

Habitat suitability of *Anopheles* vector species and association with human malaria in the Atlantic Forest in south-eastern Brazil

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Every year, autochthonous cases of *Plasmodium vivax* malaria occur in low-endemicity areas of Vale do Ribeira in the south-eastern part of the Atlantic Forest, state of São Paulo, where *Anopheles cruzii* and *Anopheles bellator* are considered the primary vectors. However, other species in the subgenus *Nyssorhynchus* of *Anopheles* (e.g., *Anopheles marajoara*) are abundant and may participate in the dynamics of malarial transmission in that region. The objectives of the present study were to assess the spatial distribution of *An. cruzii*, *An. bellator* and *An. marajoara* and to associate the presence of these species with malaria cases in the municipalities of the Vale do Ribeira. Potential habitat suitability modelling was applied to determine both the spatial distribution of *An. cruzii*, *An. bellator* and *An. marajoara* and to establish the density of each species. Poisson regression was utilized to associate malaria cases with estimated vector densities. As a result, *An. cruzii* was correlated with the forested slopes of the Serra do Mar, *An. bellator* with the coastal plain and *An. marajoara* with the deforested areas. Moreover, both *An. marajoara* and *An. cruzii* were positively associated with malaria cases. Considering that *An. marajoara* was demonstrated to be a primary vector of human *Plasmodium* in the rural areas of the state of Amapá, more attention should be given to the species in the deforested areas of the Atlantic Forest, where it might be a secondary vector.

Key words: Atlantic Forest - disease vectors - spatial distribution - *vivax* malaria

Land use change, deforestation and agricultural expansion are human modifications of the natural environment, which have had direct effects on the exacerbation of malaria in the Amazon Basin (Castro et al. 2006, Victor et al. 2006, Olson et al. 2010). Thousands of malaria cases occur annually in areas of active transmission in Brazil; however, a few autochthonous cases are reported in localities outside the Amazon Region. In the forested areas of the Atlantic coast, malarial transmission is low and irregular (Oliveira-Ferreira et al. 2010). Couto et al. (2010) reported that 444 cases of *Plasmodium vivax*-malaria occurred in the Vale do Ribeira on the south-eastern coast of the state of São Paulo (SP) between 1980-2007. Moreover, nonhuman primates (e.g., *Alouatta guariba clamitans* Cabrera) were found to be infected by *P. vivax*, *Plasmodium malariae* and *Plasmodium falciparum* (Curado et al. 2006, Duarte et al. 2008) in that region. In the forested areas of Vale do Ribeira, *Anopheles (Kerteszia) cruzii* Dyar and Knab and *Anopheles (Kerteszia) bellator* Dyar and Knab are vectors of malaria parasites (Forattini et al. 1999, Marrelli et al. 2007). In considering

that the presence of *An. cruzii* and *An. bellator* may be associated with *vivax* malaria transmission in the Atlantic Forest, it would be important to determine the potential spatial distribution of both vector species, mainly in under-sampled areas of the Vale do Ribeira.

Species of the subgenus *Nyssorhynchus* may also be involved in the dynamics of malarial transmission in areas outside the Amazon Region. Among the *Nyssorhynchus* species, it is worth mentioning *Anopheles marajoara* Galvão and Damasceno of the *Anopheles albitarsis* complex because this species is a primary vector of the human *Plasmodium* sp. in Macapá, state of Amapá, north-eastern Amazon. In that region, *An. marajoara* has replaced *Anopheles darlingi* Root as the primary vector and that event was associated with changes in the land use (Conn et al. 2002). Furthermore, species of the *An. albitarsis* complex may be involved in the dynamics of malarial transmission in certain areas of the Atlantic Forest in south-eastern Brazil. For example, *An. marajoara* was the primary vector in the municipality of Iguape, Vale do Ribeira, in the 1940s (Schiavi 1945). The author pointed out that Iguape did not provide any suitable marshy habitat for the immature members of this species and also suggested that the adults emerged from the larval habitats in Ilha Comprida, which is separated from the continent by an estuarine complex (i.e., Mar Pequeno). Considering the epidemiological importance of the species, Correa et al. (1950) conducted a mark-release-recapture experiment to verify the dispersion of *Anopheles albitarsis domesticus* (= *An. marajoara*) between Ilha Comprida and Iguape and *vice-versa*. Marked specimens released in Iguape were found in Ilha

