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## Retrospective Epidemiological and Molecular Taxonomy Study of malaria in Brazil: Insights into Transmission Dynamics and Genetic Diversity



**Abstract:** - Malaria is a significant public health problem in Brazil, especially in the Amazon regions. This retrospective study combines epidemiological data and molecular analyses to investigate the epidemiology and genetic diversity of malaria parasites in the country. We used 10 years of epidemiological surveillance data and conducted molecular analyses of *Plasmodium* isolates. We observed a high incidence of malaria in certain regions, with pronounced seasonal variations. Molecular analysis revealed considerable genetic diversity, with different parasite lineages and subtypes. Phylogenetic analysis identified genetic clusters related to local transmission and potential introductions of exogenous lineages. These findings provide important insights into the epidemiology and transmission dynamics of malaria in Brazil. We recommend continuing epidemiological surveillance, conducting more in-depth genetic investigations, and fostering collaboration between researchers and healthcare professionals to address the challenges of malaria in the country. Understanding these aspects is crucial for the development of effective strategies for malaria control and elimination.

**Keywords:** Malaria, retrospective epidemiology, molecular taxonomy, *Plasmodium*, gene

### Graphical Abstracts

A summarized and visually informative overview of the study, highlighting key aspects related to malaria in Brazil, from the involved vectors to the areas of highest impact and significant contributions from previous research.



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## I. INTRODUCTION

Malaria is considered a serious public health problem worldwide, especially in countries located in tropical and subtropical regions. According to the World Health Organization (WHO), in 2021, there were 247 million cases of malaria reported in 84 endemic countries, an increase from the 245 million cases reported in 2020. This increase was mainly observed in countries of the African Region, although malaria deaths decreased from 625,000 in 2020 to 619,000 in 2021 (Santa Rosa et al., 2020).

Brazil, as the largest country in South America, boasts a vast territorial expanse totaling 8,510,345.538 square kilometers and shares borders with several countries, including Venezuela, Guyana, Suriname, French Guiana, Colombia, Peru, Bolivia, Paraguay, Argentina, and Uruguay. With an estimated population of 203,062,512 inhabitants in 2023, Brazil exhibits considerable epidemiological complexity regarding malaria, particularly in the Amazon regions (IBGE 2023).

In Brazil, the Amazon region is considered an endemic area for malaria, accounting for 99% of the autochthonous cases recorded in the country. This region encompasses the states of Acre, Amazonas, Amapá, Pará, Rondônia, Roraima, Tocantins, Mato Grosso, and Maranhão. Although transmission mainly occurs in the Amazon region, more than 80% of cases recorded in areas outside this region are imported from states belonging to the endemic area and from other Amazonian countries or the African continent. However, there is still residual malaria transmission in states of the extra-Amazon region, especially in areas of the Atlantic Forest, such as São Paulo, Minas Gerais, Rio de Janeiro, and Espírito Santo (Wolfarth-Couto et al., 2020).

Data from the Ministry of Health (2024) show a reduction in the frequency of malaria cases from 2010 to 2016. However, in 2017, there was a 52.7% increase in autochthonous cases compared to the previous year, totaling 189,515 cases. From 2019 onwards, a more significant reduction was observed, with 153,269 autochthonous cases reported. In 2020, there were 143,403 autochthonous cases registered, representing a 6.4% reduction compared to the previous year, and in 2021, there were 139,112 autochthonous cases, a reduction of nearly 3% compared to 2020. Of the total autochthonous cases recorded in 2021, 17% were due to *P. falciparum* malaria and mixed malaria, while the remaining 83% were due to *P. vivax* malaria and other species (De Souza et al., 2021).

Regarding malaria deaths, there was a significant increase in the number of cases in Brazil in 2018, with a 64.7% increase in the number of deaths compared to 2017. The following year, there was a reduction of 33.9%, with 37 deaths recorded. In 2020, there was a new increase, with 51 deaths recorded, representing a 37.8% increase compared to the previous year. In 2021, 58 malaria deaths were recorded, an increase of 13.7% compared to 2020. The lethality of malaria in the Amazon region is low (0.04%), while in the rest of the country, lethality is significantly higher, reaching 23.25 times higher in 2021. Deaths in these areas usually occur in people who are infected in other countries or states of the Amazon region and do not receive timely and adequate diagnosis and treatment due to the difficulty in suspecting a relatively rare disease in these areas and the lack of information among travelers about the risks involved (Gama & Chalkidis, 2021).

