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Phenotypic Variability of the Amazonian Species *Rhodnius brethesi* (Hemiptera: Reduviidae)

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Abstract

The wild species *Rhodnius brethesi* (Matta, 1919) (Hemiptera: Reduviidae) is found in areas of piassabais in microregion of Rio Negro, Amazonas, Brazil. Its geographical distribution overlaps the areas of *Leopoldinia piassaba* palm. In areas where palm trees are found, transmission cycle of *Trypanosoma cruzi* is related to the extractive activity of the palm fiber, exposing workers to wild vector transmission of this parasite. The close association with the palm tree *L. piassaba* suggests that this wild triatomine has special features in its sensory system allowing specificity of ecotope. The objective of the study is to identify the antennal sensilla phenotype and morphologically characterize the size and shape of the wings of wild *R. brethesi*, and to compare with the phenotype present in individuals reared in the laboratory. From the samples taken in the field, the presence of the species *R. brethesi* was found on both banks of the Rio Negro. The techniques used to verify the morphological patterns are important resources for observations of the triatomine populations, be they in an artificial or natural habitat.

Key words: *Rhodnius brethesi*, sensilla, wing, specificity, *Leopoldinia piassaba*

Rhodnius brethesi (Matta, 1919) (Hemiptera: Reduviidae) is a triatomine species found in piassaba palm trees in the microregion of Rio Negro, state of Amazonas, Brazil (Mascarenhas 1991; Coura et al. 1994, 1999; Junqueira 2005). This species is known as the "piassaba louse" among workers who extract fiber from this palm tree (Mascarenhas 1991, Junqueira 2005, Souza 2013), which is an important economic activity in the upper and middle courses of the Rio Negro (Meira 1993, Oliete-Josa 2008) and along the Orinoco River, in the western Amazon region (Henderson 2011, Smith 2015).

In serological surveys conducted in the autochthonous human population, significant prevalence of Chagas disease was observed, with rates ranging from 6 to 13% (Coura et al. 1999, 2002; Junqueira 2005). Positive cases were attributed to continuous exposure of workers to this vector species (Coura et al. 1999, 2002). In the areas where these palm trees are distributed, the vectorial transmission of *Trypanosoma cruzi* to humans may overlap with the palm tree fiber extraction activities, exposing workers to this parasite. Therefore, extraction of *Leopoldinia piassaba* is a possible risk factor for infection with *T. cruzi*, the etiological agent of Chagas disease (Coura et al. 1994, Coura et al. 2002).

Most of the species of the genus *Rhodnius* are associated especially with palm tree habitats (Dias et al.2008, Jurberg et al. 2009,

Gonçalves et al. 2013, Abad-Franch et al. 2015). This association can be defined as generalist, in the case of species that are found to infest several types of palm trees, or specialist, when they infest only one type of palm tree, as in the case of *R. brethesi* (Abad-Franch et al. 2015). The association with the palm tree *L. piassaba* suggests that this wild triatomine presents special characteristics in its sensory system.

Triatomines perceive various stimuli through antennal sensory receptors (sensilla) that function as chemoreceptors, mechanoreceptors, thermoreceptors, and hygroreceptors (Catalá and Schofield 1994, Gullan and Cranston 2012). The most important of these are the smell and taste sensilla (chemoreceptors), which detect chemical components relating to food sources, sexual partner recognition, and habitat preferences (Guerenstein and Guerin 2001, Barrozo and Lazzari 2004, Dujardin et al. 2009). Antennal phenotypes, i.e., sets of antennal sensilla (varying in type and number), present differences that make it possible to distinguish triatomine genera, species, and even populations. Because of the large numbers of sensory receptors present in antennae, the antennal phenotype may be an indicator of the adaptation of these triatomines to ecotopes of different complexity and stability (Catalá 1997, Gracco and Catalá 2000, Catalá and Dujardin 2001, Carbajal de la Fuente and Catalá 2002, Catalá et al. 2004, Dujardin et al. 2009, Villacís et al. 2010).

