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Wild populations of *Triatoma infestans*: Compilation of positive sites and comparison of their ecological niche with domestic population niche

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ABSTRACT

Background: For several years, the wild populations of *Triatoma infestans*, main vector of *Trypanosoma cruzi* causing Chagas disease, have been considered or suspected of being a source of reinfestation of villages. The number of sites reported for the presence of wild *T. infestans*, often close to human habitats, has greatly increased, but these data are scattered in several publications, and others obtained by our team in Bolivia have not been published yet.

Methodology/principal findings: Herein is compiled the largest number of wild sites explored for the presence of *T. infestans* collected with two methods The standardized methods aimed to determine the relationship between wild *T. infestans* and the ecoregion, and the directed method help to confirm the presence/absence of triatomines in the ecoregions. Entomological indices were compared between ecoregions and an environmental niche modelling approach, based on bioclimatic variables, was applied. The active search for wild *T. infestans* in Bolivia suggests a discontinuous distribution from the Andean valleys to the lowlands (Chaco), while the models used suggest a continuous distribution between the two regions and very large areas where wild populations remain to be discovered. The results compile the description of different habitats where these populations were found, and we demonstrate that the environmental niches of wild and domestic populations, defined by climatic variables, are similar but not equivalent, showing that during domestication, *T. infestans* has conquered new spaces with wider ranges of temperature and precipitation.

Conclusions/significance: The great diversity of wild *T. infestans* habitats and the comparison of their ecological niches with that of domestic populations confirm the behavioural plasticity of the species that increase the possibility of contact with humans. The result of the geographical distribution model of the wild populations calls for more entomological vigilance in the corresponding areas in the Southern Cone countries and in Bolivia. The current presentation is the most comprehensive inventory of wild *T. infestans*-positive sites that can be used as a reference for further entomological vigilance in inhabited areas.

1. Introduction

Chagas disease, whose etiologic agent is *Trypanosoma cruzi*, is a major public health problem in Latin America, presently recognized in the group of the Neglected Tropical Diseases (NTDs) by the World Health Organization (http://www.who.int/neglected_diseases/). In the Southern Cone countries of South America, *Triatoma infestans* (Reduviidae, Triatominae) remains the main and most widespread vector, the best adapted to domestic environment. Therefore, it is the target of control campaigns using insecticide spraying. This species has long been considered almost exclusively domestic, but in recent years there is a significant increase of wild population records in Bolivia

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(Buitrago et al., 2010; Waleckx et al., 2012, 2011) and other countries (Bacigalupo et al., 2010; Ceballos et al., 2009; Rolón et al., 2011). When the Southern Cone Initiative to control and eliminate Chagas disease (INCOSUR) started in the 1990s in several countries, the possibility of recolonization of treated areas by sylvatic bugs was discarded, mostly because wild populations of *T. infestans* were only reported in the Cochabamba valley in Bolivia. As a result of the INCOSUR, Brazil, Chile and Uruguay, and part of Argentina, Bolivia and Paraguay have certified the interruption of *T. cruzi* transmission by domiciliated *T. infestans* (Dias, 2007). In parts of Argentina, Paraguay, and Bolivia, reinfestations of human dwellings continue to occur in several provinces or departments (Cecere et al., 1997; Lardeux et al., 2010; Quisberth et al., 2011; Lardeux et al., 2015). Therefore, the assumption of movement of *T. infestans* populations between wilderness environment and human habitats has received more attention (Noireau et al., 2005). Population genetics studies have recently evidenced gene flow between sylvatic and intra-peridomestic *T. infestans* populations in Argentina (Piccinali et al., 2011) and Bolivia (Brenière et al., 2013), suggesting that sylvatic populations may be involved in the reinfestation observed in different places.

In this context, it is becoming increasingly important to thoroughly determine the geographic distribution of *T. infestans* wild populations and to assess their epidemiological role. Currently, the reports of wild populations of *T. infestans* are scattered in several publications, and other numerous searches and collections carried out by our team in Bolivia between 2008 and 2011 have not been published. Moreover, environmental niche modelling based on the characterization of environmental variables of spaces occupied by a species helps establish its geographic distribution, as previously reported (Gorla and Noireau, 2010).

To improve the knowledge of wild *T. infestans* populations, this report compiles the geographic coordinates of all the sites investigated by our team for the presence of wild populations in Bolivia, and the positive sites reported in other countries. Then the geographic distribution of sylvatic *T. infestans* populations is estimated with bioclimatic variables, and the environmental niches of sylvatic and domestic populations are compared.

