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Research paper

Plasmodium infection in *Kerteszia cruzii* (Diptera: Culicidae) in the Atlantic tropical rain forest, southeastern Brazil

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ABSTRACT

In Southeastern Brazil, *Kerteszia cruzii* (former *Anopheles cruzii*), a bromeliad mosquito species, is considered an efficient human *Plasmodium* spp. vector. In this region, recent studies showed asymptomatic or sub-patent *Plasmodium falciparum* infection. In areas of the Atlantic coast in Rio de Janeiro, *Plasmodium simium* infection was recently reported in both human and howler monkey. Considering that (1) few malaria cases are reported each year in areas across the tropical Atlantic rain forest in southeastern Brazil; (2) malaria elimination in Atlantic forest is challenged by circulation of *P. falciparum* and *P. simium* in humans; (3) the complexity of malaria epidemiology in this region; and (4) the public health importance of *Kerteszia cruzii* as a sylvatic vector; the major goal of this study is to evaluate *Plasmodium* infection in *Ke. cruzii*. Mosquito sampling collections were conducted in Esteiro do Morro and Sítio Itapuan, in Cananeia municipality, and Tapiraí municipality in Ribeira Valley, southeastern São Paulo state, Brazil. Influence of climate and landscape factors in *Plasmodium* infection in *Ke. cruzii* was addressed. Among the 1719 mosquitoes tested, 3 females collected in Sítio Itapuan and three from Tapiraí were found infected with either *P. vivax* or *P. simium*. Results of statistical analyses did not demonstrate association between *Plasmodium* infection in mosquito and the landscape. Mosquito infection was found in two landscape clusters, with *Plasmodium* detected in forest fringe mosquitoes. This finding shows that *Ke. cruzii* can facilitate transmission among human and non-human primates. *Plasmodium falciparum* was not identified in the samples analyzed. Spatiotemporal variation in local malaria incidence, low prevalence of *Plasmodium*, variations in humidity and temperature can explain the absence of mosquitoes infected with *P. falciparum* in the study.

1. Introduction

Malaria threatens the public health in Brazil, with approximately 200,000 cases reported in 2018 (Ministério da Saúde, http://portalsaude.saude.gov.br/). The ecology of *Plasmodium* transmission encompasses multiple anopheline vectors and parasites species. Furthermore, the risk and incidence of malaria depends on environmental and socio-economic factors (Castro et al. 2006; Chaves et al. 2018). In Brazil, most malaria occur in the Amazon region, where 99% of cases are reported. The primary vector is *Nyssorhynchus darlingi* (previously known as *Anopheles darlingi*) and *Plasmodium vivax* is the most prevalent parasite. Conversely, outside Amazon region, malaria occurs in areas of Atlantic Forest. The bromeliad mosquito *Kerteszia cruzii* (formerly known as *Anopheles cruzii*) is abundant and well-known vector in areas in south and southeast Brazil (de Pina-Costa et al. 2014). In this region, malaria is caused by either *Plasmodium vivax* or *Plasmodium malariae* (Carlos et al. 2019).

The importance of *Ke. cruzii* as a vector of *Plasmodium* in southeastern areas of Atlantic Forest in Brazil has been demonstrated in several studies. In the 1940s, sporozoites were found in the salivary glands of this species Rachou (1958). Subsequently, this species was registered naturally infected with *P. vivax* in São Vicente and Juquitiba municipalities (Branquinho et al. 1997), and with *P. vivax* and *P. malariae* in Parelheiros district, São Paulo city (Duarte et al. 2013). Recently, *Ke. cruzii* was found naturally infected with either *P. vivax* or *Plamodium. simium* in the state of Espírito Santo (Buery et al. 2018), and *P. vivax* and *P. malariae* in Juquitiba and Palestina, São Paulo state (Kirchgatter et al. 2014). In the Ribeira Valley, other anopheline species can be local vectors of both *P. vivax* and *P. falciparum*, such as *Ke. cruzii*, *Nyssorhyncus strodei*, *Nyssorhyncus triannulatus*, and *Nyssorhyncus galvaoi* (Laporta et al. 2015).