In various triatomine species, a series of morphological and genetic changes associated with adaptations from wild habitats to domestic and laboratory conditions have been observed (Catalá et al. 2004, Abrahan et al. 2008, Hernández et al. 2008, Dujardin et al. 2009). Vector species populations kept in laboratories can undergo antennal phenotype modifications in which the number of sensilla may vary, depending on the rearing conditions provided and the number of generations spent in the artificial environment (Catalá et al. 2004). Such changes indicate the degree of phenotypic plasticity exhibited by the species. This plasticity has been observed through geometric morphometry in comparisons among individuals bred under different laboratory conditions (Riaño et al. 2009).

Geometric morphometry is based on quantitative description of morphological variations between structures. It arose as a low-cost methodology that presented promising results for detecting sources of phenotypic variability among organisms from different species, development stages, and environments. It has contributed toward characterizing the interaction dynamics among wild, peridomestic, and domestic populations (Catalá et al. 2004, Borges et al. 2005, Gurgel-Gonçalves et al. 2011). However, only a few studies have provided information about the phenotypic variability found between wild triatomines and those bred under artificial laboratory conditions (Jaramillo et al. 2002, Riaño et al. 2009). The phenotypic variability of wild and laboratory specimens of *R. brethesi* has not previously been evaluated through wing geometric morphometry and neither has their antennal phenotype been evaluated.

The objective of the present study was to identify the antennal sensilla phenotype and morphologically characterize the size and shape of the wings of wild *R. brethesi*, and to compare these with the phenotypes present in specimens sampled from the same wild area and bred in the laboratory for several generations. A comparative quantitative and qualitative analysis on these two structures in the natural environment and in the laboratory was proposed as an initial investigation on the phenotypic plasticity of this species, i.e., the capacity of the genotype to produce different phenotypes in response to changes in environmental conditions (Pigliucci 2001).

The hypothesis of the present study was that the antennal phenotype and wings would be found to present morphological characteristics intrinsic to the specific species of *R. brethesi*. The pattern of sensilla has higher density and length of chemoreceptors for recognition and perception of ecotopes. Wild specimens must have greater sensilla density than laboratory specimens. The wings should also reflect the change of ecotopes, and reduced size in laboratory specimens. Antennal morphology may be directly involved in the specificity of this wild species to a single palm tree species where these insects develop. In combination with the geometric morphometry of the wings, it may be capable of distinguishing individuals according to the ecotope.

Materials and Methods

Study Area
The specimens used in the present study were caught in areas of fiber extraction from the palm tree *L. piassaba* (piassaba palm tree) in the municipalities of Barcelos (00° 58′30″ S, 62° 55′26″ W) located in the microregion of the Negro River, state of Amazonas, Brazil. In Barcelos (right bank of the Negro River), specimens were caught along the Acuquaia creek (igarapé) of the Padauirí River.

Triatomines
In addition to wild triatomines, specimens of *R. brethesi* belonging to the 21st generation bred in the Laboratory of Parasitic Diseases,

IOC/Fiocruz, were also used. The parental generation of the laboratory specimens had been caught in the same area where the wild individuals were caught.

Capture of Wild Triatomines
Triatomines were caught in the wild environment with the aid of two types of traps. To catch nymphs, Noireau traps fixed on 23 piassaba palm trees (*L. piassaba*) were used (Noireau et al. 2002), with mice as bait (License: LW-56/12). The Malaise light trap (adapted from Townes 1972) was used for catching adult specimens. Specimens thus caught were sent to the laboratory and identified through using a dichotomous identification key, in accordance with Lent and Wygodzinsky (1979).

Antennal Phenotype and Statistical Analysis
Antennae from 92 adult individuals of *R. brethesi* were removed and preserved in 70% alcohol. The methodology used by Catalá et al. (2005) for cuticle whitening was used with the objective of identifying and counting sensilla. One antenna from each specimen was placed between a slide and a cover slip, on the ventral side, for identification under a microscope (400×). A clear chamber was used to count bristle mechanoreceptors (BR) and chemoreceptors: thin-walled trichoid (TH), thick-walled trichoid (TK), and basiconic (BA; Fig. 1; Catalá and Schofield 1994). The TH and TK chemoreceptors were measured from the base to the apex, with the aid of micrometric ocular lenses (10× magnification; ZEISS) coupled to an optical microscope.

