Available online at www.sciencedirect.com

ScienceDirect

Acta Tropica 102 (2007) 47–54

ACTA TROPICA

www.elsevier.com/locate/actatropica

Sylvatic *Triatoma infestans* (Reduviidae, Triatominae) in the Andean valleys of Bolivia

Mirko Rojas Cortez[a,b,c], Laure Emperaire[d], RominaV. Piccinali[e], Ricardo E. Gürtler[e], Faustino Torrico[b], Ana Maria Jansen[f], François Noireau[b,d,*]

[a] *Departamento de Entomologia, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil*
[b] *Centro Universitario de Medicina Tropical, Facultad de Medicina, Universidad Mayor San Simon, Cochabamba, Bolivia*
[c] *Programa Nacional de Control de Chagas (PNCCH), Ministerio de Salud, La Paz, Bolivia*
[d] *UR 016, Institut de Recherche pour le Développement (IRD), Montpellier, France*
[e] *Laboratorio de Eco-Epidemiología, Departamento de Ecología, Genética y Evolución, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina*
[f] *Departamento de Protozoologia, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil*

Received 24 June 2006; received in revised form 24 February 2007; accepted 26 February 2007
Available online 1 March 2007

Abstract

Triatoma infestans is the main vector of Chagas disease in the Southern Cone countries. Wild populations of *T. infestans* appear widespread throughout the Andean valleys of Bolivia. In Cotapachi (2750 m asl), all sorts of rocky outcrops, regardless of their size, provided good refuges for *T. infestans*. Of the 1120 ecotopes investigated, 330 (29.5%) contained triatomines and 92% of the collected insects were nymphal instars. In the cold season, triatomine densities were similar in small and large outcrops. During the hot season, bug densities were higher in the larger outcrops, particularly in those located in peridomestic sites. *T. infestans* populations apparently produced one generation per year. Over half the sampled bugs were positive for *T. cruzi* infection. At Mataral (1750 m asl), a site located in the inter-Andean Chaco, a new morph of *T. infestans* was detected in a sylvatic environment.

Keywords: *Triatoma infestans*; Sylvatic populations; *Trypanosoma cruzi*; Andean valley; Bolivia

1. Introduction

Triatoma infestans (Klug) is the main vector of *Trypanosoma cruzi* (Kinetoplastida: Trypanosomatidae), the causative agent of Chagas disease, in the Southern Cone countries. Thereby, it is the target of a regional elimination program. Bolivia is the only country where the existence of wild *T. infestans* is documented (Noireau et al., 2005). Sylvatic colonies were first reported in a stony hill situated in the outskirts of Cochabamba (2600 m asl), an important Andean city (Torrico, 1946). Other sylvatic populations of *T. infestans* were found elsewhere in the Cochabamba region (Dujardin et al., 1987; Bermudez et al., 1993). In the late 1990s, *T. infestans* was found in sylvatic habitats some 120 km east of La Paz (2500 m asl) and in the lowlands of the Boreal Chaco at 350 m asl (Noireau et al., 1997, 2000). The Andean wild insects occurred amongst rock-piles and exhibited a chromatic pattern similar to the domestic bugs. In contrast, the wild *T. infestans* from the Chaco were arboreal and exhibited an overall darker coloration

---

[*] Corresponding author at: UR 016, Institut de Recherche pour le Développement (IRD), Montpellier, France. Tel.: +33 4 6741 6178; fax: +33 4 6754 2044.
E-mail address: francois.noireau@ird.fr (F. Noireau).

0001-706X/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.actatropica.2007.02.014

that distinguished them from the domestic *T. infestans*. A wider distribution of the wild *T. infestans* throughout the Andean valleys and the Chaco was suggested by the fact that our recent surveys have often been successful in detecting sylvatic populations. Therefore, in order to select a new study area in the Cochabamba valley, an attempt was made at Cotapachi, an area of dry hills covered with rocks. Some rocks were turned over and *T. infestans* colonies were found. Another focus was found near the small community of Mataral, where residents pointed out the occurrence of sylvatic triatomines in a rocky zone. The area was investigated and wild *T. infestans* were found. In summary, using the characteristics of the landscape (i.e. dry area covered with rocks) and villagers' reports as selection criteria, two new wild foci of *T. infestans* were detected in the Bolivian Andes.