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Plasmodium simium caused an outbreak of malaria in a forest fragment area in Rio de Janeiro. Patients were preliminary diagnosed with *P. vivax* (Brasil et al. 2017). Subsequently, the parasite was identified as *P. simium* based on two SNPs in a mitochondrial gene (Brasil et al. 2017; de Alvarenga et al. 2018). Because there is a controversy regarding species identification (Buery et al., 2017), it is premature to affirm that malaria is enzootic and caused by *P. simium*. An alternative hypothesis is that *P. vivax* is circulating in both human and non-human primates in southeastern Brazil. Additionally, further studies will be necessary to verify whether *P. simium* is a valid species as proposed by Grigg and Snounou (2017).

Considering that (1) malaria has a low endemicity in areas of the Atlantic tropical rain forest in southeastern Brazil; (2) *Ke. cruzii* is the main vector in this region; and (3) the recent reports of *P. simium* infection in humans, this study aims to verify the vector status of *Ke. cruzii* in Tapiraí and in Cananeia, São Paulo state, southeastern Brazil. We also addressed the effect of landscapes on *Plasmodium* infections in *Ke. cruzii*. The effect of the climate on *Plasmodium* infection was accessed employing both Laporta et al. (2015) and the newly data.

2. Material and methods

2.1. Field collections

Mosquitoes were collected between July 2016 and December 2017 (Table 1) using white cotton Shannon traps with 1.3 m width x 3.0 m length x 2.0 m height of main tent, and 2 lateral arms of 0.6 m × 3.0 m × 1.0 m each. LED lamps were used as a source of light. Resting mosquitoes were aspirated from the Shannon trap walls by two collectors using battery power manual aspirator. Collections were done in one site per each locality in three landscapes described in Laporta et al. (2015), including Esteiro do Morro (−24.809933, −47.860367) and Sítio Itapuan (−24.888583, −47.851667) in Cananeia municipality; and Tapiraí (−24.006220, −47.500060) municipality (Fig. 1). Both Esteiro do Morro and Sítio Itapuan are situated on the coastal plain. Sítio Itapuan landscape encompass a estuarine region, with mangrove, restinga, tropical rain forest, interspaced by small farms, roads, fresh water rivers and salt water. Esteiro do Morro landscape is composed of 65.37% forest (Fig. 2B) and 34.63% encroached rural areas (Laporta et al. 2015). Tapiraí landscape is mostly forest and capoeira vegetation (Fig. 2A). Whenever possible, field collections were conducted on the border and inside the forest, between 6:00 PM and 10:00 PM (Table 1). Mosquitoes were euthanized with ethyl acetate, stored on silica gel, and identified morphologically to species using entomological keys (Consoli and Lourenço-de-Oliveira, 1994; Forattini 2002), later confirmed by *COI* barcode sequence using the protocol proposed by Bourke et al. (2018). The mosquitoes were bisected in head/thorax and abdomen as recommended in Foley et al. (2012). Only the head/the thorax were tested for *Plasmodium* detection. During the dissection process, mosquitoes were kept in ice to avoid DNA degradation. Head/thorax were stored at −70 °C until *Plasmodium* identification.

Genomic DNA was extracted using the salt method detailed in Miller et al. (1998) and Laporta et al. (2015). Extractions were mostly performed individually, except for one collection in Tapiraí (March 2017) in which genomic DNA was extracted in pools of 5 head/thorax. The DNA was quantified using a Qubit® fluorometer (Life Technologies, Carlsbad, California, USA).

Table 1
Field collection data.

Date	Locality	Munipality	Number of collected mosquitoes	
			Inside the forest	Forest border
19/07/2016	Sítio Itapuan	Cananeia	212	not performed
20/07/2016	Sítio Itapuan	Cananeia	not performed	136
21/07/2016	Esteiro do Morro	Cananeia	not performed	2
07/09/2016	Tapiraí	Tapiraí	0	5
28/09/2016	Sítio Itapuan	Cananeia	72	19
07/11/2016	Esteiro do Morro	Cananeia	46	6
08/11/2016	Tapiraí	Tapiraí	0	184
08/03/2017	Tapiraí	Tapiraí	54	206
04/12/2017	Tapiraí	Tapiraí	not performed	525
05/12/2017	Esteiro do Morro	Cananeia	not performed	252
			384	1335

