Analysis of antenal sensilla patterns of *Rhodnius prolixus* from Colombia and Venezuela

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Antennal sensilla patterns were used to analyze population variation of domestic Rhodnius prolixus *from six departments and states representing three biogeographical regions of Colombia and Venezuela. Discriminant analysis of the patterns of mechanoreceptors and of three types of chemoreceptors on the pedicel and flagellar segments showed clear differentiation between* R. prolixus *populations east and west of the Andean Cordillera. The distribution of thick and thin-walled trichoids on the second flagellar segment also showed correlation with latitude, but this was not seen in the patterns of other sensilla. The results of the sensilla patterns appear to be reflecting biogeographic features or population isolation rather than characters associated with different habitats and lend support to the idea that domestic* R. prolixus *originated in the eastern region of the Andes*

Key words: Chagas disease - sensilla patterns - *Rhodnius prolixus* - Triatominae - Colombia - Venezuela

Triatominae (Hemiptera, Reduviidae), the insect vectors of Chagas disease (American trypanosomiasis) have been recorded from 30 of the 32 departments of Colombia, from the coast to 2000 m.a.s.l. (Molina et al. 2000). Over 20 species have been reported, of which *Rhodnius prolixus* (Stål) is the most frequently encountered in domestic habitats, representing 95.4% of domestic Triatominae collected, with house infestation rates above 20% in many municipalities (Corredor et al. 1990, Angulo et al. 1997). *R. prolixus* is also widespread in Venezuela where, in spite of extensive control campaigns since 1966, it continues to be the most important domestic vector of Chagas disease (Aché & Matos 2001, Feliciangeli et al. 2003).

Historical reconstruction, combined with biogeographical studies and available genetic comparisons, suggests that *R. prolixus* may have been first domesticated in the savanna-like areas (llanos) of Venezuela, and subsequently spread by accidental human intervention (Dujardin et al. 1998, Schofield & Dujardin 1999, Zeledón 2004). In general however, populations of *R. prolixus* tend to show low levels of genetic or allozyme variability (Dujardin et al. 1998, Monteiro et al. 2000, 2003, Jaramillo et al. 2001), and the aim of the present study was to examine phenetic variability between Colombian and Venezuelan populations using antennal sensilla patterns, which have been shown to be informative characters in studies of population structuring in other species of Triatominae (Gracco & Catalá 2000, Catalá & Dujardin 2001, Carbajal et al. 2002).

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MATERIALS AND METHODS

Areas of study - Adults *R. prolixus* were collected from domestic habitats in six departments from three biogeographical regions of Colombia selected on the basis of previous reports and the national entomological survey (Angulo et al. 1997, Molina et al. 2000, Ministerio de Salud 2000). These represented the Andean zone (departments of Santander and Tolima), the Sierra Nevada (departments of Magdalena and Guajira), and the llanos (department of Arauca). *R. prolixus* was also collected from houses in the Venezuelan state of Barinas, which neighbours the department of Arauca (Fig. 1) and from where acute cases of Chagas disease have recently been reported (Añéz et al. 1999).

Santander and Tolima in the Andean zone west of the Cordillera are mountainous woodland regions with altitudes from 1000 to 5500 m.a.s.l. The average annual temperature in Santander is 18ºC, compared to 28ºC in Tolima. Magdalena and Guajira in the Sierra Nevada de Santa Marta, mountain at the Caribbean coast in the North of Colombia, isolated from the rest of the Andean range, with a wide diversity of habitats. The lower slopes are used for agriculture, cattle-ranching and tourism and the upper slopes are inhabited by the indiginous Arhuaco and Kogi cultures. The llanos of Arauca and Barinas are part of an extensive savanna-like plain extending east of the Andes in Colombia and Venezuela. The climate is humid-tropical, with average temperatures of 22-28ºC and extensive woodlands with numerous stands of palm trees.

The majority of rural houses in the studied areas are of adobe or 'bahareque' (woven stick and mud) with roofs of palm thatch or corrugated metal or fibre-cement sheets. *R. prolixus* was collected from infested houses during 2003-2005, by timed manual collection (using forceps and a torch to see into crevices in the house structure) (Table I). The collected insects were maintained live or placed into 70% ethanol for transportation to our laboratory. From each collection, adults were selected at random until at least 10 with complete antennae could be included from each of the departments. Magdalena and Guajira were taking as the same department and included in the same biogeographical regions (Fig. 1).

