Acta Tropica 176 (2017) 228–235

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

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

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ARTICLE INFO

Keywords:
Chagas vector
Geographical distribution
Environmental niche model
Climatic variables
Triatoma infestans
Wild

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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http://dx.doi.org/10.1016/j.actatropica.2017.08.009
Received 19 May 2017; Received in revised form 26 July 2017; Accepted 10 August 2017
Available online 15 August 2017
0001-706X/

(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.

2.4. Data analysis

Four entomological indices were calculated for each collection site: (i) the infestation index was the number of positive traps/number of traps set (%); (ii) the colonization index was the number of traps with at least one nymph/number of positive traps × 100; (iii) the crowding index was the number of collected *T. infestans*/number of positive traps; and (iv) the abundance index was the number of *T. infestans*/number of traps set × 100, stating the number of collected *T. infestans* for 100 traps. Ranges, averages and standard deviations of these indices were calculated per ecoregion. The odds ratio (OR), their 95% confidence interval and the *p*-value of the significant test were calculated using the MedCalc statistical software at the web site https://www.medcalc.org/calc/odds_ratio.php.

2.5. Analysis of the geographic distribution of wild T. infestans

Based on previous studies that showed the relation between geographic distribution and of Triatominae species and bioclimatic factors such as temperature, humidity, precipitation, and altitude (Gorla et al., 1997; Carcavallo, 1999; Ramsey et al., 2000), the potential distribution of sylvatic *T. infestans* populations was explored using the environmental niche modelling approach, using all the presence points reported in this paper. Nineteen bioclimatic environmental variables of the Worldclim database (www.worldclim.org) plus altitude with a nominal spatial resolution of 1 km^2 at the equator (Hijmans et al., 2005) were used to fit a generalized linear model (glm). Although the maximum entropy method is currently the most frequently used because of its ease of use in the Maxent software, a recent study showed that the Maxent algorithm is essentially a Poisson regression model with a LASSO penalty (Aarts et al., 2012; Fithian and Hastie, 2013; Renner and Warton, 2013). Given that glm assumptions are transparent and that its longer history of use gives better knowledge of methods limitations, we selected a glm model for this analysis. Occurrence (n = 89) and absence (n = 66) (coded 1 and 0) location points came from field data collected by the research team and published reports (S1, S2 and S3 Tables).

As preliminary fits of the glm using recorded absences were not significant, it was decided to use background points selected through random sampling (n = 500 points) over the maximum previously estimated area covered by *T. infestans* (Gorla, 2002), probably a more reasonable selection because no wild population of *T. infestans* has ever been recorded in vast areas of Uruguay, Brazil and southern Argentina. All values of the 20 environmental variables from presence and background locations were extracted using the *raster* R package to build a data matrix. Collinearity between environmental variables is a common problem and a potential source of model overfitting. To overcome this problem, the variance inflation factor (vif, calculated with the R car package) of each independent variable was calculated. The glm was first fit with the 20 environmental variables. The vif was calculated and the variable with the highest vif was dropped from the model. The process was iterated until there were no variables in the model with vif > 10. After variable selection to eliminate variable collinearity, the model selection was based on the Akaike Information criteria (AIC). Another common problem of fitting models to estimate environmental niches using presence-only data is that the model should not be calculated with the same data set used to evaluate the error of the model predictions. To overcome this problem, a cross validation was carried out, using the "leave-one-out" process with the *cv.glm* of the R boot package. The *cv.glm* is a bootstrap method that fits the model using "number of presence points −1" to fit the model and the remaining points to evaluate the prediction error, repeated the same number of times as there are presence points in the data set, 89 in this case. The goodness of fit is usually evaluated using the AUC value (area under the curve), although it has recently received strong criticism (see (Lobo and Jimenez Valverde, 2008)). An alternative estimation of the goodness of fit was estimated by a partial AUC using the *pROC* program of the R package.

2.6. Comparison of ecological niches of T. infestans

Two comparisons were carried out in this study. Firstly, we compared the niches of wild (population locations in S1, S2 and S3 Tables) and domestic populations of *T. infestans* located by random samples over the maximum area covered by the species, according to Gorla (2002). Secondly, we compared the niches of the Andean and non-Andean wild populations of *T. infestans*, located as shown in S1 and S2 Tables.