2.2. Real time PCR

PCR reactions were carried out with DNA pools from three to five head/thorax, using up to 15 ng of genomic DNA. Real time PCR was performed following Bickersmith et al. (2015). Genus specific primers and a probe (Table 2) were employed, using a final concentration of 0.15 µM of each primer and 0.05 µM of the probe in order to assess *Plasmodium* infection. Subsequently, samples were re-tested using primer and a probe (Table 2) for identification of *P. vivax/P. simium* in a final concentration of 0.3 µM and 0.1 µM, respectively. If samples were positive for *Plasmodium* and negative for *P. vivax/P. simium*, specific primers and a probe for *Plasmodium falciparum* were employed with concentrations of 0.3 µM and 0.1 µM, respectively. Each real time PCR was carried out in a final volume of 20 µL, using TaqMan® Fast

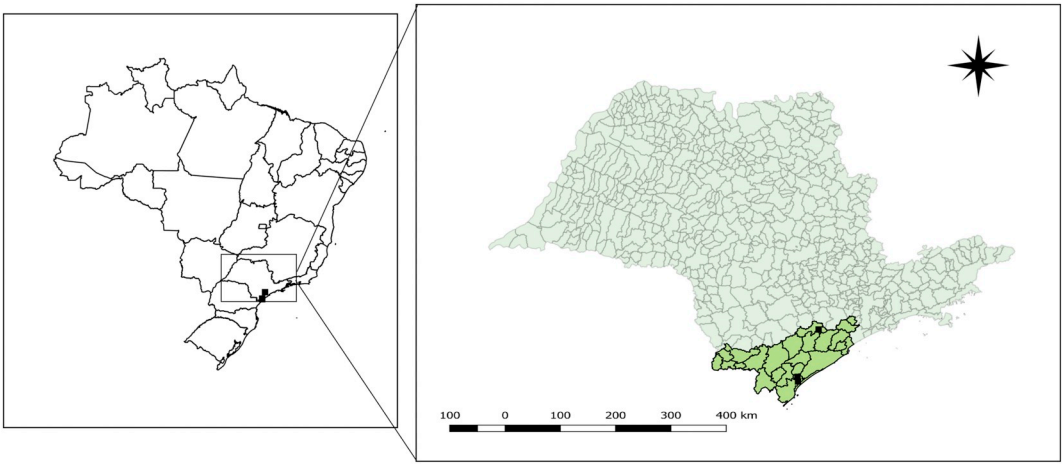

Fig. 1. Map showing localities studied in the Ribeira Valley region, southeastern São Paulo state, Brazil.

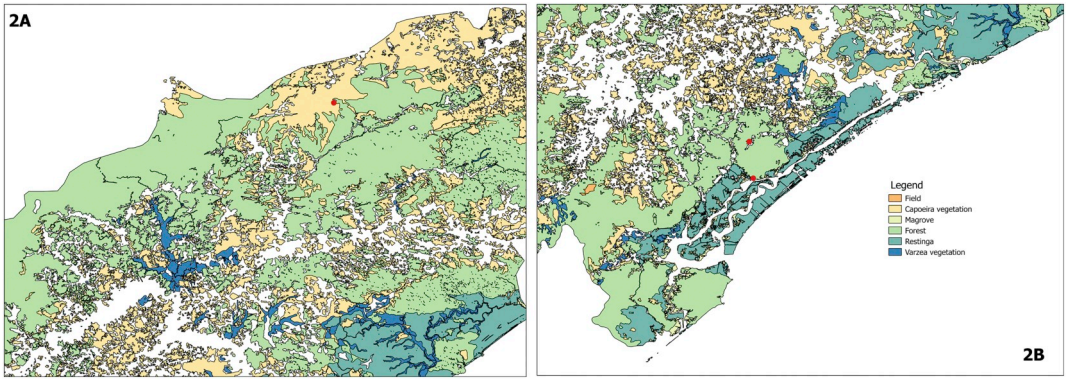

Fig. 2. Map of vegetation cover in the Ribeira Valley. (2A) shows the north of the region, where Tapiraí municipality is located; the red dot indicates the collection locality in a transition area of capoeira and forest. (2B) shows the coastal Atlantic Ocean region; the red dots indicate the two sites of collection in Cananéia municipality. The most southerly point indicates Sítio Itapuan, an area of transition among mangrove, forest and resting. The northerly point indicates Esteiro do Morro locality, in a forest-rural transition area. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Primers and probes employed in real time PCR for *Plasmodium* detection.

