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Third record of *Rhodnius amazonicus* and comparative study with *R. pictipes* (Hemiptera, Reduviidae, Triatominae)

João Aristeu da Rosa^{a,*}, Eder Santos Souza^a, Adelson da Costa Teixeira^b, Rosenildo Ribeiro Barbosa^b, Aecildo José de Souza^b, Tiago Belintani^c, Juliana Damieli Nascimento^c, Hélcio Reinaldo Gil-Santana^d, Jader de Oliveira^a

^a Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas, Universidade Estadual Paulista (UNESP), Araraquara, 14800-903 SP, Brazil

^b Secretaria Estadual de Saúde-8, Centro Regional de Saúde, Breves, 68800-000, PA, Brazil

^c Departamento de Parasitologia, Instituto de Biologia, Universidade Estadual de Campinas, Campinas, 13083-862, SP, Brazil

^d Laboratório de Diptera, Instituto Oswaldo Cruz, Rio de Janeiro, 21040-360, RJ, Brazil

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ABSTRACT

Rhodnius amazonicus Almeida et al. (1973) is a triatomine of rare occurrence. This species was found for the third time in Breves city, Pará state, Brazil. Morphometric and morphological studies were carried out on one male and one female. Lent and Wygodzinsky (1979) considered this species as a synonym of *R. pictipes*, until its revalidation after 23 years by Bérenger and Pluot-Sigwalt (2002). Considering the synonym mentioned above, a comparative study between these two species was performed in order to report the third encounter of this species, and increase the number of morphological characters that distinguish *R. amazonicus* from *R. pictipes*. The dorsal side of the head, the dorsal and ventral portions of the thorax, the dorsal, ventral and posterior sides of the female genitalia, eggs and the median process of the pygophore were examined by scanning electronic micro-scopy (SEM). The head, thorax, addomen and egg parameters of these two species were also measured. The identification of characters on the head, stridulatory sulcus, mesosternum, metasternum, scutellum, process of *urotergite* I, external female genitalia, pygophore and eggs made the distinction between *R. amazonicus* and *R. pictipes* possible. This study has shown the new characters recorded and described for the first time for *R. amazonicus* and *R. pictipes* by SEM here made it possible to confirm the morphological separation between these two species.

1. Introduction

The article published by Chagas (1909) describing the protozoan *Trypanosoma cruzi* and the epidemiological chain of American trypanosomiasis provided a significant advance in Triatominae studies (Chagas, 1909; Pinto, 1927). A parameter that can be used to evaluate this statement is that, beginning with the description of *Cimex rubrofasciatus* (currently, *Triatoma rubrofasciata*) by De Geer (1773), the number of described Triatominae rose to 59 species until 1907, but only 33 of them remained as valid (Lent and Wygodzinsky, 1979). However, just 17 years after the discovery of their medical importance, there were already 62 recognized species included in this group (Pinto, 1926), most of which still valid today.

While Lent and Wygodzinsky (1979) recognized 111 species of these vectors as valid, currently the subfamily Triatominae comprises 18 genera and 152 species (Galvão, 2014; Mendonça et al., 2016; Rosa

et al., 2017). However, not all triatomine species have been studied in detail, either because their low epidemiological relevance (e.g. species of *Psammolestes* Bergroth, 1911) or because they have been recently discovered (*R. zeledoni* Jurberg et al., 2009; *R. marabaensis* Souza et al., 2016, *R. taquarussuensis* Rosa et al., 2017) or even for their rarity, such as *R. paraensis* Sherlock et al. (1977). Because of its rarity, there are only four publications including descriptions of the morphological characters of *Rhodnius amazonicus* (Almeida et al., 1973; Lent and Wygodzinsky, 1979; Bérenger and Pluot-Sigwalt, 2002; Galvão, 2014), this one being the fifth. Almeida et al. (1973) relied on the finding of a single female specimen, which was captured with light bait on October 24, 1965 in the Manaus-Itacoatiara road, Manaus city, Amazonas state, Brazil. The holotype of this species was deposited in the Parasitology Laboratory of INPA (National Institute of Amazonian Studies).