The knowledge of the sylvatic ecology and the potential role of wild *T. infestans* in the transmission of *T. cruzi* to humans is still scarce in spite of the number and diversity of foci recorded. In the current study, wild *T. infestans* populations were investigated in three areas located in the Department of Cochabamba. Two of them (Cotapachi and Mataral) are described for the first time, whereas the third one (Jamach'uma) was re-surveyed after more than 10 years of the initial detection of sylvatic *T. infestans* there (Bermudez et al., 1993).

2. Materials and methods

2.1. Study areas

The three study areas were located in the central zone of the eastern cordillera in the Cochabamba Department. The climate is dry, hot during the summer and temperate in the winter. At Cochabamba city, the mean annual temperature is 16.3 °C and the mean annual rainfall is 362 mm (from November to February). Average summer and winter temperatures differ by less than 5 °C. The vegetation of the Andean valleys is generally disturbed because of the human occupation. It is dominated by thickets with scarce arboreal components (principally shrubs). The inhabitants of rural zones belong to the Quechua ethnic group. Their dwellings are mainly of wattle and daub construction and roofed with tiles.

Fig. 1. Landscape of the sylvatic focus of *T. infestans* at Cotapachi (2750 m asl), Cochabamba valley.

Cotapachi (2750 m asl; 17°26′S, 66°17′W) is a peri-urban area located in the outskirts of Quillacollo, a city that spread out with the extension of Cochabamba, the capital of the Department. The study area is formed by a semicircle of hills open to the east that surrounds a valley and peaks at some 60 m above it. Some dwellings and cultivated areas (market-gardening and maize) are located in the small valley. In the peridomestic environment, various structures accommodate cows, horses, sheep and goats, and some patches of Cactaceae (*Opuntia ficus-indica*) are cultivated. Guinea pigs are bred in cages placed on stony bases near the houses. The zone located on the foot and slopes of hills is more homogeneous. It is covered by rocky outcrops of different sizes and displays a low diversity of vegetation dominated by shrubs of Mimosoideae. We performed insect collections in three sylvatic sites located in an area of ≈1 km^2: the slope of hills (Fig. 1) and two far apart rocky outcrops made of large blocks and located in the valley (the first, situated some 100 m from the nearest house, is named Inca wall by the local population for its likeness to a human construction; the second, quite close to a house, here is named peridomestic outcrop).

Jamach'uma (2800 m asl; 17°31′S, 66°07′W) is also situated in the Cochabamba valley some 25 km southeast of Cotapachi. It is a narrow valley overhanging a wide lake. The vegetation is a mosaic of open and dense shrub formations, dominated by *Schinus* sp. (Anacardiaceae), Mimosaceae and Cactaceae. *T. infestans* was searched in small rocky outcrops and walls made of stony piles that run across the valley.

Mataral (1750 m asl; 18°36′S, 65°07′W) is situated some 200 km southeast of Cochabamba city and pertains to the semi-arid formation of the inter-Andean Chaco. The mean annual temperature is 18.8 °C and the mean annual rainfall is 540 mm. The collecting site was a secondary valley located 3 km far from the locality. The vegetation presents an herbaceous discontinuous stratum, with spots of Cactaceae and Bromeliaceae covered by deciduous shrubs. The southern slope of the valley, which exhibits piles of large rocky blocks in some places, was investigated.

2.2. Collection of triatomines

The area of Cotapachi was investigated nine times between June 2002 and June 2005. Jamach'uma was surveyed in May 2005 and Mataral three times, in January 2003, May 2004 and May 2005. *T. infestans* was searched in cracks between rocks and nests or shelters of small mammals located under the rocks. Baited traps as described by Noireau et al. (1999) were used to capture the insects. The stage structure of *T. infestans* populations at Cotapachi was investigated by collecting triatomines during four periods of the year 2003 (January, April, July and October).

