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Short communication

Molecular characterization of *Rhodnius robustus* specimens, potential vectors for Chagas disease in French Guiana, South America

Christian Barnabé[a,*], Simone Frédérique Brenière[a,b], Jean-François Guégan[c], Denis Blanchet[d], Christine Aznar[d]

[a] INTERTRYP, CIRAD, IRD, TA A-17/G, International Campus in Baillarguet, Montpellier, France
[b] Centro de Investigación para la Salud en América Latina (CISeAL), Pontificia Universidad Católica del Ecuador, Av. 12 de Octubre 1076 y Roca, Campus Nayon, Quito, Ecuador
[c] UMR MIVEGEC IRD-CNRS-Université de Montpellier, centre IRD de Montpellier, 34394 Montpellier cedex 5, France
[d] Université des Antilles et de la Guyane, Campus Saint Denis, 97300 Cayenne, French Guiana

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ABSTRACT

Insects of the genus *Rhodnius* are broadly involved in Chagas disease transmission. In French Guiana, where the disease remains a public health problem, *R. robustus* and *R. pictipes* are vectors, but so far few genetic analyses of these local species have been reported. Here, we explored three mitochondrial genes (*Cytb*, *Lsu-rRNA*, and *ND1*) and one nuclear gene (*D2*) in 49 adult specimens morphologically characterized as *R. robustus*. We analyzed genetic polymorphisms and haplotype distributions, and we built phylogenetic trees using the available GenBank sequences from *R. robustus* and related species. The molecular taxonomy analysis confirmed that 35 insects, closely related to Brazilian species and separated by a few mutations, truly belong to *R. robustus*; two others were attributed to the *R. prolixus* complex and for 12 no sequence was obtained. The geographical haplotype distribution indicates a likely geographical structuring and evidenced true differentiation between the two main urban centers, Cayenne and Saint-Laurent-du-Maroni.

1. Introduction

The genus *Rhodnius* (Larrousse, 1927), mostly associated with palm trees, is widely involved in transmission of Chagas disease in the Amazonian regions (Pavan et al., 2013; Pavan and Monteiro, 2007), including French Guiana (Abad-Franch et al., 2009). It is divided into three species complexes (Dujardin et al., 1999): (i) *R. pallescens* complex (*R. pallescens* and *R. ecuadoriensis*), (ii) *R. pictipes* complex (*R. pictipes*, *R. stali* and *R. brethesi*) and (iii) *R. prolixus* complex (*R. prolixus*, *R. robustus*, *R. neglectus*, *R. nasutus*, *R. domesticus*, and *R. neivai*). Within this last complex, *R. robustus* and *R. prolixus* are the most closely related species, often morphologically indistinguishable (Monteiro et al., 2003). Eleven species belonging to *Rhodnius*, *Panstrongylus*, *Triatoma*, and *Eratyrus* genera coexist in French Guiana (Berenger et al., 2009). Twenty specimens of *R. robustus* have been reported while previous reports of *R. prolixus* are considered doubtful and mislabeled as *R. robustus* (Berenger et al., 2009; Péneau, 2014). Previous analysis of one mitochondrial gene (*Cytb*) proposed that *R. prolixus* is monophyletic, while *R. robustus* appears paraphyletic and divided into four groups (Monteiro et al., 2003). Until now, only one specimen from Cayenne has been assigned to *R. robustus* group IV using this gene (Monteiro et al., 2003; Pavan et al., 2013; Pavan and Monteiro, 2007). In this context, we have undertaken molecular characterization of 49 putative *R. robustus* specimens from French Guiana previously identified by morphology (Lent and Wygodzinsky, 1979).

2. Methods

The *R. robustus* specimens were collected between 2004 and 2013 and preserved in alcohol at the University of French Guiana School of Medicine in Cayenne. Eleven specimens were collected in the forest (inland forest) during light trap sessions; all others were collected by inhabitants inside or near houses in two littoral areas: Cayenne ($n = 17$) and Saint-Laurent-du-Maroni ($n = 21$) (Fig. 1). DNA was extracted from legs using a cetyltrimethylammonium bromide procedure (Brenière et al., 2013). Three mitochondrial gene fragments and one nuclear gene were examined. The primers were for *Cytb* according to (Monteiro et al., 2003), *Lsu-rRNA* according to (Lyman et al., 1999), and *ND1* and *D2* (variable domain of the 28S RNA gene) according to (Brenière et al., 2017). The PCR cycles conditions were slightly

* Corresponding author.
E-mail address: christian.barnabe@ird.fr (C. Barnabé).