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Comprida, whereas specimens from Ilha Comprida were captured in Iguape. Wilkerson et al. (1995), employing randomly amplified polymorphic DNA polymerase chain reaction, confirmed the presence of *An. marajoara* in Ilha Comprida, whereas *An. albitarsis* s.s. and *An. albitarsis* B (i.e., *Anopheles oryzalimnetes* Wilkerson and Motoki) were found in the municipality of Registro, in the Vale do Ribeira (Motoki et al. 2009). *An. marajoara* was the only species of the *An. albitarsis* complex present in Ilha Comprida, which corroborates with Schiavi (1945) and Correa et al. (1950). However, the distribution of *An. marajoara* in other areas of the Vale do Ribeira is unknown mainly because of under-sampling as well as problematic species identification based mainly on morphological characteristics. Considering the importance of *An. marajoara* as a primary vector of human *Plasmodium* in Macapá (Conn et al. 2002), it is reasonable to assume that this species may be involved in the dynamics of human malarial transmission in areas outside the Amazon, such as in the Vale do Ribeira.

The distribution of *An. marajoara* may have been underestimated by Wilkerson et al. (1995) because some areas of the Vale do Ribeira possess adequate habitat and ecological conditions for the proliferation of the *An. albitarsis* species complex. For example, Forattini et al. (1995) demonstrated that irrigated rice fields provide a suitable habitat for the immature *An. albitarsis* l.s. Consequently, to establish the potential distribution of *An. marajoara* in the Vale do Ribeira, it may be helpful to understand the spatial dynamics of human malaria in areas of low-endemicity in the Atlantic Forest.

Considering that the spatial distributions of the five primary vectors of human *Plasmodium* are not completely known in Africa, Ayala et al. (2009) applied predictive distribution modelling based on the presence of a species for constructing habitat suitability maps for *Anopheles gambiae* Giles, *Anopheles funestus* Giles, *Anopheles arabiensis* Patton, *Anopheles nili* (Theobald), and *Anopheles moucheti* Evans in Cameroon. *An. gam-*

biae and *An. funestus* were ecologically more tolerant, whereas *An. arabiensis*, *An. moucheti* and *An. nili* were more specialized. A similar approach was employed in the Malaria Atlas Project to establish the potential distributions of the dominant mosquito vector species of human *Plasmodium* (Hay et al. 2010, Sinka et al. 2010).

Considering that the majority of the Atlantic Forest is under-sampled for mosquito assemblages, ecologic niche modelling is a powerful tool that can both predict the potential spatial distribution of the vectors and assess the potential risk of exposure to human *Plasmodium* sp. circulating in that region. The objectives of the present study are: (i) to apply habitat suitability models to predict the potential spatial distribution of *An. bellator*, *An. cruzii* and *An. marajoara*; (ii) to associate autochthonous cases of human malaria with the potential spatial distribution of these mosquitoes in the Vale do Ribeira in south-eastern Atlantic Forest, Brazil.

MATERIALS AND METHODS

The study encompassed 23 municipalities of the Vale do Ribeira, which occupies 11,635 km² within the Serra do Mar in south-eastern Brazil (Fig. 1). The region comprises the largest continuous preserved forest (approximately 1,100 million ha) in the Atlantic Forest biome (Ribeiro et al. 2009), with a hydrographic estuarine system that is intermixed with mosaics of human occupation, as well as a rich assemblage of mosquito species (Forattini & Massad 1998). The climate is characterized as humid subtropical (*Cfa* in the Köppen classification).

Adult mosquito sampling was carried out monthly from May 1996-April 2000 in nine collection sites (Fig. 1) totaling 108 sampling units. To determine the habitat suitability for *An. cruzii*, *An. bellator* and *An. marajoara*, the total number of individuals of each species collected in the Shannon traps each month at each collection site was used as a single measurement. These values were further correlated to abiotic and biotic variables to assess the predictors of potential habitat suitability.