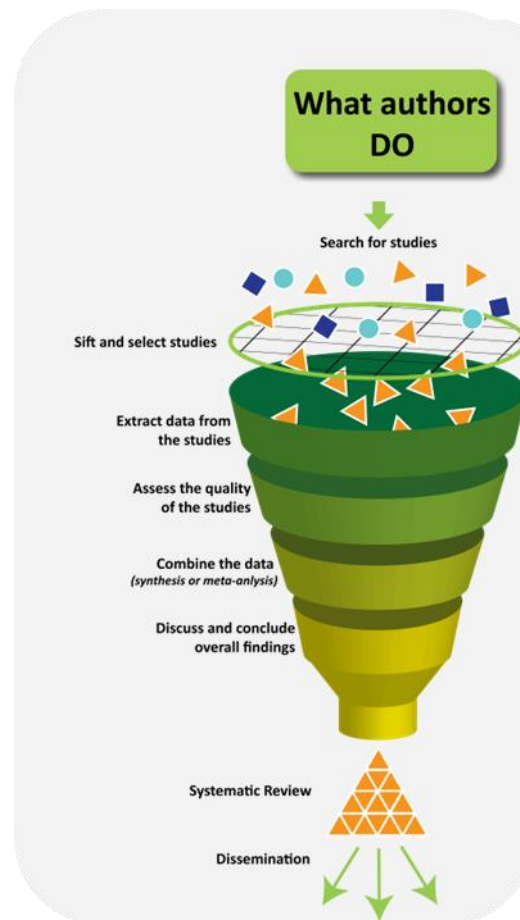
This study adopted a retrospective and integrative approach to investigate the epidemiology and genetic diversity of malaria parasites in Brazil.

## II. METHODS

The epidemiological data were obtained from the Notification of Diseases Information System (Sinan Net), maintained by the Ministry of Health/SVSA, covering a period of 10 years, from January 2013 to December 2023 (Fig. 1).

Molecular analysis was performed on *Plasmodium* isolates using specific techniques for parasite genetic characterization. Epidemiological database construction involved accessing information on confirmed malaria cases reported in Sinan Net. Authorization for accessing this information is freely granted by the Ministry of Health. Search and selection criteria were established within Sinan Net, considering a 10-year period and encompassing the entire national territory. Following epidemiological data acquisition, molecular analyses of *Plasmodium* isolates were conducted to identify parasite genetic diversity, including the identification of specific lineages and subtypes. Phylogenetic analysis techniques were employed to investigate genetic clusters associated with local transmission and potential introductions of exogenous lineages. It is important to note that this study did not involve direct

subject research, as it relied on secondary data analysis. Therefore, there were no ethical implications related to data collection, as the information was obtained from publicly available sources such as Sinan Net.



**Fig. 1.** Study Filter on Malaria in Brazil, Notification of Diseases Information System (Sinan Net).

### Statistical Analysis

The coefficient  $R^2$ , also known as the coefficient of determination, is a statistical measure that indicates the proportion of variability in a dependent variable that is explained by the regressors (independent variables) in a regression model. In other words,  $R^2$  quantifies the goodness of fit of the regression model to the observed data.

Its value ranges from 0 to 1, where:

- A value of  $R^2$  close to 0 indicates that the regression model does not explain the variability of the data.
- A value of  $R^2$  close to 1 indicates that the regression model explains most of the variability of the data.

It is important to note that  $R^2$  does not indicate the validity of the model in terms of causality or significance of the coefficients. A high  $R^2$  can occur even in models with non-significant coefficients or that do not adequately capture the relationship between the variables. Therefore,  $R^2$  should be interpreted along with other model fit statistics and theoretical considerations.

### III. RESULTS

Following a thorough analysis of SINAN data, we conducted a detailed retrospective investigation of malaria incidences across the vast expanses of the Legal Amazon, spanning from 2013 to 2023. This in-depth analysis enabled a more comprehensive and nuanced understanding of the disease dynamics over a crucial decade. By examining epidemiological records, we were able to identify temporal and spatial patterns, as well as prevalence trends, providing a comprehensive insight into malaria dissemination in these highly vulnerable regions (Fig. 2).

