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Genomic surveillance of SARS-CoV-2 in the State of Mato Grosso, Midwest Brazil

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ABSTRACT

The emergence of SARS-CoV-2 in December 2019 impacted global public health as the most severe modern pandemic ever recorded. Remarkably, it was possible to monitor its evolution in real-time, including viral evolution and the emergence of new lineages, thanks to technology and the speed of communication via the internet. Understanding the circulating viral variants and their characteristics within the population was crucial for effectively guiding public health actions in combating the disease. In this study, we conducted detailed phylogenetic analyses as a result of a recently established genomic surveillance hub at LACEN-MT. The samples were sequenced and subsequently deposited on the GISAID platform between October 21, 2021 and March 31, 2023. The findings of this study suggest that the SARS-CoV-2 epidemic in Mato Grosso State was characterized by the successive introduction of new strains from different countries, leading to the generation of waves of infection. Overall, this study underscores the importance of investigating the spatiotemporal evolution of the SARS-CoV-2 epidemic and how its variants spread across the state, contributing to pandemic peaks in the region. It also highlights the significance of disseminating this information to the management team in Mato Grosso to help implement monitoring strategies that could reduce cases and deaths in the state.

1. Introduction

At the end of 2019, the world was struck by the SARS-CoV-2 pandemic, resulting in severe social, economic, and political consequences (Li et al., 2020; WHO, 2020). During the pandemic, multiple epidemiological waves were observed, driven by viral evolution and the introduction of new variants, which were distinguished by the infections they caused and the corresponding immune responses (Li et al., 2021; Harvey et al., 2021). SARS-CoV-2 is an RNA virus with a 30 kb genome that encodes four structural proteins: the spike (S) protein, the envelope protein, the membrane (M) protein, and the nucleocapsid (N) protein (Stoicescu et al., 2021). This virus continuously accumulates polymorphisms in its genome, leading to the emergence of new variants with mutations in the Spike protein, raising significant concerns among public health authorities worldwide (Tao et al., 2021). As the viral genome acquires nucleotide alterations, we can analyze genomes using epidemiological tools that enable the early phenotypic characterization of new variants, providing insights into the potential significance of SARS-CoV-2 viral infection (Robishaw et al., 2021). Given this scenario, understanding the molecular evolution of SARS-CoV-2 is crucial, as this knowledge is a key factor in predicting the pandemic's trajectory and in designing more effective treatments to combat it in the future, whether

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through vaccines, antivirals, or other technologies to be developed by science (Tao et al., 2021; Singh and Yi, 2021; Agrawal et al., 2021). Additionally, the current review of the system emphasizes that more than 14 million SARS-CoV-2 genomes have been deposited in GISAID, facilitating the identification of phenotypic and molecular relationships, guiding the selection of the most appropriate therapies for each variant, and observing the emergence and establishment of variants that are of concern for the future (González-Vázquez and Arenas, 2023). In Brazil, as of October 2022, 106 studies have reported the genomic surveillance of SARS-CoV-2, describing 96 different strains across all regions of the country (Menezes et al., 2022). In the state of Mato Grosso/Brazil (MT), the first positive case of COVID-19 was reported in March 2020, with samples analyzed by the Central Laboratory of the State of Mato Grosso (LACEN-MT). In the early stages of the COVID-19 pandemic, LACEN-MT faced numerous challenges, including limited access to resources for identifying the circulating viral variant, which impacted the intervention process of the administration. Through significant efforts in establishing national and international partnerships and local infrastructure development, LACEN-MT successfully established genomic surveillance programs via genome sequencing. By October 21, 2021, LACEN-MT was already capable of generating genomic data through the sequencing of the SARS-CoV-2 genome. Furthermore, it is important to highlight that studies conducted in Mato Grosso since the beginning of the pandemic have addressed various epidemiological aspects of the disease's spread in the state (Silva et al., 2020), case prediction (Espinosa et al., 2020), seroprevalence epidemiological surveys, occupational situations of healthcare professionals during the pandemic (Nascimento et al., 2020a; Nascimento et al., 2020b; Nascimento et al., 2020c; Nascimento et al., 2021; Santos Caló et al., 2020), comorbidities and hospitalization (Oliveira et al., 2021), mortality analyses (Muraro et al., 2022), mental health (Terças-Trettel et al., 2022), and population behavior in online searches (Nascimento et al., 2021; Nascimento et al., 2022). However, until now, there have been no approaches focusing on the genomic monitoring of the virus in the state, underscoring the importance of the present proposal. This study aims to describe detailed genetic, phylogenetic, and phylogeographic analyses of samples sequenced by LACEN-MT and deposited on the GISAID platform between October 21, 2021 and March 31, 2023.

