MEASURING THE RELATIONSHIP BETWEEN BIODIVERSITY, *TRYPANOSOMA CRUZI* PREVALENCE AND TRIATOMINAE BLOOD MEAL SOURCES IN SECONDARY FORESTS AND OIL PALM PLANTATIONS IN THE EASTERN LLANOS OF COLOMBIA

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Measuring the relationship between biodiversity, *Trypanosoma cruzi* prevalence and triatominae blood meal sources in secondary forests and oil palm plantations in the Eastern Llanos of Colombia

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Abstract

The 'dilution effect hypothesis' proposes the existence of an inverse relationship between biodiversity and the risk of disease transmission. In Chagas disease, the parasite (*Trypanosoma cruzi*) exclusively infects mammals, although birds are an important source of blood for vectors. The aim of this study was to determine if the composition of blood sources of *Rhodnius prolixus*, the main vector of Chagas disease in Colombia, and the diversity of birds are related to the prevalence of *T. cruzi* in the vectors in an oil palm plantation (PPP) and three fragments of secondary forest (FBS). Additionally, the prevalence of *T. cruzi* in small mammals in a PPA and a FBS was determined. Since PPA are less diverse than the BSF, we hypothesize that the prevalence of *T. cruzi* in the PPA would be higher than in the FBS. Overall, 371 nymphs of *R. prolixus* were captured. There were no differences between the number (H = 1.460, p = 0.834) or the proportion (H = 2.167, p = 0.705) of infected nymphs that feed on bird, mammal or both. Parasite prevalence in rodents was higher in PPP (0.31) than in FBS (0.07). Bird diversity was higher in FBS (S = 58, n = 321) than in PPA (S = 21, n = 138). Although the prevalence of *T. cruzi* in the nymphs was higher in the less diverse sites, there was no correlation between bird richness and prevalence of *T. cruzi* in the nymphs (r = -0.900, p = 0.09) or number of infected nymphs (r = -0.936, p = 0.06) nor between the abundance of birds and the prevalence of T. cruzi in the nymphs (r = -0.780, p = 0.220) or the

number of infected nymphs (r = -0.851, p = 0.149). The progressive increase in the PPA and low diversity of non-competent hosts in these agroecosystems suggest greater human exposure to Chagas disease. Further studies on variation in competence of rodents and opossums in the context of Chagas disease transmission-biodiversity interaction, are strongly suggested.

Keywords: Blood meal sources, Chagas disease, *Elaeis guineensis*, Land use changes, *Trypanosoma cruzi*

Resumen

La hipótesis del "efecto de dilución" propone la existencia de una relación inversa entre la biodiversidad y el riesgo de transmisión de enfermedades. En la enfermedad de Chagas, el parásito causante (*Trypanosoma cruzi*) infecta exclusivamente mamíferos, si bien las aves constituyen una importante fuente de alimentación de los vectores. En este estudio se determinó, en una plantación de palma africana (PPA) y en tres fragmentos de bosque secundario (FBS), si la composición de las fuentes de alimentación de *Rhodnius prolixus*, el principal vector de la enfermedad de Chagas en Colombia, y la diversidad de aves se relacionan con la prevalencia de *T. cruzi* en los vectores. Asimismo, se determinó la prevalencia de *T. cruzi* en pequeños mamíferos en una PPA y en un FBS. Dado que las PPA son menos diversas que los FBS, hipotetizamos que la prevalencia de *T. cruzi* sería mayor en la PPA que en los FBS. En total, 371 ninfas de *R. prolixus* fueron capturadas. No se hallaron diferencias entre el número (H=1,460, p=0,834) ni la proporción (H=2.167, p=0.705) de ninfas infectadas que se alimentaron de ave, mamífero o de ambos. La prevalencia del parásito en roedores fue mayor en la PPA (0,31) que en el FBS (0,07). La diversidad de aves fue mayor en los FBS (S=58, n=321) que en las PPA (S=21, n=138). Aunque la prevalencia de *T. cruzi* en las ninfas fue mayor en los sitios menos diversos, no hubo correlación entre la riqueza de aves y la prevalencia de *T. cruzi* en las ninfas (r=-0,900, p=0,09) o el número de ninfas infectadas (r=-0,936, p=0,06), ni entre la abundancia de las aves y la prevalencia de *T. cruzi* en las ninfas (r=-0,780, p=0,220) o el número de ninfas infectadas (r = -0,851, p = 0,149). El aumento progresivo de las PPA y la baja diversidad de hospederos no competentes en estos

agroecosistemas, sugieren una mayor exposición humana a la enfermedad de Chagas. Futuros estudios deben considerar la variación de la competencia de los hospederos.

Palabras clave: Cambios en el uso del suelo, Enfermedad de Chagas, *Elaeis guineensis*, Fuentes sanguíenas, *Trypanosoma cruzi*.

Introduction

Anthropogenic habitat degradation and land use transformation have generated species loss, changes in the dynamics of wild populations, diminished resilience of ecosystems, and functional changes in communities around the world [1, 2]. Since reduced disease transmission is considered as an ecosystem service provided by biodiversity [3], it has been stated that anthropogenic generated declines in biodiversity could increase both human and wildlife diseases [4, 5]. As host competence differs between species, the 'dilution effect hypothesis' proposes the existence of an inverse relationship between biodiversity and the risk of disease transmission [3, 6, 7]. Different experimental and descriptive studies in this context have documented lower rates of infection in ecosystems with high diversity, whereas hosts with low competence (those with low capacity to infect vectors) reduce the encounter probability between parasites and highly competent hosts [4, 5, 8−10]. In these cases, low-competence hosts and non-susceptible hosts (those incapable to infect vectors) [11] would "dilute" the risk of infection [7]. The different ability of hosts to transmit a parasite may be related to phenotypic plasticity among and within species, and with specific life history traits that mediate immune system functions, which in turn influence epidemiological dynamics in multihost-parasite systems [13, 14]. Thus, parasite transmission rates vary according to the spatial and temporal scale, the interacting species, and the stage of the parasite involved [15]. In fact, the existence of positive relationships between biological diversity and the infection risk or the prevalence of disease in some ecosystems makes evident the need to study the dynamic of diseases in different ecological contexts [16, 17].

