activates transcription of *pfap2-g*. Similarly, identification of the environmental factors that trigger SCC and NCC respectively and how these connect to transcriptional and epigenetic regulators will be crucial to better understanding commitment.

While many questions remain, the results of the study by Bancells *et al*. [7] require that we rethink how *Plasmodium* parasites commit to sexual differentiation. In light of the critical role that the gametocyte plays in malaria transmission, efforts to target any aspect of gametocyte commitment, conversion, or maturation will remain high priorities in antimalarial elimination strategies.

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Forum

Increasing Complexity Threatens the Elimination of Extra-Amazonian Malaria in Brazil

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Extra-Amazonian malaria has been reported to be endemic in Brazil since the end of the 19th century. Currently, only a few cases are reported annually. However, recent findings of unexpected *Plasmodium* infections with uncertain transmission cycles in the Extra-Amazonian region could pose a threat to the malaria elimination agenda in Brazil.

Malaria in the Extra-Amazonian Region

Extra-Amazonian malaria has been reported to be endemic in Brazil since the end of the 19th century [1]. In the region, autochthonous cases of malaria have two profiles (introduced and indigenous) that occur in very distinct areas. The cycles consist primarily of the transmission of *Plasmodium* by *Anopheles* mosquitoes from the *Kerteszia* and *Nyssorhynchus* subgenus [2]. The Brazilian Extra-Amazonian region comprises 17 states and the Federal District (DF) and is home to approximately 86% of the Brazilian population. From 2007 to 2017, 806 autochthonous cases of malaria were confirmed in the Extra-Amazonian region (Figure 1). Moreover, recent reports are indicating an upsurge in the incidence of Extra-Amazonian malaria with a substantial increase in the number of cases, totaling 103 confirmed cases from January to July 2018, an increase of 35% in relation to the same period in 2017 [3].

There are two main autochthonous malaria transmission cycles in the Extra-Amazonian region:

(i) Introduced malaria cases, secondary from imported cases (e.g., malaria acquired in the Amazon region) occurring in plains, lowlands, and plateau areas where malaria was formerly endemic. Introduced malaria cases are caused by the introduced *Plasmodium* and are usually transmitted by *Anopheles* species from subgenus *Nyssorhynchus* (*Anopheles darlingi* as the primary vector). Although there were tremendous sanitary improvements and landscape changes in the Extra-Amazonian region since malaria was first considered nonendemic, the majority of Brazil is still highly receptive for malaria transmission [1,2].

(ii) Autochthonous bromeliad-malaria transmission cycle occurring in the Atlantic Forest biome. Bromeliad-malaria consists of the transmission of *Plasmodium vivax* and *Plasmodium malariae* to humans primarily by *Anopheles* (*Kerteszia*) *cruzii*, and to a lesser extent by other *Kerteszia* species, namely *Anopheles bellator* and *Anopheles homunculus*. These mosquito species almost exclusively lay their eggs in the water accumulated in phytotelmata bromeliads, which are found abundantly in the Atlantic Forest biome. Bromeliad-malaria also includes simian malaria transmission (*Plasmodium brasilianum* and

