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Spatial distribution of arboviral mosquito vectors (Diptera, Culicidae) in Vale do Ribeira in the South-eastern Brazilian Atlantic Forest

Distribuição espacial de mosquitos (Diptera, Culicidae) vetores de arbovírus no Vale do Ribeira, sudeste da Mata Atlântica, Brasil

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Abstract

Mosquitoes are vectors of arboviruses that can cause encephalitis and hemorrhagic fevers in humans. Aedes serratus (Theobald), Aedes scapularis (Rondani) and Psorophora ferox (Von Humboldt) are potential vectors of arboviruses and are abundant in Vale do Ribeira, located in the Atlantic Forest in the southeast of the State of São Paulo, Brazil. The objective of this study was to predict the spatial distribution of these mosquitoes and estimate the risk of human exposure to mosquito bites. Results of the analyses show that humans are highly exposed to bites in the municipalities of Cananéia, Iguape and Ilha Comprida. In these localities the incidence of Rocio encephalitis was 2% in the 1970s. Furthermore, Ae. serratus, a recently implicated vector of yellow fever virus in the State of Rio Grande do Sul, should be a target for the entomological surveillance in the southeastern Atlantic Forest. Considering the continental dimensions of Brazil and the inherent difficulties in sampling its vast area, the habitat suitability method used in the study can be an important tool for predicting the distribution of vectors of pathogens.

Disease Vectors; Arboviruses; Statistical Model

Introduction

A comparison of 25 major infectious diseases around the world which are the leading causes of mortality and/or morbidity among humans, showed that eight out of ten of these diseases in the tropics are vector-borne. In contrast, in temperate regions only two out of 15 of these diseases are vector-borne ¹. Furthermore, of the diseases that emerged globally between 1940 and 2004, approximately 23% were vector-borne and caused epidemics in tropical countries ². In addition, several previously unknown arboviruses from the Amazon Basin in Brazil have been described over the past decades ³. Due to these facts, Brazil is considered a sanctuary for latent arboviruses ⁴.

The ecological and environmental conditions of the Atlantic Forest support a large and diversified assemblage of mosquitoes including species that are potential vectors of both human and animal pathogens. Among the medically important mosquitoes, *Aedes scapularis*, *Aedes serratus* and *Psorophora ferox* have been identified as potential vectors of arboviruses associated with severe human diseases ^{5,6,7}. *Ae. scapularis* and *Ps. ferox* were incriminated as the vectors of the Rocio virus (ROCV), which caused 1,100 cases of encephalitis between 1975 and 1983 in the regions of Baixada Santista and Vale do Ribeira in the State of São Paulo ^{8,9,10,11}. In addition, *Ae. serratus* was found to be naturally infected with yellow fever

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virus (YFV)¹², and it is possible that it played a role as a secondary vector of this virus in the State of Rio Grande do Sul during the epidemics and epizootics that occurred in 2008¹³.

The transmission of arboviruses is determined by the presence of a competent mosquito species and also by ecological interactions between reservoir/host, pathogens, and mosquito communities¹⁴. The presence and spatial distribution of mosquito vector species are determined by both abiotic (e.g., temperature, water disposal, and topography) and biotic factors (e.g., abundance of vertebrate hosts and availability of larval habitats). Data on these abiotic and biotic factors can be obtained by remote sensing and correlated to mosquito presence/abundance data using habitat suitability modeling. This approach can be used to identify the spatial distribution of a species over a variety of geographical ranges^{15,16}. A starting point to perform habitat suitability modeling is the framework proposed by Soberon¹⁷ that looks at the potential occupied area based on favorable habitat factors such as: abiotic variables (e.g., climate, topography), biotic variables (e.g., vegetation cover) and metapopulation structure (e.g., source-sink dynamics). An example of the application of habitat suitability modeling to surveillance and intervention activities is the work of Diuk-Wasser et al.¹⁸ that created maps to assess the spatial distribution of West Nile virus mosquito vectors in New Haven County, Connecticut, USA.