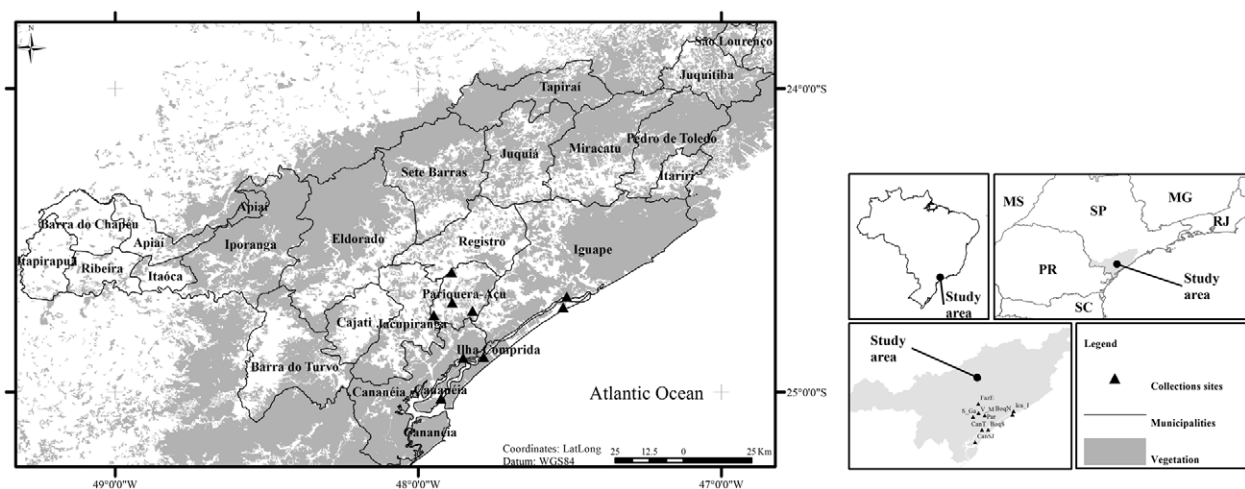


Fig. 1: twenty three municipalities of the study area Vale do Ribeira, south-eastern of the state of São Paulo, Brazil. The region is mainly covered with forest remnants in the Atlantic Forest biome. Mosquito collections were performed from nine sites. MG: state of Minas Gerais; MS: state of Mato Grosso do Sul; PR: state of Paraná; RJ: state of Rio de Janeiro; SC: state of Santa Catarina; SP: state of São Paulo.

Terrain slope, bioclimatic variables and vegetation cover were the habitat variables that were quantified for each collection site by applying distinct routines in the GRASS 6.4 GIS software (grass.osgeo.org). These variables were assumed to represent ecologic niches because each species is potentially adapted for specific ranges of the habitat variables. The topographically variable terrain slope, derived from the Shuttle Radar Topography Mission (SRTM) 1.4 elevation map, was interpolated to a 30-meter spatial resolution, which was assumed to be the home range of a mosquito population. Climate data from 1950-2000 with a 900-meter spatial resolution were acquired from the WorldClim database (worldclim.org). For the purpose of the analyses, the average maximum temperature in the warmest month was defined as Bio5, and the average annual precipitation as Bio12. Although the area encompassed in a 900-meter survey is bigger than that assumed for the mosquito population, the climate variables represented an accurate approximation for the study area. The accuracy was assessed by comparing Bio5 and Bio12 estimates to the values obtained from the local meteorological stations in the municipalities of Registro and Pariquera-Açu (cuiagro.sp.gov.br). The landscapes were characterized by the percentage of vegetation cover estimated within a 200-meter radius around each sampling site. A similar approach was used for chironomids (Roque et al. 2010), birds (Boscolo & Metzger 2009) and carnivore mammals (Lyra-Jorge et al. 2010) in the fragmented landscapes of SP. A natural vegetation map was derived from the visual interpretation of a 30-meter spatial resolution Landsat-TM imagery (Kronka et al. 2005).

To select statistically significant abiotic and biotic variables, which can explain the potential habitat suitability of each species, a preliminary analysis was performed. In this work, the number of individuals of *An. bellator*, *An. cruzii* and *An. marajoara* were defined as the dependent variables, whereas terrain slope, Bio5, Bio12 and vegetation cover were the independent variables. Subsequently, negative binomial regressions were run with the software R-2.9.2 (r-project.org), with the MASS package, to determine if the contribution of each variable was significant ($p < 0.15$).

Using the variables selected in the aforementioned steps, the potential habitat suitability for *An. cruzii*, *An. bellator* and *An. marajoara* was estimated by employing the MAXENT algorithm (Phillips et al. 2006) implemented in the openModeller 1.1.0 software (openmodeller.sourceforge.net). Firstly, the MAXENT was used to estimate the coefficient value of the presence of a mosquito species as predicted by the prevailing abiotic and biotic variables. Then the coefficient values were used in 500-iteration runs to estimate the maximum likelihood (ML) of an expected binomial distribution (0, pseudo-absence; 1, presence). The ML probability of the presence of a species was estimated for each collection site, which represented the potential habitat suitability of this species. These values varied from 0% for an unsuitable habitat to 100% for suitable habitats and were used to construct maps with a 30-meter spatial resolution that covered the entire Vale do Ribeira region. The receiver

operating characteristic (ROC) method was used to validate the potential habitat suitability maps, which were generated under stochastic modelling. The area under the ROC curve (AUC) provides a measure of accuracy (Fielding & Bell 1997). The AUC values varied from 0-1, where 0 meant that the model failed to establish the potential habitat suitability of a species, while a score of 1 indicated that the selected model was accurate. An AUC value of 0.9 was used as the cut-off for determining model adequacy (Metz 1986). In considering that a given species did not have a random spatial distribution because its habitat was not randomly distributed (Hanski 1994), a second validation process was performed based on the concept of a metapopulation structure (Soberón 2007). To do this validation, a Poisson distribution model was used. A species potential distribution map was considered as validated when the potential habitat suitability of a specific collection site was higher than 80%. An 80% cut-off was used as a standard because it represented those localities in which the species reached the optimum habitat and thus could be involved in the transmission of *Plasmodium*.

