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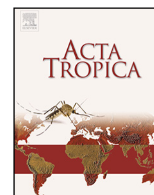

Landscape ecology of *Trypanosoma cruzi* in the southern Yucatan Peninsula

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ABSTRACT

Landscape interactions of *Trypanosoma cruzi* (Tc) with *Triatoma dimidiata* (Td) depend on the presence and relative abundance of mammal hosts. This study analyzed a landscape adjacent to the Calakmul Biosphere Reserve, composed of conserved areas, crop and farming areas, and the human community of Zoh Laguna with reported Chagas disease cases. Sylvatic mammals of the Chiroptera, Rodentia, and Marsupialia orders were captured, and livestock and pets were sampled along with *T. dimidiata* in all habitats. Infection by *T. cruzi* was analyzed using mtDNA markers, while lineage and DTU was analyzed using the mini-exon. 303 sylvatic specimens were collected, corresponding to 19 species during the rainy season and 114 specimens of 18 species during dry season. Five bats *Artibeus jamaicensis*, *Artibeus lituratus*, *Sturnira lilium*, *Sturnira ludovici*, *Dermanura phaeotis* (Dp) and one rodent *Heteromys gaumeri* were collected in the three habitats. All but Dp, and including *Carollia brevicauda* and *Myotis keaysi*, were infected with predominately TcI in the sylvatic habitat and TcII in the ecotone. *Sigmodon hispidus* was the rodent with the highest prevalence of infection by *T. cruzi* I and II in ecotone and domestic habitats. *Didelphis viginiana* was infected only with TcI in both domestic and sylvatic habitats; the only two genotyped human cases were TcII. Two main clades of *T. cruzi*, lineages I (DTU Ia) and II (DTU VI), were found to be sympatric (all habitats and seasons) in the Zoh-Laguna landscape, suggesting that no species-specific interactions occur between the parasite and any mammal host, in any habitat. We have also found mixed infections of the two principal *T. cruzi* clades in individuals across modified habitats, particularly in livestock and pets, and in both haplogroups of T. *dimidiata*. Results are contradictory to the dilution hypothesis, although we did find that most resilient species had an important role as *T. cruzi* hosts. Our study detected some complex trends in parasite transmission related to lineage sorting within the matrix. Intriguingly, TcIa is dominant in terrestrial small wildlife in the sylvatic habitat and is the only parasite DTU found in *D. virginiana* in the domestic habitat, although its frequency remained constant in sylvatic and ecotone vectors. Bats have a key role in TcVI dispersal from the sylvatic habitat, while dogs, sheep, and humans are drivers of TcVI between domestic and ecotone habitats. Overall, our results allow us to conclude that *T. cruzi* transmission is dependent on host availability within a highly permeable landscape in Zoh Laguna.

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1. Introduction

Landscape heterogeneity can influence ecological processes such as animal movement, population persistence, and species interactions (Turner, 2005). In anthropogenic landscapes, community assemblages are in continuous transition, even though they end up with few resilient species, the so called "winners". These species survive at the expense of those that cannot tolerate human-associated disturbance, the "losers" (McKinney and Lockwood, 1999). It has been argued that landscape changes due to anthropogenic land use (deforestation, agriculture, and livestock introduction) are the main drivers of disease outbreaks in humans

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since they modify pathogen dynamics (Patz et al., 2004). The principal mechanisms for these changes may be associated with metacommunity processes resulting from changes in landscape heterogeneity (i.e., changes in land cover and resource availability), through secondary or alternative hosts, pathogen amplification or selection, and differential dispersal by resilient hosts (Turney et al., 2014). These ecological processes are not, however, the only factors provoking an increase in disease risk, since vulnerability due to socio-cultural practices affect the human population in all fragments where they interact (Gurtler and Yadon, 2015; Valdez-Tah et al. 2015a).

Natural landscapes of the Yucatan Peninsula (YP) have been modified since expansion of Mayan groups at least until 900 A.D., and subsequently the Spanish conquest, mainly in the north and east region between Yucatan and Quintana Roo states (Carranza et al., 1996). Conversely, the southern region of the YP in Campeche state contains the largest natural reserve and one of the only remaining conserved tropical rainforests in Mexico, Calakmul (Martínez and Galindo-Leal, 2002; Ibarra-Manríquez et al., 2002). In 1989, the Calakmul Biosphere Reserve (CBR) was established to preserve and regulate this important ecosystem, since in previous decades, transportation routes provoked increasing modification dividing the reserve into north and south regions (Domínguez and Folan, 1996). The Spanish conquest in the XVI century dominated social and environmental development in the YP based on timber exploitation (Culbert, 1973), which was followed by intensive gum and sisal plantations until the last century. This economic development was accompanied by new human settlements that exploited wildlife resources surrounding the CBR and cleared land for small-scale agriculture and farming from 1890 to 1940 (Konrad, 1992). The result has been the irreversible landscape modification of the CBR region by the late twentieth century, with subsequent natural rehabilitation following lumber, sisal, and gum industry failures (Culbert, 1973; Rice, 1986; Braswell et al., 2004) and significant reduction of deterioration of the remaining conserved regions (Folan et al., 1995; Martínez-Galindo, 2002). These historical changes in use and transformation of the Calakmul region and current land use and socio-cultural practices have had an important impact on biodiversity.

Trypanosoma cruzi is transmitted in southern Mexico, principally via the *Triatoma dimidiata* complex although other triatomines are present and sporadically collected even in anthropic habitats: *Panstrongylus rufotuberculatus*, *Eratyrus cuspidatus*, *Triatoma nitida*, *Triatoma hegneri* (Ramsey et al., 2015). There are three haplogroups of the *dimidiata* complex in Mexico (Bargues et al., 2008), two of them (haplogroups 2 and 3) without complete phenotypic differentiation (i.e., morphometry or hydrocarbons), and haplogroup 1 (Marcilla et al., 2001; Lehmann et al., 2005; Ramirez et al., 2005; Bargues et al., 2008; Dorn et al., 2009). Several studies have now reported extension of natural distributions and sympatry for all three haplogroups, h1 and h2 have both been found in the Yucatan Peninsula (YP) (Bargues et al., 2008; Dorn et al., 2009; Gómez-Palacios and Triana 2014), and h2 and h3 in northern Chiapas (Pech-May, personal communication). *Triatoma dimidiata* infests between 8% and 61% of houses in the YP, with colonization indices between 5% and 25% (Guzmán-Marín et al., 1990; Dumonteil et al., 2002; Hernández et al., 2010; Monteón et al., 2013). Many natural hosts of the *dimidiata* complex from modified habitats (i.e., domestic and ecotone) have been reported, such as the common opossum, fox, coati, and rodent pest species (Zavala-Velázquez et al., 1996; Jiménez-Coello et al., 2008; Rebollar-Tellez et al., 2009). Between 17% and 33% of domestic and periurban mammal species have been reported infected with *T. cruzi* in the YP (Zavala-Velázquez et al.,1996; Ruiz-Piña and Cruz-Reyes, 2002; Jiménez-Coello et al., 2008; Rebollar-Tellez et al., 2009). Livestock and pets also have similar infection prevalence between 5% and 34% (Hernández et al., 2010; Jiménez-Coello et al., 2010, 2012a,b; Monteón et al., 2013; Carrillo-Peraza et al., 2014). The human population in the YP has a heterogeneous *T. cruzi* prevalence, from 0.1% to 18% (Farfán-Ale et al., 1992; Barrera-Pérez et al., 1992; Dumonteil, 1999; Zavala-Velázquez, 2003; Balan et al., 2011; Alducin-Téllez et al., 2011). *T. cruzi* transmission is clearly zoonotic and anthropozoonotic, and Chagas disease is endemic to the YP, as it is in all of Mexico (Gamboa-León et al., 2014; Ramsey et al., 2014).

T. cruzi transmission is an ideal model to analyze the ecological processes that affect human disease risk. Recent studies on the ecological connectivity of vector and parasite host communities within anthropogenic landscapes are providing novel insights into the spatial epidemiology linked to *T. cruzi* transmission, vector population dynamics, and mammal host metacommunities (Ramsey et al., 2012; Gottdenker et al., 2014; Fernández et al., 2014). Metacommunities resulting from habitat filtering in anthropogenic landscapes influence bloodmeal use by triatomines, and generally increase *T. cruzi* prevalence (Gottdenker et al., 2012; Ramsey et al., 2012; Gurtler et al., 2014). Furthermore, regional-scale phylogeographic studies of *T. cruzi* highlight the influence of sylvatic habitats surrounding human-transformed land as sources of parasite diversity (Ocaña-Mayorga et al., 2010; Lima et al., 2014a). However, there have been no fine-scale (landscape) studies to analyze relationships between parasite diversity based on direct measurement, without the bias of isolate selection, of host metacommunities and vectors in a human-disturbed gradient. The present study's goals were to analyze the ecological components of *T. cruzi* transmission dynamics in all habitats within a gradient of landscape transformation that contains sylvatic patches, crops, livestock, and a human community, and to analyze the association of host community and vector persistence on *T. cruzi* genetic diversity. The southern region of the YP was chosen, due to the fact that it has highly conserved regions (CBR) bordering on human-fragmented landscape which would allow for a more complete contrast of zoonotic vs. anthropozoonotic transmission. This study was conducted in parallel with analysis of socio-cultural representations and practices related to vectors and associated with the landscape published elsewhere (Valdez-Tah et al., 2015b).

