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Phenotypic Variability of *Rhodnius ecuadoriensis* Populations at the Ecuadorian Central and Southern Andean Region

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ABSTRACT *Rhodnius ecuadoriensis* is an important vector of Chagas disease in Ecuador. Whereas only sylvatic and peridomestic populations are common in Manabí province, this species occupies domestic, peridomestic, and sylvatic habitats in Loja province where high reinfestation of houses was observed. To explore the existence of phenetic changes linked to the domiciliation of the species, this study set out to analyze the wing and antennal phenotypes of *R. ecuadoriensis* in these two provinces where the vector presents different affinity for domestic habitats. The antennal phenotype and the wing size and shape distinguish the two geographical populations of *R. ecuadoriensis*. In Manabí, sylvatic and peridomestic specimens were very similar. In Loja, sylvatic and nonsylvatic (domestic and peridomestic) populations showed distinctive characteristics. Remarkable sexual dimorphism of wing and antenna, exclusive of domestic specimens, and high metric disparity in the wing shape of the domestic females point out the existence of a particular situation in this habitat. The results of this phenotypic analysis and previous evidence of behavioral differences support the hypothesis of disruptive selection acting upon *R. ecuadoriensis* populations.

KEY WORDS Chagas disease, *Rhodnius ecuadoriensis*, wing morphometry, antennal phenotypes, Ecuador

Trypanosoma cruzi, the causative agent of Chagas disease, is one of the most serious health problems in Latin America. In Ecuador, an estimated 25% of the population (6.2 million people) is at risk for infection (WHO 1991, PAHO 2006) and 230,000 people are infected (PAHO 2006). The principal route of transmission is via insects of the subfamily Triatominae (Hemiptera: Reduviidae) that serve as vectors (WHO 1991). At least 16 species of triatomines have been reported in Ecuador (Abad-Franch et al. 2001). These bloodsucking insects occupy a wide range of climatic areas (Abad-Franch et al. 2001) and different habitats (Schofield 1994). The main vectors in Ecuador are *Triatoma dimidiata* and *Rhodnius ecuadoriensis*. Populations of *R. ecuadoriensis* are widely distributed in the central and southern coastal regions, the southern Andean region of Ecuador, and northern Perú (Abad-Franch et al. 2002).

In the coastal region of Ecuador, *R. ecuadoriensis* was previously reported mostly in association with *Phytelephas aequatorialis*, the main ecotope of this triatomine (Abad-Franch et al. 2001, 2005; Cuba Cuba et al. 2002). One of the reasons for the prevalence of *R. ecuadoriensis* in the coastal region could be the

pervasiveness of the *P. aequatorialis* palm, also known as “tagua palm” or “cade palm” in Manabí and Santo Domingo de los Tsáchilas provinces. This palm is economically important because its leaves are widely used for thatch roof construction and the nuts are used for handicrafts and button manufacturing (Henderson et al. 1995, Southgate 1997, Abad-Franch et al. 2005). *P. aequatorialis* is less abundant in the southern coastal region (El Oro province), and is absent at the temperate valleys of the southern Andes region (Loja province) (Cuba Cuba et al. 2002), where the vegetation becomes dry forest. However, recent reports find an association between this species of triatomine and the Guayaquil squirrel (*Sciurus stramineus*) in both the coastal and southern Andean regions (Grijalva and Villacís 2009, Suarez-Davalos et al. 2010). The recent finding of *R. ecuadoriensis* in different habitats (domestic, peridomestic, and sylvatic) in two provinces of Ecuador (Loja and Manabí) with two different climatic conditions (Grijalva et al. 2005, Grijalva and Villacís 2009, Suarez-Davalos et al. 2010) highlights the importance of increasing our understanding of the relationship between different populations of this species.

Previous report of genetic differences in southern and northern populations of this species suggested that the two groups may be incipient species and indicated that control programs can target them independently (Abad-Franch et al. 2005). The purpose of this study was to analyze the phenotypes of *R. ecuadoriensis* in Loja and Manabí provinces, two areas

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Table 1. Geographical location and altitude of studied communities in Loja province, Ecuador

Province	County	Community	Range of altitude ^a	Latitude	Longitude
Manabí	Portoviejo	San Gregorio	74–220	–01.031°	–80.237°
		Maconta Abajo	68–144	–01.081°	–80.413°
		Jesús María	65–400	–01.020°	–80.228°
Loja	Célica	Bejuco	65–400	–0.9495°	–80.331°
		La Ciénega	580–870	–04.196°	–80.104°
	Quilanga	Galápagos	1,200–1,400	–04.356°	–79.730°
		Santa Rosa	730–850	–04.160°	–80.080°
	Catamayo	La Extensa	1,207–1,295	–04.032°	–79.3705°
	Paltas	Naranjo Dulce	1,007–1,532	–04.059°	–79.694°
Ashimingo		870–1,150	–04.030°	–79.730°	
		Vega del Carmen	1,121–1,683	–04.127°	–79.604°

^a Miles above sea level.

of Ecuador where the vector presents different affinity for domestic habitats, to explore the existence of phenotypic changes linked to the domiciliation of the species.

Morphology is clearly modulated by ecological factors (Schofield and Galvão 2009), and phenotypic variation can arise in response to different environments. Many species of Triatominae show evidence of phenotypic flexibility, which is proposed as an intraspecific feature with potential for evolutionary changes (Dujardin et al. 2009).

Quantitative morphology analyzes the interaction between the genotype and the environment, and assists in the interpretation of the functional relationships, adaptations, and evolution of the species (Adams et al. 2004). Wing veins provide well-defined morphological landmarks and are excellent material for morphometric analysis (Gumiell et al. 2003) in taxonomic studies, sexual differentiation (Matias et al. 2001), and to understand the population's spatial structuring at different habitats (Schachter-Broide et al. 2004, Feliciangeli et al. 2007, Hernández et al. (2010)).