Analysis of antennal sensilla - Antennae were cut from the head using fine forceps, and stored in 70% ethanol prior to diaphanisation in 4% NaOH. After clearing, they were individually slide-mounted in glycerine and examined at 40×. Sensilla were classified according to Catalá and Schofield (1994), and counted along the entire length of the pedicel (P) and the two flagellar segments (F1 and F2). Only one antenna was examined from each specimen. Four sensilla types were used for analysis: BR, mechanoreceptive bristles, BA, basiconics, TH, thin walled trichoids, TK, thick-walled trichoids; these sensilla have showns to be significant in differentiating Triatominae populations (Catala & Dujardin 2001, Catalá et al. 2004, 2005).

TABLE I

Source of *Rhodnius prolixus* populations used in the analysis

Municipalities	Department/State	Latitude (deg/min)	Longitude (deg/min)	Altitude (masl)
Santa Marta	Magdalena	11º09’	73º18’	300
Dibulla	Guajira	11º09’	73º18’	297
Albania	Santander	5º45’	73º55’	1900
Sucre	Santander	5º55’	73º47’	1640
Jesús Maria	Santander	5º52’	73º48’	1950
Coyaima	Tolima	3º47’	75º12’	350
Arauca	Arauca	6º98’	71º12’	130
Bolívar	Barinas	8°47’	70°30’	990
Barinas	Barinas	7°51’	71°15’	460
Ezequiel Zamora	Barinas	7°52’	71°14’	160