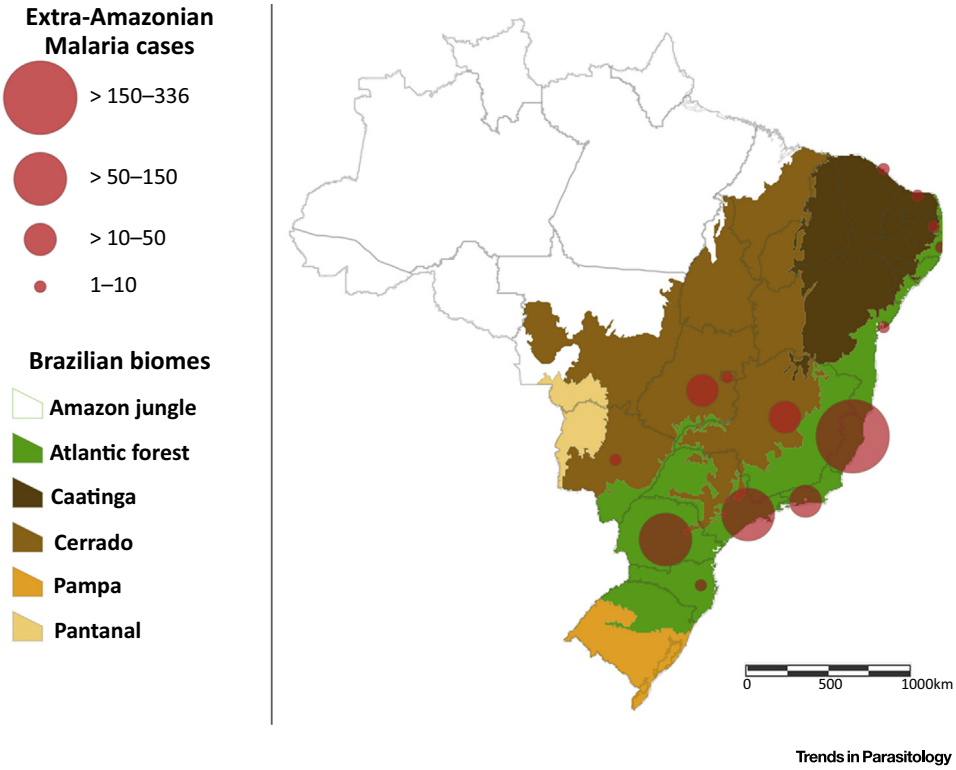

Figure 1. Map of Brazil Displaying Biomes and Number of Confirmed Extra-Amazonian Malaria Cases from 2007 to 2017. Confirmed malaria cases data were collected from *Sistema de Informação de Agravos de Notificação* (SINAN/DATASUS; http://portalsinan.saude.gov.br/malaria). All cases were caused by *Plasmodium* species reported in the Extra-Amazonian region. Biomes: Amazon Jungle, the largest tropical moist forest on the planet; Atlantic Forest, extends along the Atlantic coast of Brazil, and is characterized by high biodiversity and endemism; Caatinga, has dry soils and vegetation, suffers risk of desertification; Cerrado, savannah with highest biodiversity in the world; Pampa, composed mainly of plains and plateaus, and covered mostly by gramineaceae; Pantanal, the world's largest tropical wetland environment (https://brasilemsintese.ibge.gov.br/territorio.html). The map was created with ArcGIS 10.2 (Esri, Redlands, CA) using layers freely available at https://mapasinterativos.ibge.gov.br/arcgis/rest/services/BIOMA/MapServer.

Plasmodium simium) to nonhuman primates [1,2].

Bromeliad-malaria used to be one of the most important vector-borne diseases in Brazil in the 20th century. During the 1940s there were around 40,000 cases of bromeliad-malaria every year. However, due to an aggressive malaria-elimination campaign, which involved the indiscriminate use of dichloro-diphenyl-trichloroethane (DDT) and great environmental devastation by the destruction of bromeliads by manual removal, and also copper sulfate spraying, the number of cases decreased to 500 cases per year in the 1980s. Residual malaria transmission in the Atlantic Forest was considered eliminated in 1988 [4]. However, it is currently possible to acknowledge that residual malaria cases occurring in the Atlantic Forest have never been eliminated.

Detection of *Plasmodium falciparum* Infections in the Atlantic Forest

Officially, *P. falciparum* is hardly thought to be responsible for human cases of malaria outside the Amazon region and has not been considered important for the epidemiology of malaria in the Atlantic Forest. However, the epidemiology of bromeliad-malaria transmission has become a matter of debate.

A recent study has caused a stir in the scientific community by finding asymptomatic *Plasmodium* infections in human blood samples from a blood bank in São Paulo, Brazil. Many donors, who did not have a history of traveling to endemic areas, tested positive for malaria; there were 57 infected with *P. falciparum,* 25 infected with *P. vivax*, and two blood donors infected with both *P. falciparum* and *P. vivax* [5]. These findings were questioned by Mendrone *et al*. (2014) [6], who criticized the methodology used to survey the human blood donors as well as the public health implications of the findings; this led to a prompt response by Sallum *et al*. (2014) [7] who provided evidence supporting the circulation of *P. falciparum* or *P. falciparum*-like species in the Atlantic Forest.

Despite the skepticism among the scientific community regarding the high rates of

Plasmodium infection in humans, this was not the first report of asymptomatic plasmodial infections in the Atlantic Forest, highlighting the importance of investigating the cycle of transmission of this parasite in the Extra-Amazonian region [8].

Moreover, a previous study using semi-nested multiplex PCR found preliminary evidence of *P. falciparum* infection in nonhuman primates in the southeastern Atlantic Forest [9]. It has long been suggested that malaria in the Atlantic Forest is a zoonosis and that nonhuman primates are the reservoir hosts and thus responsible for the maintenance of *Plasmodium* in natural habitats [4]. A subsequent study discovered *An. cruzii* and other species of the *Nyssorhynchus* subgenus (*Anopheles strodei*, *Anopheles triannulatus*, and *Anopheles galvaoi*), infected with both *P. vivax* and *P. falciparum* in the Atlantic Forest region in areas intermixing natural and human-modified environments. Furthermore, the authors observed a higher prevalence of *An. cruzii* mosquitoes infected with *P. falciparum* (82%) than those infected with *P. vivax* (18%) in the surveyed areas [10].