The type and quantity of sensilla in each segment (pedicel P, flagellum-1 F1, and flagellum-2 F2) of the antenna was analyzed and compared between the groups of *R. brethesi*. The comparison between wild and laboratory specimens was made according to sex. The lengths of TH and TK chemoreceptors of *R. brethesi* were measured and compared between these two groups. Levene's test was used for determining the homogeneity among variables. Those that presented homogeneous values were analyzed using t tests ($P < 0.05$). For those that presented heterogeneity, the nonparametric Mann–Whitney U test was used. Discriminant analysis was performed using PADWIN software (Dujardin 2004), with the aim of identifying morphological differences between groups. The statistical significance of such differences was estimated through Mahalanobis distance values for nonparametric studies by means of a permutation test (permutation 1,000), after the Bonferroni correction test. These distances were used in cluster analyses by means of an unweighted pair-group method with arithmetic average (UPGMA), in order to produce dendrograms (neighbor-joining/ UPGMA method version 3.65; negative branch lengths allowed).

Wing Geometric Morphometry and Statistical Analysis
Measurement Data
In total, 106 right wings of wild and laboratory specimens of *R. brethesi* were removed at the level of the joint with the thorax and were placed between two slides in order to flatten them. They were then photographed with identification and scale, using a digital camera coupled to a Luxeo 4D stereo microscope with 0.8 objective lenses (8× magnification). Nine type I anatomical points (landmarks) were selected (Bookstein 1991; Fig. 2).

Size Variation
To compare wing sizes between groups, we used the isometric estimator known as centroid size (CS), which is derived from coordinate data. Centroid size is defined as the square root of the sum of the
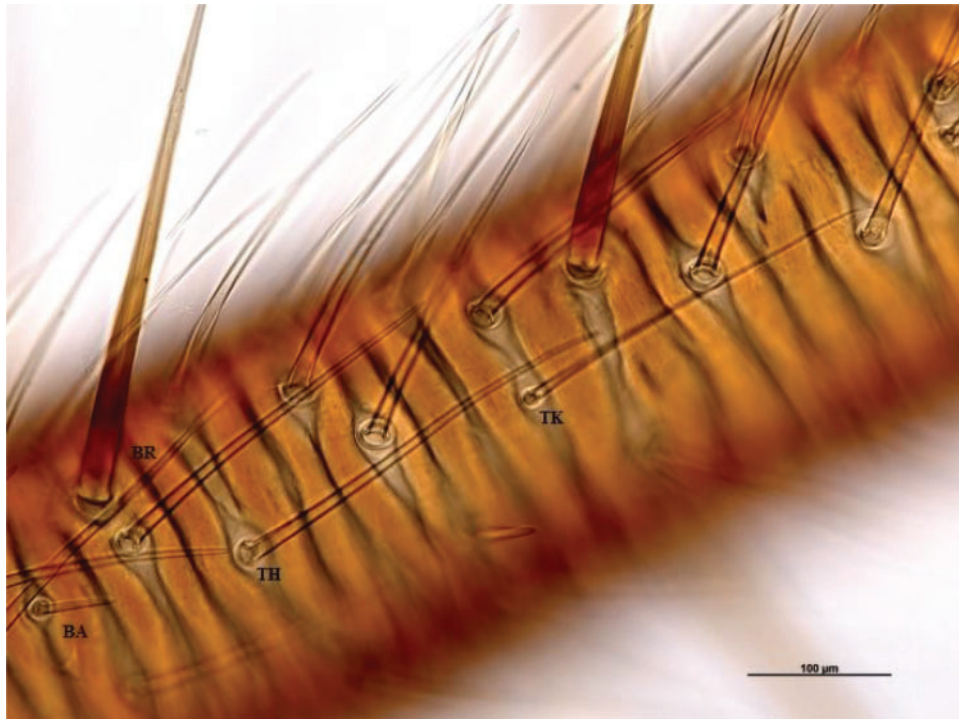
Fig. 1. Optical microscopy of sensilla on antennae of *R. brethesi*. BR, bristles; TH, thin-walled sensilla trichoidea; TK, thick-walled sensilla trichoidea; BA, basiconica.
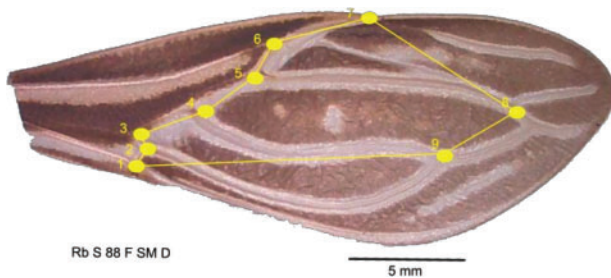