Chagas disease (or American Trypanosomiasis) is one of the neglected tropical diseases (NTDs) in which the dilution effect is possible, due to its strong dependence upon a sylvatic transmission cycle. This zoonosis is caused by *Trypanosoma (Schizotrypanum) cruzi* [18] a flagellated protozoan vectored by hematophagous insects (Hemiptera: Triatominae) that feed mainly on blood [19−23]. While more than 150 species of mammals have been found to be reservoirs of *T. cruzi* [24], birds have proved to be refractory and thus, resistant to the infection [25, 26]. In endemic areas, the principal mode of transmission is vectorial, but the parasite also can be transmitted to humans by blood transfusions, organ transplants, congenital infections, and by oral transmission, which has been recently recorded as the deadliest form, responsible for several outbreaks in Latin America [27−30]. Currently, there are about six to seven million people worldwide infected with *T. cruzi*, mainly in Latin America, where this disease is endemic.

Several studies have determined differential levels of competence for *T. cruzi* between mammals, and an increase in the abundance of more frequently infected hosts in human-modified landscapes, an essential condition for a dilution effect to exist. Thus, some *T. cruzi* reservoirs, such as the nine-banded armadillo *(Dasypus novemcinctus)* and the white-eared opossum *(Didelphis albiventris),* are more infectious to vectors than other species of armadillos, small opossums and sigmodontine rodents [31]. Similarly, sigmodontine rodents have been found to be hosts of *T. cruzi* but have very low infectiousness to triatomine bugs, as stated by xenodiagnosis [32]. Although there are no experimental or descriptive studies including birds as part of the transmission cycle of *T. cruzi,* it has been suggested, in accordance with the dilution effect too, that in "natural conditions" the infection rate of *T. cruzi* should increase when "susceptible animals" (such as *Didelphis* spp.) are part of the cycle, and diminish when triatomine bugs feed on refractory animals, such as birds [33]. On the other hand, birds may increase populations of triatomine bugs in areas with more nests in palm trees [34]. Thus, diversity of birds and mammals and the species composition of mammal community may drive the course of the infection in a *T. cruzi* transmission scenario. In this context, Vaz and collaborators [35] found a positive relationship between the prevalence of *T. cruzi*, the abundance of opossums (*Didelphis aurita* and *Philander frenata),* and the seasonal variation of the host community in fragmented habitats in Brazil. Another related study

documented higher prevalence of *T. cruzi* in transformed and less diverse habitats [36]. Conversely, Oda and collaborators [37] did not find any correlation between small mammal diversity and the risk of *T. cruzi* transmission in semi-arid regions in Chile.

Oil palm plantations (*Elaeis guineensis* Jacq: Arecaceae) are one of the crops driving habitat transformation worldwide in the tropics, with an increasing cultivated land in several countries [38]. In Colombia, the first producer of oil palm in America, this agroindustry has dramatically changed the landscape in vast part of Orinoco basin [39]. The estimated planted area with this palm tree species in the country is 450 131 ha [40], and prediction models incorporating government biodiesel blending targets estimate a total cultivated land of 930 000 ha in year 2020 [41]. Since oil palm plantations can be colonized by the triatomine bug *Rhodnius prolixus* [42, 43] this agroindustry represents an increasing suitable habitat for the establishment of *T. cruzi* transmission cycle and a subject of concern from the public health perspective.

In this context, the aim of this study was to determine if the diversity of blood sources of *R. prolixus* is related to the prevalence of *T. cruzi* in mammals and triatomines in two different ecosystems (oil palm plantations and secondary forests) in the Eastern Llanos of Colombia. In addition, we made comparisons of the relative abundances of mammals and the diversity of birds (as potential blood sources for triatomines) between habitats and determined its relationship with *T. cruzi* infection and triatominae population densities in the studied ecosystems. As host competence (i.e. the ability of a host infected with the pathogen to make the pathogen available to a vector) differs between species, diverse ecosystems such as forests should harbor a less competent host community than monocrops. We hypothesized that oil palm plantations harbor less diversity of mammals and birds than forests do, so that *T. cruzi* prevalence in mammals and triatomine bugs will be higher in these agroecosystems than in forests.

Methods

Ethics statement

Ethical approval for sampling procedures to animals captured in the field was obtained from the ethical review board of the Universidad de los Andes (CICUAL– C.FUA 14-026).

Study Site

Fieldwork was performed in Tauramena (Casanare, Colombia), a municipality located in a highly endemic transmission area for Chagas disease [44] in the Orinoco region. Dry season occurs in the first and third trimester of the year, and rainy season between May and July, when 50.7% to 88.5% of overall annual precipitation is recorded [45]. Sampling was performed in three oil palm plantations and three secondary forest fragments with different sampling efforts. The main sampling area was located in La Candelaria (El Güira district) and was performed by a fieldwork team enrolled in a long-term project. This sampling area included a secondary forest fragment (<10 ha) and a contiguous oil palm plantation (c.a. 24 ha). Both habitats were sampled along five nights each, in May, July and December 2015, and March 2016. Mammal sampling was performed as described below, and prevalence of the parasite both in vectors and hosts was assessed. Additionally, the diversity of birds as potential blood source for triatomine bugs in both ecosystems was evaluated. A complementary small-scale sampling was conducted in two sites in Iquía district, to assess spatial variation in triatominae blood sources and parasite infection within the municipality. One sampling site included an oil palm plantation (6 years old) and one adjacent secondary forest (<10 ha) in May 2015; the other was an isolated secondary forest (<10 ha) and one oil palm plantation (12 years old) sampled in June 2015 (Table 1, Figure 1).

