Vector/Pathogen/Host Interaction, Transmission

OXFORD

Trypanosoma cruzi Infection in *Rhodnius pallescens* (Heteroptera: Reduviidae) Infesting Coyol Palms in the Dry Arch of Panamá

Indra G. Rodríguez,[1,]* Azael Saldaña,[2,3,]* Kadir González,[2] Vanessa Pineda,[2] Milixa Perea,[2] Ana M. Santamaría,[2] Carmen C. de Junca,[3] Luis F. Chaves,[4,]* and José E. Calzada[2,5]

[1]Programa Centroamericano de Maestría en Entomología, Universidad de Panamá, Ciudad de Panamá, República de Panamá, [2]Departamento de Investigación en Parasitología, Instituto Gorgas de Estudios de la Salud (ICGES), Ciudad de Panamá, República de Panamá, [3]Centro de Investigación y Diagnóstico de Enfermedades Parasitarias (CIDEP), Facultad de Medicina, Universidad de Panamá, Panamá, [4]Programa de Investigación en Enfermedades Tropicales (PIET), Escuela de Medicina Veterinaria, Universidad Nacional, Heredia, Costa Rica, and [5]Corresponding author, e-mail: jcalzada@gorgas.gob.pa.

*Contributed equally to the study.

Subject Editor: Ricardo Gürtler

Received 3 September 2017; Editorial decision 5 December 2017

Abstract

Ecoepidemiological scenarios for *Trypanosoma cruzi* Chagas transmission are partially shaped by kissing bug vector ecology. The presence of *Attalea butyracea* Kunth, the 'royal palm', is a major risk factor for Chagas disease transmission in Panamá given their frequent infestations by *Rhodnius pallescens* Barber, a major neotropical *T. cruzi* vector. It was assumed that in Panamá this relationship was very close and unique, limiting the niche of *R. pallescens* to that of *Att. butyracea*. However, here we present observations about *T. cruzi*-infected *R. pallescens* infesting coyol palms, *Acrocomia aculeata* Jacquin, in Pedasí district, Los Santos Province, Panamá. Between May 2015 and August 2016, we sampled kissing bugs from 83 coyol palms using mice-baited traps placed at the crown of each palm during the dry and wet season. We collected 62 *R. pallescens* and one *Eratyrus cuspidatus* Stål kissing bugs. Using logistic regression, we found that the probability of kissing bug infestation in coyol palms increased during the rainy season, with infructescence number and palm height. We examined adult *R. pallescens* bugs ($n = 30$) and found *T. cruzi* in 67% of the samples. We were able to isolate and characterize *T. cruzi* from parasites present in the feces from *R. pallescens*, all parasites belonging to the TC I lineage. We found that green fronds number and house proximity increased *T. cruzi* infection probability in kissing bugs collected in coyol palms. These results highlight coyol palms as a potential risk factor for Chagas disease transmission in the dry arch of Panamá.

Key words: palms, mice baited trap, perfect separation, Chagas disease, *Trypanosoma rangeli*

Recognition of *Rhodnius pallescens* importance as a dominant *Trypanosoma cruzi* vector in Panamá started with the observations by Dunn (1933) working at the Gorgas Memorial Research Lab. This early report, made soon after the first report of Chagas disease in Panamá (Miller 1931), documented the invasion of rural huts with thatched roofs near Ciudad de Panamá by *R. pallescens*. Then, observations followed about the preponderance of *T. cruzi*-infected *R. pallescens* in rural homes all over the Republic of Panamá (Pipkin 1968, Sousa and Johnson 1973). Nevertheless, it took nearly 50 years to make the connection between *Attalea butyracea* royal palms, *R. pallescens*, and the risk of *T. cruzi* transmission, following the nationwide kissing bug survey in palms by Whitlaw and Chaniotis (1978), which showed *R. pallescens* had an ecology similar

to that previously described for *Rhodnius prolixus* Stål in Venezuela (Gómez Núñez 1963, Gómez-Núñez 1969), where both kissing bug species have as natural habitats the royal palm, where they blood-feed on the many mammal and bird wildlife species nesting or visiting, palms' crowns, and which include major *T. cruzi* reservoirs like the common opossum, *Didelphis marsupialis* L. (Tonn et al. 1976, Christensen and de Vasquez 1981, Rabinovich et al. 2011).

Recent epidemiological and ecological studies have furtherly highlighted the importance of *R. pallescens* as the main vector of *T. cruzi* in Panamá, as well as its close association with royal palms (Gottdenker et al. 2011, 2012, 2016), whose presence near domiciles has been recognized as a major risk factor for the transmission of Chagas disease in humans (Vasquez et al. 2004;

Saldaña et al. 2005; Calzada et al. 2006, 2010; Hurtado et al. 2014) and domestic animals, e.g., dogs (Saldaña et al. 2015) in the Republic of Panamá. In fact, it has been suggested that royal palms are the main component of the habitat templet defining the ecological niche of *R. pallescens* (Arboleda et al. 2009) and that, in general, palms are a major risk factor for the infestation of rural houses by kissing bugs and Chagas disease transmission in the neotropics (Romaña et al. 1999; Abad-Franch et al. 2000, 2005, 2010, 2015; Feliciangeli et al. 2007; Grijalva et al. 2017; Poveda et al. 2017).

In Panamá, most epidemiological studies on Chagas disease have been carried out in the area bordering the Canal de Panamá (Calzada et al. 2010) or in areas where royal palms are the dominant native palm species in the landscape (Whitlaw and Chaniotis 1978, Hurtado et al. 2014), and the same has been the case for ecological studies of *R. pallescens* in Panamá (Gottdenker et al. 2011, 2016) and Colombia (Pizarro and Romaña 1998), which have been focused on *Att. butyracea*. This might have biased the understanding of *R. pallescens* ecology by grounding the assumption that, at least in Panamá, *R. pallescens* is uniquely and specifically associated with royal palms.

However, few cases of Chagas disease have been registered by the Panamá's Ministry of Health in areas where *Att. butyracea* is absent or infrequent. This is the case of the area known as the 'dry arch', a strip of land with a long dry season located in the midwest coast of the isthmus. In this region, the ecological dominance of *Att. butyracea* has been replaced by *Acrocomia aculeata* (coyol palm), a palm with a physiognomy similar in many aspects to that of the royal palm. Here, we present results from a study that asked if coyol palms, locally known in Panamá as corozo palms, are natural habitats for *R. pallescens*. We also studied potential risk factors associated with the infestation, colonization, and *T. cruzi* infection of *R. pallescens* found in *Acr. aculeata* palms at Pedasí district, Los Santos province, Panamá, during the dry and rainy seasons of 2015 and 2016.

Materials and Methods

Study Site

Our study was done in Pedasí district, Los Santos Province, República de Panamá (Fig. 1). Pedasi district has a surface of 378.3 km² and a population of 4,481 inhabitants according to the 2010 census. Before the Spanish colonial period, this area was a tropical dry forest, which subsequently was deforested for cattle ranching and agriculture. Pedasí is within the 'dry arch' of Panamá, a dry area, with relative low rain when compared with the rest of the Isthmus (Autoridad Nacional del Ambiente 2010). According to the Kôppen climatic classification, Pedasí has a Tropical Savanna climate, with a rainy season from mid-May until the end of November and a dry season the rest of the year (Autoridad Nacional del Ambiente 2010). Annual average temperature oscillates between 25 and 27°C, and there is an annual average precipitation of 1,354.1 mm (Autoridad Nacional del Ambiente 2010). Currently, the main economic activity in the area is tourism, mainly for recreational use of the beaches but also for marine wildlife sighting, mainly of turtles and whales.