Primer	Probe	Citation
Plasmo1-F: GTTAAGGGAGTGAAGACGATCAGA Plasmo2-R: AACCCAAAGACTTTGATTTCTCATAA	Plasprobe: FAM-TCGTAATCTTAACCATAAAC-MGBNFQ	Rougemont et al. 2004, Shokoples et al. 2009
Vivax-F: GACTAGGCTTTGGATGAAAGATTTTAA	Vivaxprobe: NED-ATAAACTCCGAAGAGAAAA-MGBNFQ	Bickersmith et al. 2015
Falc-F: GACTAGGTGTTGGATGAAAGTGTTAAA	FalciProbe: VIC-TGAAGGAAGCAATCTAAAAGTCACCTCGAAAGA-QSY	Bickersmith et al. 2015

Advanced Master Mix Uracil N-glycosylase (UNG), ROX (Applied 144 Biosystem, Foster City, California, USA). Thermocycling conditions were: 5 min at 45 °C for UNG-activation with a 2 min denaturation step at 95 °C, followed by 45 cycles of 95 °C denaturation for 15 s and 60 °C annealing/elongation for 1 min. The samples from the positive pools were individually re-tested and confirmed by Sanger sequencing.

2.3. Landscape and Plasmodium infection

A binomial distribution test was conducted to test the effect of the landscape on the distribution of *Plasmodium* infection in *Ke. cruzii* in accordance to Laporta et al. (2015). Analysis was conducted in STATA/IC 15.1 software StataCorp. (2017) using the bitesti function. Firstly, we calculated the overall prevalence of infected mosquitoes; subsequently the probability of having at least one infected mosquito (the assumed probability – 1-P) in each locality, including the border and inside the forest. Following, we estimated the observed probability (P) of the infected mosquitoes in the study (k) in each landscape and collection site. The test was considered statistically significant if $P < 0.05$.

2.4. Climate versus infectivity

2.4.1. Spearman's rank correlation test

To address the correlation between climate and *Plasmodium* infection, the newly data was concatenated with those by Laporta et al. (2015). Decision was guided by: (1) field collections were conducted in the same localities; (2) data set included collections from two periods of time; (3) data was more robust for testing the hypothesis. Data was concatenated considering the collection date and time; the collection methods (CDC or Shannon); mosquito species (in Laporta et al. 2015) other Anophelinae besides *Ke. cruzii* were tested for *Plasmodium* infection); and number of infected mosquitoes (Additional File 1). Correlations among response variables (number of mosquitoes infected by *P. vivax* or *P. falciparum*) and climate explanatory variables, we applied Spearman's rank correlation (rho). Explanatory variables are described in Table 3. Scatterplots were produced to aid data visualization (Additional File 2).

Table 3
Description of the variables employed in the Spearman correlation test.

Explanatory variables	Description
Tmaxdia	Maximum temperature in the day of collection
Tmaxm4	Average maximum temperature in the previous 4 days before collection
Tmindia	Minimum temperature in the day of collection
Tminm4	Average minimum temperature in the previous 4 days before collection
Prec7	Cumulative rainfall during the week of the collection
Prec14	Cumulative rainfall 14 days before the collection
URdia	Relative humidity at collection day
URm4	Average relative humidity 4 days before the collection

2.4.2. Bayesian multinomial regression approach– confirmatory analyses

Two ordered multinomial probit models were applied with the following response variables: (1) the number of mosquitoes infected by *P. falciparum* per day of collection and (2) the number of mosquitoes infected by *P. vivax* per day of collection in function of important explanatory variables. Important explanatory variables were selected from the Spearman correlation testing when the *p*-values of rho were lower than 0.2.

The expected count of infected mosquitoes under a given explanatory variable is estimated by:

$$E[w_i \mid x_i] = \sum_{j=1}^{J} u_j \left[\Phi(b_j - x_i^T\beta) - \Phi(b_{j-1} - x_i^T\beta)\right]$$

where w_i = the response variable, x_i = the explanatory variables, u_j = the ordered unique values of w_i, $\Phi()$ = the cumulative density function of a standard normal distribution, b = breaks to be estimated, $b_j = \infty$, $b_0 = -\infty$, x_i^T = explanatory variables and 1 as intercept, β = regression coefficients.