In addition, the antennal phenotype (type and number of sensilla present in the antenna) is a quantitative morphological characteristic closely linked to adaptive genetic characteristics, as demonstrated in Catalá and Dujardin (2007); Hernández et al. (2008, 2010); Dujardin et al. (2009); and Martínez-Hernández et al. (2010). The sensory organs are essential for survival; in insects, the antennal phenotype reflects ancestral patterns modified by specific adaptations related to the exploitation of different hosts and habitats (McIver 1987). The triatomines antenna holds 90% of all nonvisual organs (sensilla) in the body. Comparative studies of Triatominae antennal phenotypes reveal clear taxonomic differences in sensilla patterns at specific and supraspecific levels as well as a correlation with their habitat range and their dispersive capacities (Catalá 1997; Catalá and Dujardin 2001, 2007; Moreno et al. 2005; Catalá and Schofield 1994; Gracco and Catalá 2000; Catalá et al. 2005; Esteban et al. 2005; Arroyo et al. 2006; Abraham et al. 2008; Carbajal de la Fuente et al. 2008; Hernández et al. 2008).

This work analyzes the geometric morphometry of wings and the antennal phenotypes of *R. ecuadoriensis* collected in Loja and Manabí provinces (Ecuador),

and discusses the significance and the implication of their differences.

Materials and Methods

Study Area. Specimens were collected from five rural communities in Manabí (central coastal region) and seven in Loja (southern region) in Ecuador (Table 1, Fig. 1). The central coastal region consists of flat terrain and low hills with a mixture of subtropical dry and tropical humid forest (INAMHI 2008) with an average annual rainfall of 563 mm/yr. Agriculture is the main economic activity in this region with a predominance of sugar cane (*Saccharum officinarum*), oranges (*Citrus sinensis*), banana (*Musa paradisiaca*), yucca (*Manihot esculenta*), corn (*Zea mays*), and rice (*Oryza sativa*). In addition, some palms such as cade



Fig. 1. Map of Ecuador, showing the distribution of *R. ecuadoriensis* (gray shade, Abad-Franch et al. 2001) and location of studied communities in Loja and Manabí provinces.

Table 2. Mean (standard deviation) of the number of antennal sensilla of *Rhodnius ecuadoriensis*, from Loja and Manabí, Ecuador

Sex	Pedicel		Flagellum 1				Flagellum 2			
	BR	BR	TH	TK	BA	BR	TH	TK	BA	
Males	63.58	23.94	44.62	76.41	18.10	13.72	43.46	92.11	24.28	
<i>n</i> = 51	(12.12)	(6.09)	(16.99)	(29.94)	(7.58)	(3.81)	(11.11)	(27.09)	(7.60)	
Females	67.48	23.81	48.01	83.67	18.84	13.36	44.01	95.84	24.78	
<i>n</i> = 48	(13.83)	(6.12)	(17.80)	(34.85)	(7.94)	(3.53)	(10.83)	(30.01)	(7.19)	

BA, basiconic; BR, bristles; TH, thin-walled trichoidea; TK, thick-walled trichoidea.

or tagua (*P. aequatorialis*) and coconuts (*Cocos nucifera*) are cultivated.

The Province of Loja, in the southern Andean region, is characterized by a mix of hilly and mountainous topography. The region includes inter-Andean temperate valleys and has an average rainfall of 400 mm/yr with two rainy seasons: February to May and October to November, and two dry seasons: June to September and December to January (INAMHI 2008). The inhabitants of these communities normally cultivate sugar cane, coffee, corn, peanuts, as well as fruit trees, such as papaya (*Carica papaya*), chirimoya (*Annona cherimola*), and orange.

Collection of *R. ecuadoriensis*. Triatomines were collected, as previously described by Grijalva et al. (2005), in domestic and peridomestic habitats (active search one man/h), and in sylvatic areas (Grijalva and Villacis 2009) in Loja and Manabí provinces. A total of 734 houses was visited (*n* = 330 in Manabí and *n* = 404 in Loja). The domestic searches included all the rooms inside each house. The peridomestic searches included chicken and pigeon nests, accumulations of stones, wood, bricks, piles of agricultural products, storage buildings, and other structures near the houses. In Manabí, *R. ecuadoriensis* was not found in the intradomestic habitat.

The sylvatic searches were performed within a 1.5-Km radius around the communities in Loja (communities: La Ciénega, Santa Rosa, and Galápagos) trees and shrubs, where the squirrel nests can be found (Grijalva and Villacis 2009). In the community of Naranjo Dulce, Loja province, the sylvatic searches were done in 600 × 600-m² quadrants (Suarez-Davalos et al. 2010). The sylvatic searches in Manabí were carried out in randomly chosen *P. aequatorialis* palm trees located near the communities, as previously described (Abad-Franch et al. 2000, Noireau et al. 2002).

Analysis of Antennal Phenotype. The antennae of 99 adult *R. ecuadoriensis* (48 female and 51 males) collected in domestic, peridomestic, and sylvatic habitats in the two provinces were analyzed following the methodology described in Abrahan et al. (2008).

Comparative Statistics of Antennal Phenotype. The number and type of antennal sensilla in each segment were recorded, and descriptive statistics were calculated. Homogeneity of variances was checked by the Levene test. Homoscedastic variables were analyzed using analysis of variance, whereas heteroscedastic variables were analyzed using the Kruskal-Wallis non-parametric test. The PAD software version 94 (Dujardin 2008) was used to perform discriminant analysis

(DA) and estimate the statistical significance (with Bonferroni correction) by the values of Wilks and Mahalanobis distances, using a nonparametric test (1,000 permutations). A cross-check classification was used to validate the classification of the individuals in the DA. The unweighted pair-group method with arithmetic average tree based on Mahalanobis distances was constructed using the PHYLIP package (<http://evolution.genetics.washington.edu/phylip.html>) and a TREEDYN module (<http://www.treedyn.org>).