Palm Sampling

Palm sampling sites (Fig. 2) consisted of four main core areas and a few solitary palms interspersed in the study area (Fig. 2A). We sampled palms in peridomiciliary environments, i.e., within 100 m from the nearest houses (Fig. 2B) and palms embedded in grassland associated with cattle ranching (Fig. 2C). In two sites, we mapped all palms within defined polygonal areas. One for peridomiciliary palms (Fig. 2D) and one for grassland palms (Fig. 2E). In these core areas, we did not observe any royal palm. Each palm was georeferenced using a Garmin GPS 60CSx. For each palm, we counted the number of fronds, keeping track of how many fronds were green and dead/ dry, as well as, the number of infructescences. We also recorded the presence of birds, birds' nests, wildlife mammals, such as opossums and epiphytic vegetation for each palm. Additionally, we recorded if in the surrounding environment of each palm (up to 100 m), there were households and domestic animals, more specifically dogs, horses, and cattle.

Kissing Bug Collection

From May 2015 to August 2016, we collected kissing bugs four times. We sampled the bugs twice during the dry season (May 2015 and April 2016) and twice during the rainy season (July 2015, August 2016). In each palm, we put two mice baited box traps (Abad-Franch et al. 2000, 2005) and added approximately three pellets of mice
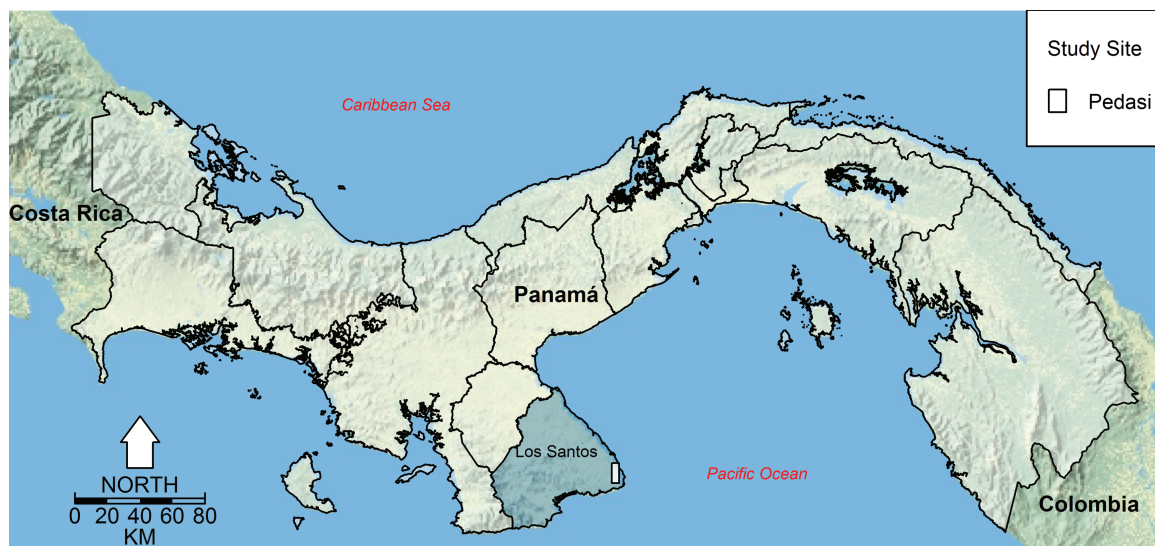

Fig. 1. Study site. This map shows the location of Panamá, between Costa Rica and Colombia. As shown in the map, the study area in Pedasí was located in Los Santos Province in the Pacific Basin of Panamá.

food (~5 g) for feeding during the sampling (Fig. 3A). Traps were set using a 6 m stair and a hanger (Fig. 3B). Traps were put inside each palm crown, in opposite sides of the crown from 18:00 to 06:00 for three consecutive nights. Traps were simultaneously deployed in each core area, and the sampling effort was split between the dry ($n = 54, 65\%$) and rainy ($n = 29, 35\%$) seasons. Collected bugs were placed in identified 100-ml plastic containers (Sarstedt, Numbrecht, Germany), with filter paper strips. Kissing bugs were identified using the taxonomic key by Lent and Wygodzinsky (1979). Additionally, each kissing bug was identified by stage and sex (for adults). The information on kissing bug abundance, by stage, was then summarized by palm.

Fig. 2. Study area and core sites. (A) The study area highlighting the four core areas where palms were sampled. Core areas, identified by numbers next or inside the highlighted polygons corresponding to each core area are magnified in Panels B to E. Each point corresponds to a palm. Please refer to the inset legends for identification of infested and uninfested palms and for color coding used to identify infested palms with infected bugs. (B) Magnification of core area 1, (C) magnification of core area 2, (D) magnification of core area 3, a peridomiciliary area where all palms were mapped, and (E) magnification of core area 4, a grassland area where all palms were mapped.

Fig. 3. Mice baited trap setting. (A) Mouse baited trap with an adult *Rhodnius pallescens*, (B) setting the mouse baited trap in the palm crown with a hanger.

Kissing Bug Infection With *Trypanosoma* spp.

All adults and fifth- and fourth-stage nymphs were examined for *T. cruzi* and *Trypanosoma rangeli* infection. We did not test first-, second-, and third-stage nymphs due to their low abundance and the technical difficulty to extract feces for microscopic examination. We started by extracting kissing bug feces by pushing a capillary tube toward the bug rectum. The extract was diluted in 100 µl of 1× saline solution, and a sample (10 µl) of this dilution was directly examined by microscopy for the presence of trypanosomes at a total 400× magnification. A subsample of randomly selected kissing bugs (*n*=10, five positive and five negative by microscopic examination) was then furtherly processed using the protocol by Calzada et al. (2006) where each collected kissing bug is completely triturated using sterile scissors, in 500 µl of 0.01 M phosphate-buffered saline solution (PBS, pH 7.2). Samples were then centrifuged at 15,000 × *g* for 10 m. Next, pellets were resuspended in 510-µl PBS and once more centrifuged at 400 × *g* for 5 m. The supernatant was collected and a final centrifugation at 15,000 × *g* for 20 m was done. DNA was then extracted from the pellets using a commercial kit (Promega, Madison, WI). Finally, we employed the multiplex PCR by Chiurillo et al. (2003) for specific and simultaneous detection of *T. cruzi* and *T. rangeli*.

Isolation and Strain Characterization of *T. cruzi*

To isolate and characterize circulating *T. cruzi* in the study area, we selected bugs with a high-parasitic load, as determined by the microscopic analysis of bug fecal samples. From the selected bugs, we employed 0.1 ml of PBS diluted fecal samples and intraperitoneally inoculated white mice. The parasitemia of these mice was followed daily by taking blood samples that were microscopically examined, until a peak was reached. At this time, mice were sacrificed and their blood was cultivated in biphasic Senekjie's medium with physiological saline as overlay. These media were weekly monitored for 2 mo. Isolated parasites from this medium were then genotypified using the multiplex-PCR assay based on the amplification of the nontranscribed spacer of the mini-exon gene described by Fernandes et al. (2001).