For the comparison of the ecological niches, we used the values of the environmental variables of the Worldclim set mentioned above, through the approach suggested previously (Broennimann et al., 2012). Even if two or more niches are identical, there will be some differences in the data purely by chance. To rule out detecting two niches as different when they only differ due to sampling variation, niches should be compared statistically to determine whether the same probability distribution describes the niche of two species (or populations as in the present case), or whether there is evidence of some difference (Geange et al., 2011). The *T. infestans* population niches were compared using the index of niche overlap D (Schoener, 1970) (varying between 0 and 1, no niche overlap and identical niche, respectively), which is used to perform niche equivalency and similarity tests. We followed the ordination method (PCA-env) previously described (Broennimann et al., 2012), because the proposed framework disentangles the dependence of species occurrence from frequency of different climatic conditions (correcting for relative availability of environments) and from environmental data resolution (Warren et al., 2008). For the niche equivalency test, all occurrences were pooled and randomly split into two data sets, maintaining the number of occurrences as in the original data sets, and the niche overlap statistic D was calculated; the process was repeated × 100. If the observed value of D fell within the density of 95% of the simulated values, the null hypothesis of niche equivalency could not be rejected. For the niche similarity test, we randomly shifted the entire observed density of occurrences in one range and calculated the overlap of the simulated niche with the observed niche in the other range. The test of niche similarity was also based on 100 repetitions. If the observed overlap is greater than 95% of the simulated values, it means that the entity occupies environments in both of its ranges that are more similar to each other than expected by chance. All calculations were carried out using an appropriately modified R script (Broennimann et al., 2012).

3. Results

3.1. Positive sites with wild T. infestans in Bolivia

From February 2008 to June 2011, 111 collection sites were explored in Bolivia using a total of 8398 mice-baited adhesive traps. The geographical characteristics and the results of the triatomine captures in the sites studied are detailed in S1 and S2 Tables for the standardized and directed methods, respectively. Overall positive and negative sites are reported on the map of Bolivia presented in Fig. 1. *T. infestans* specimens were identified in 50 of the 111 collection sites (45%) belonging to three of the seven ecoregions that cover the study area (BSIA, PP and GC). The comparison of the occurrence of wild populations of *T. infestans* among the ecoregions was performed with the data of the standardized method (Table 1).

According to these data that include 47 sites and 2221 traps, the highest rates of positive sites and positive traps were observed in the BSIA ecoregion. The rates were significant ($p < 0.01$) for traps (95% confidence interval odds ratio, 1.81–31.55 compared to GC and 2.42–42.09 compared to PP), whereas they were not significant ($p > 0.05$) for sites (95% confidence interval odds ratio, 0.14–8.99

