RESEARCH ARTICLE

A mathematical model for zoonotic transmission of malaria in the Atlantic Forest: Exploring the effects of variations in vector abundance and acrodendrophily

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Abstract

Transmission foci of autochthonous malaria caused by *Plasmodium vivax*-like parasites have frequently been reported in the Atlantic Forest in Southeastern and Southern Brazil. Evidence suggests that malaria is a zoonosis in these areas as human infections by simian *Plasmodium* species have been detected, and the main vector of malaria in the Atlantic Forest, *Anopheles* (*Kerteszia*) *cruzii*, can blood feed on human and simian hosts. In view of the lack of models that seek to predict the dynamics of zoonotic transmission in this part of the Atlantic Forest, the present study proposes a new deterministic mathematical model that includes a transmission compartment for non-human primates and parameters that take into account vector displacement between the upper and lower forest strata. The effects of variations in the abundance and acrodendrophily of *An. cruzii* on the prevalence of infected humans in the study area and the basic reproduction number (R_0) for malaria were analyzed. The model parameters are based on the literature and fitting of the empirical data. Simulations performed with the model indicate that (1) an increase in the abundance of the vector in relation to the total number of blood-seeking mosquitoes leads to an asymptotic increase in both the proportion of infected individuals at steady state and R_0; (2) the proportion of infected humans at steady state is higher when displacement of the vector mosquito between the forest strata increases; and (3) in most scenarios, *Plasmodium* transmission cannot be sustained only between mosquitoes and humans, which implies that non-human primates play an important role in maintaining the transmission cycle. The proposed model contributes to a better understanding of the dynamics of malaria transmission in the Atlantic Forest.

Author summary

The etiological agents of malaria are protozoa of the genus *Plasmodium* that are transmitted to humans and other vertebrate hosts by mosquitoes. In the Atlantic Forest in Southeastern and Southern Brazil, human infections by simian *Plasmodium* species have been detected, showing that in certain situations malaria can be transmitted zoonotically in this region. *Anopheles cruzii*, a sylvatic mosquito, is considered the main vector of malaria parasites in the Atlantic Forest. The fact that this species can feed on humans and monkeys reinforces the hypothesis of zoonotic transmission. Here we present a new mathematical model to explain the dynamics of zoonotic transmission of malaria in the Atlantic Forest. Simulations performed with the model showed that the prevalence of human cases and the basic reproduction number for malaria can be strongly influenced by variations in the abundance of *An. cruzii* and the frequency with which these mosquitoes move between the ground and the forest canopy to feed on humans and monkeys, respectively. The proposed model contributes to a better understanding of the dynamics of malaria transmission in the Atlantic Forest.

Introduction

Autochthonous cases of malaria are recorded every year in the Atlantic Forest in Southeastern and Southern Brazil, [1,2]. In these areas, different species of *Plasmodium* are transmitted to humans by mosquitoes (Diptera:Culicidae) of the genus *Anopheles*, which includes *Anopheles* (*Kerteszia*) *cruzii*, considered the main vector of human and simian malaria in the region [3,4]. Because immature forms of the subgenus *Kerteszia* develop in water that accumulates in the leaf axils of bromeliads (Bromeliaceae), autochthonous malaria in the Atlantic Forest is known as "bromeliad-malaria". There has been a low incidence of autochthonous malaria outbreaks in the Atlantic Forest in recent decades, and most cases have been asymptomatic with low circulating parasite loads [5–7]. *Plasmodium vivax*-like parasites and, less frequently, *P. malariae* and *P. falciparum* have been involved in the majority of cases [1].

   In Brazil, primates from the families Atelidae and Cebidae have been found infected with two species of *Plasmodium*: *P. brasilianum* and *P. simium* [8–11]. These are morphologically indistinguishable from the species that infect humans: *P. brasilianum* is identical to *P. malariae*, and *P. simium* to *P. vivax* [8]. This similarity has been confirmed by molecular studies, which showed a high identity between the genomes in each of these pairs of plasmodia [12–15], suggesting recent speciation and reinforcing the possibility of *Plasmodium* transmission from monkeys to humans and vice versa [15–17].

   Human infection by simian *Plasmodium* was considered rare or accidental until recently [18–21]. However, molecular tests on blood samples from 208 malaria patients in Malaysia between 2000 and 2002 revealed that 58% of the patients had been infected with *P. knowlesi*, a parasite commonly found in *Macaca fascicularis* and *Macaca nemestrina* [22]. After this finding, other cases of human infection with *P. knowlesi* were detected in other Southeast Asian countries [23–27]. In Brazil, the first recorded case of human infection by simian *Plasmodium* occurred in 1966 in the Serra da Cantareira, in the metropolitan region of São Paulo, where simian malaria is highly enzootic. On that occasion, a forest guard who performed mosquito collections in the tree canopies presented with bouts of fever and chills at two-day intervals, and *P. simium* was detected in his blood [19]. In a study with autochthonous cases of malaria in the state of Rio de Janeiro between 2015 and 2016, molecular analysis revealed that all the individuals concerned had been infected with *P. simium*. This was the first evidence, more than 50 years since the first

report, of human infection with a simian *Plasmodium* species in the Atlantic Forest. The authors suggest that malaria occurs zoonotically in the Atlantic Forest and that many cases of infection with *P. simium* may have been misidentified as *P. vivax* infection [2]. Howler monkeys (*Alouatta clamitans*) are probably the main reservoir of malarial parasites (*P. simium / P. vivax*) that cause zoonotic infections in humans in the Atlantic Forest [11]. Analysis of the *P. simium* genome revealed that these zoonotic parasites underwent host-switching adaptations, including switching (1) from European humans carrying *P. vivax* to New World monkeys during the first centuries of Brazilian colonization and (2) from New World monkeys carrying *P. simium* (a descendent form of *P. vivax*) to modern humans engaging in forest activities [15].

Vector species that feed at ground level and in the forest canopy enable pathogens to circulate between human and non-human primates (NHPs) [8]. A number of studies have shown *An. cruzii* to exhibit acrodendrophily (a preference for living and feeding in tree canopies) [28–33] although depending on location and environmental and climatic factors, it can bite at ground and canopy level or even predominantly ground level. As a result, this species can feed on humans and other primates, making circulation of *Plasmodium* species between these hosts possible [30,32,34]. In studies of simian malaria transmission in Brazil, Deane et al. [8,35] found that *An. cruzii* appears to transmit only simian malaria in some places while in others it transmits simian and human malaria. The authors also observed that this vector can behave differently, biting almost exclusively in the canopy in areas where only simian malaria occurs and biting at both ground and canopy level where simian and human malaria occur.

Few studies have sought to explain the dynamics of malaria transmission in the Atlantic Forest with mathematical models [7,36,37]. Laporta et al. [37] developed a model to show the impact of host and vector diversity on the risk of malaria transmission. Using a biodiversity-oriented model developed from a modification of the Ross-Macdonald model, the authors showed that (1) the presence of non-susceptible vertebrate hosts (dilution effect), (2) competition for blood meal sources between vector and non-vector mosquitoes (diffuse competition) and (3) host defensive response to an increased number of bites may reduce the risk of infection and better explain malaria dynamics in regions of high biodiversity, such as the Atlantic Forest. However, none of the models proposed to date have sought to explain and simulate the dynamics of zoonotic malaria transmission in the Atlantic Forest. This would require not only the inclusion of NHPs in the transmission cycle, but also an understanding of how variations in vector displacement between the upper and lower strata of the forest can affect the dynamics of transmission of malaria pathogens between monkeys and humans.

Considering the increasing importance of NHPs as malaria reservoirs of human zoonotic infections in the Atlantic Forest, the present study proposes a deterministic mathematical model that includes (1) a transmission compartment for NHPs and (2) parameters that take into account variations in the acrodendrophily of the vector. The aim was to (1) analyze the transmission dynamics of malaria in the Atlantic Forest by simulating a zoonotic scenario and (2) evaluate the effects of variations in the abundance and acrodendrophily of *An. cruzii* on the prevalence of infection in the local human population and the basic reproduction number for malaria in the Atlantic Forest. Simulations with the model indicate that, in addition to vector abundance, variations in vector acrondendrophily can play a determining role in the prevalence of human infection and the basic reproduction number.

Methods

Description of the model

The mathematical model proposed for this study is an SIS epidemic model (susceptible, infected, susceptible) as individuals who recover from *Plasmodium* infection do not become

immune to new infections. Based on the biodiversity-oriented model proposed by Laporta et al. [37], an infection compartment for NHPs (dI_P/dt) and parameters that take into account vector displacement between the upper (tree canopies) and lower (ground level) strata of the forest (F_{mc} and F_{mg}) were included. The proposed model is deterministic and initially includes 19 parameters and six variables. The number of parameters can be reduced to 14 if the abundance of non-vector mosquitoes and non-host vertebrates and host defensive behavior are disregarded.

The six variables are:

I_P = number of infected NHPs;

$S_P = N_P - I_P$ = number of susceptible NHPs (where $N_P = I_P + S_P$ is the total population of NHPs, which is assumed to be constant);

I_H = number of infected humans;

$S_H = N_H - I_H$ = number of susceptible humans (where $N_H = I_H + S_H$ is the total human population, which is assumed to be constant);

I_M = number of infected mosquitoes; and $S_M = M - I_M$ = number of susceptible mosquitoes (where $M = I_M + S_M$ is the total mosquito population, which is assumed to be constant).

A susceptible *An. cruzii* female (S_M) bites at a certain daily rate (b) depending on its gonotrophic cycle and whether or not there is gonotrophic discordance. When a susceptible vector blood feeds on an infected host (I_P and I_H), there is a certain probability that it will become infected: this is equal to T_{PM} when the susceptible vector bites an infectious monkey and T_{HM} when it bites an infectious human. The probability of a vector biting a monkey or a human will depend on how often it bites in the upper stratum of the forest, where there are simian hosts (F_{mc}–relative biting frequency of a mosquito in the canopy), and how often it bites near the ground, where there is human activity ($F_{mg} = 1 - F_{mc}$). An infectious mosquito (I_M) may take a new blood meal on a vertebrate host in the forest canopy or at ground level. The rate at which mosquitoes infect a new host in the monkey population (N_P) or local human population (N_H) will depend on (1) the number of susceptible monkeys and humans (S_P and S_H, respectively), (2) the probability of *Plasmodium* transmission from a mosquito to a monkey or human host (T_{MP} and T_{MH}, respectively), (3) the daily biting rate (b), (4) the biting frequency of the mosquito in the canopy or at ground level (F_{mc} and F_{mg}, respectively), (5) the number of infected mosquitoes (I_M) and (6) the mortality rate (μ) of the mosquito population (M). Monkeys and humans recover from the infection at certain rates (τ and γ, respectively) and become susceptible again.