The terrain slope, Bio5, Bio12, vegetation cover and potential habitat suitability of *An. bellator*, *An. cruzii* and *An. marajoara* data were extrapolated for the entire region of the Vale do Ribeira. The extrapolation was conducted by correlating potential habitat suitability with abiotic and biotic variables, which were aggregated for each municipality. The mean pixel values of each variable were estimated by using the spatial analyst extension of ArcMap 10 software (esri.com). The Spearman's rank test was used to estimate the correlation coefficient between the pixel mean of potential habitat suitability (dependent variable) and each abiotic and biotic variable ($p < 0.10$).

Finally, an association between the prevalence of mosquito bites and malaria cases was assessed. Prevalence to mosquito bites was assumed to be positively linked to the potential habitat suitability values. Poisson regressions were applied using the numbers of autochthonous cases of malaria that were registered for each municipality from 1996-2000 as dependent variables. The number of malaria cases was obtained from Couto et al. (2010). Potential habitat suitability values aggregated per municipality were transformed into a binary variable (0 = absence; 1 = presence) for each species and later used as independent variables. A species was considered present in a specific locality when the value of the habitat suitability was $\geq 80\%$, whereas a $< 80\%$ cut-off value meant absence. Cases of malaria were tested to ascertain whether they had a Poisson distribution using the Moran Global Autocorrelation test. Considering that malaria cases have a Poisson distribution, the regression coefficients were estimated using a generalized linear model to estimate the prevalence ratio (PR) of the disease associated with multiple independent variables. The variables were altered into dummy variables as follow: $X_1 = An. bellator$ (model 1); $X_2 = An. cruzii$ (model 2); $X_3 = An. marajoara$ (model 3); $X_4 =$ both *An. bellator* and *An. cruzii* (model 4); $X_5 =$ both *An. bellator* and *An. marajoara* (model 5); $X_6 =$ both *An. cruzii* and *An. marajoara* (model 6); $X_7 =$

An. bellator, *An. cruzii* and *An. marajoara* (model 7). The PR and the confidence interval (95%) were estimated in the software R-2.9.2 with the *epicalc* package to identify mosquito species that were positively associated with malaria (PR > 1; p < 0.05).

RESULTS

A total of 8,288 females of *An. bellator*, *An. cruzii* and *An. marajoara* were captured in Shannon traps in nine localities of the Vale do Ribeira. Collection sites, mosquito species and both abiotic and biotic variables are shown in Table I. The mean and standard deviations of the number of each type of mosquito collected per

month are in the Supplementary data. The total number of mosquitoes showed a negative binomial distribution in which abiotic and biotic variables were added to estimate the coefficients of the univariate regressions. The significant coefficient indicated that the terrain slope and vegetation cover (*An. cruzii*), Bio12 and vegetation cover (*An. bellator*) and Bio5 and vegetation cover (*An. marajoara*) were the most explicative variables; thus they were selected for the potential habitat suitability modelling for each species (Table II).

Potential habitat suitability maps are shown in the Supplementary data for *An. bellator* (range: 4-88%, AUC: 0.95), *An. cruzii* (range: 51-97%, AUC: 0.91) and

TABLE I
Collection sites, mosquito species and abiotic and biotic variables in the south-eastern Atlantic Forest of Brazil

Sites	<i>Anopheles bellator</i>	<i>Anopheles cruzii</i>	<i>Anopheles marajoara</i>	Terrain slope (°)	Average annual precipitation (mm)	Average maximum temperature in the warmest month (°C)	Vegetation cover (%)
BoqN	18	194	126 ^a	0	2,116	29.9	15
BoqS	1,796 ^a	509	21	1	2,279	30.5	64
CanSJ	523 ^a	454	4	9	2,469	30.6	27
CanT	316	2,751 ^a	0	13	2,272	30.2	77
FazE	0	352	9	2	1,915	30.9	3
S_Ga	11	611	0	4	2,043	30.5	81
Ica_I	36	543	0	0	2,096	29.5	93
Par	0	14	0	1	2,060	30.6	0
V_M	0	0	0	0	2,012	30.8	0

a: higher mosquito frequency as expected in a Poisson distribution. These collections sites were herein considered as the source of a mosquito-specific species, i.e., a location with ecological characteristics that positively influence the population growth. These localities were the control sites utilized for the validation of potential habitat suitability modelling. Mosquito collection sites: BoqN: Ilha Comprida (North); BoqS: Ilha Comprida (South); CanSJ: Cananéia; CanT: Sítio Itapuã; FazE: Fazenda Experimental; S_Ga: Sítio Galiléia; Ica_I: Icapara; Par: Pariquerá-Mirim; V_M: Vila Maria.