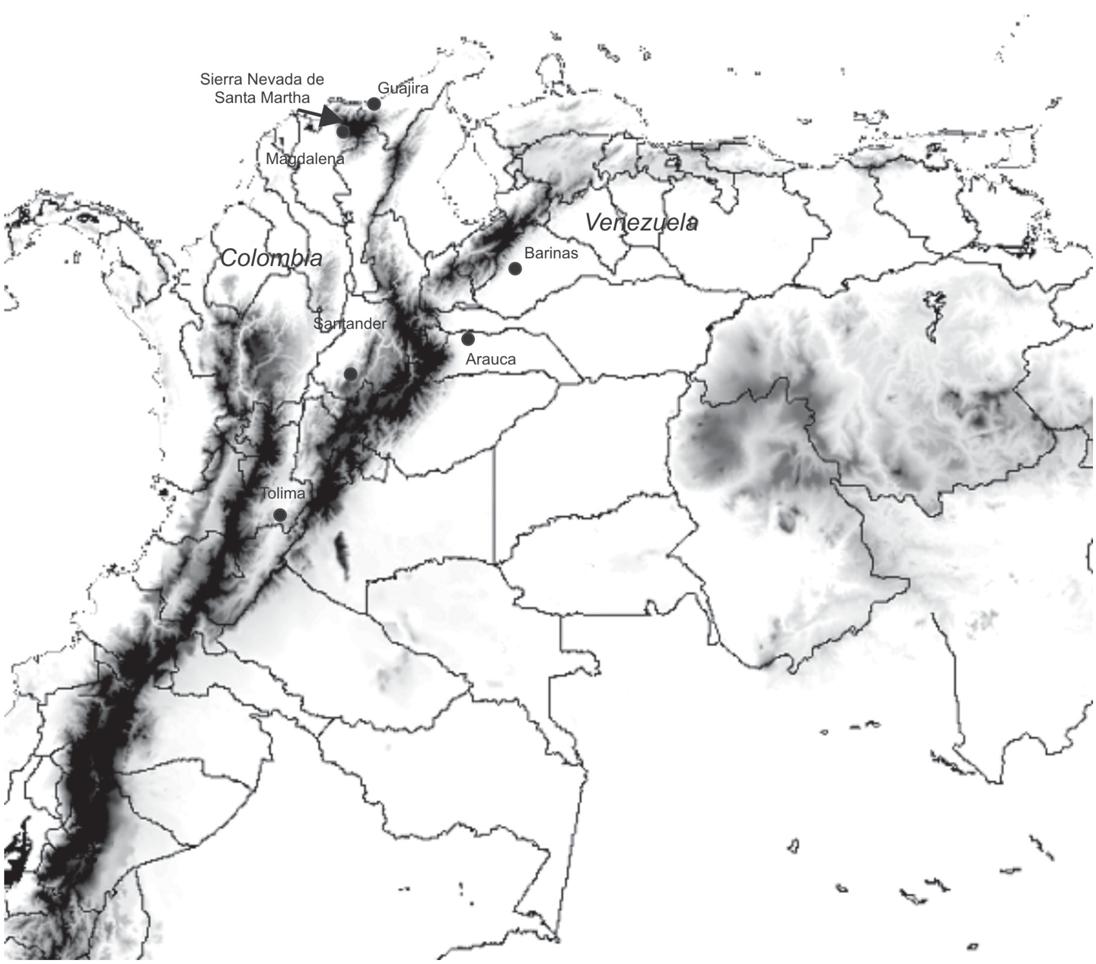

Fig. 1: map of Colombia and Venezuela showing the collection places of *Rhodnius prolixus* in Tolima, Santander, Magdalena, Guajira, and Arauca in Colombia and Barinas in Venezuela.

A total of 53 insects (27 males and 26 females) was included in the analysis. For each population, mean sensilla density (±SD) was calculated for each segment (Table II). Logs of these variables were compared by ANOVA or Kruskal-Wallis, with significance of differences estimated by Newman-Keuls with Bonferroni correction for continuity (Sokal & Rohlf 1997) using STATISTICA v6. Discriminant analysis were made between the five populations, and also between populations from the three biogeographical regions, using PADWIN v65 (http://www.mpl.ird.fr/morphometrics). Latitudinal variation, which has been observed in populations of *Triatoma dimidiata* (Catalá et al. 2005), was also examined by linear correlation with TH and TK densities on the flagellar segments.

RESULTS

ANOVA between sexes showed no significant differences between sexes for any sensilla pattern in any of the departments studied (data not shown) so that subsequent analysis were made on groups of 10 (5 males and 5 females) or 11 insects (5 males and 6 females) for each department (Table II).

Univariate analysis showed significant differences in densities of pedicel mechanoreceptors (PBR) and flagellar chemoreceptors (F1BA, F1TK, F2TK, F1TH, F2TH), especially those of the first flagellar segment (F1) (Table III). Insects from the Andean region differed from those of the Sierra Nevada only in numbers of F2TH. However, insects from the llanos differed significantly from those of the Andes and those from la Sierra Nevada by densities of five receptor types (Table III).

Multivariate discriminant analysis using all variables except F2BA and F1BR (due to heteroscedascity) correctly reclassified 86% of all individuals to their respective department. The population from Magdalena-Guajira was 100% correctly reclassified, followed by those of Barinas (90%), Arauca (88%), Santander (80%), and Tolima (72%). Of the four discriminant functions obtained, the first two contributed 94% of the observed variation (63% for the first, and 31% for the second), and the two variables with highest contribution to variability were PBR and F1TH. Plotting individuals within the discriminant space defined by the first two discriminant functions revealed clear groupings according to biogeographical region (Fig. 2). Similar levels of significance were shown for the Mahalanobis distances between these biogeographical groupings, but with insignificant differences between those of Tolima-Santander or Arauca-Barinas; the Magdalena-Guajira population showed no significant grouping with either of these two.

Regression analysis of sensilla density on latitude showed significant increase in TH of the second flagellar segment in accordance with increasing north latitude (Fig. 3A) with a similar decrease in TK (Fig. 3B). However, no such correlation was found for other sensilla types or for TH and TK on the first flagellar segment (data not shown).