2.3. Genetic characterization of *T. infestans*

In order to confirm the status of the first wild adult bug collected in Mataral as *T. infestans*, a 661 bp fragment of the mitochondrial gene COI was sequenced using primers S1718 and A2442 (Normark, 1996) and compared with other triatomine sequences available at GenBank (García and Powell, 1998; Gaunt and Miles, 2002) and two sequences from domestic *T. infestans* from Bolivia and Argentina (Piccinali et al., unpublished results). Most parsimonious trees were found with a heuristic search with 500 random addition sequences and tree bisection-reconnection branch swapping (mult*max*) using NONA 2.0 (Goloboff, 1999). All nucleotide positions were considered to have the same weight and all characters were regarded as unordered. Statistical support for the clades in the trees was assessed by means of bootstrap methods (Felsenstein, 1985).

2.4. Parasitological analysis of Triatominae

Feces were obtained by gently squeezing the live insects. The fecal droplets were then mixed with phosphate buffered saline and examined for the presence of flagellates by direct microscopic observation at 400× magnification.

2.5. Statistical analysis

Two standard indices of triatomine density were used: percentage of positive traps and mean number of insects per positive trap (Noireau et al., 2000). Results of insect numbers per positive trap were also expressed as medians. At Cotapachi, the differences in frequencies between sites according to season were analyzed using χ^2 tests. The Mann–Whitney and Kruskal–Wallis tests were used to compare the median numbers of triatomines per positive trap according to collection site and season.

3. Results

Of 1120 bait-traps placed amongst the rocks at Cotapachi, 330 (29.5%) collected live triatomines (Table 1). With the exception of two *Triatoma sordida* specimens, all the others were identified as *T. infestans*. The great majority were nymphal instars (797 of 868, 92%).

Triatomine density in each collecting site was compared from March to June (cold season), and from September to January (hot season). The data corresponding to the three survey years were pooled for both seasons and presented in Table 1. All the sites investigated were positive for wild *T. infestans*. In the cold season, the

Table 1
Apparent density of *T. infestans* in rocks at Cotapachi (2002–2005)

	Hill slopes		Large outcrop (Inca wall)		Large peridomestic outcrop		Total	
	Cold season[a]	Hot season[b]	Cold season[a]	Hot season[b]	Cold season[a]	Hot season[b]	Cold season[a]	Hot season[b]
No. of traps	473	297	88	144	85	33	646	474
No. (%) of positive traps	105 (22.2)[c]	94 (31.6)[d]	18 (20.5)[c]	65 (45.1)[d]	25 (29.4)[c]	23 (69.7)[d]	148 (22.9)	182 (38.4)
No. of triatomines	186	259	32	135	68	188	286	582
Mean no. (± S.D.) of triatomines per positive trap	1.8 (1.2)	2.7 (3.9)	1.8 (0.8)	2.1 (1.7)	2.7 (2.8)	8.2 (11.0)	1.9 (1.6)	3.2 (5.2)
Median catch (first and thid quartiles) per positive trap	1 (1–2)[e]	1 (1–3)[f]	2 (1–2)[e]	1 (1–3)[f]	1 (1–3)[e]	4 (2–7.5)[f]	1 (1–2)	2 (1–3)

[a] Cold season extends from March (late summer) to June (early winter).

[b] Hot season extends from September (late winter) to January (summer).

[c] χ^2-test, d.f. = 2, $P = 0.29$.

[d] χ^2-test, d.f. = 2, $P \leq 0.001$.

[e] Kruskal-Wallis test, d.f. = 2, $P = 0.64$.