2. Material and methods

2.1. Ethical statement

All ethical aspects of the research were adhered to in accordance with Resolution 466/2012 of the National Health Council (CNS), and the project was approved by the Ethics Committee of the State University of Mato Grosso (Report: CAAE: 65072322.3.0000.5166).

2.2. Study area

A genomic surveillance study was conducted based on samples sent to LACEN-MT by municipalities in the health macro-regions of Mato Grosso, conditioned by a state resolution for the coordination and management of health care related to COVID-19. The areas defined as health macro-regions coincide with the socio-spatial organization and geographic network of the state of Mato Grosso, a state in the Brazilian Legal Amazon whose capital is Cuiabá. Mato Grosso is located in the Central-West Region, being the third largest state in the country in terms of territorial extension, where it covers three main biomes: Cerrado, Amazon and Pantanal.

2.3. Molecular screening

Oropharyngeal swabs from all patients with symptoms of COVID-19 from municipalities in the state of Mato Grosso were collected between October 2021 and March 2023 for molecular diagnosis at the Central Public Health Laboratory of the State of Mato Grosso (LACEN-MT) in central-western Brazil. After molecular analysis, 650 positive samples were selected for sequencing based on the Ct value ($\leq$30). The samples were subjected to nucleic acid purification using the MagMAX pathogen RNA/DNA kit and the KingFisher Flex purification system (Thermo Fisher), following the manufacturer's recommendations. Detection of Sars-Cov-2 RNA by RT-qPCR was performed using the Allplex 2019-nCoV kit (Seegene) targeting the envelope (E), RNA-dependent RNA polymerase (RdRp) and nucleocapsid (N) genes, following the manufacturers' instructions.

2.4. cDNA synthesis and whole-genome nanopore sequencing

cDNA was synthesized using the SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA. Cat. No. 11754050) according to the manufacturer's instructions using 10.5 μL of RNA. Libraries were prepared on the Ion Chef system as described in Ion AmpliSeq library preparation in the Ion Chef system user guide. Samples were amplified for 18 cycles with an extension and annealing time of 4 min. The Ion AmpliSeq SARS-CoV-2 Research Panel, provided by Thermo Fisher Scientific for this study, contains pool A and pool B. Amplified samples were then sequenced on the Ion S5 system (Thermo Fisher Scientific) as described in the Ion S5 instrument user guide using Ion 530 chips (Thermo Fisher Scientific).

2.5. Generation of consensus sequences

Sequencing data were processed using Genome Detective. Lineage assignment was conducted using the Pangolin COVID-19 Lineage Assigner Tool. We used Nextclade (Nextstrain, https://clades.nextstrain.org) to assess genome quality and classification.

Nucleotide sequences were aligned using MAFFT and submitted to IQ-TREE2 for maximum likelihood (ML) phylogenetic analysis.

Additionally, phylogeographic analysis was performed using the Bayesian method implemented in BEAST v1.10.4 (Suchard et al., 2018) to infer the evolutionary history and dispersal of viral lineages in the state.

3. Results

This study analyzed a total of 1194 SARS-CoV-2 genome sequences from the state of Mato Grosso, collected between October 21, 2021 and March 31, 2023 (Fig. 1 panel A). Of these, 650 samples were sequenced and processed by the Central Public Health Laboratory of the State of Mato Grosso (LACEN-MT), which were subsequently included in our analysis (Fig. 1 panel B). The sequencing effort covered 82 out of the 141 municipalities in Mato Grosso, demonstrating a broad regional sampling effort.