Morphometric Analyses of Wings. A total of 230 right hemelytra (front wings) of 114 females and 116 males collected from domestic, peridomestic, and sylvatic habitats in Loja province and from peridomestic and sylvatic habitats in Manabí Province was analyzed (Table 2). The wings were mounted on microscope slides, and a digital image of each wing was obtained using a MiScope-MIP (www.zarbeco.com). Eight landmarks were identified for each wing using the software Collection of COordinates (COO) (Fig. 2). In addition, the Software Table Space Tabulations (TET), Morphometrics Geometrics (MOG), COV (Mancova), and PAD (Dujardin 2008) were used. These programs permitted the computation of shape variables and centroid sizes derived from data coordinates. The centroid size (CS) is defined as the square root of the sum of the square distances between the center of the configuration of landmarks and each individual landmark (Dujardin 2008). The statistical significance of CS differences was assessed by using a nonparametric test based on permutations (1,000 runs).

Shape variables were obtained through the generalized procrustes analysis superimposition algorithm and the subsequent projection of the procrustes re-



Fig. 2. Dorsal view of the right wing of *R. ecuadoriensis*. The white points indicate the landmarks used for the analysis. Numbers indicate the order of landmark capture. (Online figure in color.)

siduals into a Euclidean space. Both nonuniform (partial warps) and uniform components were used as shape variables. The uniform component describes global variation such as stretching and compression, whereas the nonuniform component corresponds to local variations. These two components describe the differences in shape as deviations from an average configuration of landmarks (Dujardin 2008).

A DA was performed on shape variables to examine relationships between the two geographical populations of *R. ecuadoriensis* (Manabí and Loja), among the three habitats (sylvatic, peridomestic, and domestic) and gender (males and females). To examine the allometric content of shape variables, a multivariate regression test computed with size as the independent variable and shape as the dependent variable was used (Henry et al. 2010). The "different versus common slope model" function of COV software (Dujardin 2008) was used to verify that different subpopulations were growing according to the same allometric model. In the case of a common model, the size influence was removed from shape variables, and then Euclidean distances between subpopulations were recomputed and their significance verified by nonparametric analysis. The unweighted pair-group method with arithmetic average tree based on Mahalanobis distances was constructed using the PHYLIP package (<http://evolution.genetics.washington.edu/phylip.html>) and TREEDYN module (<http://www.treedyn.org>).

Results

Antennal Phenotype of *R. ecuadoriensis*. A description of the antennal phenotype is necessary because this is the first time that a large number of specimens (99) was studied. Like other triatomines, the antenna of *R. ecuadoriensis* consists of four segments, as follows: scape; pedicel; and two flagellar segments, flagellum 1 (F1) and flagellum 2 (F2). As is typical for the antennal typology of Rhodniini, the pedicel presents mechanoreceptors (trichobotrias and bristles), and it lacks the sensilla chemoreceptors of trichoidea and basiconic type (Catalá and Schofield 1994). In F1 and F2, bristles (BR), thin-walled trichoidea (TH), thick-walled trichoidea (TK), and basiconic were observed. Table 2 shows the average and standard error (SE) of the number of antennal sensilla in both sexes without discriminating by region or habitat. Other sensilla of much smaller sizes ($\sim 1 \mu\text{m}$) were not considered in this work, but they have been described by Catalá and Schofield (1994).

Sexual Dimorphism of the Antennal Phenotype. In general, *R. ecuadoriensis* does not show sexual differences in the antennal phenotype (Table 3). However, the existence of sexual dimorphism in the antennal phenotype of the specimens collected in the intradomestic habitat was verified in Loja province.

Geographical Variation of Antennal Phenotype. Specimens from Manabí province presented a higher number of BR (pedicel), TH, and TK (F1 and F2) than those collected in Loja, showing a significant difference ($P < 0.001$; Fig. 3, Table 4). These differences

Table 3. *Rhodnius ecuadoriensis* sexual dimorphism of antennal phenotype and wing size and shape

	Sexual dimorphism		
	Wing shape	Wing size	Antennal phenotype
Total	Yes	Yes	No
Manabí	Yes	Yes	No
Loja	Only in domestic habitat	Only in domestic habitat	Only in domestic habitat

remained constant even when specimens of both provinces were compared by habitat ($P < 0.005$).

Antennal Phenotype Variation by Habitat. In Manabí province, differences in the antennal phenotype attributed to the habitat were not detected. However, in Loja populations, the univariate analysis detected a higher number of TK in F2 of the peridomestic females with respect to the sylvatic and domestic females ($P < 0.01$). The domestic females presented a lower number of TH and TK in F1 and F2 than males ($P < 0.05$). In peridomestic and domestic habitats, the males presented a larger number of BR (pedicel) and the TK F2 than those of the sylvatic habitat ($P < 0.05$; Table 4).

Two multivariate analyses of the antennal phenotype, including TH and TK from F1 and TK from F2, were performed. The principal components analysis for females from the three different habitats indicated that the first component represents 77.2% of the variation and the second 16.0%. Fig. 4 shows the phenotypic amplitude of sylvatic specimens, whereas peridomestic and domestic females appear grouped in opposed ends of function 1. The DA with the same variables confirmed significant distances of Mahalanobis ($P < 0.01$) between the domestic and peridomestic females and the absence of differentiation of both with the sylvatic phenotype. By cross-check classification, 83% of the correct classification was obtained for the domestic phenotype, 80% for peridomestic phenotype, and 43% for the sylvatic phenotype.

Wing Size Variation (CS)

Sexual Dimorphism of Wing Size and Shape. A noticeable sexual dimorphism of the wing CS was verified ($P < 0.00001$). The wings of the females were larger than those of the males (Fig. 5, Table 3). Sexual differences were more remarkable in peridomestic ($P = 0.00004$) than in sylvatic habitats of Manabí ($P = 0.0213$). The analysis of the wing shape in individuals from Loja showed sexual dimorphism only in the intradomestic habitat.

Geographical Variation of Wing Size (CS). Significant differences in the wing size of the individuals from Manabí and Loja were observed ($P = 0.000009$), as the wings of *R. ecuadoriensis* from Manabí were larger than the wings from both sexes of Loja specimens (both sexes; Fig. 5, Table 4).