TABLE II

Mean (standard deviation) of antennal sensilla of domestic *Rhodnius prolixus* from Colombia and Venezuela

Segment	Sensilla type [a]	Magdalena Guajira	Santander	Tolima	Arauca	Barinas
		Number of specimens				
		(11)	(10)	(11)	(11)	(10)
Pedicel	PBR	137 (13,36)	128 (15,68)	135 (10,27)	176 (20,51)	166 (24,14)
1st flagellar segment	F1BR	30 (4,85)	27 (5,10)	27 (2,16)	38 (22,62)	36 (5,32)
	F1TH	104 (18,95)	77 (15,74)	92 (20,62)	133 (18,64)	131 (23,10)
	F1TK	155 (21,13)	173 (30,86)	163 (21,14)	212 (42,78)	178 (34,41)
	F1BA	26 (6,50)	31 (7,81)	31 (7,59)	39 (7,60)	35 (10,83)
2nd flagellar segment	F2BR	14 (8,09)	11 (2,01)	11 (2,02)	21 (2,60)	15 (3,06)
	F2TH	81 (12,55)	47 (12,74)	43 (12,27)	57 (12,45)	66 (13,59)
	F2TK	203 (33,38)	225 (27,87)	242 (39,44)	265 (42,02)	210 (30,89)
	F2BA	34 (8,98)	38 (5,65)	38 (6,80)	46 (19,78)	35 (12,52)

a: mecanoreceptors: PBR, F1BR and F2BR; chemoreceptors: thin-walled trichoids (TH), thick-walled trichoids (TK), basiconics (BA). Following Catalá and Schofield (1994).

TABLE III

Univariate analysis of *Rhodnius prolixus* sensilla patterns

	Pedicel	1st flagellar segment				2nd flagellar segment			
ANOVA[a]	PBR	F1BR	F1TH	F1TK	F1BA	F2BR	F2TH	F2TK	F2BA
Between departments	0,000*	0,0318	0,000*	0,002*	0,004*	0,134	0,000*	0,003*	0,563
Llanos vs Andes	0,000*	0,000*	0,000*	0,031	0,041	0,003*	0,000*	0,784	0,705
Andes vs Sierra Nevada	0,249	0,130	0,038	0,170	0,073	0,307	0,000*	0,019	0,176
Sierra Nevada vs llanos	0,000*	0,028	0,001*	0,003*	0,000*	0,330	0,001*	0,072	0,396

a: significant value after being examined with Bonferroni, were marked with *.

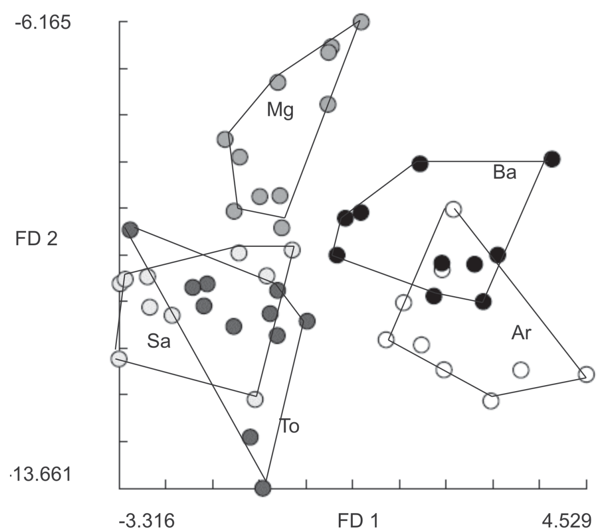

Fig. 2: discriminant analysis using PBR, F1TH, F1TK, F1BA, F2BR, F2TH, F2TK, Each point represents one individual insect on the canonical axes (FD1, FD2), with polygons encompassing the individuals from Arauca (Ar), Barinas (Ba), Santander (Sa), Tolima (To), and Magdalena-Guajira (Mg).

