

New Discoveries of Sylvatic *Triatoma infestans* (Hemiptera: Reduviidae) Throughout the Bolivian Chaco

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Abstract. Sylvatic populations of *Triatoma infestans* might be involved in the recolonization of human dwellings. We report here the discoveries of new *T. infestans* sylvatic foci in the Bolivian Chaco. Eighty-one triatomines were caught, 38 of which were identified as *T. infestans*. *Triatoma sordida* and *Panstrongylus geniculatus* were the other species collected. One *T. infestans* and one *T. sordida* were infected with *Trypanosoma cruzi* TcI; one *T. infestans* was infected with TcII. These discoveries add to the debate on the geographic distribution of sylvatic *T. infestans* populations, the geographic origin of the species, and the epidemiological role of these populations.

In South America, *Triatoma infestans* remains the main vector of *Trypanosoma cruzi*, the causative agent of Chagas disease. This species has been considered almost exclusively domestic for a long time: despite old reports of occasional findings of *T. infestans* in sylvatic areas in Argentina, Brazil, and Paraguay,^{1–5} it was thought that true sylvatic foci were restricted to the Andean valleys of Cochabamba in Bolivia, where sylvatic populations had repeatedly been detected.^{6–8} This belief led to the hypothesis that the origin and initial domestication of the species occurred in the Bolivian Andes.^{9,10} Moreover, when the vector control programs started, the possibility of a recolonization of treated areas by sylvatic bugs was mainly discarded. Recently, it has been shown that Andean sylvatic populations in Bolivia are distributed in larger areas than the Cochabamba valleys.^{11–14} In addition to the Bolivian Andean valleys, dark morphs of *T. infestans* were reported in 1997 in one sylvatic area of the Bolivian Chaco,¹⁵ and recently, other sylvatic foci were discovered in the Argentinean and Paraguayan Chaco and in Chile,^{16–19} challenging the hypothesis that the species originated in the Bolivian Andes. All of these data show that sylvatic *T. infestans* populations are much more widespread than previously thought and that their role in the recolonization of treated areas has to be considered.^{13,20} Indeed, domestic *T. infestans* persists primarily in the Bolivian Andean valleys and in the Gran Chaco ecoregion, precisely where sylvatic populations have been found.²¹

Here, we report the discovery of new sylvatic foci in the Bolivian Chaco, highlighting the wide distribution of sylvatic *T. infestans* in this region and questioning the geographic origin of the species, its domestication process, and the epidemiological role played by sylvatic populations.

The search for triatomines was conducted in October and November 2009, using mouse-baited adhesive traps in 14 forest sites located in 11 different sylvatic areas (Table 1) of the Bolivian Chaco (Figure 1).²² Two search methods were used.

In the first method, five sylvatic sites were randomly pre-selected (GC1–GC5), and traps were placed along transects (200–500 m) into the forest, in hollows of live (35.1%) or dead (42.2%) trees, burrows (13.4%), and other locations, such as under woodpiles (9.3%). The second method focused the search on nine forest sites selected according to the information provided by inhabitants (Y01 to Y09), and traps were placed mostly in hollows of live trees (88.2%). Traps were set in the afternoon and inspected the next morning. The triatomines caught were first identified with morphological keys,²³ and then sequencing of the rDNA ITS-2 region according to previous descriptions.^{14,24}

A total of 580 traps were set. Forty-six were positive for triatomines (7.9%) in nine different forest sites (64%, Table 1). Eighty-one triatomines belonging to *T. infestans*, *Triatoma sordida*, and *Panstrongylus geniculatus* were collected. Only five adults were found: four were morphologically and genetically identified as *T. infestans* and exhibited the dark phenotype¹⁵; one was identified as *T. sordida*. The other specimens were nymphs and were identified only after sequencing. Complete or partial ITS-2 sequences (386 bp at least) were resolved for 57 specimens. Thirty-eight were characterized as *T. infestans*: 1) 27 presented the ITS2Hap2 haplotype, previously found in sylvatic *T. infestans* from the Bolivian Chaco (accession no. HQ333212)¹⁴; 2) one, collected in GC03, presented the ITS2Hap1 haplotype (accession no. HQ333211), previously reported as the only Andean haplotype in sylvatic *T. infestans* from Bolivia¹⁴; and 3) 10, for which the resolved sequences were partial, presented 99% or 100% identity with various sequences deposited for *T. infestans* (accession nos. HQ333214, HQ333212, HQ333211, AY860388, AY860387, AJ582025, AJ582024, AJ576055, AJ576054, AJ576052, AJ576051). Seventeen specimens were characterized as *T. sordida* (accession no. AJ576063, 99% identity) and two as *P. geniculatus* (accession no. AJ306543, 99% identity). *T. infestans* and *T. sordida* specimens were all collected in hollow trees (Figure 2); the two *P. geniculatus* were captured in burrows.