Wing Size Variation by Habitat. When compared by habitat within each province, differences in the size of the wing were not detected between females and

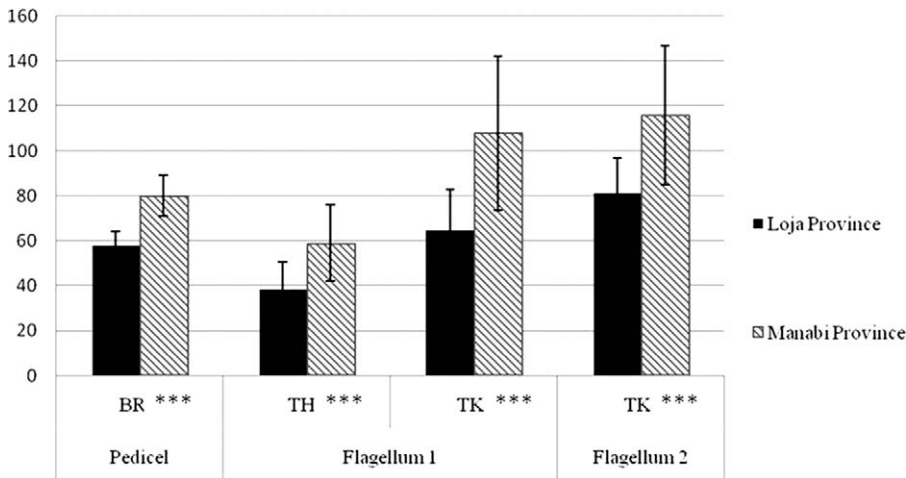


Fig. 3. Mean number and standard deviation of four antennal sensilla of *R. ecuadoriensis* from Loja and Manabí provinces. BR, bristles of pedicel; F1TH, thin-walled trichoids (first flagellar segment); F1TK, thick-walled trichoids of the first flagellar segment; F2TK, thick-walled trichoids (second flagellar segment). All differences between the two provinces were statistically significant.

males from Loja. However, in Manabí, greater wing size was found in peridomestic females than in sylvatic females ($P < 0.05$). The males' wing size did not show differences between the peridomestic and sylvatic habitats.

Wing Shape Variation

Geographic Variation of Wing Shape. The contribution of size to wing shape differentiation (first discriminant factor) was 45% for males and 52% for females ($P = 0.000$). The wing shape showed significant differences between populations from both provinces. A common allometric model was accepted (males, females), and the distances computed from allometry-free partial warps were significant ($P < 0.001$; Table 4).

Variation of Wing Shape by Habitat. Comparison of the wing shape between individuals from different habitats in each region showed no difference in either sexes in Manabí, but some variations in Loja. The specimens collected in the sylvatic habitat differed in wing shape compared with the individuals obtained

from peridomestic and domestic sites (both sexes) of Loja. Fig. 6 shows the DA and phenetic trees from male and female wing shape.

The metric disparity (MD) of the wing shape was analyzed for both sexes and habitats in the populations of the two provinces. This analysis showed the shape variance under the hypothesis of limitation in the diversity of forms by genetic or environmental factors. It was observed that the individuals of both sexes from Manabí have total minor MD than those from Loja ($P < 0.05$). When the wing shape between habitats was compared, a significantly smaller MD was observed in the males of the sylvatic habitat of Manabí, and a significantly greater MD was found in the females of the domestic habitat of Loja (Table 5).

Discussion

In Triatominae, it is frequently observed that populations of the same species occupying different geographical regions present distinctive phenotypic expression (Catalá et al. 2005, Abrahan et al. 2008,

Table 4. Comparison of phenotypic variables between provinces and among habitats, according to sex

	Sex	Habitats ^a	Wing		Antennal phenotype
			Shape	Size	
Between provinces	Males	All	***	***	***
Loja vs Manabí	Females	All	***	***	***
Between habitats	Males	S vs P	—	—	—
Manabí	Females	S vs P	—	*	—
Between habitats	Males	S vs P	***	—	—
Loja		S vs D	***	—	—
		D vs P	—	—	—
	Females	S vs P	***	—	—
		S vs D	***	—	—
		D vs P	—	—	***

Statistical significant difference: *, $P < 0.05$; **, $P = 0.05-0.01$; ***, $P < 0.01$; —, no difference.
^a D, domestic; P, peridomestic; S, sylvatic.

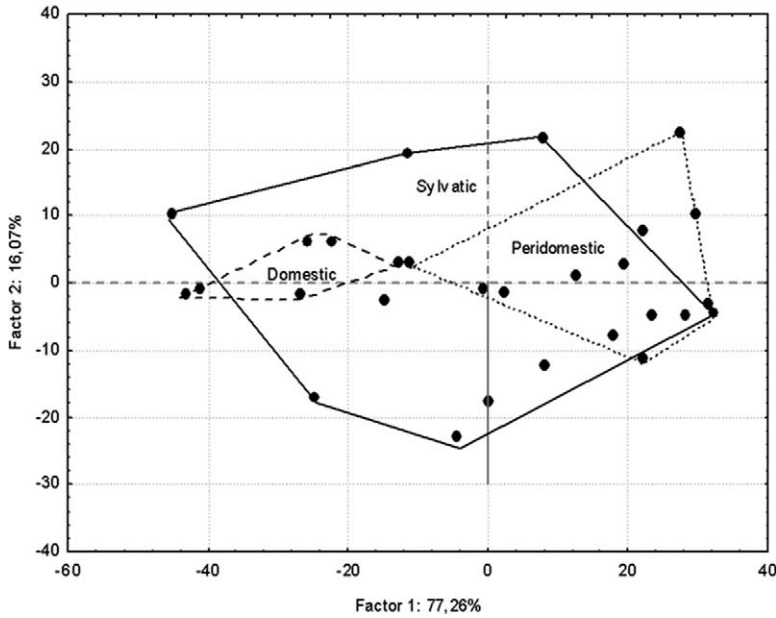


Fig. 4. Principal component analysis with three variables of the antennal phenotype of females from three different habitats in Loja province, as follows: domestic, peridomestic, and sylvatic. DA with the same variables confirmed significant distances of Mahalanobis ($P < 0.01$).