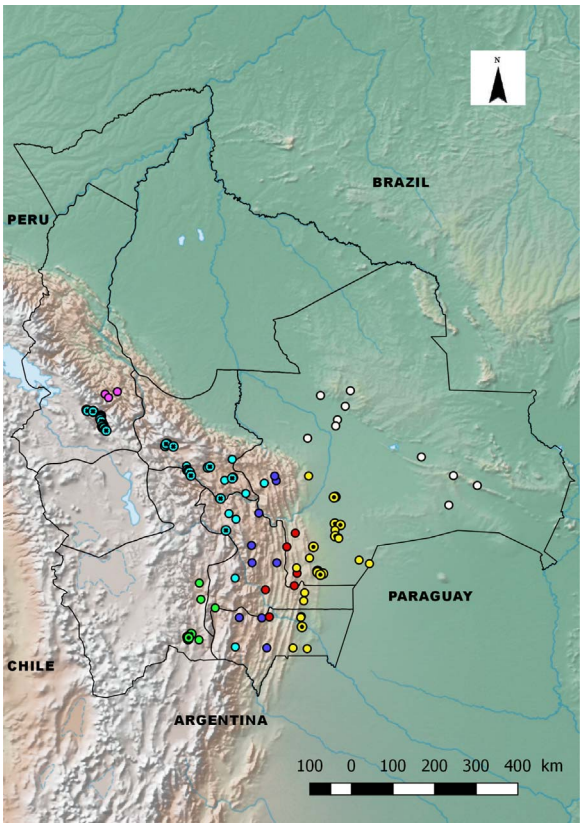

Fig. 1. Map of Bolivia locating the 111 positive or negative sites explored for wild *T. infestans* between 2008 and 2011 and some other sites explored before. See also Tables S1 and S2. For all captures mice-baited adhesive traps were used. Sites are depicted according to the presence (⊙) or absence (○) of wild *T. infestans*. The seven ecoregions explored are BSC (Dry Chiquitano forest, white ○), BTB (Tucuman-Bolivian forest, dark blue ○), BSIA (Interandean Dry Forest, light blue ○), CS (Chaco Serrano, red ○), GC (Gran Chaco, yellow ○), PP (Prepuna, green ○) and YUN (Yungas, pink ○). Map created with Natural Earth. Free vector and raster map data @ naturalearthdata.com. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Identification of positive sites with wild *T. infestans* in Bolivian ecoregions using two capture methods.

Ecoregion	No. of explored sites	Positive sites		No. of traps	Positive traps	
		No.	%		No.	%
Standardized method						
BSC	4	0	0	192	0	0
BTB	8	0	0	384	0	0
BSIA	14	6	42.8	653	39	6.0
CS	6	0	0	288	0	0
GC	5	2	40.0	240	2	0.8
PP	7	1	14.3	320	2	0.6
YUN	3	0	0	144	0	0
Total	47	9	19.1	2221	43	1.9
Directed method						
BSC	6	0	0	186	0	0
BTB	1	0	0	43	0	0
BSIA	32	29	90.6	3794	851	22.4
GC	22	9	40.9	2104	47	2.2
PP	3	3	100	50	19	38.0
Total	64	41	64.0	6177	917	14.8

BSC, Bosque Seco Chiquitano (Dry Chiquitano forest); BTB, Bosque Tucumano-Boliviano (Tucuman-Bolivian forest); BSIA, Bosques Secos Interandinos (Dry interandean forests); CS, Chaco Serrano; GC, Gran Chaco; PP, Prepuna; YUN, Yungas.

Table 2
Entomological indices of positive collection sites with *T. infestans* per ecoregion.

Variables[a]		Ecoregions BSIA	PP	GC
Total number of positive collection sites (referred to S1 and S2 Tables)		35	4	11
Infestation index (%)				
	Range	0.6–83.7	6.3–65	1.3–16.7
	Median	22.5	17.5	4.2
	1st quartile	12.7	9.1	2.8
	3rd quartile	35.0	35.0	7.6
Colonization index (%)				
	Range	0–100	50–100	50–100
	Median	95.5	100	100
	1st quartile	88.2	87.5	84.3
	3rd quartile	100	100	100
Crowding index (no. of specimens/positive trap)				
	Range	1–10.0	1.5–7.4	1–3.3
	Median	3.0	3.6	1.5
	1st quartile	2.2	1.9	1.0
	3rd quartile	4.3	5.8	2.0
Abundance (no. of specimens/100 traps)				
	Range	0.6–797	9.4–340.0	1.3–50
	Median	81.7	102.5	7.4
	1st quartile	45.6	17.3	2.8
	3rd quartile	117.3	223.8	17.7

[a] See Materials and Methods for the definition of the indices. BSIA, Bosques Secos Interandinos (Interandean Dry Forest); GC, Gran Chaco; PP, Prepuna.

compared to GC and 0.42–47.99 compared to PP). The results from the directed capture method consolidated the previous ones obtained with the standardized method and no more ecoregion was revealed as positive for the presence of wild triatomines. The directed method gave higher rates of positive sites and traps than standardized one (Table 1). High variability of the entomological indices was detected between the overall positive sites explored with the standardized and directed capture methods (Table 2).