2. Materials and methods

2.1. Study area

The study was conducted in the landscape surrounding the town of Zoh Laguna (elevation 190m, 18°35′32″N, – 89°25′02″W) and bordering on the CBR in southeastern Campeche (Fig. 1). The climate is sub-humid tropical with summer rains (Aw1 and Aw2) and annual mean precipitation of 1076.2 mm. The rainy season extends from June to November (<190 mm/month rain), with very little precipitation during the dry season (December to May, <70.9 mm/month) (García and March, 1990). The annual mean temperature is 25 °C, with a range from 19 °C to 32 °C.

Sylvatic areas are characterized by medium forest vegetation interspersed with flooded lowland and deciduous forest (García and March, 1990). The most common plant species in the mixed lowland forests are *Cameraria latifolia* ("chechén blanco" in Spanish; "sac cheechem" in Maya), *Haematoxylum campechianum* ("tinto" in Spanish), *Manilkara zapota* ("chico zapote" in Spanish), *Lonchocarpus xuul* ("xu'ul" in Maya), and *Platymiscium yucatanum* ("granadillo" in Spanish; "subin che" in Maya) (Martínez and Galindo-Leal, 2002). Ecotone areas have predominately cleared lands for goats, sheep or cattle grazing, and maize, bean, or pumpkin crops. The town of Zoh Laguna has 265 houses, 13 public buildings, and 1074 inhabitants (INEGI, 2010); it is located between the north and south regions of the CBR (Fig. 1).

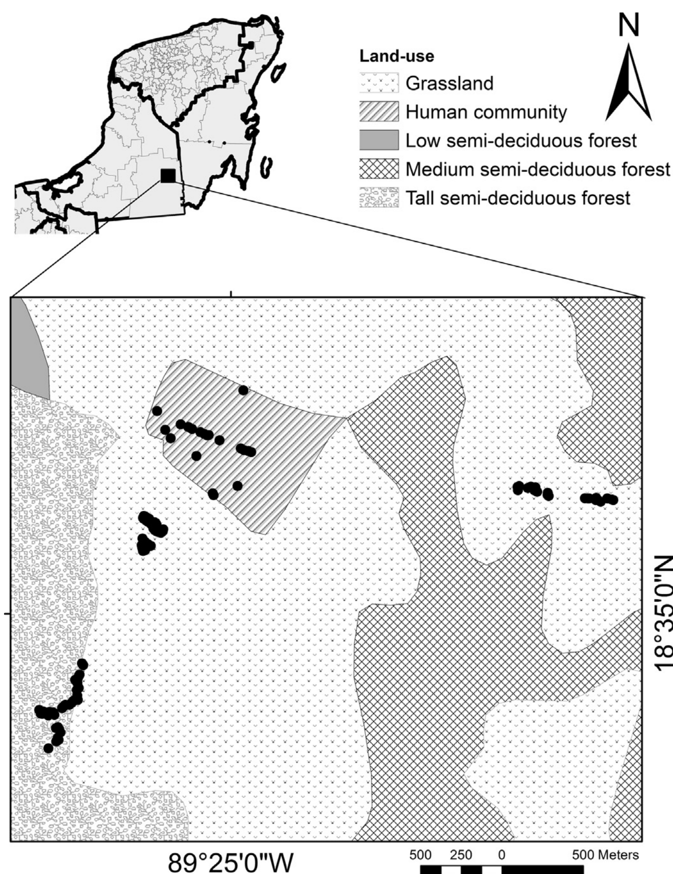

Fig. 1. Location of the Zoh Laguna landscape in the Yucatan Peninsula, in relation to the Calakmul Biosphere Reserve (CBR) with principal vegetation patches noted. Black dots represent georeferenced wildlife traps.

2.2. Mammal communities

Small and medium-sized terrestrial and flying mammals were collected using Sherman (H. B. Sherman Traps, Tallahassee, FL, USA) and Tomahawk (Tomahawk Live Traps, Tomahawk, WI, USA) traps or mist nets, along transects within each of three habitat types: sylvatic (conserved), ecotone (modified with crop or livestock pasture), and domestic (clusters of permanent human housing). All wildlife collections were conducted during the 2010 rainy season (July–November) and the 2011 dry season (February–March). Bats were collected between 20:00 and 01:00 h using three mist nets (12 m wide by 2 m high.) set across paths and water causeways with a minimum of 100 m between them, for five consecutive nights in domestic and ecotone habitats, and for 11 nights in sylvan areas (360 m^2/d). Rodents were sampled using 80 Sherman traps, set along terrestrial transects in each habitat, for five consecutive days for a total sampling effort of 400 traps/night/habitat. Traps were baited with a mixture of oatmeal and vanilla essence. Mid-sized mammals were sampled using 15 Tomahawk traps baited with a sardine and vanilla mixture and set along linear transects close to burrows, for six consecutive nights for a sampling effort of 75 traps/night/habitat. All mammals were collected according to the guidelines of the Mexican Secretary of Environment and Natural Resources (SEMARNAT, 2001), using a collection permit to JMRW. All animals collected were identified to species using keys from Reid (1997) and Medellín et al. (2008), and were weighed, measured for standard mammal museum measurements, sexed, and aged. All mammals collected were anesthetized and peripheral blood, and cardiac, and striated muscular and soft tissues extracted. Blood was preserved at a 1:1 dilution of guanidine buffer (Britto et al., 1995) and tissues were immediately cut into fine tissue blocks (2×2 mm^2), and preserved in ethanol (90%). Preserved whole wildlife specimens in ethanol were deposited as vouchers in the mammal collection of the Centro Regional de Investigación en Salud Pública/INSP or El Colegio de la Frontera Sur (ECOSUR) in San Cristobal de las Casas, Chiapas.

A census of domesticated animals (livestock and pets) was carried out along with other community activities on Chagas disease during the rainy season (RS). Following informed consent by owners, a blood sample was obtained by venipuncture and immediately preserved in guanidine buffer. All samples were preserved at 4 °C and transported to the laboratory for processing and *T. cruzi* molecular analysis.

Sampling rarefaction curves were plotted to determine sampling sensitivity for all wild mammal collections. The relative abundance of all species collected in both rainy and dry seasons was used to calculate species diversity using the Clench Equation with 100 randomized repetitions (Moreno and Halffter, 2001). These analyses were conducted using EstimateS version 8.2 (Colwell, 2012) and Statistica version 10 (StatSoft, 2011).

Community structure (species composition and abundance) of the sampled habitats and seasons were independently compared using a one-way Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2001). PERMANOVA was conducted on Bray–Curtis similarity matrices of square-root transformed data of wildlife mammal abundances with 9999 permutations. The Bray–Curtis coefficient was used to compare community similarity among the six combinations of habitats × seasons in a non-metric multidimensional scaling (NMDS) ordination to graphically represent the assemblagesí patterns (Legendre and Legendre, 1998). The stress coefficient was used as a criterion for goodness of embedding, which varies between 0 and 1, with values near to 0 indicating better fit. These analyses were conducted in PAST version 3.05 (Hammer et al., 2001).

2.3. Triatomines

Triatoma dimidiata and *Triatoma nitida* were the only triatomine species collected in the Zoh Laguna landscape; only one specimen of the latter was collected from the sylvatic habitat. Triatomines were collected from sylvatic and ecotone habitats using four black light traps (2 m × 3 m) for 4 h each of 5 consecutive nights, totaling 120 m^2/habitat/season of light trapping. In the domestic habitat, houses and yards were searched during 40-min periods both in the dry and rainy season. Additionally, householders collected triatomines in periodic inspections or when they were fortuitously observed. All bugs were immediately preserved in 90% EtOH and transported to the laboratory for taxonomic identification (Lent and Wygodzinsky, 1979), midgut extraction, and molecular analyses.

In order to verify the haplogroup of *T. dimidiata* collected in Zoh Laguna, the ND4 was amplified using methods previously reported (Harris, 2003). PCR products were purified using the QIAquickTM PCR purification kit (QIAGEN, Valencia, CA) and sequencing was carried out on an Applied Biosystems 3730xl (Macrogen, Korea). Amplified sequences were aligned and edited manually using BioEdit v.7.0.9 (Hall, 2004) and Mega v.6 (Tamura et al., 2013). The sequences were compared with a database of *T. dimidiata* from Mexico which is in the process of publication (Pech-May, personal communication). The best-fit model of evolution was estimated using the Akaike Information Criterion (AIC) (Akaike, 1974) as implemented in JModeltest v.0.1.1 software (Posada, 2008). Intra-specific phylogeny was analyzed using maximum likelihood (ML) with the Phyml v.3.0 software (Guindon and Gascuel, 2009) based on 500 bootstrap replicates.

The bloodmeal source of all *T. dimidiata* was identified using PCR as previously described (Mota et al., 2007; Ramsey et al., 2012). The

Table 1
List of mammal species collected within the Zoh-Laguna landscape specifying habitat and season collection number and relative abundance adjusted per collection effort. Bold lettering and numbers indicate most abundant species.

