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Description of *Rhodnius montenegrensis* n. sp. (Hemiptera: Reduviidae: Triatominae) from the state of Rondônia, Brazil

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Abstract

We present here a multisource approach that takes advantage of several disciplines to address a taxonomic issue. A triatomine related to *Rhodnius robustus* Larrousse, 1927 was recently found in the state of Rondônia, Brazil. The name *Rhodnius montenegrensis* **n. sp.** is suggested because it was found in the municipality of Monte Negro. The main differences between these two species can be detected in the female and male genitalia, but there are also noticeable differences in their eggs. Molecular analysis using PCR-RFLP technique and Bayesian inferences based on a fragment of the Cytochrome *b* (Cyt *b*) gene corroborated the morphological findings. We used this integrative approach to address the taxonomic decision for a new *Rhodnius* species and its relationship with others of this genus. Results obtained herein stress that morphology must be used as the major approach for obtaining phenotypic information, and molecular data should be taken as a complementary tool.

Key words: new species, *Rhodnius montenegrensis, Rhodnius robustus*

Introduction

Triatomines have epidemiological importance because they are responsible for the transmission of *Trypanosoma cruzi* (Chagas 1909) to humans, and also because they are a fundamental link for keeping the zoonotic cycle of such a protozoan among wild animals in peridomestic and domestic habitats.

There are 18 acknowledged genera of triatomines (Galvão *et al.* 2003; Forero *et al.* 2004). The genera *Panstrongylus*, *Rhodnius,* and *Triatoma* are those with the highest number of species and contain the most prevalent and relevant species regarding the transmission of *T. cruzi* to humans.

Among the described triatomines, the genus *Rhodnius* is the easiest to identify by external morphological examination; however, the specific separation involves several difficulties, as already noticed by Neiva and Pinto: “The genus *Rhodnius* is well characterized, which does not happen to the species that constitute it" (Neiva & Pinto 1923).

In 2007, samples of a *Rhodnius* were collected in 100 properties within the municipality of Monte Negro, state of Rondônia, including 75 males, 61 females, 53 fifth instars, 46 fourth instars, 70 third instars, 23 second instars, and 3 first instars. These specimens raised some doubts regarding their specific definition, but they were assumed

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to be *Rhodnius robustus* Larrouse, 1927 because of their geographical distribution. Then, on September 18th, 2008, another eight samples were collected in the same municipality, with the same morphological features. To clarify the doubts, we decided to keep a colony of these *Rhodnius*.

The description of this new *Rhodnius* species was made by comparing some structures with a phenotypically closely related species, *R. robustus*. The characters used were morphology of female genitalia, median process of the pygophore and phallus of the male genitalia, eggs, first urotergite, scutellum, morphometry, head, thorax, and abdomen. Molecular analyses, such as mitochondrial Cytochrome *b* (Cyt *b*) gene sequencing and PCR-RFLP techniques, were also carried out.

Material and methods

The specimens used for the morphological, morphometric, and molecular description were obtained from the colonies kept at the Triatominae Insectarium of the Department of Biological Sciences of the Faculty of Pharmaceutical Sciences, UNESP, Araraquara, São Paulo, Brazil. The colony named CTA 088 (Triatominae Colonies of Araraquara—originally abbreviated as CTA) consisted of the first eight *Rhodnius* sp. specimens collected on September 18th, 2008, from *babassus* (*Orbignya phalerata*) within a pasture area located at C – 35 line, coordinates S: 10° 10´ 05.1” and W: 63° 24’ 09.1,” in the municipality of Monte Negro, Rondônia, Brazil (Fig. 1). The morphological features of the species matched those previously observed in the 136 adult specimens from the same municipality (Fig. 2).

For the comparative study, specimens from four *R. robustus* colonies (CTA 083, 084, 085, and 086) were examined. The CTA 083 and 084 colonies originated from specimens brought from Peru, and have been kept since August 1973. The CTA 085 colony was also brought from Peru, and has been kept since February 1972. The CTA 086 colony came from Madre de Diós, Peru, and has been kept since August 1973 (Fig. 3). This municipality is located in the Amazon jungle bordering Brazil, which is an area known for the occurrence of this species (Lent and Wygodzinsky 1979). The comparisons between the new species of *Rhodnius* (males and females) and *R. robustus* were based on the characteristics of the latter found in the dichotomous key of Lent and Wygodzinsky 1979. The descriptions of *R. stali* Lent, Jurberg & Galvão, 1993; *R. colombiensis* Mejia, Galvão & Jurberg, 1999; *Rhodnius milesi* Valente *et al*., 2001; and *R. zeledoni* Jurberg, Rocha & Galvão, 2009 were also considered.

All morphological features analyzed herein for *R. robustus* from CTA colonies were compared and confirmed with samples of this same species which came from the National and International Laboratory of Reference in Triatominae Taxonomy from the Oswaldo Cruz Institute. These specimens had their taxonomy confirmed by using molecular and morphological approaches (Cleber Galvão, pers. comm.).

A Leica MZ APO stereoscopic microscope and the Motic Images Advanced software, version 3.2, were used for measurements and to study the male phallic structures and the process of the first urotergite.

Fifteen males and 15 females were measured together with 15 eggshells (Naegele *et al.* 2003; Rosa *et al.* 2003). The characters measured were: total length of the triatomines (TL), length of the head (HL), length of the antenna segments (A1, A2, A3, and A4) and rostrum segments (R1, R2, and R3), inner distance between eyes (IE), anteocular (AO) and post-ocular distances (PO), diameter of the eye (DE), maximum abdomen (MWA) and thorax widths (MWT), eggshell area, length, width, and diameter of the opercular aperture (Lent and Wigodzinsky 1979; Dujardin *et al.* 1999a; Rosa *et al.* 2000).

To obtain images of eggs, external female genitalia, scutellum, and first urotergite of 35 specimens of the new species of *Rhodnius* and 32 specimens of *R. robustus*, samples were examined by scanning electron microscopy under a Topcon SM-300 microscope (Barata 1981; Rosa *et al.* 1999; Quintero 2002; Obara *et al.* 2007; Rosa *et al.* 2010).

Geometric morphometric techniques were applied to wings to evaluate whether this morphotype exhibited any differences in shape compared to *R. robustus*, according to Campos *et al.* (2011) and Almeida *et al.* (2012). Seven anatomical landmarks (Gurgel-Gonçalves *et al.* 2008) (Fig. 4) were collected at intersections between venations and processed by the same researcher using modules available at the CLIC (Collection of Landmarks for Identification and Characterization, http://www.mpl.ird.fr/morphometrics/clic/index.html (Dujardin *et al*. 2010), and the COOWin software (Dujardin 2004), as described by Dujardin (2008). Right forewings of 18 females of *R. robustus* and 23 of new *Rhodnius* species were used to evaluate the shape variables. Analyses were computed as nonuniform (partial warps) and uniform components, which describe regional and global deformations of the wing architecture (Bookstein 1991). Prior to the generalized procrustes analysis, an isometric estimator of size variation (centroid-size) was calculated as the square root of the sum of the squared distances between the center of the configuration of landmarks and each individual landmark (Bookstein 1991). A factorial map was built to illustrate the variation, which resulted from the first and second principal components of the analysis, representing 95% of the shape.

The phylogenetic reconstruction of the new *Rhodnius* species was developed using primers according to Lyman *et al*. (1999). Cyt *b* gene fragments from insects of the CTA insectarium and the new *Rhodnius* species were aligned with some fragments downloaded from GenBank. The dendrogram was constructed by Bayesian analyses with the MrBayes software (Huelsenbeck & Ronquist 2001) using the alignment of a 369pb of the Cyt *b* gene. The support of the clades was estimated using a Markov chain Monte Carlo (MCMC) algorithm performed by the MrBayes software, version 3.1.2. The first analysis was run for 1,000,000 generations, with sampling of every 250 generations. Estimates of likelihood settings were calculated using MrModeltest (Nylander *et al.* 2004), and the Akaike information criterion (AIC) was chosen to obtain the best model to be applied to the gene fragment. For Cyt *b*, the general model of DNA substitution with gamma rate variation across sites (HKY + G) was used, with two substitution types that only distinguish between transitions and transversions (nst=2). *Rhodnius brethesi* Matta, 1919; *R. ecuadoriensis* Lent & León, 1958; *R. neglectus* Lent, 1954; *R. pallescens* Barber, 1932; *R. pictipes* Stal, 1872; *R. prolixus* Stal, 1859; and *R. robustus* were compared with the new *Rhodnius* species. *Triatoma infestans* (Klug, 1834) and *Panstrongylus megistus* (Burmeister, 1835) were used as outgroups.