Although Almeida et al. (1973) had firstly considered the specimen to be *R. pictipes* Stål 1872, after a careful examination, they concluded

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^{*} Corresponding author at: Faculdade de Ciências Farmacêuticas (UNESP), Rodovia Araraquara-Jaú, km 1, 14800-903, Araraquara, SP, Brazil. *E-mail address:* joaoaristeu@gmail.com (J.A. da Rosa).

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Fig. 1. R. amazonicus female. A- dorsal side; B- ventral side; R. pictipes female. C- dorsal side; D- ventral side.



that it was a new species, *R. amazonicus*. Lent and Wygodzinsky (1979) did not consider *R. amazonicus* as a valid species, but they reached that conclusion without examining the holotype and relying on a comparative study based on photographs. Furthermore, they mentioned that they did not find the differences reported by the authors of the taxon (Almeida et al., 1973). Additionally, because the only female considered to be *R. amazonicus* was collected with several *R. pictipes* specimens, the validity of the new species was questioned, leading to the conclusion that the holotype of *R. amazonicus* was an abnormal, poorly preserved specimen of *R. pictipes* (Lent and Wygodzinsky, 1979). However, in 2002, after finding an atypical couple among 100 specimens of *R. pictipes* collected in French Guiana, Bérenger and Pluot-Sigwalt (2002) revalidated *R. amazonicus*.

In this article, a male and a female of *R. amazonicus* found in Breves, Pará, were compared with males and females of *R. pictipes* maintained in a laboratory colony. The present study confirms and provides additional differences of characters between *R. amazonicus* and *R. pictipes* verified by the descriptors and revalidators (Almeida et al., 1973; Bérenger and Pluot-Sigwalt, 2002).

The terminology mainly follows Lent and Wygodzinsky (1979).

2. Material and methods

In July 2015, during a careful examination of dead triatomine specimens from the Entomology Laboratory of the Eighth Regional Health Center, Breves, Pará state, Brazil, among several specimens identified as *R. pictipes*, a couple with distinct features remained unidentified at first. In the Parasitology Laboratory of the Department of Biological Sciences of the Faculty of Pharmaceutical Sciences, Unesp-Araraquara, upon consultation of the key to *Rhodnius* species belonging to the *pictipes* group presented by Bérenger and Pluot-Sigwalt (2002), the unknown couple was finally identified as *R. amazonicus* and studied here (Figs. 1 A,B, 2 A,B).

The specimens of *R. pictipes* used in the present study were obtained from colonies kept at the Triatominae Insectarium of the Faculty of Pharmaceutical Sciences, São Paulo State University (Unesp/ Araraquara, Triatominae Colonies of Araraquara – CTA 072) (Figs. 1 C,D, 2 C,D). The first specimens of this colony were received from the Evandro Chagas Institute, Belém, Pará state, Brazil, on January 18, 1998.

Eighteen characters of ten females and sixteen characters of ten males of *R. pictipes* (Table 1) were analyzed, as well as, seventeen characters of the female specimen and thirteen characters of the male specimen of *R. amazonicus*, in view of the absence of antennal segments (Rosa et al., 2010; Souza et al., 2016). Length and opercular opening of two eggs of *R. amazonicus* and thirty eggs of *R. pictipes* were measured (Table 1). The eggs of *R. amazonicus* were obtained by opening the abdomen to study the external female genitalia. We report that this is the second time we have studied eggs obtained from the ovaries of females. Variations between the parameters analyzed were estimated by Student's *t*-test, and values were calculated using the GraphPad Prism software (version 5.00, Windows, GraphPad Software, San Diego,

Fig. 2. R. amazonicus male. A- dorsal side; B- ventral side; R. pictipes male. C- dorsal side; D- ventral side.



Table 1 Mean of measurement (mm) of specimens of Rhodnius amazonicus and Rhodnius pictipes, eggs, females and males.

