Open access • Posted Content • DOI:10.1101/2020.06.17.158006

Genomic surveillance of SARS-CoV-2 reveals community transmission of a major lineage during the early pandemic phase in Brazil — Source link

Paola Cristina Resende, Edson Delatorre, Tiago Gräf, Daiana Mir ...+19 more authors

Institutions: Oswaldo Cruz Foundation, Universidade Federal do Espírito Santo, University of the Republic

Published on: 18 Jun 2020 - bioRxiv (Cold Spring Harbor Laboratory)

Genomic surveillance of SARS-CoV-2 reveals community transmission of a major lineage during the early pandemic phase in Brazil

Paola Cristina Resende[1a], Edson Delatorre[2], Tiago Gräf[3], Daiana Mir[4], Fernando do Couto Motta[1], Luciana Reis Appolinario[1], Anna Carolina Dias da Paixão[1], Maria Ogrzewalska[1], Braulia Caetano[1], Mirleide Cordeiro dos Santos[5], Jessylene de Almeida Ferreira[5] , Edivaldo Costa Santos Junior[5], Sandro Patroca da Silva[5], Sandra Bianchini Fernandes[6], Lucas A Vianna[7], Larissa da Costa Souza[8], Jean F G Ferro[9], Vanessa B Nardy[10], Júlio Croda[11,12], Wanderson K Oliveira[13], André Abreu[14], Gonzalo Bello[15a*], Marilda M Siqueira[1*]

[*] Both authors contributed equally to this work.

[a] Correspondent authors

Paola Cristina Resende - paola@ioc.fiocruz.br

Gonzalo Bello - gbellobr@gmail.com

Affiliation

[1]Laboratory of Respiratory Viruses and Measles, Oswaldo Cruz Institute (IOC), FIOCRUZ, Rio de Janeiro, Brazil. SARS-CoV-2 National Reference Laboratory for the Brazilian Ministry of Health (MoH) and Regional Reference Laboratory in Americas for the Pan-American Health Organization (PAHO/WHO).

[2]Departamento de Biologia. Centro de Ciencias Exatas, Naturais e da Saude, Universidade Federal do Espirito Santo, Alegre, Brazil.

[3]Instituto Gonçalo Moniz, FIOCRUZ, Salvador, Brazil.

[4]Unidad de Genomica y Bioinformatica, Centro Universitario Regional del Litoral Norte, Universidad de la Republica, Salto, Uruguay.

[5]Instituto Evandro Chagas, Belem, Para

[6]Laboratorio Central de Saúde Publica do Estado de Santa Catarina (LACEN-SC), Florianopolis, Santa Catarina, Brazil.

[7]Laboratorio Central de Saude Publica do Estado Espirito Santo (LACEN-ES). Vitoria, Espirito Santo, Brazil.

[8]Laboratorio Central de Saude Publica do Distrito Federal (LACEN-DF). Brasilia, Distrito Federal, Brazil.

[9]Laboratorio Central de Saude Publica de Alagoas (LACEN-AL). Maceio, Alagoas, Brazil.

[10]Laboratorio Central de Saude Publica da Bahia (LACEN-BA). Salvador, Bahia, Brazil.
[11]Fiocruz Mato Grosso do Sul, Campo Grande, Mato Grosso do Sul, Brazil.
[12]Universidade Federal de Mato Grosso do Sul – UFMS, Campo Grande, MS, Brazil.
[13]Hospital das Forças Armadas, Ministério da Defesa, Brasília, Distrito Federal, Brazil.
[14]Coordenadoria Geral de Laboratórios – Ministério da Saúde, Brasilia, Distrito Federal, Brazil.
[15]Laboratório de AIDS e Imunologia Molecular, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil.