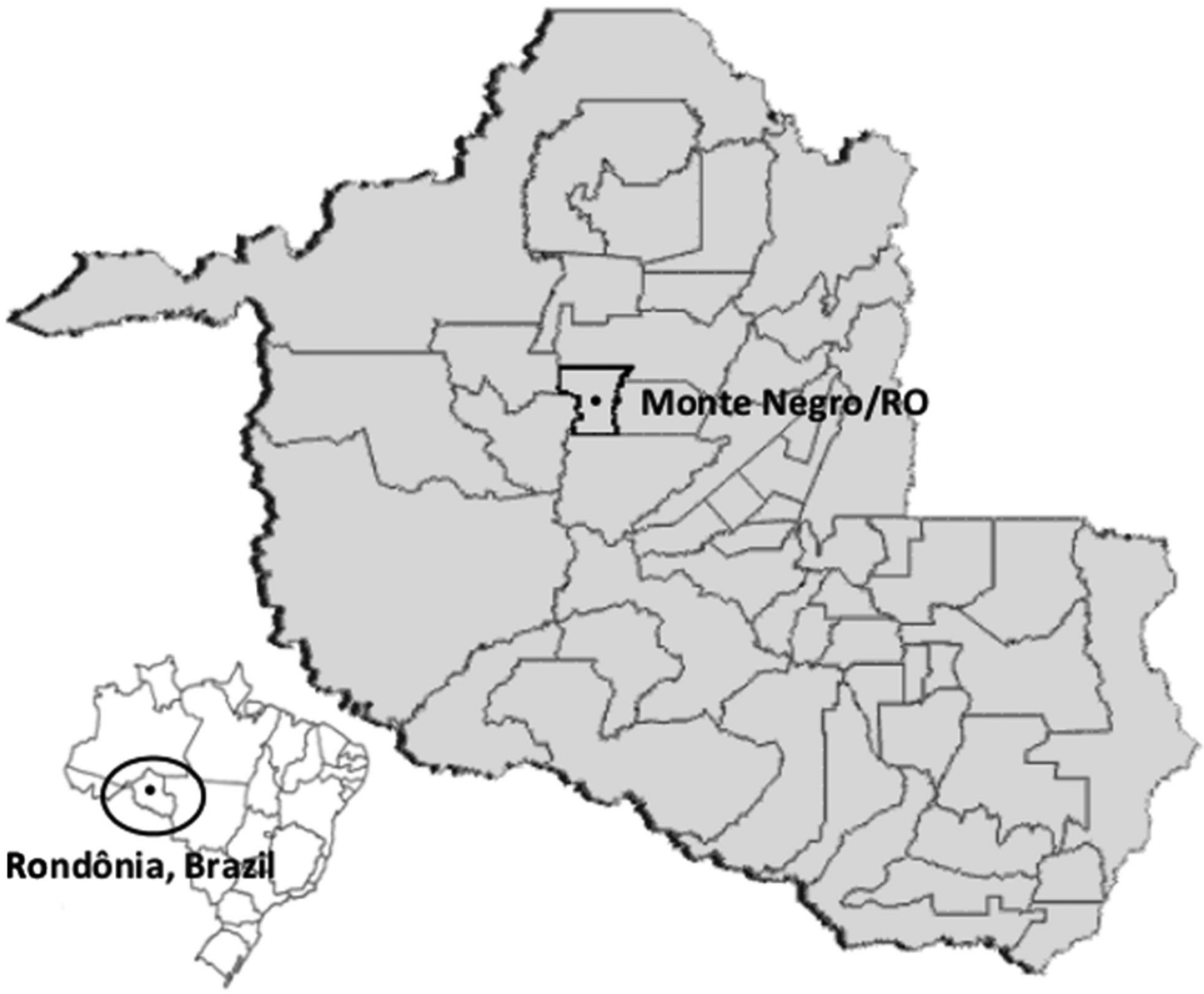

FIGURE 1. Localization of Monte Negro municipality where is collected *R. montenegrensis* **n. sp.** (S: 10° 10´ 05,1” and W: 63° 24’ 09,1”).

Systematics

Rhodnius montenegrensis n. sp.

Holotype. ♀, BRAZIL: Triatominae Insectarium of the Faculty of Pharmaceutical Sciences, Unesp, Araraquara, SP, Rodovia Araraquara-Jaú km 1. 2012.

Paratypes. BRAZIL: 8♀, 8♂, Oswaldo Cruz Institute, Rio de Janeiro, RJ, Avenida Brasil, 4365. 2012.

Etymology. The name *Rhodnius montenegrensis* was chosen because this species was found in the municipality of Monte Negro, state of Rondônia, Brazil.

Description. Length of male 18.49 ± 0.82 mm, of female 21.54 ± 0.75 mm, width of pronotum of male 3.99 ± 0.32 mm, of female 4.54 ± 0.26 mm; width of abdomen of male 6.16 ± 0.39 mm, of female 6.95 ± 0.39 mm (Table 1).

TABLE 1. Mean of measurement (mm) of 15 specimens of *Rhodnius montenegrensis* and *Rhodnius robustus*, females and males.

	Female		**Male**	
	R. montenegrensis	*R. robustus*	*R. montenegrensis*	*R. robustus*
HL	**4.81**	**4.06**	**4.45**	**3.80**
IE	**0.63**	**0.68**	0.59	0.61
AO	**2.97**	**2.39**	**2.73**	**2.23**
PO	**0.83**	**0.79**	**0.78**	**0.73**
DE	**1.22**	**1.08**	**1.16**	**0.99**
R1	**0.82**	**0.97**	0.85	0.90
R2	**3.81**	**3.29**	**3.56**	**3.03**
R3	0.93	0.96	**0.85**	**0.92**
TL	**21.54**	**18.96**	19.29	18.49
MWT	**4.54**	**4.14**	**3.99**	**3.99**
MWA	**6.95**	**6.56**	6.16	6.09
A1	0.44	0.38	**0.49**	**0.37**
A2	**3.94**	**3.19**	**4.00**	**3.28**
A3	**2.63**	**2.39**	**2.54**	**2.30**
A4	1,53	1.61	1.53	1.54

HL, head length; **IE**, inner distance between eyes; **AO**, anteocular distance; **PO**, postocular distance (excluding neck); **DE**, diameter of the eye; **R1**, **R2,** and **R3**, lengths of first, second, and third rostral segments, respectively; **TL**, Total length of the Triatominae; **MWT**, maximum width of the thorax; **MWA**, maximum width of the abdomen; **A1**, **A2**, **A3** and **A4**, 1st, 2nd, 3rd, and 4th antennal segments, respectively. The values in bold were significant at α = 0.05, using unpaired t-test.

General color yellow with longitudinal dorsal black stripes on pronotum, wings, and connexivum (Fig. 2A).

Head of male and female with no spots, from clypeus to neck, with a central yellow stripe located between two (1+1) continuous brown stripes. Neck with a brown mark and a yellow central circular mark (Fig. 2A). Head length, inner distance between eyes, anteocular distance, postocular distance (excluding neck), diameter of the eye, lengths of 1st, 2nd, and 3rd-rostral segments, respectively, and 1st, 2nd, 3rd, and 4th-antennal segments, respectively, are shown in Table 1.

Mesothoracic wings with well-demarcated veins, notably subcostal (Sc) vein with its yellow color (Fig. 4). Legs: yellow, except for a black stripe posteriorly to tibia, near tarsus (Fig. 2). Mesosternum with lateral stripes and one central stripe that delimits two dark areas. Metasternum with two yellow stripes between coxa that delimit a dark central area (Fig. 2). First urotergite: brown and with distinct transverse groove (Fig. 5A). Scutellum with a long glabrous space between pronotum and beginning of a semi-circular base that supports central depression with sensillae. Lateral edges of scutellum slightly curved and with fine apex (Fig. 6A).

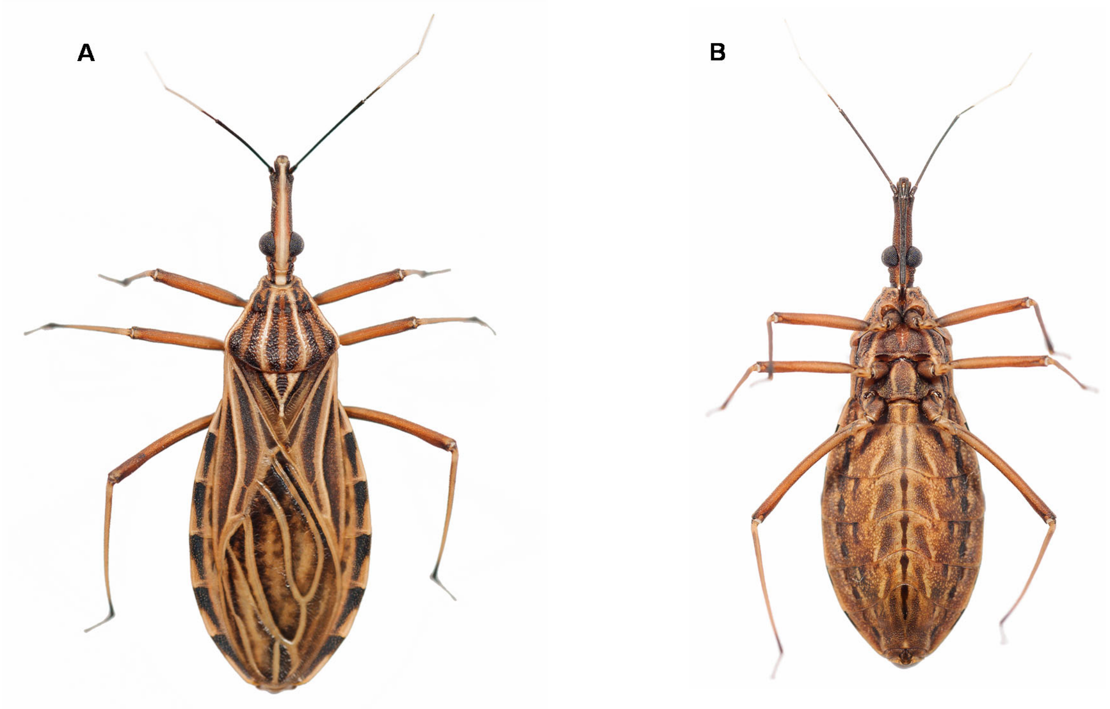

FIGURE 2. *R. montenegrensis* **n. sp.** female. A, dorsal side; B, ventral side.