Fig. 2. Dorsal view of the right wing of *R. brethesi*. The points indicate the nine landmarks used for the analysis.

squared distances between the center of the configuration of landmarks and each individual landmark (Bookstein 1991).

Shape Variation

Shape variables (partial warps) were obtained using a superimposition algorithm within generalized Procrustes analysis (Rohlf 1996). This method was based on superimposition of each individual using least squares, thereby eliminating the effects of scale, orientation, and position of the objects. The shape variables defined the positional changes at each landmark in relation to a consensus shape. Mahalanobis distances were derived from the selected set of relative warps and then used to construct unweighted pair-groups with an arithmetic-average dendrogram using the NEIGHBOR module of the PHYLIP package (Felsenstein 2005). The relationship between CS and shape variation (residual allometry) was estimated by means of multivariate regression (Good 2000).

Data Analysis

A nonparametric comparison employing Wilcoxon was used to examine differences in CS for sexual dimorphism and ecotope. The

Mahalanobis distances derived from shape variables were used to explore the closeness of shapes between groups. Their statistical significance was calculated through permutation tests (1,000 runs each) and was corrected by means of the Bonferroni method. These distances were used in an UPGMA cluster analyses to produce dendrograms. Discriminant analyses were performed to evaluate the existence of significant differences at habitat level and to reclassify the individuals using the discriminant functions and Mahalanobis distances.

Software

For morphometric analysis, we used COO for landmark digitization; MOG for Procrustes superimposition and generation of CS, PW, and RW; PAD for discriminant analyses, nonparametric permutation tests, and reclassification tests; and PHYLIP (version 3.5c) for the UPGMA tree construction. The modules MOG and PAD, which were developed by J.P. Dujardin, were included in the CLIC package (available at www.mpl.ird.fr/morphometrics). The statistical analysis was done using JMP v4 (SAS Institute, Inc, Cary, NC).

Results

Antennal Phenotype of Wild *R. brethesi*

The antennal phenotype presented the general characteristics previously found in other species of Rhodniini, in which the absence of trichoid and basiconic chemoreceptors on the pedicels characterizes the first difference with other tribes of Triatominae. Table 1 presents the mean values, standard deviations, and medians of the numbers of antennal sensilla in males and females of wild specimens of *R. brethesi*. The comparative analysis on the number of sensilla in male and female specimens showed the presence of sexual dimorphism in two types of sensilla present in F2. The males presented a higher number of TH ($P = 0.02$) in F2; and females, of TK ($P = 0.02$).

Table 1. Mean, standard deviation, and median of the comparative analysis of the antennal phenotype of specimens of *R. brethesi*

	n	F1-BR	F1-TH	F1-BA	F1-TK	F2-TH
Lab_F	20	18.55 (2.70) 19	109.35 (20) 109	16.50 (5.58) 16	x	28.70 (10.12) 27
W_F	24	15.00 (4.05) 15.5	81.25 (11.78) 83.5	23.54 (4.97) 24	x	23.58 (4.63) 23
Lab_M	23	18.52 (3.52) 18	98.43 (20.81) 94	x	64.21 (33.01) 49	x
W_M	26	15.92 (3.68) 17.5	81.69 (11.42) 80.5	x	80.11 (19.19) 80.5	x

M, male; F, female; L, laboratory; W, wild; *n*, number of individuals used in the analysis.

