Acta Tropica 120 (2011) 103–109

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Morphometric and molecular differentiation of a *Rhodnius robustus*-like form from *R. robustus* Larousse, 1927 and *R. prolixus* Stal, 1859 (Hemiptera, Reduviidae)

Edna Márquez[a,*], Nicolás Jaramillo-O[b], Andrés Gómez-Palacio[b], Jean-Pierre Dujardin[c]

[a] *Facultad de Ciencias, Universidad Nacional de Colombia, Medellín, Colombia*
[b] *Universidad de Antioquia, Medellín, Colombia*
[c] *MIVEGEC, IRD (France), presently at the Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand*

ARTICLE INFO

Article history:
Received 16 February 2011
Received in revised form 19 June 2011
Accepted 23 June 2011
Available online 6 July 2011

Keywords:
Geometric morphometrics
Rhodnius robustus
Rhodnius prolixus
Triatominae
Robustus group
Cytochrome b
mtDNA

ABSTRACT

In Triatominae, "robustus" group constitutes a cluster of species with great haplotypic divergences but high similarities at morphological and nuclear DNA levels. Given these similarities, species identification generates a frequently problematic issue. In northwestern Amazonia, *Rhodnius robustus* cohabit with an apparently new species, cryptic with *R. robustus* (Abad-Franch and Monteiro, 2005). In this region (municipality of Puerto Asís, Department of Putumayo, Colombia), we collected insects classified as *R. robustus* by traditional keys. We compared this sample with specimens of *R. robustus* from Venezuela, and of *R. prolixus* from Colombia and Venezuela. The comparisons used landmark-based geometric morphometrics, and analyses of mitochondrial cytochrome b gene and of D2 variable region of the 28S RNA. The shape of the wings from Puerto Asís specimens disclosed clear-cut divergence from the shape of the wings as found for *R. prolixus* specimens from Venezuela and Colombia, and diverged from the shape of *R. robustus* from Venezuela. Thus, morphometric analyses suggested that the Puerto Asís collection could represent a new taxon. Using *R. pallescens* as an outgroup, a tentative phylogenetic tree based on the geometry of the wing showed the *Rhodnius* from Puerto Asís more similar to the *R. prolixus* from Colombia than their congeners from Venezuela. In contrast, the molecular classification clustered Colombian *R. prolixus* and Venezuelan *R. robustus* with published GenBank sequences, but it gave the insects from Puerto Asís a basal position to the "*robustus*" group. This outcome suggests that the Puerto Asís haplotype could be the one found by Abad-Franch and Monteiro (2005). Thus, both morphometric and molecular markers used here, although differing in the phylogenetic classification of samples, could differentiate the Puerto Asís sample from the morphologically similar *R. prolixus* and *R. robustus*. This could represent a valuable help in the entomological surveillance related to the control of Chagas disease in the South of Colombia and North of Ecuador.

1. Introduction

The subfamily Triatominae (Hemiptera-Reduviidae) includes 141 species of hematophagous insects taxonomically arranged in five tribes and 15 genera (Schofield and Galvao, 2009; Jurberg et al., 2009). Most species are potential vectors of the protozoan parasite *Trypanosoma cruzi*, the causative agent of American trypanosomiasis, or Chagas disease. The '*robustus* group', a major lineage of the tribe Rhodniini (Abad-Franch and Monteiro, 2007), comprises members with principally cis-Andean (*prolixus*, *robustus*, *nasutus*, *neglectus*, *milesi*, *dalessandroi*, *domesticus*, and the *Psammolestes*) and only one trans-Andean species (*R. neivai*). *Rhodnius prolixus*, the main vector of Chagas disease in Colombia and Venezuela, is essentially a domestic species most of its range. However, this species has been found occupying the same sylvatic habitat with its closely related and morphologically similar species *R. robustus*, which has a minor epidemiological importance (Feliciangeli et al., 2007; Fitzpatrick et al., 2008). The frequently colonization of houses by sylvatic populations of *R. prolixus* and their confusion with *R. robustus*, difficult surveillance and control practices (Feliciangeli et al., 2007; Fitzpatrick et al., 2008).

R. robustus exhibits high morphological and nuclear DNA similarities with *R. prolixus* (Harry et al., 1992a,b; Harry, 1993; Barrett, 1996; Solano et al., 1996; Monteiro et al., 2000), but divergences in mitochondrial DNA and known ecological adaptations are used as an argument to considered them as different species

---

* Corresponding author at: Facultad de Ciencias, Universidad Nacional de Colombia, Calle 59A No 63–20 Bloque 19 A Laboratorio 310, Medellín, Colombia. Tel.: +57 4 4309072; fax: +57 4 4309116.
E-mail address: ejmarque@unal.edu.co (E. Márquez).

0001-706X/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2011.06.009

Table 1
List of samples submitted to morphometric analysis. (–) indicated absent data. Environmental mean temperature: 27 °C (Puerto Asís), 27.6 °C (Coyaima).