[f] Kruskal-Wallis test, d.f. = 2, $P \leq 0.0001$.

three sites had similar percentages of positive traps ranging from 20.5% to 29.4% (χ^2-test d.f. = 2, P = 0.29; medians of triatomine catch per positive trap: 2 and 1, respectively, Kruskal–Wallis test d.f. = 2, P = 0.64). In the hot season, the hills had the lowest percentage of positive traps (31.6%, χ^2-test d.f. = 2, $P \leq 0.001$), whereas the large peridomestic outcrop had a significantly higher index than the Inca wall (69.7% versus 45.1%, χ^2-test d.f. = 1, P = 0.019). In the hot season, the triatomine catch per positive trap in the large peridomestic outcrop was significantly higher than in the other sites (Kruskal–Wallis test d.f. = 2, $P \leq 0.0001$). Regarding seasonal variations of bug density, the percentage of positive traps in the three sites was always higher in the hot rather than in the cold season (χ^2-test d.f. = 1, P = 0.0046). Similarly, the catch of triatomines per positive trap increased markedly in all the sites but the large peridomestic outcrop was the only one that showed significant seasonal variations in density (Mann–Whitney test, P = 0.0038).

The stage structure of *T. infestans* populations at Cotapachi showed that all the stages were collected throughout the year (Fig. 2). Catches of first and second instars peaked in summer (January) and were very low in winter (July).

Fecal samples of 233 triatomines collected at the three sites in Cotapachi were examined microscopically for *T. cruzi* (Table 2). A total of 136 bugs (58.4%) were positive for *T. cruzi*. The infection prevalence increased with developmental stage from 20% in first/second instar nymphs to 80% and 94% in male and female *T. infestans* (adult average, 91.1%).

Results of captures performed in the areas of Mataral and Jamach'uma are summarized in Table 3. The percentages of positive traps varied between 3.1% and 13.0%, depending on site and collection period. Surprisingly, the two sylvatic adult forms collected in Mataral were morphologically distinct from the domestic *T. infestans* collected in the same area, and also differed from those collected in the Cochambamba valley. They were larger and had a yellowish connexivum with transverse black marks on the dorsum (Fig. 3). On the other hand, the sylvatic nymphs collected in Mataral were similar to the domestic ones. The phylogenetic analysis strongly supported that the single sylvatic Mataral bug analyzed belonged to *T. infestans*. Its COI sequence was exactly the same as the one of a

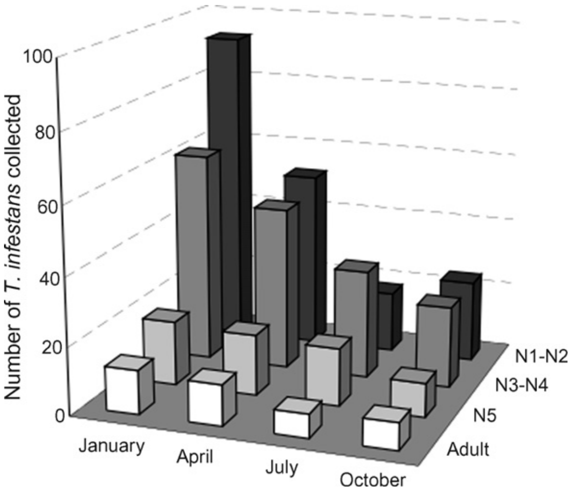

Fig. 2. Seasonal variations of sylvatic *T. infestans* numbers according to developmental stage at Cotapachi, Cochabamba valley. The numbers of collected adult and nymphal stages were pooled for all collecting sites and plotted for each 3-month period of the year 2003. The nymphal stages 1 and 2, and 3–4 were pooled, respectively.

Table 2
Infection by *T. cruzi* in *T. infestans* according to stage (Cotapachi, 2002–2003)

	N1–N2	N3	N4	N5	Males	Females	Total
No. examined	25	79	33	39	40	17	233
No. positive	5	36	19	28	32	16	136
% Positive	20.0	45.6	57.6	71.8	80.0	94.1	58.4

Table 3
Capture of wild *T. infestans* at Mataral and Jamach'uma

Study site	Collection period	No. of traps	No. (%) of positive traps	Total bugs collected	
				Nymphs	Adults
Mataral	January 2003	60	4 (7.7)	5	0
	May 2004	26	3 (11.5)	3	1
	May 2005	32	1 (3.1)	0	1
Jamach'uma	May 2005	23	3 (13.0)	3	1