Table 1. Field sampling location

Habitat	Locality – District	Abbreviation	Coordinates
Large scale monitored sites (triatomines, mammals, and birds)			
Secondary forest 1	Candelaria – El Güira	BC	N 04°40'36.9" W 72°34'35.6"
Oil palm plantation 1	Candelaria – El Güira	PC	N 04º 40’ 26.9” W 72º 34’ 39.4”
Complementary sampling sites (triatomines and birds)			
Secondary forest 2	Remanso – Iquía	BR	N 04º 59’ 03.6” W 72º 38’ 08.6”
Oil palm plantation 2	Remanso – Iquía	PE	N 04º 59’ 03.8” W 72º 38’ 03.5”
Secondary forest 3	Potrillos – Iquía	BP	N 04°59'14.4" W 72°36'19.8"
Oil palm plantation 3	Remanso – Iquía	BR	N 04°59'01.7" W 72°37'56.1"

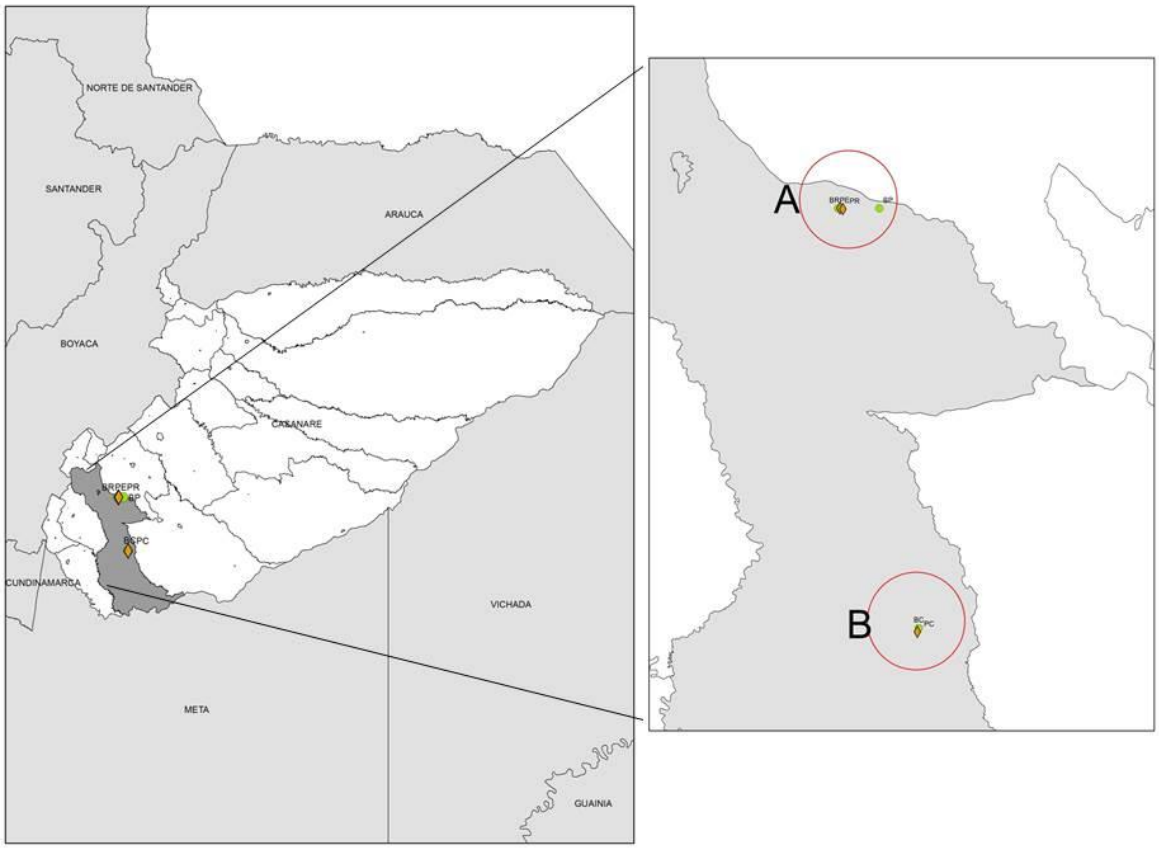


Figure 1. Map of the study sites showing (A) small scale sampling sites where birds and triatomine bugs where sampled once, and (B) the place where monitoring of infection took place in Casanare (Orinoco basin, Colombia).

Triatomine bug sampling

Sylvatic triatomine bugs were collected using traps (32 x 18 x 24 cm) baited with a hen [46]. Traps were placed at 17:00 h at the crown of the palm trees, and revised for triatomines the next day at 08:00 h. Collected triatomines were placed in ethanol 70% until laboratory procedures took place at Centro de Investigaciones en Microbiología y Parasitología Tropical (CIMPAT) (Universidad de los Andes, Bogotá D.C.).

Non-flying mammal sampling and blood collection

Non-flying mammals were sampled using 111 traps (87 Sherman, 24 Tomahawk) in one secondary forest (CF) and one adjacent oil palm plantation (CP) at La Candelaria during five consecutive nights in May, July and December 2015. We followed a web sampling design described by Parmenter *et al.* [47]. Sampling points were distributed in 12 concentric transects with nine traps each. In each transect, the first three traps were spaced by five meters, and the distance between the next six traps was 10 m. The 4^{th} and 9^{th} traps located in each transect were Tomahawk traps, and the rest were Sherman traps. Additionally, three Sherman traps were placed in the center of the web. All traps were baited using a mixture of hazelnut cream, oat, banana, and tuna fish, and were checked each morning at 8:00 h. Overall sampling effort was 2220 trap-nights/habitat.

Captured animals were transported to a field lab for blood collection. For each captured individual, intramuscular anesthetic Zoletil® was administered (0.05 ml/40 g). Once the animal was completely asleep, blood was collected by cardiac puncture using 0.5 ml sodium citrate in a 3-ml needle. All samples were placed in guanidine hydrochloride buffer (3:1 volume of blood collected), and stored at room temperature. Each captured individual was tagged in the ear, then standard measurements and photographs to further support species identification were taken. All individuals were assigned to order based on measurements (total length, tail length, feet length) and coloration of the fur.

Bat sampling and blood collection

Bats were captured using two mist nets set between 18:00 and 21:00 h during two nights in each habitat, totalizing a sampling effort of 24 mist-net hours per habitat. Blood samples of

bats were collected as described above for non-flying mammals. Individuals were assigned to morphospecies based on body measurements (total length, tail length, forearm length and tragus length), presence/absence (and length) of nose-leaf, the form of the uropatagium, and the position of the tail (enclosed or not). Identification to species was carried out by sequencing, as is described in molecular methods.