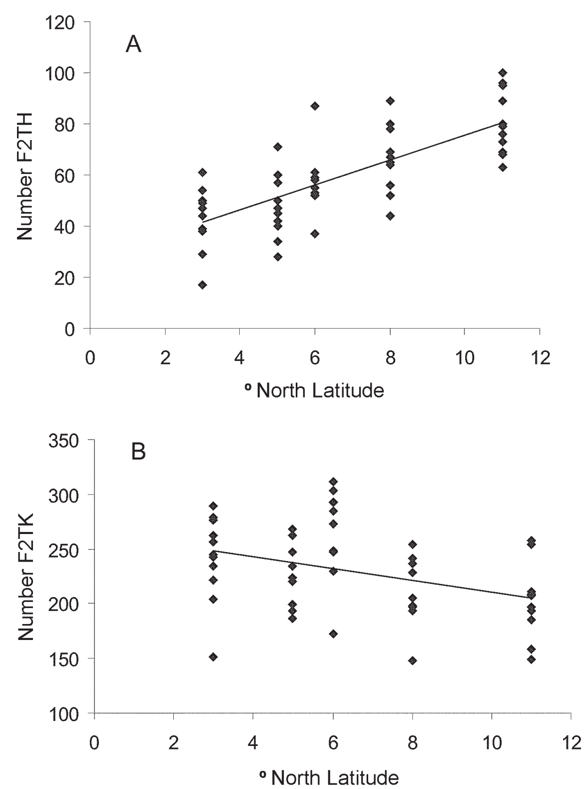

Fig. 3: latitudinal variation of the thin walled trichoids TH (A) and the thick walled trichoids (TK) (B) of the flagellum 2 in both sexes of populations of *Rhodnius prolixus* from Colombia and Venezuela (for Barinas, the highest latitude was taken from Table I).

DISCUSSION

The lack of overt sexual dimorphism in *Rhodnius*, and the relatively simple sensilla patterns, for example shown by the absence of chemoreceptors on the pedicel has been suggested to relate to the abundance of these insects in stable habitats such as domestic environments and palm tree crowns (Schofield 1988, Catalá 1997, Carbajal de la Fuente 2002). As shown by Dujardin et al. (1999), Triatominae tend to show reduced sexual dimorphism in domestic populations, compared to those occupying silvatic habitats. In the case of *R. prolixus*, the high densities attained in domestic habitats (Rabinovich et al. 1995, Sandoval et al. 2000) presumably facilitate encounters between sexes, reducing any necessity for sexual dimorphism or for specific means of sexual identity.

Populations of Triatominae occupying similar biogeographical regions will presumably evolve similarly, so that their antennal sensilla patterns can be expected to show similar features (Catalá & Dujardin 2001, Carbajal & Catalá 2002, Jaramillo et al. 2002). In the present study, the high degree of correct reclassification of *R. prolixus* populations in accordance with their biogeographical origin supports the idea that sensilla patterns can act as specific indicators for particular populations. However, since all populations examined were taken from similar domestic habitats, the sensilla patterns appear to be reflecting biogeographic features or population isolation rather than characters associated with different habitats.

The analysis of sensilla patterns indicates clear differentiation between *R. prolixus* populations to the east (Arauca and Barinas) and west (Santander and Tolima) of the Andean Cordillera, as has also been indicated by comparative head morphometrics (Esteban et al. 2000). Populations from Magdalena-Guajira do not group directly with either the west or eastern forms, but appear to be more similar to the western forms in the sense that they are differentiated from the western forms only by the distribution of TH on the second flagellar segment (F2TH). These groupings lend support to the idea that the original forms of domestic *R. prolixus* developed to the east of the Andes, in the region of the llanos of Venezuela and Colombia, being subsequently spread westwards presumably by accidental human intervention along a route following the pass between the Sierra Nevada and the northern tip of the Andean Cordillera (Schofield & Dujardin 1999). The direction of this presumed dispersal is further supported by the fact that for all sensilla types on all segments (except for the F2TH of the Magdalena-Guajira populations); density is reduced from east to west.

The latitudinal variation in the number of F2TK and F2TH, reported for the first time by *R. prolixus*, was also observed in first flagellar segment in populations of *T. dimidiata*, and explained by the authors in relation to the greater housing capacity to Ecuador (Catalá et al. 2005)

If this interpretation is correct, that domestic *R. prolixus* has been dispersed westwards into Colombia presumably by accidental human intervention and that the Andean Cordillera remains as a biogeographical barrier between the eastern populations of the llanos and the western populations of the Magdalena valley, then this could suggest that control interventions would be more successful against the western populations, since these are no longer within the original biogeographical region of the species. There is evidence that *R. prolixus* populations in Central America were also carried there from a Venezuelan source (Dujardin et al. 1998, Zeledón 2004). By contrast, in the llanos of Venezuela and eastern Colombia which are characterized by extensive palm tree stands where silvatic forms of *R. prolixus* have been reported (Gamboa 1963) the biology of domestic *R. prolixus* may require additional studies to reorientate binational control interventions.

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