The vector mosquito can also feed on other vertebrates that live in the tree canopy (B_c) or on the ground (B_g). This can produce a pathogen dilution effect in the environment since these animals are dead ends, in which the parasite would be unable to develop and be transmitted to another vector. In addition, the vector-mosquito population (M) competes for blood meal sources with non-vector mosquitoes that circulate in the forest canopy or at ground level (C_c and C_g, respectively), leading to an increase in the number of mosquitoes per host (C_{th}) and triggering a defensive response (h) by the hosts [37].

The proposed model, which is based on this depiction of the transmission dynamics, consists of a system of six nonlinear differential equations to express the variation per unit of time in the number of susceptible monkeys (1), infected monkeys (2), susceptible humans (3), infected humans (4), susceptible mosquitoes (5) and infected mosquitoes (6):

$$\frac{dS_P}{dt} = -\frac{bT_{MP}S_P I_M F_{mc}}{N_P + \zeta_c} + \tau I_P \tag{1}$$

$$\frac{dI_P}{dt} = \frac{bT_{MP}S_P I_M F_{mc}}{N_P + \zeta_c} - \tau I_P \tag{2}$$

$$\frac{dS_H}{dt} = -\frac{bT_{MH}S_H I_M F_{mg}}{N_H + \zeta_g} + \gamma I_H \tag{3}$$

$$\frac{dI_H}{dt} = \frac{bT_{MH}S_H I_M F_{mg}}{N_H + \zeta_g} - \gamma I_H \tag{4}$$

$$\frac{dS_M}{dt} = \mu I_M - \frac{bT_{PM}S_M I_P F_{mc}}{N_P + \zeta_c} - \frac{bT_{HM}S_M I_H F_{mg}}{N_H + \zeta_g} \tag{5}$$

$$\frac{dI_M}{dt} = \frac{bT_{PM}S_M I_P F_{mc}}{N_P + \zeta_c} + \frac{bT_{HM}S_M I_H F_{mg}}{N_H + \zeta_g} - \mu I_{M,} \tag{6}$$

where

$$\zeta_c = \frac{B_c h + M\,F_{mc} + C_c}{h} \text{ and } \zeta_g = \frac{B_g h + M\,F_{mg} + C_g}{h}.$$

The parameters ζ_c and ζ_g should be considered weighting values that represent the effect of parameters B_c, B_g, C_c, C_g and h on the *Plasmodium* transmission dynamics (for more details see S1 Text).

The main assumptions in the proposed model are that (1) the human and monkey populations are constant, i.e., the birth and immigration rates perfectly balance the mortality and emigration rates. This is generally a good approximation over short timescales; (2) the only local vector of *Plasmodium* is *An. cruzii*, which can bite humans, monkeys and other vertebrates with the same frequency in the absence of acrodendrophily; (3) the *P. vivax* infection parameters found in the available literature are valid; (4) the parameter h, i.e., the number of bites per day before a host exhibits defensive behavior, is similar for different host species; (5) there is no mortality due to infection and no cure as a result of treatment as most cases tend to be asymptomatic or oligosymptomatic; (6) vector abundance (M) is constant [birth rate (α) equals mortality rate (μ), i.e., $\mu I_M + \mu S_M = \alpha M$] and there is a constant ratio of *An. cruzii* females to all female mosquitoes per host ($\frac{M}{C_{th}}$, $M \leq C_{th}$). The same is considered to be the case for non-vector mosquitoes; (7) the NHP is the howler monkey (*Alouatta clamitans*) given the importance of this species as reservoirs of malaria parasites in the Atlantic Forest [8]. Despite some evidences of natural infection, the role of other NHP species in maintaining *Plasmodium* circulation is still unclear [8,38–40]; and (8) pathogen latency periods can be ignored. Fig 1 shows the malaria transmission dynamics in the Atlantic Forest based on the proposed model.

Parameter values

Parameter values were obtained from the literature or by estimation when no information was available. Arbitrary values were assigned to human and monkey population sizes, vector and non-vector mosquito abundances and vector frequencies in the upper and lower strata of the forest. Simulations were conducted to investigate the effects of variations in the values of these parameters on the basic reproduction number (R_0) for malaria and on the proportion of infected hosts or vectors when the system is in equilibrium. Table 1 shows the parameters, estimated or assigned values and available references.

For parameters b, μ, T_{MH}, T_{HM}, γ and h, the same values used by Laporta et al. [37] were assigned. Parameter b represents the daily biting rate of *An. cruzii* and can be calculated based
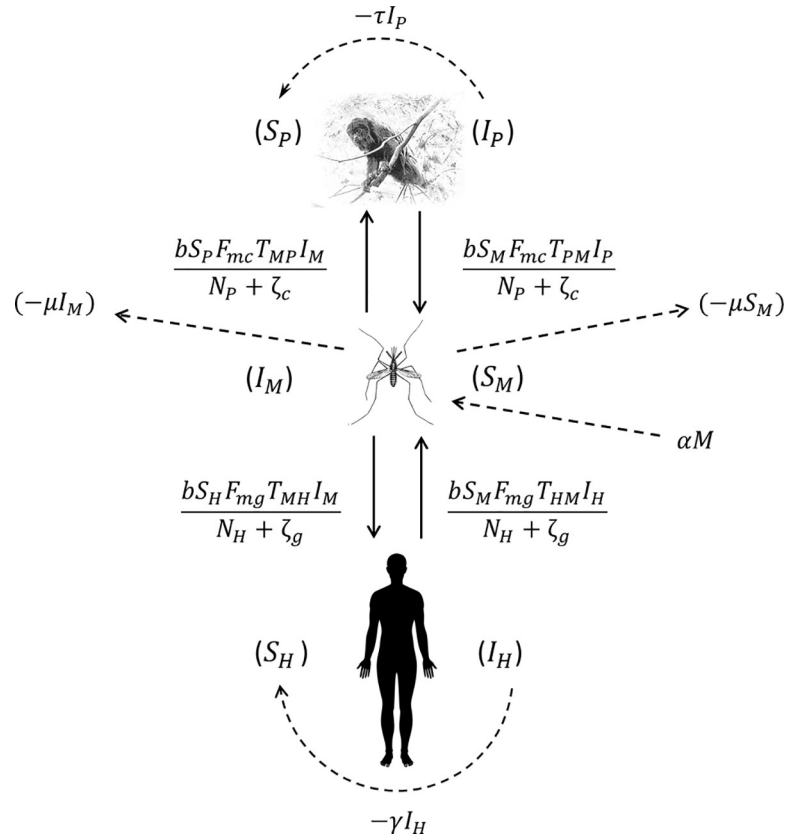
Fig 1. Schematic model of the dynamics of *Plasmodium* transmission among humans, mosquitoes and monkeys.
The solid arrows and the formulae beside them indicate vector-host and host-vector transmission and the rate at which
this transmission occurs. The curved dashed arrows and the formulae beside them indicate host recovery, i.e., transfer
from the infected to the susceptible compartment, and the rate at which this occurs. The straight dashed arrows
indicate the population dynamics of the vector.

on the number of blood feeds a female performs on average over a gonotrophic cycle. Because
of the gonotrophic discordance of this species, *An. cruzii* was considered to bite on average
twice per gonotrophic cycle, which is approximately 4 days [38], giving $b = \frac{2}{4} = 0.5$. If we con-
sider that *An. cruzii* mortality is independent of density, the mortality rate (μ) can then be cal-
culated from the daily survival rate (θ), where $\mu = -\log(\theta)$. The daily survival rate of *An. cruzii*
was estimated by Santos [41] as approximately 0.45, giving $\mu = -\log(0.45) \cong 0.8$. Chitnis et al.
[42] performed sensitivity analyses on a mathematical model of malaria transmission to deter-
mine the relative influence of the parameters used in the model on predictions of disease trans-
mission and prevalence. Following a review of a number of papers, the authors assigned the
following values for low-transmission areas: $T_{MH} = 0.022$, $T_{HM} = 0.24$ and $\gamma = 0.0035$ (9.5
months). In the same study, Chitnis et al. [42] assigned a ten-fold lower value for T_{HM} (0.024)
considering a contact between a recovered (asymptomatic carrier) human to a susceptible
mosquito. Thus, it was considered an alternative value for this parameter (see S1 Table and
S3–S6 Figs). For parameter γ an alternative value of 0.0055 was considered based on the study
of Alencar et al [7] with asymptomatic carriers of *Plasmodium* in the Atlantic Forest region of
the state of Espírito Santo (see S1 Table and S4 and S6 Figs).

As defined by Laporta et al. [37], *h* is a phenomenological parameter that reflects the host's
functional response to mosquito density. It is assumed that a host will tolerate a maximum

Table 1. Parameters used in the model, estimated or assigned values and references.