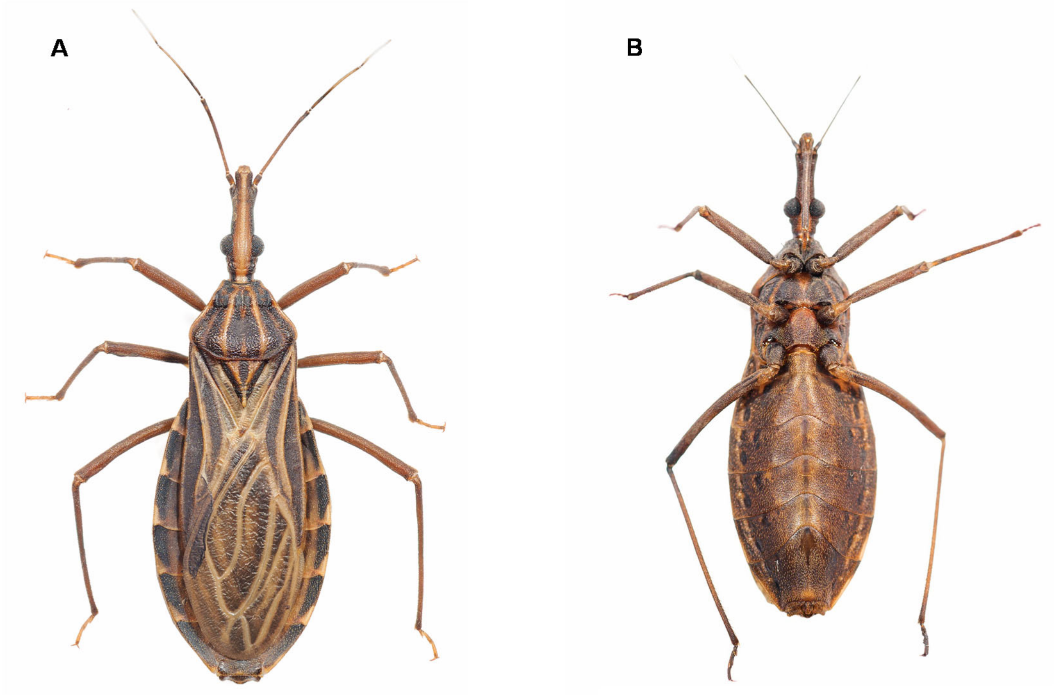

FIGURE 3. *R. robustus* female. A, dorsal side; B, ventral side.

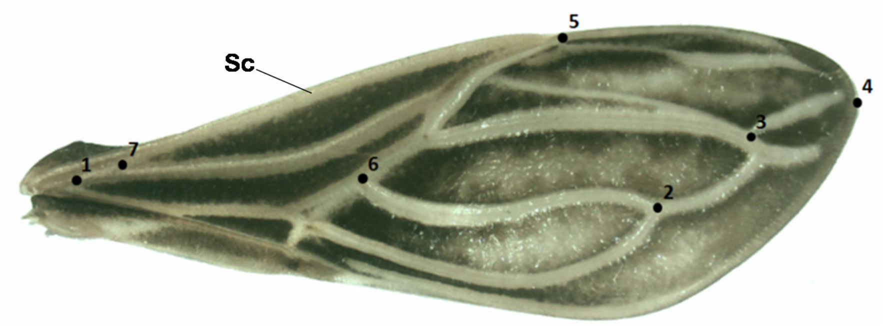

FIGURE 4. Wing of *R. montenegrensis* **n. sp.** with the seven landmarks used in morphometric analysis. According to Gurgel-Gonçalves *et al.* (2008). Sc, subcosta vein.

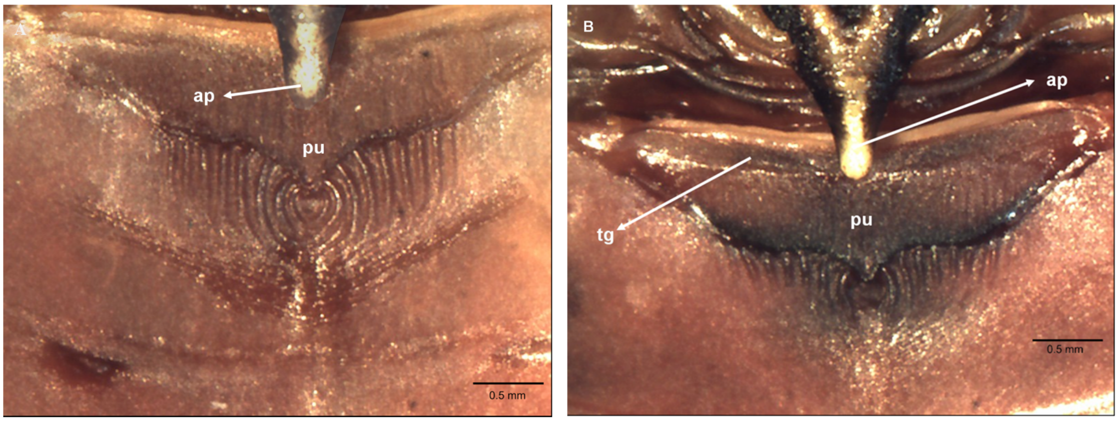

FIGURE 5. Process of the I urotergite. A, *R. montenegrensis* **n. sp.**; B, *R. robustus.* ap, apex of scutellum; pu, process of the I urotergite; tg, transverse groove.

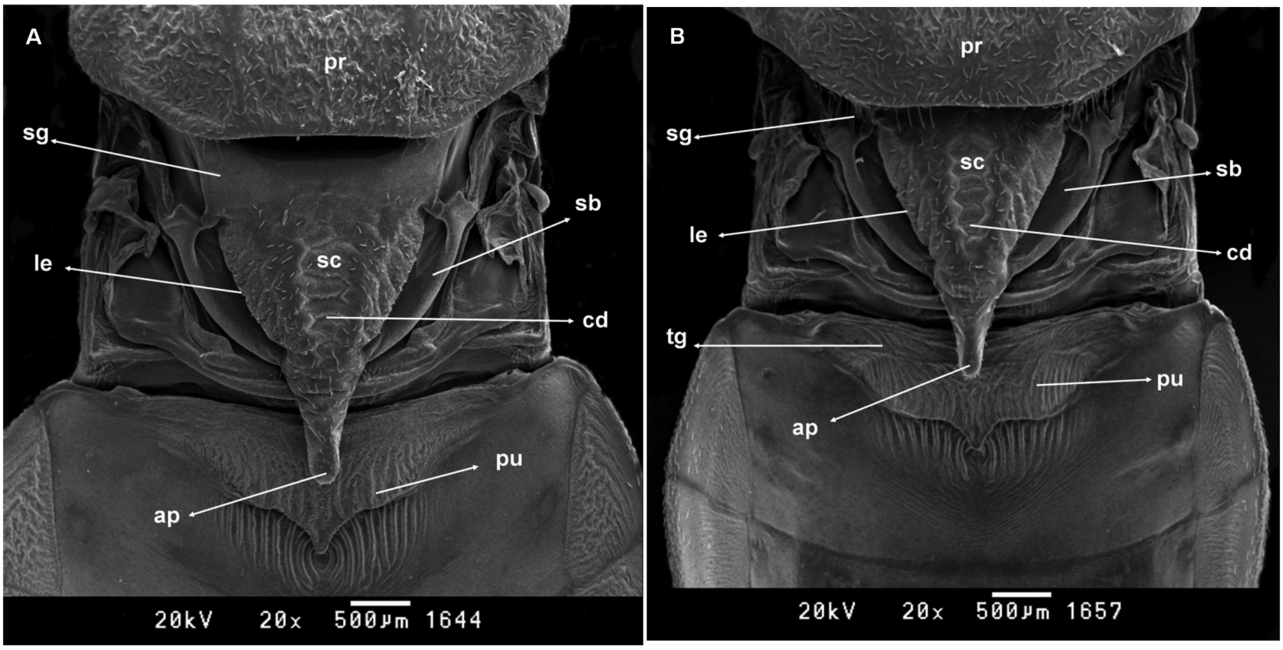

FIGURE 6. Scutellum by SEM. A, *R. montenegrensis* **n. sp.**; B, *R. robustus.* pr, pronotum; sc, scutellum; sb, semi-circular base; sg, glabrous space; cd, central depression; le, lateral edge; ap, apex of scutellum; pu, process of the I urotergite; tg, transverse groove.

Ventral abdomen with yellow and dark areas anteriorly to posteriorly. Dorsal connexivum with a dark spot occupying about 1/3 of each segment, wider on anterior portion, and narrowing towards posterior portion. Internal and external borders of connexivum with a noncontinuous yellow stripe. Externally yellow stripe not continuous. First abdominal segment resembling metasternum, but smaller (Fig. 2).

Median length of the basal plate (EPlb) does not involve endosomes. Basal plate (Plb) has a regular arc shape. Process of phallosoma (PrPh) is the most intensely chitinized structure of phallus, contains two cylindrical pieces with well-defined contours (Fig. 7A, B, C) Median process of the pygophore inserted in a wide triangular base surrounded by a groove-shaped cuticle, anterior 2/3 wider than the posterior 1/3, which narrows to a gross point (Fig. 8A).