Bird sampling

Bird records were obtained through observation with binoculars 10X50 and recording of vocalizations with a digital recorder, in circular points (50 m in diameter) [48] sampled between 06:00 and 10:00 h spaced by 50-100 m and located at a minimum distance of 50 m from the edge. Identification of observed birds was made following the descriptions and illustrations provided by Hilty & Brown [49] and McNish [50]. Vocalizations were identified by contrasting records and spectrograms obtained in the field with those in the sound database Bird Sounds of the Americas (<http://www.xeno-canto.org/>). Additionally, three mist nets were placed in open spaces in forests and oil plantations to obtain blood from brachial vein of passerine birds to build a DNA reference collection following standardized procedures [51]. Mist nets and birds were manipulated following methods described by Ralph *et al.* [52]. Taxonomy and nomenclature of bird species follow Remsen *et al.* [53].

Parasite detection in triatomine bugs and mammals

DNA was extracted from blood of triatomine bug nymphs and mammals, using a DNA extraction kit (ZR Tissue & Insect DNA MiniPrep®) following the manufacturer's instructions with some modifications for triatomine DNA extraction. Preparation of triatomines for DNA extraction included previous cleaning with a mercuric-based solution for 10 minutes and then in PBS for two minutes. To achieve mechanical destruction of the exoskeleton of the insects, the procedure included **(a)** freezing, **(b)** lyophilization during 12 hours, and **(c)** vigorous agitation by vortex of each individual in a Disruptor Genie™ holder assembly for at least three minutes.

To assess the presence/absence of *T. cruzi*, both in triatomine bugs and mammals, the primers 121 (5′-AAATAATGTACGG(T/G)GAGATGCATGA-3′) and 122

(5′GGGTTCGATTGGGGTTGGTGT-3’), targeting the kinetoplast minicircle, were employed to obtain an amplicon of 330 bp [54]. The PCR cycling conditions included an initial denaturation at 94ºC for 5 minutes followed by 5 cycles of 94ºC for 1 minute, 68ºC for 1 minute, 72ºC for 1 minute, and 35 cycles of 94ºC for 45 seconds, 64ºC for 45 seconds and 72ºC for 45 seconds. PCR ended with a final extension of 72ºC for 10 minutes. PCR products were loaded into a 2% agarose gel. It was included, in each case, a positive control for *T. cruzi*, a negative control for DNA extraction, and a negative control for PCR.

Triatomine bugs identity

Species identification of triatomine bugs collected in palms was based both on morphological and molecular analysis. The morphological identification was based on keys, illustrations and descriptions provided by Lent & Wygodzinsky [20]. For molecular identification, a sample of DNA from two random selected bugs per palm was subject to PCR with the primers 7432(f) (5’-GGACG(AT)GG(AT)ATTTATTATGGATC-3’) and 7433(r) (5’-GC(AT)CCAATTCA(AG)GTTA(AG)TAA-3’) to amplify a 682 bp fragment of the *cytb* gene [55]. The PCR cycling conditions included an initial denaturation at 95ºC for 5 minutes, followed by 35 cycles of 95ºC for 30 seconds, 56ºC for 45 seconds, and 72ºC for 45 seconds, and a final extension at 72ºC for 5 minutes. PCR products were loaded into a 2% agarose gel. It was included, in each case, a positive control for *R. prolixus*, a negative control for DNA extraction, and a negative control for PCR. PCR products were sequenced using both forward and reverse primers at Sequencing Laboratory of Universidad de los Andes. The resulting sequences were edited in Sequencher® version 4-2.1.4 (Gene Codes Corporation), and analyzed by the NCBI BLAST.

Blood meal sources of triatomine bugs

Molecular identification of blood meals was carried on by nested PCR [56]. Avian DNA obtained in the blood meals of nymphs was analyzed initially using a conventional PCR with the Avian-3 and Avian-4 primers, followed by a nested PCR with the primers Avian-3 and Avian-8. For the detection of mammalian DNA, a conventional PCR was initially carried out using the primers Mammalian-1 and Mammalian-2, followed by a PCR with the primers Mammalian-7 and Mammalian-2 and the product of the first PCR as template [56]. Resulting

PCR products were run on a 2% agarose gel. In each case a negative control for DNA extraction was included, as well as a negative control for PCR, and positive controls (DNA from bird or mammal, and a mix of both). Positive controls for bird DNA were extracted from blood by puncture of the brachial vein *of Dendroplex picus, Pipra filicauda, Ramphocelus carbo, Cantorchilus leucotis and Tolmomyias flaviventris* captured in the field with mist nets. Positive controls for mammal DNA were obtained from blood of opossums and rodents captured in Sherman traps.

Molecular identification of mammals

To identify the particular species of flying mammals, it was carried out a conventional PCR using two primers to amplify the 16S ribosomal RNA region: VerU-1 (f) (5'-AAGACGAGAAGACCCYATGGA-3') and VerU-2 (r) (5'-CCTGATCCAACATMGAGGTCGTA-3') [56]. The PCR cycling conditions included an initial denaturation at 95^{o}C for 2 minutes, 35 cycles of 95^{o}C for 30 seconds, 55^{o}C for 30 seconds, and 72^{o}C for 1 minute, and finally an extension at 72^{o}C for 5 minutes. Positive and negative controls were included in each case. A 25 µl reaction volume was prepared with 12.5 µl of Gotaq Green Master Mix, 1 µl of each primer (10 µM), 8.5 ul of water and 2 ul of template DNA. PCR products were loaded into a 2% agarose in searching for an amplicon of 280 bp [56]. The PCR products were sequenced with both forward and reverse primers at Sequencing Laboratory of Universidad de los Andes. The resulting sequences were edited with Sequencher® version 4-2.1.4 (Gene Codes Corporation), and compared with those in GenBank (NCBI http://blast.ncbi.nlm.nih.gov). The performance of the described molecular-based assay was validated by isolating DNA from the blood of two previously identified mammals (*Desmodus rotundus),* subjecting the samples to PCR, sequencing, edition and BLAST analysis. This allowed us to establish the minimum level of confidence to assign the taxonomic identity to a DNA sample of vertebrate found in the blood meals of triatomine bugs. Consequently, the sequences with the lowest E values and of sequence identity ≥95% were identified as the more likely species. The occurrence and geographical distribution in Colombia of the identified species were verified with data from Alberico *et al.* [57] and Solari *et al.* [58]. Taxonomy and nomenclature of mammalian species are according to Solari *et al.* [58]. Barcoding assays are in course to stablish the taxonomic identity of the captured

individuals and to evaluate the relationship between mammal diversity and prevalence of infection in hosts and vectors.