Order	Family	Species	Rainy						Dry					
			Sylvatic		Ecotone		Domestic		Sylvatic		Ecotone		Domestic	
			N	RA	N	RA	N	RA	N	RA	N	RA	N	RA
Chiroptera	Mormopidae	*Mormops megallophylla*	0	0.00	0	0.00	0	0.00	1	0.02	0	0.00	0	0.00
		Pteronotus parnellii	0	0.00	0	0.00	0	0.00	0	0.00	1	0.02	0	0.00
	Phyllostomidae	***Artibeusjamaicensis***	**15**	**2.79**	**13**	**2.42**	**30**	**5.58**	1	0.02	1	0.02	2	0.04
		Artibeuslituratus	**10**	**1.86**	3	0.56	**11**	**2.05**	2	0.04	2	0.04	5	0.11
		Carollia brevicauda	3	0.56	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
		Dermanura phaeotis	1	0.19	1	0.19	4	0.74	0	0.00	4	0.09	0	0.00
		Desmodus rotundus	1	0.19	0	0.00	0	0.00	0	0.00	0	0.00	0	0.00
		Glossophaga soricina	0	0.00	1	0.19	1	0.19	0	0.00	0	0.00	0	0.00
		Sturniralilium	**29**	**5.40**	**11**	**2.05**	8	1.49	2	0.04	1	0.02	0	0.00
		Sturnira ludovici	5	0.93	5	0.93	4	0.74	0	0.00	4	0.09	0	0.00
	Vespertilionidae	*Myotis keaysi*	3	0.56	0	0.00	0	0.00	2	0.04	2	0.04	0	0.00
Marsupial	Didelphidae	*Didelphis marsupialis*	1	0.15	0	0.00	2	0.29	2	0.29	0	0.00	0	0.00
		Didelphis virginiana	**8**	**1.17**	0	0.00	1	0.15	0	0.00	0	0.00	0	0.00
		Marmosa mexicana	0	0.00	0	0.00	0	0.00	1	0.15	0	0.00	0	0.00
		Philander opossum	2	0.29	1	0.15	0	1.17	0	0.00	1	0.15	0	0.00
Rodentia	Heteromydae	***Heteromysgaumeri***	**35**	**4.29**	**9**	**1.10**	2	0.25	**19**	**2.57**	**10**	**1.35**	1	0.14
		Orizomys couesi	1	0.12	0	0.00	0	0.00	1	0.14	0	0.00	0	0.00
		Ototylomysphyllotis	**10**	**1.23**	0	0.00	0	0.00	1	0.14	0	0.00	0	0.00
	Muridae	*Mus musculus*	0	0.00	3	0.37	6	0.00	0	0.00	0	0.00	0	0.00
		Peromyscusyucatanicus	3	0.37	6	0.74	0	0.00	**32**	**3.92**	6	0.81	0	0.00
		Rattusrattus	0	0.00	0	0.00	**8**	**0.98**	0	0.00	0	0.00	1	0.14
		Reithrodontomys gracilis	0	0.00	0	0.00	0	0.00	0	0.00	3	0.41	0	0.00
		Sigmodonhispidus	0	0.00	**38**	**4.66**	**8**	**0.98**	0	0.00	5	0.68	1	0.14

proportion of bugs containing human blood was used to calculate the adjusted prevalence of single or mixed human bloodmeals in each habitat. The frequency of single or mixed human blood meals in *T. dimidiata* was compared between habitats using Chi square analyses of frequency.

2.4. *Trypanosoma cruzi* infection

Genomic DNA was extracted from all mammal tissues, blood, and bug midguts using DNAzol (Invitrogen, San Diego, California, USA), following manufacturer's instructions. Extracted DNA was re-suspended in 80 μL of nuclease-free water, and maintained at 20 °C prior to amplification protocols. All samples were first analyzed for the presence of *T. cruzi* using oligonucleotide primers from the conserved region of the kinetoplast minicircle, S34 5-ACA CCA ACC CCA ATC GAA CC-3 and S67 5-TGG TTT TGG GAG GGG SSK TC-3 (Sturm et al., 1989; Ramsey et al., 2012), resulting in a 125 bp product. PCR amplicons were separated on 3% agarose gel, stained with ethidium bromide and visualized under UV light. A DNA sample of Cari06 *T. cruzi* strain (Breniere et al., 2003) was used as positive control in all amplification reactions. Due to low expected parasite loads in tissues and blood from mammals, all samples negative for amplicons in the first PCR were processed using a second amplification. Agarose from the first PCR at the expected weight (125 bp) was excised and re-amplified using the same previous primers (S34/S67) and conditions. PCR products were purified as above and products from all positive samples were sequenced to confirm *T. cruzi* homology using a Prisma 310 ABI (High-Throughput Genomics Center, University of Washington, Department of Genome Sciences).

Infection prevalence for *T. cruzi* was calculated for each species (mammal, bug) and taxonomic or guild group, in each habitat and for the entire landscape. The proportion of infected species (at least one individual in any season or habitat infected) among all those collected was calculated for taxonomic groups or guilds. The proportion of all specimens collected from infected species was calculated for taxonomic groups or guilds in order to adjust prevalence based on relative abundance, for each habitat and the entire landscape. Infected bug prevalence was calculated according to season and stage (adult or nymph). We analyzed the relationship between relative abundance and infection by using a linear regression of the Log10 transformed data. This analysis was conducted in JMP version 9.0.1 (SAS Institute Inc.)

2.5. *Trypanosma cruzi* lineages and population structure

All samples positive for *T. cruzi* using S34/S67 kDNA were amplified using the inter-genic region of the mini-exon gene to identify lineage type (Fernándes et al., 1998; Botero et al., 2007). A volume of 1.5 μl of the total 80 μl DNA from *T. cruzi* positive samples from mammals or triatomines was used as template for a final volume of 25 μl, with 1x Master mix, Taq polymerase in a 200 mM buffer at pH 8.5 of each dNTP and 1.5 mM MgCl (Promega Corporation, Madison, Wisconsin, USA), and 10 μM of each primer. Initial DNA denaturalization was carried out at 94 °C for 3 min, followed by 27 cycles of DNA denaturalization (94 °C for 30 sec), oligo alignment (55 °C for 30 s), and chain elongation (72 °C for 30 s), ending with a final elongation period at 72 °C for 10 min. PCR products (350 bp for TcI and 300 bp for TcII) were separated and visualized on 3% agarose gel stained with ethidium bromide and observed under UV light (the Cari06 strain was used as control for TCI and MV4 V strain for TCII). In order to optimize mini-exon gene amplification from positive samples, once again a secondary PCR using the same mini-exon primers was run under the same conditions (Botero et al., 2007). If bands were faint or not present in the first PCR, gels were cut at the expected weight, purified, and amplified again. All amplicons (from first and second amplifications) were purified as described above and sequenced to verify homology with *T. cruzi* from GenBank using BLASTN. A total of 92% of bands at 300 or 350 bp had homology (>90%) with previously reported *T. cruzi* mini-exon (TcI or TcII). Amplified sequences were aligned and edited manually as previously described. A total of 19 haplotypes, five sequences of TcI (TcIa AM259467, TcIb AM259469, TcIc AM259472, TcId AM259473, TcIe EF576840), and five sequences from GenBank (accession numbers AY367125, Tu18; AY367126, M5631; AY367123, CanIII; AY367128, MN; and AF510513, CLBrener) were used for phylogenetic anal-

Table 2
One-way PERMANOVA based on Bray–Curtis distance matrix of mammal community among habitats and seasons. *P*-Values are based on 9999 permutations.

Factor	Total sumof squares	Within-groupsum of squares	*F*	*P*
Season	0.8669	0.6104	1.681	0.19
Habitat	0.8669	0.3894	1.8	0.068

yses. The best-fit model of evolution was estimated using the Akaike Information Criterion (AIC) (Akaike, 1974) as implemented in JModeltest v.0.1.1 software (Posada, 2008). The DNAsp v5 (Rozas, 2009) program was used for haplotype analysis and phylogenetic relationships were reconstructed using Network version 4.6.1.3 (Bandelt et al., 1999) with the median-joining (MJ) network method for multi-state data analysis. Intra-specific phylogeny was analyzed using MrBayes 3.2 (Ronquist et al., 2012), four Markov Chain Monte Carlo (MCMC) were run for 10 million generations (sampled every 1000 generations) to allow adequate time for convergence ($\leq$0.008). The first 25% of sampled trees were considered as burn in. The tree was visualized with FigTree V1.4. (http://tree.bio.ed.ac.uk/software/figtree/).

3. Results

3.1. Wild and domesticated mammal communities

A total of 417 wild mammal specimens from three orders, seven families, and 23 species were collected across the Zoh laguna landscape (Table 1). The mammal community from the complete landscape was satisfactorily represented according to the sampling sensitivity based on rarefaction curves (r^2 = 0.99). There were no statistical differences among habitats or between seasons for wild mammals (Table 2, Fig. 2). The assemblage patterns had a landscape similarity gradient, with the ecotone community between sylvatic and domestic communities, although only one cluster was formed using the annual (rainy and dry season) communities in the three habitats (95% confidence in accordance with PERMANOVA). The domestic community in the dry season had a greater relative distance as compared to the other five groups, indicating a trend for community differentiation.

Five bat *Artibeus jamaicensis* (AJ), *Artibeus lituratus* (Al), *Dermanura phaotis* (Dp), *Sturnira lilium* (Sl), *Sturnira ludovici* (Slu) and one rodent species (*Heteromys gaumeri*, Hg) were present in all habitats, and most in both seasons (Table 1). In contrast, four bat (*Carollia brevicauda* Cb, *Desmodus rotundus* Dr, *Glossophaga soricina* Gs, and *Mormops megallophylla* Mm) and two rodent species (*Ototylomys phyllotis*, Op and *Oryzomys couesi* Oc) were only collected in the sylvatic habitat. Only *Rattus ratus* (Rr) was uniquely found in the domestic habitat. Apart from these extremes in generalists and specialists, respectively, all remaining species were found in at least two habitats. *Pteronotus parnelli* (Pp) and Mm were only collected in the dry season, as were the majority of *Myotis keaysi* (Mk) individuals. Temporal trends associated with seasons were also registered in the two *Artibeus* (Aj and Al) species and in Sl, with significant decreases during the dry season. Two rodent species increased their relative abundance in the dry season (Py and Hg). Didelphids were more abundant in sylvatic habitats, particularly in the rainy season, although only one species was collected exclusively in both seasons but only in the ecotone (*Philander opossum*, Po). While *Didelphis virginiana* (Dv) was the most frequent opossum in the sylvatic habitat in the rainy season, its populations plummet in the dry season, when *Didelphis marsupialis* (Dm) is the most abundant. In the domestic habitat, in the rainy season, Dm is twice as abundant as Dv, although in the dry season neither was collected.