Despite the controversy of which *Plasmodium* species are circulating in the Extra-Amazonian region, which mosquito species are involved in their transmission, and whether or not there are ongoing malaria outbreaks, one thing is certain: there is a paucity of knowledge of the epidemiology of malaria in the Extra-Amazonian region. In our opinion, more studies with more robust experimental designs are needed to address these questions.

Is the Atlantic Forest a Focus for Zoonotic Malaria?

An. cruzii mosquitoes are known for their acrodendrophilous behavior (i.e., preference for inhabiting forest canopy). In areas where *An. cruzii* can be found abundantly only at the tree canopies, but not at ground level, human cases of malaria are rarely reported despite a high prevalence of malaria among nonhuman primates. On the other hand, where *An. cruzii* is found in tree canopies and also at ground level, a high prevalence of both human and simian malaria would be expected. This behavior is well documented for *An. cruzii* and represents an increased risk for zoonotic transmission of simian *Plasmodium* to humans [11].

A recent study showed the unforeseen presence of *P. simium* infecting humans who have visited some parts of the Atlantic Forest in the state of Rio de Janeiro [12]. The cases had been initially erroneously reported as being caused by *P. vivax* due to high degrees of morphological, genetic, and immunologic similarity between the two species. After publication of the study, Grigg and Snounou (2017) [13] hypothesized that malaria transmission in the Atlantic Forest could represent an anthropozoonosis, revealing the importance of these findings and a possible threat to malaria elimination in the country.

Considering the unique epidemiological characteristics of the many different species of *Plasmodium* circulating in the Extra-Amazonian region, and to effectively guide malaria-control operations and improve policy, it is imperative to obtain the full genome of *P. simium* [13]. Additionally, we would like to extend this statement saying that it is also imperative to obtain the full genome of *P. falciparum* from infected humans, nonhuman primates, and mosquitoes of the Atlantic Forest to confirm that it is, in fact, the same parasite. Considering the new findings regarding malaria transmission in the Atlantic Forest, it is possible to assume that a complex malaria transmission scenario is currently occurring.

Future Perspectives

The Brazilian Atlantic Forest is one of the most endangered biomes in the world, and most of the forest was reduced to small fragments surrounded by urbanized areas. As a consequence of fragmentation, humans, nonhuman primates, and mosquitoes are likely to share the same environments and therefore have increased contact. There is growing evidence that the traditional bromeliad-malaria transmission model is not able to explain the current panorama of malaria transmission in the Atlantic Forest [7]. The epidemiological importance of asymptomatic *Plasmodium*-infected humans and nonhuman primates in the maintenance and transmission of malaria is currently poorly understood and largely ignored, rendering unclear the real extent of the problem.

Furthermore, asymptomatic plasmodial infections in humans in the Atlantic Forest region may be an indicator of a silent outbreak, since asymptomatic infections are likely detected only on epidemiological surveys, mainly by molecular methods [14]. Malaria-control strategies in the Extra-Amazonian region are based on precise diagnosis using microscopy by thick blood smear, treatment, use of insecticides, and barriers to vectors [9]. However, it is important to note that only 19% of all infections are detected and rapidly treated in the Extra-Amazonian region, and that the diagnosis is not always adequately made due to the lack of awareness of health professionals who are not used to diagnosing malaria outside the endemic Amazonian region [2].

Moreover, despite the importance of species of *Anopheles* native to Brazil in the transmission of malaria in the Extra-Amazonian region, very little information is available on which species are the most important vectors, as well as how their ecology, behavior, and adaptation processes are being driven by the increased anthropogenic changes of their natural habitats, including deforestation and clandestine settlements. It is likely that the environmental disturbance caused by anthropogenic changes may

affect the phenotype of *An. cruzii* [15], as well as favor malaria transmission [10].

In our view, the current research aims that should be prioritized regarding Extra-Amazonian malaria are: (i) to address the importance of the asymptomatic *Plasmodium*-infected human and nonhuman primates in the maintenance of malaria in the Extra-Amazonian region; (ii) to determine what vectors are responsible for the transmission of Extra-Amazonian malaria; and (iii) to discover to what extent *P. falciparum* and *P. simium* are a threat to the human population and the malaria elimination agenda (Box 1).