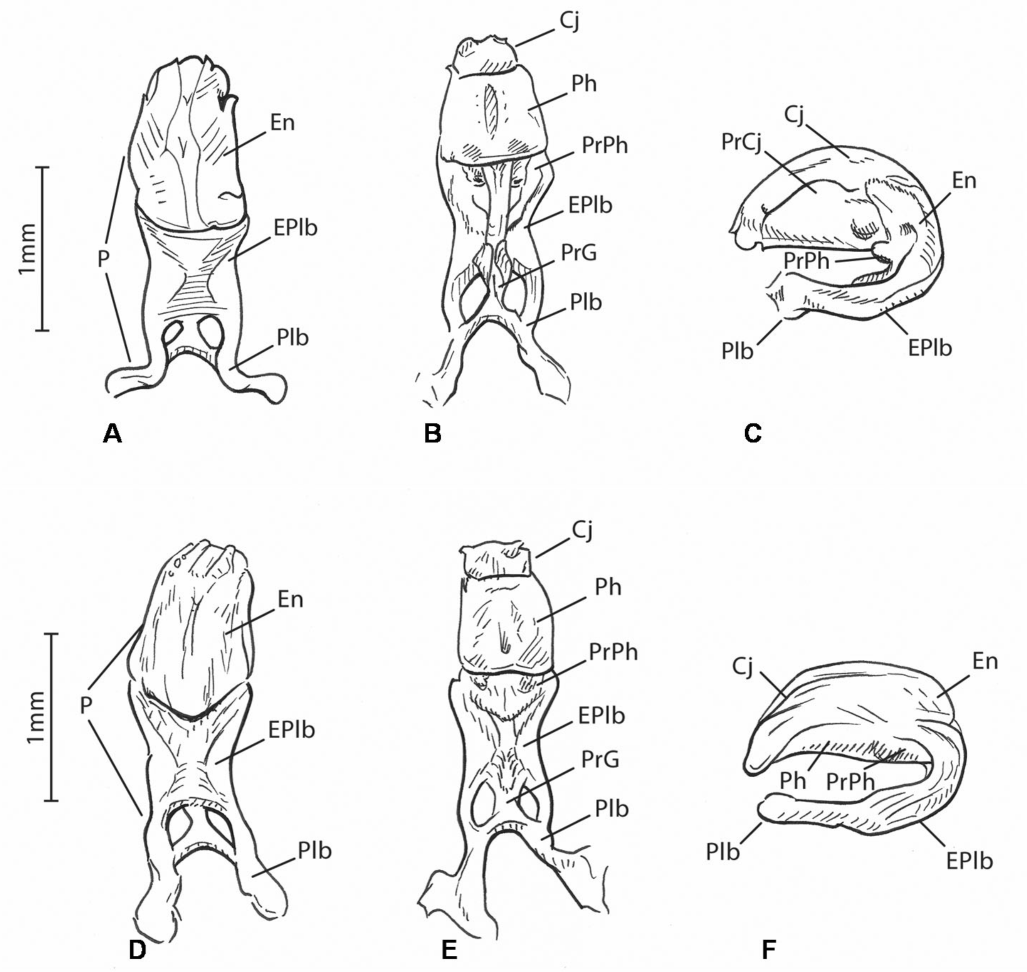

FIGURE 7. Phallus of *R. montenegrensis* **n. sp.** (A, dorsal view; B, ventral view; C, lateral view) and *R. robustus* (D, dorsal view; E, ventral view; F, lateral view). Cj, conjunctive; En, endosome; EPlb, median extension of basal plate; P, phallus; Plb, basal plate; PrG, gonopore process; PrPh, phallosoma process; Ph, phallosoma; PrCj, conjunctive process.

Ventral view: female external genitalia with two small lateral saliencies on 9th sternite and a circular line (Fig. 9A) on 7th sternite, which borders gonocoxites. Dorsal view: 7th tergite with a trapezoidal shape ending on two tips on posterior portion (Fig. 10A). Posterior view: female external genitalia with small 9th tergite; large opening limiting X segment with gonapophyses VIII and gonocoxites VIII (Fig. 11A).

Eggshell length 1.58 ± 0.080 mm, width 0.99 ± 0.035 mm, area 1.26 ± 0.061 mm. Egg featuring a collar, an exochorion with a grainy surface, tapered holes regularly distributed and no cell demarcation (Figs. 12A, 13A).

Classic and geometric morphometrics. Statistical analyses based on unpaired t-test show significant values ($\alpha=0.05$) for some parameters between females (HL, IE, AO, PO, DE, R1, R2, TL, MWT, MWA, A2 and A3) and between males (HL, AO, PO, DE, R2, R3, MWT, A1, A2 and A3) (Table 1).

Eggshell measurements and the statistical analyses based on unpaired t-test show significant values (α=0.0001) for eggshell area and length, whereas the others parameters were not significant.

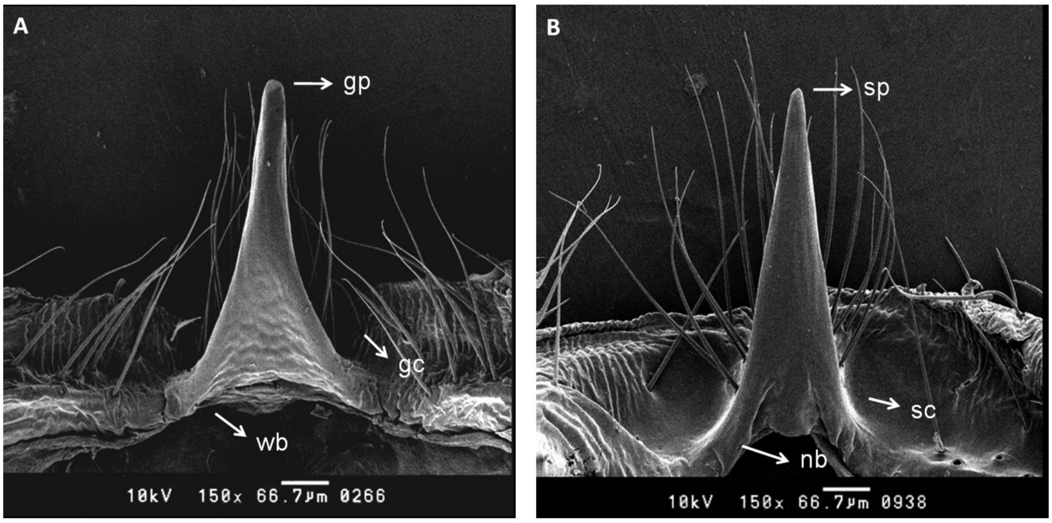

FIGURE 8. Median process of the pygophore by SEM. A, *R. montenegrensis* **n. sp.**; B, *R. robustus*. gc, groove cuticle; gp, gross point; nb, narrow triangular base; sc, smooth cuticle; sp, slender point; wb, wide triangular base.

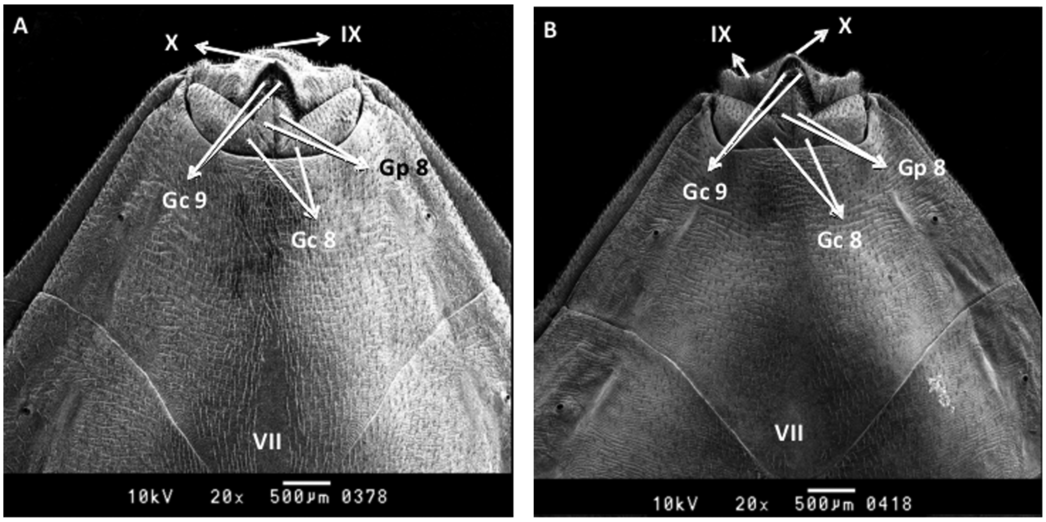

FIGURE 9. Female external genitalia by SEM, ventral side. A, *R. montenegrensis* **n. sp.**, B, *R. robustus*. Gc 8, gonocoxite VIII; Gc 9, gonocoxite IX; Gp 8, gonapophyse VIII; VII, IX, esternites; X, segment (Rosa *et al*. 2010).

For the geometric morphometrics the factorial map built with 18 specimens of *R. robustus* and 23 specimens of *R. montenegrensis* **n. sp.** distinguished both species in well-defined groups. The Mahalanobis distance between the two species was 3.85. Considering the shape variation components, the contribution of the first principal (PC1) component accounted for 39% of the total variation, whereas the second principal component (PC2) accounted for 35% (Fig. 14).