Hernández et al. 2008, Dujardin et al. 2009, Schofield and Galvão 2009). Whatever the level of exchange between populations, if local selection produces different phenotypes (i.e., overcomes the effects of migration), then mechanisms described as genetic assimilation or genetic accommodation may act progressively to induce real genetic changes (Dujardin et al. 2009). Several differences in the mitochondrial DNA of *R. ecuadoriensis* have been found by Abad-Franch and Monteiro (2005) in a study of mitochondrial cytochrome *b* (mt *cyt b*) from five populations of *R. ecuadoriensis*, four from Ecuador (Manabí area), and one from the north of Perú (South of Loja). Peruvian bugs presented a markedly divergent haplotype, whereas all Ecuadorian sequences

(nine haplotypes) were similar. These authors suggested that the two clades represent discrete phylogroups (or even incipient species), and suggested that control programs can target them independently. The fact that *R. ecuadoriensis* is well domiciliated in extensive areas of southern Ecuador (Loja) and northern Perú, but not in more humid regions (Manabí), where it associates strongly with sylvatic habitats (Abad-Franch et al. 2001, 2005; Grijalva and Villacís 2009; Suarez-Davalos et al. 2010), points out the need to investigate the regional characteristics of their populations to explore phenetic changes linked to domiciliation.

The results of this study demonstrate strong phenotypic differences between populations from both

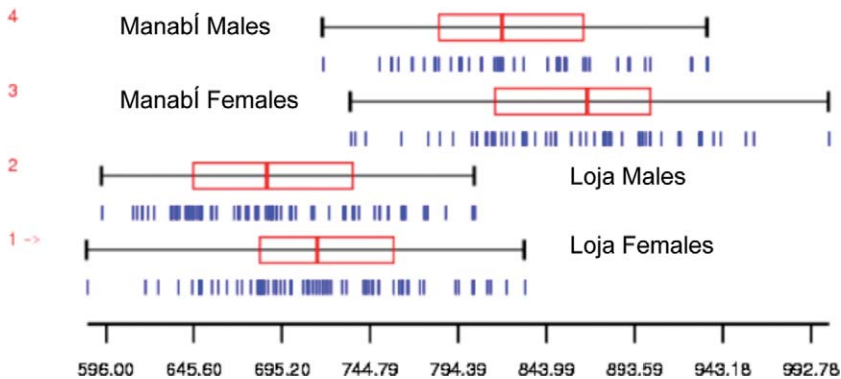
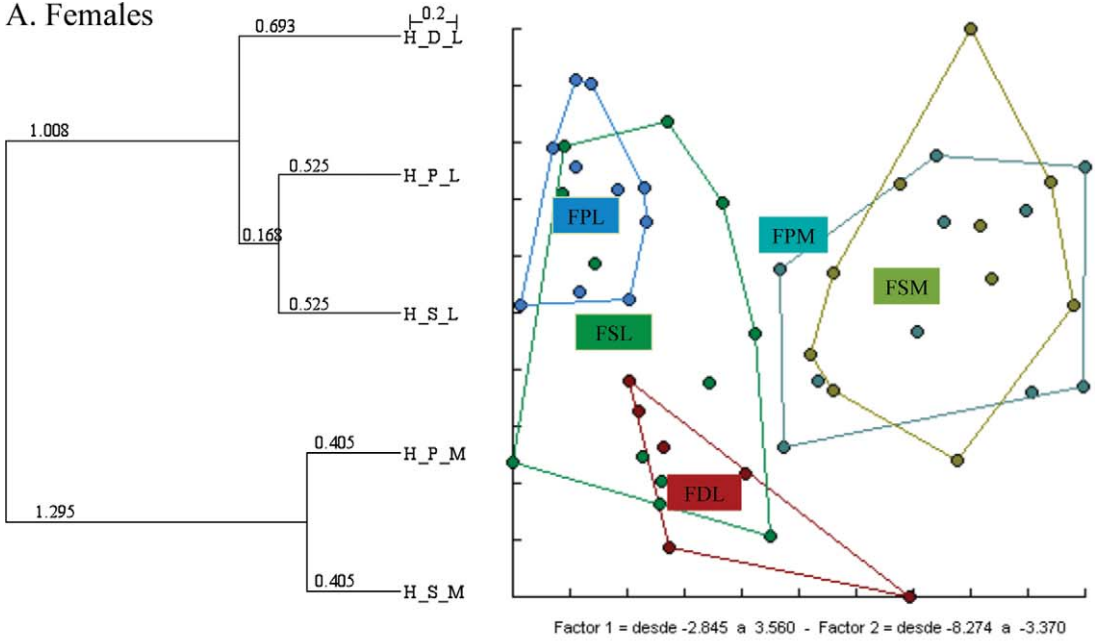


Fig. 5. Sexual and geographical wing size variation of *R. ecuadoriensis* individuals. Differences in geographical variation between the two areas ($P = 0.000009$) were observed. Sexual variation was only observed in Manabí province. (Online figure in color.)