Wild *T. infestans* populations appear to be less abundant in GC with lower infestation and crowding indices. However, both indices presented a large range in the other Andean ecoregions (BSIA and PP). The colonization index did not differ among seasons throughout the period studied ($p > 0.05$, glm with a quasibinomial link, because of overdispersion). Nymphs were collected in all the positive collection sites, except BSIA03 where only one adult specimen was captured (S1 Table), and the colonization index of the different sites was very high for all ecoregions. The age structure of the populations was broadly similar in the three ecoregions, with most of the bugs being, overall population, young nymphs (1st, 2nd and 3rd nymphs; 67.6%), 4th and 5th less abundant (23.6%) and the adults accounting for around 10%. This age structure was similar to the one reported for *T. infestans* domestic populations growing under natural climatic conditions during the warm season, in central Argentina (Gorla and Schofield, 1989). As previously reported, the 11 adults from GC were darker (dark morph) than the Andean specimens (wild and domestic specimens). The male-to-female ratio was not significantly different from the unit in the three ecoregions ($p > 0.05$).

Thirteen of the collection sites explored using the directed capture method were visited more than once (S2 Table). Seven of them were visited in the same month in different years. The infestation and crowding indices were generally similar but significant variations were observed for the site VIZ02 (no. 87) where the very high infestation rate, reaching around 80% in 2008, dropped by half 2 years later. Other collection sites were sampled during different months (sites QUI no. 96, THA no. 101, SAP no. 81 and TUN01 no. 77), but no clear seasonal variation trend in infestation rates or number of captured *T. infestans*

was observed.

3.2. Habitat of wild T. infestans

The habitat of wild *T. infestans* varied radically between highlands (Andean valleys) and lowlands (Gran Chaco region). The Andean populations of wild *T. infestans* found in BSIA and PP ecoregions, mainly had a rupicolous habitat, as already reported (Cortez et al., 2007). However, between 2008 and 2011, they were also captured in new habitats that were not previously suspected such as fields of prickly pears and cactus, cliffs and deep cracks in the sedimentary ground (Buitrago et al., 2010). Sapini (SAP − no. 81, S1 Table) is a good example of a site with *T. infestans* in sedimentary cliffs that are very abundant in Andean landscapes (S1 Figure): the traps placed in the cliffs 50–400 m away from houses presented 20% positivity with around 2.4 *T. infestans* per positive trap. Close to the village, other ecotopes were also infested, such as walls and piles of stones distributed between agricultural fields and a thorny wild hill.

The collection sites of the Gran Chaco (GC ecoregion) were generally characterized by dense and high vegetation cover, with little low vegetation mainly composed of bromeliads that characterize the Chaco forest (S2 Figure). In this kind of forest, most of the putative ecotopes for triatomines were in trees; they consisted of holes in the trunk of live and dead trees, holes at the foot of trees between the roots, and bird nests. Of 489 traps set in 13 different collection sites where the detailed information of each ecotope was properly noted, 60.7% were placed in tree trunk holes, 17.4% in dead trunks, 9.6% in dry trees and to a lesser extent between dry branches, tree roots at their foot, below felled trees, in burrows, etc. The most infested habitat was “pile of branches on the ground” with 12.5% (one positive out of eight), but this ecotope was only occasionally found in the Chaco forest. Then, 8.7% and 8.5% of the “holes in tree trunk” and “dry tree” ecotopes were positive, respectively, accounting for 88.2% of positive traps overall (Fig. 2). According to the biology of the triatomines, their association with mammals living in burrows is expected; very few holes in the ground (possible burrow) were detected by the research team during the searches in the different collection sites visited in the Chaco, because they may be hard to see. Note however, that two traps placed in an armadillo burrow were positive, but with *Panstrongylus geniculatus* specimens. Of 520 trap locations for which the information was properly noted by the field team, only 35 (6.7%) presented signs of mammal presence (mammal faeces, fur, seeds) and in two of these cases, bird feathers and a snake moulting were found sticking to the trap. Our results confirm the arboreal habitat of wild *T. infestans* in the Bolivian Chaco.