Species	Code	Collection site		Field/ Colony	Domestic/ Sylvatic	Date collected	Females	Males
		Country	State/Municipality					
R. robustus-like	roCOL	Colombia	Putumayo/Puerto Asís	F	S	1999	7	5
	rCc	Colombia	Putumayo/Puerto Asís	C	S	1999	31	39
	roVE	Venezuela	Trujillo/Pampanito	F	S	1997	5	7
R. prolixus	prCOL	Colombia	Tolima/Coyaima	F	D	1991	5	6
			Casanare/San Luis	F	D	1995	15	14
	pCc	Colombia	Tolima/Coyaima	C	D	1991	20	20
	prVE	Venezuela	Barinas/–	F	D	–	12	18
R.pallescens	paCOL	Colombia	Diferent sites	F	S	–	25	24

(Monteiro et al., 2003). Furthermore, *R. robustus* presents five moderately divergent allopatric groups of haplotypes (I–V) with different geographical location from Amazonian region to Colombian and Venezuelan Plains (Monteiro et al., 2003; Abad-Franch et al., 2009): Haplotype I is sympatric with *R. prolixus* in the Orinoco region (Colombian and Venezuelan Plains), haplotype II is in Amazonian region from Ecuador and Brazil, haplotypes III and IV are parapatric in Northeast of Brazil (Amazon forest region) and Southern French Guyana (Pavan and Monteiro, 2007). Finally, an apparently new species, morphologically indistinguishable to *R. robustus* was found in palm trees in the Ecuadorian Amazon (Abad-Franch and Monteiro, 2005). This outcome was based on genetic distance of Kimura 2-parameters substitution model among cytochrome b (mtCyt b) gene sequences of mitochondrial DNA and the basal position of that haplotype to the lineage "*robustus*" (*R. prolixus*, *R. neglectus*, *R. robustus* and *Psammolestes* sp.).

The genetic and biogeographic heterogeneity of specimens morphologically indistinguishable from *R. robustus*, may suggest differences in ecological and evolutionary dynamics among members of the "*robustus*" group, especially in their abilities to colonize human dwellings (Abad-Franch et al., 2009). In this context, the use of inexpensive identification tools, such as the one based on Geometric Morphometrics, is of epidemiological relevance. Since the goal is to discriminate *R. prolixus* for other species morphologically similar but with less epidemiological importance, the relevant question is to discriminate rather than to classify. However, this approach requires determine whether the morphometric variation found in *Rhodnius sp.* address taxonomical differences or whether this variation correspond to plastic responses to environmental changes. Metric changes according to habitat are known to occur indeed, such as those described in the transition from sylvatic to domestic or laboratory habitat (Dujardin et al., 1997, 1998, 1999a,b,c; Jaramillo et al., 2002; Feliciangeli et al., 2007; Rodríguez et al., 2007; Caro-Riaño et al., 2009; Dujardin et al., 2009). This issue is important because morphological variation patterns of sylvatic *R. prolixus* in Colombian (Guhl, 1999, 2003; Pinto et al., 2005; Angulo-Silva, 2000) and Venezuelan Plains (Gamboa, 1963; Zeledon and Rabinovich, 1981; Feliciangeli et al., 2007; Fitzpatrick et al., 2008) may be confounded with *R. robustus,* making difficult surveillance and control practices (Feliciangeli et al., 2007; Fitzpatrick et al., 2008).

Given the importance to get a fast and low cost tool in the entomological surveillance of the vectors of Chagas disease (Dujardin et al., 2007), especially in tracking possible house re-infestation by *R. prolixus* from palm trees (Feliciangeli et al., 2007), we verified in this work the relevance of the morphometric identification approach. This was possible thanks to the concomitant application on the same specimens of molecular techniques based on nucleotide sequences comparison of a fragment of the mitochondrial cytochrome b (mtCyt b) gene and of D2 variable region of the 28S RNA (D2).

2. Materials and methods

2.1. Insects

A total of 82 Colombian specimens of *Rhodnius* from municipality of Puerto Asís, department of Putumayo, and 40 *R. prolixus* from municipalities of Coyaima and San Luis, from the departments of Tolima and Casanare, respectively, were submitted to geometric morphometric analysis and compared with other specimens previously genotyped by Monteiro et al. (2003) that included 12 *R. robustus* haplotype I from Venezuela and 18 individuals of *R. prolixus* from the same country. In addition, 24 Colombian specimens of *R. pallescens* were used as outgroup for the phylogenetic study. Comparison among taxa was performed separately for specimens obtained from the field and laboratory (descendants from those field insects). Detailed information on the samples used in this study is given in Table 1. The morphological diagnostic based on traditional keys (Lent and Wygodzinsky, 1979), assigned the Puerto Asís sample to *R. robustus* (Laboratório Nacional e Internacional de Referência em Taxonomia de Triatomíneos, Departamento de Protozoologia, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brazil).

One Colombian specimen of *Rhodnius* from Puerto Asís and one of *R. prolixus* were sequenced and compared with GenBank data for *R. robustus*, *R. prolixus*, *R. neglectus and Psammnolestes coreodes.* For the phylogenetic classification tree, GenBank sequences of species of "pictipes lineage" (*R. pictipes, R. brethesi, R. ecuadoriensis, R. pallescens, R. colombiensis*) were used as an outgroup (Table 2).

2.2. Geometric morphometrics

Right wings of *R. pallescens* and of all specimens reared in laboratory (*Rhodnius* from Puerto Asís and *R. prolixus* from Coyaima) were dissected and mounted by standard techniques. These wings were photographed using a Nikon 990 digital camera fitted to a Nikon SMS 800 stereomicroscope. The Colombian specimens collected in the field, are part of the biological collections of BCEI Laboratory. For this reason, their wings were only photographed leaving the specimens intact without a dissection procedure. Both *R. prolixus* and *R. robustus* from Venezuela (Pampanito), were dissected, mounted in Hoyer, and photographed ten years ago by using a digital camera that was manually adapted to one ocular of the binocular microscope. On the other hand, the taxonomical status of these two species was confirmed by genotyping of mtCyt b and D2 genes by Monteiro et al. (2003).

Nine landmarks of type I (Bookstein, 1991), were identified on each wing on digital photographs (Fig. 1). The coordinates of these landmarks were digitized by using the software COO V. 39 (Dujardin, 2010). The isometric estimator known as centroid size (CS) was used for size comparisons. CS is defined as the square root of the sum of the squared distances between the center of the con-