We sampled 388 pets and livestock: *Ovis aries* (Oa), *Bos taurus* (Bt), *Sus domesticus* (Sd), *Capra hircus* (Ch), *Equus caballus* (Ec), *Felis catus* (Fc) and *Canis familiaris* (Cf). Sampling proportions were variable according to species, ranging from 92.8% for Oa to 15.2% for Fc. The most abundant species were Oa (39.9%) and Cf (45.4%). Medium-sized domestic species (Oa, Sd, and Cf) were more abundant (47.9%) than the larger domestic livestock (Bt, Ec; 3.6%). Almost 50% of domestic mammals grazed in the ecotone habitat or made frequent incursions to ecotone areas for grazing or hunting activities (dogs). All other livestock or pets remained in the domestic habitat.

3.2. Sensitivity and specificity of molecular techniques to detect single or multiple lineages of *Trypanosoma cruzi*

Modification of PCR protocols resulted in an important increase in *T. cruzi* detection sensitivity and specificity (Table 3). Using one amplification step, only 32.3% of wildlife, 11.1% of livestock and 43.6% of bug confirmed infections were detected, although the homology confirmation of *T. cruzi* sequences was less efficient in second vs first amplifications (24.4% and 24.6% vs. 71.4% and 66.7%, respectively). However, both first and second PCR yielded similar proportional confirmation of *T. cruzi* bands in bug samples and therefore specificity (89.5% and 95.7%, respectively). The PCR protocol allowed us to identify mixed lineages in samples from wildlife, livestock/pets, and bugs (13.3%, 61.1%, and 20.5%, respectively). Samples having more than one lineage were principally identified following secondary amplification: 100.0% of mixed samples from wildlife and livestock/pets, and 87.5% of those from bugs. However, mini-exon amplification efficacy was lower in bug samples as compared to wildlife and livestock (79.5% vs. 93.3% and 100.0%, respectively).

3.3. *Trypanosoma cruzi* in wildlife, domesticated mammals, and *Triatoma dimidiata*

Overall, 52.2% of all wildlife species collected had detectable infection with *T. cruzi* (Table 4). Bats had the highest proportion of infected species which had greatest prevalence, 97.4% of all specimens collected. In contrast, specimens collected from infected marsupial species were only 47.4% of all marsupial specimens. 50.0% of rodent species had 88.0% of all specimens. Assuming equal success and representation of collected infected individuals across taxonomic or guild groups, highest adjusted infection was in marsupials (15.8%), followed by bats with 10.6%, rodents with 7.7%, and livestock/pets with 4.6%. Bats had the highest proportion of infected species, yet prevalences were not statistically different as compared to terrestrial mammals in the complete Zoh Laguna landscape (bat prevalence = 10.9%, confidence intervals at 95% = 7–16%; terrestrial prevalence = 9.8%, 6.4–15%). Species groups had contrasting prevalence patterns within the landscape. For instance, bats had higher infection prevalence than rodents in the sylvatic habitat, while marsupials had higher prevalence than rodents and bats in the domestic habitat (Table 4). In livestock and pets, 33.3% of species were infected, representing 94.4% of all specimens from the ecotone, and 92.2% of all specimens from the domestic habitat. All wildlife and livestock/pets in the domestic habitat had similar infection prevalence (5.7% vs. 5.1%, respectively), while in the ecotone, infection prevalence of wildlife (12.4%) was double that in livestock/pets (4.9%) and the previous. The overall infection prevalence in wildlife was similar between sylvatic and ecotone habitats (12.4% vs. 12.9%, respectively). Bats were significantly more infected in the sylvatic habitat than in either the ecotone or the domestic (Table 4), while rodents were four times more infected in the ecotone (16.9%) as compared to the sylvatic habitat (3.4%), and were not positive in the domestic habitat. Livestock and pets were equally infected in ecotone and domestic habitats (4.9% and 5.1%, respectively).

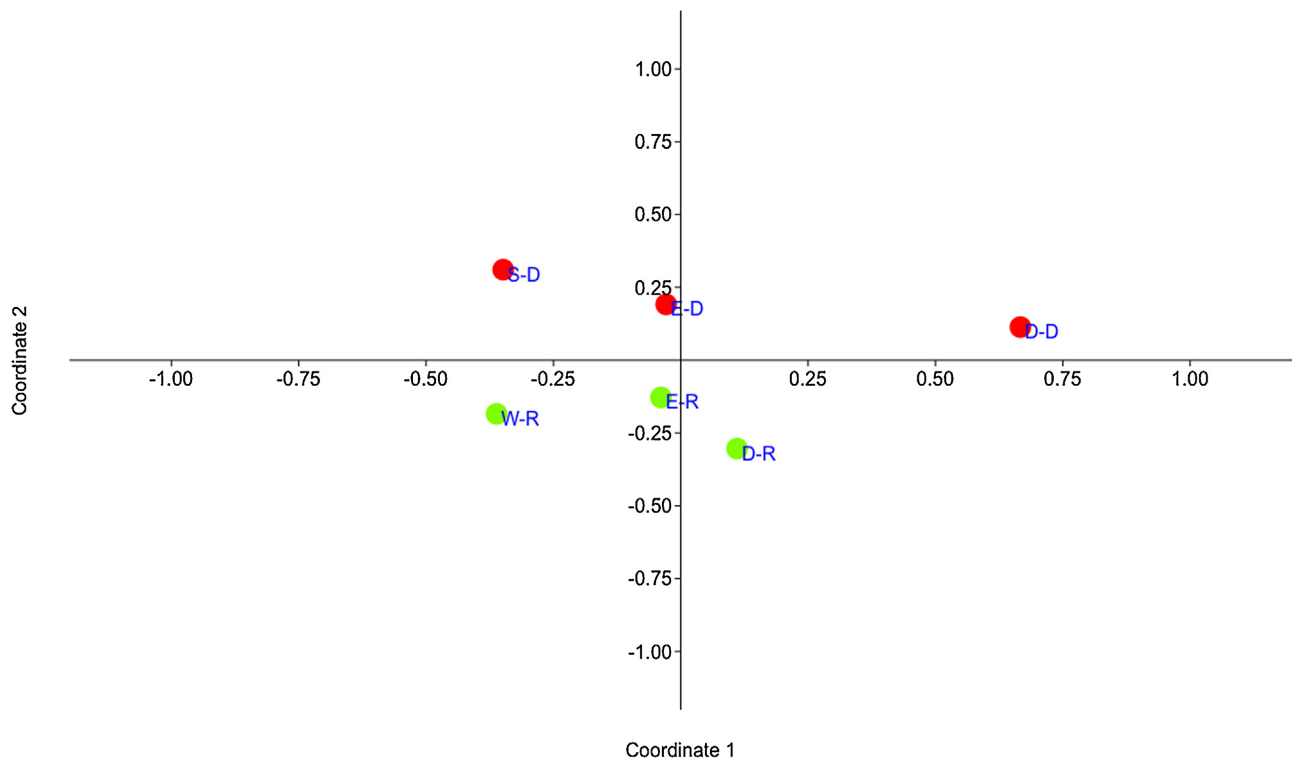

Fig. 2. Nonmetric multidimensional scaling (NMDS) of ordination of mammal host community in sylvatic, ecotone, and domestic habitats of the Zoh-Laguna landscape in rainy and dry seasons. Sylvatic–Dry = S–D; Sylvatic–Rainy S–R,; Ecotone–Dry = E–D; Ecotone–Rainy = E–R; Domestic–Dry = D––D; and Domestic–Rainy = D–R. Stress = 0.

Table 3
Sensitivity and specificity of *T. cruzi* infection detection using single or doble PCR for diagnosis (Dx, S34/S67 kDNA), and lineage analysis (single or mixed) using the mini-exon gene, in samples from different host groups. DxTc Seq/PCR 1 = proportion samples diagnosed (confirmed by sequencing) after first PCR (PCR1); DxTc Seq/PCR2 = proportion samples diagnosed (confirmed by sequencing) after second PCR only (PCR2); Dx Mix PCR1 = proportion of mixed lineage samples identified in first PCR; Dx Mix PCR2 = proportion of mixed lineage samples identified in second PCR; Mix TcI + TcII = total proportion of samples with both TcI and TcII; Dx ME/Dx S34/S67 = proportion samples positive in PCR with mini-exon among all confirmed using DxS34/S67/Seq.

Host group (N)	DxTc Seq/PCR1 % (N)	DxTc Seq/PCR2 % (N)	Dx PCR2 (%)	Dx Mix PCR1 % (N)	Dx Mix PCR2 % (N)	Mix TcI + TcII %	Dx ME/Dx S34/S67 % (N)
Wildlife (417)	71.4 (14)	24.4 (82)	66.7	0 (3)	100.0 (3)	13.3	93.3 (30)
Livestock/pets (388)	66.7 (3)	24.6 (65)	88.9	0 (11)	100.0 (11)	61.1	100.0 (18)
T. dimidiata (56)	89.5 (19)	95.7 (23)	56.4	12.5 (8)	87.5 (8)	20.5	79.5 (39)

Table 4
Trypanosoma cruzi infection in taxonomic or guild groups of mammals, according to habitat. Proportion of positive species are those with at least one specimen positive. Proportion of specimens from infected species is the total number of specimens from all infected species over the total number of specimens collected. Wilson score confidence intervals (CI) at 95% are showed in parenthesis.