In the Atlantic Forest, the ecological requirements of *Ae. scapularis*, *Ae. serratus*, and *Ps. ferox* are provided mainly by way of temporary ground pools (larval habitats) and forest cover (shelter and presence of hosts)^{5,6} that can be measured by remote sensing and thus inputted into the habitat suitability model.

Considering the abundance of competent mosquito vectors^{5,6}, the circulation of several arboviruses^{19,20,21} and the presence of natural ecosystems (dense ombrophilous forest, “restinga” forest and mangrove²²), it is possible that Vale do Ribeira shelters pathogens that might accidentally emerge in humans. Since the role that some determinants of mosquito ecology can play on human epidemiology has already been studied in this region^{5,6}, the analyses were designed to be spatially discrete so as to avoid quantitative extrapolation of the results. Habitat suitability modeling permits extrapolation of the potential spatial distribution of mosquito vectors in continuous and quantitative geographical maps. Considering that habitat suitability modeling can be a useful input to the entomological surveillance system, the objectives of the present study are: (1) to estimate the potential spatial distribu-

tion of *Ae. scapularis*, *Ae. serratus* and *Ps. ferox* using habitat suitability modeling; and (2) to identify the municipalities in Vale do Ribeira where potential exposure of the human population to bites of *Ae. scapularis*, *Ae. serratus* and *Ps. Ferox* is greatest. The results of this study show how potential spatial distribution maps can aid in the surveillance of vector-borne diseases in Brazil.

Materials and methods

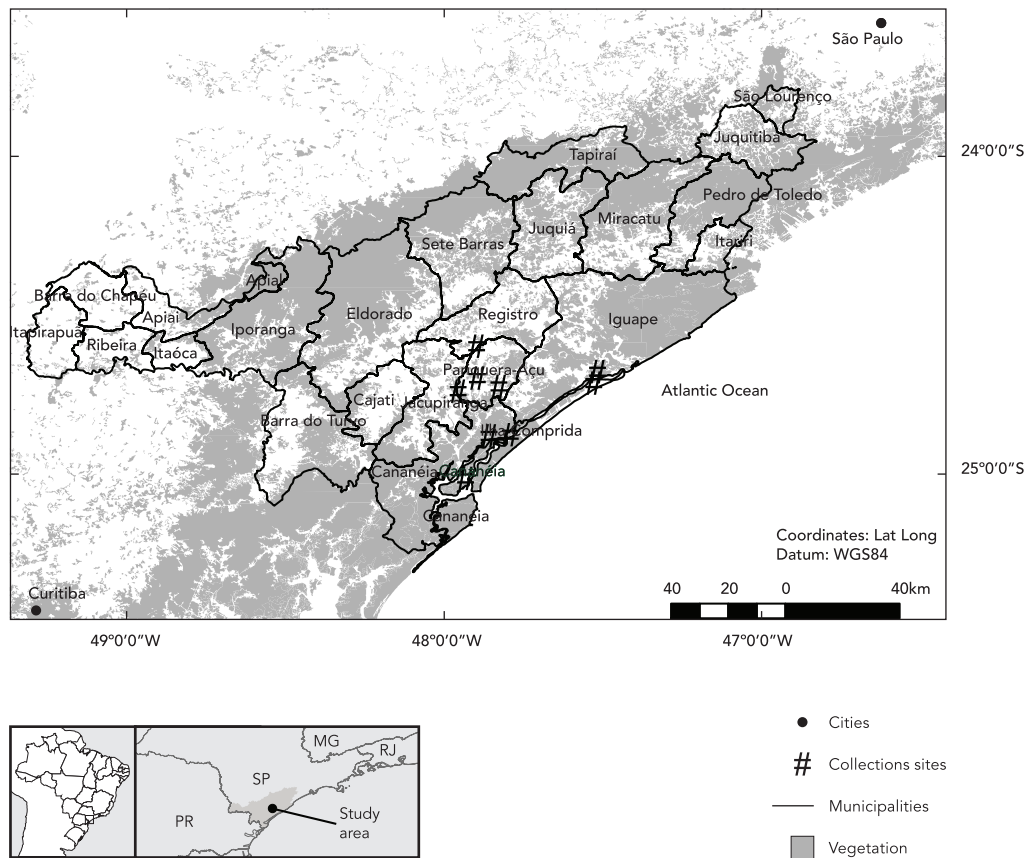
The study encompassed 23 municipalities in Vale do Ribeira that covers an area of approximately 11,635km² within the Serra do Mar mountain range in the Southeast Region of Brazil (Figure 1). The climate is humid subtropical (*Cfa* in the Köppen classification) and the region comprises the largest continuous area of preserved Atlantic Forest²². The landscape is made up of forest, river systems and mosaics of human occupation and a large assemblage of mosquito species⁶.