F1, flagellum first segment; F2, flagellum second segment; BR, bristles; TH, thin-walled trichoidea; TK, thick-walled trichoidea; BA, basiconica.

Antennal Phenotypic Changes Induced by Rearing of *R. brethesi* in the Laboratory

The comparison between male and female phenotypes did not show any sexual dimorphism among the specimens of *R. brethesi* ($P < 0.05$) bred in the laboratory. Males of *R. brethesi* bred in the laboratory presented a higher density of F1-BR ($P = 0.01$) and F1-TH ($P = 0.00$), and lower quantity of the receptor F1-TK ($P = 0.00$), in comparison with wild males (Table 2).

Comparison of the antennal phenotypes of females bred in the laboratory and wild females showed statistically significant values for three sensilla from F1, and one from F2. The laboratory females presented a higher density of receptors of F1-BR ($P = 0.00$), F1-TH ($P = 0.00$), and F2-TH ($P = 0.00$), and a lower number of F1-BA ($P = 0.00$), in comparison with wild females (Table 2).

Multivariate Analysis of the Antennal Phenotype of *R. brethesi*

In this analysis, all the receptors studied were used (P-BR, F1-BR, F1-TH, F1-TK, F1-BA, F2-BR, F2-TH, F2-TK, and F2-BA). Mahalanobis distance (between females: 2.82; between males: 1.89) showed that there was a clear phenotypic separation among groups from different ecotopes, and it was more accentuated between females (Fig. 3).

Wing Size Variation

Sexual dimorphism was observed in wild and laboratory *R. brethesi* specimens. Both wild females' wings (Wilcoxon test q = 1.95; $\alpha = 0.05$; $P < 0.0002$) and laboratory females' wings (Wilcoxon test q = 1.95; $\alpha = 0.05$; $P < 0.0001$) were significantly larger than the males' wings (Fig. 4).

There were no significant differences in wing size between laboratory females (median CS = 521.97; Q25 = 504.32; Q75 = 538.87) and wild females (median CS = 525.01; Q25 = 514.91; Q75 = 544.26), and the same was observed between laboratory males (median CS = 491.17; Q25 = 478.49; Q75 = 505.30) and wild males.

Variation of Wing Shape According to Sex and Ecotope

The Mahalanobis distances showed significant differences among the groups studied, except between wild and laboratory males (Table 3). The UPGMA tree drawn up from the Mahalanobis distances presented similarities between individuals in the wild group. However, the laboratory males and females were placed in separated clades, which suggested that greater modification had occurred among the laboratory-bred females (Fig. 5).

The simple reclassification of individuals was considered to be moderate to acceptable, ranging from 61% to 89%. The contributions of canonic factors resulted in 59%, 37%, and 4% for the first, second, and third factors, respectively. The multivariate regression analysis on the shape variables and CS did not present any significant effect (Wilks's lambda = 0.7805; approximate $F = 0.7690$; df 1 = 30; df 2 = 261.90; $P = 0.8038$).

Discussion

The present study showed the particular features of the antennal phenotype of the wild species of *R. brethesi* in its natural environment for the first time, and compares this characteristic with laboratory-bred individuals. The study supported the hypothesis that this species had greater olfactory capacity for choosing *L. piassaba* as its ecotope, regardless of the complex environment of the piassaba palm tree. This pattern and the geometric morphometry of the wings could be used to distinguish different populations of *R. brethesi*.

The complete absence of trichoid sensilla (chemoreceptors) on the pedicel of species of the genus *Rhodnius* is a characteristic shared with species of the genus *Psammolestes*, but not with species of the genera *Triatoma*, *Dipetalogaster*, or *Panstrongylus* (Catalá and Schofield 1994). The data obtained from *R. brethesi* showed that the pedicel segment presented bristles (i.e., mechanoreceptor sensilla) and absence of chemoreceptors, thus confirming this peculiarity within the genus *Rhodnius*.