The geographic distribution of the sequenced samples revealed significant insights into the viral spread within the state. Our results indicate that the SARS-CoV-2 epidemic in State of Mato Grosso, central region of Brazil, was marked by multiple introductions of different viral lineages, which subsequently dispersed both locally and regionally. Notably, the capital city of Cuiabá had the highest number of samples, with 297 genomes sequenced, accounting for 45.69 % of the total. This high concentration suggests a significant viral activity in this area, likely influenced by population density and the presence of more developed healthcare infrastructure, facilitating greater sample collection and testing. Other municipalities with notable sample counts include Rondonópolis (44 samples, 6.77 %), Cáceres (36 samples, 5.54 %), and Guarantã do Norte (15 samples, 2.31 %). The relatively high number of samples from Cuiabá and Rondonópolis underscores the influence of urban centers on the spread and detection of SARS-CoV-2 within the state.

Demographic analysis revealed a higher percentage of cases among female individuals, with 388 cases (59.7 %) compared to 262 (40.3 %) in

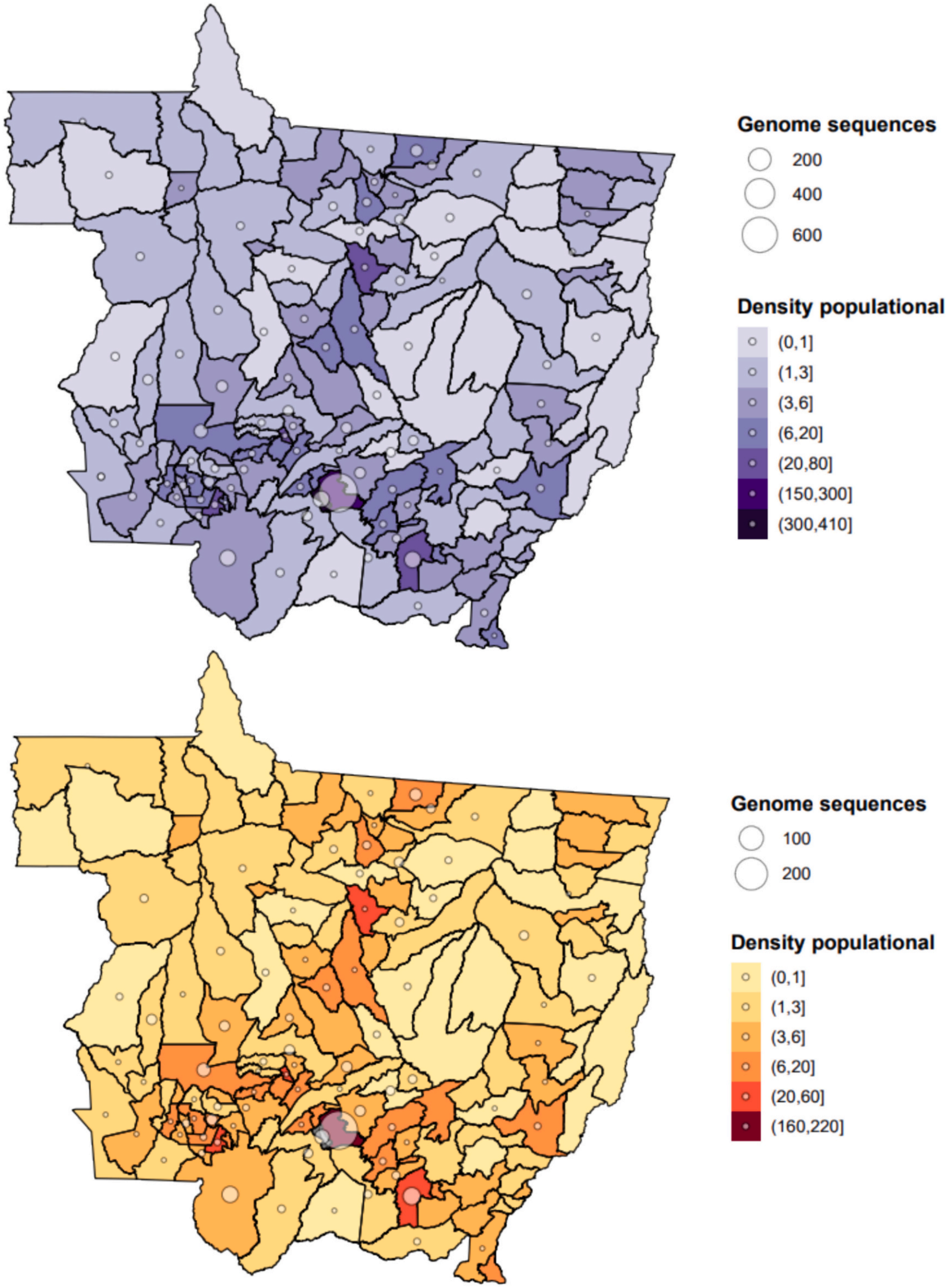

Fig. 1. Geographic distribution and genomic surveillance of samples from the State of Mato Grosso, sequenced between October 21, 2021, and March 31, 2023. The left panel shows the number of genome sequences (circles) across different municipalities, overlaid on population density, indicated by varying shades of blue. Darker shades represent higher population densities. The right panel provides a similar genomic distribution visualization but with population density ranges in shades of yellow to red, where deeper red regions indicate the highest population density. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

males. This trend may be influenced by several factors. Women are overrepresented in healthcare and caregiving professions, which increases their occupational exposure to SARS-CoV-2 (Sobotka et al., 2020). Moreover, women are generally more likely to seek healthcare and undergo diagnostic testing, which could lead to higher detection rates (Wang et al., 2020). Biologically, women tend to mount a stronger immune response to viral infections due to hormonal and genetic factors, although this does not necessarily correlate with a higher susceptibility to infection (Gadi et al., 2020). However, these are hypotheses and not conclusive findings; more targeted studies are needed to establish causal relationships.

Regarding age distribution, individuals over 50 years accounted for the highest proportion of cases (33.4 %), which aligns with global literature indicating that older age is a risk factor for both infection and disease severity due to immunosenescence — the natural decline of immune function with age (Márquez et al., 2020).