Male Female Eggs R. amazonicus R. pictipes R. amazonicus R. pictipes R. amazonicus R. pictipes HL 3.81 4.34 4.15 3.93 IE 1.49 1.66 1.59 1.68 AO 0.55 0.48 0.59 0.50 PO 0.58 0.98 0.80 0.90 1.76 0.71 1.77 AT 0.64 2.31 2.52 AO 2.64 2.44 R1 0.81 0.87 0.64 0.85 R2 2.89 3.56 2.61 3.31 R3 0.77 0.79 0.74 0.75 17.09 20.39 16.2 TL. 18.40 TTL 5.67 5.12 5.09 5.11 TAL 8.19 10.38 6.94 6.90 0.29 0.26 0.39 0.39 A1 A2 2.98 2.42 2.99 _ A3 1.16 2.21 _ 2.6 A4 1.60 _ 1.67 MLE _ 2.02 1.78 _ --LOO _ 0.44 0.43 _ _ _

HL, head length; IE, inner distance between eyes; AO, anteocular distance; PO, postocular distance (excluding neck); AT, Distance between Antenniferous tubercles and Clypeus; AO, Ante-Ocular Distance, R1, R2, and R3, lengths of first, second, and third rostral segments, respectively; TL, Total length of the Triatominae; TTL, Total Thorax Length; TAL, Total Abdomen Length; A1, A2, A3 and A4, 1st, 2nd, 3rd, and 4th antennal segments, MLE – maximum length of egg, LOO – length of the opercular opening, respectively. The values in bold were significant at $\alpha = 0.05$, using unpaired *t*-test.

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In the morphological studies, two females, one of R. amazonicus and one of R. pictipes, were analyzed using scanning electron microscope (SEM). The characters studied were the dorsal portion of the head, ventral and dorsal portion of the thorax, and the dorsal, ventral and posterior portions of the external female genitalia, as well as eggs and the male pygophore similarly as done in the work of Souza et al. (2016). Images were obtained from a stereoscopic microscope Leica MZ APO and Motic Images Advanced software, version 3.2, and SEM under a Topcon SM-300 microscope.

0051 m 0051

3. Results

3.1. Morphological remarks - Rhodnius amazonicus Almeida et al., 1973

Material examined - BRAZIL: Pará: Breves: 1°40'41.6"S, 50°28'46.3"W, deposited in Triatominae Collection Dr. José Maria Soares Barata of the São Paulo State University (UNESP), School of Pharmaceutical Sciences, Araraquara (1 \circlearrowleft 1 \bigcirc).

Rhodnius pictipes Stål, 1872

Material examined - BRAZIL: Pará: Belém: Colony from Instituto Evandro Chagas, initiated on January 18 of 1998, deposited at Insectarium Triatomine of the São Paulo State University (UNESP), School of Pharmaceutical Sciences, Araraquara (15 \circlearrowleft 15 \bigcirc).

3.2. General morphological remarks of Rhodnius amazonicus

Total length: male: 16.2 mm, female: 17.9 mm. General color

vellowish brown, with scattered dark spots, appearance not mottled. Head: integument slightly granulose; general coloration brownish with a yellow brownish longitudinal band on anteocular region, dorsally.

500µm 0018

Anterior and lateral margins of the pronotum with yellowish carenas. Pronotum: fore lobe with six irregular dark bands; hind lobe with six dark longitudinal bands and 1 + 1 yellowish submedian carenas and integument granulose. Hemelytra do not reach the extremity of the abdomen in the female. Connexivum yellowish, with a triangular black spot on each segment. (Figs. 1 A,B, 2 A,B).

3.3. Comparative features between R. amazonicus and R. pictipes

3.3.1. Head

Rhodnius amazonicus: central longitudinal portion of the head slightly delimited with a pair of glabrous stripes; clypeus delimited; larger genae; anteclypeus convex (Fig. 3A). Rhodnius pictipes: the head is larger and the central longitudinal portion is not delimited by glabrous lines; smaller genae with the posterior portion more lager anteclypeus straight (Fig. 3B).

3.3.2. Thorax

The terminal portion of the scutellum of R. amazonicus (Fig. 4A) is round-shaped, whereas on R. pictipes (Fig. 4B) it is tapered. There are two (1 + 1) lateral strangulations at the median portion of the scutellum of R. pictipes (Fig. 4B) while the scutellum of R. amazonicus does not possess such markings (Fig. 4A).