Abstract

Despite all efforts to control the COVID-19 spread, the SARS-CoV-2 reached South America within three months after its first detection in China, and Brazil became one of the hotspots of COVID-19 in the world. Several SARS-CoV-2 lineages have been identified and some local clusters have been described in this early pandemic phase in Western countries. Here we investigated the genetic diversity of SARS-CoV-2 during the early phase (late February to late April) of the epidemic in Brazil. Phylogenetic analyses revealed multiple introductions of SARS-CoV-2 in Brazil and the community transmission of a major B.1.1 lineage defined by two amino acid substitutions in the Nucleocapsid and ORF6. This SARS-CoV-2 Brazilian lineage was probably established during February 2020 and rapidly spread through the country, reaching different Brazilian regions by the middle of March 2020. Our study also supports occasional exportations of this Brazilian B.1.1 lineage to neighboring South American countries and to more distant countries before the implementation of international air travels restrictions in Brazil.

Keywords: COVID-19; SARS-CoV-2; Brazil; coronavirus; genetic lineages; community transmission.

Introduction

COVID-19, the disease caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), is leading to high rates of acute respiratory syndrome, hospitalization, and death [1,2]. Brazil, the second most hit country in the world so far, has reported 923.189 cases and 45.241 deaths (last update 17th June 2020) [3]. The first positive case of SARS-CoV-2 infection in Brazil was reported on 26th February 2020 in an individual traveling from Europe to Sao Paulo metropolitan region [4], and during the following two weeks, the virus was detected in all country regions [5]

The rapid worldwide genomic surveillance of SARS-CoV-2, mainly shared via the GISAID (https://www.gisaid.org/) databank that provides public access to genomic sequence and patient's metadata, is being crucial for managing this healthcare emergency enabling the tracking of viral transmission patterns as the epidemic progresses. The SARS-CoV-2 has diversified in several phylogenetic lineages while it spread geographically across the world [6-8]. A SARS-CoV-2 lineage previously designated as "G" or "B.1" clade, was initially identified as the most common variant in Europe and is currently one of the predominant viral lineages in North America [6-8]. Inspection of SARS-CoV-2 genomic sequences from South America available on GISAID revealed that the clade B.1 is also the most prevalent (82%) SARS-CoV-2 variant circulating in South America (**Fig. 1A**).

Genomic epidemiology has been a useful tool to track the community transmission of SARS-CoV-2 in different geographical settings. Previous studies revealed that SARS-CoV-2 epidemics in Australia [9,10], Belgium [11], Denmark [12], France [13], Iceland [14], Israel [15], Netherlands [16] Spain [17] and the United States (US) [18-20], resulted from multiple independent introductions, followed by community dissemination of some viral strains that resulted in the emergence of national (or local) transmission clusters. Early phylogenetic analyses of SARS-CoV-2 complete genomes from the Brazilian states of Minas Gerais [21], Sao Paulo [22] and Amazonas [23], revealed multiple independent viral importations and limited local spread during the initial stage of the SARS-CoV-2 epidemic in Brazil. Even so, the SARS-CoV-2 genomes analyzed in those previous studies were mostly recovered from individuals returning from international travel, and thus might not have recovered the genetic diversity of SARS-CoV-2 strains linked to community transmission in Brazil.

To investigate the SARS-CoV-2 strains circulating in Brazil, we recovered 95 whole-genomes collected from 10 different Brazilian states during the first two months of the COVID-19 epidemic. New SARS-CoV-2 Brazilian viral sequences were combined with other Brazilian and global reference sequences available in GISAID and subjected to maximum likelihood (ML) and Bayesian coalescent analyses.

Methods

Sampling and ethical aspects

Nasopharyngeal-throat combined swabs were collected from clinically ill individuals between the first and the eleventh day after their first symptoms, or from asymptomatic individuals suspicious of SARS-CoV-2 infection. Samples were conserved in the viral transport medium at 4°C to 8°C

up to processing. This study was approved by the FIOCRUZ-IOC Ethics Committee (68118417.6.0000.5248) and the Brazilian Ministry of Health SISGEN (A1767C3).