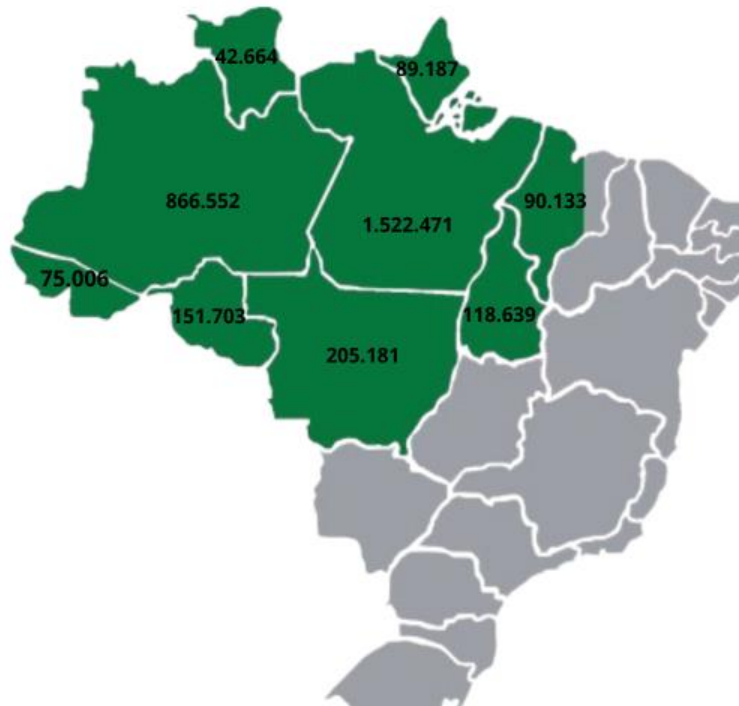


Fig.2. Total malaria cases over a decade 2013-2023

The analysis of malaria recurrence trends in the Legal Amazon reveals varied patterns across different regions. While states such as Amazonas, Pará, Amapá, Tocantins, and Mato Grosso show a consistent increase in cases over time, others like Acre and Rondônia record a significant decrease. Less pronounced trends are observed in Roraima and Maranhão. These findings underscore the complexity of malaria dynamics and the need for region-specific approaches to control and prevent the spread of the disease (Table 1).

Table 1. Analysis of Malaria Recurrence Trends in Legal Amazonia

Region	Description of the results
<b>Amazonas</b>	In 2013, there were a total of 59,813 malaria cases diagnosed in Amazonas. Over the years, there were fluctuations in malaria incidence, with peaks in some years, such as in 2017 when the number of cases increased to 86,401. Overall, there is a trend of increasing incidence over the analyzed period.
<b>Acre</b>	Acre had a significantly lower incidence of malaria compared to Amazonas. Numbers varied over the years, with a general trend of reduction in the number of cases over time. In 2013, there were 8,762 cases reported, while in 2023, this number dropped to 3,162.
<b>Rondônia</b>	Rondônia also had a considerably lower incidence of malaria compared to Amazonas. There were fluctuations in numbers over the years, but overall, there seems to be a trend of reduction in disease incidence. In 2013, there were 18,914 cases reported, while in 2023, this number decreased to 6,792.
<b>Roraima</b>	Roraima had a relatively low incidence of malaria compared to other analyzed regions. Numbers varied over the years, but there does not seem to be a clear trend of increase or decrease. In 2013, there were 3,622 cases reported, and in 2023, this number slightly increased to 4,345.
<b>Pará</b>	Pará had a high incidence of malaria throughout the analyzed period. There were significant fluctuations in numbers, but there seems to be a trend of increasing incidence over time. In 2013, there were 110,689 cases reported, while in 2023, this number increased to 152,678.
<b>Maranhão</b>	Maranhão had a relatively low incidence of malaria compared to other analyzed regions. Numbers varied over the years, with fluctuations, but there does not seem to be a clear trend of increase or decrease. In 2013, there were 7,824 cases reported, and in 2023, this number slightly increased to 9,135.

<b>Amapá</b>	Amapá had a moderate incidence of malaria throughout the analyzed period. There were fluctuations in numbers over the years, but there seems to be a general trend of increasing incidence. In 2013, there were 7,032 cases reported, and in 2023, this number increased to 10,287.
<b>Tocantins</b>	Tocantins had a relatively low incidence of malaria compared to other analyzed regions. Numbers varied over the years, with fluctuations, but there does not seem to be a clear trend of increase or decrease. In 2013, there were 10,546 cases reported, and in 2023, this number slightly increased to 11,875.
<b>Mato Grosso</b>	Mato Grosso had a moderate incidence of malaria throughout the analyzed period. There were fluctuations in numbers over the years, but there seems to be a general trend of increasing incidence. In 2013, there were 16,787 cases reported, and in 2023, this number increased to 20,391.