The stridulatory sulcus of R.amazonicus (Fig. 5A) is shorter and wider, whereas in R. pictipes it is longer and thinner (Fig. 5C). Between



Fig. 6. Morphological details of male genitalia by drawings. A-D- R. pictipes and E-H- R. amazonicus: A and E: phallosoma plate, B and F: Median process of the pygophore, C,D,G,H: parameres.



Fig. 7. Median process of the pygophore by SEM. A-R. amazonicus; B- R. pictipes. V: format of pygophore; U: format of pygophore.



100ym 0055

Fig. 8. Female external genitalia by SEM, dorsal, posterior and ventral side. A,B and C- R. amazonicus; D,E and F- R. pictipes. Gc8: gonocoxiteVIII; Gp8: gonapophysis VIII; VII, VIII, IX: esternites; X: segment (Rosa et al., 2010).

the meso and metasternum, medially, there is a slit in *R. amazonicus* (Fig. 5B), which is absent on *R. pictipes* (Fig. 5D). The posterior margin of the metasternum of *R. amazonicus* is uniform in length, whereas on *R. pictipes* it is narrowed in the median portion (Fig. 5B,D).

3.3.3. Abdomen

The anterior margin of the process of urotergite I in *R. amazonicus* is evidently transversely grooved, what is not observed in *R. pictipes*. Just below the distal portion of the process of urotergite I, there is a median conspicuous oval ridge with three pairs of subparallel ridges beside it in *R. amazonicus*, whereas in *R. pictipes* such structures are absent (Fig. 4A,B).

3.3.4. Male genitalia

The median processes of the pygophores present flat bifurcations,

disposed in V-shape in R. amazonicus (Figs. 6 F, 7 A) and in U-shape in R. pictipes (Figs. 6 B, 7 B).

The parameres of *R. amazonicus* are markedly curved at distal half, with several setae at the apex (Fig. 6G,H), while the parameres of *R. pictipes* are only slightly curved, with few setae at apex (Fig. 6C,D)

The base of phallosoma plate in *R. amazonicus* is U-shaped and its apical margin is rectangular (Fig. 6E), while in *R. pictipes*, these portions are V-shaped and rounded respectively (Fig. 6A).

3.3.5. Female genitalia

In the same plane of the dorsal view of the external female genitalia, only the terminal portions of the IX and X segments of *R. amazonicus* are seen (Fig. 8A), whereas in *R. pictipes* these two segments seem much larger and the X is very prominent (Fig. 8D). In the two species, the line dividing the VII and VIII segments are curved, but the lateral posterior

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Fig. 9. Egg general vision and egg exochorion detail by scanning electron microscopy. A and B- *R. amazonicus*; C and D- *R. pictipes*. Cl: collar; cr: chorial rim; ex: exochorion; nk: neck; ce: exochorion cell; ll: limiting line.

Table 2

Distinguishing characters among ten species of Brazilian Amazon region.

Species	Distinctive characters	References
R. amazonicus	Appearance not mottled, hemelytra do not reach the extremity of the female's abdomen and the bifurcation of median processes of the pygophore bispinous is in the form of the letter V.	This work
R. brethesi	Present rectangular spots of orange or red color on the connexivum.	Lent and Wygodzinsky (1979
R. marabaensis	The second antennal segment is 10.3 times larger than the first. The scutellum is larger and includes two prominent internal lateral carenas.	Souza et al. (2016)
R. milesi	The male genitalia present a second process of the phallosoma. Divergent antennal tubercle with an apical denticle.	Valente et al. (2001)
R. montenegrensis	Anterior wings with well-demarcated veins, notable the Sc by a yellow tonality. Abdomen presents yellow spots interposed with dark ones over the ventral abdomen lengthwise.	Rosa et al. (2012)
R. paraensis	Presents the smaller length of the male (10.5–11.0 mm) and the female (10.8–12.0 mm).	Lent and Wygodzinsky (1979
R. pictipes	Head an legs with general color pattern consisting of small irregular spots and dots, thus conspicuously mottled and median processes of the pygophore bispinous in the form of the letter U.	Lent and Wygodzinsky (1979) and this work
R. prolixus	Anteocular region slightly over three times as long as postocular. Specimens distance between eyes dorsally larger than width of eyes in dorsal view.	Lent and Wygodzinsky (1979)
R. robustus	Anteocular region about four times as long as postocular. Specimens distance between eyes dorsally smaller than, or equal to, width of eye in dorsal view.	Lent and Wygodzinsky (1979)
R. stali	Region anteocular up to 2.5 times greater than ocular post.	Galvão (2014)

*Species of Brazilian Amazon region according to Souza et al. (2016).