Feces of 43 bugs were microscopically observed, and no flagellates were found. The infection was then determined for 24 bugs (11 *T. infestans*, 11 *T. sordida*, and the two *P. geniculatus*) using the mini-exon multiplex polymerase

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TABLE 1
Geographical localization of the investigated sylvatic areas in the Bolivian Chaco and triatomine captures

Sylvatic area	Department	Nearest village	Forest site code	Distance to nearest human habitat (m)	Latitude (S)	Longitude (w)	Altitude (m)	Number of traps	Number positive trap (%)	Number and species of caught triatomines					Total
										<i>T. infestans</i>	<i>T. sordida</i>	<i>P. geniculatus</i>	Undetermined species*		
1	Tarija	Caiza	Y01	6,850	21°50'50.6"	63°14'51.6"	442	40	4 (10.0)	1	0	3	4		
2	Tarija	La Quinta-Caiza	Caiza GC01	850	21°49'56.0"	63°33'15.2"	605	48	0 (0.0)	0	0	0	0		
3	Tarija	Cueva de Leon	GC02	810	21°22'37.0"	63°21'34.8"	347	61	8 (13.1)	4	0	1	9		
4	Tarija	Taiguati	Y04	570	21°10'34.0"	63°23'05.5"	471	41	2 (4.9)	2	0	0	2		
5	Chquisaca	Machareti	Y02	760	21°09'25.3"	63°22'30.7"	488	20	1 (5.0)	1	0	0	1		
6	Santa Cruz	San Antonio	Y03	2,840	20°49'06.4"	63°19'18.8"	646	20	0 (0.0)	0	0	0	0		
			Y07	110	20°15'06.8"	62°57'46.5"	581	45	4 (8.9)	2	0	2	12		
			Y08	8,555	20°12'02.3"	63°01'21.4"	578	49	7 (14.3)	8	0	3	11		
			Y09	1,500	20°10'15.9"	63°01'22.7"	615	60	11 (18.3)	17	1	13	31		
7	Santa Cruz	17 de Noviembre	Y05	170	20°00'48.3"	61°54'11.2"	352	22	0 (0.0)	0	0	0	0		
8	Santa Cruz	Yanahigua	Y06	260	19°56'09.1"	62°07'35.8"	385	15	0 (0.0)	0	0	0	0		
9	Santa Cruz	San Lorenzo	GC03	450	19°38'54.3"	63°06'46.1"	697	63	6 (9.5)	1	0	1	7		
10	Santa Cruz	Estancia Basilio	GC04	2,200	18°06'55.9"	63°12'57.5"	543	48	0 (0.0)	0	0	0	0		
11	Santa Cruz	Sinat	GC04	1,200	17°56'24.0"	62°65'6.07"	272	48	3 (6.3)	1	2	1	4		
Total							580	46 (7.9)	38	17	2	24	81		

* All the triatomines in this column were nymphs whose species have not been genetically confirmed.

chain reaction method after DNA extraction from the digestive tracts,²⁵ and the genetic characterization of parasites was completed by sequencing of the glucose-6-phosphate isomerase gene. Only three bugs were positive: two *T. infestans* (one male and one 4th instar captured in Y08 and GC02, respectively) and one *T. sordida* (4th instar captured in GC05). The *T. infestans* male was infected with TcII. The two other bugs were infected with TcI. This report is the first to document the presence of TcII in a sylvatic *T. infestans* specimen.

Sylvatic *T. infestans* were captured in three areas (3, 6, and 9) farther south (130–320 km) than Tita, the only area with sylvatic *T. infestans* previously recorded in the Bolivian Chaco (Figure 1).¹⁵ These discoveries, and the recent finding of sylvatic foci in the Argentinean and Paraguayan Chaco,^{17,19} highlight the wide distribution of sylvatic *T. infestans* in the Gran Chaco ecoregion. Interestingly, the Y01 and Y08 sites were distant from human habitat (≈ 8 km). As in the Tita area, this supports the primary occurrence of sylvatic populations in these areas from the Gran Chaco and makes the hypothesis of a secondary colonization of the sylvatic environment by bugs derived from domestic populations unlikely. Different studies have recently shown that the Andean origin of *T. infestans* was not unequivocally supported and that a Gran Chaco origin could not be rejected.^{14,26,27} Among the arguments used to support the hypothesis of an Andean origin is the scarcity of *T. infestans* sylvatic foci in the Gran Chaco; this hypothesis is now weakened given the new foci reported here and those reported in the Argentinean and Paraguayan Chaco.^{17,19}