TABLE II
Preliminary analysis: equations of negative binomial regression models and significance of coefficient β_1 of the independent variable X_1 (p < 0.15)

Species	Terrain slope (°)	Average annual precipitation (mm)	Average maximum temperature in the warmest month (°C)	Vegetation cover (%)
<i>Anopheles bellator</i> Coefficient β_1	5.59 + 0.03 X_1 z = 0.17, p < 0.87 ^a	-7.09 + 0.006 X_1 z = 49.88, p < 0.01	-45.29 + 0.17 X_1 z = 0.86, p < 0.40 ^a	3.20 + 0.05 X_1 z = 2.37, p < 0.02
<i>Anopheles cruzii</i> Coefficient β_1	5.39 + 0.18 X_1 z = 69.47, p < 0.01	-1.80 + 0.004 X_1 z = 1.27, p < 0.21 ^a	44.83 - 0.13 X_1 z = -1.01, p < 0.28 ^a	4.88 + 0.03 X_1 z = 0.03, p < 0.02
<i>Anopheles marajoara</i> Coefficient β_1	3.36 - 0.34 X_1 z = -1.35, p < 0.18 ^a	4.16 - 0.001 X_1 z = -0.10, p < 0.92 ^a	49.77 - 0.16 X_1 z = -9.5, p < 0.001	3.40 - 0.04 X_1 z = -1.56, p < 0.12

a: this variable was not selected to potential habitat suitability modelling of the given species (p > 0.15). X_1 : independent variable *An. bellator*, *An. cruzii* and *An. marajoara*. The results printed in bold represent significant outcomes under the null hypotheses ($B_1 = 0$; p < 0.15).

An. marajoara (range: 3-95%, AUC: 0.95). Collections sites in which the numbers of mosquitoes were higher than that expected within a Poisson distribution were then assumed to be population sources. Boqueirão Sul and Morro do São João (*An. bellator*), Sítio Itapuã (*An. cruzii*) and Boqueirão Norte (*An. marajoara*) (Table I) showed $\geq 80\%$ potential habitat suitability for *An. bellator* (Fig. 2A), *An. cruzii* (Fig. 2B) and *An. marajoara* (Fig. 2C). Abiotic and biotic values varied from 0-50° (terrain slope), 1,400-2,792 mm (Bio12), 22.3-31.4°C (Bio5) and 0-100% (vegetation cover). The higher values were 13° of terrain slope in Iporanga, 2,411 mm of Bio12 in Cananéia, 30.7°C of Bio5 in Registro and 94% of vegetation cover in Tapiraí. *An. cruzii* potential habitat suitability was positively correlated to terrain slope ($\rho = 0.93$, $p < 0.001$, $df = 21$), high vegetation cover (e.g., 86% in Iporanga), low Bio12 (e.g., 1,602 mm in Iporanga) and Bio5 (e.g., 25.6°C in Juquitiba). *An. bellator* was posi-

tively correlated to Bio5 ($\rho = 0.62$, $p < 0.002$, $df = 21$) and Bio12 ($\rho = 0.76$, $p < 0.001$, $df = 21$) and negatively correlated to terrain slope ($\rho = -0.43$, $p < 0.05$, $df = 21$). *An. marajoara* was positively correlated to Bio5 ($\rho = 0.93$, $p < 0.001$, $df = 21$) and negatively correlated to vegetation cover ($\rho = -0.35$; $p < 0.10$, $df = 21$).

Malaria cases followed a Poisson distribution in which spatial autocorrelation was not significant (Moran Z score = 1.71, $p < 0.09$). The disease was distributed by municipalities with the following frequencies: one case each in Cananéia, Pariqueira-Açu, Registro, Juquiá and Tapiraí, two cases each in Iguape and Pedro de Toledo, three in Miracatu and eight in Sete Barras (Supplementary data). Associations between malaria and dummy variables showed that models 1, 4, 5 and 7, which include *An. bellator* as a vector, were not significant. On the other hand, models 3 (= malaria cases ~ *An. marajoara*) and 6 (= malaria cases ~ both *An. cruzii* and *An. marajoara*) were significant, showing associations between exposure to those mosquitoes and disease. The values obtained were 12.21 (1.64-91.24, $p < 0.02$) and 4.36 (1.46-13.05, $p < 0.01$), respectively (Table III).