Parameter	Description	Estimated or Assigned values	References
N_H	Size of the human population–individuals	$10N_P$, $5N_P$, N_P	Simulated values
N_P	Size of the monkey population–individuals	$\frac{1}{10}N_H$, $\frac{1}{5}N_H$, N_H	Simulated values
C_{th}	Total no. of mosquitoes per host (females only)–individuals	20	Simulated value
M	Vector abundance (*An. cruzii* females)–individuals	$0.01C_{th}$ to $0.99C_{th}$	Variation in simulated values
C_c	Abundance of non-vector species at canopy level (females only)–individuals	$(C_{th}-M)\frac{1}{2}$	Variation in simulated values
C_g	Abundance of non-vector species at ground level (females only)–individuals	$(C_{th}-M)\frac{1}{2}$	Variation in simulated values ($C_c = C_g$)
F_{mc}	Probability of *An. cruzii* bites at canopy level	0.9, 0.7, 0.5	Simulated values
F_{mg}	Probability of *An. cruzii* bites at ground level	$1-F_{mc}$	Simulated values
b	Biting rate of each *An. cruzii* female on a given host—day^{-1}	0.5	[37,41]
μ	Mortality rate of female vector population—day^{-1}	0.8	[37,41]
T_{MH}	Probability of *Plasmodium* transmission from *An. cruzii* to humans in low-endemicity areas	0.022	[42]
T_{HM}	Probability of *Plasmodium* transmission from humans to *An. cruzii* in low-endemicity areas	0.24 or 0.024	[42]
γ	Human recovery rate—day^{-1}	0.0035 or 0.0055	[7,42]
T_{MP}	Probability of *Plasmodium* transmission from *An. cruzii* to monkeys	0.034 (0.025–0.063), 0.029 (0.022–0.064), 0.026 (0.019–0.03)	Estimated values and credible interval of 95%. The parameters values were estimated to $N_H = 10N_P$, $N_H = 5N_P$, and $N_H = N_P$, respectively. These parameters were estimated assuming $T_{HM} = 0.24$ and $\gamma = 0.0035$.
T_{PM}	Probability of *Plasmodium* transmission from monkeys to *An. cruzii*	0.428 (0.105–0.471), 0.342 (0.249–0.479), 0.261 (0.241–0.331)	
τ	Monkey recovery rate—day^{-1}	0.0044 (0.0016–0.0055), 0.0044 (0.0037–0.0057), 0.0038 (0.0032–0.0044)	
h	Number of bites per day before a host starts to exhibit defensive behavior divided by *An. cruzii* biting rate	$20 = 10/b$	[37]
B_g	Abundance of birds and mammals in the lower forest stratum–individuals	0	Value not estimated
B_c	Abundance of birds and mammals in the upper forest stratum–individuals	0	Value not estimated

number of bites per day before exhibiting a defensive response. The value $h = 20$ proposed by the authors refers to the average number of bites that a host tolerates per day (10 bites) multiplied by the *An. cruzii* daily biting rate, $b = 0.5$. Two simplifications were made: that all hosts have the same tolerance and that the biting rate is the same for vector and non-vector mosquitoes.

The parameters F_{mc} and F_{mg} represent the variation in the acrodendrophily of *An. cruzii*. The two parameters range from 0 to 1 and are complementary, i.e., $F_{mc} = 1-F_{mg}$. These parameters do not define the size of the population in each stratum of the forest, as it is assumed that there is a single vector population (M) in panmixia. F_{mc} and F_{mg} can be defined as the rate of displacement of mosquitoes between forest strata and can be interpreted as the probability of a single mosquito feeding, or attempting to feed, in the upper forest stratum (F_{mc}) or near the ground (F_{mg}) in a given unit of time. Another way of interpreting these parameters would be to view them as the probability of two successive mosquito bites, one in the canopy and the

other at ground level or vice versa. Therefore, the maximum probability (or maximum displacement between strata) occurs when $F_{mc} = F_{mg}$, as shown in S2 Fig.

Because of the lack of information about the transmission parameters related to a simian host, τ, T_{MP}, and T_{PM}, plausible values were estimated by fitting the steady state of the model (calculated in the Equilibrium points and stability analysis Subsection) to empirical data, which are assumed to be in equilibrium. Since the equations are not analytically solvable in terms of the parameters, they were estimated by sampling methods using Markov Chain Monte Carlo simulation with adaptive Metropolis algorithm [43,44] implemented in the modMCMC function in the FME package in R [45]. The best set of parameters were obtained using 2,000 iterations and a 95% highest posterior density interval was calculated. The values obtained for each simian parameter are presented in the Table 1. Details of the strategy and data used to obtain these values are given in S2 Text. Alternative values of simian parameters considering different values for human transmission parameters T_{HM} and γ were also estimated (see S1 Table and S3–S6 Figs).

For simulation purposes, we considered a scenario of forest fragmented due to human activity with settlements in small villages and rural properties at the forest edge. In this scenario, the humans (N_H) live close to a population of howler monkeys (N_P) in an environment where *Plasmodium* circulate, and the vector mosquito population (M) represents a given proportion of the total number of mosquitoes per host (C_{th}). The non-vector mosquitoes are equally distributed between the forest canopy (C_c) and ground level (C_g).

The parameters related to the presence of other (dead-end) vertebrates at canopy and ground level were considered null, i.e., $B_c = 0$ and $B_g = 0$. Thus, the transmission dynamics simulated considered local mosquitoes attempting to obtain blood repasts only from humans and monkeys. Therefore

$$\zeta_c = \frac{M\,F_{mc} + C_c}{h} \text{ and } \zeta_g = \frac{M\,F_{mg} + C_g}{h}.$$

Basic reproduction number

The equation for the basic reproduction number (R_0) was determined by computing the spectral radius (the largest eigenvalue in absolute value) of the next-generation matrix using the method proposed by Diekmann et al. [46,47]. For details on how the equation for R_0 was derived see S3 Text. It can be seen from the next generation matrix that R_0 is composed of

$R_{MP} = \frac{bT_{MP}N_P F_{mc}}{\mu(N_{P+}\zeta_c)}$ = the number of secondary infections generated by an infected mosquito in simians in a disease-free system;

$R_{MH} = \frac{bT_{MH}N_H F_{mg}}{\mu(N_{H+}\zeta_g)}$ = the number of secondary infections generated by an infected mosquito in humans in a disease-free system;

$R_{PM} = \frac{bT_{PM}M F_{mc}}{\tau(N_{P+}\zeta_c)}$ = the number of secondary infections generated by an infected simian in vectors in a disease-free system; and $R_{HM} = \frac{bT_{HM}M F_{mg}}{\gamma(N_{H+}\zeta_g)}$ = the number of secondary infections generated by an infected human in vectors in a disease-free system and is given by

$$R_0 = \sqrt{R_{MP}R_{PM} + R_{MH}R_{HM}}$$

$$= \sqrt{\frac{bT_{MP}N_P F_{mc}}{\mu(N_{P+}\zeta_c)}\frac{bT_{PM}M F_{mc}}{\tau(N_{P+}\zeta_c)} + \frac{bT_{MH}N_H F_{mg}}{\mu(N_{H+}\zeta_g)}\frac{bT_{HM}M F_{mg}}{\gamma(N_{H+}\zeta_g)}}$$

$$= \sqrt{\frac{F_{mc}{}^2 M\, N_P T_{PM} T_{MP} b^2}{(N_{P+}\zeta_c)^2 \mu\tau} + \frac{F_{mg}{}^2 M\, N_H T_{HM} T_{MH} b^2}{(N_{H+}\zeta_g)^2 \mu\gamma}}.$$

Note that the average number of secondary infections generated by an infected human host in the susceptible human population is given by

$$R_0^H = R_{MH} R_{HM}$$

and that the average number of secondary infections generated by an infected simian host in the susceptible simian population is given by

$$R_0^P = R_{MP} R_{PM}.$$

From the next generation matrix (K) it also follows that the average number of secondary infections generated by an infected simian host in the susceptible human population is given by

$$R_0^{PH} = R_{PM} R_{MH}$$

and, analogously, that the average number of secondary infections generated by an infected human host in the susceptible simian population is given by

$$R_0^{HP} = R_{HM} R_{MP}$$

Equilibrium points and stability analysis

The equilibrium conditions for the system of differential equations analyzed here, considering only the infection compartments, are

$$\frac{dI_P}{dt} = 0 = \frac{b T_{MP}(N_P - I_P^*) I_M^* F_{mc}}{N_P + \zeta_c} - \tau I_P^*$$

$$\frac{dI_H}{dt} = 0 = \frac{b T_{MH}(N_H - I_H^*) I_M^* F_{mg}}{N_H + \zeta_g} - \gamma I_H^*$$

$$\frac{dI_M}{dt} = 0 = \left(\frac{b T_{PM}(M - I_M^*) I_P^* F_{mc}}{N_P + \zeta_c} + \frac{b T_{HM}(M - I_M^*) I_H^* F_{mg}}{N_H + \zeta_g} \right) - \mu I_M^*,$$

where I_P^*, I_H^*, and I_M^* represent the variables at equilibrium. The solutions found for each variable were

$$I_P^* = 0,\ I_H^* = 0 \text{ and } I_M^* = 0$$

when in disease-free equilibrium, i.e., the pathogen is not circulating and the disease is absent in populations involved in the transmission cycle;
or

$$I_P^* = \frac{b T_{MP} N_P I_M^* F_{mc}}{b T_{MP} I_M^* F_{mc} + (N_P + \zeta_c)\tau},$$

$$I_H^* = \frac{bT_{MH}N_H I_M^* F_{mg}}{bT_{MH}I_M^* F_{mg} + (N_H + \zeta_g)\gamma},$$

$$I_M^* = \frac{b\left(T_{PM}MI_P^* F_{mc}\left(N_H + \zeta_g\right) + T_{HM}MI_H^* F_{mg}(N_P + \zeta_c)\right)}{b\left(T_{PM}I_P^* F_{mc}(N_H + \zeta_g) + T_{HM}I_H^* F_{mg}(N_P + \zeta_c)\right) + \mu(N_P + \zeta_c)\left(N_H + \zeta_g\right)}$$

when in the endemic equilibrium condition, i.e., the pathogen is circulating and the disease is established endemically in the populations concerned.

From the equilibrium condition it can be deduced that

$$S_P^* = N_P - I_P^*,\ S_H^* = N_H - I_H^*\ \text{and}\ S_M^* = M - I_M^*.$$

The stability of the equilibrium points, i.e., whether the system will approach or move away from the equilibrium points when it is in their vicinity (stable and unstable equilibrium points, respectively), depends on the threshold $R_0 = 1$ (bifurcation). When $R_0 < 1$, only the disease-free equilibrium point that is asymptotically stable can occur. If $R_0 > 1$, both equilibrium points occur, the endemic equilibrium being asymptotically stable and the disease-free equilibrium unstable [48,49]. S1 Fig shows the system bifurcation when R_0 exceeds the threshold value 1.