Monthly sampling of adult mosquitoes was carried out in nine collection sites between May 1996 and April 2000 (Figure 1) resulting in a total of 108 independent collections. The correlation between the months in which collections were made (i.e., seasonality) and mosquito abundance were similar throughout all collection sites. To determine habitat suitability for *Ae. scapularis*, *Ae. serratus* and *Ps. ferox*, the total number of individuals of each species collected monthly in Shannon traps for each collection site was used as a single variable. These values were further correlated to abiotic and biotic variables to assess the significant habitat suitability determining factors.

Topographic, climate and landscape variables were quantified for each collection site using the GRASS 6.4 GIS software (<http://grass.osgeo.org/>). The topographic variable slope, derived from SRTM 1.4 elevation map (<http://www2.jpl.nasa.gov/srtm/>), was interpolated to a 30-meter spatial resolution, representing availability of larval habitats. Climate data (mean maximum temperature in the warmest month and annual precipitation) with a 900-meter spatial resolution was acquired from the WorldClim database (<http://www.worldclim.org/>). The landscape variable “percentage of vegetation cover” (with a 30-meter spatial resolution) was obtained by calculating the proportion of remaining vegetation within a radius of 200m around each collection site. Similar approaches were used in studies of chironomid²³, birds²⁴ and carnivorous mammals²⁵ conducted in fragmented landscapes in the State of São Paulo. The map of vegetation cover used in this step of the

Figure 1

Map of the study area of Vale do Ribeira adapted from Laporta et al.³³. The region is mainly covered with Atlantic Forest remnants. Mosquito collections were performed in nine sites.



process was derived from visual interpretation of Landsat TM images²⁶.

A preliminary analysis was performed to assess highly important abiotic and biotic determining factors of spatial distribution of species. To determine whether the contribution of each regression coefficient was significant ($p < 0.15$), a negative binomial regression analysis was performed using R 2.9.2 software (<http://www.r-project.org/>). For the purpose of this analysis, the total number of individuals of *Ae. scapularis*, *Ae. serratus* and *Ps. ferox* were dependent variables, while slope, temperature, precipitation and vegetation were explanatory variables. This step allowed us to assess better correlated environmental variables for incorporation into the habitat suitability model.

Using the variables selected in the previous analysis, correlations between species presence

data and environmental variables were assessed using the MAXENT algorithm²⁷ of the OpenModeller 1.1.0 software (<http://openmodeller.sourceforge.net/>), thus obtaining a statistical estimation of maximum entropy. A predicted value for each pixel was used to assess a species' potential spatial distribution (with a 30-meter spatial resolution), which could vary from 0 (meaning the worst habitat scenario) to 100 (the best habitat scenario).

The map of potential spatial distribution, generated using estimates of maximum entropy, is a result of a stochastic model and thus needs to be validated. The receiver operating characteristic (ROC) method was used to validate the models. A ROC plot was obtained for each species by plotting all sensitivity values (e.g., mosquito presence fraction) against their equivalent ($1 - \text{specificity}$) values (e.g., mosquito absence fraction).

