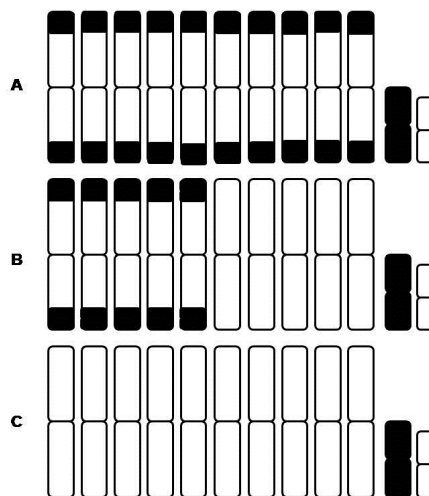


Figure 2. Ideogram of the male meiotic karyotype of *Rhodnius palleescens*, *R. colombiensis*, and *R. ecuadoriensis*, showing the distribution and evolution of the heteropyknotic and C-positive heterochromatin blocks in one or both ends of the autosomes. **A.** *R. palleescens*. Note that all of the autosomes have heteropyknotic and C-positive heterochromatin. **B.** *R. colombiensis*. There has been a significant loss of heteropyknotic and C-positive heterochromatin regions (approximately 50%). **C.** *R. ecuadoriensis*. Note that none of the autosomes have heterochromatin or heteropyknotic blocks.

Table 2. Cytogenetic characteristics of pallescens group.

Techniques and cytogenetic characteristics	<i>R. colombiensis</i>	<i>R. ectadforiensis</i>	<i>R. pallescens</i>
LACTO-ACETIC ORCEIN			
Karyotype (2n)	20A + XY	20A + XY	20A + XY
Heteropyknotic pattern in prophase	One chromocenter and heteropyknotic blocks in chromatin	One heteropyknotic chromocenter	One chromocenter and heteropyknotic blocks in chromatin
Heteropyknotic pattern in chromosomes	Heteropyknotic blocks in 5 autosomal bivalents	Absent	Heteropyknotic blocks in all autosomal bivalents
C-BANDING			
Heterochromatinic pattern in prophase	One C-positive chromocenter and C-positive heterochromatinic blocks in chromatin	One C-positive chromocenter	One chromocenter and C-positive heterochromatinic blocks in chromatin
Heterochromatinic pattern	C-positive heterochromatinic blocks in 5 autosomal bivalents	Without C-positive blocks in autosomal bivalents	C-positive heterochromatinic blocks in 1 or both ends of almost all autosomes
FISH	X chromosome	X and Y or small signal in Y	X and Y or X chromosome

DISCUSSION

The divergence of *R. colombiensis* from *R. pallescens* is associated with the emergence of the Panama Isthmus (Díaz et al., 2014), and the time since the divergence of *R. ecuadoriensis* from its Colombian relatives (*R. pallescens* and *R. colombiensis*) roughly coincides with the uplift of the Andes in the Pliocene (Abad-Franch and Monteiro, 2007).

Pre-zygotic (infeasibility of the reproductive organs) and post-zygotic (errors in meiotic pairing) barriers have been observed in an experimental hybrid cross between the sister species *R. colombiensis* and *R. pallescens* (Díaz et al., 2014). We suggest that the divergence of *R. colombiensis* from *R. pallescens* led to a significant loss of heteropyknotic and heterochromatic regions in the former karyotype (approximately 50%). *R. ecuadoriensis* is the most cytogenetically differentiated species within the group, because it has lost all of the heterochromatin and heteropyknotic blocks in the autosomes.

Panzer et al. (2004) suggested that the loss of heterochromatin is related to adaptive genomic changes that contribute to the capacity to survive, reproduce, and disperse in different environments. Abad-Franch and Monteiro (2007) proposed that the current distribution of *Rhodnius* is related to the effects of adaptive radiation and vicariance. Schreiber and Pellegrino (1950) suggested that heteropyknotic pattern differences in the autosomes might be related to triatomine speciation. We are of the opinion that during speciation in the *pallescens* group the loss of heteropyknotic blocks and constitutive heterochromatin in the autosomes was related to adaptation to different environments.

By conducting cytogenetic and molecular analyses, Gómez-Palacio et al. (2008, 2012) detected polymorphisms in *R. pallescens*. Morielle-Souza and Azeredo-Oliveira (2007) and Pita et al. (2013) analyzed *R. pallescens* at different locations using the *in situ* hybridization (FISH) technique, which probed 45S and obtained variable results (Table 1). Pita et al. (2013) detected intraspecific variation in the location of 45S ribosomal DNA clusters in *R. ecuadoriensis* from Ecuador and Peru (Table 1). The great polymorphism detected for *R. pallescens* confirms the possible origin of the *pallescens* group from this species.

In conclusion, our results suggest that *R. colombiensis* and *R. ecuadoriensis* have lost heteropyknotic blocks and C-positive heterochromatin during speciation. Furthermore, this loss could be related to adaptation to different environments.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Abad-Franch F and Monteiro FA (2007). Biogeography and evolution of Amazonian triatomines (Heteroptera: Reduviidae): implications for Chagas disease surveillance in humid forest ecoregions. *Mem. Inst. Oswaldo Cruz* 102: 57-70.