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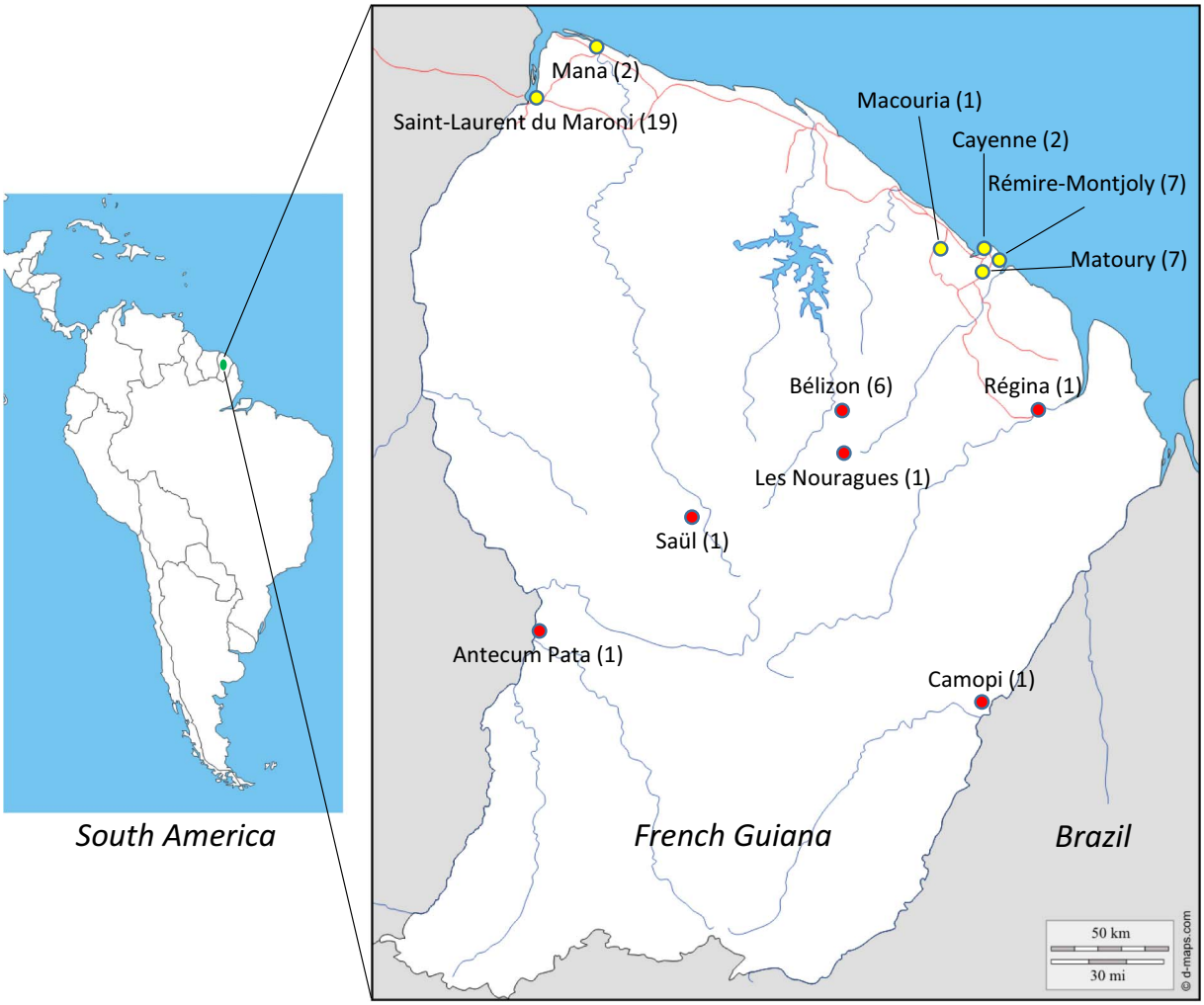