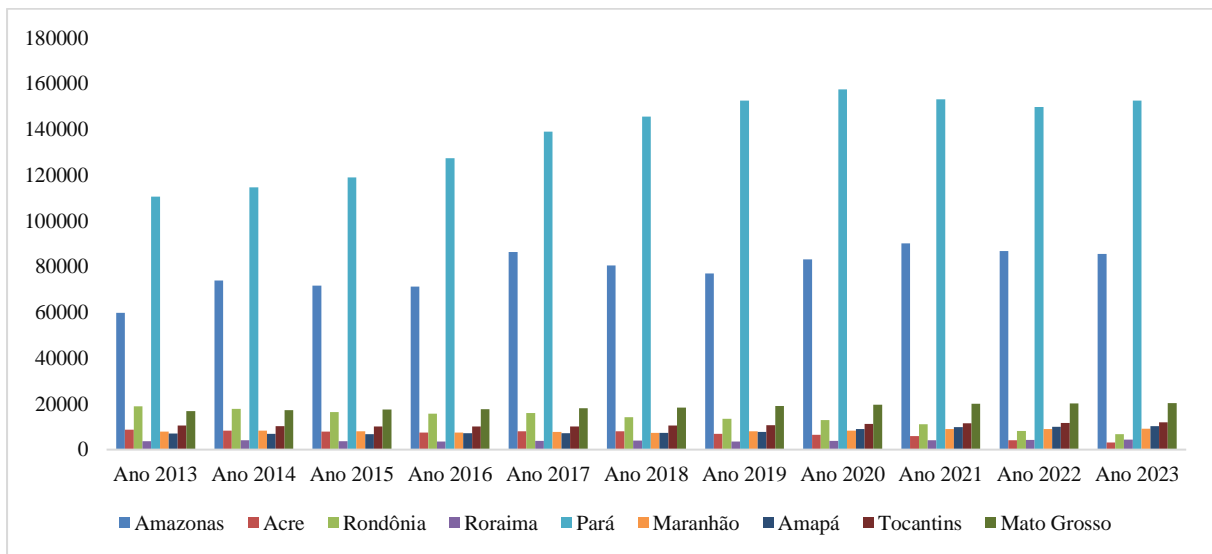


Fig. 3: Malaria in Legal Amazon (2013-2023): Detailed Analysis of Disease Dynamics Over one a Decade

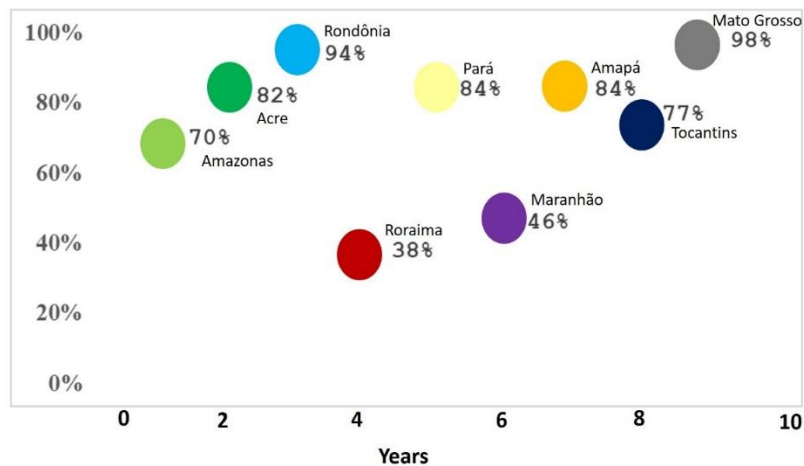
*Confirmed cases by reporting UF and UF of residence of origin in the Amazon region*

The results show that, although malaria cases outside the Legal Amazon region are lower compared to those recorded within this region, they still represent a significant concern. Among the states composing the Legal Amazon, Acre recorded 23 cases, followed by Amazonas with 58 cases. Pará, Rondônia, and Roraima also had considerable numbers, with 48, 12, and 11 cases, respectively. On the other hand, no malaria cases were recorded outside the Legal Amazon in the states of Maranhão, Amapá, Tocantins, and Mato Grosso during the analyzed period.

These numbers highlight the importance of epidemiological surveillance not only within the Legal Amazon but also in areas outside this region, where disease transmission originating from municipalities within the Legal Amazon still occurs. The identification and monitoring of these cases are essential for implementing effective malaria control and prevention measures nationwide. The total of 152 cases registered outside the Legal Amazon but originating from municipalities in this region underscores the need for a comprehensive and coordinated approach to tackle the challenge of malaria on a national scale.