To calculate both sensibility and specificity, we crossed predictions of mosquito presence and pseudo-absence against mosquito presences and absences data obtained from other field collections carried out in the study region. The area under the ROC curve (AUC) provides a measure of overall accuracy²⁸. The AUC values vary from zero to one, where zero means that the model has failed in establishing habitat suitability for a specific species and one indicates that the model is suitable for defining habitat suitability. AUC values ≥ 0.90 were used hereafter as the cut-off to classify a model as valid²⁹. In the case of a given species not having a random spatial distribution because its habitat was not randomly distributed³⁰, a second validation process was performed based on the concept of metapopulation structure¹⁷. For the purposes of this process, a Poisson distribution model was used. A specific species' potential spatial distribution map was validated when the habitat suitability of a specific collection site (i.e., the population source) was greater than 80%. This percentage was adopted as a cut-off because it represents those localities in which the species reached the optimum habitat.

A post-processing analysis was performed in which a zonal statistic, i.e., the sum of pixel values divided by the total number of pixels in a polygon (e.g., the area within the boundary of each municipality), was inputted into the spatial analyst Arcmap 10 program (<http://www.esri.com/>). The zonal statistic assigned a mean pixel value for each of the following rasters: slope, temperature, precipitation, and vegetation (environmental layers), *Ae. scapularis*, *Ae. serratus* and *Ps. ferox* (potential spatial distribution maps) in each of the 23 municipalities. Each of these 7 raster layers were transformed by processing the zonal statistic with 7 other variables with 23 values, i.e., mean pixel values for each municipality. Spearman's rank correlation coefficient was used to assess important determining factors (slope, temperature, precipitation, or vegetation) for each potential spatial distribution map ($p < 0.20$).

To estimate the man-biting rate, the potential spatial distribution map for each mosquito species (range: 0-100%) was transformed into a binary variable (0, absence and 1, presence), with a habitat suitability cut-off of 80%. Mosquito presence in a 30-meter spatial resolution was assumed to represent a proxy of the home range of a mosquito population. Predicted values of mosquito presence were summed and divided by the number of inhabitants to represent the proportion of people at risk of being bitten. A value ≥ 1 means a risk of human exposure to potentially infective mosquito bites.

Results

A total of 14,895 females of the species *Ae. scapularis*, *Ae. serratus* and *Ps. ferox* were captured in Shannon traps in 9 areas located in the Vale do Ribeira. The data regarding the total number of individuals of *Ae. scapularis*, *Ae. serratus* and *Ps. ferox* did not fit a Poisson distribution; upper outliers represent collection sites with suitable habitats for a given species. For each collection site, estimates of environmental variables were found that may be associated with variations in the total number of individuals of *Ae. scapularis*, *Ae. serratus* and *Ps. ferox* (Table 1). In the preliminary analysis, all abiotic (slope, temperature and precipitation) and biotic (vegetation) variables were selected to generate *Ps. ferox* and *Ae. serratus* potential spatial distribution maps; a precipitation layer was not used in the model when analyzing the distribution of *Ae. scapularis* because this variable was not significant (Table 2).

Potential spatial distribution maps of *Ae. scapularis* (range: 38-91%; AUC: 0.91; Figure 2a), *Ps. ferox* (range: 25-88%; AUC: 0.90; Figure 2b) and *Ae. serratus* (range: 24-86%; AUC: 0.90; Figure 2c) showed habitat suitability $\geq 80\%$ for the control sites, i.e., where there is a high probability of presence of the species: Boqueirão Sul, Fazenda Experimental and Pariquera-Mirim (*Ae. scapularis*), and Boqueirão Sul and Icapara (*Ps. ferox* and *Ae. serratus*) (Table 1, Figure 2).