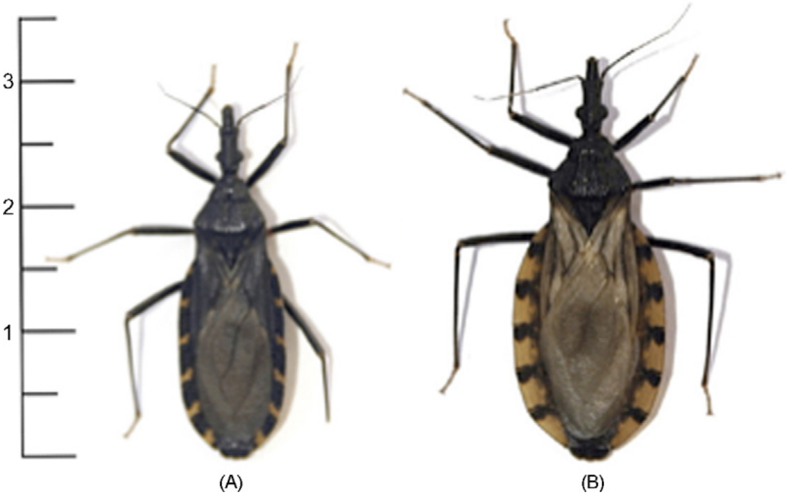

Fig. 3. Morphological and chromatic differences between domestic and sylvatic *T. infestans* collected in Mataral: (A) Domestic female and (B) sylvatic female.

domestic *T. infestans* collected in Mataral village, and formed a well supported clade together with the *T. infestans* sequences from Argentina (Fig. 4). Bolivian and Argentinian haplotypes of *T. infestans* group were separated by only 11 or 12 mutational steps, a level of intraspecific variation that was very similar to the values observed between sequences of *T. guasayana* (15 changes) or *T. brasiliensis* (17 changes).

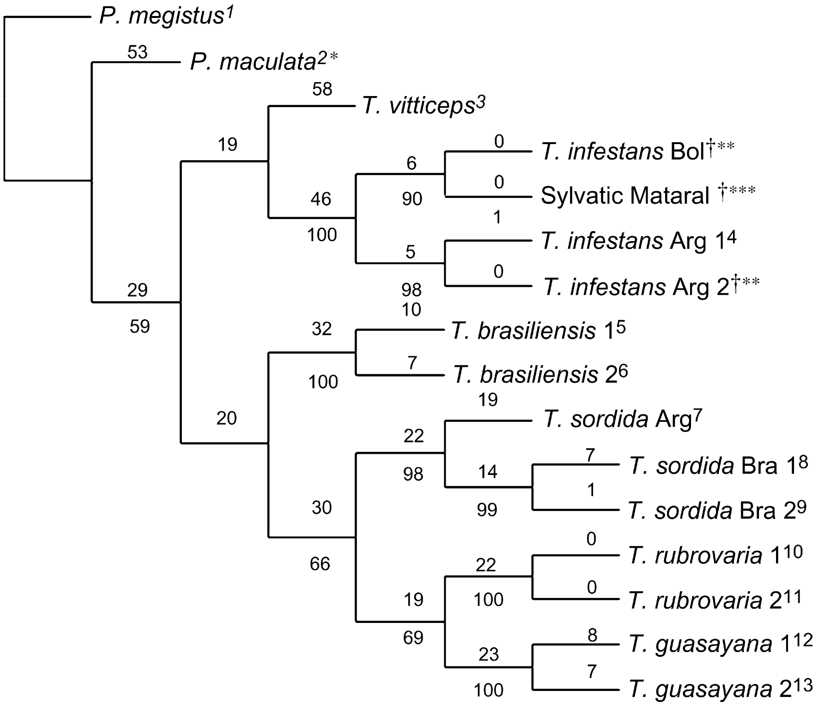

Fig. 4. Most parsimonious tree based on COI sequences for several *Triatoma* species, including the Mataral sylvatic bug. Tree length: 511. Consistency index: 0.59. Retention index: 0.72. Numbers above branches: number of changes (ACCTRAN optimization). Numbers below branches: boostrap values above 50%. *Gaunt and Miles (2002). **Piccinali et al. (unpublished data). ***Present work. All the remaining COI sequences are from García and Powell (1998). GeneBank accession number: [1]AF021179, [2]AF449139, [3]AF021219, [4]AF021199, [5]AF021184, [6]AF021186, [7]AF021210, [8]AF021213, [9]AF021216, [10]AF021204, [11]AF021206, [12]AF021193, [13]AF021195, †Submitted and waiting for an accession number.