2. Materials and methods

2.1. Study area

To establish the geographic distribution of *T. infestans* wild populations, field searches were intensified in Bolivia between 2008 and 2011 in the area covering the distribution of *T. infestans* domestic populations. This area corresponds to the villages identified by the National Chagas disease Program (PNCH) in 2007 (Rojas Cortez, 2007) and it includes seven entire or partial ecoregions (Ibish et al., 2008), four of them included in the Andean zone Bosque Tucumano-Boliviano (BTB, Tucuman-Bolivian forest); Bosques Secos Interandinos (BSIA, Interandean Dry Forest); Prepuna (PP) and Yungas (YUN); and three others considered as in no-Andean zones: Bosque Seco Chiquitano (BSC, Dry Chiquitano forest); Chaco Serrano (CS); and Gran Chaco (GC).

2.2. Sampling methods

Two sampling methods were used: the “standardized” method was aimed at determining a relation between the presence of wild *T. infestans* and the ecoregion. Triatomines were searched in randomly selected points with a sample size proportional to the surface of the ecoregion included in the study area. The “directed” method aimed at verifying the real presence/absence of triatomines in the ecoregions, searching insects in points selected because of their high probabilities of encountering triatomines. This last method could also increase the number of points with presence of *T. infestans* in order to improve the ecological niche modelling. In each collection site, before to set traps, the team made contact with local residents, preferably the head of the community, to inform the activity and make it clear that the traps were left in place overnight to be recovered the next day. The conversation has led to exchange information regarding the knowledge of triatomines and the danger they represent. This activity was always well received, and the villagers and/or the head of the local community granted oral consent.

2.2.1. Standardized method

Having divided the ecoregion areas into squares of 110 km^2, a number of squares approximately proportional to the surface of each studied ecoregion was chosen at random. In each square an accessible village was selected as collection site. In each site, a total of 48 mice-baited adhesive traps (see below) were set in the sylvatic environment surrounding the village, along three 200-m transects (16 traps by transects, 4 traps each 50 m), starting at fixed distances from the edge of the peridomestic area (50 m from the periphery of the houses). In some sites, a few additional traps were set around the transects. This method was applied in 47 collection sites (S1 Table, sites 1–47).

2.2.2. Directed method

The choice of these additional no-randomly-selected collection sites was based on information given by health workers and inhabitants, and on landscape features considered by the researchers as a potential habitat for wild populations. With this method, 64 additional collection sites were investigated. Some of these sites were explored several times, in different years or/and in different season. The geographic coordinates of each collection site were recorded, as well as the ecoregion (S2 Table, sites 48–111). All the ecoregions were covered by this method, except YUN (Yungas) and CS (Chaco Serrano) because of logistical issues.

2.2.3. Insects

All the triatomines were caught using mice-baited adhesive traps, whatever of the sampling method used (Noireau et al., 1999). These were positioned in different potential habitats such as small burrows, shelters under stones and vegetation, cliffs and deep cracks, hollows of live or dead trees, and other locations such as under woodpiles. They were set in the afternoon and inspected the next morning. Trapped insects were gently detached from the traps, grouped per trap to be and transported alive to the laboratory. In the laboratory, sex and stages of the bugs were determined according to morphological criteria (Lent and Wygodzinsky, 1979). After their identification the bugs were usually dissected, to examine the presence of parasites in the feces by microscopic observation, to extract the digestive tract to analyse the origin of the blood meals, and to collect the legs for DNA extraction and genetic analysis. Much of these results have been published yet (Buitrago et al., 2010; Waleckx et al., 2012; Brenière et al., 2013; Brémond et al., 2014; Brenière et al., 2012a,b; Buitrago et al., 2013, 2016, 2012; Waleckx et al., 2011). A site or a trap was considered positive if at least one specimen of *T. infestans* (nymph or adult) was caught.

2.3. Compilation of the sites explored for the presence of wild T. infestans

S1 Table refers to Bolivian explored sites between 2008 and 2011 for the presence/absence of wild triatomines with the standardized method. Details based on capture information for each trap allowed the analysis of several entomological indices (see Data analysis below). S2 Table lists additional sites explored for the presence/absence of wild *T. infestans* in Bolivia using the directed method between 2008 and 2011, while S3 Table lists the sites explored in Bolivia before 2008 by Dr. F. Noireau’s team† and others in Argentina, Paraguay and Chile.