Nucleic acid isolation and RT–qPCR

The Viral RNA was extracted manually from 140 µl of clinical samples using QIAamp Viral RNA Mini kit (QIAGEN, Hilden, Germany) or automatedly using the 300 µl of sample and Perkin-Elmer Chemagic machine/chemistry, according to the manufacturer's instructions. SARS-CoV-2 positive cases were confirmed by real-time RT–PCR assays using the SARS-COV-2 Molecular E/RP Kit (Biomanguinhos, Rio de Janeiro, Brazil) based on the protocol previously designed by Corman et al (2020) [24]. Amplifications were conducted in ABI7500 platform using the following conditions: reverse transcription (50°\C, 15 min), reverse transcriptase inactivation and DNA polymerase activation (95 °C, 2 min), followed by 45 cycles of DNA denaturation (95 °C, 20 s) and annealing–extension (58 °C, 30 s). The fluorescence data was collected in the annealing-extension step and all samples with sigmoid curves crossing the threshold line up to cycle 40 were named positive. Negative and positive controls were included in each extraction and real time RT–PCR batch.

SARS-CoV-2 whole-genome amplification and sequencing

Total RNA from positive samples presenting Ct values up to 30,0 for gene E was reverse transcribed using SuperScript™ IV First Strand Synthesis System (Invitrogen). Two multiplex PCR reactions using the primer scheme previously described [25] (Pool A = nine amplicons and Pool B = eight amplicons), were performed using the Q5® High-Fidelity DNA Polymerase (NEB). Amplicons were purified using Agencourt AMPure XP beads (Beckman Coulter™) and the DNA quantified with Qubit 4 Fluorometer (Invitrogen) using the Qubit dsDNA HS Assay Kit (Invitrogen) and sequenced using Illumina MiSeq or NextSeq (San Diego, CA, USA) and Nanopore (Oxford, UK) platforms. Illumina short reads DNA libraries were generated from the pooled amplicons using Nextera XT DNA Sample Preparation Kit (Illumina, San Diego, CA, USA) according to the manufacturer specifications. The size distribution of these libraries was evaluated using a 2100 Bioanalyzer (Agilent, Santa Clara, USA) and the samples were pair-end sequenced (Micro V2, 300 cycles) on a MiSeq equipment (Illumina, San Diego, USA) in around 18 hours. The Nanopore library protocol is optimized for long reads (2 kb amplicons)[25]. Library preparation was conducted using Ligation Sequencing 1D (SQK-LSK109 Oxford Nanopore Technologies (ONT) and Native Barcoding kit 1 to 24 (ONT), according to the manufacturer's

instructions. After end repair using the NEBNext® Ultra™ II End Repair/dA-Tailing Module (New England Biolabs, NEB) the native barcodes were attached using a NEBNext® Ultra™ II Ligation Module (NEB). Up to 23 samples were pooled for sequencing in the same flow cell (FLOMIN106 flow cell R9.4.1), and a negative mock sample was loaded in each run for validation. The sequencing was performed for 12 hours using the high accuracy base calling in the MinKNOW software, however, the run was monitored by RAMPART (https://github.com/articnetwork/rampart) allowing us stop the assay after 2 hours, when ≥ 20x depth for all amplicons was achieved.

Data analysis to recover the SARS-CoV-2 whole-genome consensus sequences

Demultiplexed fastq files generated by Illumina sequencing were used as the input for the analysis. Reads were trimmed based on quality scores with a cutoff of q30, in order to remove low quality regions and adapter sequences. The reads were mapped to Wuhan Strain MN908947. Duplicate reads were removed from the alignment and the consensus sequence called at a threshold of 10x. The entire workflow was carried out in CLC Genomics Workbench software version 20.0. For the Oxford Nanopore sequencing data, the high accuracy base called fastq files were used as an input for analysis. The pipeline used was an adaptation of the artic-ncov2019 medaka workflow (https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html). We used an earlier version of the workflow which used Porechop to demultiplex the reads. The mapping to the Wuhan reference sequence (MN908947) was done using Minimap2 with Medaka used for error correction. This was all carried out within the artic-ncov2019-medaka conda environment (https://github.com/artic-network/artic-ncov2019).

SARS-CoV-2 genotyping

New Brazilian genome sequences of SARS-CoV-2 were assigned to viral lineages according to the nomenclature proposed by Rambaut *et al* [7], using the pangolin web application (https://pangolin.cog-uk.io). A matrix with the count of each possible character at each position of the alignment of the B.1.1 sequences available in GISAID as of June 6, was computed using the R package SeqinR [26].