Molecular analysis. For the Bayesian analysis, the alignment of a 369pb of Cyt *b* gene was obtained, and the phylogenetic tree showed that the *R. montenegrensis* **n. sp.** is a sister to *R. robustus*. This tree also included other six *Rhodnius* species (*R. prolixus*, *R. neglectus*, *R. pallescens*, *R. ecuadoriensis*, *R. pictipes*, *R. brethesi*). Phylogenetic reconstruction also certified the taxonomic status of insects belonging to CTA 88 (Fig. 15).

Based on this, an additional molecular methodology was applied to differentiate both species. Therefore, 5.8S/ITS-2 genes from nuclear DNA were amplified by PCR and generated a fragment of 1,200 base pairs (Marcilla *et al.* 2001). This gene were treated by restriction enzyme BstUI by PCR-RFLP (Naegele *et al.* 2006) and generated two fragments of 1,000 and 150 pair of bases for *R. robustus* and there was no digestion for *R. montenegrensis* (Fig. 16).

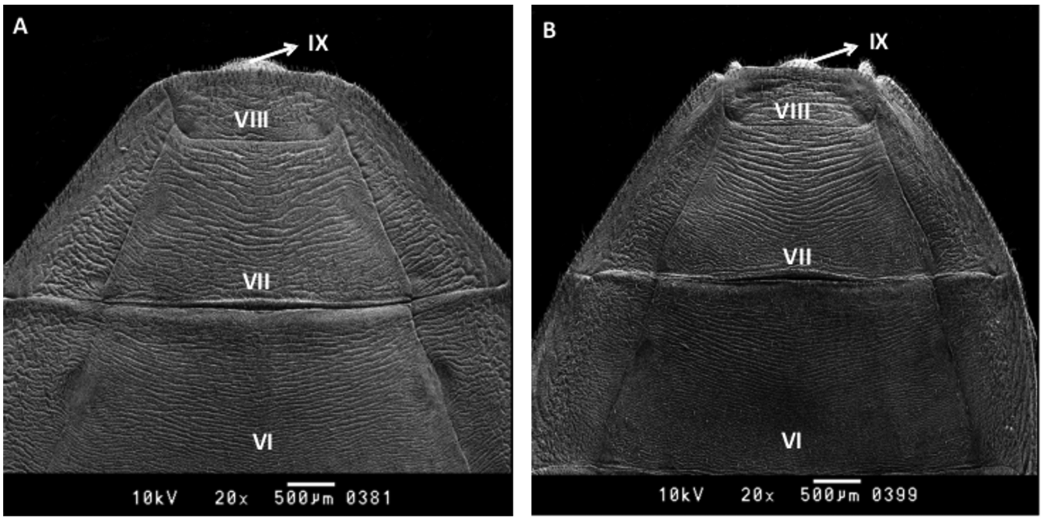

FIGURE 10. Female external genitalia by SEM, dorsal side. A, *R. montenegrensis* **n. sp.**; B, *R. robustus*. VI, VII, VIII, IX, tergites (Rosa *et al*. 2010).

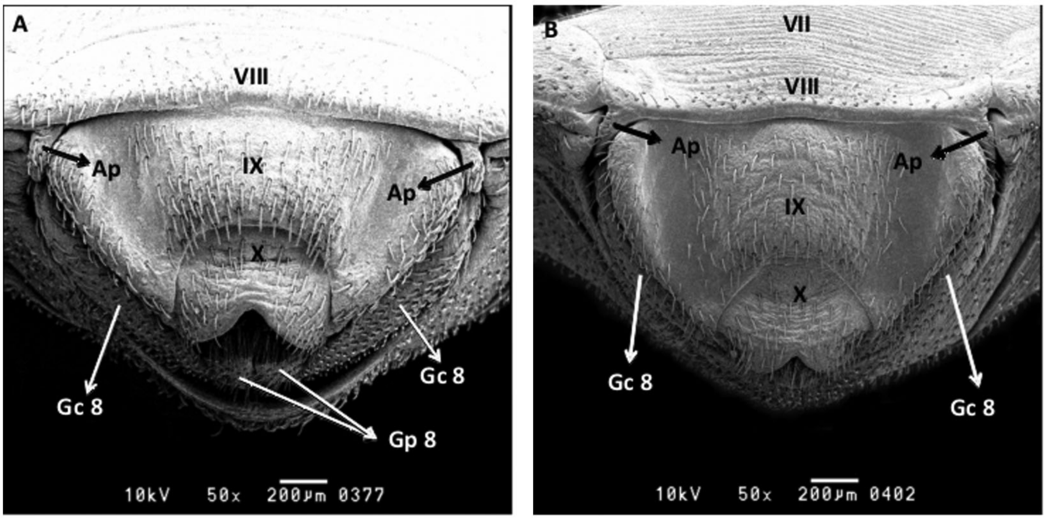

FIGURE 11. Female external genitalia by SEM, posterior side. A, *R. montenegrensis* **n. sp.**; B, *R. robustus*. Ap, appendices; Gc 8, gonocoxite VIII; Gp 8, gonapophyse VIII; VII, VIII, IX, tergites; X, segment (Rosa *et al*. 2010).

Discussion

Distinction between *Rhodnius* species by means of a stereoscopic microscope caused many difficulties, as it had already been reported by many authors who had studied this genus (Neiva & Pinto 1923; Valente *et al*. 2001). Nevertheless, even under a macroscopic view, it was noticed that a yellow tonality is prevailing, and reflects itself in a lighter overall color in the case of *R. montenegrensis*, while amber prevails in the case of *R. robustus* (Figs. 2, 3).

By using a stereoscopic microscope, it can be noticed that in *R. montenegrensis* **n. sp.** there are yellow spots interposed with dark ones over the ventral abdomen lengthwise, while in *R. robustus* such spots are not noticed (Figs. 2B, 3B). Respiratory spiracles at the ventral abdomen are surrounded by yellow in *R. robustus*, but absent in *R. montenegrensis* (Fig. 2B, 3B). *R. robustus* legs present a brown tonality and did not have a black stripe at the posterior tibial portion *R. robustus* exhibits two lateral saliencies on the 9th sternite (Fig. 3). *R. robustus* first urotergite is black and notably distinct from those exhibited by *Rhodnius montenegrensis* **n. sp.** (Fig. 5). These characters *per se* allow the taxonomic identification of *R. montenegrensis* without the aid of sophisticated techniques. In addition, it must be stressed that the taxonomic definition based on chromatic patterns has been widely proposed for other species of triatomines, such as the members of the *Triatoma brasiliensis* species complex (Costa *et al*. 2006; Costa & Felix 2007) and *phyllosoma* complex (Lent and Wygodzinsky 1979; Martínez *et al*. 2006; Martínez-Ibarra *et al.* 2009), and these studies have also been strongly supported by biological and molecular approaches.

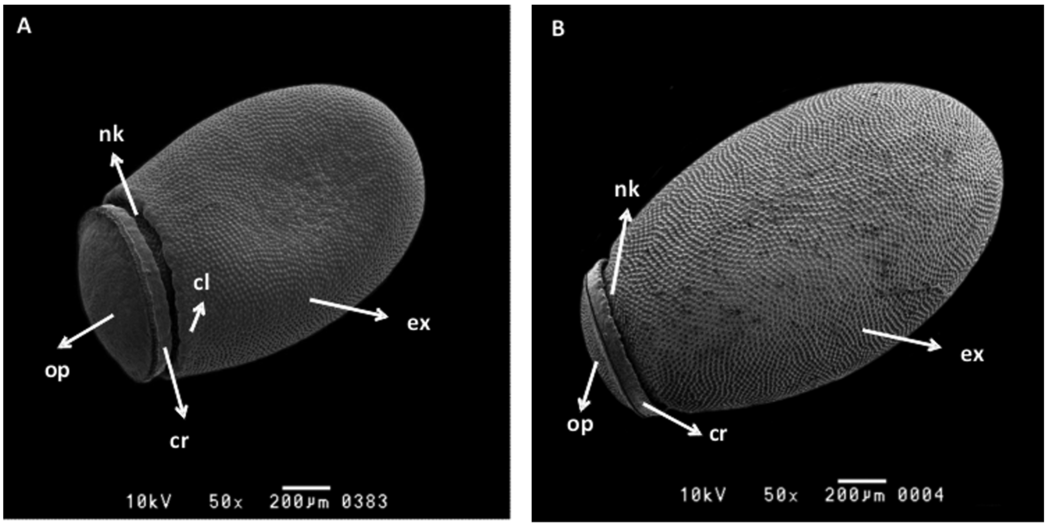

FIGURE 12. Egg general vision by scanning electron microscopy. A, *R. montenegrensis* **n. sp.**; B, *R. robustus.* cl, collar; cr, chorial rim; ex, exochorion; nk, neck; op, operculum (Barata 1981).