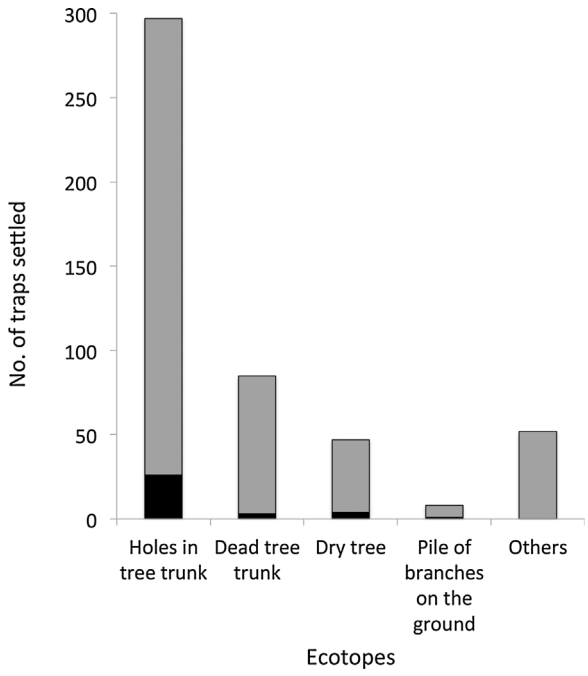

Fig. 2. Positive and negative ecotopes with wild *T. infestans* in the Bolivian Chaco ecoregion. Grey, negative traps; black, positive traps.

3.3. Distribution of wild populations of T. infestans

The first modelling approach used to compare the areas with similar environments to those where wild populations of *T. infestans* occurred, within the largest possible area ever occupied by *T. infestans*, showed that after the selection process using the vif < 10 criteria, and keeping the significant criteria ($p < 0.05$), three variables that remained in the model were related to temperature variation and temperature of driest month, and four were related to precipitation (low and high extremes of humidity and temperature) (S4 Table).

Mean diurnal range of temperature (Bio2) and precipitation of the driest month (Bio14) were the variables with highest weight in the model (i.e. higher coefficient values). The goodness of fit of the model is high, as the leave-one-out cross-validation process showed the model produced an error of 8.88% (± 1.4e-5) and the partial AUC = 85% (77.8–91.2%).

Using the model and rasters of the environmental variables selected, a map was produced to identify the areas with an environment similar to the one where wild *T. infestans* populations were found. The model identified areas in the arid Chaco, some areas in western and southwestern Argentina and a few fragmented areas in the Brasilian Cerrado and Caatinga (Fig. 3a). The prediction model of wild *T. infestans* occurrence correctly describes 95.5% (85/89) of the observed presence point set. Four points were erroneously described as absences south of the Potosi Department (Bolivia).

The second modelling approach aiming at analysing the distribution of the Andean and Chaco (non-Andean) wild populations of *T. infestans* showed that terrain elevation on its own is able to fully describe the distribution of the two populations that each correspond to a different cytotype (Panzera et al., 2014, 2004). To further analyse the effect of the other climatic variables, terrain altitude was eliminated from the environmental variables list, and the other 19 bioclimatic variables were used to fit glms to the distribution of the two populations. The final model (after the variable selection process) included only two variables: Bio3 (isothermality = ratio of diurnal temperature and annual ranges that was negatively related to Chaco wild *T. infestans* occurrence) and Bio6 (minimum temperature of the coldest month that was positively related to Chaco wild *T. infestans* occurrence) (Fig. 3b).

3.4. Comparison of sylvatic and domestic niches of T. infestans

The PCA-env procedure for the comparison of niches showed that niches of wild and domestic *T. infestans* were similar but not identical (Fig. 4). Niche overlap was D = 0.365, and the randomization estimation of niche equivalence indicated that niche equivalency can be rejected ($p < 0.05$), although not for niche similarity ($p > 0.05$). Together, the first two principal components explain 68.7% of the variation (45.3% and 23.4% for components 1 and 2, respectively). The environmental niche space defined by these first two components showed that from its sylvatic template, *T. infestans* expanded its niche to more variable temperature (Bio2, Bio3) and wetter areas (Bio14). Although it is frequently considered that insect species are limited by temperature and rainfall, *T. infestans*, unlike sylvatic populations, occupy an annual temperature–precipitation space similar to the domestic populations at its maximum expansion. The ecological niche model that analysed the distribution of domestic and wild *T. infestans* populations showed that the diurnal range of temperature (Bio2) and rainfall during the driest month (Bio14) defines the space that shows the greatest change from sylvatic to domestic. The domestication process seems to