DISCUSSION

Potential habitat suitability modelling revealed that the spatial distribution of *An. cruzii* is correlated with areas of ombrophilous dense forest that are present in the mountainous areas of the Vale do Ribeira (Fig. 2B), whereas *An. bellator* spatial distribution was determined by high Bio5 and Bio12 on the coastal low lands where the vegetation is the Atlantic Coast Restinga forest (Fig. 2A). Moreover, *An. marajoara* spatial distribution is correlated with high Bio5 and open areas of the Restinga or deforested lands associated with transformations of the natural ecosystem (Fig. 2C) (Forattini et al. 1995, Conn et al. 2002). Although differences among the values of

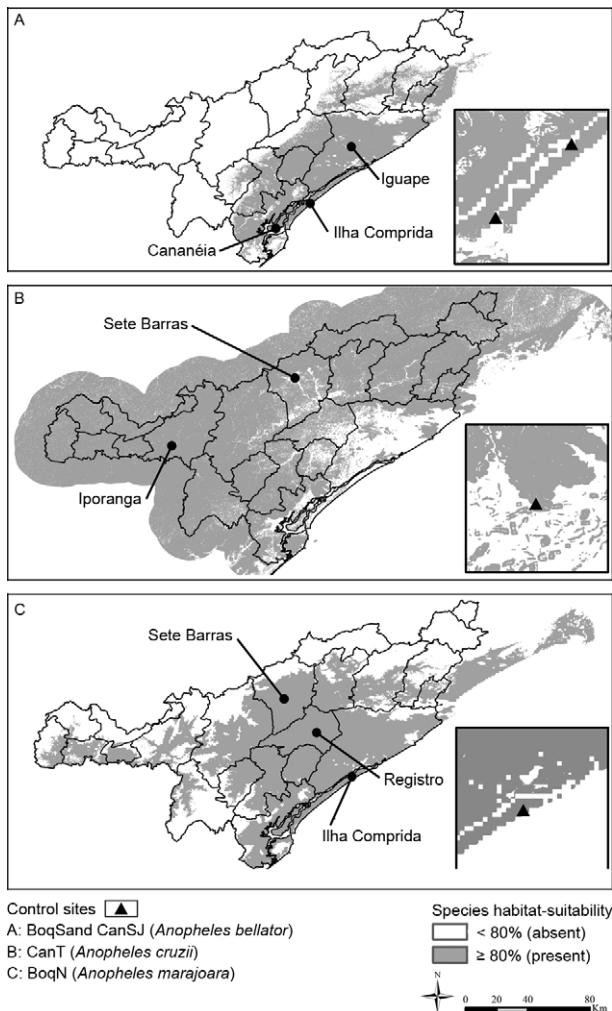


Fig. 2: binary potential habitat suitability maps (control sites have potential habitat suitability $\geq 80\%$). A: *Anopheles bellator* [area under the receiver operating characteristic curve (AUC) 0.95, range 4-88%]; B: *Anopheles cruzii* (AUC 0.91, range 51-97%); C: *Anopheles marajoara* (AUC 0.95, range 3-95%).

TABLE III

Poisson regression of malaria cases in function of binary potential habitat suitability $\geq 80\%$ (presence)/ $< 80\%$ (absence)

Models (malaria cases ~ Poisson)	PR (95% CI)	p (Wald's test)
1: ($\lambda = e^{-0.31+0.43 \times X_1}$)	1.53 (0.64-3.7)	$p < 0.35$
2: ($\lambda = e^{-0.00-0.16 \times X_2}$)	0.85 (0.25-2.9)	$p < 0.80$
3: ($\lambda = e^{-2.2+2.50 \times X_3}$) ^a	12.21 (1.64-91.24)	$p < 0.02$
4: ($\lambda = e^{-0.25+0.43 \times X_4}$)	1.54 (0.59-4.01)	$p < 0.39$
5: ($\lambda = e^{-0.31+0.43 \times X_5}$)	1.53 (0.64-3.7)	$p < 0.35$
6: ($\lambda = e^{-1.01+1.47 \times X_6}$) ^a	4.36 (1.46-13.05)	$p < 0.01$
7: ($\lambda = e^{-0.25+0.43 \times X_7}$)	1.54 (0.59-4.01)	$p < 0.39$

^a: significant model, prevalence ratio (PR) > 1 and ($p < 0.05$). Independent variables: X_1 : *Anopheles bellator*; X_2 : *Anopheles cruzii*; X_3 : *Anopheles marajoara*; X_4 : both *An. bellator* and *An. cruzii*; X_5 : both *An. bellator* and *An. marajoara*; X_6 : both *An. cruzii* and *An. marajoara*; X_7 : *An. bellator*, *An. cruzii* and *An. marajoara*; CI: confidence interval. The results printed in bold represent significant outcomes under the null hypotheses (PR = 1; $p < 0.05$).