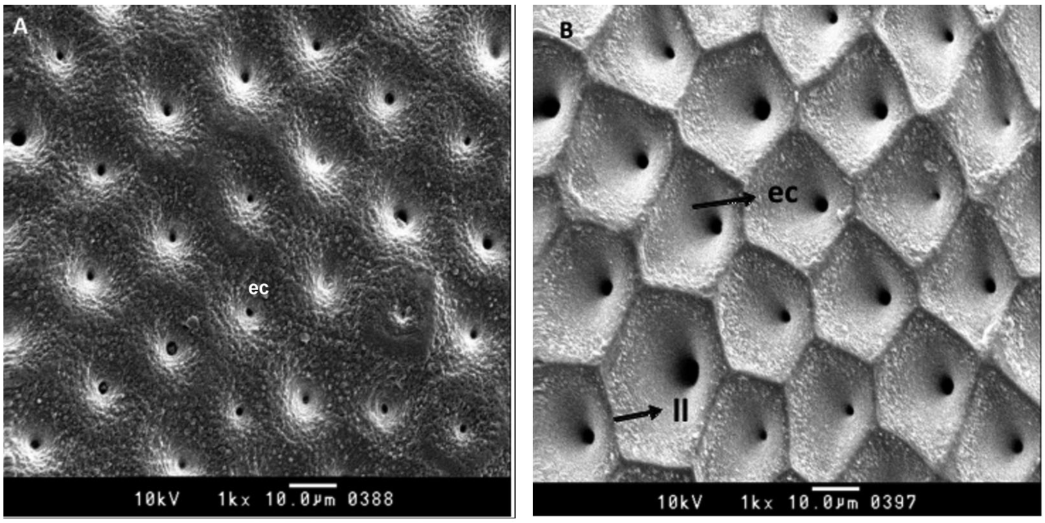

FIGURE 13. Egg exochorion detail by scanning electron microscopy. A, *R. montenegrensis* **n. sp.**; B, *R. robustus.* ec, exochorion cell; ll, limiting line (Barata 1981).

Using classic morphometric approaches, significant distinguishing characteristics were observed between *R. montenegrensis* **n. sp.** and *R. robustus*, mainly in the proboscis and antennae, but also in head and egg shape. However, the most prominent distinguishing characteristics between these two species were noticed by means of morphometry and scanning. In both males and females of *R. montenegrensis* **n. sp.**, the total length of the second segment of the proboscis, second segment of the antennae, and interocular distance are significantly larger than in *R. robustus* (Table 1). On the other hand, length and area of eggs are significantly larger in *R. robustus* than in *R. montenegrensis* **n. sp.** (Fig. 12). Geometric morphometrics revealed that the shape of *R. montenegrensis* **n. sp.** wings was clearly different from that of *R. robustus*. The degree of shape divergence noticed in this study was greater than those observed by Márquez *et al*. (2011) among populations of *R. robustus*.

By means of scanning, it was noticed that *R. montenegrensis* **n. sp.** eggs bear a collar, and the exochorion cells feature a grainy surface, tapered holes that are regularly distributed, with no cell demarcation (Fig. 12). According to Barata (1981), in *R. robustus* there is no collar, and the exochorion cells are well demarcated and tapered, each of them delimited by a central hole, mostly hexagonally shaped (Fig. 13). The most noticeable differences have also been observed by dorsal, posterior and ventral views of the female genitalia (Rosa *et al.* 2010), as well as of the median process of pygophore (Lent and León 1958; Harry 1993) and phallosoma (Figs. 7–11). Male genitalia of *R. robustus* is described in accordance with the description provided by Lent and Jurberg (1969).

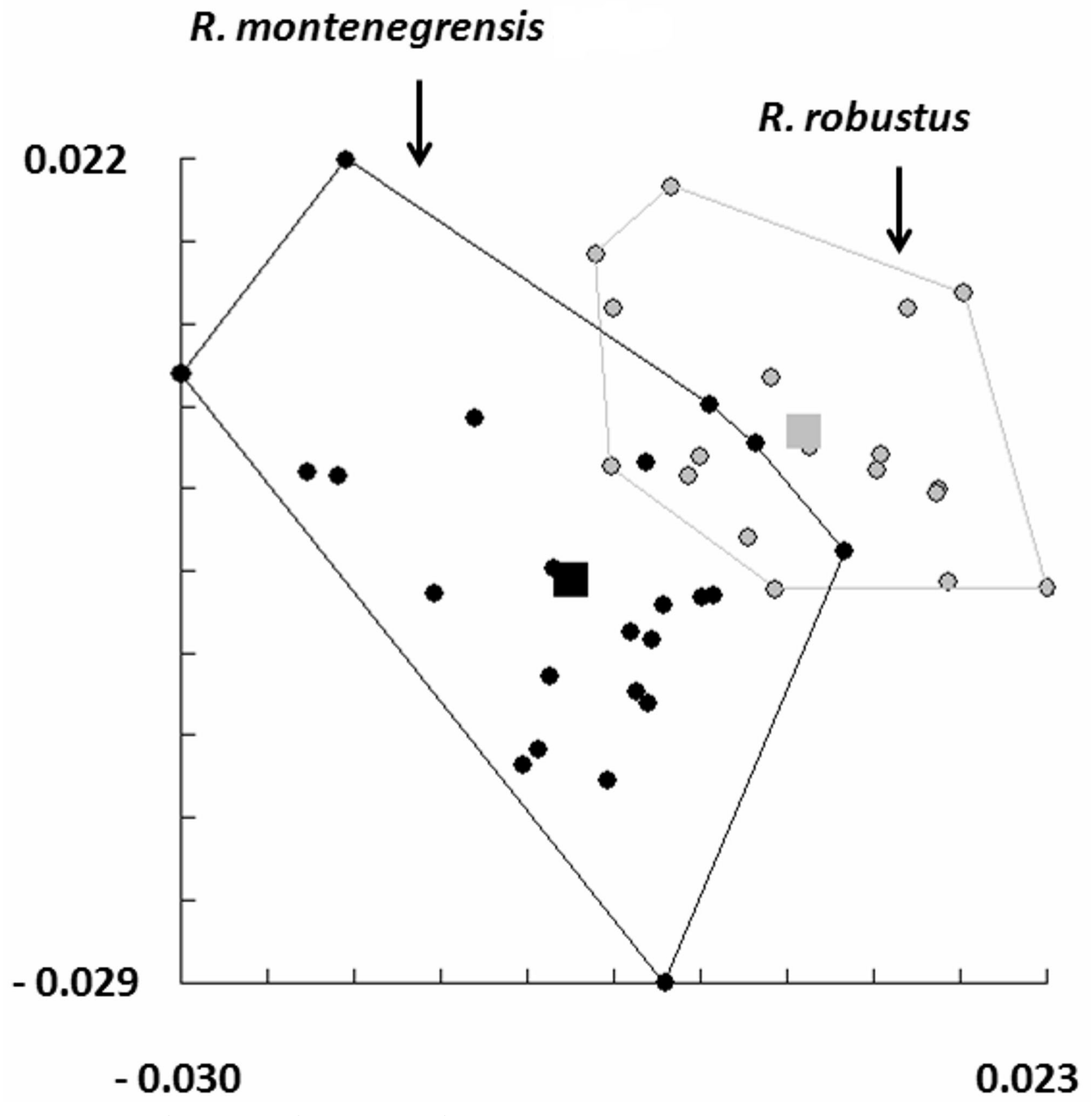

FIGURE 14. The factorial map of the wing shape for the specimens *R. robustus* and *R. montenegrensis* **n. sp.**

R. montenegrensis **n. sp.** and *R. robustus* scutellum showed three distinct features, which are detailed in Figure 6.

Phylogenetic analysis showed a close relationship between the new described species and *R. robustus* (Fig. 15). The digestion product of the 5.8S/ITS-2 genes by B*st*UI enzyme also showed distinct profiles between them (Fig. 16). Even though molecular approaches have corroborated morphological evidences for *R. montenegrensis* **n. sp.** as a new species, being closely related to *R. robustus*, the dataset obtained herein stress that the morphology must be the major approach and the species under study should be carefully examined before defining a new evolutionary entity. Therefore, morphology must be used for providing phenotypic information to define the species, and molecular data work as a complementary approach to support morphological findings (Schlick-Steiner *et al*. 2010).

After describing *R. montenegrensis* **n. sp.**, 146 Triatominae species are currently acknowledged; out of which 18 belong to the genus *Rhodnius* (Jurberg *et al*. 2009; Schofield & Galvão 2009).

The "prolixus" complex comprises *R. prolixus*, *R. domesticus*, *R. robustus*, *R. neglectus*, and *R. nasutus* (Barrett 1991; Harry 1993; Barrett 1996; Dujardin *et al*. 1999b). *R. montenegrensis* **n. sp.** is clearly a new member of this species complex, and a taxonomic key for specific identification is currently being developed and will be presented with a revision based on the female genital structures.