Data analysis

Normality tests were performed and correlations between bird diversity, blood meal sources, and prevalence in triatomine bug nymphs were computed using R [59]. Kruskal-Wallis One Way Analysis of Variance on Ranks was employed for comparison of triatomine bug infection grouped by blood meal source. Species accumulation curves were constructed to estimate the diversity of birds and mammals in each site and habitat using the Chao 1 and 100 iterations without replacement [60]. To compare the species richness between sites and between forests and plantations, a rarefaction analysis was carried out based on individuals [61], using 100 iterations without replacement. The Shannon Index was also calculated using EstimateS [60], and Wilcoxon tests in R were used to compare diversity among study sites. Finally, Pearson correlations (previous Shapiro-Wilk normality tests) were performed to test the relationship between *T. cruzi* infection in triatomine bugs and bird species richness and abundance. All analyses were made excluding adults since this flying stage of triatomine bugs may lead to confounding results as dispersion ability between habitats may confound the results.

Results

Triatomine bug abundance and prevalence of *T. cruzi* infection

Overall, 371 *R. prolixus* nymphs were captured, 257 of them in three secondary forests and 114 in one oil palm plantation (Table 2). A sample of 315 nymphs was examined for infection. Overall prevalence of *T. cruzi* in triatomines at the municipality level was 0.60 but there were no statistical differences between the proportion of infected nymphs in secondary forests and oil palm plantation, as indicated by a one-way ANOVA ($F=0.0628$, $p = 0.808$). The proportion of infected bugs in all nymphal stages both in secondary forests and oil palm plantation was ≥ 0.40. The number of individuals in both ecosystems was concentrated in nymph two and three (Table 3).

Table 2. Total number of *R. prolixus* nymphs captured in the study sites in Casanare (Orinoco Basin, Colombia).

Stage	Secondary forests	Oil palm plantation	Total
N1	24	23	47
N2	73	34	107
N3	111	39	150
N4	40	12	52
N5	9	6	15
Total	257	114	371

Table 3. Number of examined *R. prolixus* nymphs grouped by habitat and prevalence of *T. cruzi* in the study sites in Casanare (Orinoco Basin, Colombia).

	Secondary forests				Oil palm plantation				Total	
Stage	n	negative	positive	prevalence	n	negative	positive	Prevalence	N	Prevalence
N1	19	7	12	0.63	15	9	6	0.40	34	0.53
N2	66	28	38	0.58	27	13	14	0.52	93	0.56
N3	94	36	58	0.62	34	12	22	0.65	128	0.63
N4	29	14	15	0.52	19	4	15	0.79	48	0.63
N5	6	3	3	0.50	6	0	6	1.00	12	0.75
Total	214	88	126	0.59	101	38	63	0.62	315	0.60

Non-flying mammal diversity and *T. cruzi* infection

Ninety individuals of two orders were captured: Didelphimorphia (24 mouse opossums and two common opossums - *Didelphis marsupialis*) and Rodentia (64 individuals). All didelphids were captured in the secondary forest fragment, where its relative abundance was higher than the relative abundance of rodents (Table 4). The molecular analysis revealed higher proportion of infected mammals in oil palm plantation (0.31) than in secondary forest (0.22). Sixteen out of 64 rodents examined were infected, and the prevalence of the parasite in this taxon was higher in oil palm plantation (0.31) than in the secondary forest (0.07). Six out of 24 mouse opossums were not infected, while the two *D. marsupialis* captured in the secondary forest were positive for *T. cruzi* (Table 4).

Table 4. Number of captures, relative abundance and *T. cruzi* prevalence in non-terrestrial mammals in the study sites in Casanare (Orinoco Basin, Colombia). Numbers in parenthesis represent infected individuals.

Taxon	secondary forest			Oil palm plantation			
	captures	relative abundance	*T. cruzi* prevalence	captures	relative abundance	*T. cruzi* prevalence	Overall Prevalence
DIDELPHIMORPHIA							
Didelphis marsupialis	2(2)	0.05	1.00				1.00
mouse opposum	24(6)	0.59	0.25				0.25
RODENTIA	15(1)	0.37	0.07	49(15)	1.00	0.31	0.07
Total	41(9)		0.22	49(15)		0.31	0.27

Bat diversity and *T. cruzi* infection.

With 26 captures (22 three of them analyzed for *T. cruzi* infection), we recorded nine species of bats of two families (Phyllostomidae and Vespertilionidae). Although number of captures was higher in forests, species richness did not differ between habitats. The bat species most abundant in secondary forest was *Carollia perspicillata* (*n*=8) while in oil palm plantation was cf. *Phyllostomus hastatus* (*n*=4). Parasite infection was detected only in two individuals of cf. *Phyllostomus hastatus* (Table 5).

Table 5. Number of captures, infected individuals (shown in parenthesis), and prevalence of *T. cruzi* in bats from La Candelaria study sites (Orinoco Basin, Casanare, Colombia).

FAMILY / Bat species	Secondary forest	Oil palm plantation	Total	Prevalence
PHYLLOSTOMIDAE				
Artibeus sp.	1		1	0.00
Carollia perspicillata	8	1	9	0.00
cf. Carollia perspicillata	1		1	0.00
cf. Phyllostomus hastatus		4 (2)	4 (2)	0.50
Desmodus rotundus		1	1	0.00
Phyllostomus hastatus		2	2	0.00
Platyrrhinus brachycephalus		1	1	0.00
Tonatia (Lophostoma) brasiliense	1		1	0.00
Sp 1.	1		1	0.00
VESPERTILIONIDAE				
Myotis riparius	2		2	0.00
Total	14	9 (2)	23 (2)	0.09

Bird diversity

Bird species richness was higher in secondary forests than in oil palm plantations (Figure 3, Appendix 1). As indicated by the upper limit of the 95% confidence interval (Chao2), estimated richness reached 116 species in forests and 28 species in oil palm plantations (Figure 4). Higher diversity was found in the secondary forest at La Candelaria, and the least diverse site was the oil palm plantation at the School (Figure 5).

