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Research Article

Multiple introductions and country-wide spread of DENV-2 genotype II (Cosmopolitan) in Brazil

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Abstract

Dengue virus serotype 2, genotype Cosmopolitan (DENV-2-GII), is one of the most widespread DENV strains globally. In the USA, DENV-2 epidemics have been dominated by DENV-2 genotype Asian-American (DENV-2-GIII), and the first cases of DENV-2-GII were only described in 2019, in Peru, and in 2021 in Brazil. To gain new information about the circulation of DENV-2-GII in Brazil, we sequenced 237 DENV-2 confirmed cases sampled between March 2021 and March 2023 and revealed that DENV-2-GII is already present in all geographic regions of Brazil. The phylogeographic analysis inferred that DENV-2-GII was introduced at least four times in Brazil, between May 2020 and August 2022, generating multiple clades that spread throughout the country with different success. Despite multiple introductions of DENV-2-GII, analysis of the country-wide laboratory surveillance data showed that the Brazilian dengue epidemic in 2022 was dominated by DENV-1 in most states. We hypothesize that massive circulation of DENV-2-GIII in previous years in Brazil might have created a population immune barrier against symptomatic homotypic reinfections by DENV-2-GII, leading to sustained cryptic circulation in asymptomatic cases and localized outbreaks of this new genotype. In summary, our study stresses the importance of arboviral genomic surveillance to close monitoring and better understanding the potential impact of DENV-2-GII in the coming years.

Keywords: dengue virus serotype 2; genotype Cosmopolitan; Brazil; genomic surveillance; phylogeography.

Research letter

Dengue virus serotype 2 (DENV-2) can be divided into five endemic/epidemic (nonsylvatic) genotypes: American, Cosmopolitan, Asian-American, Asian-II, and Asian-I (Twiddy et al. 2002), also known as genotypes I–V, respectively. In the USA, the DENV-2 American genotype (DENV-2-GI) was the first to

be identified in the 1940s. However, it was later replaced by the Asian-American genotype (DENV-2-GIII) in the 1980s, and cases of DENV-2-GI were not found since then (Allicock et al. 2012). In Brazil, DENV-2-GIII was first introduced in the country in the 1990s and at least three other introductions happened between 1990 and 2014, all with origin in Caribbean or Northern South America countries (de Jesus et al. 2020; Brito et al. 2021). Besides DENV-2, in Brazil, all four DENV serotypes were already identified, presenting a complex dynamics of introductions, co-circulation, and alternation of dominance, which is associated with an increasing number of severe dengue cases and deaths in recent years (Fares et al. 2015).

In 2021, the first cases of DENV-2 Cosmopolitan genotype (DENV-2-GII) were identified in the Brazilian states of Goiás (GO) (Giovanetti et al. 2022) and Acre (AC) (Amorim et al. 2023), located in the Central-Western and Northern country regions, respectively. Phylogenetic analysis suggested that the Brazilian DENV-2-GII sequences derived from an outbreak of this genotype in Peru in 2019 (García et al. 2022) representing the first documented introduction of DENV-2-GII in the American continent. DENV-2-GII is one of the most widespread genotypes, circulating in the Asia–Pacific, the Middle East, Africa, and Oceania, substantially contributing to the global dengue burden (Yenamandra et al. 2021). Here, we present new data on the DENV-2-GII spread in Brazil, showing that shortly after multiple introductions in the country, it is already present in all geographical regions.

The genomic surveillance for DENV was performed in the Brazilian states of Amazonas (AM), Pernambuco (PE), São Paulo (SP), Paraná (PR), Santa Catarina (SC), and Rio Grande do Sul (RS) by each Central State Laboratories (LACEN) and Fiocruz laboratories. A selection of DENV-2-positive samples in molecular tests was submitted for whole-genome amplification and sequencing using Illumina's Viral Surveillance Panel or COVIDseq Test adapted to DENV-2. The genomes were assembled in ViralFlow software (Dezordi et al. 2022) or using Geneious Prime 2022, and consensus sequences were genotyped using online tools (methods detailed in Supplementary text).