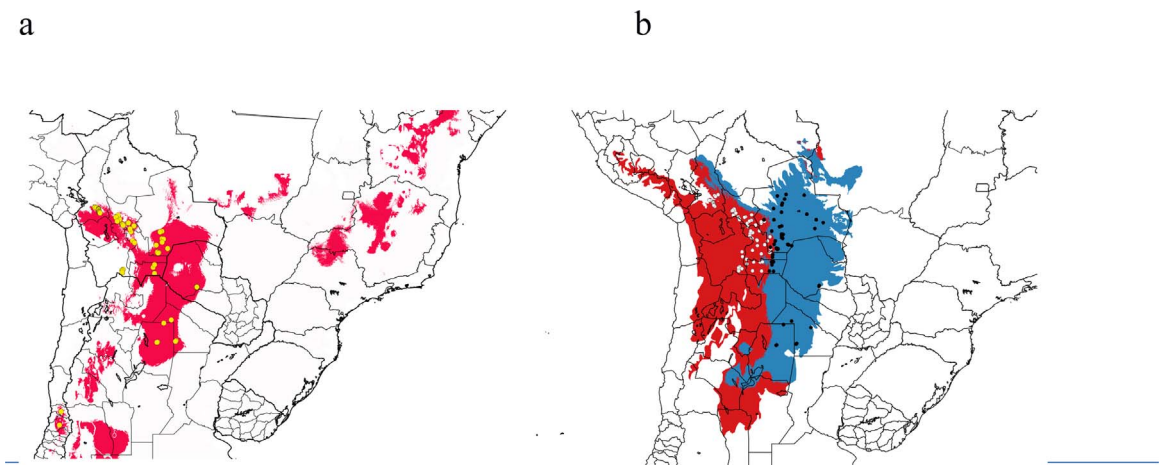

Fig. 3. Modelling approaches. a) Area estimated by the environmental niche model showing places where wild *T. infestans* populations might potentially exist (red). Yellow circles indicate the sites included in the model where wild populations of *T. infestans* were found. b) Area estimated by the environmental niche model showing places where wild Andean-Puna *T. infestans* populations might potentially exist (red) and places where wild lowland *T. infestans* populations might potentially exist (blue). White circles indicate the positive and negative sites of the Andean-Puna areas included in the model, black circles similarly indicate those of the lowland areas included in the model. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

provide *T. infestans* the possibility of colonizing areas with wider temperature ranges, both diurnal and annual (Bio2, Bio3), and a higher rainfall regime during the driest period (Bio14).

4. Discussion

Chagas disease is commonly associated with rural areas and poverty, mainly with inhabitants living in human dwellings of mud with straw and palm roofs. Indeed, the transmission of the disease culminated when the colonization of human dwellings by the vectors was at its maximum expansion, subjecting the inhabitants to vector bites during the night and thus allowing transmission of *T. cruzi*, the agent of the disease, at a very high incidence level.

Is this model currently unchanged? What are the factors that have respectively turned this paradigm?

The main factors that have changed the epidemiology of Chagas disease over the last 10 years are vector control by massive insecticide spraying of human dwellings and home improvement. The Southern Cone countries, where the transmission due to *Triatoma infestans* was the highest, first initiated national vector control programs grouped in the INCOSUR initiative launched in 1991. This initiative was followed by subregional programs, the IPA (Iniciativa de los Países Andinos de Control de la Transmisión Vectorial y Transfusional de la Enfermedad de Chagas), the IPCA (Iniciativa de los Países de América Central para el Control de la Transmisión Vectorial, Transfusional y la Atención Médica de la Enfermedad de Chagas) and the AMCHA (Iniciativa de los Países Amazónicos para la Vigilancia y el Control de la Enfermedad de Chagas). These initiatives have eliminated vector colonies from human dwellings in most places, and together with additional control measures, transmission interruption was officially declared in various regions wherever the mean human dwelling infestation rate was below 3%. However, in several regions, human dwelling infestation persists or reinfestation occurs after control programs. Different factors that may hinder the elimination of vectors have been inferred: (i) insecticide resistance of triatomines, (ii) other biological and structural factors reducing the effectiveness of pyrethroids, (iii) social and economic factors and (iv) incursion of wild bugs and subsequent colonization. This latter phenomenon has raised great interest and appears to be