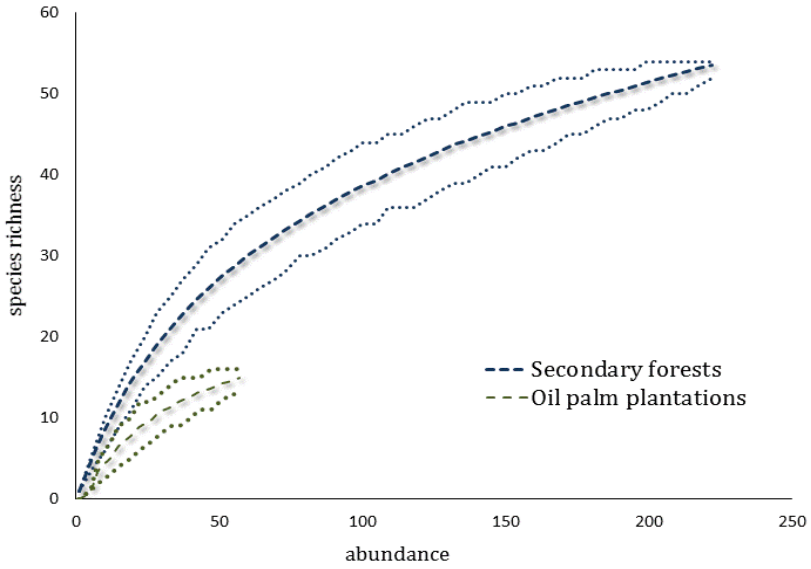
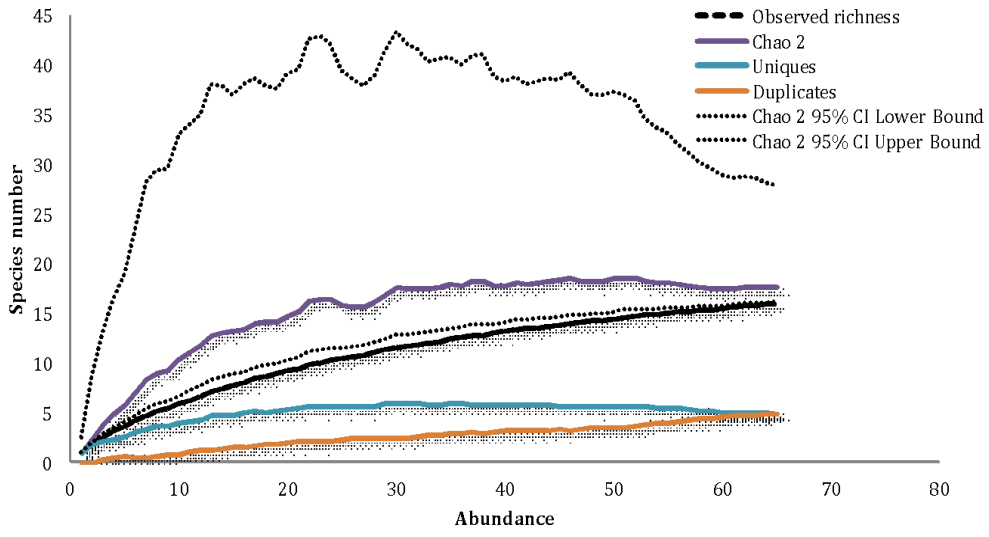


Figure 3. Comparison by rarefaction of bird species richness between oil palm plantations and secondary forests in Casanare (Orinoco basin, Colombia).

(a)



(b)

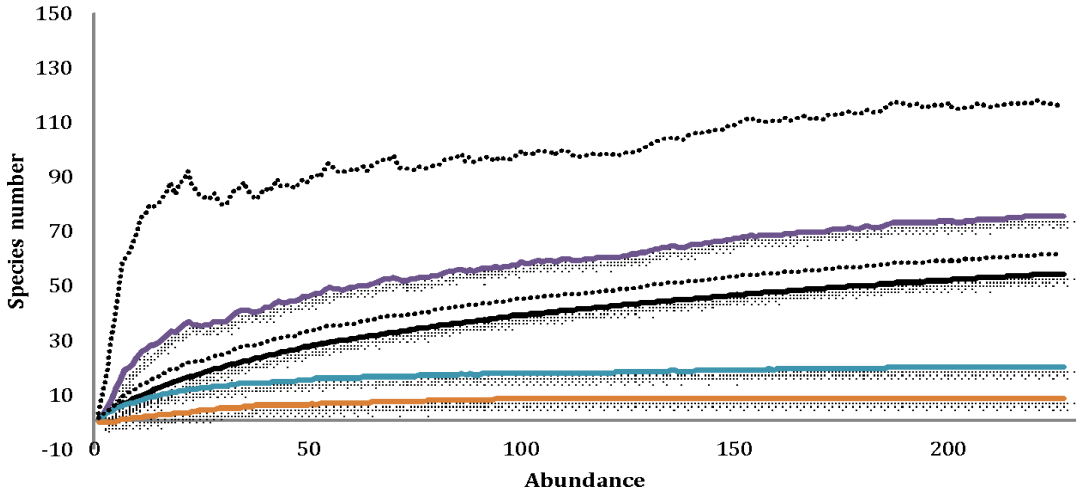


Figure 4. Estimated species richness of birds in (a) oil palm plantations and (b) secondary forests in Casanare (Orinoco basin, Colombia).

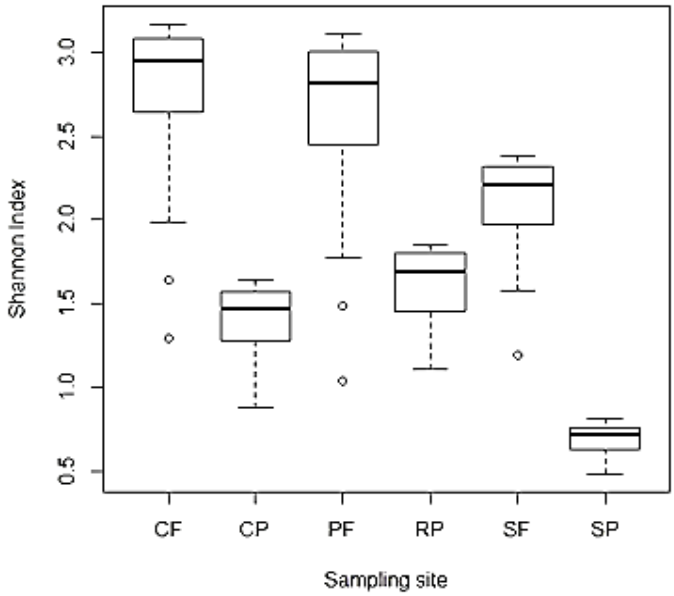


Figure 5. Mean diversity of birds in sampling sites: Candelaria Forest (CF), School Forest (SF), Potrillos Forest (PF), Candelaria oil palm plantation (CP), School oil palm plantation (SP) and Remanso oil palm plantation (RP).