The number of iterations used for the Gibbs sampler was 10,000. The number of iterations discarded as burn-in was 5000. The prior variance for regression coefficients was equal to 1. The associated R code for reproducing theses analyses can be found in (Valle et al. 2019).

Table 4
Binomial test results.

Landscape	Positive (k)	Tested (n)	(P)	1-P
Sítio Itapuan	3	439	0.13	0.785
Esteiro do Morro	0	306	0.342	0.657
Tapiraí	3	974	0.2187	0.967
Inside forest	1	384	0.18	0.99
Forest border	5	1335	0.351	0.7398

3. Results

One thousand nineteen mosquitoes were tested for *Plasmodium* infection and a total of 6 were found infected with *Plasmodium vivax/simium*, 3 collected in Tapiraí and 3 in Sítio Itapuan. The overall prevalence of infected mosquitoes was 0.34%. One infected mosquito was collected inside the forest and 5 were collected at forest edge environment.

Results of the binomial test showed that *Plasmodium* infection in *Ke. cruzii* was randomly distributed in the landscape. The correlation between both landscapes and collection localities was not significant (Table 4).

The results from the Spearman correlation test for *P. vivax* and *P. falciparum* are in Table 5. Accordingly, only the maximum daily temperature (Tmaxdia) was correlated with the probability of finding mosquitoes infected with *P. falciparum*, and the accumulated precipitation one-week before (Prec7) and two-weeks before (Prec14) were associated with *Plasmodium vivax* infection.

The response variables (Table 6), i. e., number of mosquitoes infected with *P. falciparum* or *P. vivax* per collection date, were: (1) over dispersed employing the Poisson model (sd / mean = 2.66 – *P. vivax*; sd / mean = 4.37 – *P. falciparum*), and (2) zero-inflated (85% of 0 s per collection – *P. vivax*; 89% - *P. falciparum*). Thus, a confirmatory analysis was employed with a multinomial regression method for overdispersion and zero-inflated data.

Accordingly, the Bayesian multinomial regression approach was applied (Table 7). After the application of this method, the only variable that remained statistically significant was the maximum daily temperature (Tmaxdia). This variable showed association with mosquitoes infected with *P. falciparum*. The estimation of its coefficient means that for each increase of 1 °Celsius in the maximum daily temperature, the number of *Ke. cruzii* infected with *P. falciparum* increased by up to 1 unit (i.e., one *P. falciparum*-infected *Ke. cruzii*). None of the variables tested was associated with the probability of finding *Ke. cruzii* infected with *P. vivax* in the landscapes studied.

4. Discussion

Kerteszia cruzii is a phytotelmata bromeliad mosquito that occur in the areas of the Atlantic Forest (Marques et al. 2012). The species has been found naturally infected with *P. vivax/P. simium*, *P. malariae*, and recently with *P. falciparum* Apicomplexa parasites (Duarte et al. 2013; Kirchgatter et al. 2014; Laporta et al. 2015; Buery et al. 2018). Results of this study corroborate the relevance of *Ke. cruzii* in the maintenance of *Plasmodium* propagation in areas of the Atlantic tropical rain forest. The sampling localities for this study were previously investigates by Laporta et al. (2015). Distinctly from the mentioned study, *Ke. cruzii* was not found infected with *P. falciparum*.

The spatiotemporal distribution of malaria distribution is highly variable, primarily in areas with low transmission rate as in Atlantic forest (Ernst et al. 2006; Alemu et al. 2013; Xia et al. 2015; Rejeki et al. 2019). Malaria prevalence in this region is low, with most infections either asymptomatic or oligosymptomatic. Human infection is local and associated with ecological tourism and human encroachment in forest areas with presence of *Ke. cruzii* (de Pina-Costa et al. 2014). Variation in the number of malaria cases fluctuate depending on the introduction of either humans or non-humans primates hosts of *Plasmodium* in an area (Sallum et al. 2019). Consequently, it is plausible to hypothesize that the incidence of malaria can vary depending on the introduction of parasites, presence of susceptible hosts and vectors, and the contact rate between *Ke. cruzii* vector and humans. In a forest reserve in the city of São Paulo, Brazil, Medeiros-Sousa et al. (2019) showed that increased abundance in *Ke. cruzii* depends on the proportion of forest cover, whereas the total forest-edge length increases the activity of this species at ground level. Thus, anthropogenic changes in the Atlantic Forest landscape can cause a reduction in the abundance of *Ke. cruzii*. However, these changes can lead to increased contact rate between vectors, simians and humans living in areas bordering the edge of forests, or by entering the forest environment.