In addition to the geographic distribution, demographic analysis of the data revealed that a higher percentage of cases were observed in female individuals, with 388 cases (59.7 %), as compared to 262 cases (40.3 %) in male individuals (Table 1). The 95 % confidence interval for female cases was between 55.85 % and 63.48 %, while for male cases, it was between 36.52 % and 44.15 %. These findings may suggest that women are either more exposed or more likely to seek testing and diagnosis, or possibly more susceptible to infection. Previous studies suggest that although men are more likely to experience severe disease outcomes, women may present with higher infection rates due to occupational and behavioral factors (Sobotka et al., 2020; Tu et al., 2020).

The age distribution of cases also showed variability, with the age groups of 21–30 years and above 50 years presenting the highest number of cases. Specifically, the 21–30 years age group had 83 cases (12.8 %), while the over 50 years age group had 217 cases (33.4 %). The high incidence among older individuals may reflect increased vulnerability due to comorbidities and a weakened immune system. Laboratory data analysis revealed that the median time from symptom onset to diagnosis was 3.47 ± 0.852 days, indicating a relatively prompt identification of cases following symptom onset. The mean CT (cycle threshold) value in RT-PCR analysis was 24.04 ± 0.415, reflecting the viral load in the samples analyzed. These laboratory findings provide critical insights into the dynamics of infection and the effectiveness of testing strategies employed during the study period.

The analysis of 650 SARS-CoV-2 genomes from various municipalities in Mato Grosso provided a detailed understanding of the viral spread and variant distribution within the state (Fig. 2). Temporal analysis (Fig. 2A) revealed the presence and cocirculation of multiple SARS-CoV-2 lineages over time, driven by repeated introductions and subsequent local transmissions. A significant peak in variant diversity was observed in 2021, corresponding to the second wave of COVID-19 in Brazil. This period was dominated by the emergence of the Gamma variant (B.1.1.28.1, also known as P.1), which was first identified in December 2020 in the Amazonas state. The P.1 variant was characterized by mutations of biological significance, including E484K, K417T, and N501Y.

Subsequent to the dominance of Gamma, the Delta and Omicron variants emerged, leading to the third wave of infections between December 2021 and May 2022. This wave was marked by the rapid spread of Omicron and its subvariants, which continue to circulate within the state, surpassing the previous variants, including Alpha, Beta, Gamma, and Delta (Fig. 2B). The XBB variant, a descendant of Omicron, was detected around August 2022 and has rapidly become the predominant variant globally by late February 2023. In Mato Grosso, this variant was sequenced in early 2023, showing a significant proportion among the genomes analyzed.