4. Discussion

The Southern Cone Initiative against Chagas disease relies primarily on the interruption of transmission by elimination of domestic *T. infestans* populations (Schofield and Dias, 1998). In Bolivia, control activities have made substantial progress even in the face of recent detection of insecticide resistance in the vector (PAHO, 2005). The expected success of the elimination program was based on the assumption that *T. infestans* is an almost exclusive domestic vector. Recent fieldwork revealed that wild populations were much more widespread throughout Bolivia and also in neighboring countries than previously thought (Noireau et al., 2005). Up to date in Bolivia, wild *T. infestans* populations were found in three Andean departments (Cochabamba, La Paz and Potosi) and in the Chaco region (Department of Santa Cruz). Outside of Bolivia, wild *T. infestans* were recently detected in Santiago del Estero and Chaco Provinces, Argentina (L.A. Ceballos and R.E. Gürtler, unpublished data) and in the Metropolitan region of Chile (Bacigalupo et al., 2006).

To date, Cotapachi is the more widespread focus of wild *T. infestans* found in the Andean valleys. It covers several hectares, and various rocky outcrops, regardless of their size, provide good refuges for triatomine bugs. The indices of triatomine density used in this study were based on trap catches. Because the live-baited system is likely to attract and capture starved bugs, the observed stage structure of the population may be biased (Noireau et al., 2000). In the cold season, the densities of bugs were similar among the small rocky outcrops located in the hills and in the large ones situated in the valley. During the hot season, triatomine densities increased consistently across all sites, but the median abundance of *T. infestans* in peridomestic outcrop increased four-fold. Overall, the greatest catches were in the large outcrop close to a house. In addition to providing good shelter for wild animals (rodents and marsupials) as shown by Cortez et al. (2006), these large rocks may offer temporary refuge for domestic animals (poultry, dogs and cats) from the neighboring houses. The abundance of host-feeding sources would favor the population growth of triatomines. Although the large rocky outcrop ($\approx 30\,\mathrm{m}^3$) located near the house is undoubtedly peridomestic in location, it is a natural site little modified by human activities. In our opinion, we consider it as a natural site close to a house. In contrast, the hill slopes and Inca wall were clearly sylvatic based on the distance to the nearest house and, more specifically, by the absence of human-made alteration.

The insects collected in the rocks were found infected by *T. cruzi*. Genotyping of trypanosomes using the mini-exon approach and multilocus enzyme electrophoresis has confirmed that they were *T. cruzi* I (Cortez et al., 2006). The infection rate (58.4%) was similar to that observed in another Andean *T. infestans* wild population (73%; Bermudez et al., 1993), and higher than that observed in dark morph populations of *T. infestans* from the Chaco (2.5%; Noireau et al., 2000).

The stage structure of *T. infestans* wild populations exhibited strong seasonal variations. The younger developmental stages (nymphs 1 and 2) peaked during the hot season and the peak of adults occurred over the previous 3-month period. As in central Argentina (Gorla and Schofield, 1989), the yearly pattern of population stage-structure at Cotapachi is characterized by only one emergence peak of young nymphs and corresponds to the production of one generation a year. In contrast, domestic habitats enable *T. infestans* to produce two generations a year in the warmer climate of central Brazil (Schofield, 1980). In addition to weather-related factors, fluctuations in host availability in wild habitats may put bugs at a disadvantage and explain the increased developmental times.