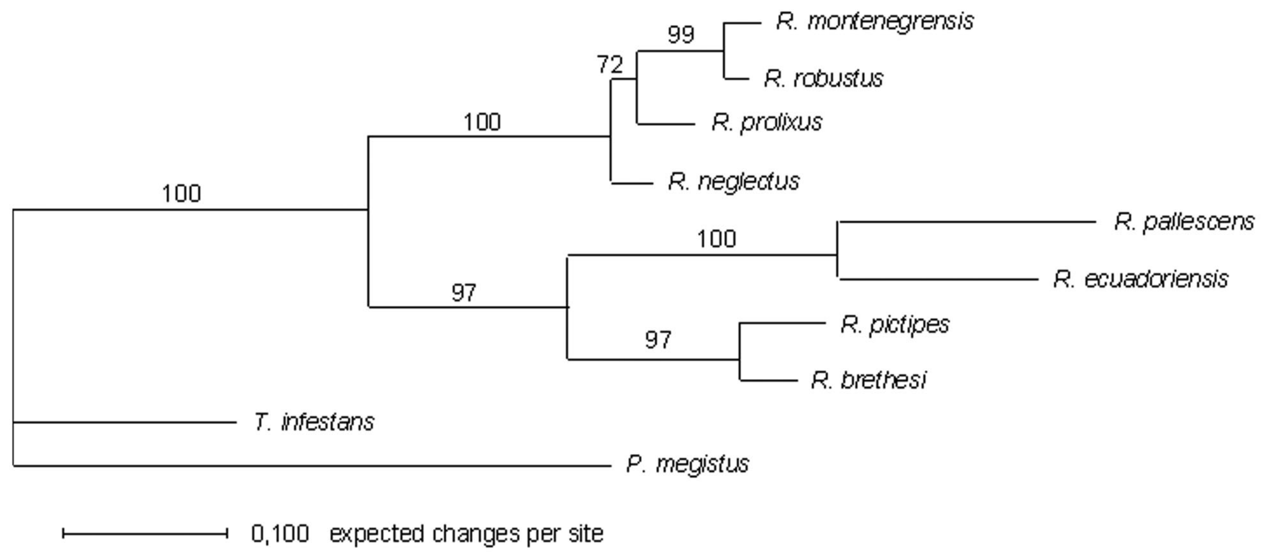

FIGURE 15. Bayesian analyze based on Likelihood parameter among seven *Rhodnius* species and *R. montenegrensis* **n. sp.** (CTA 088), based on Cyt *b* gene of the mtDNA sequences. *Triatoma infestans* and *P. megistus* were using by outgroup.

Summary of the main characters that distinguish *R. montenegrensis* n. sp.

Head: head length of females and males of *R. montenegrensis* **n. sp.** is significantly larger than that of *R. robustus*. There are also differences in other eleven characters of that portion (Table 1).

Thorax: anterior wings with well-demarcated veins, notably the Sc by a yellow tonality in *R. montenegrensis* **n. sp.**, but not in *R. robustus* (Fig. 4). *R. montenegrensis* **n. sp.** exhibits yellow legs, and overall that is the insect's color, except for a black stripe on the posterior tibial portion, at the tarsus limit. Mesosternum presents two lateral stripes and one central stripe, which delimits two dark areas. Metasternum features two yellow stripes between the medial coxa that delimits a dark area in the center (Fig. 2). *R. robustus* legs present brown color and do not exhibit a black stripe at the posterior tibial portion (Fig. 3).

Abdomen: *R. montenegrensis* presents yellow spots interposed with dark ones over the ventral abdomen lengthwise, while no such spots are noticed in *R. robustus*. Respiratory spiracles at the ventral abdomen are surrounded by yellow for *R. robustus*, differing from those of *R. montenegrensis*, which are darker. In *R. robustus* there are two lateral saliencies on the 9th sternite (Figs. 2B, 3B).

Eggs: *R. montenegrensis* **n. sp**. have a collar, and exochorion cells feature a grainy surface, tapered holes that are regularly distributed, with no cell demarcation (Fig. 12). In *R. robustus*, there is no collar, and the exochorion cells are well demarcated and tapered, being all of them delimited by a central hole, mostly hexagonally shaped (Fig. 13).

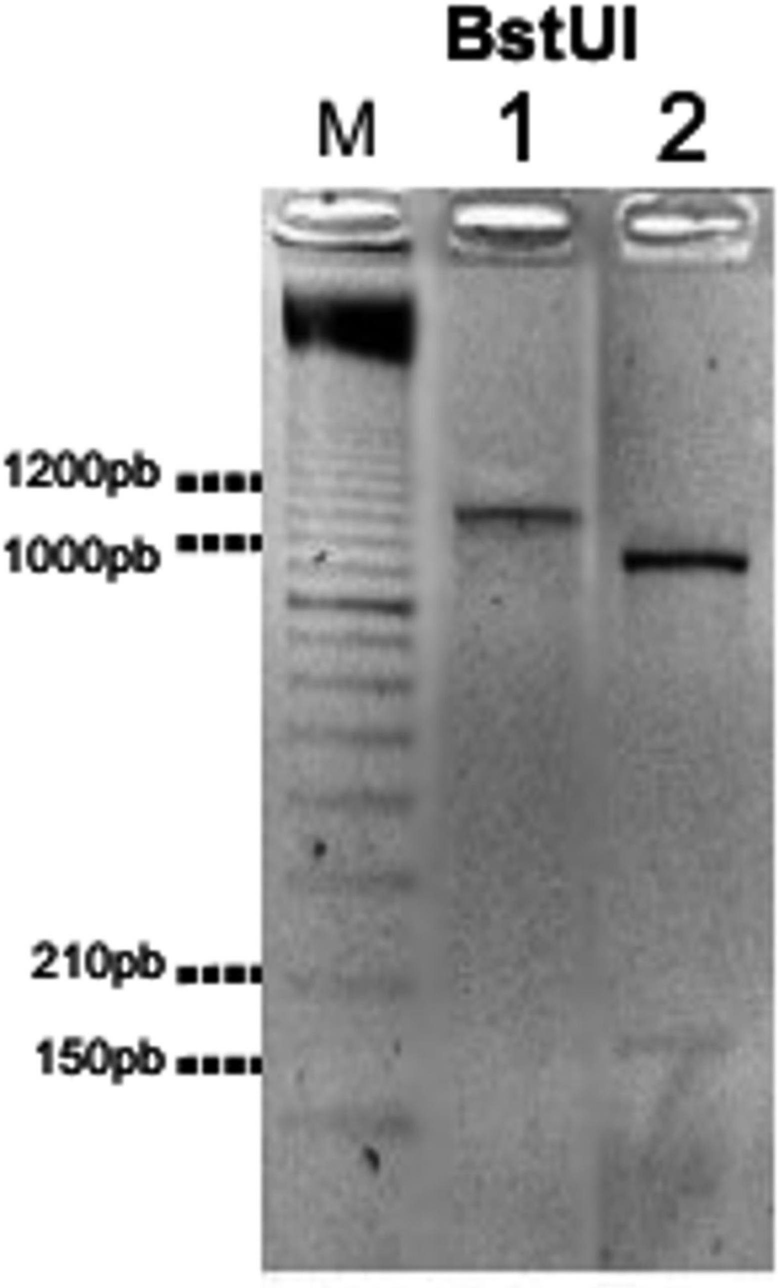

FIGURE 16. Fingerprint of the triatominae species produced by PCR-RFLP with BstUI enzyme on 2% agarose gel. M, 100bp DNA ladder marker; 1, *R. montenegrensis* **n. sp.**; 2, *R. robustus*.

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References

Almeida, C.E., Oliveira, H.L., Correia, N., Dornak, L.L., Gumiel, M., Neiva, V.L., Harry, M., Mendonça, V.J., Costa, J. & Galvão, C. (2012) Dispersion capacity of *Triatoma sherlocki*, *Triatoma juazeirensis* and laboratory-bred hybrids. *Acta Tropica*, 122, 71–79.

Barata, J.M.S. (1981) Aspectos morfológicos de ovos de Triatominae II. Características macroscópicas e exocoriais de dez espécies do gênero *Rhodnius* Stal, 1859 (Hemiptera, Reduviidae). *Revista de Saúde Pública,* 15, 490–542.

Barrett, T.V. (1991) Advances in triatominae bug ecology in relation to Chagas disease. *Advances Disease Vector Research*, 8, 143–176.

Barrett, T.V. (1996) Species interfertility and crossing experiments in triatomine systematics. In: Proceedings of the International Workshop on Population Genetics and Control of Triatominae (eds SchofieldCJ et al.). INDRE, Mexico City, pp. 72–77.

Bookstein, F.L. (1991) *Morphometric tools for landmark data: geometry and biology.* Cambridge Univ. Press: New York. 435 pp.

Campos, C., Botto–Mahan, C., Coronado, X., Jaramillo, N., Panzera, F. & Solari, A. (2011) Wing shape differentiation of *Mepraia* species (Hemiptera: Reduviidae). *Infecttion Genetics and Evolution,* 11, 329–333.

Chagas, C.J.R. (1909) Nova tripanossomíase humana. Estudos sobre a morfologia e o ciclo evolutivo de *Schizotrypanum cruzi* n.sp., agente etiológico de nova entidade mórbida do homem. *Memórias do Instituto Oswaldo Cruz*, 1,159–218.

Costa, J., Argolo, A.M. & Felix, M. (2006) Redescription of *Triatoma melanica* Neiva & Lent, 1941, new status (Hemiptera: Reduviidae: Triatominae). *Zootaxa*, 1385, 47–58.

Costa, J. & Felix, M. (2007) *Triatoma juazeirensis* sp. nov. from the state of Bahia, Northeastern Brazil (Hemiptera: Reduviidae: Triatominae). *Memórias do Instituto Oswaldo Cruz,* 102, 87–90.

Dujardin, J.P., Steindel, M., Chavez, T., Machane, M. & Schofield, C.J. (1999a) Changes in the Sexual Dimorphism of Triatominae in the Transition from Natural to Artificial Habitats. *Memórias do Instituto Oswaldo Cruz*, 94(4), 565–569.

Dujardin, J.P., Chavez, T., Moreno, J.M., Machane, M., Noireau, F. & Schofield, C.J. (1999b) Comparison of isoenzyme electrophoresis and morphometric analysis for phylogenetic reconstruction of the Rhodniini (Hemiptera: Reduviidae: Triatominae). *Journal of Medical Entomology*, 36, 653–659.