Group	Proportion positive species	Proportion specimens infected species	*T. cruzi* infection						
			Sylvatic		Ecotone		Domestic		Landscape
(N species)	% (N)	%	% (CI)	N	% (CI)	N	% (CI)	N	% (CI)
Bats (7)	63.6 (11)	97.4	21.9 (14–33)	73	6.4 (1–18)	47	1.6 (0–8)	64	10.9 (7–16)
Rodents (4)	50.0 (8)	88.0	3.4 (1–9)	89	16.9 (10–27)	77	0 (0–17)	18	8.7 (5–14)
Marsupials (1)	25.0 (4)	47.4	25.0 (7–59)	8	nc	0	100.0 (21–100)	1	33.3 (12–65)
Total wildlife	52.2 (23)	78.9	12.4 (8–18)	170	12.9 (8–20)	124	6 (3–13)	83	11.9 (9–16)
Livestock/pets (3)	33.3 (9)	93.3	nc	0	4.9 (3–9)	184	5.1 (3–9)	178	5 (3–8)

Bat infections were unequal between habitats, 80.0% were sylvatic, 15.0% from the ecotone, and 5.0% from the domestic habitat (Table 5). Uninfected bat species had lowest relative abundance from only two species of Mormoopidae, *Desmodus rotundus*, and *Glossophaga soricina.* The only species collected from the Vespertilionidae, *Myotis keasyi* (Mk), was one of the two with highest *T. cruzi* prevalence (14.3%). Both bat species not collected in the domestic habitat, *Carollia brevicauda* (Cb, 66.7%) and Mk, had highest *T. cruzi* infection prevalence of all bats. *Dermanura phaeotis* also had high infection prevalence (10.0%), principally collected in ecotone and domestic habitats. The remaining four infected species (*Artibeus* spp, and *Sturnira* spp.), which represent 87.7% of bat specimens, had infection prevalence below 5.9% (*Sturnira lilium*, Sl), the lowest having 3.0% (*Artibeus lituratus*, Al). There was a significantly lower infection in domestic specimens of *Artibeus jamaicensis* (Aj) as compared to ecotone or sylvatic. Only Mk and Al had *T. cruzi* lineage II (TcII), in single (Mk) or mixed (Al) infections. All other isolates from bats were TcI.

Four rodent species (50.0% of species) were infected with *T. cruzi*, 81.3% from the ecotone and 18.7% from the sylvatic habitat (Table 5). Uninfected rodent species had low relative abundance and only 2-11 collected specimens. *Sigmodon hispidus* (Sh) collected

Table 5
Trypanosoma cruzi infection in small and medium-size wildlife and domesticated animals in the Zoh Laguna landscape. Values represent prevalence from all (rainy + dry season) collected specimens for each species. Nc = not collected. Dogs classified as ecotone commonly moved daily between domestic and ecotone habitats, while those classified as domestic did not leave that habitat. Livestock were classified according to the habitat where they were maintained. *T. cruzi* samples with single lineages indicate if the specimen was sylvatic (S), ecotone (E), or domestic (D).

	Prevalence				*T. cruzi* lineage		
Species	Sylvatic% (N)	Ecotone% (N)	Domestic% (N)	Total% (N)	TcI	TcII	Mixed
Artibeus jamaicensis	6.3 (16)	7.1 (14)	0.0 (32)	3.2 (62)	1S	–	–
Artibeus lituratus	0.0 (12)	0.0 (5)	6.3 (16)	3.0 (33)	–	–	1D
Carollia brevicauda	66.7 (3)	nc	nc	66.7 (3)	2S	–	–
Dermanura phaeotis	0.0 (1)	20.0 (5)	0.0 (4)	10.0 (10)	1E	–	–
Myotis keaysi	20.0 (5)	0.0 (2)	nc	14.3 (7)	–	1S	–
Sturnira lilium	6.5 (31)	8.3 (12)	0.0 (8)	5.9 (51)	2S + 1E	–	–
Sturnira ludovici	20.0 (5)	0.0 (9)	0.0 (4)	5.6 (18)	1S	–	–
Heteromys gaumeri	3.7 (54)	5.3 (19)	0.0 (3)	3.9 (76)	2S + 1E	–	–
Peromyscus yucatanicus	2.9 (35)	8.3 (12)	nc	4.3 (47)	1S + 1E	–	–
Mus musculus	nc	33.3 (3)	0.0 (6)	11.1 (9)	1E	–	–
Sigmodon hispidus	nc	23.3 (43)	0.0 (9)	19.2 (52)	7E	1E	2E
Didelphis virginiana	25.0 (8)	nc	100.0 (1)	33.3 (9)	2S	–	–
Ovis aries	nc	2.3 (128)	11.1 (27)	3.9 (155)	–	2D	3D, 1E
Suis domesticus	nc	nc	3.2 (31)	3.2 (31)	2D	–	1D
Canis familiaris	nc	10.7 (56)	4.2 (120)	6.3 (176)	1E	1E, 2D	3D, 3E

Table 6
Trypanosoma cruzi infection of *Triatoma dimidiata* according to season (rainy or dry) and age (adult or nymph) and bloodmeal source of *T. dimidiata* in Zoh Laguna. Nc = not collected.

	T. cruzi infection% (N)						
	Season		Stage		Bloodmeal source% (N)		
Habitat	Rainy	Dry	Adults	Nymphs	Human	Non-human	Mixed
Sylvatic	nc	100.0 (2)	100.0 (2)	nc	0.0 (1)	100.0 (1)	0.0 (1)
Ecotone	75.0 (12)	100.0 (3)	86.0 (14)	0.0 (1)	0.0 (10)	90.0 (10)	10.0 (10)
Domestic	65.6 (32)	57.1 (7)	70.0 (34)	20.0 (5)	8.3 (24)	87.5 (24)	4.1 (24)
Landscape	68.0 (44)	75.0 (12)	76.0 (50)	16.7 (6)	5.7 (35)	88.5 (35)	5.7 (35)

only in ecotone and domestic habitats, had significantly higher infection prevalence than other rodent species (19.2%); only those from the ecotone were infected. Similarly, infected specimens of *Mus musculus* (Mm) (11.1%) were only collected in the ecotone. *Heteromys gaumeri* (Hg) was the only infected rodent (3.9%) collected in all three habitats. Specimens from sylvatic and ecotone habitats of both Hg and Py were infected with *T. cruzi*. TcII was only collected from the two rodent species with highest prevalence (Sh and Mm), in single or mixed infections (all ecotone). Both Py and Hg only had TcI, in both sylvatic and ecotone habitats. It is important to note that single infections of TcI and TcII, and mixed infections of both were all identified only in Sh.

Although four species of didelphids were collected in the entire landscape, only *Didelphis virginiana* (Dv), the species with highest annual relative abundance (although only collected in the rainy season) and only in sylvatic and domestic habitats, was infected with *T. cruzi* (Table 5). Specimens from both sylvatic and domestic habitats of Dv were infected (33.3%); only TcI in sylvatic specimens was confirmed.

The most abundant domesticated species were infected with *T. cruzi*: *Ovis aries* (Oa, 3.9%), *Suis domesticus* (Sd, 3.2%), and *Canis familiaris* (Cf, 4.2%) (Table 5). Infection in Oa was significantly higher in the domestic habitat than in the ecotone (OR 5.20, CI 0.99–27.36, *p* 0.051), while the reverse occurred in dogs: in Cf, the prevalence in the ecotone (10.7%) was more than double that in the domestic habitat (4.2%). Single and mixed infections of both TcI and TcII were found in both Oa and Cf, in both ecotone and domestic samples. Only TcI was isolated from Sd.

Two *T. dimidiata* haplogroups were present in Zoh Laguna, haplogroup 1 (h1, 63.6%) and haplogroup 2 (h2, 36.4%); infections in both haplogroups were comparable (4/4 and 2/3, respectively). Only 62.5% of collected bugs had midgut contents, and overall, 5.7% had only human blood (all from domestic habitat) while 4.1% had mixed human and non-human mammal blood (Table 6). In the ecotone, 10% of bugs had mixed human and non-human bloodmeals. Overall, 69.6% of *T. dimidiata* in the landscape were infected with *T. cruzi*; 100.0% of sylvatic, 80.0% of ecotone, and 64.1% of domestic collections, although these differences are not significant and are principally due to greater abundance of infected nymphs in domestic and ecotone habitats (12.8% and 6.7%, respectively) (Table 6). Adult *T. dimidiata* were four times more infected (76.0%) than nymphs (16.7%). All h2 individuals were collected from the domestic habitat, 75.0% of them infected, while only 42.9% of h1 were domestic, 85.7% of them infected with *T. cruzi*. TcI alone was identified in 59.0% of bugs, and TcII was only identified from mixed infections (20.5%) (Table 7). The overall adjusted TcI prevalence was 66.0%, similar among all habitats. Overall prevalence of TcII was 17.0% although in the domestic habitat (8.0%), it was significantly lower than in ecotone or sylvatic habitats (41.7% and 50.0%, respectively). In both *T. dimidiata* haplogroups, there was a predominance of single TcI infection, and one sample of each in domestic and ecotone habitats had mixed infection with TcII.

The relative abundance of infected speciesí populations by taxonomic or guild host group was significant yet inversely associated with infection prevalence in both bats and rodents, while not significant in marsupials or livestock (Fig. 3). Lack of association with marsupials may be specifically related to the fact that only one species was infected. Domesticated mammals share the fact that they have been genetically selected and may no longer conserve an immune response related with tolerance.