The wild *T. infestans* collected in Cotapachi were morphologically and chromatically similar to domestic bugs from the same area. In contrast, the adult forms of the wild *T. infestans* from Mataral (inter-Andean Chaco; 1750 m asl) are morphologically and chromatically very different from the sylvatic specimens collected in Cochabamba (2750 m asl) and the dark morphs from the Bolivian Chaco (350 m asl) (Noireau et al., 1997). In particular, their size was greater and the marks on the connexivum were clearly different (Fig. 2). Nevertheless, the characterization of a fragment of COI and its comparison with other triatomines confirmed them as *T. infestans*. Moreover, the particular haplotype of the analyzed insect has been found also in domestic *T. infestans* from Mataral as well as in sylvatic *T. infestans* from Cotapachi (Piccinali et al., unpublished results).

The Andean wild *T. infestans* is assumed to be the most ancient form (Dujardin et al., 1998; Panzera et al., 2004; Bargues et al., 2006). Nevertheless, the detection of wild *T. infestans* in the boreal Chaco challenged this opinion and suggested an ancestral population native to this ecogeographic region (Carcavallo et al., 2000). The detection of a new morph of *T. infestans* in the inter-Andean Chaco (i.e. intermediate between the high Andean valleys and the Chaco) adds to the complexity of the question and makes necessary further studies incorporating the Mataral morph. Regardless of the ancestral form of *T. infestans* (Andean or Chaco), the other wild populations could be a consequence of a geographic expansion of the primitive wild population or derivatives from domestic insects recolonizing wild habitats.

Current knowledge on wild populations of *T. infestans* is scarce. Therefore, it is necessary to expand this research and investigate its geographic distribution across the Southern Cone. A landscape ecology approach of the known foci and spatial analysis using GIS and remote sensing is indicated to predict its geographic distribution (Gorla, 2002). However, the fundamental question is whether wild *T. infestans* may play a role in the reinfestation of insecticide treated houses. A detailed analysis of gene flow between wild and domestic populations is needed to test whether it occurs and if so, how intense it is.

Acknowledgements

This study benefited from international collaboration through the European Community—Latin America Triatomine Research (ECLAT) network and received financial support from CAPES and CNPq (Brazil), and the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (grant A50678). R. Rodriguez gave invaluable assistance in the fieldwork; L. Ceballos kindly assisted fieldwork during 2005. Special thanks to the National Program of Chagas Control (PNCCH), to SEDES Cochabamba and SENAMIH Bolivia. Participation of R.V. Piccinali and R.E. Gürtler was supported by awards from the National Institutes of Health/National Science Foundation Ecology of Infectious Disease program award R01TW05836 funded by the Fogarty International Center and the National Institute of Environmental Health Sciences (Uriel Kitron and REG, PI), the Agencia Nacional de Promoción Científica y Técnica (Argentina) and the University of Buenos Aires. R.E. Gürtler is member of CONICET Researcher's Career and R.V. Piccinali is a CONICET postdoctoral fellow.

References

Bacigalupo, B.A., Segura, M.J.A., Garcia, C.A., Hidalgo, C.J., Galuppo, G.S., Cattan, P.E., 2006. Primer hallazgo de vectores de la enfermedad de Chagas asociados a matorrales silvestres en la Región Metropolitana, Chile. Rev. Méd. Chile 134, 1230–1236.

Bargues, M.D., Klisiowicz, D.R., Panzera, F., Noireau, F., Marcilla, A., Perez, R., Rojas, M.G., O'connor, J.E., Gonzáles-Candelas, F., Galvão, C., Jurberg, J., Carcavallo, R.U., Dujardin, J.P., Mas-Coma, S., 2006. Origin and phylogeography of the Chagas disease main vector *Triatoma infestans* based on nuclear rDNA sequences and genome size. Infect. Gen. Evol. 6, 46–62.

Bermudez, H., Balderrama, F., Torrico, F., 1993. Identification and characterization of wild foci of *Triatoma infestans* in Central Bolivia. Am. J. Trop. Med. Hyg. 49 (Suppl.), 371.

Carcavallo, R.U., Jurberg, J., Lent, H., Noireau, F., Galvão, C., 2000. Phylogeny of the Triatominae (Hemiptera: Reduviidae) proposals for taxonomic arrangements. Entomol. Vect. 7, 1–99.