An increase in the number of mechanoreceptors BR on flagellum 1 of both sexes of *R. brethesi* created in the laboratory was observed. According to *Triatoma infestans* Klug data presented by Catalá et al. (2004), our results may be related to the high density of insects per rearing breeding flask, once believed that this sensillum detects vibration, variations in air current, and contact stimuli. The creation of the *R. brethesi* began with about 20 adults, 20 nymphs of all stadium, and 100 eggs, all the specimens from the same study site. The colony has been kept >15 yr in the laboratory. According to Catalá et al (2004), the changes observed in the sensilla model are not related to the time of breeding in the laboratory.

Specimens of *R. brethesi* presented TH receptor density greater than or equal to TK density on the first flagellum segment (F1). Types of trichoid sensilla (TH and TK) of *R. brethesi* were significantly larger than in the other 10 species of *Rhodnius* studied. This antennal phenotype characteristic is different from what was found in other species of the tribe Rhodniini (Catalá and Schofield 1994) and other species of triatomines (Catalá 1997, Moreno et al. 2005).

The ability to perceive odors may be an adaptation for ecotopes where low availability of hosts and search for partners require refined olfactory perception. Increases in the length and number of TH sensilla provide greater porous surface, which suggests that greater efficiency of perception of odor molecules is attained. Other insects (e.g., *Antheraea polyphemus* (Cramer) and *Bombix mori* (L.)) have also increased olfactory surface of their antennae in order to have a high number of multiporous sensilla that can detect small quantities of pheromones from great distances (Steinbrecht 1999).

McIver (1987) showed that other hematophagous insects have also an increased number of TH sensilla in their antennae related to

Table 2. Mean, standard deviation, and median of receptors present in wild individuals of the species *R. brethesi*

Sex	P-BR	F1-BR	F1-TH	F1-TK	F1-BA	F2-BR	F2-TH*	F2-TK*	F2-BA
Male (n = 26)	56.46 (7.70) 55	15.92 (3.68) 17	81.69 (11.42) 78	80.11 (19.19) 74	21.26 (5.11) 21	7.76 (1.92) 8	30.26 (6.70) 32	59.46 (8.62) 60	25.96 (7.27) 27
Female (n = 24)	58.50 (7.54) 58	15.00 (4.05) 15.5	81.25 (11.78) 83.5	76.58 (17.20) 70.5	23.54 (4.97) 24	8.00 (2.02) 8	23.58 (4.63) 23	64.87 (7.28) 65.5	28.83 (5.51) 28

P-BR, pedicel-bristles; F1-BR, flagellum1-bristles; F1-TH, flagellum1-thin-walled trichoidea; F1-TK, flagellum1-thick-walled trichoidea; F1-BA, flagellum1-basiconica; F2-BR, flagellum2-bristles; F2-TH, flagellum2-thin-walled trichoidea; F2-TK, flagellum2- thick-walled trichoidea; F2-BA, flagellum2-basiconica.
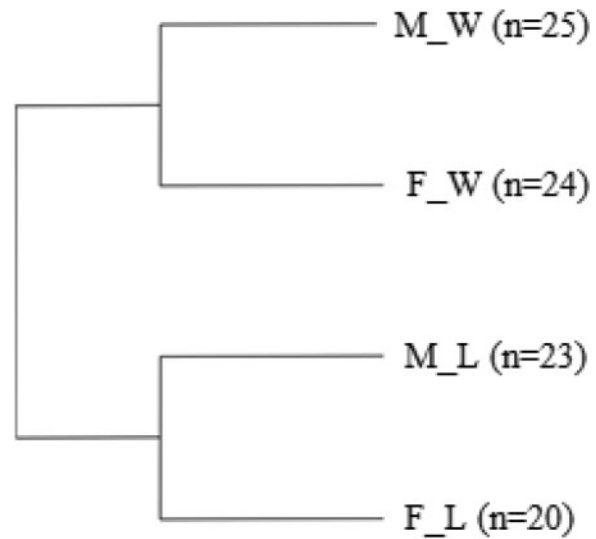
*Significant values $P < 0.05$.