Among the 237 DENV-2 sequenced samples, we identified 60 DENV-2-GII genomes (Supplementary Table S1) and 177 DENV-2-GIII, representing 0.05–0.9 per cent of all DENV-2 cases detected in the studied Brazilian states between March 2021 and March 2023 (Supplementary Table S2). DENV-2-GII genomes were aligned with a representative global dataset, and the maximum likelihood phylogenetic analysis revealed that all Brazilian genomes, from this and previous studies (Giovanetti et al. 2022; Amorim et al. 2023), clustered in a monophyletic clade with sequences from Peru and Bangladesh (Supplementary Fig. S1). Our phylogeographic analysis estimated that DENV-2-GII was introduced from Bangladesh into Peru between May 2017 (December 2016—September 2017, 95 per cent high posterior density [HPD]) and April 2019 (November 2018—August 2019, 95 per cent HPD), and from there, it was introduced in Brazil at least four times (Fig. 1A). The first introduction occurred through the AC state (North) between February 2020 (June 2019—September 2020, 95 per cent HPD) and May 2020 (June 2019—November 2020, 95 per cent HPD), and from AC, DENV-2-GII disseminated to all other four Brazilian regions represented by the states of GO (Central-West), PE (Northeast), SP (Southeast), and SC (South). The oldest DENV-2-GII Brazilian samples collected in 2021 in AC (February to March), PE (July), and GO (November) clustered within this clade. A second introduction of DENV-2-GII happened in the state of SP between June 2020 (November 2019—January 2021, 95 per cent HPD) and March 2021 (September 2020—July 2021, 95 per cent HPD), and from there, DENV-2-GII spread southwards to the states of PR, SC, and RS. A third introduction of DENV-2-GII occurred between February 2020 (June 2019—September 2020, 95 per cent HPD) and January 2022 (October 2021—March 2022, 95 per cent HPD) in the state of RS, without evidence of further dissemination to other Brazilian states. Finally, the most recent introduction happened in AM state between May 2020 (July 2019—March 2021, 95 per cent HPD) and August 2022 (June 2022—October 2022, 95 per cent HPD), causing an outbreak in cities close to the border with Peru and Colombia (Naveca 2023). A previous study supports that aerial transportation of humans and/or vector mosquitoes is an important driving force for the spatial spread of DENV between Brazilian states (Nunes et al. 2014). Despite the air travel restrictions being implemented during the first year of the COVID-19 pandemic in Brazil to restrict human mobility, the introduction and dispersion of the first DENV-2-GII viruses in Brazil probably occurred in that time frame (May 2020—March 2021). This supports that new DENV strains could be introduced from abroad and establish local transmission chains in Brazil even during the periods of more restricted air flux of both international and national passengers.

To assess the potential of DENV-2-GII to spread and trigger relevant outbreaks in Brazil, we analyzed the dynamics of DENV serotypes' circulation in the country as identified through molecular tests by the laboratory surveillance and informed in the national system of diseases of compulsory notification (SINAN). Between 2018 and 2022, the Brazilian dengue epidemic was dominated by DENV-1 and DENV-2, with significant regional differences (Fig. 1B). A predominance of DENV-2 after 2020, which could indicate a genotype II outbreak, was observed in the northern states of AC (where genomic surveillance detected DENV-2-GII circulation), Rondônia and Roraima, in the southeastern state of Rio de Janeiro (RJ), and in the northeastern states of Ceará (CE), Paraíba (PB), Rio Grande do Norte (RN), Sergipe (SE), and PE. In the latter, only one sequence of DENV-2-GII was found compared to eighty sequences of DENV-2-GIII in 2021, indicating that the dominance of DENV-2 in PE at that time was not caused by genotype II, which could also be the case for the neighboring northeastern states. A high prevalence of DENV-2 was observed in several states between 2018 and 2020, but there was no evidence of circulation of DENV-2-GII in Brazil before 2021 (Supplementary Figure S2). In other states, including AM and RS, where genomic surveillance detected recent introductions of DENV-2-GII in 2022, DENV-1 was responsible for most dengue cases in the whole period supporting that DENV-2-GII only caused localized outbreaks in those states. In fact, in 2022, DENV-1 was responsible for more than 80 per cent of the dengue cases in seventeen out of twenty-five Brazilian states with data available (Supplementary Figure S3). Finally, it is important to note that our study sampled only a fraction of Brazilian states, and a more comprehensive sampling regarding DENV-2, both in time and space, will be necessary to unveil the complete picture of genotype II spread and to assess the implications in future outbreaks.

In summary, this study confirms that DENV-2-GII is circulating nationally in Brazil, expanding the findings of previous localized studies (Giovanetti et al. 2022; Amorim et al. 2023). Our phylogenetic analysis unveiled multiple introductions of this genotype into Brazil from Peru, generating multiple clades that spread in the country with different success. Despite the wide geographic dispersion of DENV-2-GII, dengue epidemics in 2021–2 in most Brazilian states were dominated by DENV-1, supporting that circulation of DENV-2-GIII in previous years probably created a higher population immunity barrier against homotypic reinfections by DENV-2-GII than against heterotypic reinfections by