Scenarios exploration

Simulations were performed to evaluate the effect of variations in the parameters M and F_{mg} on the proportion of infected individuals in each population at equilibrium and on the basic reproduction numbers R_0, R_0^H, R_0^P, R_0^{PH} and R_0^{HP}. For each simulation three different scenarios were tested for the ratio of humans to monkeys: $N_H = 10N_P$, $N_H = 5N_P$ and $N_H = N_P$.

Data analysis was performed with the *rootSolve* [50] and *FME* [45] packages in R. Algebraic manipulations were performed with the *Maxima* computer algebra system [51].

Results

For the chosen values of the defined parameters, the numerical simulations show that the proportions of infected individuals in the monkey (I_P), human (I_H) and mosquito (I_M) populations at steady state change as the abundance of the vector population (M) changes. For a low ratio of M to C_{th} (with $C_{th} = 20$) the system remains in disease-free equilibrium. With an increase in this ratio, a threshold is reached where the disease-free equilibrium becomes unstable (when $R_0 > 1$) and a stable endemic equilibrium is reached. From this point on, the proportion of infected monkeys, humans and mosquitoes in the respective populations increases monotonically as the ratio M to C_{th} increases, as shown in Fig 2.

The threshold representing the bifurcation between disease-free and endemic equilibrium also changes with N_H: N_P and F_{mg} (or F_{mc}). For similar values of N_H and N_P an endemic equilibrium can occur with smaller values of M. As F_{mg} tends toward 0.5 (corresponding to maximum displacement between strata), the proportion of infected humans at steady state in the three different scenarios for N_H: N_P increases (Fig 2D, 2E and 2F). Similarly, the proportion of infected monkeys and mosquitoes in endemic equilibrium increases at higher values of F_{mg} when $N_H = 10N_P$ and $N_H = 5N_P$ (Fig 2A, 2B, 2G and 2H). For $N_H = N_P$ the proportions of infected monkeys and mosquitoes vary more with F_{mg} as *the* ratio of M to C_{th} increases (Fig
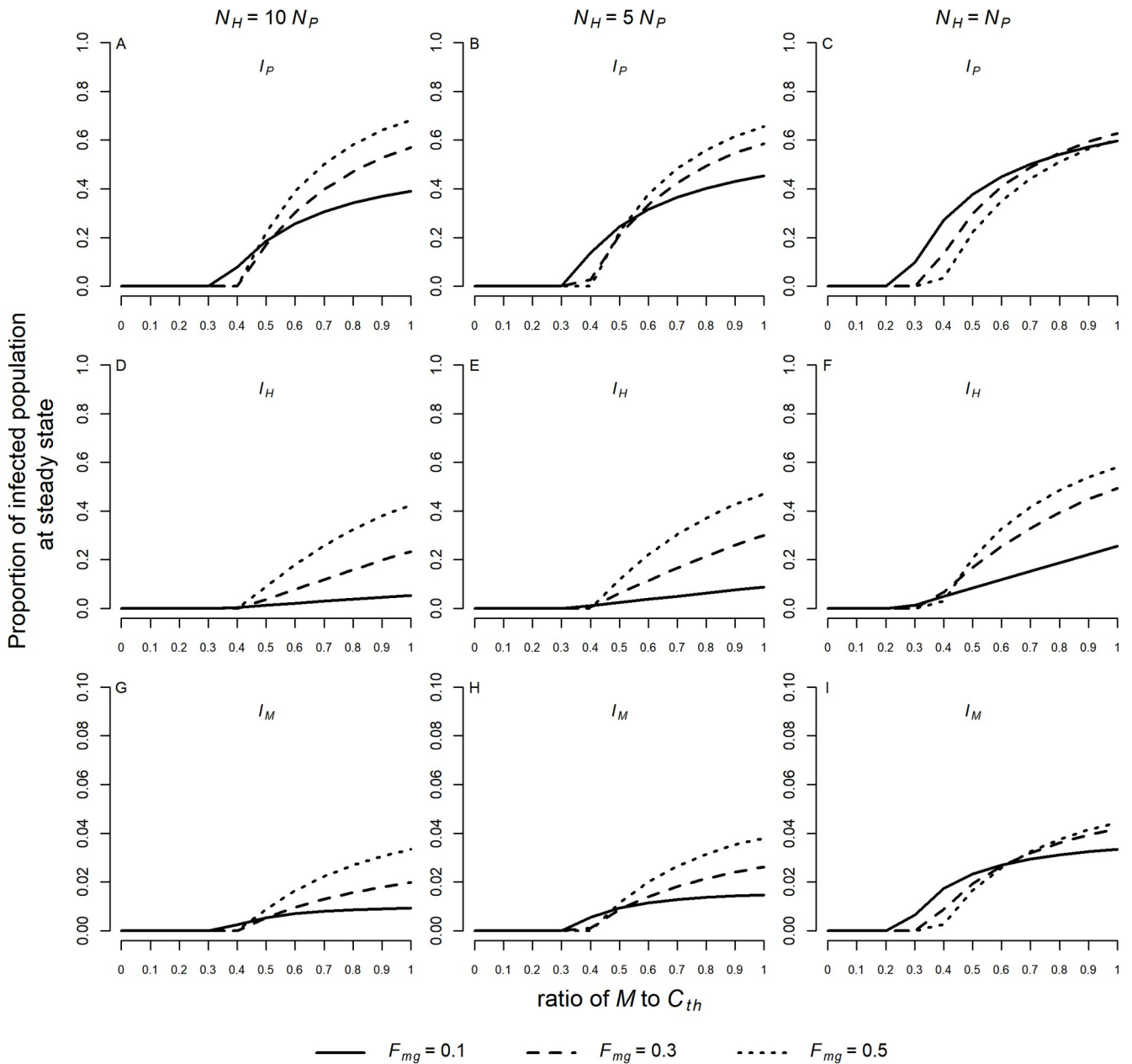
Fig 2. Proportions of infected individuals at steady state in the monkey (A, B, C), human (D, E, F), and mosquito (G, H, I) populations. Simulations were performed for a ratio of M to C_{th} varying from 0.01 to 0.99 and for $F_{mg} = 0.1$, 0.3 and 0.5. Three different scenarios were considered: $N_H = 10N_P$ (A, D, G), $N_H = 5N_P$ (B, E, H), and $N_H = N_P$ (C, F, I). The following values were assumed to simian parameters: $\tau = 0.0044$, $T_{MP} = 0.034$, and $T_{PM} = 0.428$ when $N_H = 10N_P$; $\tau = 0.0044$, $T_{MP} = 0.029$, and $T_{PM} = 0.342$ when $N_H = 5N_P$; $\tau = 0.0038$, $T_{MP} = 0.026$, and $T_{PM} = 0.261$ when $N_H = N_P$. The values of the other parameters used in the model were fixed: $C_{th} = 20(N_H + N_P)$, $C_c = (C_{th} - M)\frac{1}{2}$, $C_g = C_c$, $\mu = 0.8$, $\gamma = 0.0035$, $T_{MH} = 0.022$, $T_{MP} = 0.044$, $T_{HM} = 0.24$, $b = 0.5$, $h = 20$, $B_c = 0$ and $B_g = 0$.

2C and 2I). Different scenarios where the proportion of infected monkeys and mosquitoes in endemic equilibrium increases at lower values of F_{mg} can be seen in Supporting Information S3 and S4 Figs.

The epidemic threshold ($R_0 > 1$) tends to be exceeded as the ratio of M to C_{th} increases. As N_H becomes similar to N_P the epidemic threshold occurs at a lower ratio of M to C_{th}, and,

similarly, for lower F_{mg} the epidemic threshold is also exceeded at a lower ratio. Nevertheless, the values of R_0 vary little in relation to the different values of F_{mg} (Fig 3A, 3B and 3C).

Although R_0^H increases with higher values of M and F_{mg}, it only exceeds the epidemic threshold when $F_{mg} = 0.5$ and the ratio of M to C_{th} exceeds 0.8. In all other cases, it remains below the epidemic threshold (Fig 3D, 3E and 3F), suggesting that under conditions similar to those simulated, secondary cases of malaria after a human index case in the susceptible human population would be unlikely to occur. Conversely, lower values of F_{mg} (higher F_{mc}) favor transmission among monkeys; hence, lower values of M are needed for the epidemic threshold of R_0^P to be exceeded, especially when N_H is similar to N_P (Fig 3G, 3H and 3I).

Comparison of R_0^P with R_0^{PH} reveals that for $N_H = 10N_P$ and as M and F_{mg} increase, an infected monkey will generate more new cases in the susceptible human population than in the susceptible monkey population itself (Fig 3G and 3J). As N_H becomes similar to N_P this pattern is reversed and an infected monkey generates more new cases in the monkey population than in the susceptible human population (Fig 3H, 3L, 3I and 3M).

Finally, when R_0^{PH} and R_0^{HP} are compared for different values of M and F_{mg}, we find that for $N_H = 10N_P$ and $N_H = 5N_P$ the values of R_0^{PH} are higher than those of R_0^{HP} (Fig 3J, 3N, 3L and 3O). Conversely, when $N_H = N_P$ the simulated values of R_0^{PH} are lower than those of R_0^{HP} (Fig 3M and 3P). This indicates that in the first two cases an infected monkey may generate on average more new cases in the susceptible human population than an infected human generates in the susceptible monkey population whereas in the latter case the opposite may occur. In S5 and S6 Figs scenarios are presented where the role of humans in the generation of secondary cases in the human population itself (R_0^H) and in the simian population (R_0^{HP}) are irrelevant.

Discussion

Our simulations indicate that the dynamics of zoonotic transmission of malaria in the Atlantic Forest can vary depending on the abundance and acrodendrophily of the vector mosquito. Of particular note are the following findings: (1) an increase in the abundance of the vector in relation to the total number of blood-seeking mosquitoes leads to an asymptotic increase in the proportion of infected individuals at steady state and R_0; (2) the proportion of infected humans at steady state increases with increasing displacement of the vector mosquito between the forest strata; (3) in most scenarios, *Plasmodium* transmission would not be sustained between mosquitoes and humans alone, implying that NHPs play an important role in maintaining the transmission cycle.