The spatial distribution of malaria is complex and dynamics. The transmission cycle involves several components, including those from environment, human hosts, mosquito vectors, and *Plasmodium* (Cohen et al. 2017). Tropical forests landscapes are considered hot beds for transmission because they provide adequate conditions for *Plasmodium* dispersion. For example, local proportion of forest cover, temperature, rainfall and humidity determine the spatiotemporal distribution and

Table 5
Results of Spearman's rank correlation for each climate variable tested for *Plasmodium falciparum* and *P. vivax/simium* in the concatenated data with Laporta et al. (2015).

Plasmodium falciparum			*Plasmodium vivax/simium*		
Variable	Spearman rho	P	Variable	Spearman rho	P
Tmaxdia	0.46	**< 0.05**	Tmaxdia	0.14	0.5
Tmaxm4	0.07	0.73	Tmaxm4	−0.1	0.63
Tmindia	0.3	0.15	Tmindia	−0.06	0.77
Tminm4	0.23	0.26	Tminm4	−0.05	0.81
Prec7	0.31	0.13	Prec7	0.51	**< 0.01**
Prec14	0.25	0.22	Prec14	0.46	**< 0.05**
URdia	0.32	0.12	URdia	−0.11	0.58
URm4	−0.21	0.3	URm4	−0.19	0.37

Statistically significant *P* values are in bold.

Table 6
Collection date and number of Anophelinae mosquitoes infected with *Plasmodium vivax/simium* (Pv) and *P. falciparum* (Pf), respectively, in the concatenated data with Laporta et al. (2015).

Collection	Date	Pv	Pf
1	13 Aug 2012	0	0
2	14 Aug 2012	0	0
3	15 Aug 2012	0	0
4	16 Aug 2012	0	0
5	20 Aug 2012	0	0
6	21 Aug 2012	0	0
7	22 Aug 2012	0	0
8	23 aug 2012	0	0
9	12 Sept 2012	0	0
10	17 Out 2012	0	2
11	19 July 2016	1	0
12	20 July 2016	2	0
13	21 July 2016	0	0
14	28 Sept 2016	0	0
15	7 Nov 2016	0	0
16	5 Dec 2016	0	0
17	27 Aug 2012	0	0
18	28 Aug 2012	0	0
19	29 Aug 2012	0	0
20	19 Sept 2012	0	0
21	24 Out 2012	0	1
22	28 Nov 2012	4	18
23	7 Sept 2016	0	0
24	8 Nov 2016	0	0
25	8 Mar 2017	0	0
26	4 Dec 2017	3	0

Table 7
Results from the Bayesian multinomial regression derived from the concatenated data with Laporta et al. (2015). The table shows the effect of each climate variable in *Plasmodium* infectivity in Anophelinae mosquitoes.

Plasmodium falciparum			*Plasmodium vivax/simium*		
Explanatory variable	CI 0.025-0.975	Interpretation	Explanatory variable	CI 0.025-0.975	Interpretation
Tmaxdia	0.1, 1,16	Positive effect	Tmaxdia	0, 0.49	No clear effect
Tmaxm4	−1.08, 0	No clear effect	Tmaxm4	−0.4, 0.04	No clear effect
Tmindia	−0.04, 0.85	No clear effect	Tmindia	−0.5, 0	No clear effect
Prec7	0, 0	No clear effect	Prec7	−0.24, 0.01	No clear effect
Prec14	0, 0	No clear effect	Prec14	0, 0.04	No clear effect
URdia	0, 0	No clear effect	URdia	0, 0.03	No clear effect
URm4	−0.1, 0	No clear effect	URm4	0, 0	No clear effect

survival of *Plasmodium* vectors, and the human-vector contact rate (de Pina-Costa et al. 2014; Kar et al. 2014; Medeiros-Sousa et al. 2019). Variations on temperature, precipitation, land use, distribution of mosquito habitats are associated to prevalence of vectors, whereas temperature range modifies the extrinsic incubation period of the *Plasmodium* parasite (Stresman et al. 2019).