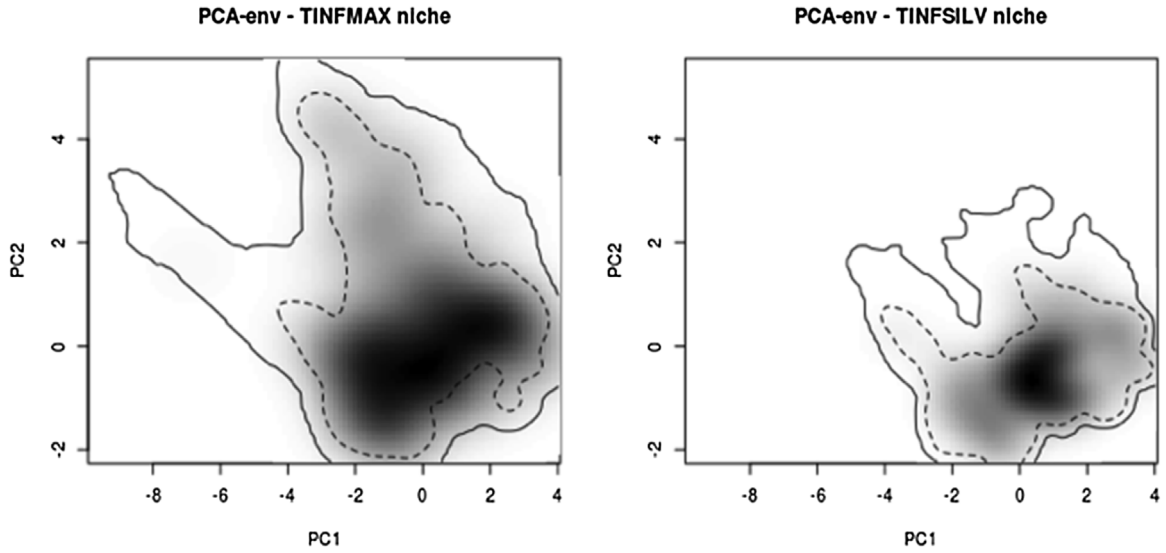

Fig. 4. The *T. infestans* niche in climatic space, calculated with the PCA − env method. The surfaces in the plots represent the climatic niche along the first two axes of the PCA, the historical maximum expansion of domestic populations (left) and the wild populations (right). Grey shading indicates the density of occurrences. Solid and dashed lines represent, respectively, 100% and 50% of the available environment.

related to urbanization of wild areas and increasing anthropization of peripheral urban areas (forest destruction), together with global warming, which should not be ruled out because temperature is a major factor enabling the movement and dispersion of adult triatomines. With bloodsucking on mammals mandatory for triatomine vectors, the destruction of flora and some wildlife requires the vectors to find alternative blood sources. Consequently, the bugs can move to the human habitat where they will find food and shelter. In this context, the challenge is therefore to understand the wild populations of vectors better and be vigilant about the occurrence, even sporadic, of wild vector incursions in human dwellings and peridomestic areas.

The first useful task is to define the geographical distribution of wild populations of triatomines. On the basis of areas with environmental variables similar to those where wild *T. infestans* populations were found, the current map draws a large continuous area from the Andean valleys to the arid Chaco region. This area belongs to Bolivia, Paraguay and Argentina. In addition, other discontinuous areas have been identified farther south on both sides of the Cordillera (Chile and Argentina) and also in Brazil in the Cerrado and Caatinga areas. In these Brazilian areas, there is no report of wild *T. infestans* capture, while numerous other species of triatomines have been described (Gurgel-Goncalves et al., 2012; Costa et al., 2014). In Chile, the only two reports of *T. infestans* wild foci (Bacigalupo et al., 2010, 2006) coincide with the model, although this area appears isolated from the large continuous Andes–Chaco area. Molecular characterizations of these Chilean populations have made it possible to propose a dual-origin of these *T. infestans*, one centred in Peru and Bolivia and the second one to Argentina (Torres-Perez et al., 2011). The active field research conducted in Bolivia suggests a discontinuous distribution of wild populations between the Andean valleys and the Gran Chaco region. Indeed, despite search efforts in intermediate ecoregions between that BSIA and GC, the explorations proved negative (Brenière et al., 2012a). The study of genetic data from wild populations of Andean valleys and Chaco (Bolivia, Paraguay, and Argentina) supports a dichotomy of *T. infestans* compatible with a long-lasting geographical isolation (Waleckx et al., 2011). Other studies have estimated the divergent time between *T. infestans* from the Andes and Gran Chaco at around 59,000 years (Bargues et al., 2006), long before the arrival of humans on the continent, which is generally thought to have occurred in North and South America 15,000 years ago (Guhl, 2005). This discrepancy is probably due to the geographical separation of two populations for a long time, by geographic or bioclimatic barriers. Consequently, molecular information more likely fits with a discontinuous geographic distribution, allowing genetic divergence between populations.