According to empirical observations, there are at least two hypotheses that could explain the maintenance of human malaria foci in areas of the Atlantic Forest. The first is that *Plasmodium* species circulate enzootically between NHPs and mosquitoes, and that in certain circumstances zoonotic transmission of these parasites between NHPs and humans occurs, a possibility considered in the model described here. This hypothesis has gained greater support in recent years from studies that have proven the role of NHPs as reservoirs of *P. simium* and the finding of infected humans in close proximity to forests where the reservoir and vector are present [2,6–8,11]. The second hypothesis, which does not exclude the first, is that asymptomatic human infections are responsible for maintaining transmission foci [52] and that alternative vector species may participate in the transmission cycle in areas where *An. cruzii* is less abundant [53]. However, the role of humans in the maintenance of malaria foci in the Atlantic Forest has been questioned as humans infected with *P. simium* have low parasitemia, may no longer show signs of infection after several days and may not have relapses. In addition, not only have the human cases detected in the last decades been in individuals who had to go into forests or who live on the edges of forests where enzootic transmission cycles occur, but also
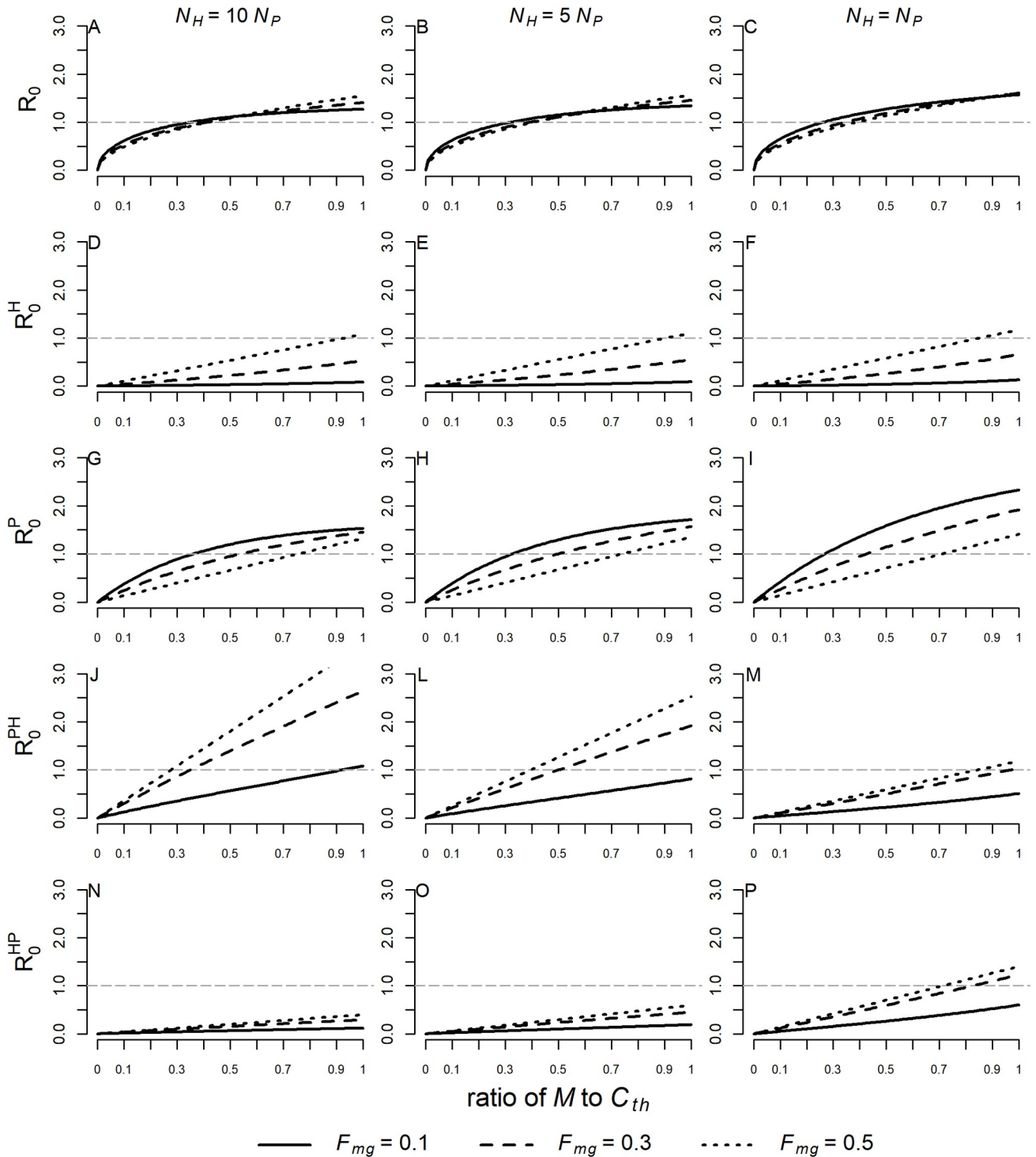
Fig 3. Variations in R_0, R_0^H, R_0^P, R_0^{PH} and R_0^{HP} with the ratio of M to C_{th} and F_{mg} (or $1-F_{mc}$). The dashed line represents the epidemic threshold, above which more than one new case will be generated in the susceptible population by an infected individual. Values were simulated for ratios of M to C_{th} ranging from 0.01 to 0.99 and for F_{mg} = 0.1, 0.3, and 0.5. Three different scenarios were considered: $N_H = 10N_P$ (A, D, G, J, N), $N_H = 5N_P$ (B, E, H. L, O) and $N_H = N_P$ (C, F, I, M, P). The following values were assumed to simian parameters: τ = 0.0044, T_{MP} = 0.034, and T_{PM} = 0.428 when $N_H = 10N_P$; τ = 0.0044, T_{MP} = 0.029, and T_{PM} = 0.342 when $N_H = 5N_P$; τ = 0.0038, T_{MP} = 0.026, and T_{PM} = 0.261 when $N_H = N_P$. The values of the other parameters were fixed: $C_{th} = 20(N_H + N_P)$, $C_c = (C_{th} - M)\frac{1}{2}$, $C_g = C_c$, μ = 0.8, γ = 0.0035, T_{MH} = 0.022, T_{MP} = 0.044, T_{HM} = 0.24, b = 0.5, h = 20, B_c = 0 and B_g = 0.

no secondary cases derived directly from a human case have been detected outside these sylvatic foci [2,11]. In this sense, the present model supports the assumption that infected humans may not be able to produce cases of secondary infection in the same human population as it predicts that in most of the simulated scenarios it would not be possible for a human index case to be directly responsible for more than one new case in average in the local human population. Nonetheless, the model assumes that humans have enough parasitemia to infect mosquitoes and allow maintenance of the zoonotic cycle, i.e., humans were not considered dead ends.

The predictions made by the model showed that vector abundance is a determining factor for outbreaks and maintenance of the transmission cycle. In fact, autochthonous malaria in the Atlantic Forest has frequently been associated with a high abundance of *An. cruzii* [8,33,53–55]. Mosquitoes of this species usually occur in greater abundance in humid forests on coastal slopes, especially in the region known as Serra do Mar, an extensive mountain range in Southeastern Brazil that harbors the largest remnant of Atlantic Forest as well as many species of NHPs and several human settlements and tourist sites, making it a very favorable setting for autochthonous malaria outbreaks resulting from zoonotic transmission [1,2,56]. For simplicity and clarity, the model described here considers a constant abundance for *An. cruzii*; however, climatic and environmental variations that influence the larval development rate, reproduction rate and longevity of vector mosquitoes can be of great importance when determining the temporal dynamics of *Plasmodium* transmission [57].

Vector displacement between the upper and lower forest strata (variations in acrodendrophily) is an important parameter that should be considered in the dynamics of *Plasmodium* transmission between and circulation among NHPs and humans. As predicted by the model, zoonotic transmission of *Plasmodium* and the prevalence of infection in the local human population are strongly influenced by this vertical movement of the vector, a hypothesis raised decades ago by Deane et al. [8,30,35] based on his field observations. It is not known for certain which factors may lead to greater or lesser displacement of *An. cruzii* between the canopy and ground level, but genetic and morphological variations between different populations have been found in some studies, suggesting that this mosquito may actually represent a complex of cryptic species [58–60]. A recent study indicates that in preserved areas with a moderate human presence the edge effect may favor activity of this mosquito at ground level, possibly because of a greater supply of blood from humans and domestic animals [34]. This suggests that the landscape matrix may influence mosquito acrodendrophily and feeding behavior.

The changes in the landscape promoted by humans and the consequent loss of biodiversity have been shown to be important factors for the emergence of malaria outbreaks in recently deforested areas in the Amazon region, a phenomenon that has been called "frontier malaria" [61–63]. Similarly, agricultural expansion and forest fragmentation have been associated with increased risks of exposure to *P. knowlesi* infection in Malaysia [64,65]. The Atlantic Forest biome has historically undergone an intense process of urbanization and agricultural expansion that allowed the economic growth of the region, but, on the other hand, led to a drastic reduction and fragmentation of natural habitats [66]. The loss and fragmentation of natural habitats has several negative impacts on NHP populations and favors greater proximity of these animals with human-modified and occupied areas [67,68]. This scenario may favor the establishment of zoonotic cycles of malaria in conditions where the circulation of *Plasmodium* exists and the vector mosquito is abundant and feeds on both humans and NHP [8]. Although variations in the landscape and the spatial dynamics of transmission have not been modeled in the present study, the proposed model includes parameters that are directly relevant to investigate the effects of biodiversity loss and variations in vector abundance and behavior on the dynamics of malaria in the Atlantic Forest. It should be mentioned that the prevalences of

infection in the vector and host populations predicted in the simulations in the present study are similar to those reported in empirical studies as a high prevalence of *Plasmodium* infection in howler monkeys and a lower prevalence in mosquito and human populations has often been observed in the Atlantic Forest. Deane et al. [35] report that in locations in Southeastern and Southern Brazil the proportion of infected howler monkeys ranged from 31 to 62%, and between 0.7 and 2% of mosquitoes had *Plasmodium* sporozoites in their salivary glands. More recent studies using molecular techniques indicate a prevalence of 25 to 35% in howler monkeys and a minimum infection rate of 0.01 to 1% in *An. cruzii* [11,33,52,53,55,69,70]. A prevalence of around 2 to 3% has been observed in human populations tested in autochthonous malaria foci of Southeastern Brazil [5–7].