Blood meal sources of triatomine bugs

Thirty-two insects were found engorged inside the hen trap, which impeded the analysis of blood meals. Thirty-one nymphs fed on bird blood, 67 on mammalian blood, and 8 ingested blood from both. Forty-seven infected insects fed on mammalian blood, 22 infected insects fed on birds, and five infected insects fed on both mammal and bird blood. Mammalian DNA was found in 17 non-infected individuals, bird DNA in 7, and mixed blood of both vertebrates in three insects. There were no differences between the number (Kruskal-Wallis One Way Analysis of Variance on Ranks: $H = 1.460$ $df = 4$, $p = 0.834$) nor proportion Kruskal-Wallis One Way Analysis of Variance on Ranks $H = 2.167$ $df = 4$, $p = 0.705$) of infected triatomine bugs that fed on mammals, birds, or both.

There were no correlations between bird species richness and prevalence of infection in *R. prolixus* nymphs ($r = -0.900$, $p = 0.09$) or number of infected nymphs ($r = -0.936$, $p = 0.06$) nor between bird relative abundance and prevalence of infection in nymphs ($r = -0.780$, $p = 0.220$) or number of infected nymphs ($r = -0.851$, $p = 0.149$). No correlation was found between estimated species richness and prevalence of infection ($r = -0.570$, $p = 0.430$) or number of infected nymphs ($r = -0.251$, $p = 0.749$). Nonetheless, the prevalence of *T. cruzi* in nymphs was higher at School forest and Candelaria oil palm plantation, the less diverse habitats where triatomine bugs were captured and analyzed.

Discussion

The infection prevalence and number of triatomine bugs captured in our study sites were similar to that found by Rendón *et al.* [62] in the Casanare region, where *T. cruzi* natural infection in 269 *R. prolixus* reached 60.2%. The number of captured mammals in the forest was higher to that found by Vaz *et al.* [35] in forest fragments < 10 ha in size, where after a sampling effort of 1920 traps-night, 45 individuals of small rodents and opossums were captured. The prevalence of infection in mammals was higher in our study sites compared with 11.9% reported in the same region [62]. Nonetheless, it is important to note the low number of infected opossums that were captured in our study sites. Particular taxa of small opossums (*Marmosa, Marmosops, Monodelphis*) have been consistently found less infected

than marsupials of the genus *Didelphis*, rodents and bats [35, 63]. Moreover, the absence of infection in *Marmosa*, *Marmosops* and *Monodelphis* has been previously reported [35].

The low diversity of birds found in oil palm plantations in this study agrees with studies concerning biological diversity in this agroecosystems around the world [64−68], and confirm the limited conservation value of this agroecosystems in the context of biodiversity conservation in the Eastern Llanos landscapes of Colombia. While nearly asymptotic species accumulation curves were achieved in oil palm plantations (suggesting that bird diversity in this agroecosystem is not significantly higher than observed), the non-asymptotic shape of accumulation curve in the secondary forests indicates that bird diversity in this ecosystem is still higher than observed. Rarefaction analyses confirmed the observed differences in bird species richness between both ecosystems. As expected, secondary forests were more diverse than oil palm plantations, and the prevalence of the parasite in mammals was lower in the secondary forest compared to the oil palm plantation.

It is possible that higher diversity of blood sources may contribute to increase *R. prolixus* colonies and populations, because it has been proved that those palms with nests of birds and mammals are inhabited by more triatomine bugs than palms without nests or with only nests of birds or mammals [69]. Nonetheless, triatomines inhabiting bird nests are less prone to acquire the parasite. For example, Gamboa [19] found that none of the 759 *R. prolixus* collected in nests of *Mycteria americana* (Ciconiiformes: Ciconiidae) was infected with *T. cruzi* and Gurgel-Gonçalves *et al.* [70] suggested that the high frequency of birds in *M. flexuosa* palms could be related with the low infection rate of triatomine bugs (8%). Another study found that despite the fact that nests found in 37 *Mauritia flexuosa* palms were infested by *R. neglectus* (85%) and *Psammolestes tertius* (53%), none of the 177 captured triatomines there were infected with *T. cruzi* nor with *T. rangeli* [71]. Similarly, although relative abundance of *R. neglectus* and *P. tertius* was higher in areas with more nests in palm trees, none of 96 collected triatomine bugs in them was infected with *T. cruzi* or *T. rangeli* [34].

Vector feeding preferences may be a key component of transmission dynamics [72]. In the case of Chagas disease, the dilution effect can be drived by the feeding patterns of *R. prolixus*.

Birds are an incidental blood meal source in this triatomine bug, and consequently, meal bloods on birds may have not exerted a determinant buffer effect in the parasite prevalence in this triatomine bug. Although it has been proved in short term experiments that the presence of chickens can decrease the prevalence of *T. cruzi* in triatomine bugs, the high rate of triatomine host change would result in higher prevalence infection rates at longer exposure experiments [11]. Moreover, the eventual preference of *R. prolixus* for blood of competent hosts (such as rodents) over blood of non-competent hosts (such as birds or mouse opossums) could be a key element in the maintenance and amplification of the disease in the studied ecosystems. Thus, although certain mammals in the study sites were less infected than others, it is necessary to determine whether they are competent hosts of *T. cruzi*. Nonetheless, it is probable that mammals such as mouse opossums, may limit the amplification of the disease in secondary forests, while its absence in oil palm plantations may facilitate the maintenance of the parasite in these ecosystems. Moreover, the low bird species richness and relative abundance in oil palm plantations would contribute to *R. prolixus* feeding more frequently on blood of mammals, thus enhancing the probability of infection. Mixed blood meals both from refractory animals and hosts of parasites are found in *R. prolixus* [23] and these are not mutually exclusive, and may cause that a triatomine bug that has frequently feed on blood from birds has previously infected or ingest the parasite after feeding on infected hosts. Thus, the time elapsed since the initial colony establishment, the blood feeding sources availability for triatomine bugs, and the eventual preference for mammalian blood would determine the course of infection maintenance in oil palms and forest fragments at our study sites.