In the study region, abiotic and biotic pixel values varied from 0 to 50° (slope), 1,400 to 2,792 mm (precipitation), 22.3 to 31.4°C (temperature), and 0 to 100% (vegetation). The highest mean values among municipalities were 13° (slope) in Iporanga, 2,411 mm (precipitation) in Cananéia, 30.7°C (temperature) in Registro and 94% (vegetation) in Tapiraf. The potential spatial distribution of *Ae. scapularis* was negatively correlated with slope ($\rho = -0.88$; $p < 0.001$; $n = 23$) and positively correlated with precipitation ($\rho = 0.61$; $p < 0.002$; $n = 23$) and temperature ($\rho = 0.52$; $p < 0.02$; $n = 23$). The potential spatial distribution of *Ps. ferox* and *Ae. serratus* were negatively correlated with slope ($\rho = -0.31$ and -0.24 ; $p < 0.15$ and 0.27 ; $n = 23$; respectively) and positively correlated with precipitation ($\rho = 0.77$ and 0.78 ; both $p < 0.001$; $n = 23$; respectively) and temperature ($\rho = 0.63$ and 0.58 ; $p < 0.002$ and 0.004 ; $n = 23$; respectively). It is interesting to note that correlation between vegetation and the potential spatial distribution of *Ae. serratus* and *Ps. ferox* was stronger ($\rho = 0.36$ and 0.29 ; $p < 0.10$ and 0.19 ; $n = 23$; respectively) than with the potential spatial distribution of *Ae. scapularis* ($\rho = 0.12$; $p < 0.58$; $n = 23$). According to these results, localities with steeper slopes,

Table 1

Collection sites, mosquito frequencies, and environmental variables. Vale do Ribeira, State of São Paulo, Brazil.

Site	<i>Aedes scapularis</i> (n)	<i>Aedes serratus</i> (n)	<i>Psorophora ferox</i> (n)	Terrain slope (°)	Annual precipitation (mm)	Maximum temperature (°C)	Vegetation cover (%)
BoqN	1,073	89	72	0	2,116	29.9	15
BoqS	2,244 *	368 *	209 *	1	2,279	30.5	64
CanSJ	831	99	58	9	2,469	30.6	27
CanT	646	48	22	13	2,272	30.2	77
FazExp	4,157 *	46	6	2	1,915	30.9	3
S_Galiléia	528	61	30	4	2,043	30.5	81
Ica_Iguape	887	287 *	227 *	0	2,096	29.5	93
Par_Mirim	1,899 *	90	55	1	2,060	30.6	0
V_Maria	756	34	13	0	2,012	30.8	0

Mosquito collection sites: BoqN, Ilha Comprida (Norte); BoqS, Ilha Comprida (Sul); CanSJ, Cananéia; CanTaki, Sítio Itapuã; FazExp, Fazenda Experimental; S_Galiléia, Sítio Galiléia; Ica_Iguape, Icapara; Par_Mirim, Pariquera-Mirim; V_Maria, Vila Maria.

* Higher mosquito frequency as expected in a Poisson distribution. These collection sites were hereafter considered as the source of a mosquito-specific species i.e., a location with ecological characteristics that positively influence population growth. These localities were the control sites where species are present in the context of habitat-suitability modeling.

Table 2

Preliminary Analysis: significance of coefficient ($p < 0.15$) in negative binomial regression.

Species	Terrain slope (°)	Annual precipitation (mm)	Maximum temperature in the warmest month (°C)	Vegetation cover (%)
<i>Aedes scapularis</i>	$p < 0.11$	$p < 0.19 *$	$p < 0.09$	$p < 0.07$
<i>Psorophora ferox</i>	$p < 0.13$	$p < 0.13$	$p < 0.04$	$p < 0.03$
<i>Aedes serratus</i>	$p < 0.13$	$p < 0.12$	$p < 0.12$	$p < 0.02$

* This variable was not selected for habitat suitability modeling of the given species ($p > 0.15$).