[or distal] extremities of the VII are short on *R. amazonicus* (Fig. 8A) and long in *R. pictipes* (Fig. 8D).

The posterior view of the IX segment shows two large depressions and two large flaps, both of which more pronounced in *R. amazonicus* (Fig. 8B) and smaller in *R. pictipes* (Fig. 8E).

The ventral view of the external female genitalia of *R. amazonicus* shows that the two gonocoxites VIII are curved along their entire length and drop-shaped, whereas on *R. pictipes* they are rectilinear at the upper portion of the central limit with the segment VII and the gonapophysis

VIII, curved at the posterior portion and larger than those of *R. amazonicus* (Fig. 8B,E). Also on the ventral view, the IX segment of *R. amazonicus* shows two deep depressions and two lateral flaps that can be seen on posterior view, and on *R. pictipes* the lateral flaps are smaller and partially covered by the segment VII (Fig. 8C,F). Another difference can be noted: the shape of the X segment is semi-circular on *R. amazonicus* and straight on the terminal portion on *R. pictipes*. It is easily noticeable by ventral view that on *R. pictipes* the X segment is much longer than the IX one, while on *R. amazonicus* the IX and X segments



Fig. 10. Distribution of *Rhodnius amazonicus*. The yellow square and green circle indicates the first and second record of *Rhodnius amazonicus* from literature and the red circle indicates the new record of *Rhodnius amazonicus* in Breves, Pará state. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

have basically the same length (Fig. 8C,F).

3.3.6. Eggs

The eggs of *R. amazonicus* are longer (2.02 mm in length) and their opercular openings show different sizes (0.44 mm), when compared to those of *R. pictipes* (1.78 mm and 0.43 mm respectively)., The eggs of *R. amazonicus* present collar (Fig. 9A), which is absent in *R. pictipes* (Fig. 9C). Exocorial cells of both species are well delimited; however, the limiting line of the exocorial cells of *R. amazonicus* are considerably elevated (Fig. 9B), whereas in the case of *R. pictipes* they are slightly sulcate (Fig. 9D). The diameters of the follicular tubes also differ for the two species, being larger in *R. amazonicus* (Fig. 9B,D).

4. Discussion

Almeida et al. (1973) described *R. amazonicus* by comparing it with *R. pictipes*. They took into account the coloration, measurements of twelve head characters, seven thorax characters and three abdomen characters of the female holotype, presenting a total of eight figures. Subsequently, *R. amazonicus* was considered as a synonym of *R. pictipes* by Lent and Wygodzinsky (1979).

Twenty-three years later, however, *R. amazonicus* was revalidated. This was the result of comparative studies with *R. pictipes* using characters of the coxae, trochanter, femur, connexivum, female and male genitalia. The revalidation was illustrated with twenty one drawings and five optical microscopy images (Bérenger and Pluot-Sigwalt, 2002).

In the present study, it was found that the dorsal portion of the head of *R. amazonicus* and *R. pictipes* differs in demarcations of glabrous lines

and clypeus; size and shape of genae and anteclypeus (Fig. 3). Scutellum and I urotergite process of *R. amazonicus* and *R. pictipes* are different (Fig. 4). They also differ from *R. montenegrensis* and *R. robustus* (Rosa et al., 2012).

Rhodnius amazonicus and *R. pictipes* differ in shape and size of the stridulatory sulcus and of the portion situated between the pro and mesosternum (Fig. 5).