Ethical Statement

All procedures were made in accordance with research protocols of the ICGES. The use of mice-baited traps and the use of mice for isolation and strain characterization of *T. cruzi* was evaluated and approved by ICGES Institutional Animal Care and Use Committee (CIUCAL-ICGES, 2015-01).

Statistical Analysis

Summary Statistics

Data collected from the palms in grasslands and peridomiciles were first analyzed using descriptive statistics. For normally distributed variables, we estimated the mean and SD by land use type (grassland or peridomicile). For binary and count variables we show, respectively, the median and frequency/proportion, and their variability is shown through the interquartile range (Zar 1998). We also estimated several entomologic indices useful for a preliminary Chagas disease transmission risk assessment (World Health Organization 2003), which included the number of infested palms, infestation index (proportion of palms with kissing bug at any stage), kissing bug density (average number of kissing bugs per palm), kissing bug abundance (average number of kissing bugs per infested palm), and the colonization index (proportion of infested palms with nymphs).

Palm Spatial Patterns

To detect any potential differences between palm spatial arrangement under peridomiciliary (Fig. 2D) and grassland (Fig. 2E) land use, we analyzed the palm location point patterns in the two core areas where all palms were sampled. For the analysis, we employed the *L* function, a distance method used to test whether a point pattern is random, as inferred from the observed pairwise distances between points in a defined polygonal area (Diggle 2013). The *L* function, for a given distance *r*, is a transformation of Ripley's *K* function (Baddeley and Turner 2006):

$$L(r) = \sqrt{\frac{K(r)}{\pi}} \qquad (1)$$

where *K* is expected, under a homogeneous Poisson process, to be:

$$K_{\text{theo}}(r) = \pi r^2 \qquad (2)$$

Thus making *L,* under a homogeneous Poisson process, i.e., by substituting (2) in (1), a straight line:

$$L_{\text{theo}}(r) = r \qquad (3)$$

For inference, an estimated $\hat{L}$ function above the straight line defined in equation (3) suggests a clustering pattern, and one below the straight line a regular pattern, the null hypothesis being that points are distributed randomly over the studied area (Baddeley 2010). We also chose the *L* function, over Ripley's *K*, because of the variance stabilization in $\hat{L}$ that emerges from the square root transformation presented in equation (1) (Baddeley 2010). We estimated $\hat{L}$ employing the following formula:

$$\hat{L} = \sqrt{\frac{1}{\hat{\lambda}^2 \text{Area}(W)\pi} \sum_i \sum_{j \neq i} \left\{ x_i - x_j \leq r \right\} e\left(x_i, x_j; r\right)} \qquad (4)$$

Where W is the polygon area under study, $\hat{\lambda}$ the point intensity in the polygon, and the function e() an edge correction weight (Baddeley 2010). We employed the package spatstat in the statistical software R to estimate the $\hat{L}$ function (Baddeley and Turner 2005). For the estimation, we used the 'border' edge correction (Baddeley and Turner 2005). To make inferences about $\hat{L}$, we estimated a global envelope, a.k.a., simultaneous critical bands, using the maximum deviation from the expected Poisson *L* function. The maximum deviation is estimated through an *m* number of simulations of a homogenous point process with parameter $\hat{\lambda}$ (Baddeley 2010). For the analysis, we set *m* = 39, to find bands where the probability of an estimated $\hat{L}$ falling outside the bands is exactly 5% (2.5% for being above or below) under the null hypothesis that points, meaning in this case palms, are randomly distributed.

Palm Infestation by Kissing Bugs

To analyze the importance of different environmental factors on kissing bug palm infestation, we employed logistic regressions, considering infestation, defined as the simple presence of any stage kissing bugs, as a success (Venables and Ripley 2002). We performed three infestation analyses using logistic regressions. First, we considered the infestation by kissing bugs in any stage, i.e., including nymphs and adults. We then performed a similar analysis exclusively for nymphs (a.k.a., colonization) and then one for adults only. For the abundance fourth and fifth instar nymphs, as well as, adults, we fitted Poisson generalized linear models. All models included as covariates: the sampling season (dry or rainy), land use

(peridomicile or grassland), palm height, number of dead/dry fronds, number of green fronds, number of infructescences, epiphyte plant presence, bird presence (yes or no), and mammal presence (including the following categories: no mammals, opossums, dogs, horses, or cattle).

Kissing Bug Infection With Trypanosoma spp

We used Poisson rate generalized linear models (Faraway 2006) to study *T. cruzi* and *T. rangeli* infection rates in the subset of kissing bug infested palms. In these models, the response variable was the number of infected kissing bugs, and covariates included an offset variable with the logarithm of the total number of kissing bugs examined in the palm, the number of houses present in a 10, 50, and 100 m radius from each focal palm, in addition to the set of covariates used in the infestation analysis.

Variable Selection

In both infestation and infection models, we selected covariates using a process of backward elimination (Kuhn and Johnson 2013). Briefly, in backward elimination, a full model that includes all covariates is fitted and then simplified in an stepwise manner by leaving outside one covariate at a time and then comparing the AIC (Akaike Information Criterion, a metric that helps to select models by minimizing the trade-off between number of parameters and model fit) of the resulting set of models with an equal number of parameters, the model selected at each step being the one that minimizes the AIC, and repeating the process until there is no further decrease in AIC values. When the model minimum AIC value was selected, models were furtherly simplified if removing a statistically not significant covariate ($P > 0.05$) did not increase the AIC by more than two units, as the simpler model would not be significantly different ($P > 0.05$) from the model with the extra covariate (Faraway 2004).

Handling the Problem of Perfect Separation in Logistic and Poisson Regression

Adult kissing bugs were only collected during the rainy season. This produced an almost perfect separation in models, since without the need of a model, the data indicate that in the dry season adult bugs were not observed in the palms, and they were more likely to be present during the rainy season. However, separation makes unreliable parameter estimation with a generalized linear model, since the error for parameter estimates will be inflated, tending to infinite (Albert and Anderson 1984). To solve this problem, we estimated parameters for the best logistic and Poisson regression models for adults, which included sampling season as a significant predictor, using penalized generalized linear models (Friedman et al. 2010). We specifically employed elasticnet regression and chose the penalty parameter (lambda), using cross validation, i.e., we chose a value that minimized the deviance between our observations and the model fit (Kuhn and Johnson 2013). One of the drawbacks of this method is that standard errors, and subsequently confidence intervals, cannot be estimated because of the bias introduced by the penalty parameter (Faraway 2004, Friedman et al. 2010). Thus, for inference about parameter estimates, we employed likelihood ratio tests between the best model and models where the covariates were dropped (Faraway 2006).

Spatial Independence Assumption of Generalized Linear Models

Given the spatial nature of our data and the need to fulfill error independence assumptions for a sound statistical inference (Brunsdon and Comber 2015), we tested the spatial independence assumption in the regression by estimating the global Moran's I index for residuals of all models selected as best. We considered a maximum nearest neighbor distance between palms of up to 600 m when calculating the spatial weights matrix used for the Moran's I index estimation (Brunsdon and Comber 2015), since 600 m was the longest minimum distance to the nearest neighbor recorded in our study. Under a similar argument, the maximum distance was raised to 720 m for residuals from the model studying kissing bug infection, which only considered kissing bug infested palms. The significance of Moran's *I* index was tested with a 1,000 replications Monte Carlo simulation (Brunsdon and Comber 2015).