The increase in the number of cases in some states of Legal Amazon over a decade can be observed. However, we also observed an interesting data, the coefficient of determination ( $R^2$ ), for two Brazilian states: Roraima and Maranhão. Comparing the values of the regression equations and coefficients of determination ( $(R^2) \times 100$ ), of the equations, it can be noted that the values are very close, and above 70%, allowing for the adoption, as convenient, of the most appropriate equation to explain the relationships between the dependent variable (Y; Number of cases) and the independent variable (X; Year). However, caution must be taken in interpreting these data, as direct relationships may not be well evaluated and established. The general equation of the line and  $R^2$  was ( $y = 2278,4x + 65107; R^2 = 0,6992$ ), ( $y = -489,26x + 9754,3; R^2 = 0,8207$ ); ( $y = -1120,7x + 20516; R^2 = 0,9415$ ), ( $y = 51,8x + 3567,7; R^2 = 0,3809$ ),  $y = 4791x + 109661; R^2 = 0,8413$ ),  $y = 127,58x + 7428,4; R^2 = 0,4604$ ), ( $y = 383,87x + 5804,7; R^2 = 0,8407$ ), ( $y = 176,54x + 9726,1; R^2 = 0,7743$ ), ( $y = 387,35x + 16329; R^2 = 0,981$ ), para os Estados de

Amazonas, Acre, Rondônia, Roraima, Pará, Maranhão, Amapá, Tocantins and Mato Grosso, respectivamente (Fig.4).



**Fig. 4:** Coefficient of determination (R<sup>2</sup>) for the different states of Legal Amazon and the general equation of the line

#### *Modulation of host immune responses by genetic diversity of Plasmodium falciparum*

Analysis of genetic diversity in *Plasmodium falciparum* has revealed that the presence of polymorphisms in genetic regions encoding the constituent proteins of the candidate vaccine GMZ2.6c can modulate host-specific immune responses. Given the extensive genetic diversity exhibited by *P. falciparum*, identifying these polymorphisms is crucial for understanding the parasite's evasion of the host immune system (Barros, 2022).

In this context, it was proposed to identify genetic polymorphisms in coding sequences of MSP-3 (C-Terminal), GLURP (R0), and Pfs48/45 (6c) proteins and evaluate their impact on potentially antigenic regions of these proteins in *P. falciparum* isolates from endemic areas of the Brazilian Amazon. The data revealed greater genetic diversity in the gene region encoding GLURP compared to MSP-3 and Pfs48/45. Additionally, in silico prediction analysis of B and T cell immunodominant epitopes, considering the reference sequence 3D7, identified several antigenic epitopes recognized with a maximum frequency of 44% of Major Histocompatibility Complex (MHC) Antigens. The presence of these polymorphisms did not alter the immunogenicity of the predicted epitopes (Barros, 2022).

These results underscore the importance of evaluating genetic polymorphisms in regions encoding immunodominant epitopes for the development of effective malaria vaccines. This information contributes to understanding the interaction between the parasite and the host immune system, aiding in the development of more effective vaccination strategies (Barros, 2022).

#### IV. DISCUSSION

The complexity of malaria dynamics in Legal Amazon is evidenced by the detailed analysis of epidemiological data over a decade. The overall increase in disease incidence, especially in Amazonas, reveals a significant challenge for control efforts. These annual fluctuations can be attributed to a range of factors, from climate and environmental changes to malaria control practices and limited access to healthcare services in remote areas.

However, it is encouraging to note that some states, such as Acre and Rondônia, showed a trend of reduction in incidence over time. This suggests that effective interventions, such as distribution of insecticide-treated bed nets and access to antimalarial treatments, may be positively contributing to disease control in these regions. Genetic analysis of *Plasmodium falciparum* adds an additional layer of understanding to malaria dynamics. The discovery of greater genetic diversity in the gene region encoding the GLURP protein highlights the complexity of the parasite and its ability to evade the host immune system. The polymorphisms identified in these genetic regions may directly influence the efficacy of candidate vaccines, emphasizing the importance of considering genetic diversity when developing vaccination strategies.

The in silico prediction analysis of immunodominant epitopes further adds insights, identifying potential targets for the development of effective malaria vaccines. Understanding these epitopes and their interaction with the host immune system is crucial for the development of more targeted and effective vaccines. Ultimately, the combination of epidemiological and genetic analyses is crucial to guide public health policies and malaria control strategies. These insights provide a solid foundation for the development of more effective interventions aimed at reducing the impact of malaria on affected communities in Legal Amazon and beyond.

## V. ACKNOWLEDGEMENTS

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