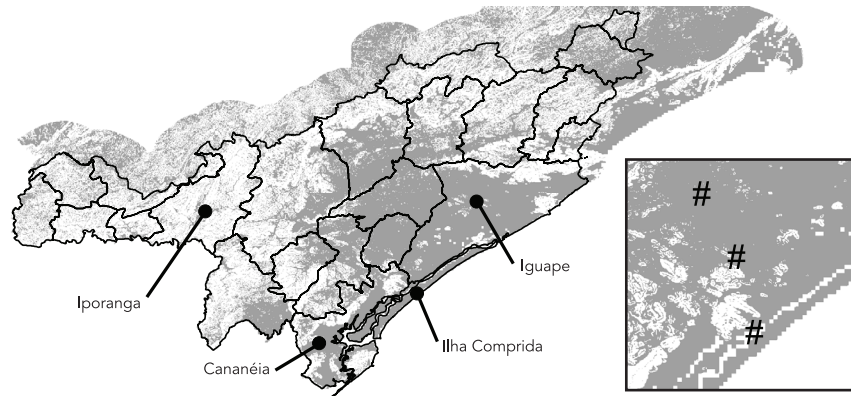
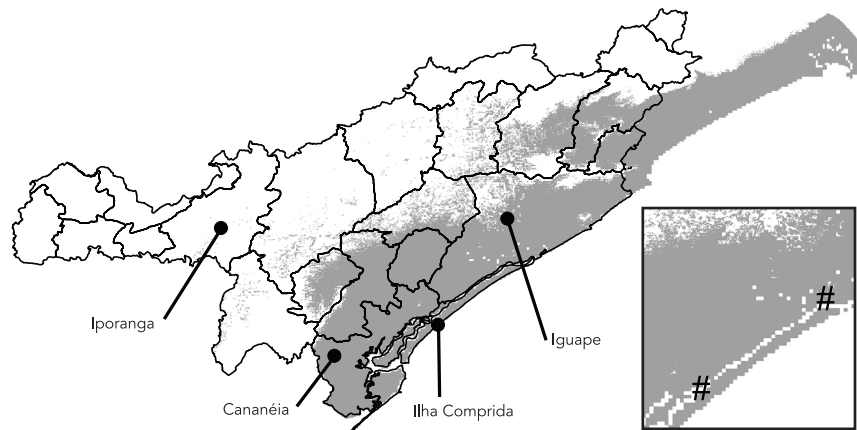
which are usually located in mountainous areas (e.g., Iporanga), represent a less-suitable habitat, whereas in areas on the coast (e.g., Cananéia) and on flatter grounds (e.g., Registro) the variables precipitation and temperature show high values, making these areas suitable habitats for these mosquitoes. As a result the inhabitants of Iporanga municipality are not exposed to these mosquitoes and the highest risk of human exposure to mosquito bites occurs in the municipalities of Cananéia, Iguape and Ilha Comprida (Table 3).

Discussion

Potential spatial distribution maps of *Ae. scapularis* (Figure 2a), *Ps. ferox* (Figure 2b) and *Ae. serratus* (Figure 2c) indicate that these species are widely distributed in Vale do Ribeira, as shown by Forattini et al. ⁵ and Forattini & Massad ⁶. Abiotic and biotic determining factors were assessed using habitat suitability modeling to obtain geographical maps that may represent potential favorable habitats for mosquitoes ¹⁷. However, an external validation of the potential spatial distribution models may be necessary i.e., a sampling effort in highly favorable areas identified by the maps. Despite this, since this study area has been sampled over time, there is room for improvement in these models.

Figure 2

Aedes scapularis (2a; AUC: 0.91; range: 38-91%), *Psorophora ferox* (2b; AUC: 0.90; range: 25-88%) and *Aedes serratus* (2c; AUC: 0.90; range: 24-86%) potential spatial distribution maps. Control sites have habitat-suitability $\geq 80\%$.