Software

All statistical analyses and Geographic Information Systems procedures, including the elaboration of maps, were done using the statistical language R.

Results

We sampled a total of 83 palms (Table 1), of which 19 (23%) were infested by kissing bugs. In general, most entomological indices were similar between the two land use types (Table 1), the major difference being a larger number of bugs in the grassland with a higher proportion of adults (Table 1). Palm physiognomy was also very homogenous across grassland and peridomiciliary palms (Table 1), showing no significant differences between the two sites.

A total of 63 bugs were collected, of which 62 were *R. pallescens* and one was *Eratyrus cuspidatus*. The *E. cuspidatus* sample was free of *Trypanosoma* spp infection. Thus, the rest of the results will only refer to observations about *R. pallescens*. We processed a total of 30 kissing bugs to detect *Trypanosoma* spp infection by microscopy (14 adults, eight fifth instar nymphs and eight fourth instar nymphs). From these, 20 individuals (67%) had *Trypanosoma* spp. infections. Further molecular analysis, from the subsample (*n*=10) of randomly selected microscopically positive and negative bugs, showed that bugs were infected by *T. cruzi* and/or *T. rangeli*. More specifically, we were able to confirm five positives (four *T. cruzi*, one was a *T. cruzi–T. rangeli* mixed infection), and five negative samples. Based on this result, we will assume that all infections were due to *T. cruzi* in subsequent analyses. We were also able to isolate *T. cruzi* from two infected bugs. The mini-exon gene analysis in both isolates showed the characteristic PCR product (350 bp) of *T. cruzi* Trypanosoma cruzi Lineage I (TcI) lineage (Supp. Fig. 1 [online only]).

Figure 4 shows the expected and fitted *L* function for the peridomiciliary area polygon. The pattern indicates that palm spatial distribution in this area was random, as it was not significantly different from what is expected under a homogenous spatial Poisson random process, meaning that palms are neither clustered nor uniformly distributed. This pattern is contrasting with the one observed for the grassland area polygon (Fig. 5), where palms were clustered at a distance of around 100 m, this difference being statistically significant ($P < 0.05$).

Although the *L* function analysis suggested that palms had different spatial arrangement patterns according to the land use type, the logistic regression analyses suggested that sampling season was a major factor explaining the infestation of palms by kissing bugs of any stage. Infestation odds increased 50 times for bugs of any stage, 25 times for nymphs, and over 17,000 times for adults, during the rainy season (Table 2). Another important factor was the number of infructescences where each infructescence increased the odds of infestation by 24% and 39%, respectively, by any stage bugs or by

Table 1. Summary of entomological indices and palm physiognomic traits according to land use in "coyol" palms (*Acrocomia aculeata*) from Pedasí district, Los Santos province, Panamá

Entomological variables	Peridomicile	Grassland	Total
Palms sampled	44	39	83
Palms infested[a]	9	10	19
Infestation index (%)[b]	20.4	25.6	22.9
Number of triatomines captured	26	36	62
Adults captured	4	10	14
Nymphs captured	22	26	48
Total nymph:adult ratio	5.5	2.6	3.4
Density[c]	0.59	0.95	0.75
Crowding[d]	2.9	3.7	3.3
Colonization index[e]	88.9	90.0	89.4
Ecological variables			
Palms height (M ± SD)	6.27 ± 1.74	6.66 ± 2.28	6.46 ± 2.01
Green fronds (Me - IQR)	23 (19.3–29)	27 (19–33)	25 (19–30)
Dry fronds (Me - IQR)	23.5 (15.3–34)	30 (20–38)	25 (17–36)
Infructescences (Me - IQR)	6 (4–9.5)	8 (5–12)	7 (4–10)
Epiphytic plants (Ratio)	31/44	32/39	63/83

Land use included peridomicile and grassland.

IQR (interquartile range), M (mean), Me (median), SD (standard deviation)

[a]Palms infested: no. of palms with triatomines.

[b]Infestation index: no. of palms with triatomines/no. of palms sampled.

[c]Density: no. of triatomines/no. of palms sampled.

[d]Crowding: no. of triatomines/no. of palms infested.

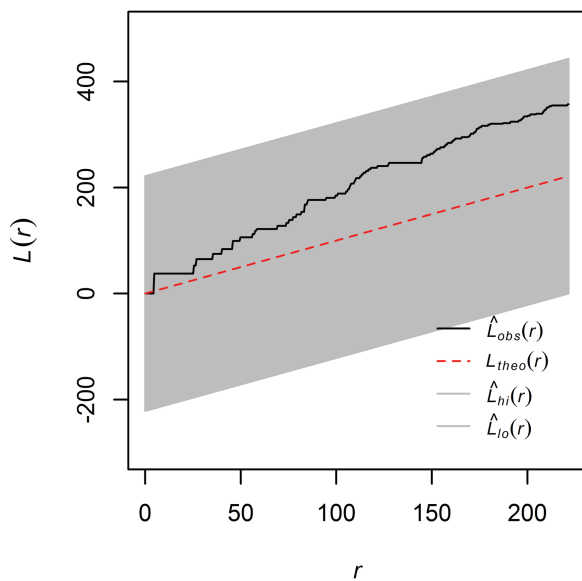[e]Colonization index: no. of palms with nymphs/no. of palms with triatomines.

Fig. 4. *L* function of palms in a peridomiciliary area of Pedasí, Panamá. The inset legend shows the lines for the theoretical *L* function expected under a homogenous spatial $\hat{L}$ Poisson process ($L_{theo}[r]$), the $\hat{L}$ function estimated from the observations $\hat{L}_{obs}(r)$ and the highest $\hat{L}_{hi}(r)$ and lowest $\hat{L}_{lo}(r)$ critical bands of the global envelope.

nymphs only (Table 2). Adult bugs were 93% less likely to be present in peridomiciliary palms and increased three times their odds of infesting a palm for each meter of height the palm had (Table 2). Meanwhile, for each green frond, nymphs were 13% less likely to be infesting a palm (Table 2).

Following a similar pattern to the one observed with palm infestation by kissing bugs, sampling season had a major impact on kissing bug abundance, fourth and fifth instar nymphs (iny) were 146 times, and adults were over 4,000 times more abundant during the

rainy season (Table 3). The fourth and fifth iny and adults, increased around two and three times, respectively, their abundance with each meter of height a given palm had (Table 3). The abundance of fourth and fifth iny increased around 30% by the presence of each infructescence in the palms, while it decreased around 13% by the presence of each green frond (Table 3). Meanwhile, adult abundance decreased around 84% in peridomiciliary palms when compared with grassland palms (Table 3). Kissing bug infection rate with *T. cruzi* in infested palms increased about five times for each house within 10 m of the palm (Table 4). The infection rate increased around 12% for each green frond in the palm, while it decreased 11% for each dead/dry frond (Table 4), suggesting greener palms near houses, if infested, were more likely to have infected bugs with *T. cruzi*.