3.4. Analysis of *T. cruzi* population structure in the landscape

The overall proportion of TcI and TcII, adjusted for mixed infections decreased according to degree of habitat modification in both wildlife and livestock/pets (Table 7, Fig. 4). TcI prevalence

Table 7
Trypanosoma cruzi lineages according to habitat and host group: wildlife, domesticated (livestock and pets), and *T. dimidiata.* Adjusted overall lineage prevalence (including from mixed infections) in parentheses.

Habitat	Host (N)	TCI % (%)	TCII % (%)	MIX
Sylvatic	Wildlife (12)	91.7	8.3	0.0
	Livestock/pets (0)	–	–	–
	T. dimidiata (2)	50.0 (66.7)	0.0 (33.3)	50.0
Ecotone	Wildlife (16)	68.8 (73.7)	6.3 (21.1)	18.8
	Livestock/pets (7)	14.3 (50.0)	14.3 (50.0)	71.4
	T. dimidiata (12)	50.0 (64.7)	0.0 (29.4)	41.7
Domestic	Wildlife (2)	0.0 (33.3)	0.0 (33.3)	50.0
	Livestock/pets (11)	18.2 (43.8)	36.4 (58.8)	54.5
	T. dimidiata (25)	64.0 (66.7)	0.0 (7.4)	8.0

decreased according to an increase in habitat modification, from sylvatic to domestic habitats ($R^2 = 0.953$), as did that in livestock and pets (Fig. 4A). However, single TcI infection prevalence in bugs was constant along the disturbance gradient. The proportions and presence of TcI and TcII in single or mixed infections was distinct between host guilds (wildlife, livestock/pets, *T. dimidiata*) and habitat (Table 7, Fig. 4A). The proportion of single TcI infections diminished from conserved to modified habitats in favor of mixed infections, while single TcII infections remained similar between sylvatic and ecotone habitats. Livestock and pets, only present in ecotone and domestic habitats, had principally mixed infections in the ecotone (57.1%), three times more than in wildlife from the same habitat (18.8%) (Fig. 4B). Single TcII infections in domestic livestock and pets (36.4%) was two and a half times that in the ecotone (14.3%), and significantly higher than the lack of single TcI infection in wildlife or in bugs.

Bayesian phylogenetic inference was constructed with the GTR + G model as the most appropriate for the data (−INL = 2241.438, Delta AIC = 3.310, AIC = 4830.8759) with gamma of 0.1830. Analysis provided maximum support (1.0 posterior probability). The population structure of *T. cruzi* across all host species in Zoh Laguna indicates the presence of two primary DTUs from each of the two principal lineages TcI and TcII (Figs. 5 and 6, Table 1). All TcI were phylogenetically homologous to TcIa, with one primary and multiple additional haplotypes. All TcII had homology with the DTU group II/V/VI using a Bayes analysis, although the network analysis clearly placed the samples with the TcVI outgroup haplotype (CLBrener). Principal haplotypes of TcIa and TcVI were identified in wildlife, livestock/ pets, and bugs, and all hosts had additional additional haplotypes of both DTUs. Similarly, both DTUs were found in all habitats, and all habitats had haplotypes. *T. cruzi* from two residents of Zoh Laguna were identical to the primary haplotype of TcVI. While proportions of TcIa and TcVI prevalence in bugs and other hosts were very similar, the prevalence from different host groups were distinct. While about half of the dominant haplotype samples of TcIa were from bugs, only about a quarter of TcVI samples were identified in bugs. The dominant TcVI haplotype prevalence in livestock was double that of TcIa.

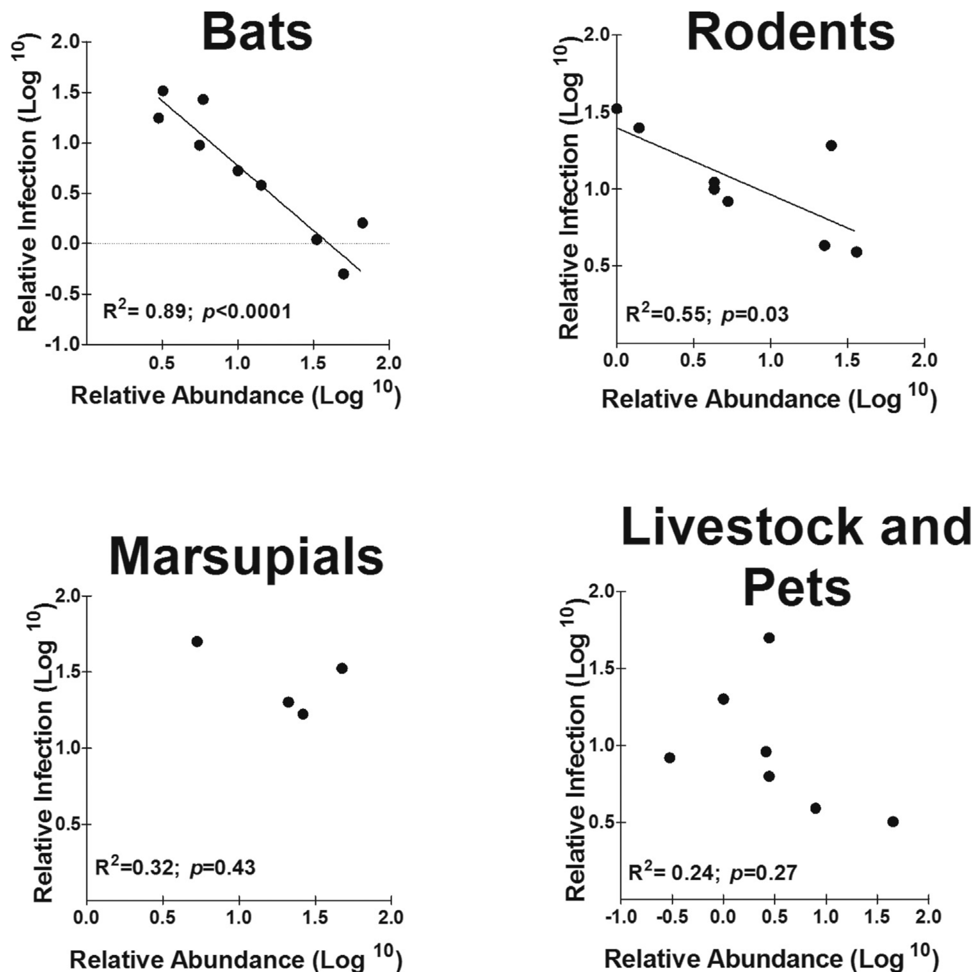

Fig. 3. Association of relative abundance and *T. cruzi* infection in wildlife and livestock/pet species based on taxonomic or guild group.

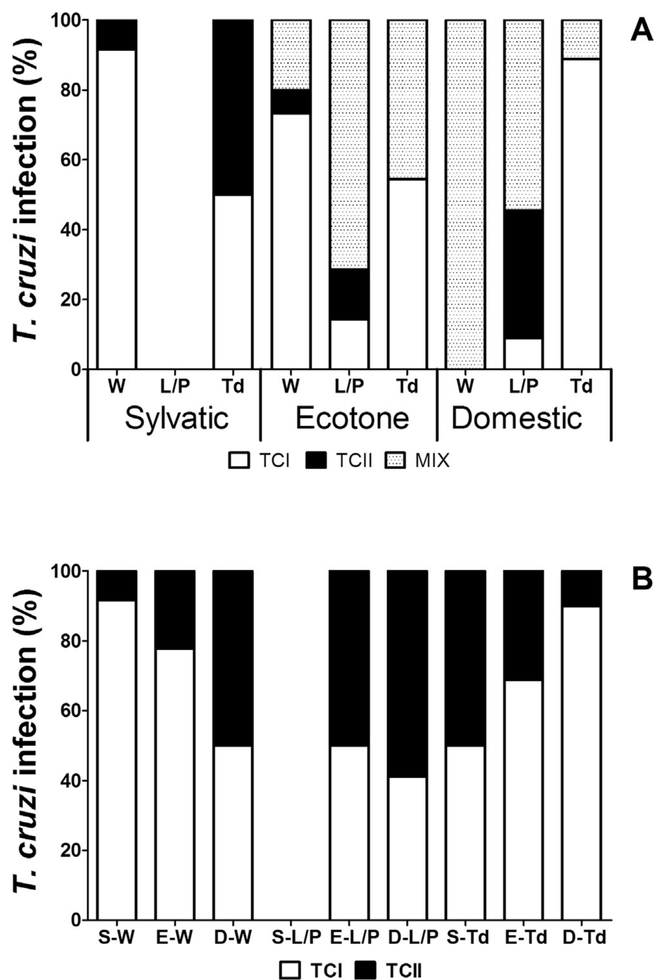

Fig. 4. *Trypanosoma cruzi* infection according to habitat and reservoir groups (wildlife, livestock/pets, bugs). (A) Proportion of single or mixed infection with TcI and TcII, in wildlife (W), livestock or pets (L/P), and *T. dimidiata* (*Td*) in sylvatic (S), ecotone (E), and domestic (D) habitats. (B) Adjusted infection proportions of lineages TcI and TcII (includes single and mixed infections) in sylvatic, ecotone, and domestic habitats, from wildlife (W), livestock or pets (L/P), and *T. dimidiata* (*Td*).