Fig. 1. Map of French Guiana indicating the collection areas of the 49 specimens of *Rhodnius robustus* sampled. Yellow dots represent collection from littoral areas (Littoral-Saint-Laurent-du-Maroni = Saint-Laurent-du-Maroni and Mana; Littoral-Cayenne = Cayenne, Macouria, Matoury, and Rémire-Montjoly); red dots, inland forest. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

different for the different genes: for *Cytb* and *Lsu-rRNA,* 94 °C for 5 min, 35 cycles (94 °C 30 s, 47 °C 30 s, 72 °C 60 s), and 72 °C for 7 min; for *ND1* the conditions were 94 °C for 2 min, 35 cycles (94 °C 30 s, 50 °C 30 s, 72 °C 60 s) and 72 °C for 4 min; for *D2* 94 °C for 5 min, 35 cycles (94 °C 60 s, 50 °C 120 s, 72 °C 120 s) and 72 °C for 7 min. Purification and double-stranded sequencing were performed at Eurofins Genomics (Ebersberg, Germany). Maximum-likelihood (ML) and maximum-parsimony (MP) trees were built using MEGA6 (Tamura et al., 2013), with the best substitution model for the ML trees (models with the lowest Bayesian information criterion are considered to describe the best substitution pattern) and a bootstrap procedure with 100 replications. Indices of DNA or nucleotide variability were calculated by DnaSP5 (Librado and Rozas, 2009). Fisher's exact tests were applied to haplotype distributions using the "*stats*" package of R (R Core Team, 2017).

3. Results and discussion

Out of the 49 *R. robustus* specimens, 30 sequences were obtained for *Cytb*, 29 for *Lsu-rRNA*, eight for *ND1*, and 25 for *D2* (see Tables S1 and S2). The corresponding GenBank accession numbers are MF966277-306 for *CytB*, MF966332–60 for *Lsu-rRNA*, MF966361-68 for *ND1*, and MF966307-31 for *D2*. For 12 specimens no sequence was obtained for any of the genes while for the other specimens, in most cases genes were sequenced in both directions but some were sequenced only in a single direction. The haplotype numbers were 7, 3, 3, and 2 for *Cytb, Lsu-rRNA, ND1*, and *D2*, respectively. Among the four gene fragments, *Hd* varied from 0.46 (*ND1*) to 0.75 (*Cytb*) and *Pi* varied from 0.0012 (*ND1*) to 0.0023 (*Cytb*) (see Table S2 for details). ML and MP trees were built with the current haplotypes and all available GenBank sequences of the *R. prolixus* complex species using those of the *R. pictipes* complex (*R. pictipes*, *R. stali*, and *R. brethesi*) as the outgroup. In the *Cytb* ML tree, as well as in the MP tree, the five French Guiana haplotypes that are closely related to each other were clustered (bootstrap value of 97% and 99%, respectively) with 18 other *R. robustus* haplotypes belonging to *R. robustus* types II, III, and IV, most of them from Brazil, and others from Bolivia and Ecuador (Fig. 2), without formally belonging to any of these types. There was, however, a tendency to cluster with type IV with which the genetic p-distances were the lowest. For the *Lsu-rRNA* tree, poor structuring of the species was observed within the *R. prolixus* complex, making it difficult to assign species to the three French Guiana haplotypes even if they were closely related to two *R. robustus* haplotypes (99% identity) – one from Beni, Bolivia (KT805173), the other from Ecuador (AF045705). Only four sequences of the *R. prolixus* complex were available in GenBank for the *ND1* gene, and the three French Guiana haplotypes were clustered (bootstrap value, 94%), close but separate from *R. prolixus* and *R. robustus* haplotypes. Similarly, few sequences of the *D2* gene were available from GenBank, and no significant cluster was identified within the *R. prolixus* complex, including the two French Guiana haplotypes. However, one of these two haplotypes was identical to a *R. robustus* from Brazil (AF435859), and both