2a) *Aedes scapularis*2b) *Psorophora ferox*

(continues)

The findings presented reflect the ecological characteristics of the mosquito species considered by this study and the distribution of cases of Rocio virus (ROCV) encephalitis in Vale do Ribeira. According to the results of the correlation tests and Figure 2b, *Ps. ferox* is present along the coastal humid lowlands⁵ and might have played a role in maintaining the enzootic cycle of ROCV during the 1976 epidemic⁸ in the municipalities of Cananéia, Iguape and Ilha Comprida (Table 3). The non-significant correlation between the potential spatial distribution of *Ae. scapularis* (Figure 2a) and vegetation cover ($p < 0.58$) showed

that this species has a tendency to be present in environments modified by human activities⁶. This mosquito has a high vector competence for transmitting ROCV among humans^{9,10}. It is interesting to note that people are highly exposed to this mosquito in Cananéia, Iguape and Ilha Comprida (Table 3), reflecting the high incidence rate (2%) of Rocio virus encephalitis in 1976¹¹.

The risk of YFV transmission still remains low in the Atlantic Forest¹². However, individuals of *Ae. serratus* naturally infected by YFV were found and, although other arboviruses were isolated from this species in the Amazon Basin³¹, people

Figure 2 (continued)

2c) *Aedes serratus*

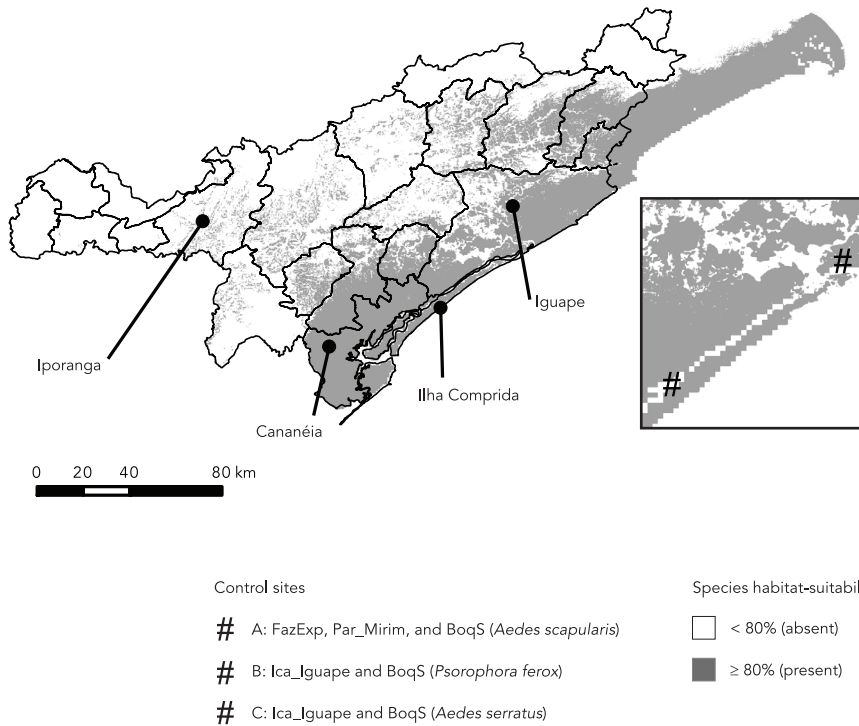


Table 3

Risk of human exposure to mosquito vectors, Vale do Ribeira, State of São Paulo, Brazil.

Municipalities	<i>Aedes scapularis</i>	<i>Aedes serratus</i>	<i>Psorophora ferox</i>
Cananéia	28.47 (349,188 * / 12,267 **)	69.03 (846,781 / 12,267)	68.91 (845,322 / 12,267)
Iguape	46.23 (1,267,072/27,410)	31.93 (875,152 / 27,410)	42.11 (1,154,344 / 27,410)
Ilha Comprida	18.23 (121,266 / 6,653)	16.03 (106,619 / 6,653)	16.74 (111,367 / 6,653)
Iporanga	0.72 (3,296 / 4,563)	0.70 (3,202 / 4,563)	0.48 (2,197 / 4,563)