An important limitation of the present model is that it does not consider the natural variability and randomness of some important processes in the transmission dynamics, such as temporal changes in the abundance of the vector, which is influenced by seasonal climatic and environmental variations and the movement of humans and monkeys in the forest. Future models could also: include the latency period of *Plasmodium* in the vector and hosts; differentiate between symptomatic and asymptomatic human cases; distinguish between infections with different *Plasmodium* species; and include auxiliary vector species in the transmission dynamics. In addition, more accurate estimates of vector and host transmission parameters, especially those related to simian reservoirs, should be determined from empirical data as this would ensure more realistic, reliable predictions. Despite these limitations, the model proposed here provides a basis for other models to be developed and further studies carried out in order to better understand and more accurately predict zoonotic transmission of malaria in the Atlantic Forest.

Conclusion

The transmission dynamics of a simulated zoonotic scenario was modeled with a new mathematical model. The results show that variations in the abundance and acrodendrophily of the main malaria vector (*An. cruzii*) significantly affect the prevalence of human malaria infection and the basic reproduction number for malaria in the Atlantic Forest.

Supporting information

S1 Text. The parameters ζc and ζg.
(PDF)

S2 Text. Estimation of the parameters related to the simian host.
(PDF)

S3 Text. Obtaining the basic reproduction number.
(PDF)

S1 Table. Estimation of simian parameters and 95% credible interval (highest posterior density) obtained by Markov Chain Monte Carlo sampling method. Alternative values for each parameter were estimated for different sets of human parameters.
(DOCX)

S1 Fig. Bifurcation plots showing the change in stability between disease-free and endemic equilibria when $R_0 = 1$: A—proportion of infected non-human primates at equilibrium, B —proportion of infected humans at equilibrium, C—proportion of infected mosquitoes at equilibrium. The R_0 values were obtained by the variation of M (from 0.013 to $0.91C_{th}$). The other parameters were set at: $b = 0.5$, $\mu = 0.8$, $T_{MH} = 0.022$, $T_{HM} = 0.24$, $\gamma = 0.0035$, $h = 20$, F_{mc}

= 0.58, $F_{mg} = 1 - F_{mc}$, $N_H = 300$, $N_P = 30$, $C_{th} = 20(N_P + N_H)$, $C_c = (C_{th} - M)^{\frac{1}{2}}$, $C_g = C_c$, $B_c = 0$, $B_g = 0$, $\tau = 0.0044$, $T_{MP} = 0.034$, and $T_{PM} = 0.428$.
(TIF)

S2 Fig. Graphical representation of *An. cruzii* displacement between canopy and ground level. The vertical axis corresponds to the probability that the same mosquito bites first in the canopy and then at ground level (or vice versa), which equals $2F_{mc}F_{mg}$. The dotted line represents the maximum probability, which occurs when $F_{mc} = F_{mg} = 0.5$.
(TIF)

S3 Fig. Proportions of infected individuals at steady state in the monkey (A, B, C), human (D, E, F), and mosquito (G, H, I) populations when THM = 0.024. Simulations were performed for a ratio of M to Cth varying from 0.01 to 0.99 and for Fmg = 0.1, 0.3 and 0.5. Three different scenarios were considered: NH = 10NP (A, D, G), NH = 5NP (B, E, H), and NH = NP (C, F, I). The following values were assumed to simian parameters: $\tau = 0.0027$, TMP = 0.04, and TPM = 0.38 when NH = 10NP; $\tau = 0.0027$, TMP = 0.03, and TPM = 0.346 when NH = 5NP; τ = 0.0028, TMP = 0.026, and TPM = 0.291 when NH = NP. The values of the other parameters used in the model were fixed: Cth = 20(NH + NP), $C_c = (C_{th} - M)^{\frac{1}{2}}$, $C_g = C_c$, $\mu = 0.8$, $\gamma = 0.0035$, $T_{MH} = 0.022$, $T_{MP} = 0.044$, $b = 0.5$, $h = 20$, $B_c = 0$ and $B_g = 0$.
(TIF)

S4 Fig. Proportions of infected individuals at steady state in the monkey (A, B, C), human (D, E, F), and mosquito (G, H, I) populations when THM = 0.024 and $\gamma = 0.0055$. Simulations were performed for a ratio of M to Cth varying from 0.01 to 0.99 and for Fmg = 0.1, 0.3 and 0.5. Three different scenarios were considered: NH = 10NP (A, D, G), NH = 5NP (B, E, H), and NH = NP (C, F, I). The following values were assumed to simian parameters: $\tau = 0.0025$, TMP = 0.041, and TPM = 0.348 when NH = 10NP; $\tau = 0.0031$, TMP = 0.04, and TPM = 0.306 when NH = 5NP; $\tau = 0.003$, TMP = 0.028, and TPM = 0.308 when NH = NP. The values of the other parameters used in the model were fixed: Cth = 20(NH + NP), $C_c = (C_{th} - M)^{\frac{1}{2}}$, $C_g = C_c$, $\mu = 0.8$, $T_{MH} = 0.022$, $T_{MP} = 0.044$, $b = 0.5$, $h = 20$, $B_c = 0$ and $B_g = 0$.
(TIF)

S5 Fig. Variations in R_0, R_0^H, R_0^P, R_0^{PH} and R_0^{HP} when $T_{HM} = 0.024$. The dashed line represents the epidemic threshold, above which more than one new case will be generated in the susceptible population by an infected individual. Values were simulated for ratios of M to C_{th} ranging from 0.01 to 0.99 and for $F_{mg} = 0.1$, 0.3, and 0.5. Three different scenarios were considered: $N_H = 10N_P$ (A, D, G, J, N), $N_H = 5N_P$ (B, E, H, L, O) and $N_H = N_P$ (C, F, I, M, P). The following values were assumed to simian parameters: $\tau = 0.0027$, $T_{MP} = 0.04$, and $T_{PM} = 0.38$ when $N_H = 10N_P$; $\tau = 0.0027$, $T_{MP} = 0.03$, and $T_{PM} = 0.346$ when $N_H = 5N_P$; $\tau = 0.0028$, $T_{MP} = 0.026$, and $T_{PM} = 0.291$ when $N_H = N_P$. The values of the other parameters used in the model were fixed: $C_{th} = 20(N_H + N_P)$, $C_c = (C_{th} - M)^{\frac{1}{2}}$, $C_g = C_c$, $\mu = 0.8$, $\gamma = 0.0035$, $T_{MH} = 0.022$, $T_{MP} = 0.044$, $b = 0.5$, $h = 20$, $B_c = 0$ and $B_g = 0$.
(TIF)

S6 Fig. Variations in R0, R_0^H, R_0^P, R_0^{PH} and R_0^{HP} when THM = 0.024 and $\gamma = 0.0055$. The dashed line represents the epidemic threshold, above which more than one new case will be generated in the susceptible population by an infected individual. Values were simulated for ratios of M to C_{th} ranging from 0.01 to 0.99 and for $F_{mg} = 0.1$, 0.3, and 0.5. Three different scenarios were considered: $N_H = 10N_P$ (A, D, G, J, N), $N_H = 5N_P$ (B, E, H, L, O) and $N_H = N_P$ (C, F, I, M, P). The following values were assumed to simian parameters: $\tau = 0.0025$, $T_{MP} = 0.041$, and $T_{PM} = 0.348$ when $N_H = 10N_P$; $\tau = 0.0031$, $T_{MP} = 0.04$, and $T_{PM} = 0.306$ when $N_H = 5N_P$; τ

= 0.003, T_{MP} = 0.028, and T_{PM} = 0.308 when $N_H = N_P$. The values of the other parameters used in the model were fixed: $C_{th} = 20(N_H + N_P)$, $C_c = (C_{th} - M)\frac{1}{2}$, $C_g = C_c$, $\mu = 0.8$, $T_{MH} = 0.022$, $T_{MP} = 0.044$, $b = 0.5$, $h = 20$, $B_c = 0$ and $B_g = 0$.
(TIF)

Acknowledgments

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References

1. de Pina-Costa A, Brasil P, di Santi SM, de Araujo MP, Suárez-Mutis MC, Santelli ACF e. S, et al. Malaria in Brazil: What happens outside the Amazonian endemic region. Mem Inst Oswaldo Cruz. 2014; https://doi.org/10.1590/0074-0276140228 PMID: 25185003

2. Brasil P, Zalis MG, de Pina-Costa A, Siqueira AM, Júnior CB, Silva S, et al. Outbreak of human malaria caused by *Plasmodium simium* in the Atlantic Forest in Rio de Janeiro: a molecular epidemiological investigation. Lancet Glob Heal. 2017; 5: e1038–e1046. https://doi.org/10.1016/S2214-109X(17)30333-9

3. Marrelli MT, Malafronte RS, Sallum MAM, Natal D. *Kerteszia* subgenus of *Anopheles* associated with the Brazilian Atlantic rainforest:current knowledge and future challenges. Malar J. 2007; 6: 127. https://doi.org/10.1186/1475-2875-6-127 PMID: 17880709

4. Harbach R. Mosquito Taxonomic Inventory. In: Anopheles Meigen, 1818 [Internet]. 2020 [cited 13 Apr 2020]. Available: http://mosquito-taxonomic-inventory.info/simpletaxonomy/term/6047.