Fig. 3. Dendrogram of UPGMA derivate of the distance of Mahalanobis of the antennae of *R. brethesi* grouped according to ecotope and sex. Analysis with the same variables confirmed significant distances of Mahalanobis ($P < 0.05$). M, male; F, female; L, laboratory; W, wild. The value in parenthesis indicates the number N of individuals used in the analysis.
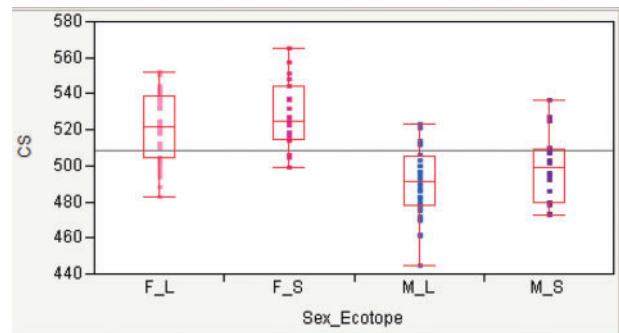

Fig. 4. Centroid size (CS) of the wings of *R. brethesi* grouped according to ecotope and sex. L, laboratory; W, wild; F, female; M, male. The boxes show the median in each group, the centerline separates the quartiles 25 and 75 and the extreme lines (10 and 90). The axis denotes the large middle and points in the centers of distribution boxes of individuals.

host search and flight autonomy. This phenomenon is very well-known among females of mosquito species that feed on blood; in contrast, a lower number of TH is observed among males that need plant fluids (McIver 1982).

Thick-walled trichoid sensilla are also longer, but their function in triatomines remains unknown. In the insect *Cimex lectularius* L., their function relates to perception of specific pheromones (Steinbrecht and Muller 1976) and detection of molecules through contact, thus acting as olfactory sensilla (Steinbrecht 1999).

Several modifications to the antennal phenotype were observed in laboratory-bred specimens of *R. brethesi*, which made it possible to differentiate them from wild insects. The changes observed related to the environment and food sources (Catalá et al. 2004), and to inbreeding. Their natural microenvironment, i.e., palm trees, is certainly different from the controlled environment of a laboratory, with stable humidity and temperature. Instead of becoming

established in the microclimate created by the fibers of the piassaba palm tree, in the laboratory, they live in large glass flasks with filter paper to absorb urine and feces. They also undergo abrupt feeding changes, such that they no longer feed on the blood of wild animals, such as reptiles (Monte 2010): their only food source becomes chicken blood.

Wild males of *R. brethesi* were found to have a larger number of TH on the flagellum than do females. This sexual dimorphism had already been observed in other species of triatomines (Catalá 1997, Catalá et al. 2005). However, the antennal phenotype of laboratory-bred individuals also presented an increase in the number of TH on the first segment of the females' flagellum.

These results suggest that wild specimens present greater olfactory perception, which would be related to high efficiency in detecting molecules of importance for sex encountering and for flight dispersal in search of new habitats. This characteristic is very important in the case of *R. brethesi*, with its high habitat specificity, in which greater olfactory capacity is required for detecting *L. piassaba* among other palm tree species.

It was possible to distinguish the specimens of *R. brethesi* according to both sex and ecotope, through antennal phenotype analysis. Nonetheless, it was sought to complement this through using geometric morphometry on the wing structures.

Morphometric differences have also been observed among triatomines from different habitats, in an attempt to explain the origin of morphological alterations (Batista et al. 2012). These differences may be related to adaptation to a certain ecotope (Dujardin et al. 1997) or to different food sources (Nattero et al. 2013), but they may also have a genetic base (Schachter-Broide et al. 2009) and were already used to assess evolutionary process (Costa et al. 2009). Some authors observed changes to the phenotypic characteristics of wing and head structures in species of triatomines, in the transition from wild habitats to laboratory conditions, and identified individuals that were undergoing a domestication process (Dujardin et al. 1999, Riaño et al. 2009, Gurgel-Gonçalves et al. 2011). Use of this technique has been used to elucidate the taxonomic status of Triatominae (Villegas et al. 2002, Carbajal de La Fuente et al. 2011,

Monte Gonçalves et al. 2013), and the tendency for populations to separate within their geographic distribution zones (Dias et al. 2011).