* Number of pixels in which species is present (habitat suitability ≥ 80%);

** Human population (2000 IBGE census; <http://www.ibge.gov.br/>).

are highly exposed to this mosquito in Vale do Ribeira (Table 3). Unfortunately, the role of this species in the epidemiology of yellow fever is unknown. Although the potential spatial distribution of *Ae. serratus* represents a past scenario (1996-2000), it is reasonable to assume that this distribution is applicable to the present day because only minor vegetation cover changes oc-

curred between the 1980s (58% in Iversson¹¹) and the 2000s (53% in Ribeiro et al.²²). This assumption is based on the correlation between this species and vegetation cover ($\rho = 0.36$; $p < 0.10$). Furthermore, Pardini et al.³² showed that the abundance of specialist species may not respond to vegetation loss in a fragmented landscape with ≥ 50% remaining forest.

Potential spatial distribution maps of vectors are important instruments for the surveillance of arboviruses in Brazil, where dormant or non-pathogenic viruses can emerge due to the infestation of densely populated cities by mosquitoes^{4,7}. According to Wolfe et al.¹ and Jones et al.², conditions in the Vale do Ribeira region seem to favor the emergence of vector-borne diseases. The results of the habitat suitability modeling process show that careful attention should be given to the mosquito assemblages in Vale do Ribeira, particularly in the municipalities of Cananéia, Iguape and Ilha Comprida, as suggested by Forattini & Massad⁶. However, the main contribution of the present study is to show that habitat suitability modeling is an important tool for estimating the potential spatial distributions of species and can thus make a significant contribution to mosquito surveillance, as demonstrated in North America by Diuk-Wasser et al.¹⁸.

Estimates of the number of people at risk considered total population but did not take account of immune responses to arbovirus infec-

tion. It is possible that a fraction of people might have become refractory to a new infection because of past exposure to the disease. It is therefore important to consider this segment of the population to ensure a more accurate picture of the number of people at risk.

Considering that habitat suitability modeling can be applied to a wider area than that of this study, this approach is recommendable in tropical regions (particularly Latin America) where sample data is often unavailable. We believe that the potential spatial distribution maps presented here can contribute to defining strategic areas for monitoring mosquito populations and evaluating the risk of the emergence of arboviruses throughout Brazil, optimizing the use of resources and also minimizing efforts spent on surveillance. Combined with assessments of the presence of viruses in mosquito samples, habitat suitability modeling can be used in surveillance programs, providing important inputs for risk assessment, decision-making and adoption of preventive measures.

Resumo

Mosquitos são vetores de arbovírus que podem causar encefalites e febres hemorrágicas em humanos. Aedes serratus (Theobald), Aedes scapularis (Rondani), e Psorophora ferox (Von Humboldt) são vetores potenciais de arbovírus e são abundantes no Vale do Ribeira, Mata Atlântica, sudeste do Estado de São Paulo, Brasil. O objetivo desse estudo foi inferir a distribuição espacial desses mosquitos e estimar o risco da exposição humana às picadas de mosquitos. Os resultados das análises indicaram que os humanos estão altamente expostos às picadas nos municípios de Cananéia, Iguape e Ilha Comprida. Nessas localidades a incidência de encefalite Rocio foi 2% na década de 1970. Adicionalmente, Ae. serratus, que foi recentemente implicado vetor do vírus da febre amarela no estado do Rio Grande do Sul, deveria ser alvo da vigilância entomológica no sudeste da Mata Atlântica. Considerando a extensão territorial do Brasil e as inerentes dificuldades em amostrar esse vasto território, a modelagem de habitat empregada nesse trabalho poderia ser utilizada para a vigilância de vetores de patógenos.

Vetores de Doenças; Arbovirus; Modelos Estatísticos

Contributors

G. Z. Laporta, D. G. Ramos and M. A. M. Sallum conceived and designed the study. G. Z. Laporta performed the statistical analysis with contributions by M. C. Ribeiro. All authors wrote the paper.

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