In this study, several forest sites positive for *T. infestans* were close to human habitat (< 500 m), supporting the possibility of incursion events of sylvatic specimens into houses. The traditional hypothesis to explain the current distribution of domestic *T. infestans* in South America puts forward an initial and major vector domiciliation in the Bolivian Andes, followed by a recent passive human-mediated spread.^{9,10} This hypothesis has been further supported by the genetic similarities between domestic and sylvatic *T. infestans* populations in the Bolivian Andes.^{7,28} In fact, the same genetic argument can be applied to the Bolivian Chaco, where ITS-2 and mtCytB haplotypes are shared between domestic and sylvatic *T. infestans*.^{14,24} The diversity of ecoregions and ecotopes in which *T. infestans* sylvatic populations have been found to date and the propensity of this species to diversify feeding sources suggest a long evolutive process that allowed it to acquire a strong adaptive ability and to survive in a great variety of environments. Consequently, domestication could be an opportunity rather than a costly adaptive change. In this way, new human settlements intruding into the sylvatic environment would provide an opportunity for *T. infestans* to feed on new mammal species (humans, domestic animals) that are more stable (an easy feeding source) than sylvatic mammals. Easy domestication of *T. infestans* sylvatic populations suggests that their epidemiological role has to be considered carefully because they can infest dwellings after vector control and new human settlements in sylvatic environments.

In the current study, the infection rate of sylvatic *T. infestans* was low (18.2%) but not negligible. In contrast, in the Andes, sylvatic *T. infestans* populations have a high infection rate.¹³ The low infection reported here agrees with previous results in the Gran Chaco.^{17,29} It might be related to the main ecotope investigated (hollow trees), perhaps occupied by birds (which are not *T. cruzi* reservoirs). This result might strengthen the

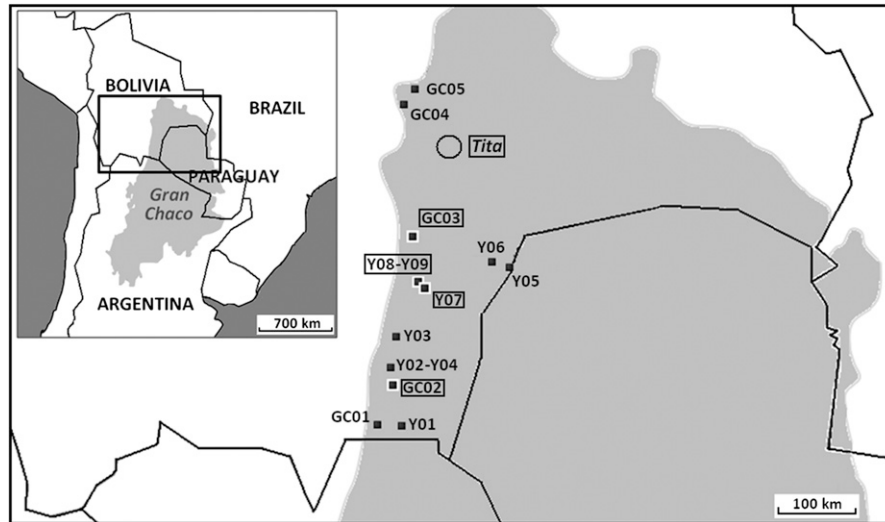


FIGURE 1. Forest sites in which the search for sylvatic triatomines was carried out in the Bolivian Chaco (see also Table 1). Sylvatic areas: 1 = Y01; 2 = GC01; 3 = GC02; 4 = Y02 + Y04; 5 = Y03; 6 = Y07 + Y08 + Y09; 7 = Y05; 8 = Y06; 9 = GC03; 10 = GC04; 11 = GC05; Tita = only one with sylvatic *Triatoma infestans* previously recorded in the Bolivian Chaco.¹⁵ Circled names: positives for sylvatic *T. infestans*.

hypothesis of an ornithophilic feeding behavior of these populations. Nevertheless, most of the hollow trees in which *T. infestans* were found during the current fieldwork did not seem to be inhabited by birds. Consequently, other blood sources (lowly infected small mammals, reptiles) and the



FIGURE 2. Hollow tree of the Bolivian Chaco, positive for *Triatoma infestans*. Typical ecotope in which sylvatic triatomines were collected in the current fieldwork.

occurrence of hemolymphagy cannot be discarded. Future studies determining the blood sources of *T. infestans* in the Gran Chaco will clarify the *T. infestans* ecology in this region.

Extensive searches for sylvatic *T. infestans* in the Gran Chaco must be pursued to determine the distribution of sylvatic populations in this ecoregion more accurately. Future genetic studies of these sylvatic populations with Andean populations will help determine the geographical origin and dispersion routes of the species. Other molecular studies comparing the genetic characteristics of sylvatic and domestic bugs are also needed to clarify the epidemiological role of sylvatic populations and to improve our understanding of the domestication processes of *T. infestans*.

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