- Abad-Franch F, Monteiro FA, Jaramillo ON, Gurgel-Gonçalves R, et al. (2009). Ecology, evolution, and the long-term surveillance of vector-borne Chagas disease: a multi-scale appraisal of the tribe Rhodniini (Triatominae). *Acta Trop.* 110: 159-177.
- Abad-Franch F, Pavan MG, Jaramillo N, Palomeque FS, et al. (2013). *Rhodnius barretti*, a new species of Triatominae (Hemiptera: Reduviidae) from western Amazonia. *Mem. Inst. Oswaldo Cruz* 108: 92-99.
- Alevi KCC, Mendonça PP, Pereira NP, Rosa JA, et al. (2012). Karyotype of *Triatoma melanocephala* Neiva and Pinto (1923). Does this species fit in the Brasiliensis subcomplex? *Infect. Genet. Evol.* 12: 1652-1653.
- Alevi KCC, Rosa JÁ and Azeredo-Oliveira MTV (2013). Mini review: Karyotypic survey in Triatominae subfamily (Hemiptera, Heteroptera). *Entomol. Ornithol. Herpetol.* 2: 106. Doi: 10.4172/2161-0983.1000106.
- De Vaio ES, Grucci B, Castagnino AM, Franca ME, et al. (1985). Meiotic differences between three triatomine species (Hemiptera: Reduviidae). *Genetica* 67: 185-191.
- Díaz S, Panzera F, Jaramillo-Ocampo N, Pérez R, et al. (2014). Genetic, cytogenetic and morphological trends in the evolution of the *Rhodnius* (Triatominae: Rhodniini) trans-Andean group. *PLoS One* 9: e87493. Doi:10.1371/journal.pone.0087493.
- Dujardin JP, Schofield CJ and Panzera F (2002). Los vectores de la enfermedad de Chagas. *Académie Royale des Sciences D'Outre-Mer, Bruxelles*, 189.
- Gómez-Palacio A, Jaramillo-Ocampo N, Caro-Riaño H, Díaz S, et al. (2012). Morphometric and molecular evidence of intraspecific biogeographical differentiation of *Rhodnius pallescens* (Hemiptera: Reduviidae: Rhodniini) from Colombia and Panama. *Infect. Genet. Evol.* 12: 1975-1983.
- Gómez-Palacio A, Jaramillo-Ocampo N, Triana-Chávez O, Saldaña A, et al. (2008). Chromosome variability in the Chagas disease vector *Rhodnius pallescens* (Hemiptera, Reduviidae, Rhodniini). *Mem. Inst. Oswaldo Cruz* 103: 160-164.
- Jurberg J, Cunha V, Cailleaux S, Raigorodski R, et al. (2013). *Triatoma pintodiasi* sp. nov. do subcomplexo *T. rubrobaria* (Hemiptera, Reduviidae, Triatominae). *Rev. Pan-Amaz. Saúde* 4: 43-56.
- Lent H and Wygodzinsky P (1979). Revision of the Triatominae (Hemiptera: Reduviidae) and their significance as vector of Chagas disease. *Bull. Am. Mus. Nat. Hist.* 163: 123-520.
- Mejia JM, Galvão C and Jurberg J (1999). *Rhodnius colombiensis* sp. n. da Colômbia, com quadros comparativos entre estruturas fáticas do gênero *Rhodnius* Stal, 1859 (Hemiptera, Reduviidae, Triatominae). *Entomol. Vect.* 6: 601-617.
- Morielle-Souza A and Azeredo-Oliveira MTV (2007). Differential characterization of holocentric chromosomes in triatomines (Heteroptera, Triatominae) using different staining techniques and fluorescent *in situ* hybridization. *Genet. Mol. Res.* 6: 713-720.
- Panzera F, Dujardin JP, Nicolini P, Caraccio MN, et al. (2004). Genomic changes of Chagas disease vector, South America. *Emerg. Infect. Dis.* 10: 438-446.
- Panzera Y, Pita S, Ferreira MJ, Ferrandis I, et al. (2012). High dynamics of rDNA cluster location in kissing bug holocentric chromosomes (Triatominae, Heteroptera). *Cytogenet. Genome Res.* 138: 56-67.
- Panzera F, Scvortzoff E, Pérez R, Panzera Y, et al. (1998). Cytogenetics of Triatomines. In: Atlas of Chagas disease vectors in the Americas (Carcavallo RU, Galíndez-Girón I, Jurberg J, Lent H, eds.). Editora Fiocruz, Rio de Janeiro, 621-664.
- Pita S, Panzera F, Ferrandis I, Galvão C, et al. (2013). Chromosomal divergence and evolutionary inferences in Rhodniini based on the chromosomal location of ribosomal genes. *Mem. Inst. Oswaldo Cruz* 108: 376-382.
- Poinar Jr G (2013). *Panstrongylus hispaniolae* sp. n. (Hemiptera: Reduviidae: Triatominae), a new fossil triatomine in Dominican amber, with evidence of gut flagellates. *Palaeodiversity* 6: 1-8.
- Schofield CJ and Dujardin JP (1999). Theories on the evolution of *Rhodnius*. *Actual. Biol.* 21: 183-197.
- Schreiber G and Pellegrino J (1950). Eteropicnosi di autosomi come possibile meccanismo di speciazione. *Sci. Genet.* 3: 215-226.