5. Curado I, dos Santos Malafronte R, de Castro Duarte AMR, Kirchgatter K, Branquinho MS, Bianchi Galati EA. Malaria epidemiology in low-endemicity areas of the Atlantic Forest in the Vale do Ribeira, São Paulo, Brazil. Acta Trop. 2006; https://doi.org/10.1016/j.actatropica.2006.09.010 PMID: 17126279

6. Cerutti C, Boulos M, Coutinho AF, Hatab MDCLD, Falqueto A, Rezende HR, et al. Epidemiologic aspects of the malaria transmission cycle in an area of very low incidence in Brazil. Malar J. 2007; https://doi.org/10.1186/1475-2875-6-33 PMID: 17371598

7. de Alencar FEC, Malafronte RDS, Cerutti Junior C, Natal Fernandes L, Buery JC, Fux B, et al. Assessment of asymptomatic *Plasmodium* spp. infection by detection of parasite DNA in residents of an extra-

Amazonian region of Brazil. Malar J. 2018; 17: 113. https://doi.org/10.1186/s12936-018-2263-z PMID: 29540186

8. Deane LM. Simian malaria in Brazil. Mem Inst Oswaldo Cruz. 1992; 87: 1–20. https://doi.org/10.1590/s0074-02761992000700001 PMID: 1343676

9. Duarte AMR de C, Porto MAL, Curado I, Malafronte RS, Hoffmann EHE, Oliveira SG, et al. Widespread occurrence of antibodies against circumsporozoite protein and against blood forms of *Plasmodium vivax*, *P. falciparum* and *P. malariae* in Brazilian wild monkeys. J Med Primatol. 2006; 35: 87–96. https://doi.org/10.1111/j.1600-0684.2006.00148.x PMID: 16556295

10. Yamasaki T, Duarte AMRC, Curado I, Summa MEL, Neves DVDA, Wunderlich G, et al. Detection of etiological agents of malaria in howler monkeys from Atlantic Forests, rescued in regions of São Paulo city, Brazil. J Med Primatol. 2011; https://doi.org/10.1111/j.1600-0684.2011.00498.x PMID: 21933192

11. Abreu FVS de, Santos E Dos, Mello ARL, Gomes LR, Alvarenga DAM de, Gomes MQ, et al. Howler monkeys are the reservoir of malarial parasites causing zoonotic infections in the Atlantic Forest of Rio de Janeiro. Fuehrer H-P, editor. PLoS Negl Trop Dis. 2019; 13: e0007906. https://doi.org/10.1371/journal.pntd.0007906 PMID: 31815937

12. Goldman IF, Qari SH, Millet PG, Collins WE, Lal AA. Circumsporozoite protein gene of *Plasmodium simium*, a *Plasmodium vivax*-like monkey malaria parasite. Mol Biochem Parasitol. 1993; 57: 177–180. https://doi.org/10.1016/0166-6851(93)90257-x PMID: 8426613

13. Lim CS, Tazi L, Ayala FJ. Plasmodium vivax: Recent world expansion and genetic identity to *Plasmodium simium*. Proc Natl Acad Sci. 2005; 102: 15523–15528. https://doi.org/10.1073/pnas.0507413102 PMID: 16227436

14. Lalremruata A, Magris M, Vivas-Martínez S, Koehler M, Esen M, Kempaiah P, et al. Natural infection of *Plasmodium brasilianum* in humans: Man and monkey share quartan malaria parasites in the Venezuelan Amazon. EBioMedicine. 2015; 2: 1186–1192. https://doi.org/10.1016/j.ebiom.2015.07.033 PMID: 26501116

15. Mourier T, Alvarenga DAM de, Kaushik A, Pina-Costa A de, Guzmán-Vega FJ, Douvropoulou O, et al. The genome of the zoonotic malaria parasite *Plasmodium simium* reveals adaptations to host-switching. bioRxiv. 2019; https://doi.org/10.1101/841171

16. Li J, Collins WE, Wirtz RA, Rathore D, Lal A, McCutchan TF. Geographic Subdivision of the Range of the Malaria Parasite, *Plasmodium vivax*. Emerg Infect Dis. 2001; 7: 35–42. https://doi.org/10.3201/eid0701.010105 PMID: 11266292

17. Tazi L, Ayala FJ. Unresolved direction of host transfer of *Plasmodium vivax* v. *P. simium* and *P. malariae* v. *P. brasilianum*. Infect Genet Evol. 2011; 11: 209–221. https://doi.org/10.1016/j.meegid.2010.08.007 PMID: 20849978

18. Chin W, Contacos PG, Coatney GR, Kimball HR. A Naturally Acquired Quotidian-Type Malaria in Man Transferable to Monkeys. Science (80-). 1965; 149: 865–865. https://doi.org/10.1126/science.149.3686.865 PMID: 14332847

19. Deane LM, Deane MP, Ferreira Neto J. Studies on transmission of simian malaria and on a natural infection of man with *Plasmodium simium* in Brazil. Bull World Health Organ. 1966; 35: 805. https://doi.org/7/4/245 [pii] PMID: 5297817

20. Most H. *Plasmodium cynomolgi* malaria: Accidental human infection. Am J Trop Med Hyg. 1973; 22: 157–158. https://doi.org/10.4269/ajtmh.1973.22.157 PMID: 4631763

21. Ta TH, Hisam S, Lanza M, Jiram AI, Ismail N, Rubio JM. First case of a naturally acquired human infection with *Plasmodium cynomolgi*. Malar J. 2014; 13: 68. https://doi.org/10.1186/1475-2875-13-68 PMID: 24564912

22. Singh B, Sung LK, Matusop A, Radhakrishnan A, Shamsul SSG, Cox-Singh J, et al. A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. Lancet. 2004; 363: 1017–1024. https://doi.org/10.1016/S0140-6736(04)15836-4 PMID: 15051281

23. Vythilingam I, NoorAzian YM, Huat T, Jiram A, Yusri YM, Azahari AH, et al. *Plasmodium knowlesi* in humans, macaques and mosquitoes in peninsular Malaysia. Parasit Vectors. 2008; 1: 26. https://doi.org/10.1186/1756-3305-1-26 PMID: 18710577

24. Ng OT, Ooi EE, Lee CC, Lee PJ, Ng LC, Pei SW, et al. Naturally Acquired Human *Plasmodium knowlesi* Infection, Singapore. Emerg Infect Dis. 2008; 14: 814–816. https://doi.org/10.3201/eid1405.070863 PMID: 18439370

25. Luchavez J, Espino F, Curameng P, Espina R, Bell D, Chiodini P, et al. Human Infections with *Plasmodium knowlesi*, the Philippines. Emerg Infect Dis. 2008; 14: 811–813. https://doi.org/10.3201/eid1405.071407 PMID: 18439369

26. Eede P Van Den, Van HN, Van Overmeir C, Vythilingam I, Duc TN, Hung LX, et al. Human *Plasmodium knowlesi* infections in young children in central Vietnam. Malar J. 2009; 8: 249. https://doi.org/10.1186/1475-2875-8-249 PMID: 19878553

27. Jongwutiwes S, Buppan P, Kosuvin R, Seethamchai S, Pattanawong U, Sirichaisinthop J, et al. *Plasmodium knowlesi* Malaria in Humans and Macaques, Thailand. Emerg Infect Dis. 2011; 17: 1799–1806. https://doi.org/10.3201/eid1710.110349 PMID: 22000348

28. Garnham PCC, Harper JO, Highton RB. The mosquitos of the Kaimosi forest, Kenya colony, with special reference to yellow fever. Bull Entomol Res. 1946; 36: 473–496. https://doi.org/10.1017/s000748530002410x PMID: 21015624

29. Forattini OP, Lopes OS, Rabello EX. Investigações sobre o comportamento de formas adultas de mosquitos silvestres no Estado de São Paulo, Brasil. Rev Saude Publica. 1968; https://doi.org/10.1590/S0034-89101968000200002

30. Deane LM, Ferreira Neto JA, Lima MM. The vertical dispersion of *Anopheles* (*Kerteszia*) *cruzi* in a forest in southern Brazil suggests that human cases of malaria of simian origin might be expected. Mem Inst Oswaldo Cruz. 1984; 79: 461–463. https://doi.org/10.1590/s0074-02761984000400011 PMID: 6533421

31. Guimarães AE, Arlé M, Machado RNM. Mosquitos no Parque Nacional da Serra dos Orgãos, Estado do Rio de Janeiro, Brasil: II. Distribuição vertical. Mem Inst Oswaldo Cruz. 1985; 80: 171–185. https://doi.org/10.1590/S0074-02761985000200008

32. Ueno HM, Forattini OP, Kakitani I. Distribuição vertical e sazonal de *Anopheles* (*Kerteszia*) em Ilha Comprida, SP. Rev Saude Publica. 2007; https://doi.org/10.1590/S0034-89102007000200014 PMID: 17384803

33. Buery JC, Rezende HR, Natal L, Da Silva LS, De Menezes RMT, Fux B, et al. Ecological characterisation and infection of anophelines (Diptera: Culicidae) of the Atlantic Forest in the Southeast of Brazil over a 10 year period: Has the behaviour of the autochthonous malaria vector changed? Mem Inst Oswaldo Cruz. 2018; https://doi.org/10.1590/0074-02760170225 PMID: 29236924

34. Medeiros-Sousa AR, de Oliveira Christe R, de Castro Duarte AMR, Mucci LF, Ceretti-Junior W, Marrelli MT. Effects of anthropogenic landscape changes on the abundance and acrodendrophily of *Anopheles* (*Kerteszia*) *cruzii*, the main vector of malaria parasites in the Atlantic Forest in Brazil. Malar J. 2019; 18: 110. https://doi.org/10.1186/s12936-019-2744-8 PMID: 30940142

35. Deane LM, Deane MP, Ferreira Neto JA, Barbosa de Almeida F. On the transmission of simian malaria in Brazil. Rev Inst Med Trop Sao Paulo. 1971; 13: 311–319. PMID: 5162258

36. Oliva WM, Sallum EM. Periodic dynamic systems for infected hosts and mosquitoes. Rev Saude Publica. 1996; 30: 218–223. https://doi.org/10.1590/s0034-89101996000300003 PMID: 9110466