4. Discussion

The present study has attempted to analyze as many of *T. cruzi* metapopulations present in all hosts in the entire Zoh Laguna landscape, in order to focus future studies on specific components of the parasite's population dynamics. The complete landscape ecology of *T. cruzi* is rarely studied, principally due to the multiple methodological problems related with representative collections of all hosts and fragments in a landscape, to vector sampling in sylvatic (conserved) or ecotone (modified) habitats, and to low parasite loads in most mammals, especially wildlife. Several multi-host landscape or regional level analyses have in recent years begun to give a more complete perspective of parasite dynamics at these levels (Marcili et al., 2009a,b; Ocaña-Mayorga et al., 2010; Carrasco et al., 2012; Orozco et al., 2013; Fernández et al., 2014), while a growing literature provides a heterogeneous profile of proposed host and geographic associations of *T. cruzi* DTUs at different scales. However, the majority of these previous studies are based on parasite isolates and analyze only a subset of available hosts. Finding a single method to detect and typify parasites in all host systems and tissues, implies sensitivity and specificity across issues of quantity, competing amplicons (*T. cruzi* specific, i.e., DTUs, or non-specific), and study goals.

Two main clades of *T. cruzi*, lineages I (DTU Ia) and II (DTU VI), were found to be sympatric (all habitats and seasons) in the Zoh-Laguna landscape, and no species-specific interactions occur between the parasite and any mammal host, in any habitat. Other studies reported similar results when analyzing *T. cruzi* diversity among hosts and landscapes in several regions of South America (Lima et al., 2014b; Llewellyn et al., 2009; Ocaña-Mayorga et al., 2010). We have also found mixed infections of these two clades in individuals across habitats, particularly in livestock and pets, and in vectors. Overall, our results allow us to conclude that *T. cruzi* transmission depends on host availability within a highly permeable landscape. Two inhabitants in Zoh Laguna were found to be infected with only one of these clades (TcVI), suggesting that the host assemblage properties of the landscape drive the disease risk of *T. cruzi* transmission. Our study also detected some complex trends in parasite transmission related to lineage sorting within the matrix. The frequency ratio between lineages I/II changed between habitats from 9:1 in sylvatic to almost 1:1 in domestic habitats and the ratio of mixed/single infections changed from null in sylvatic to all in the domestic habitat. Intriguingly, as the frequency of lineage I decreased in wildlife and to a lesser degree in livestock toward the domestic habitat, its frequency remained constant in vectors in all habitats. Although the mechanisms behind this outcome remain obscure, mammal and vector host filters are probably all occurring. These patterns could also be related to inter-infrapopulation interactions of *T. cruzi* lineages within hosts, since these interspecific interactions could be density dependent, in that the abundance of one parasite population affects the fitness of a co-infecting species. If interactions are immune-mediated, the effect of the interaction might be temporally delayed, or depend on host condition (Pedersen and Fenton, 2007). They also could be related to differential dispersal abilities of particular hosts as we have analyzed species with different spatial ranges, including more mobile hosts such as bats, and more restricted ones such as rodents (and bugs), in addition to species for which movement is relatively human-controlled, as in the case of livestock. These differences in dispersal capacity are likely to affect vector-host contact rates and specific lineage sorting. The epidemiological repercussions of these patterns remain poorly understood, for instance what are the consequences of mixed infections in anti-parasitic patient treatment or in congenital transmission, to cite a few very important examples (Burgos et al., 2010; Mantilla et al., 2010; Ortiz et al., 2012; Monje-Rumi et al., 2013).

The method we have optimized to detect the presence of *T. cruzi* in wildlife tissues, in livestock, pet, or human blood, and in the vector midgut has provided the most complete representation of landscape meta-populations in Mexico, without which genetic diversity and gene flow cannot be quantitatively analyzed at the host and landscape level. The optimized DNA amplification method has the sensitivity and specificity to detect multiple parasite populations, despite potential differential amplification of multiple DTUs when encountered together in a sample (Laurent et al., 1997). Confirmation of TcVI homology will require analysis with at least a second locus in order to ascertain appropriate designation of samples within the TcII family group (Burgos et al., 2010), although differences are statistically significant. We may not have identified all populations/DTUs present in wildlife host tissues and ongoing analyses of infra-populations will provide a broader perspective of *T. cruzi* meta-populations in these hosts. Future studies will need to improve the sensitivity of PCR detection using sequential samples from livestock or humans, since low numbers of circulating parasites are expected. The mixed infections identified in Zoh Laguna were most prevalent in both bugs and livestock, in contrast to wildlife and humans. Previous studies have reported detection of mixed TcIa and TcVI in human samples in Rio (Sangenis et al., 2015) and in dogs and bugs in northwestern Argentina and the Atlantic

forest of Brazil (Marcet et al., 2006; Lima et al., 2014a). Other DTU mixes of TcI and TcIV have been identified in the Brazilian Amazon (Lima et al., 2014b), the United States (Roellig et al., 2013), and in Venezuela (Carrasco et al., 2012); TcI and TcII or TcV have also been reported in mixed infections in Amazonia (Lima et al., 2012). These few studies suggest, however, only minor structuring of *T. cruzi* on broad geographic scales. Although analysis using real time PCR could be a potential improvement in sensitivity and to quantify parasite loads and proportional content of multiple DTUs, this method is still not as sensitive or specific as the present method described, specifically with wildlife from the present study (Cura et al., 2015).

Analysis of parasite dynamics, prevalence, and diversity depends on representative sampling in all host systems and differentially according to available host communities (and habitat types). Parasite load is not the same in wildlife as in domesticated mammals, or even humans, given evolutionary interactions and mechanisms which develop either tolerance or active selection processes. Recently domesticated mammals which have not co-evolved with the parasite may not be as competent to filter parasite populations as wildlife (i.e., in blood). Some wildlife studies use parasites directly from tissues, others from blood, and yet others culture and select parasites prior to typification, despite the bias of these latter samples for metapopulation studies (Marcili et al., 2009b; Lo Presti et al., 2014). Given the paucity of information regarding *T. cruzi* genetic diversity, population structure, or prevalence in Mexico, and evidence that both in wildlife and humans, parasite populations may segregate anatomically or temporally in the host (Naumann, 2006; Mantilla et al., 2010; Ortiz et al., 2012; Monje-Rumi et al., 2013; Lo Presti et al., 2014), our goal has been to directly typify wildlife tissues for natural representation, thereby avoiding the bias of restricted populations from either blood, or culture-isolated samples. Ongoing analysis of parasite infra-populations in different wildlife species should provide a measure of overall parasite metapopulation representation, such as that described by Lo Presti et al. (2014).

As has been pointed out elsewhere, host metacommunities are a renewed concern in disease ecology, particularly in multi-host pathogens (Suzán et al., 2015). In this study we have identified associations of parasite transmission with host dispersal ability and habitat filtering, within the framework of altered yet similar mammal assemblages among habitats and over a progressively anthropogenic gradient. Not all hosts had the same potential role in *T. cruzi* transmission within the landscape, as we found that bats have higher prevalence in sylvatic areas, rodents were highest in the ecotone, and marsupials in domestic habitats. Whereas in bats, species richness correlated positively with infection, this same association was observed in the ecotone for rodents. These results do not support the dilution hypothesis for biodiversity and disease risk (LoGiudice et al., 2003). However, we did find that more resilient species had an important role as *T. cruzi* hosts, which is one of the assumptions of the dilution hypothesis (Keesing et al., 2006). Several synanthropic species collected in modified areas (ecotone and domestic) were infected with *T. cruzi*. The hispid cottontail rat, *Sigmodon hispidus*, a highly resilient species, had high infection rates in the disturbed habitat in Zoh Laguna, just as was found in the Chalcatzingo landscape in Morelos (Ramsey et al., 2012). *Peromyscus yucatanicus*, another crop pest specific to the YP, *Artibeus jamaicensis*, the Jamaican fruit bat, and *Didelphis virginiana* all benefit from landscape degradation and are consistently found infected with *T. cruzi*.

Host abundance was inversely related to infection in bats and rodents, but not in marsupials or livestock. This inverse relationship was reported previously in another study of *T. cruzi* transmission in an anthropogenic landscape, dealing with a different vector, *Triatoma pallidipennis* and rodents (Ramsey et al., 2012). This observed inverse relationship could be explained by two non-mutually exclusive mechanisms: host-vector saturation contact, and prevalence dilution as a result of reproductive patterns. It has been suggested that the vector feeding process associated with stercorarian transmission to hosts and blood-borne transmission to vectors is limited by the population density of vectors when dealing with rodents, but by that of hosts when dealing with raccoons and opossums (Kribs-Zaleta, 2010). Vector density in the Zoh-Laguna landscape appears to follow a gradient from low density in sylvatic to higher density in domestic habitats, and a relatively low density of opossums was also registered in the latter habitat. Our results hence support predictions by Kribs-Zaleta (2010). The fact that vector density increased in ecotone and domestic habitats, has been reported elsewhere for other Chagas disease vectors (Gottdenker et al., 2014; Ramsey et al., 2012) or vectors of other disease (McCauley et al., 2015).

Triatomines remain close to and in host nests, and principally disperse only for alternate food source, or reproduction. Their sampling in non-domestic habitats means successfully competing with available food source, or mates, in an appropriate micro-environment (near arboreal or terrestrial nest, caves/outcroppings). Although there have been many trapping methods developed for triatomines, their efficacy for some species and in certain habitats or landscapes has not been universal (Noireau et al., 2002; Angulo and Esteban, 2011) and in the case of *T. dimidiata*, not effective except in isolated reports which may be related to vector abundance and habitat (Rebollar-Tellez et al., 2009; Hernández et al., 2010). One of the limitations of the present study is the low number of bug specimens collected in sylvatic and ecotone habitats, using black light traps, the most consistent method we have tested for this species. Future studies will need to improve sample size to better describe vector density or host selection. These studies may benefit from recent information regarding aggregation molecules in *T. dimidiata* (May-Concha et al., 2015), which will hopefully now allow us, without the need for tracker dogs (Rolon et al., 2011), to develop traps for more effective and representative collections of all haplogroups, and more importantly, the parasite populations they contain. Parasite detection from bug faeces, although representing infective populations, may not be the only population in the vector. The present study analyzed a broader representation of populations acquired by hosts from the midgut, which may not be comparable to parasites effectively typified from faecal material (Marcet et al., 2006).