All best models for kissing bug infestation (Table 2), kissing bug abundance (Table 3), and kissing bug infection (Table 4) minimized the AIC, and in all cases, the null hypothesis of lack of spatial autocorrelation in model residuals was not rejected (Tables 2–4) ensuring a sound inference. In addition, in all the best models, the residual deviance was smaller than its associated degrees of freedom, which ensures that models appropriately fitted the data (Faraway 2006), not justifying the use of models for overdispersed count distributions (Venables and Ripley 2002).

Discussion

From the ten kissing bug species able to transmit *T. cruzi* present in Panamá, *R. pallescens* is by far the most abundant and geographically widespread vector species reported in endemic areas (Sousa and Johnson 1973). The close association of *T. cruzi*-infected *R. pallescens* with royal palms (Gottdenker et al. 2011) has led to the concentration of Chagas disease control activities and research in areas of Panamá where *Att. butyracea* palms are common (Vasquez et al. 2004; Saldaña et al. 2005; Calzada et al. 2006, 2010; Samudio et al. 2007; Pineda et al. 2008, 2011; Gottdenker et al. 2011). This situation has created a scenario where it was believed that *Att. butyracea*
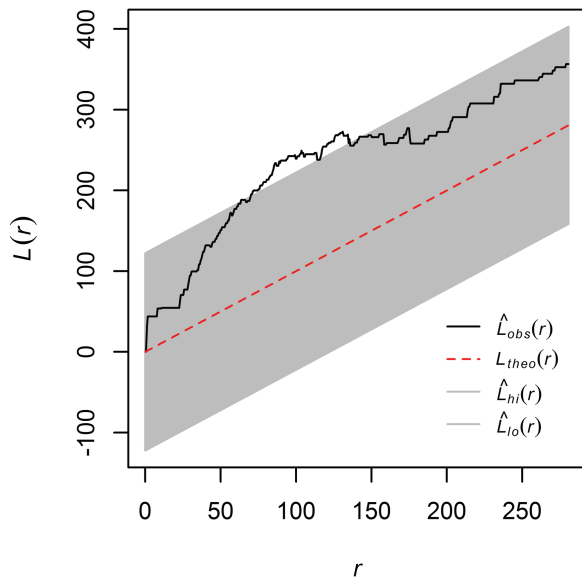
Fig. 5. *L* function of palms in a grassland area of Pedasí, Panamá. The inset legend shows the lines for the theoretical *L* function expected under a homogenous spatial Poisson process ($L_{theo}[r]$), the $\hat{L}$ function estimated from the observations $\hat{L}_{obs}(r)$ and the highest $\hat{L}_{hi}(r)$ and lowest $\hat{L}_{lo}(r)$ critical bands of the global envelope. Note that around 100 m, the observed function is significantly larger than what is expected by random.

palm presence was a major factor driving the geographic distribution of both Chagas disease (Sousa and Johnson 1973, Whitlaw and Chaniotis 1978) and *R. pallescens* (Arboleda et al. 2009), despite observations on *R. pallescens* ability to infest different palm species across Latin America (Abad-Franch et al. 2015). However, our observations suggest that *R. pallescens* infested coyol palms, *Acr. aculeata*, might maintain a sylvatic transmission cycle in the dry arch of Panamá; an area previously not considered endemic for Chagas disease transmission. We think transmission might be mainly sylvatic given the likely involvement of wildlife mammal *T. cruzi* reservoir species in transmission (Alvarado-Otegui et al. 2012). Here, it is important to highlight that although not important for our statistical models, the only wildlife mammal species that we observed during sampling was the common opossum *D. marsupialis,* which was present in two of the kissing bug uninfested palms; and our observations about opossums being the most common blood source at our other study sites in Panamá, where we have found more wildlife mammal species in crowns of royal palms (Kieran et al. 2017), reinforces this hypothesis that could be rigorously tested in future studies. This region presents some elements that have been associated with the emergence of diseases, including significant changes in land use mainly driven by the development of infrastructure (Levins et al. 1994), in this specific area for tourism (Autoridad Nacional del Ambiente 2010). In this emerging landscape, palms are kept intact due to their perceived value as ornamental and useful trees (McKillop 1996), and, in the specific case of the coyol palm for

Table 2. Odds ratios and parameter estimates for the best logistic regression models explaining palm infestation by kissing bugs of different stages

Kissing bug stage	Parameter	Odds ratio	Estimate	S.E.	z value	Pr(>|z|)
All	Dry season	—	–4.226	1.105	–3.825	0.000131*
	Rainy season	49.048	3.893	0.812	4.793	1.64E-06*
	No. infructescences	1.241	0.216	0.108	1.994	0.0462*
	Moran's I	—	–0.0678	—	—	0.907
	AIC	—	55.886	—	—	—
	AIC FM	—	62.426	—	—	—
	Δ AIC	—	6.540	—	—	—
Nymphs only	Dry season	—	–1.781	1.161	–1.534	0.125
	Rainy season	25.707	3.247	0.780	4.163	3.14E-05*
	No. green fronds	0.874	–0.13436	0.0686	–1.959	0.05
	No. infructescences	1.389	0.328	0.146	2.243	0.0249*
	Moran's I	—	–0.0717	—	—	0.912
	AIC	—	60.007	—	—	—
	AIC FM	—	65.483	—	—	—
	Δ AIC	—	5.476	—	—	—
	Parameter	Odds ratio	Estimate	df	LRT	Pr(> χ^2)
Adults only	Dry season grassland	—	–23.888	—	—	—
	Rainy season	17,440	9.767	1	31.424	2.07E-08*
	Peridomiciliary	0.0739	–2.605	1	4.763	0.0291*
	Palm height (m)	3.0272	1.108	1	9.633	0.00191*
	Moran's I	—	–0.0326	—	—	0.657
	AIC	—	20.740	—	—	—
	AIC FM	—	25.807	—	—	—
	Δ AIC	—	5.067	—	—	—
	Lambda	—	0.00238	—	—	—
	Model deviance	—	12.74	—	—	—

AIC indicates the Akaike Information Criterion of the best model, AIC FM, the AIC of the full model, and Δ AIC, the difference between AIC FM and AIC. Note that only for adults, no S.E. are shown and parameter significance is based on a χ^2 test for the likelihood ratio test (LRT) between the best model, whose deviance is shown in the table, and simplified versions without a given covariate, whose degrees of freedom (df) are also shown in the table. Lambda is the penalty parameter that minimized model deviance, via cross-validation, for adult infestation.