Overall, the results corroborate the limited value that oil palm plantations may have for biodiversity conservation**,** particularly when oil palm plantations replace native forests [73−77]. In fact, between 16, 1% and 17, 5% of oil palm plantations in Colombia has been established in areas previously occupied by natural ecosystems [41, 78]. Moreover, the colonization of oil palm plantations by *R. prolixus*, as well as the progressive increase in the cultivated land with oil palms in the Eastern Llanos of Colombia, and the low diversity and abundance of non-competent hosts in this agroecosystem suggests higher human exposure to Chagas disease in this geographical region. Low abundance of food resources and starvation may produce flight initiation in triatomines [79] and consequent migration to adjacent human

dwellings, as observed at the start of the rainy season in El Güira in March, when 46 adult *R. prolixus* were found in two houses surrounded by oil palm plantations.

Although mammals and birds are the main blood source of *R. prolixus* [21−23], amphibians and reptiles may play a role in the transmission cycle of *T. cruzi*, since its blood is eventually consumed by triatomine bugs [23, 80, 81]. Thus, further studies concerning eco-epidemiological assessments must consider the role of non-competent hosts as feeding sources of *R. prolixus* and the reservoir competence of rodents and mouse opossums in the context of Chagas disease-biodiversity interaction.

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Author Contributions

Conceived and designed the study: CG, JAMB JMC, DE, PS. Performed the field work: JAMB, DE. Analyzed the data: JAMB, CG. Contributed reagents/materials/analysis tools: CG, JMC. Wrote the paper: JAMB, CG, JMC, PS, DE.

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Appendix 1. Bird species richness and abundance in the study sites in Casanare (Orinoco Basin, Colombia).

Scientific name	Secondary forests	Oil palm plantations	Total
TINAMIFORMES			
Tinamidae			
Crypturellus cinereus	5		5
ANSERIFORMES			
Anatidae			
Dendrocygna autumnalis		2	2
GALLIFORMES			
Cracidae			
Ortalis ruficauda	21		21
PELECANIFORMES			
Ardeidae			
Ardea alba		1	1
Bubulcus ibis		39	39
Threskiornithidae			
Theristicus caudatus	2		2
CATHARTIFORMES			
Cathartidae			
Cathartes aura	1		1
Coragyps atratus	2		2
ACCIPITRIFORMES			
Accipitridae			
Rupornis magnirostris	3	1	4
GRUIFORMES			
Rallidae			
Aramides cajaneus	5	2	7
EURYPYGIFORMES			
Eurypygidae			
Eurypyga helias		2	2
COLUMBIFORMES			
Columbidae			
Patagioenas cayennensis	11		11
Leptotila sp.	10	6	16
OPISTHOCOMIFORMES			
Opisthocomidae			
Opisthocomus hoazin	4		4

Appendix 1. Continued.

Scientific name	Secondary forests	Oil palm plantations	Total
CUCULIFORMES			
Cuculidae			
Crotophaga major	2	8	10
Crotophaga ani	6	1	7
Tapera naevia	2	1	3
APODIFORMES			
Trochilidae			
Phaethornis antophilus	5		5
Glaucis hirsutus	1		1
Amazilia versicolor	1		1
TROGONIFORMES			
Trogonidae			
Trogon viridis	3		3
CORACIIFORMES			
Momotidae			
Momotus momota	14		14
PICIFORMES			
Ramphastidae			
Pteroglossus castanotis	3		3
Picidae			
Melanerpes rubricapillus	1		1
Dryocopus lineatus	1		1
Picumnus squamulatus	3		3
FALCONIFORMES			
Falconidae			
Caracara cheriway		5	5
Milvago chimachima	15	33	48
PSITTACIFORMES			
Psittacidae			
Amazona amazonica	1	4	5
Forpus conspicillatus	2		2
Eupsittula pertinax		2	2
Orthopsittaca manilata	10		10
PASSERIFORMES			
Thamnophilidae			
Sakesphorus canadensis	9		9
Thamnophilus nigrocinereus	4		4

A**ppendix 1.** Continued.

Scientific name	Secondary forests	Oil palm plantations	Total
Furnariidae			
Dendrocincla fuliginosa	1		1
Dendroplex picus	1		1
Xiphorhynchus picus	2		2
Tyrannidae			
Elaenia flavogaster	2		2
Atalotriccus pilaris	1		1
Tolmomyias flaviventris	1		1
Poecilotriccus sylvia	1		1
Myiozetetes cayanensis	4		4
Pitangus sulphuratus	11	5	16
Megarhynchus pitangua	1		1
Tyrannus melancholicus	7	1	8
Tyrannus tyrannus	5		5
Myiarchus sp.	1		1
Pipridae			
Pipra filicauda	22		22
Tityridae			
Pachyramphus polychopterus	1		1
Vireonidae			
Cyclarhis gujanensis	3		3
Vireo olivaceus	1		1
Corvidae			
Cyanocorax violaceus	12	4	16
Troglodytidae			
Cantorchilus leucotis	27		27
Troglodytes aedon	2		2
Turdidae			
Turdus nudigenis	7	1	8
Turdus ignobilis	1		1
Thraupidae			
Ramphocelus carbo	9		9
Thraupis episcopus	9		9
Thraupis palmarum	3	2	5
Tangara cayana	3		3
Saltator coerulescens	2		2
Coereba flaveola	11		11

Appendix 1. Continued.

Scientific name	Secondary forests	Oil palm plantations	Total
Icteridae			
Psarocolius decumanus		11	11
Cacicus cela	19	7	26
Quiscalus lugubris	4		4
Species richness	58	21	65
Relative abundance	321	138	459