The present study was conducted in parallel with analyzing social representations and practices in Zoh Laguna related to *T. cruzi* vector transmission in different habitats and according to the population's interactions in the landscape (appropriation and use), and their practices related to health/disease/healthcare seeking (Valdez-Tah et al., 2015a,b). As part of the original community engagement for collective "ejido" approval to conduct the present study, information was exchanged and dialogue established regarding Chagas disease, its vector, and parasite transmission. Based on self-determined risk for contact with bugs (having lived in a house with bugs at any time in life, having had contact with bugs at any place or time, and having had a chinchoma at any time in life), the population participated in a serological survey in which 3 (3.5%) out of 86 samples (8% of population) were positive with two serological tests. Parasites were typified as TcVI in single infections from two of these individuals, both men. Both samples from the inhabitants belonged to the principal TcVI haplotype. The human population in Zoh Laguna comes into contact with bugs in all habitats, according to their information, even though bug bloodmeals only detected 25% and 10% in domestic and ecotone bugs, respectively. Few bugs were collected in the sylvatic habitat, due to method insensitivity since aggressive bites are reported by men while they wait in arboreal hunting blinds for wildlife

Fig. 5. Bayesian Inference (BI) topology tree, from 226 nucleotides of the inter-genic region of the mini-exon gene of *T. cruzi* samples from all hosts in the Zoh Laguna landscape (orange, domestic; pink, ecotone; blue, sylvatic and black control sequences), inferred under the GTR + G model. Numbers on each branch (above branch) represent posterior probabilities obtained in the BI. The scale bar represents the expected number of nucleotide substitutions per site.

(deer, armadillos, wild pigs, wild turkeys) or sleep in timber camps. Hunting was reported to occur near brooks, where it is clear most mammals, and nesting bugs, would be found in the dry season. In the ecotone areas, men interact principally, also in the dry season, due to livestock care activities, charcoal production, preparing fields, and collection of natural materials (which are transported to the peridomestic area). Due to the fact that most information regarding bugs is extrapolated by the population from that received principally for mosquitos, men often sleep unprotected near their fields for several or more days in the dry season, which coincides with the 10% human bloodmeal in ecotone collected bugs. Men mostly refer to contact with triatomines in ecotone and sylvatic areas, rather than in domestic spaces, while women only refer to contact with bugs in domestic areas, and proportionally less than men. Bugs were never collected only with TcVI from any habitat, while approximately half from sylvatic and ecotone bugs had only TcIa, while the other half both DTUs. Less than 8% of domestic bugs had human bloodmeals and none with only TcVI. It would appear

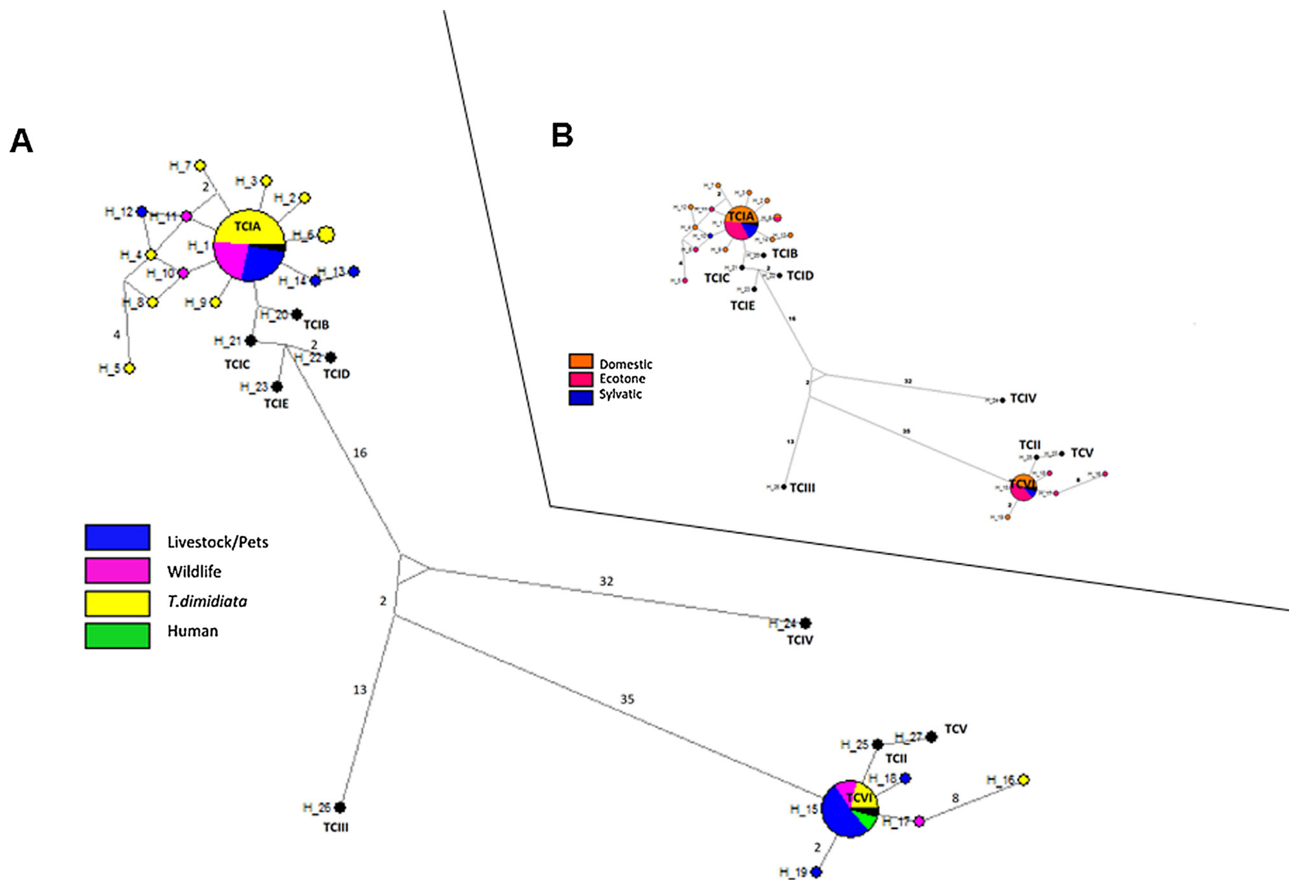

Fig. 6. Haplotype network of *T. cruzi* isolated from all wildlife, livestock, pets, and bugs in the Zoh Laguna landscape. Insert network is based on the habitat where each haplotype was isolated.

clear that information from bugs and inhabitants coincide and parasite DTU may suggest that men may have different exposure source than most women in Zoh Laguna, yet this remains to be analyzed with appropriate design and sample size.

Integrated knowledge of parasite and vector dynamics, host meta-communities, landscape structure, and sociocultural interactions not only allow us to analyze *T. cruzi* transmission risk to humans, but also to use that information for contact prevention. Oral *T. cruzi* transmission has never been reported in Mexico but there is a latent potential in the country and in the YP, given the population's traditional and current gastronomy (e.g., use of insects as food source), preparation of "pozol" or "sakíha" drink from maize and use of breadnut (Maya nut) in their diet (*Brosimum alicastrum*, common name "ramón"or "ojoche") which could be contaminated with bugs (Meiners et al., 2009; Cahuich-Campos et al., 2012; Chan-Quijano et al., 2013). The primary crop rodent pests of maize (*Sigmodon hispidus, Peromyscus yucatanicus*) were all highly infected in ecotone areas indicating presence of infected vectors which could be transported to domestic areas and contaminate prepared drinks. Bats and didelphids are also associated with the principal fruit or breadnut species in the landscape, even in the domestic habitat, which may allow this increasingly cultivated product to become contaminated with bugs. The integrated biological, ecological and social information base allows us to propose, even without a full picture, interventions to prevent or interrupt current practices which create vulnerability and risk for parasite transmission. Should there be a switch in public health programs to prioritize Chagas disease prevention in Mexico, interventions to prevent and control vector-human contact in Zoh Laguna need to specifically emphasize (1) the difference in biology and ecology of bugs from mosquitos emphasizing the importance of the dry season for contact in the whole landscape (including use of hammock-nets in the dry season in all habitats), (2) the risk particularly to men in non-domestic spaces related to all their activities (unprotected sleep day or night, hunting), (3) challenging beliefs that the more the bug bites, the greater one is vaccinated against ill-effects, particularly related to male perceptions of personal "strength", (4) livestock pens should be maintained far from houses, since they become infected with the parasite and the bugs use them as food source and can later transmit to a person; their meat should be properly cooked; (5) natural sylvatic or agricultural products from the ecotone may contain bugs or represent attraction for rodents, which are highly infected; they should be maintained away from houses, areas where these products are stocked should be continuously cleaned and controlled, and (6) the peridomestic spaces of the house are at risk for bugs year-round and these spaces should be continuously checked and maintained to prevent bug infestation. Methods to prevent or reduce interaction between humans and bugs, and hence Chagas disease risk are available and most effective when they target evidence-based transmission mechanisms and the interactions or practices that create vulnerability for transmission. At least in this landscape, risk will be continuous unless specific mechanisms are targeted to reduce human vulnerability and prevent parasite transmission based on modified practices.

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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.2015.07.021.

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