*Statistically significant ($P < 0.05$)

Table 3 Abundance changes and parameter estimates for the best Poisson models explaining kissing bugs abundance, of different stages, by palm

Stage	Parameter	Abundance change	Estimate	S.E.	z value	Pr(>|z|)
Fourth and	Dry season	—	–12.129	2.780	–4.364	1.28E-05*
fifth instar	Rainy season	146	4.986	1.004	4.964	6.91E-07*
nymphs	No. green fronds	0.870	–0.139	0.0382	–3.635	0.000278*
	Palm height (m)	2.976	1.091	0.217	5.016	5.27E-07*
	No. infructescences	1.312	0.2715	0.0736	3.687	0.000227*
	Moran's I	—	–0.0257	—	—	0.583
	AIC	—	55.425	—	—	—
	AIC FM	—	57.267	—	—	—
	Δ AIC	—	1.842	—	—	—
	Parameter	Abundance change	Estimate	df	LRT	Pr(> χ^2)
	Dry season grassland	—	–20.136	—	—	—
Adults	Rainy season	4,333	8.374	1	54.431	1.69E-13*
only	Peridomiciliary	0.163	–1.812	1	7.170	0.00741*
	Palm height (m)	2.192	0.785	1	15.743	7.25E-05*
	Moran's I	—	–0.0422	—	—	0.758
	AIC	—	40.604	—	—	—
	AIC FM	—	46.520	—	—	—
	Δ AIC	—	5.916	—	—	—
	Lambda	—	0.00137	—	—	—
	Model deviance	—	14.401	—	—	—

AIC indicates the Akaike Information Criterion of the best model, AIC FM, the AIC of the full model, and Δ AIC the difference between AIC FM and AIC. Note that only for adults, no S.E. are shown and parameter significance is based on a χ^2 test for the likelihood ratio test (LRT) between the best model, whose deviance is shown in the table, and simplified versions without a given covariate, whose degrees of freedom (df) are also shown in the table. Lambda is the penalty parameter that minimized model deviance, via cross-validation, for adult abundance.

*Statistically significant (*P* < 0.05).

Table 4 Proportional rate change and parameter estimates for the Poisson rate model explaining kissing bug infection with *Trypanosoma cruzi* in kissing bug infested Palms

Parameter	Proportional rate change	Estimate	S. E.	z value	Pr(>|z|)
Intercept	—	–2.040	0.954	–2.138	0.0325*
No. green fronds	1.117	0.110	0.0550	2.005	0.0449*
No. dry fronds	0.890	–0.116	0.0440	–2.639	0.00832*
No. houses within 10 m	5.055	1.620	0.541	2.994	0.00275*
Moran's I	—	–0.189	—	—	0.827
AIC	—	40.781	—	—	—
AIC FM	—	42.321	—	—	—
Δ AIC	—	1.540	—	—	—

AIC indicates the Akaike Information Criterion of the best model, AIC FM, the AIC of the full model, and Δ AIC, the difference between AIC FM and AIC.
*Statistically significant (*P* < 0.05).

wine production, a tradition dating back to prehispanic times (Lentz 1991).

The emerging landscape matrix where numerous isolated coyol palms are embedded within pastures or peridomiciliary areas might have enhanced conditions for Chagas disease transmission, provided our previous research has shown these isolated palms in the middle of pastures harbor more bugs (Gottdenker et al. 2011) with higher infection rates (Gottdenker et al. 2012, 2016). Indeed, we found 23% of the studied *Acr. aculeata* palms infested by *R. pallescens*, of which, 67% were infected with trypanosomes, suggesting that coyol palms might play a role on maintaining *T. cruzi* sylvatic transmission in this area of Panamá. Besides *T. cruzi,* we detected *T. rangeli* by molecular means, a nonpathogenic trypanosome that frequently infects *R. pallescens* and humans in different endemic zones from Panamá (Calzada et al. 2010). We were also able to detect the TCI lineage of *T. cruzi*, the only genotype so far described in vectors and

reservoirs and responsible for human infections in endemic regions across Panamá (Sousa et al. 2006, Samudio et al. 2007, Brandao et al. 2008). Our isolates could also be useful to characterize the substructure of TCI in the Republic of Panamá, as done in parts of Colombia (Poveda et al. 2017).

Our results also highlight the need to better understand ecological factors and palm traits that may influence palm infestation and vector abundance, information that might prove crucial for the proposal of ecologically sound strategies for the control of Chagas disease transmission. Our statistical analysis showed that sampling season had a major influence on bug abundance: adult vectors were only collected during the rainy season and nymph colonization increased over 25 times during the rainy season. It is also worth mentioning that during the sampling period the planet was facing one of the strongest El Niño/Southern Oscillation (ENSO) events on record (Levine and McPhaden 2016); which locally caused a severe drought

and higher than usual temperatures across Panamá, particularly in the studied area (Olmedo 2016). Our previous research has shown that hot ENSO phases are associated with decreases on the abundance of sand flies in Panamá (Chaves et al. 2014), especially in the dry arch where this study was carried out (Yamada et al. 2016). Thus, assuming that similar patterns could be the case for kissing bugs, it would not be a surprise that coyol palms may harbor larger numbers of kissing bugs during normal environmental conditions. The unusual hot environmental conditions could also be associated with the differences we observed in the stage structure of kissing bugs between peridomiciliary and grassland environments, provided previous research has shown more frequent kissing bug movement to areas potentially hosting more blood resources when temperature is hotter than usual (Dumonteil and Gourbiére 2004, Hernández et al. 2010), as could be the case for grassland palms, which our analysis showed to be clustered, unlike peridomiciliary palms which have a random spatial distribution. Alternatively, these hot periods could also drive migration of adult bugs from peridomiciliary palms into nearby homes (Abad-Franch et al. 2015), a hypothesis that could be tested in longitudinal studies looking at kissing bug home invasion patterns. Differences in clustering patterns of palms might also be associated with differences in *R. pallescens* infection by *T. cruzi* which might have been lower in grassland palms, i.e., when houses are absent in Table 4, due to an increased biodiversity and/or abundance of zooprophylactic hosts (Gómez Núñez 1963, Tonn et al. 1976, Chaves et al. 2007) in palm clusters, but this also might be related to the common presence of *T. cruzi* wildlife mammal reservoirs hosts in peridomiciliary environments, as we have observed with opposums in peridomestic royal palms (Pineda et al. 2008). Palm physiognomy, including height, number of green fronds, and infructescences, was also important to explain the abundance and/or infection rates of *R. pallescens*, in a fashion similar to that described by other studies looking at *Rhodnius* spp in several palm species (Abad-Franch et al. 2005, 2010, 2015; Urbano et al. 2015).

In conclusion, our study identified a new area with potential Chagas disease transmission in Panamá, and our results show coyol palms are a habitat where *R. pallescens* is infected by *T. cruzi* and *T. rangeli* in the dry arch of Panamá. These results also provide valuable information about palm physiognomy that could be useful to manage palms in ways that could reduce their risk as sources of infected *T. cruzi* vectors.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

Acknowledgments

References Cited

Abad-Franch, F., F. Noireau, A. Paucar, H. M. Aguilar, C. Carpio, and J. Racines. 2000. The use of live-bait traps for the study of sylvatic *Rhodnius* populations (Hemiptera: Reduviidae) in palm trees. Trans. R. Soc. Trop. Med. Hyg. 94: 629–630.

Abad-Franch, F., F. S. Palomeque, H. M. Aguilar, V, and M. A. Miles. 2005. Field ecology of sylvatic *Rhodnius* populations (Heteroptera, Triatominae): risk factors for palm tree infestation in western Ecuador. Trop. Med. Int. Health. 10: 1258–1266.

Abad-Franch, F., G. Ferraz, C. Campos, F. S. Palomeque, M. J. Grijalva, H. M. Aguilar, and M. A. Miles. 2010. Modeling disease vector occurrence when detection is imperfect: infestation of Amazonian palm trees by triatomine bugs at three spatial scales. Plos Negl. Trop. Dis. 4: e620.