37. Laporta GZ, Prado PIKL De, Kraenkel RA, Coutinho RM, Sallum MAM. Biodiversity Can Help Prevent Malaria Outbreaks in Tropical Forests. PLoS Negl Trop Dis. 2013; 7: e2139. https://doi.org/10.1371/journal.pntd.0002139 PMID: 23556023

38. Alvarenga DAM, Pina-Costa A, Sousa TN, Pissinatti A, Zalis MG, Suaréz-Mutis MC, et al. Simian malaria in the Brazilian Atlantic forest: first description of natural infection of capuchin monkeys (Cebinae subfamily) by *Plasmodium simium*. Malar J. 2015; 14(1): 81. https://doi.org/10.1186/s12936-015-0606-6 PMID: 25889933

39. Alvarenga DAM, Pina-Costa A, Bianco C, Moreira SB, Brasil P, Pissinatti A, et al. New potential *Plasmodium brasilianum* hosts: tamarin and armoset monkeys (family Callitrichidae). Malar J. 2017; 16(1): 1–7. https://doi.org/10.1186/s12936-016-1650-6 PMID: 28049519

40. Monteiro EF, Fernandez-Becerra C, Araujo MDS, Messias MR, Ozaki LS, Duarte AMRC, et al. Naturally Acquired Humoral Immunity against Malaria Parasites in Non-Human Primates from the Brazilian Amazon, Cerrado and Atlantic Forest. Pathogens. 2020; 9(7): 525. https://doi.org/10.3390/pathogens9070525 PMID: 32610598

41. Santos RLC dos. Medida da capacidade vetorial de *Anopheles albitarsis* e de *Anopheles* (*Kerteszia*) no Vale do Ribeira, São Paulo [Internet]. Universidade de São Paulo. 2001. https://doi.org/10.11606/T.6.2020.tde-31032020-102041

42. Chitnis N, Hyman JM, Cushing JM. Determining important parameters in the spread of malaria through the sensitivity analysis of a mathematical model. Bull Math Biol. 2008; 70: 1272–1296. https://doi.org/10.1007/s11538-008-9299-0 PMID: 18293044

43. Hastings WK. Monte Carlo sampling methods using Markov chains and their applications. Biometrika. 1970; 57: 97–109. https://doi.org/10.1093/biomet/57.1.97

44. Haario H, Saksman E, Tamminen J. An adaptive Metropolis algorithm. Bernoulli. 2001; 7(2): 223–242.

45. Soetaert K, Petzoldt T. Inverse Modelling, Sensitivity and Monte Carlo Analysis in R Using Package FME. J Stat Softw. 2010; 33. https://doi.org/10.18637/jss.v033.i03 PMID: 20808728

46. Diekmann O, Heesterbeek JAP, Metz JAJ. On the definition and the computation of the basic reproduction ratio R_0 in models for infectious diseases in heterogeneous populations. J Math Biol. 1990;28. https://doi.org/10.1007/BF00178324 PMID: 2117040

47. Diekmann O, Heesterbeek JAP, Roberts MG. The construction of next-generation matrices for compartmental epidemic models. J R Soc Interface. 2010; 7: 873–885. https://doi.org/10.1098/rsif.2009.0386 PMID: 19892718

48. van den Driessche P, Watmough J. Reproduction numbers and sub-threshold endemic equilibria for compartmental models of disease transmission. Math Biosci. 2002; 180: 29–48. https://doi.org/10.1016/s0025-5564(02)00108-6 PMID: 12387915

49. Chitnis N, Cushing JM, Hyman JM. Bifurcation Analysis of a Mathematical Model for Malaria Transmission. SIAM J Appl Math. 2006; 67: 24–45. https://doi.org/10.1137/050638941

50. Soetaert K. rootSolve: Nonlinear root finding, equilibrium and steady-state analysis of ordinary differential equations. R package version; 2009.

51. MAXIMA. Maxima, a Computer Algebra System. [Internet]. 2019. Available: http://maxima.sourceforge.net/.

52. Kirchgatter K, Tubaki RM, Malafronte R dos S, Alves IC, Lima GFM de C, Guimarães L de O, et al. *Anopheles* (*Kerteszia*) *cruzii* (diptera: culicidae) in peridomiciliary area during asymptomatic malaria transmission in the Atlantic Forest: molecular identification of blood-meal sources indicates humans as primary intermediate hosts. Rev Inst Med Trop Sao Paulo. 2014; https://doi.org/10.1590/s0036-46652014000500006 PMID: 25229220

53. Duarte AMR, Pereira DM, de Paula MB, Fernandes A, Urbinatti PR, Ribeiro AF, et al. Natural infection in anopheline species and its implications for autochthonous malaria in the Atlantic forest in Brazil. Parasit Vectors. 2013; 6: 58. https://doi.org/10.1186/1756-3305-6-58 PMID: 23497493

54. Rezende HR, Soares RM, Cerutti Jr C, Alves IC, Natal D, Urbinatti PR, et al. Entomological characterization and natural infection of anophelines in an area of the Atlantic Forest with autochthonous malaria cases in mountainous region of Espírito Santo State, Brazil. Neotrop Entomol. 2009; 38: 272–280. https://doi.org/10.1590/s1519-566x2009000200017 PMID: 19488519

55. Neves A, Urbinatti PR, Malafronte R dos S, Fernandes A, Paganini W da S, Natal D. Malaria outside the Amazon region: Natural *Plasmodium* infection in anophelines collected near an indigenous village in the Vale do Rio Branco, Itanhaem, SP, Brazil. Acta Trop. 2013; 125: 102–106. https://doi.org/10.1016/j.actatropica.2012.08.014 PMID: 22989665

56. Laporta GZ, Ramos DG, Ribeiro MC, Sallum MAM. Habitat suitability of *Anopheles* vector species and association with human malaria in the Atlantic Forest in south-eastern Brazil. Mem Inst Oswaldo Cruz. 2011; https://doi.org/10.1590/s0074-02762011000900029 PMID: 21881779

57. Kar N, Kumar A, Singh OP, Carlton JM, Nanda N. A review of malaria transmission dynamics in forest ecosystems. Parasit Vectors. 2014; 7: 265. https://doi.org/10.1186/1756-3305-7-265 PMID: 24912923

58. Rona LD, Carvalho-Pinto CJ, Gentile C, Grisard EC, Peixoto AA. Assessing the molecular divergence between *Anopheles* (*Kerteszia*) *cruzii* populations from Brazil using the timeless gene: Further evidence of a species complex. Malar J. 2009; https://doi.org/10.1186/1475-2875-8-60 PMID: 19358734

59. Lorenz C, Marques TC, Sallum MAM, Suesdek L. Altitudinal population structure and microevolution of the malaria vector *Anopheles cruzii* (Diptera: Culicidae). Parasit Vectors. 2014; 7: 581. https://doi.org/10.1186/s13071-014-0581-8 PMID: 25511160

60. de Rezende Dias G, Fujii TTS, Fogel BF, Lourenço-de-Oliveira R, Silva-do-Nascimento TF, Pitaluga AN, et al. Cryptic diversity in an Atlantic Forest malaria vector from the mountains of South-East Brazil. Parasit Vectors. 2018; 11: 36. https://doi.org/10.1186/s13071-018-2615-0 PMID: 29335015

61. Sawyer DR. Frontier malaria in the Amazon Region of Brazil: types of malaria situations and some implications for control. Brasília: PHO/WHO/TDR; 1988.

62. Castro MC, Monte-Mor RL, Sawyer DO, Singer BH. Malaria risk on the Amazon frontier. Proc Natl Acad Sci USA. 2006; 103: 2452–7. https://doi.org/10.1073/pnas.0510576103 PMID: 16461902

63. Chaves LSM, Conn JE, López RVM, Sallum MAM. Abundance of impacted forest patches less than 5 km2 is a key driver of the incidence of malaria in Amazonian Brazil. Sci Rep. 2018; 8: 7077. https://doi.org/10.1038/s41598-018-25344-5 PMID: 29728637

64. Fornace KM, Abidin TR, Alexander N, Brock P, Grigg MJ, Murphy A, et al. Association between landscape factors and spatial patterns of *Plasmodium knowlesi* infections in Sabah, Malaysia. Emerg Infect Dis. 2016; 22(2): 201. https://doi.org/10.3201/eid2202.150656 PMID: 26812373

65. Fornace KM, Brock PM, Abidin TR, Grignard L, Herman LS, Chua TH, et al. Environmental risk factors and exposure to the zoonotic malaria parasite *Plasmodium knowlesi* across northern Sabah, Malaysia: a population-based cross-sectional survey. Lancet Planet Health. 2019; 3(4): e179–e186. https://doi.org/10.1016/S2542-5196(19)30045-2 PMID: 31029229

66. Rezende CL, Scarano FR, Assad ED, Joly CA, Metzger JP, Strassburg BBN, et al. From hotspot to hopespot: An opportunity for the Brazilian Atlantic Forest. Perspect Ecol Conserv. 2018; 16(4): 208–214.

67. Chapman CA, Gillespie TR, Goldberg TL. Primates and the ecology of their infectious diseases: how will anthropogenic change affect host-parasite interactions?. Evol Anthropol. 2005; 14(4): 134–144.

68. Bicca-Marques JC, Chaves ÓM, Hass GP. Howler monkey tolerance to habitat shrinking: Lifetime warranty or death sentence?. Am J Primatol. 2020; 82(4): e23089. https://doi.org/10.1002/ajp.23089 PMID: 31912561

69. Costa DC, Cunha VP da, Assis GMP de, Souza Junior JC de, Hirano ZMB, Arruda ME de, et al. *Plasmodium simium/Plasmodium vivax* infections in southern brown howler monkeys from the Atlantic Forest. Mem Inst Oswaldo Cruz. 2014; 109: 641–653. https://doi.org/10.1590/0074-0276130578 PMID: 25099335

70. Demari-Silva B, Laporta GZ, Oliveira T, Sallum M. *Plasmodium* infection in *Kerteszia cruzii* (Diptera: Culicidae) in the Atlantic tropical rain forest, southeastern Brazil. Infect Genet Evol. 2020; 78: 104061. https://doi.org/10.1016/j.meegid.2019.104061 PMID: 31683005