Now, more than ever, the biodiversity of the Brazilian biomes must be preserved. In recent years we have seen a rise in the outbreaks of infectious diseases, including the ones transmitted by mosquito vectors. Lately, we have seen environmental crimes devastating natural areas of Brazil and, as a consequence, leading to an environmental disequilibrium and biodiversity loss of unprecedented proportions. Moreover, in the years to come we will probably face an era of flexibilization of environmental legislation and limited resources to public health in Brazil. All of

Box 1. Outstanding Questions and Perspectives Concerning the Environment, Malaria Transmission, Mosquito Vectors, and Vector Control in the Brazilian Extra-Amazonian Region

The Environment

- What features of the environment are facilitating the transmission of malaria in the Extra-Amazonian region?
- How are anthropogenic alterations in the environment, including deforestation and rural settlements, affecting the transmission of Extra-Amazonian malaria?
- Will the continuous anthropogenic environmental disequilibrium of Brazilian ecosystems facilitate the increase of malaria transmission in the Extra-Amazonian region?
- What is the threat that flexibilization of environmental legislation and limited resources to public health pose to the malaria elimination agenda in Brazil?

The reduction of nonhuman primate habitats and subsequent decrease in their populational density result in increased contact between *Anopheles* (*Kerteszia*) and humans. Ecological changes from deforestation are related to biodiversity loss, resulting in a hypothesized reduction in the availability of small mammals that may act as dead-end hosts, leading to increased transmission rates of *Plasmodium* to nonhuman primates, which facilitate spillover events into humans. Moreover, environmental changes could also increase the number of potential breeding sites for other *Anopheles* species capable of transmitting *Plasmodium*.

Future studies will necessarily have to consider habitat-based environmental features to better understand the current epidemiology of Extra-Amazonian malaria transmission.

Transmission Cycles

- What is the extent of, and what are the *Plasmodium* species involved in, malaria in the Extra-Amazonian region?
- What is the importance of nonhuman primates in the maintenance of *P. falciparum* and *P. vivax*/*P. simium* in the Extra-Amazonian region?
- What is the prevalence of asymptomatic *P. falciparum* infection in humans and nonhuman primates?
- What is the prevalence of *P. simium* infection in humans?

The real extent of malaria transmission, and which *Plasmodium* species are responsible for causing malaria in the Extra-Amazonian region, are currently poorly understood. Zoonotic plasmodial infection in humans could present mild symptoms, or even comprise spontaneously resolved infection. Undiagnosed and untreated human plasmodial infections could be responsible for sustaining malaria transmission in the Extra-Amazonian region.

Future surveillance of nonhuman primates and human plasmodial infections must be molecular-based and be able to reliably distinguish *Plasmodium* species.

Mosquito Vectors

- What *Anopheles* species are involved in the transmission of Extra-Amazonian malaria?
- What knowledge do we have about the ecology and behavior of mosquito vectors of malaria in the Extra-Amazonian region?
- How are bromeliad-specialists, especially anopheline species of the *Kerteszia* subgenus, adapting to the increasing modification of their natural habitats?

Little information is available about shifts in the ecology and behavior of *Kerteszia* species, and it is so far unknown how their biting preference and adaptation to breeding sites not related to bromeliads may be affected by anthropogenic alterations in the environment. Deforestation could create, or expand, breeding sites for *Nyssorhynchus* species, facilitating the transmission of malaria to humans by non-bromeliad *Anopheles* species.

Future studies focusing on the shifts in ecology and behavior of *Anopheles* species, especially *Kerteszia*, should be encouraged.

Vector Control

- What are the most suitable strategies for targeted mosquito control to effectively impact on the prevalence and incidence of Extra-Amazonian malaria?
- How to effectively control *Anopheles* mosquitoes in periurban areas near forest fragments, including illegal settlements?
- Is it possible to employ an effective and sustainable integrated vector management approach in malaria transmission foci of the Extra-Amazonian region?

Anopheles (Kerteszia) species move from natural areas to rural and suburban areas seeking hosts for blood feeding and then return to natural habitats to oviposition. Such behavior poses a major challenge for the development of control strategies based on integrated vector management. Management of breeding sites and improvements of environmental ordinances in natural areas are unfeasible. Furthermore, chemical intervention and destruction of bromeliads in natural habitats are not acceptable by the current environmental standards, leaving only a few options to control *Anopheles* species.

Vector-control strategies should focus on decreasing the contact between mosquito vectors and human hosts by the improvement of laws and policies to prevent deforestation of natural areas and the establishment of illegal settlements in conservation areas.

these factors, together, will likely culminate in an increase in vector-borne transmission of diseases.

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