There are several studies showing the influence of climate in the dynamics of malaria transmission (Huang et al. 2011; Diouf et al. 2013; Arab et al. 2014; Sena et al. 2015). Climate factors can directly influence malaria because higher temperature can decrease the time necessary for the development of the vectors (Chu et al. 2019), increase their survival rate, and increase the risk of pathogen transmission. In temperatures higher that 30 °C, the parasite survival decreases (Shapiro et al. 2017). Additionally, climate may indirectly influencing malaria by changing vegetation, landscape physiognomy and spatial distribution of vector habitats (Diouf et al. 2013). Many studies show a positive correlation between malaria and rainfall, but a negative correlation with temperature (Hasyim et al. 2018; Hurtado et al. 2018; Hussien 2019).

In Brazil. there is a paucity of studies associating climate and malaria. Thus, herein, the predictor positively and strongly associated with the occurrence of infected mosquitoes with *P. falciparum* was the maximum temperature on the collection day, and no climates variables tested here were associated with *P. vivax*. Considering that most *P. falciparum* infected mosquitoes were collected in Tapiraí, where the forest cover is about 65% (Laporta et al. 2015) and temperatures rarely exceed 30 °C, our results can be a consequence of mosquito activity and parasite development. A recent study with *Anopheles gambiae* demonstrated that daily temperatures between 20 and 35 °C are ideal for development of the mosquito, and the mosquitoes became active in temperatures above 10 °C and sedentary when temperatures reach 35 °C (Mukhtar et al. 2019). Additionally, *P. vivax* could develop in lower temperatures (16 °C ~18 °C), while the development of *P. falciparum* would be possible in temperatures ≥18 °C (Mironova et al. 2019).

Recently, phylogenetic analyses on mitochondrial, apicoplast, and nuclear genes indicated that *P. falciparum* evolved from an ancestor form of *Plasmodium* that circulated in gorillas in Western Africa. Probably, a speciation event originated *P. prefalciparum* and *P. falciparum* in a single evolutionary process (Liu et al. 2010). Due to the low mutation rate present in human lineages of *P. falciparum*, it is possible that this process is recent, and this event occurred in the last 10,000 years (Sundararaman et al. 2016). Due to the phylogenetic similarity, both *P. prefalciparum* and *P. falciparum* were placed in the Laverania subgenus (Liu et al. 2010). Subsequently, studies showed that at least six species encompassed in Laverania are genetically close to the *P. falciparum* human strains and were found infecting both chimpanzees and gorillas (Liu et al. 2016). Therefore, it is plausible to assume that the southeast Brazilian lineages of *P. falciparum* may be distinct from the original lineages from Western Africa. Considering that the initiators and the technique used in this study differ from those used by Laporta et al. (2015), we can assume that perhaps the positive samples of the latter belong to a close form of *P. falciparum* that the primers used in the present study did not amplify.

Although we have not found any association between landscape and *P. vivax / P. simium* infection in *Ke. cruzii*, other studies have shown how landscape can modulate malaria epidemiology. In the Brazilian Amazon and Malaysia, malaria caused by *P. falciparum* and *P. knowlesi*, respectively, are highly affected by deforestation (Fornace et al. 2016; Chaves et al. 2018).

In the same region studied herein, in southeastern Brazil, Laporta et al. (2015) found a positive association between infected *Ke. cruzii* and areas with higher level of forest cover. Even though, we did not find a statistically significant correlation between landscape and infection by *P. vivax / P. simium*, it was possible to observe that infected mosquitoes were collected in areas with higher proportion of forest cover. Notwithstanding, five of the six mosquitoes infected with *P. vivax / P. simium* were collected on forest preserved areas, suggesting that transmission is enhanced in these areas.

5. Conclusions

Findings of this study confirm the public health importance of *Ke. cruzii*, on the coastal Atlantic tropical rain forest, southeastern Brazil. The maximum daily temperature showed the strongest association with circulation of *P. falciparum* in the area, partially explaining the absence of *P. falciparum* in our samples. Although we have not found association between *Ke. cruzii* infectivity and level of forest cover, most of the infected mosquitoes identified here were collected on the border of the forest. This finding shows that the presence of *Ke. cruzii* in the ecotone may facilitate malaria transmission among monkeys and humans.

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Declaration of Competing Interest

We declare that we don't have any competing interests.

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