Bio5 and Bio12 were low, they were observed to be significant factors for distribution for *An. bellator* and *An. marajoara* (Table II). These findings may be related to the coarse 900 meter-spatial resolution of these variables for both the target mosquito population and study area. Also the significance of Bio5 and Bio12 may represent an indirect association with distribution of both species.

The AUC is not the best validation of potential habitat suitability maps when several niche factors of a species are poorly known. However, even considering the biases on the AUC related to the extrapolation of the data beyond a clustered sampled site (Lobo et al. 2008), the potential habitat suitability maps obtained are consistent with both ecological characteristics (Veloso 1958, Forattini et al. 1999, Marrelli et al. 2007) and registers of the presence of *An. bellator*, *An. cruzii* and *An. marajoara* (Forattini & Massad 1998) in Vale do Ribeira. The potential habitat suitability maps were also validated by the probability values higher than 80% for species control sites (Fig. 2, Table I).

Results of the present study were to associate mosquito potential distribution with malaria cases; these results are of further interest because they show that the spatial distribution of *An. cruzii* and *An. bellator* do not overlap at a larger geographical scale. *An. cruzii* is present on the hills, whereas *An. bellator* is present along the coastal shore of Vale do Ribeira. This lack of range overlap could be the result of either chance distribution or the competitive exclusion described by Gause (1934) because several unknown ecologic niche dimensions determine their actual distribution. Additionally, the potential habitat suitability for *An. marajoara* indicates that this species has a broader potential spatial distribution than that hypothesized by Wilkerson et al. (1995) in the Vale do Ribeira. As a result, further studies will be necessary to validate the prediction distribution models generated herein. Finally, it would be important to sample mosquitoes in areas with a high probability of the presence of the species that have never been sampled.

Results of the Poisson regression suggest that the presence of *An. bellator* is not significantly associated with malaria (Table III). In contrast, this disease is significantly associated with *An. marajoara* and the presence of both *An. marajoara* and *An. cruzii* (Table III). These results are partially in accordance with the hypothesis of Curado et al. (2006) and Duarte et al. (2008), which suggest that in areas of the Atlantic Forest, humans can become infected with human *Plasmodium* when they invade the natural foci of *An. cruzii* and the *Alouatta* spp of monkeys, which serve as the reservoirs of *Plasmodium* sp. Moreover, Curado et al. (2006) performed enzyme immunosorbent assay tests and found a prevalence of antibodies against *P. vivax* in 63 (35%) and 155 (29.7%) humans from Parque Estadual Intervalas and Parque Estadual do Alto do Ribeira, respectively. Interestingly, Curado et al. (2006) showed that the majority of the captured mosquitoes were *An. cruzii* in both areas, which are in the municipality of Iporanga. The spatial distribution modelling analyses indicated that the potential habitat suitability for this species was 95% in this municipality. On the other hand, the large and sig-

nificant association between *An. marajoara* spatial distributions with cases of malaria was not in accordance with the hypothesis of Curado et al. (2006) and Duarte et al. (2008). This contradictory result might have occurred because these authors had not done their research in the municipality of Sete Barras, which had 40% of all autochthonous malaria cases occurring in Vale do Ribeira between 1996-2000 (Supplementary data). This underscores the need to sample mosquito assemblages from this municipality.

In considering the results obtained in the present study and the association between malaria cases and the potential spatial distribution of *An. marajoara*, we hypothesize that *An. marajoara* seems to be involved in the dynamics of malarial transmission in areas of the Vale do Ribeira. Cohuet et al. (2010) hypothesized that *Plasmodium* exerts selective pressure on its vectors in order to increase its own dissemination - shaping the vector to its own benefit. In this sense, it is probable that *Plasmodium* species is circulating in the Vale do Ribeira by keeping a sylvatic cycle that involves *Anopheles* (*Kerteszia*) mosquitoes and *Alouatta* monkeys as reservoirs and occasionally humans, as well as a cycle that involves humans and mosquito species of the subgenus *Nyssorhynchus* (e.g., *An. marajoara*). This species was demonstrated to be a competent vector in Vale do Ribeira in the 1940s (Schiavi 1945). Furthermore, Conn et al. (2002) showed that *An. marajoara* from Macapá became the primary vector of *Plasmodium* because of the increasing habitation of this species in non-forested and marshy lands used for agriculture, combined with the intensive human migration of infected individuals from other locations in the Amazon where transmissions were endemic.