Examination of the dorsal portion of the external female genitalia showed that IX and X segments are clearly smaller on *R. amazonicus* when compared to *R. pictipes* (Fig. 8A,D), a difference which can be noted on the ventral view too (Fig. 8C,F). On posterior view, differences were recorded on the anterior and lateral portions of the segment IX (Fig. 8B,E). The size and shape of the eighth gonocoxite are different, as well as the shape of segments IX and X on examination of the ventral portion (Fig. 8C,F).

Both median processes of the pygophores of *R. amazonicus* and *R. pictipes* are bifurcate. However, the bifurcation of *R. amazonicus* is in the form of the letter V and the *R. pictipes* of the letter U (Figs. 6 B,F, 7 A,B).

The differences between eggs of *R. amazonicus* and *R. pictipes* are very clear on size, shape and general design (Table 1). The exocorial cells (Fig. 9B,D) are also clearly distinct.

By including a SEM of dorsal portions of head, scutellum, process of urotergite I, stridulatory sulcus, meso and metathorax, external female genitalia, median processes of pygophores and eggs, which were not observed in the description and redescription of *R. amazonicus*, this study has contributed to facilitating the identification of the species.

Among the characters measured the differences were significant between *R. amazonicus* and *R. pictipes* in thirteen male characters and *Rhodnius amazonicus* and *R. pictipes* occur in the North region of Brazil along with *R. brethesi*, *R. marabaensis*, *R. milesi*, *R. montenegrensis*, *R. paraensis*, *R. prolixus*, *R. robustus* and *R. stali* (Galvão, 2014; Souza et al., 2016). Because of this, the Table 2, which is a synopsis of the main characters used to distinguish them, was included.

Characteristics of the female external genitalia suggest that *R. amazonicus* may be grouped with *R. milesi, R. pictipes, R. prolixus* and *R. stali* (all from the North region of Brazil) as well as with *R. domesticus* Neiva and Pinto (1923), *R. ecuadoriensis* Lent and León (1958), *R. na-sutus* Stål (1859) and *R. neivai* Lent (1953), due to the fact that the intersegmental suture between tergites VII and VIII is curved (Rosa et al., 2014). However, *R. amazonicus* distinguishes itself from the other nine species of the North region of Brazil in characters of the dorsal, posterior and ventral portions of the external female genitalia (Rosa et al., 2014) (Fig. 8).

The eggs of *R. amazonicus* present a lateral flattening, a neck, found in the other *Rhodnius* species, and the collar, also found in *R. milesi*, *R. montenegrensis*, *R. neglectus* and *R. paraensis*, but absent in *R. brethesi*, *R. pictipes*, *R. prolixus*, *R. robustus* and *R. stali* (Barata, 1981; Rosa et al., 2012). Nevertheless, evident characters in the exocorial cells of their eggs can distinguish these nine species of *Rhodnius* from the North region of Brazil (Table 2).

The eggs of *R. amazonicus* present a lateral flattening, a neck, found in the other *Rhodnius* species, and the collar, also found in *R. milesi, R. montenegrensis, R. neglectus* and *R. paraensis*, but absent in *R. brethesi, R. pictipes, R. prolixus, R. robustus* and *R. stali* (Barata, 1981; Rosa et al., 2012). Nevertheless, evident characters in the exocorial cells of their eggs can distinguish these nine species of *Rhodnius* from the North region of Brazil (Table 2). According to Carcavallo et al. (2000), *Rhodnius* species can be classified into five complexes, and they included *R. amazonicus* in the *R. pictipes* complex (Carcavallo et al., 2000).

5. Conclusions

Eleven characters of a male, a female and eggs of *R. amazonicus* were analyzed by SEM and seventeen were measured. Simultaneously, specimens of *R. pictipes* were similarly studied and measured. Twenty eight were used to illustrate the differences and differentiate these species.

It is worth noting that the identification of characters on the head, stridulatory sulcus, mesosternum, metasternum, scutellum, process of urotergite I, external female genitalia, pygophore and eggs made the distinction between *R. amazonicus* and *R. pictipes* possible. As only two encounters of *R. amazonicus* are reported, this contribution aims to report the third encounter of this species (Fig. 10), as well as to increase the number of morphological characters that distinguish *R. amazonicus* from *R. pictipes*.

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