Abad-Franch, F., M. M. Lima, O. Sarquis, R. Gurgel-Gonçalves, M. Sánchez-Martín, J. Calzada, A. Saldaña, F. A. Monteiro, F. S. Palomeque, W. S. Santos, et al. 2015. On palms, bugs, and Chagas disease in the Americas. Acta Trop. 151: 126–141.

Albert, A., and J. A. Anderson. 1984. On the existence of maximum likelihood estimates in logistic regression models. Biometrika. 71: 1–10.

Alvarado-Otegui, J. A., L. A. Ceballos, M. M. Orozco, G. F. Enriquez, M. V. Cardinal, C. Cura, A. G. Schijman, U. Kitron, and R. E. Gürtler. 2012. The sylvatic transmission cycle of *Trypanosoma cruzi* in a rural area in the humid Chaco of Argentina. Acta Trop. 124: 79–86.

Arboleda, S., D. E. Gorla, X. Porcasi, A. Saldaña, J. Calzada, and N. Jaramillo-O. 2009. Development of a geographical distribution model of *Rhodnius pallescens* Barber, 1932 using environmental data recorded by remote sensing. Infect. Genet. Evol. 9: 441–448.

Autoridad Nacional del Ambiente. 2010. Atlas Ambiental de la República de Panamá. Editora Novo Art, Ciudad de Panamá.

Baddeley, A. 2010. Analysing spatial point patterns in R, pp. 232, Technical Report. CSIRO Canberra, Australia.

Baddeley, A., and R. Turner. 2005. Spatstat: An R package for analyzing spatial point patterns. J. Stat. Softw. 12: 1–42.

Baddeley, A., and R. Turner. 2006. Modelling spatial point patterns in R, pp. 23–74, Case studies in spatial point process modeling. Springer, New York.

Brandao, A., F. Samudio, O. Fernandes, J. E. Calzada, and O. E. Sousa. 2008. Genotyping of Panamanian *Trypanosoma cruzi* stocks using the calmodulin 3'UTR polymorphisms. Parasitol. Res. 102: 523–526.

Brunsdon, C., and L. Comber. 2015. An introduction to R for spatial analysis and mapping. Sage Publications LTD, London.

Calzada, J. E., V. Pineda, E. Montalvo, D. Alvarez, A. M. Santamaría, F. Samudio, V. Bayard, L. Cáceres, and A. Saldaña. 2006. Human trypanosome infection and the presence of intradomicile Rhodnius pallescens in the western border of the Panama Canal, Panama. Am. J. Trop. Med. Hyg. 74: 762–765.

Calzada, J. E., V. Pineda, J. D. Garisto, F. Samudio, A. M. Santamaria, and A. Saldaña. 2010. Human trypanosomiasis in the eastern region of the Panama Province: new endemic areas for Chagas disease. Am. J. Trop. Med. Hyg. 82: 580–582.

Chaves, L. F., M. J. Hernandez, A. P. Dobson, and M. Pascual. 2007. Sources and sinks: revisiting the criteria for identifying reservoirs for American cutaneous leishmaniasis. Trends Parasitol. 23: 311–316.

Chaves, L. F., J. E. Calzada, A. Valderrama, and A. Saldaña. 2014. Cutaneous leishmaniasis and sand fly fluctuations are associated with el niño in panamá. Plos Negl. Trop. Dis. 8: e3210.

Christensen, H. A., and A. M. de Vasquez. 1981. Host feeding profiles of Rhodnius pallescens (Hemiptera: Reduviidae) in rural villages of Central Panama. Am. J. Trop. Med. Hyg. 30: 278–283.

Diggle, P. J. 2013. Statistical analysis of spatial and spatio-temporal point patterns. CRC Press, Boca Raton, FL.

Dumonteil, E., and S. Gourbiére. 2004. Predicting *triatoma dimidiata* abundance and infection rate: a risk map for natural transmission of chagas disease in the yucatan peninsula of Mexico. Am. J. Trop. Med. Hyg. 70: 514–519.

Dunn, L. H. 1933. A natural infection of *Trypanosoma cruzi* Chagas found in *Rhodnius Pallescens* Barber in Panama. Am. J. Trop. Med. Hyg. s1–13: 471–473.

Faraway, J. J. 2004. Linear Models with R. CRC Press, Boca Raton.

Faraway, J. J. 2006. Extending the Linear Model with R: Generalized Linear. Mixed Effects and Nonparametric Regression Models CRC Press, Boca Raton.

Feliciangeli, M. D., M. Sanchez-Martin, R. Marrero, C. Davies, and J. P. Dujardin. 2007. Morphometric evidence for a possible role of Rhodnius prolixus from palm trees in house re-infestation in the State of Barinas (Venezuela). Acta Trop. 101: 169–177.

Fernandes, O., S. S. Santos, E. Cupolillo, B. Mendonça, R. Derre, A. C. Junqueira, L. C. Santos, N. R. Sturm, R. D. Naiff, T. V. Barret, et al. 2001. A mini-exon multiplex polymerase chain reaction to distinguish the major groups of *Trypanosoma cruzi* and *T. rangeli* in the Brazilian Amazon. Trans. R. Soc. Trop. Med. Hyg. 95: 97–99.

Friedman, J. H., T. Hastie, and R. Tibshirani. 2010. Regularization Paths for Generalized Linear Models via Coordinate Descent. J. Stat. Softw. 33: 22.

Gómez Núñez, J. C. 1963. Notas sobre la ecología de *Rhodnius prolixus*. Bol. Dir. Malariol. Saneam. Ambient. 3: 330–335.

Gómez-Núñez, J. G. 1969. Resting places, dispersal and survival of Co60-tagged adult *Rhodnius Prolixus*. J. Med. Entomol. 6: 83–86.

Gottdenker, N. L., J. E. Calzada, A. Saldaña, and C. R. Carroll. 2011. Association of anthropogenic land use change and increased abundance of the Chagas disease vector Rhodnius pallescens in a rural landscape of Panama. Am. J. Trop. Med. Hyg. 84: 70–77.

Gottdenker, N. L., L. F. Chaves, J. E. Calzada, A. Saldaña, and C. R. Carroll. 2012. Host life history strategy, species diversity, and habitat influence *Trypanosoma cruzi* vector infection in changing landscapes. Plos Negl. Trop. Dis. 6: e1884.

Gottdenker, N. L., L. F. Chaves, J. E. Calzada, J. K. Peterson, A. SantamarÍA, V. Pineda, and A. SaldaÑA. 2016. *Trypanosoma cruzi* and *Trypanosoma rangeli* co-infection patterns in insect vectors vary across habitat types in a fragmented forest landscape. Parasitology Open. 2: e10(1–10).

Grijalva, M. J., A. G. Villacís, A. L. Moncayo, S. Ocaña-Mayorga, C. A. Yumiseva, and E. G. Baus. 2017. Distribution of triatomine species in domestic and peridomestic environments in central coastal Ecuador. PLoS Negl. Trop. Dis. 11: e0005970.

Hernández, J. L., E. A. Rebollar-Téllez, F. Infante, A. Morón, and A. Castillo. 2010. [Indicators of infestation, colonization and infection of *Triatoma dimidiata* (Latreille) (Hemiptera: Reduviidae) in Campeche, México]. Neotrop. Entomol. 39: 1024–1031.