A. Females



B. Males

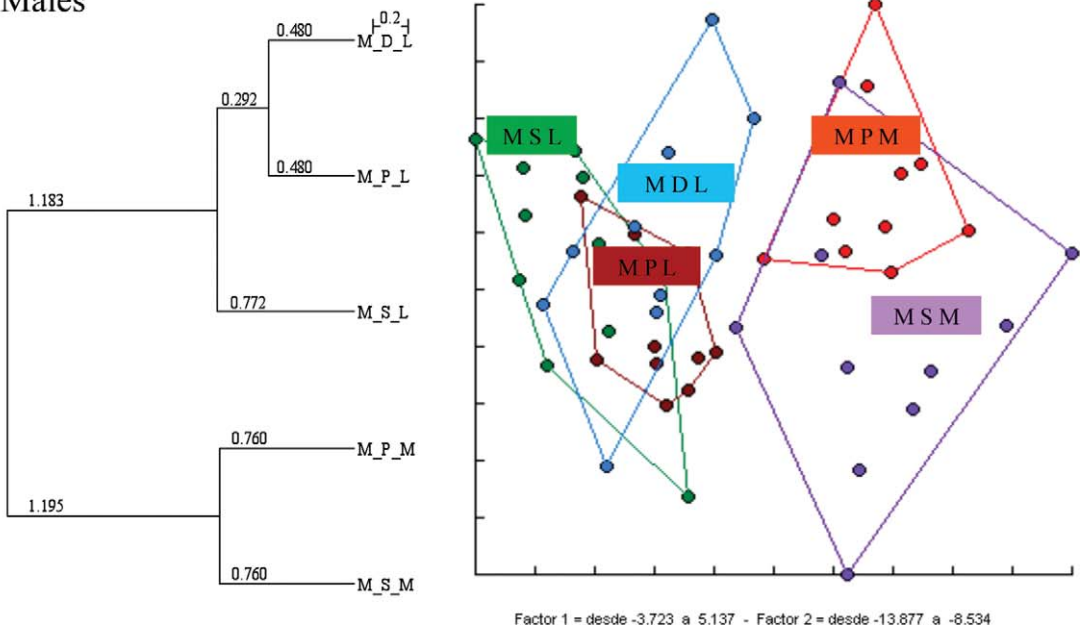


Fig. 6. Phenetic distances derived from Mahalanobis distances (from DA). (A) Females and (B) males from the two provinces in different habitats. FDL, females domestic Loja; FPL, females peridomestic Loja; FSL, females sylvatic Loja. FPM, females peridomestic Manabí; FSM, females sylvatic Manabí. MDL, males domestic Loja; MPL, males peridomestic Loja; MSL, males sylvatic Loja. MPM, males peridomestic Manabí; MSM, males sylvatic Manabí. (Online figure in color.)

provinces that parallel the differences in the behavior and size observed in individuals from Loja and Manabí (Villacís et al. 2008) and the genetic differences between *R. ecuadoriensis* populations mentioned above (Abad-Franch and Monteiro 2005). The wing size varied according to the body size because the insects from Manabí have greater dimensions than those from

Loja (Villacís et al. 2008). These size variations are generally ascribed to environmental causes such as temperature (Bergmann's rule). In this study, Bergmann's rule does not apply, as temperature is higher in Manabí than in Loja. Possible *trans*-generational effects on size cannot be excluded, as demonstrated in *Rhodnius pallescens* (Dujardin et al. 2009).

Table 5. The metric disparity of wing shape of *Rhodnius ecuadoriensis* between each habitat and sex

Province	Habitat	Males (n)	Females (n)
Manabí	Peridomestic	0.00146 (29)	0.00135 (30)
	Sylvatic	0.00109* (14)	0.00135 (21)
Total		0.00255 (43)	0.0027 (51)
Loja	Domestic	0.00162 (23)	0.00199* (18)
	Peridomestic	0.00159 (24)	0.00157 (23)
	Sylvatic	0.00145 (26)	0.00151 (22)
Total		0.00466 (73)	0.00507 (63)
Total		0.00721 (116)	0.00777 (114)

This analysis shows the shape variance under the hypothesis of limitation in the diversity of forms by genetic and/or environmental factors. *, Significant differences between habitats in the same province.

Furthermore, the wing shape of *R. ecuadoriensis* also varied significantly between provinces. Shape, considered as the geometry of a body structure defined by Cartesian coordinates of landmarks, is frequently linked to genetic factors (Dujardin et al. 2009). These results appear to complement genetic and behavioral differences previously observed, and suggest an incipient speciation process. To date, there is no evidence of an intermediate group sharing characteristics of these two populations; however, more research is needed in areas where these populations are likely to overlap.

Sylvatic and peridomestic specimens (both sexes) from Manabí are phenotypically similar, suggesting exchange of individuals between habitats (Table 4). Conversely, the individuals from the sylvatic and nonsylvatic habitats (domestic + peridomestic) of Loja showed statistical significant differences in wing shape. As shape variables are frequently driven by genetic variations and they are more stable characters in the face of environmental perturbations (Riñaño et al. 2009), low exchange of individuals may be suggested. Molecular analysis is currently being conducted to complement these morphometric findings. Alternatively, the observed similarity in wing size and antennal phenotype of Loja sylvatic and nonsylvatic individuals may be responding to similarities in the habitat requirements, as these two variables may be particularly influenced by the environment (Catalá 1997, Catalá et al. 2005, Abrahan et al. 2008, Dujardin et al. 2009). Phenotypic changes related to habitats have been observed in other Triatominae species (Schachter-Broide et al. 2004, Feliciangeli et al. 2007). For example, in *Triatoma infestans*, wing and head size and shape, as well as the antennal phenotype showed important differences when comparing two zones 80 km apart, suggesting environmental effects, low or no exchange of individuals between zones, or both. A different regional structure in the habitats they occupy was also observed (Hernández et al. 2010).

Sylvatic populations of triatomines are considered as the original forms from which domestic and peridomestic ones derive. The analysis of metric disparity of wing shape showed a progressive increase from sylvatic to human habitats in Loja populations, but not in Manabí. Moreover, a significantly reduced variabil-

ity in the wing shape of Manabí males suggests an ecological limitation to the wing diversity not observed in Loja. However, the high metric disparity in the wing shape of the domestic females of Loja could be related to the tolerance of the human habitat to phenotypic variation. In a domestic condition, where survival is probably less dependent on the ability to fly, we can expect more wing shape diversity. The wing shape differences between sylvatic and nonsylvatic specimens from Loja may support the hypothesis of some isolation between these populations. However, the specimens from domestic and peridomestic habitats showed similar wing size and shape, but special features of domestic specimens indicate the existence of a particular situation in this habitat (Table 4). Sexual dimorphism in wing shape, well evidenced in both habitats of Manabí, was only observed in domestic specimens from Loja. Studies in *Rhodnius prolixus* and *Rhodnius robustus* showed that sexual dimorphism of wings varies across the different habitats, probably induced by local regulation of growth patterns or differences in the timing of specific developmental events, known as heterochrony (Villegas et al. 2002). Interestingly, the sexual dimorphism of antennal phenotype, rarely found in species of Rhodniini, although very common in Triatomini species (Catalá 1997, Carbajal de la Fuente and Catalá 2002, Esteban et al. 2005, Moreno et al. 2005), was only observed in domestic *R. ecuadoriensis* from Loja. These regional and habitat variations on the expression of sexual differences are evidence of a diversified genic expression that may support the adaptative ability of the species (Hernández et al. 2008, 2010). Should the domestic habitat be the cause of this variation, the situation should be monitored closely because of its epidemiological implication.