Table 1
Demographic characteristics obtained from patients resulting from biological samples evaluated for COVID-19 serology in Cuiabá, Mato Grosso, Central region of Brazil.

Characteristic	Data
Gender	
Female	388 (59.7)
Male	262 (40.3)
Age range	
0–10	36 (0.55)
11–20	48 (0.74)
21–30	83 (12.8)
31–40	125 (19.2)
41–50	141 (21.7)
50–>	217 (33.4)
Median days of symptoms until diagnosis (DP*)	(0.347 ± 0.852)
Average CT value (threshold cycle) in the analysis RTq-PCR (DP*)	(24.04 ± 0.415)

N: population size, 95 %CI: 95 % confidence interval.

* Statistically significant with 95 % confidence.

Source: Own elaboration.

4. Discussion

The genomic surveillance efforts carried out by the Central Public Health Laboratory of the State of Mato Grosso (LACEN-MT) have been instrumental in providing a detailed understanding of the spread and evolution of SARS-CoV-2 within the state. The establishment of this monitoring system at LACEN-MT has significantly enhanced the state's capacity to track viral variants in real-time, enabling the timely identification of new variants and their transmission dynamics. The concentration of sequenced samples in urban centers, such as Cuiabá city, reflects not only the demographic and infrastructural realities but also underscores the strategic role of LACEN-MT as a central hub for genomic surveillance in Mato Grosso. The ability of LACEN-MT to conduct large-scale sequencing and analysis has allowed for the detection of critical variants, such as Gamma, Delta, and Omicron, which have driven significant waves of infection within the state. Moreover, the monitoring system at LACEN-MT has been crucial in identifying the emergence of the XBB variant, a descendant of Omicron, early in 2023.

This early detection highlights the lab's vital role in informing public health responses, allowing for rapid adjustments in strategies to mitigate the impact of these new variants. The integration of genomic data into public health decision-making processes has been essential in adapting to the evol-ving pandemic landscape, ensuring that interventions are based on the most current and relevant data. The success of LACEN-MT's monitoring system demonstrates the importance of having a robust, localized genomic surveillance infrastructure. This system not only supports the detection and tracking of SARS-CoV-2 variants but also contributes to the broader national and global efforts to combat the pandemic. By continuously monitoring the viral genome, LACEN-MT provides critical insights that guide public health actions, helping to contain outbreaks, prevent the spread of more transmissible or virulent variants, and protect vulnerable populations.

The greater proportion of female cases observed in this study should be interpreted as a hypothesis rather than a definitive conclusion. This trend could reflect differential exposure, health-seeking behaviors, or testing access, rather than intrinsic susceptibility. Thus, these findings highlight the importance of considering social and behavioral determinants in the interpretation of epidemiological data.

In conclusion, the genomic surveillance system established at LACEN-MT has been integral to the state's public health response to COVID-19. Its capacity to swiftly identify and characterize emerging variants has been crucial in guiding the state's efforts to control the virus and mitigate the impact of the pandemic. Looking ahead, the sustained support and expansion of these genomic surveillance capabilities will be essential for effective management of COVID-19 and for addressing future public health challenges.

CRediT authorship contribution statement

Stephanni Figueiredo da Silva: Writing – review & editing, Validation, Investigation. **Kláucia Rodrigues Vasconcelos:** Writing – review & editing, Investigation. **Julia Deffune Profeta Cidin Almeida:** Writing – review & editing, Methodology, Investigation. **Natália Rocha Guimarães:** Writing – review & editing, Formal analysis. **Luiz Marcelo Ribeiro Tomé:** Writing – review & editing, Formal analysis. **Vagner Fonseca:** Writing – review & editing, Formal analysis, Conceptualization. **Diniz Pereira Leite Júnior:** Validation, Supervision,