Wing size analysis confirmed the existence of sexual dimorphism in both ecotopes, such that both laboratory and wild females presented larger wings than did males. This characteristic is a condition of wild individuals and its loss is considered to be the beginning of a domestication process (Dujardin et al. 1999). Jaramillo et al. (2000) found a significant reduction in sexual dimorphism of the heads and wings of *Rhodnius pallescens* Barber, and showed that this phenomenon occurred in colonies with >10 generations in the laboratory. However, the results of the present study did not corroborate this finding because the laboratory-bred triatomines used had been in this artificial environment for >10 yr, and belonged to the 21st generation from the initial group sampled in the microregion of the Negro River, i.e., the same area as the wild specimens used in this study.

Body size reduction in specimens of laboratory-bred triatomines, after consecutive generations, was described by Szumlewicz (1976). High population density, greater feeding frequency, and absence of predators are the conditions found in domestic and laboratory-bred populations, thus favoring survival of smaller specimens (Riaño et al. 2009). In wild environments, it is suspected that the existence of low population density and low feeding frequency selects larger individuals due to their greater ability to endure temporary food shortage (Jaramillo et al. 2002, Rodríguez 2007). This observation can be explained by the selection hypothesis that was formulated for domestic and wild environments, in which selection was correlated with the availability of food (Riaño et al. 2009). The growth hypothesis explains this difference in the body size of triatomines, considering the longer development period of wild insects due to food shortages, thereby promoting larger insects. In domestic or laboratory-bred populations, the high population density would alter blood capture through causing competition between individuals and thus favoring smaller specimens (Dujardin et al. 1999, Jaramillo et al. 2002, Rodríguez 2007, Riaño et al. 2009).

According to the maintenance rules of the insectarium, the insects are fed every 15 d and the flasks where they are kept are frequently cleaned. The individuals are sorted according to stage, thus maintaining a low population density. Population density is highly important with regard to insect size, regardless of feeding: body size increases under low-density conditions and decreases under high-density conditions (Riaño et al. 2009). This routine may be a factor in establishing this wild characteristic in the laboratory population.

Another explanation for this fact relates to the antennae, because these are considered to be vital structures for triatomines bred in artificial environments, unlike wings. This was also observed in studies with head and wings (Jaramillo et al. 2002). No residual allometric effect was observed in the analysis on the groups, thus showing the absence of a relationship between wing size and shape. As shown by Catalá et al. (2004), the results obtained make it possible to confirm

Table 3. Mahalanobis distance for wings in populations of *R. brethesi* analyzed

Mahalanobis distance

Groups	L_M	L_F	W_M	W_F
L_M	0.00	–	–	–
L_F	2.58*	0.00	–	–
W_M	1.43	2.69*	0.00	–
W_F	3.02*	2.64*	2.21*	0.00

*Significant values $P < 0.0083$ by permutation test (1,000 runs each) after Bonferroni correction. L_M, laboratory male; L_F, laboratory female; W_M, wild male; W_F, wild female.
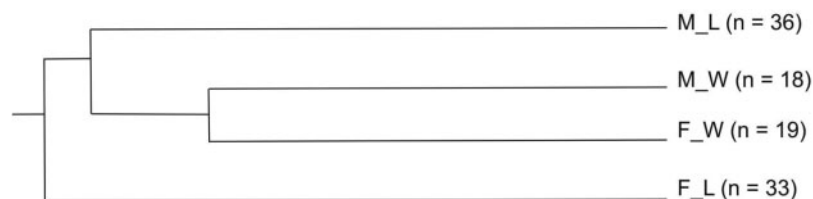

Fig. 5. Dendogram of UPGMA derivate of the distance of Mahalanobis of the wings of *R. brethesi* grouped according to ecotope and sex. M, male; F, female; L, laboratory; W, wild. The value in parenthesis indicates the number *N* of individuals used in the analysis.

that care is needed when using triatomine species bred in artificial environments for morphological and behavioral studies. The antennal phenotype, sensory markers, suggested a greater capacity for dispersal. The higher density and longer TH chemoreceptors in *R. brethesi* suggest that this species is ecotope-specific.

Acknowledgments

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