The domestic and peridomestic females from Loja showed statistically significant differences in the antennal phenotype (Table 3). Also remarkable is that the highly varied antennal phenotypic pool exhibited by the sylvatic females from Loja includes those phenotypes present on domestic and peridomestic females, although each of these habitats seems to select exclusive phenotypes (Fig. 4).

The results of this phenotypic analysis and previous studies of behavioral differences (Villacís et al. 2008) support the hypothesis of disruptive selection acting upon *R. ecuadoriensis* populations. This effect, seen in triatomines sharing domestic and sylvatic habitats or heterogeneous sylvatic habitats, is characterized by phenotypic flexibility and changes in morphological quantitative traits (Dujardin et al. 2009) as those described in this work. This situation highlights the challenges for the implementation of an effective control program in these regions, particularly in the dry areas (Loja province) where *R. ecuadoriensis* maintains sylvatic and domestic populations.

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References Cited

- Abad-Franch, F., and F. A. Monteiro. 2005. Molecular research and the control of Chagas disease vectors. *An. Acad. Bras. Ciênc.* 77: 437–454.
- Abad-Franch, F., F. Noireau, C. A. Paucar, V.H.M. Aguilar, C. C. Carpio, and V. J. Racines. 2000. The use of live-bait traps for study of sylvatic *Rhodnius* populations (Hemiptera: Reduviidae) in palm trees. *Trans. R. Soc. Trop. Med. Hyg.* 94: 629–630.
- Abad-Franch, F., A. Paucar, C. C. Carpio, C. A. Cuba Cuba, H. M. Aguilar, and M. A. Miles. 2001. Biogeography of Triatominae (Hemiptera: Reduviidae) in Ecuador: implications for the design of control strategies. *Mem. Inst. Oswaldo Cruz* 96: 611–620.
- Abad-Franch, F., H. M. Aguilar, A. Paucar, E. S. Lerosa, and F. Noireau. 2002. Observations on the domestic ecology of *Rhodnius ecuadoriensis*, vector of Chagas disease in Ecuador. *Mem. Inst. Oswaldo Cruz* 97: 199–202.
- Abad-Franch, F., F. S. Palomeque, V.H.M. Aguilar, and M. A. Miles. 2005. Field ecology of sylvatic *Rhodnius* populations (Heteroptera, Triatominae): risk factors for palm tree infestation in western Ecuador. *Trop. Med. Int. Health* 10: 1258–1266.
- Abraham, L., L. Hernández, D. Gorla, and S. Catalá. 2008. Phenotypic diversity of *Triatoma infestans* at the microgeographic level on the Gran Chaco of Argentina and the Andean valleys of Bolivia. *J. Med. Entomol.* 45: 660–666.
- Adams, D. C., F. J. Rohlf, and D. E. Slice. 2004. Geometric morphometrics: ten years of progress following the 'revolution.' *Ital. J. Zool.* 71: 5–16.
- Arroyo, C. M., L. Esteban, S. Catalá, and V. M. Angulo. 2006. Antennal phenotype variation in sylvatic, peridomestic and domestic populations of *Triatoma dimidiata* (Hemiptera: Reduviidae) from Santander, Colombia. *Rev. Biomed.* 27: 92–100.
- Carbajal de la Fuente, L., and S. Catalá. 2002. Relationship among the habitat and the antennal sensilla pattern of six species of Triatominae (Hemiptera, Reduviidae). *Mem. Inst. Oswaldo Cruz.* 97: 1121–1125.
- Carbajal de la Fuente, L., F. Noireau, and S. Catalá. 2008. Inferences about antennal phenotype: the "*Triatoma maculata* complex" (Hemiptera: Triatominae) is valid? *Acta Trop.* 106: 16–21.
- Catalá, S. 1997. Antennal sensilla of Triatominae: a comparative study of five genera. *Int. J. Insect Morphol. Embryol.* 26: 67–73.
- Catalá, S., and J. P. Dujardin. 2001. Antennal sensilla patterns indicate geographic and ecotopic variability among *Triatoma infestans* (Hemiptera: Reduviidae) populations. *J. Med. Entomol.* 38: 423–428.
- Catalá, S. S., and J. P. Dujardin. 2007. Los Triatominae: Bajo el enfoque de la morfología cuantitativa, pp 173–189. *In* Mirko Rojas Córtez (ed.), *Triatominae de Bolivia y la Enfermedad de Chagas*. La Paz, Bolivia.
- Catalá, S., and C. Schofield. 1994. Antennal sensilla of *Rhodnius*. *J. Morphol.* 219: 193–204.
- Catalá, S., C. Sachtetto, M. Moreno, R. Rosales, P. M. Salazar-Schettino, and D. Gorla. 2005. The antennal phenotype of *Triatoma dimidiata* populations and its relationship with species of the *phyllosoma* and *protracta* complexes. *J. Med. Entomol.* 42: 719–725.
- Cuba Cuba, C. A., F. Abad-Franch, R. J. Roldán, V. F. Vargas, V. L. Pollack, and M. A. Miles. 2002. The triatomines of northern Perú, with emphasis on the ecology and infection by trypanosomes of *Rhodnius ecuadoriensis* (Triatominae). *Mem. Inst. Oswaldo Cruz* 97: 175–183.
- Dujardin, J. P. 2008. Morphometrics applied to medical entomology. *Infect. Genet. Evol.* 8: 875–890.
- Dujardin, J. P., J. Costa, D. Bustamante, N. Jaramillo, and S. Catalá. 2009. Deciphering morphology in Triatominae: the evolutionary signals. *Acta Trop.* 110: 101–111.
- Esteban, L., V. M. Angulo, M. D. Feliciangeli, and S. Catalá. 2005. Analysis of antennal sensilla patterns of *Rhodnius prolixus* from Colombia and Venezuela. *Mem. Inst. Oswaldo Cruz* 100: 909–914.
- Feliciangeli, M. D., M. Sanchez-Martin, R. Marrero, C. Davies, and J. P. Dujardin. 2007. Morphometric evidence for a possible role of *Rhodnius prolixus* from palm trees in house re-infestation in the State of Barinas (Venezuela). *Acta Trop.* 101: 169–177.
- Gracco, M., and S. Catalá. 2000. Inter-specific and developmental differences on the array of antennal chemoreceptors in four species of Triatominae. *Mem. Inst. Oswaldo Cruz* 95: 67–74.
- Grijalva, M. J., and A. G. Villacís. 2009. Presence of *Rhodnius ecuadoriensis* in sylvatic habitats in the southern highlands (Loja Province) of Ecuador. *J. Med. Entomol.* 46: 708–711.
- Grijalva, M. J., F. S. Palomeque-Rodriguez, J. A. Costales, S. Dávila, and L. Arcos-Terán. 2005. High household infestation rates by synanthropic vectors of Chagas disease in southern Ecuador. *J. Med. Entomol.* 42: 68–74.
- Gumiel, M., S. Catalá, F. Noireau, A. Rojas de Arias, A. García, and J. P. Dujardin. 2003. Wing geometry in *Triatoma infestans* (Klug) and *T. melanosoma* Martínez, Olmedo & Carcavallo (Hemiptera: Reduviidae). *Syst. Entomol.* 28: 173–180.
- Henderson, A., G. Galeano, and R. Bernal. 1995. *Field Guide to the Palms of the Americas*. Princeton University Press, Princeton, NJ.
- Henry, A., P. Thongsripong, I. Fonseca-Gonzalez, N. Jaramillo-Ocampo, and J. P. Dujardin. 2010. Wing shape of dengue vectors from around the world. *Infect. Genet. Evol.* 10: 207–214.
- Hernández, M. L., L. Abraham, M. Moreno, D. Gorla, and S. Catalá. 2008. Phenotypic variability associated to genomic changes in the main vector of Chagas disease in South America. *Acta Trop.* 106: 60–67.
- Hernández, M. L., L. B. Abraham, J. P. Dujardin, D. E. Gorla, and S. Catalá. 2010. Phenotypic variability and population structure of peridomestic *Triatoma infestans* in rural areas of the Arid Chaco (Western Argentina): spatial influence of macro- and microhabitats. *Vector Borne Zoonotic Dis.* (doi: 10.1089/vbz.2009.0253).
- [INAMHI] Instituto Nacional de Meteorología en Hidrología. 2008. Climatología: características generales del clima en el Ecuador. (<http://www.inamhi.gov.ec>).
- Martínez-Hernandez, F., J. A. Martínez-Ibarra, S. Catalá, G. Villalobos, P. De la Torre, J. P. Lacleste, R. Alejandro-Aguilar, and B. Espinoza. 2010. Natural crossbreeding