In Vale do Ribeira, deforestation and changes in the land use may have affected the dynamics of malarial transmission, leading to a local succession of vector species of the subgenus *Kerteszia* and of the subgenus *Nyssorhynchus* (e.g., *An. marajoara*). In addition, adult populations of *An. albitarsis* l.s. are abundant and dominant in irrigated rice fields (Forattini et al. 1995), showing that these human-modified environments can create ecological conditions that favour species of the *Nyssorhynchus* subgenus that are vectors of *Plasmodium*.

As landscape structure is an important determinant for the dynamics of infectious diseases, man-made modifications can create ecological and microclimate conditions that favour some mosquito vector species and also the pathogens they transmit (Castro et al. 2006, Olson et al. 2010). Vittor et al. (2006) showed that deforestation increased the rate of contact between *An. darlingi* and humans in the Peruvian Amazon, thereby increasing the incidence of malaria in the region. Similarly, Castro et al. (2006) demonstrated that the early stages of frontier settlement are dominated by environmental risks of malarial transmission. Olson et al. (2010) showed that 4.3% of changes in the natural environment caused by deforestation were associated with a 48% increase in malarial incidence in the municipality of Mâncio Lima, state of Acre. Similarly, in the Vale do Ribeira, ecological conditions that favour the presence of habitats for *An. marajoara*, combined with the migration of individuals

infected with human *Plasmodium*, might change the current status of low and dispersed malarial transmission in that region. The results of the present study suggest that malarial transmission will continue and may become exacerbated if the proportion of natural forest remnants is reduced in the Atlantic Forest.

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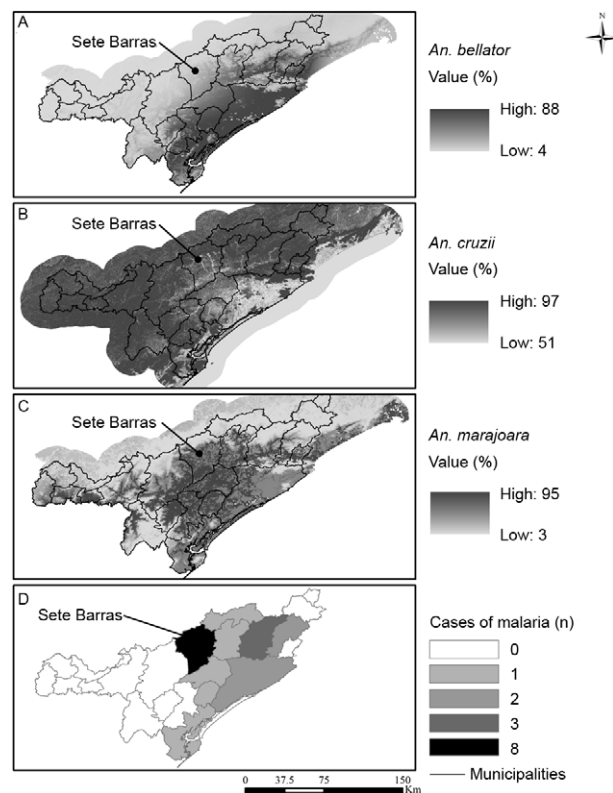
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TABLE

The mean and standard deviations of the number of mosquitoes monthly collected per species and collection site in the south-eastern Atlantic Forest of Brazil

Sites	<i>Anopheles bellator</i>	<i>Anopheles cruzii</i>	<i>Anopheles marajoara</i>
BoqN	1.50 ± 1.62	16.17 ± 5.25	10.50 ± 4.17
BoqS	149.67 ± 17.08	42.42 ± 11.24	1.75 ± 1.55
CanSJ	43.58 ± 8.03	37.83 ± 10.18	0.33 ± 0.49
CanT	26.34 ± 7.20	229.25 ± 19.24	0.00 ± 0.00
FazE	0.00 ± 0.00	29.33 ± 9.86	0.75 ± 0.75
S_Ga	0.92 ± 1.17	50.92 ± 9.62	0.00 ± 0.00
Ica_I	3.00 ± 2.26	45.25 ± 10.53	0.00 ± 0.00
Par	0.00 ± 0.00	1.17 ± 1.47	0.00 ± 0.00
V_M	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

mosquito collection sites: BoqN: Ilha Comprida (North); BoqS: Ilha Comprida (South); CanSJ: Cananéia; CanT: Sítio Itapuã; FazE: Fazenda Experimental; S_Ga: Sítio Galiléia; Ica_I: Icapara; Par: Pariquera-Mirim; V_M: Vila Maria.



Anopheles bellator (A), *Anopheles cruzii* (B), *Anopheles marajoara* (C) potential habitat suitability maps, and cases of malaria (D) in the south-eastern Atlantic Forest of Brazil. Potential habitat suitability of *An. cruzii* is beyond the slopes of Serra do Mar.