Cortez, M.R., Pinho, A.P., Cuervo, P., Alfaro, F., Solano, M., Xavier, M.C.C., d'Andrea, P.S., Fernandes, O., Torrico, F., Noireau, F., Jansen, A.M., 2006. *Trypanosoma cruzi* (Kinetoplastida Trypanosomatidae): ecology of the transmission cycle of in the wild environment of the Andean valley of Cochabamba, Bolivia. Exp. Parasitol. 114, 305–313.

Dujardin, J.P., Tibayrenc, M., Venegas, E., Maldonado, P., Desjeux, P., Ayala, F.J., 1987. Isozyme evidence of lack of speciation between wild and domestic *Triatoma infestans* (Heteroptera: Reduviidae) in Bolivia. J. Med. Entomol. 24, 40–45.

Dujardin, J.P., Schofield, C.J., Tibayrenc, M., 1998. Population structure of Andean *Triatoma infestans*: allozyme frequencies and their epidemiological relevance. Med. Vet. Entomol. 12, 20–29.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

García, B.A., Powell, J.R., 1998. Phylogeny of species of *Triatoma* (Hemiptera: Reduviidae) based on mitochondrial DNA sequences. J. Med. Entomol. 35, 232–238.

Gaunt, M.W., Miles, M.A., 2002. An insect molecular clock dates the origin of the insects and accords with palaeontological and biogeographic landmarks. Mol. Biol. Evol. 19, 748–761.

Goloboff, P., 1999. NONA (NO NAME) version 2. Tucumán, Argentina.

Gorla, D.E., 2002. Variables ambientales registradas por sensores remotos como indicadores de la distribución geográfica de *Triatoma infestans* (Heteroptera: Reduviidae). Ecol. Austral 12, 117–127.

Gorla, D.E., Schofield, C.J., 1989. Population dynamics of *Triatoma infestans* under natural climatic conditions in the Argentine Chaco. Med. Vet. Entomol. 3, 179–194.

Noireau, F., Flores, R., Gutierrez, T., Dujardin, J.P., 1997. Detection of wild dark morphs of *Triatoma infestans* in the Bolivian Chaco. Mem. Inst. Oswaldo Cruz 92, 583–584.

Noireau, F., Flores, R., Vargas, F., 1999. Trapping sylvatic Triatominae (Reduviidae) in hollow trees. Trans. R. Soc. Trop. Med. Hyg. 93, 13–14.

Noireau, F., Flores, R., Gutierrez, T., Abad-Franch, F., Flores, E., Vargas, F., 2000. Natural ecotopes of *Triatoma infestans* dark morph and other wild triatomines in the Bolivian Chaco. Trans. R. Soc. Trop. Med. Hyg. 94, 23–27.

Noireau, F., Cortez, M.R., Monteiro, F.A., Jansen, A.M., Torrico, F., 2005. Can wild Triatoma infestans foci in Bolívia jeopardize Chagas disease control efforts? Trends Parasitol. 21, 7–10.

Normark, B.B., 1996. Phylogeny and evolution of parthenogenetic weevils of the *Aramigus tessellatus* species complex (Coleoptera: Curculionidae: Naupactini): evidence from mitochondrial DNA sequences. Evolution 50, 734–745.

PAHO, 2005. http://www.paho.org/English/AD/DPC/CD/dch-cdia-II-reunion.htm.

Panzera, F., Dujardin, J.P., Nicolini, P., Caraccio, M.N., Rose, V., Tellez, T., Bermudez, H., Bargues, M.D., Mas-Coma, S., O'Connor, J.E., Perez, R., 2004. Genomic changes of Chagas disease vector, South America. Emerg. Infect. Dis. 10, 438–446.

Schofield, C.J., 1980. Nutritional status of domestic populations of *Triatoma infestans*. Trans. R. Soc. Trop. Med. Hyg. 74, 770–778.

Schofield, C.J., Dias, J.C.P., 1998. The Southern cone initiative against Chagas disease. Adv. Parasitol. 42, 1–27.

Torrico, R.A., 1946. Hallazgo de *Eratyrus mucronatus*, infestación natural de "vinchucas" de cerro y *Eutriatoma sordida* en Cochabamba. An. Lab. Central Cochabamba 1, 19–23.