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 discarded.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.10,16 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 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.

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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,22 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 phenotype; 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 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
Table 1.

Geographical localization of the investigated sylvatic areas in the Bolivian Chaco and triatomines captures.

<table>
<thead>
<tr>
<th>Department</th>
<th>Forest and Nearest village</th>
<th>Distance to nearest human habitat (m)</th>
<th>Latitude (°S)</th>
<th>Longitude (°W)</th>
<th>Altitude (m)</th>
<th>Number of captures</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tarija</td>
<td>Caiza</td>
<td>6,850</td>
<td>21°50’50.46”</td>
<td>63°14’51.6”</td>
<td>21.50’50.46”</td>
<td>63°14’51.6”</td>
<td>T. infestans</td>
</tr>
<tr>
<td>Tarija</td>
<td>La Quinta-Caiza Caiza</td>
<td>850</td>
<td>21°50’50.46”</td>
<td>63°14’51.6”</td>
<td>21.50’50.46”</td>
<td>63°14’51.6”</td>
<td>T. sordida</td>
</tr>
<tr>
<td>Tarija</td>
<td>Y04 Cueva de Leon</td>
<td>570</td>
<td>21°50’50.46”</td>
<td>63°14’51.6”</td>
<td>21.50’50.46”</td>
<td>63°14’51.6”</td>
<td>P. geniculatus</td>
</tr>
<tr>
<td>Chuquisaca</td>
<td>Machacato</td>
<td>2,200</td>
<td>20°100’63.8”</td>
<td>62°57’46.5”</td>
<td>20°100’63.8”</td>
<td>62°57’46.5”</td>
<td>T. infestans</td>
</tr>
<tr>
<td>Chuquisaca</td>
<td>Y08 San Antonio</td>
<td>8,555</td>
<td>20°120’02.3”</td>
<td>63°01’21.4”</td>
<td>20°120’02.3”</td>
<td>63°01’21.4”</td>
<td>T. sordida</td>
</tr>
<tr>
<td>Santa Cruz</td>
<td>Y09 17 de Noviembre</td>
<td>1,700</td>
<td>20°100’48.3”</td>
<td>63°01’21.4”</td>
<td>20°100’48.3”</td>
<td>63°01’21.4”</td>
<td>T. sordida</td>
</tr>
<tr>
<td>Santa Cruz</td>
<td>Y10 Estancia Basilio</td>
<td>490</td>
<td>18°56’53.1”</td>
<td>63°12’57.5”</td>
<td>18°56’53.1”</td>
<td>63°12’57.5”</td>
<td>T. sordida</td>
</tr>
<tr>
<td>Santa Cruz</td>
<td>Y11 Sina</td>
<td>2,200</td>
<td>17°56’24.0”</td>
<td>62°56’60.7”</td>
<td>17°56’24.0”</td>
<td>62°56’60.7”</td>
<td>T. sordida</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>580</td>
<td>46°7.9”</td>
<td>17°56’24.0”</td>
<td>580</td>
<td>46°7.9”</td>
<td>T. sordida</td>
</tr>
</tbody>
</table>

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

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.14,26-28 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 populations in the Bolivian Chaco.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 evolutionary 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.11 The low infection reported here agrees with previous results in the Gran Chaco.17,19 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
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 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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