- between sympatric species of the phyllosoma complex (Insecta: Hemiptera: Reduviidae) indicates the existence of only one species with morphologic and genetic variations. *Am. J. Trop. Med. Hyg.* 82: 74–82.
- Matias, A., J. X. De la Riva, M. Torrez, and J. P. Dujardin. 2001. *Rhodnius robustus* in Bolivia identified by its wings. *Mem. Inst. Oswaldo Cruz* 96: 947–950.
- McIver, S. B. 1987. Sensilla of hematophagous insects sensitive to vertebrate host-associated stimuli. *Insect Sci. Appl.* 8: 627–635.
- Moreno, M., D. Gorla, and S. Catalá. 2005. Association between antennal phenotype, wing polymorphism and sex in the genus *Mepraia* (Reduviidae: Triatominae). *Infect. Genet. Evol.* 6: 228–234.
- Noireau, F., F. Abad-Franch, and S.A.S. Valente. 2002. Trapping Triatominae in silvatic habitats. *Mem. Inst. Oswaldo Cruz* 97: 61–63.
- [PAHO] Pan American Health Organization. 2006. Technical Report: Quantitative Chagas Disease Estimates: OPS/HDMCD/425-06, Montevideo. PAHO, Montevideo, Uruguay.
- Riaño, H., C. N. Jaramillo, and J. P. Dujardin. 2009. Growth changes in *Rhodnius pallescens* under simulated domestic and silvatic conditions. *Infect. Genet. Evol.* 9: 162–168.
- Schachter-Broide, J., J. P. Dujardin, U. Kitron, and R. E. Gutler. 2004. Spatial structuring of *Triatoma infestans* (Hemiptera, Reduviidae) populations from northwestern Argentina using wing geometric morphometry. *J. Med. Entomol.* 41: 643–649.
- Schofield, C. J. 1994. Triatominae, biología y control. Euro-communic Publications, West Sussex, Reino Unido.
- Schofield, C. J., and C. Galvão. 2009. Classification, evolution, and species groups within the Triatominae. *Acta Trop.* 110: 88–100.
- Southgate, D. 1997. Alternatives for habitat protection and rural income generation: document ENV-107. Inter-American Development Bank, Washington, DC.
- Suarez-Davalos, M. V., O. Dangles, A. G. Villacís, and M. J. Grijalva. 2010. Microdistribution of silvatic triatomine populations in central-coastal Ecuador. *J. Med. Entomol.* 47: 80–88.
- Villacís, A. G., L. Arcos-Terán, and M. J. Grijalva. 2008. Life cycle, feeding and defecation patterns of *Rhodnius ecuadoriensis* (Lent & León 1958) (Hemiptera: Reduviidae: Triatominae) under laboratory conditions. *Mem. Inst. Oswaldo Cruz* 103: 690–695.
- Villegas, J., M. D. Feliciangeli, and J. P. Dujardin. 2002. Wing shape divergence between *Rhodnius prolixus* from Cojedes (Venezuela) and *Rhodnius robustus* from Mérida (Venezuela). *Infect. Genet. Evol.* 2: 121–128.
- [WHO] World Health Organization. 1991. Control of Chagas Disease: WHO Technical Report Series 811. WHO, Ginebra, Suiza.

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