In the Interandean Dry Forests ecoregion (Andean part), the first report of wild populations of *T. infestans* was the collection of *T. sordida* and *T. infestans* under rocks (Torrico, 1946). Subsequently and until now, the Andean wild *T. infestans* have been reported in rupicolous habitats (Noireau et al., 2005; Cortez et al., 2007; Bermudez et al., 1993; Cortez et al., 2006). However, diverse ecotopes were discovered with the last current searches, among others, fields of cactus and prickly pear, sedimentary cliff and scree. In the lowlands of the Gran Chaco ecoregion, the current data fully corroborate the arboreal habitat for wild *T. infestans* reported so far, not only in Bolivia but also in the Argentine and Paraguayan Chaco (Ceballos et al., 2009; Rolón et al., 2011; Noireau et al., 2000, 1997; Ceballos et al., 2011; Usinger et al., 1966). The great diversity of wild *T. infestans* habitats is probably related to the behavioural plasticity of the species, and this property has promoted the adaptation of the species to artificial ecotopes located in peridomiciles and human dwellings (domestication process). This property makes wild populations of *T. infestans* as a threat because they adapt easily to anthropized areas.

The comparison between domestic and sylvatic niche models shows that they are similar but not identical. Their significant similarity supports the notion that the wild populations of *T. infestans* have evolved to domiciliation in several places, called “multicentric colonization”, as previously proposed (Waleckx et al., 2011), and that the distribution of domestic populations is less explained by the traditional hypothesis of a passive recent human spread from the Andean areas to the Gran Chaco (Usinger et al., 1966; Schofield, 1988; Cortez et al., 2010). Indeed, in the latter case the niches should be much more dissimilar.

However, the two niche models are not equivalent. The domestic niche presents wider temperature ranges, both diurnal and annual, and a higher rainfall regime during the driest period. Presumably, some wild populations passively transported by humans or domestic animals have been able to settle in human dwellings from regions where climatic conditions varied from those where wild populations existed because of artificial indoor and outdoor ecotopes that are better protected from variations in temperature and precipitation than natural habitats, for example, the village of Otavi in Potosi department located at 3400 m where *T. infestans* had colonized the human dwellings (Brenière et al., 1991).

In conclusion, wild populations of *T. infestans* have a very wide geographical distribution and are likely implicated in the phenomenon of human dwelling reinfestation in various areas, as previously shown in Andean valleys where genetic flow was found between wild and domestic populations in three villages suffering continuous reinfestation, showing that bugs move between wild and domestic environments and suggesting that wild populations constitute a source of reinfestation post-spraying (Brenière et al., 2013). Another important point is the threat these populations represent in the countryside. Indeed, nymphs collected in wild areas have been found with human blood meal (Buitrago et al., 2013, 2016). This result can be explained by bite exposure of field workers during rest periods or other people during outdoor activities such as camping, picnicking and hiking. In this context, it is important to determine the natural distribution of *T. infestans* in order to gather information over places with a risk of Chagas disease transmission despite the control of vectors in villages.

Acknowledgments

We are particularly grateful to Marcelo Claure and Abdul Castillo, drivers at the IRD, for being with us on the Bolivian roads, in complete safety and with a lot of patience. We remember François Noireau† and Philippe Brémond† for their field dedication and we also thank colleagues and students that participate to some of the trips: Mario Charrière (agricultural engineer), Claudia Aliaga, Renata Salas and Esdenka Perez (Biologists), Fabian Benavidez (sociologist), Marcelo Monje (computer scientist), Roberto Rodriguez (entomologist technician), Hiber Calle (technician), Sergio Quisberth, Marianne Baume and Marinely Bustamante (Master students).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actatropica.2017.08.009.

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