Hurtado, L. A., J. E. Calzada, V. Pineda, K. González, A. M. Santamaría, L. Cáceres, C. Wald, and A. Saldaña. 2014. [Knowledge and risk factors related to Chagas' disease in two Panamanian communities where *Rhodnius pallescens* is the main vector]. Biomedica. 34: 260–270.

Kieran, T. J., N. L. Gottdenker, C. P. Varian, A. Saldaña, N. Means, D. Owens, J. E. Calzada, and T. C. Glenn. 2017. Blood meal source characterization using illumina sequencing in the Chagas disease vector *Rhodnius pallescens* (Hemiptera: Reduviidae) in Panamá. J. Med. Entomol. 54(6):1786–1789.

Kuhn, M., and K. Johnson. 2013. Applied predictive modeling. Springer, New York.

Lent, H., and P. Wygodzinsky. 1979. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. Bull. Am. Mus. Nat. Hist. 163: 123–520.

Lentz, D. L. 1991. Maya diets of the rich and poor: paleoethnobotanical evidence from Copan. Lat. Am. Antiq. 2: 269–287.

Levine, A. F., and M. J. McPhaden. 2016. How the July 2014 easterly wind burst gave the 2015–2016 El Niño a head start. Geophys. Res. Lett. 43: 6503–6510.

Levins, R., T. Awerbuch, U. Brinkmann, I. Eckardt, P. Epstein, N. Makhoul, C. A. de Possas, C. Puccia, A. Spielman, and M. E. Wilson. 1994. The emergence of new diseases. Am. Sci. 82: 52–60.

McKillop, H. 1996. Prehistoric Maya use of native palms: archaeobotanical and ethnobotanical evidence. *In* S. Fedick (ed.), The managed Mosaic: ancient Maya agriculture and resource use. University of Utah Press, Salt Lake City; 278–294.

Miller, J. W. 1931. Chagas' disease in Panama: report of three cases. South. Med. J. 24: 645–647.

Olmedo, B. 2016. Estado actual de las condiciones del Océano Pacífico Y su posible evolución durante el año 2015–2016., pp. 9. ETESA - Gerencia de Climatología, Panamá.

Pineda, V., E. Montalvo, D. Alvarez, A. M. Santamaría, J. E. Calzada, and A. Saldaña. 2008. Feeding sources and trypanosome infection index of *Rhodnius pallescens* in a Chagas disease endemic area of Amador County, Panama. Rev. Inst. Med. Trop. Sao Paulo. 50: 113–116.

Pineda, V., A. Saldaña, I. Monfante, A. Santamaría, N. L. Gottdenker, M. J. Yabsley, G. Rapoport, and J. E. Calzada. 2011. Prevalence of trypanosome infections in dogs from Chagas disease endemic regions in Panama, Central America. Vet. Parasitol. 178: 360–363.

Pipkin, A. C., Sr. 1968. Domiciliary reduviid bugs and the epidemiology of Chagas' disease in Panama (Hemiptera: Reduviidae: Triatominae). J. Med. Entomol. 5: 107–124.

Pizarro, J., and C. Romaña. 1998. Variación estacional de una población silvestre de *Rhodnius pallescens* Barber 1932 (Heteroptera: Triatominae) en la costa caribe colombiana. Bull. Inst. Fr. Etudes. Andines. 27: 309–325.

Poveda, C., A. Higuera, P. Urbano, and J. D. Ramírez. 2017. Ecology of *Trypanosoma cruzi* I genotypes across *Rhodnius prolixus* captured in *Attalea butyracea* palms. Infect. Genet. Evol. 49: 146–150.

Rabinovich, J. E., U. D. Kitron, Y. Obed, M. Yoshioka, N. Gottdenker, and L. F. Chaves. 2011. Ecological patterns of blood-feeding by kissing-bugs (Hemiptera: Reduviidae: Triatominae). Mem. Inst. Oswaldo Cruz. 106: 479–494.

Romaña, C. A., J. C. Pizarro, E. Rodas, and E. Guilbert. 1999. Palm trees as ecological indicators of risk areas for Chagas disease. Trans. R. Soc. Trop. Med. Hyg. 93: 594–595.

Saldaña, A., F. Samudio, A. Miranda, L. M. Herrera, S. P. Saavedra, L. Cáceres, V. Bayard, and J. E. Calzada. 2005. Predominance of *Trypanosoma rangeli* infection in children from a Chagas disease endemic area in the west-shore of the Panama canal. Mem. Inst. Oswaldo Cruz. 100: 729–731.

Saldaña, A., J. E. Calzada, V. Pineda, M. Perea, C. Rigg, K. González, A. M. Santamaria, N. L. Gottdenker, and L. F. Chaves. 2015. Risk factors associated with Trypanosoma cruzi exposure in domestic dogs from a rural community in Panama. Mem. Inst. Oswaldo Cruz. 110: 936–944.

Samudio, F., E. Ortega-Barría, A. Saldaña, and J. Calzada. 2007. Predominance of *Trypanosoma cruzi* I among Panamanian sylvatic isolates. Acta Trop. 101: 178–181.

Sousa, O. E., and C. M. Johnson. 1973. Prevalence of *Trypanosoma cruzi* and *Trypanosoma rangeli* in triatomines (Hemiptera: Reduviidae) collected in the Republic of Panama. Am. J. Trop. Med. Hyg. 22: 18–23.

Sousa, O. E., F. Samudio, C. de Juncá, and J. E. Calzada. 2006. Molecular characterization of human *Trypanosoma cruzi* isolates from endemic areas in Panama. Mem. Inst. Oswaldo Cruz. 101: 455–457.

Tonn, R. J., R. Carcavallo, R. Ortega, and B. Carrasquero. 1976. Métodos de estudio de triatominos en el medio silvestre. Bol. Dir. Malar. Sam. Amb. 16: 146–152.

Urbano, P., C. Poveda, and J. Molina. 2015. Effect of the physiognomy of *Attalea butyracea* (Arecoideae) on population density and age distribution of *Rhodnius prolixus* (Triatominae). Parasit. Vectors. 8: 199.

Vasquez, A. M., F. E. Samudio, A. Saldaña, H. M. Paz, and J. E. Calzada. 2004. Eco-epidemiological aspects of *Trypanosoma cruzi*, *Trypanosoma rangeli* and their vector (*Rhodnius pallescens*) in Panama. Rev. Inst. Med. Trop. Sao Paulo. 46: 217–222.

Venables, W. N., and B. D. Ripley. 2002. Modern applied statistics with S. Springer, New York.

Whitlaw, J. T., and B. N. Chaniotis. 1978. Palm trees and Chagas' disease in Panama. Am. J. Trop. Med. Hyg. 27: 873–881.

World Health Organization. 2003. Control de la enfermedad de Chagas: segundo informe del comité de expertos de la OMS, pp. 117, Serie de Informes Técnicos, Brasilia.

Yamada, K., A. Valderrama, N. Gottdenker, L. Cerezo, N. Minakawa, A. Saldaña, J. E. Calzada, and L. F. Chaves. 2016. Macroecological patterns of American Cutaneous Leishmaniasis transmission across the health areas of Panamá (1980–2012). Parasite Epidemiol. Control. 1: 42–55.

Zar, J. H. 1998. Biostatistical analysis. Prentice Hall, San Francisco.