Dujardin, J.P. (2004) *Anatomical landmarks collection (COO)*. Avaiable from http://www.mpl.ird.fr/morphometrics. (accessed 3 june 2012).

Dujardin, J.P. (2008) Morphometrics applied to Medical Entomology. *Infection, Genetics and Evolution*, 8, 875–890.

Dujardin, J.P., Kaba, D. & Henry, A.B. (2010) The exchangeability of shape. *BMC Research Notes*, 3, 266.

Forero, D., Weirauch, C. & Baena, M. (2004) Synonymy of the reduviidae (Hemiptera: Heteroptera) genus *Torrealbaia* (Triatominae) with *Amphibolus* (Harpactorinae), with notes on *Amphibolus venator* (Klug, 1830). *Zootaxa*, 670, 1–12.

Galvão, C., Carcavallo, R., Rocha, D.S. & Jurberg, J. (2003) Checklist of the current valid species of the subfamily Triatominae Jeannet, 1919 (Hemiptera, Reduviidae) and their geographical distribuition, with nomenclatural and taxonomic notes. *Zootaxa*, 202, 1–36.

Gurgel-Gonçalves, R., Abad-Franch, F., Ferreira, J.B.C., Santana, D.B. & Cuba, C.A.C. (2008) Is *Rhodnius prolixus* (Triatominae) invading houses in Central Brazil? *Acta Tropica,* 107, 90–98.

Harry, M. (1993) Use of the median process of the pygophore in the identification of *Rhodnius nasutus, R. neglectus, R. prolixus* and *R. robustus* (Hemiptera: Reduviidae). *Annals of Tropical Medicine and Parasitology,* 87, 277–82.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17(8), 754–755.

Jurberg, J., Rocha, D.S. & Galvão, C. (2009) *Rhodnius zeledoni* sp. nov. afim de Rhodnius paraensis Sherlock, Guitton & Miles, 1977 (Hemiptera, Reduviidae, Triatominae). *Biota Neotropica,* 9 (1), 123–128.

Lent, H., Jurberg, J. & Galvão, C. (1993) *Rhodnius stali* sp. n., afim de *Rhodnius pictipes* Stal, 1872 (Hemiptera, Reduviidae, Triatominae). *Memórias do Instituto Oswaldo Cruz,* 88, 605–614.

Lent, H. & Jurberg, J. (1969) O gênero *Rhodnius* Stal, 1859, com um estudo sobre a genitália das espécies (Hemiptera, Reduviidae, Triatominae). *Revista Brasileira de Biologia,* 29, 487–560.

Lent, H. & León, L.A. (1958) Notes on an interesting structure of the pygophore of *Rhodnius* Stal, 1859 (Hemiptera, Reduviidae). *Revista Brasileira de Biologia*, 18 (4), 381–383.

Lent, H. & Wygodzinsky, P. (1979) Revision of the triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas’s disease. *Bulletin of the American Museum of Natural History*, 163 (3), 123–520.

Lyman, E.D., Monteiro, F.A., Escalante, A.A., Cordon-Rosales, C., Wesson, D.M., Dujardin, J.P. & Beard, C.B. (1999) Mitochondrial DNA sequence variation among triatomine vectors of Chagas' disease. *American journal of tropical medicine and hygiene*, 60, 377–386.

Marcilla, A., Bargues, M.D., Ramsey, J.M., Magallon-Gastelum, E., Salazar-Schettino, P.M., Abad-Franch, F., Dujardin, J.P., Schofield, C.J. & Mas-Coma, S. (2001) The ITS-2 of the nuclear rDNA as a molecular maker for populations, species, and phylogenetic relationships in Triatominae (Hemiptera-Reduviidae), vectors of Chagas disease. *Molecular Phylogenetics and Evolution*, 18, 136–142.

Márquez, E., Jaramillo-O, N., Gómez-Palacio, A. & Dujardin, J.P. (2011) Morphometric and molecular differentiation of a *Rhodnius robustus*-like form from *R. robustus* Larousse, 1927 and R. prolixus Stal, 1859 (Hemiptera, Reduviidae). *Acta Tropica*, 120, 103–109.

Martínez-Ibarra, J.A., Salazar-Schettino, P.M., Nogueda-Torres, B., Vences, M.O., Tapia-González, J.M. & Espinoza-Gutiérrez, B. (2009) Occurrence of hybrids and laboratory evidence of fertility among three species of the Phyllosoma complex (Hemiptera: Reduviidae) in Mexico. *Memórias do Instituto Oswaldo Cruz,* 104, 1125–1131.

Martínez, F.H., Villalobos, G.C., Cevallos, A.M., Torre, P. L., Laclette, J.P., Alejandre-Aguilar, R. & Espinoza, B. (2006) Taxonomic study of the *Phyllosoma* complex and other triatomine (Insecta: Hemiptera: Reduviidae) species of epidemiological importance in the transmission of Chagas disease: using ITS-2 and mtCytB sequences. *Molecular Phylogenetics and Evolution*, 41, 279–87.

Mejia, J.M., Galvão, C. & Jurberg, J. (1999) *Rhodnius colombiensis* sp.n. da Colômbia com quadros comparativos entre as estruturas fálicas do gênero *Rhodnius* Stål, 1859 (Hemiptera, Reduviidae, Triatominae). *Entomologia y Vectores*, 6 (6), 601–617.

Naegele, M.P., Barata, J.M.S., Cilense M. & Rosa, J.A. (2003) Estudo morfológico e morfométrico de pernas de ninfas de 1º e 2º estádios de *Mepraia spinolai* (Porter, 1934), *Panstrongylus megistus* (Burmeister, 1835), *Rhodnius prolixus* (Stal, 1859) e *Triatoma infestans* (Klug, 1834) (Hemiptera, Reduviidae). *Entomologia y Vectores,* 10 (2), 223–235.

Naegele, M.P., Costa, P.I. & Rosa, J.A. (2006) Polymorphism of the ITS-2 region of the ribosomal DNA of the Triatominae *Rhodnius domesticus, R. pictipes, R. prolixus* and *R. stali*. *Medical and Veterinary Entomology*, 20, 353–357.

Neiva, A. & Pinto, C. (1923) Estado actual dos conhecimentos sobre o gênero *Rhodnius* Stal, com a descrição de uma nova espécie. *Brasil-Médico*, 37, 20–24.

Nylander, J.A.A., Ronquist, F., Huelsenbeck, J.P. & Nieves, Aldrey, J.L. (2004) Bayesian phylogenetic analysis of combined data. *Systematic Biology*, 53, 47–67.

Obara, M.T., Rosa, J.A., Ceretti Junior, W., Urbinatti P.R., Quintero, L.O., Barata, J.M.S., Galvão, C. & Jurberg, J. (2007) A study of the scutellum in eight Chagas disease vector species from genus *Triatoma* (Hemiptera, Reduviidae) using optical and scanning electron microscopy. *Memórias do Instituto Oswaldo Cruz*, 102 (4), 463–468.

Quintero, L.O. (2002) *Avaliação do valor sistemático do processo do I urotergito em machos de onze espécies de importância em saúde pública da subfamília Triatominae (Hemiptera, Reduviidae).* Doctoral Thesis, Faculdade de Saúde Pública, Universidade de São Paulo, São Paulo.

Rosa, J.A., Barata, J.M.S., Cilense, M. & Neto, F.M.B. (1999) Head morphology of 1st and 5th instar nymphs of *Triatoma circumaculata* and *Triatoma rubrovaria* (Hemiptera, Reduviidae). *International Journal of Insect Morphology and Embryology*, 28, 363–375.

Rosa, J.A., Justino, H.G. & Barata, J.M.S. (2003) Diferença no tamanho de cascas de ovos de colônias de *Panstrongylus megistus*. *Revista de Saúde Pública,* 37(4), 528–530.

Rosa, J.A., Mendonça, V.J., Rocha, C.S., Gardim, S. & Cilense, M. (2010) Characterization of the external female genitalia of six species of Triatominae (Hemiptera: Reduviidade) by scanning electron microscopy. *Memórias do Instituto Oswaldo Cruz*, 105, 286–292.

Rosa, J.A., Tres, D.F.A., Santos, J.L.F. & Barata, J.M.S. (2000) Estudos morfométrico dos segmentos antenais de ninfas e adultos de duas colônias de *Triatoma rubrovaria* (Blanchard, 1843) (Hemiptera, Reduviidae). *Entomologia y Vectores*, 7 (3), 255–264.

Schlick-Steiner, B.C., Steiner, F.M., Seifert, B., Stauffer, C., Christian, E. & Crozier, R.H. (2010) Integrative Taxonomy: A Multisource Approach to Exploring Biodiversity. *Annual Review of Entomology,* 55, 421–38.

Schofield, C.J. & Galvão, C. (2009) Classification, evolution, and species groups within the Triatominae. *Acta Tropica*, 110, 88–100.

Stal, C., (1859) Monographie der Gattung Conorhinus and Werwandten. *Berliner Entomologische Zeitschrift*, 3, 99–117.

Valente, V.C., Valente, S.A.S., Carcavallo, R.U., Rocha, D.S., Galvão, C. & Jurberg, J. (2001) Considerações sobre uma nova espécie do gênero *Rhodnius* Stal, do Estado do Pará, Brasil (Hemiptera, Reduviidae, Triatominae). *Entomologia y Vectores*, 8 (1), 65–80.