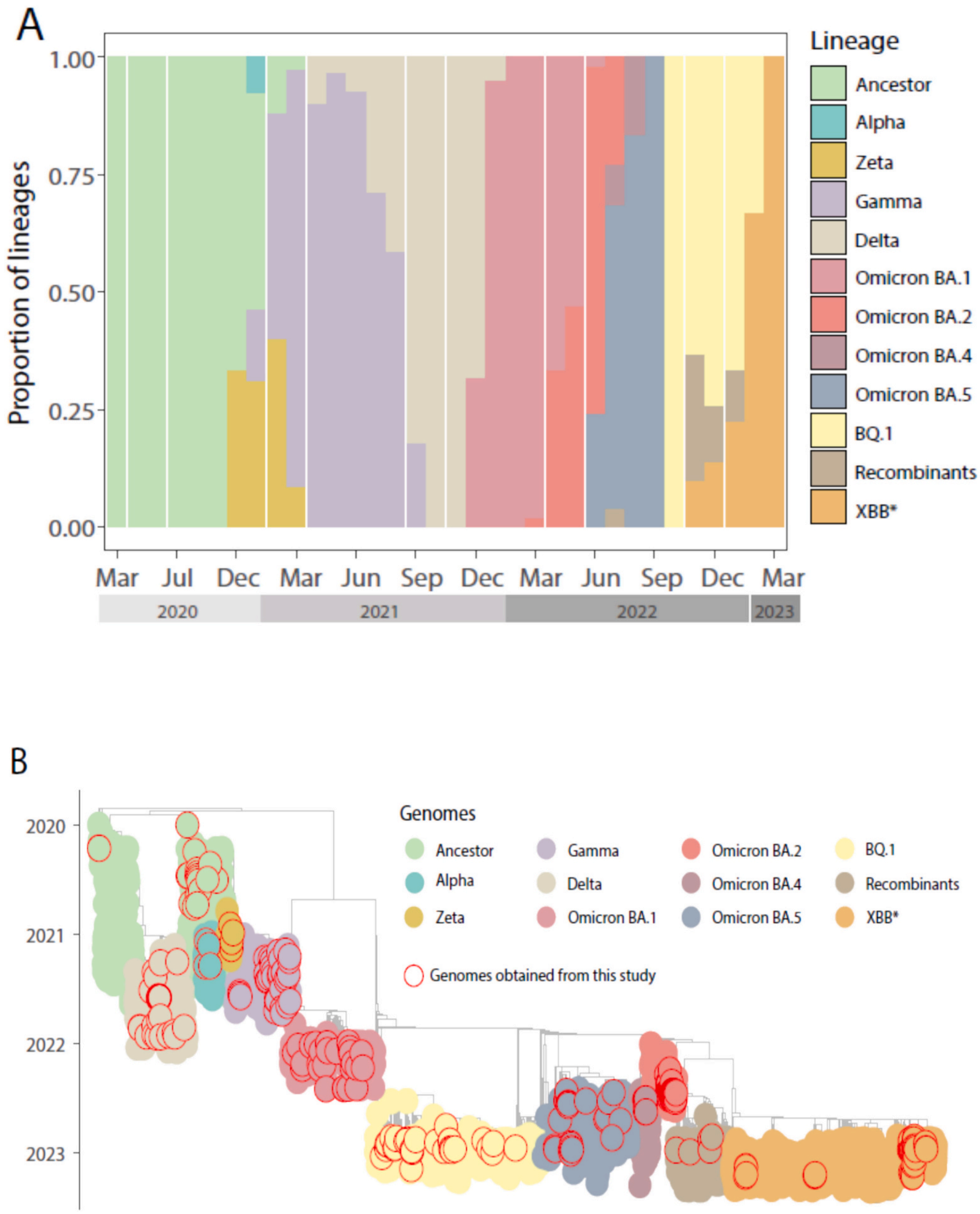

Fig. 2. Temporal and phylogenetic analysis of SARS-CoV-2 variants in Mato Grosso. A) Temporal distribution of SARS-CoV-2 lineages in Mato Grosso from March 2020 to March 2023, showing the proportion of different variants over time. B) Phylogenetic tree illustrating the evolutionary relationships of SARS-CoV-2 genomes from this study, with an emphasis on the emergence and spread of different lineages.

Conceptualization. **Marta Giovanetti:** Writing – review & editing, Visualization. **Elaine Cristina de Oliveira:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Luiz Carlos Júnior Alcântara:** Writing – review & editing, Formal analysis.

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Declaration of competing interest

The authors declare no conflict of interest.

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Data availability

Data will be made available on request.

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