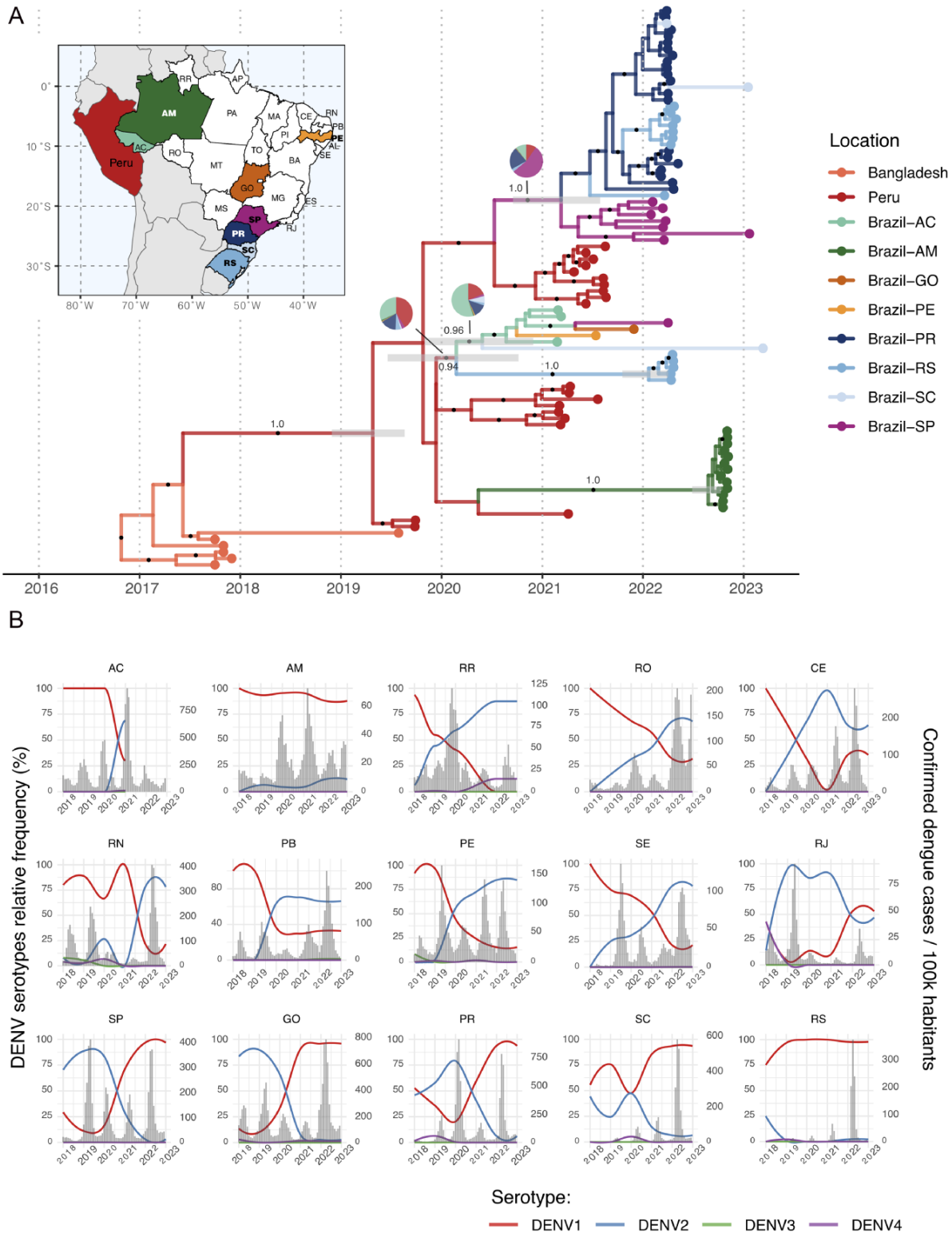

Figure 1. Spatiotemporal evolution of DENV-2 genotype II (Cosmopolitan) in South America and circulation dynamics of DENV serotypes in Brazil in recent years. (A) Time-scaled phylogenetic tree of eighty-eight DENV-2-GII genomes with ancestral locations inferred by the phylogeographic model. The branches are colored according to the most likely ancestral location, and pie charts show the posterior probability of each location for key nodes with high uncertainty. Error bars show the 95 per cent HPD of node heights related to introductions to Brazil. The dots on branches indicate a posterior probability higher than 0.9, and key branches are annotated with the posterior support. Inset map depicts the Brazilian states from which DENV-2 sequences were sampled. Colors on the map and phylogenetic tree correspond and the states directly sampled in this study are highlighted in bold. (B) The relative frequency of each DENV serotype (lines) and confirmed dengue cases per 100k habitants (histogram) for a selection of Brazilian states between 2018 and 2022. The acronyms for depicted states: AC—Acre, AM—Amazonas, RR—Roraima, RO—Rondônia, CE—Ceará, RN—Rio Grande do Norte, PB—Paraíba, PE—Pernambuco, SE—Sergipe, RJ—Rio de Janeiro, SP—São Paulo, GO—Goiás, PR—Paraná, SC—Santa Catarina, and RS—Rio Grande do Sul.

DENV-1. At the same time, the existence of asymptomatic homotypic reinfections (Murphy and Whitehead 2011; Tan et al. 2018; López et al. 2022) combined with the heterogeneous population immune landscape across Brazilian regions could have created an epidemic scenario for sustained cryptic circulation, rapid spread, and localized outbreaks of DENV-2-GII across the country's territory. Indeed, DENV-2-GII was possibly responsible for most DENV-2 infections in Brazil in 2022, and this warrants close monitoring to better understand the potential impact of this genotype in the coming years.

Data availability

The data for this article are available at https://github.com/akograf/Multiple_introductions_and_spread_of_DENV-2-GII_in_Brazil.git.

Supplementary data

Supplementary data are available at *Virus Evolution* online.

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Conflict of interest: The authors report there are no competing interests to declare.

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