Maximum Likelihood phylogenetic analyses

SARS-CoV-2 B.1.1 complete genome sequences (> 29 Kilobases) with appropriate metadata were retrieved from GISAID (https://www.gisaid.org/) as of 4th June. After excluding low quality

genomes (> 10% of ambiguous positions), we obtained a final dataset of 7,674 sequences. Because most sequences recovered (75%) were from the United Kingdom (UK), we generate a "non-redundant" global balanced dataset by removing very closely related sequences (genetic similarity > 99.99%) from the UK. To achieve this aim, sequences from the UK were grouped by similarity with the CD-HIT program [27] and one sequence per cluster was selected. With this sampling procedure, we obtained a balanced global reference B.1.1 dataset containing 3,764 sequences that were aligned with the new B.1.1 Brazilian sequences generated in this study using MAFFT v7.467 [28] and then subjected to maximum-likelihood (ML) phylogenetic analyses. The ML phylogenetic tree was inferred using IQTREE v1.6.12 [29], under the GTR+F+I+G4 nucleotide substitution model as selected by the ModelFinder application [30] and the branch support was assessed by the approximate likelihood-ratio test based on a Shimodaira–Hasegawa-like procedure (SH-aLRT) with 1,000 replicates. The ML tree was visualized using the FigTree v1.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Analysis of temporal signal and phylogeographic structure

A ML tree of the B.1.1.EU/BR and B.1.1.BR dataset was inferred as explained above and the temporal signal was assessed by performing a regression analysis of the root-to-tip divergence against sampling time using TempEst [31]. The degree of phylogeographic structure was then investigated using the BaTS program [32] which estimates phylogeny-trait associations in a posterior sampling of Bayesian trees. Bayesian trees were generated with BEAST package [33] as explained below, but without incorporation of a phylogeographic model. Phylogenetic clustering by sampling location in the posterior sampling of trees was then assessed by calculating different metrics including the Association Index (AI), the Parsimony Score (PS) and the Maximum Clade (MC) and compared to a null hypothesis generated by tip randomization. Results were considered significant for $P < 0.01$.

Bayesian phylogeographic analyses

The age of the most recent common ancestor (T_{MRCA}) and the spatial diffusion pattern of the B.1.1.EU/BR and B.1.1.BR lineages were jointly estimated using a Bayesian Markov Chain Monte Carlo (MCMC) approach implemented in BEAST 1.10 [33], using the BEAGLE library v3 [34] to improve computational time. Time-scaled Bayesian trees were estimated by using a strict molecular clock model with a fixed substitution rate (8×10^{-4} substitutions/site/year) based on previous estimates, the HKY+I+G nucleotide substitution model, and the Bayesian skyline

coalescent prior [35]. Viral migrations across time were reconstructed using a reversible discrete phylogeographic model [36] with a CTMC rate reference prior [37]. Two MCMC chains were run for 100 million generations and then combined to ensure stationarity and good mixing. Stationarity (constant mean and variance of trace plots) and good mixing (Effective Sample Size >200) for all parameter estimates were assessed using TRACER v1.7 [38]. The maximum clade credibility (MCC) tree was summarized with TreeAnnotator v1.10 and visualized using the FigTree v1.4 program.

Results

In this study, we analyzed 95 viral whole-genomes (>99% coverage) obtained from individuals with confirmed SARS-CoV-2 infection, who underwent testing and genomic sequencing at the Laboratory of Respiratory Viruses and Measles, Oswaldo Cruz Institute (IOC)-FIOCRUZ, in Rio de Janeiro, and the Evandro Chagas Institute, in Para, Brazil [25]. Samples were collected between 29th February and 28th April 2020 from individuals that reside in 10 different Brazilian states from the Southeastern (Rio de Janeiro and Espirito Santo), Central-Western (Distrito Federal), Northern (Acre, Amapa and Para), Northeastern (Alagoas, Bahia and Maranhao) and Southern (Santa Catarina) regions. (**Supplementary Table 1**). The median age of patients with COVID-19 illness was 42-year-old (range 0 to 85 years) and 54 (57%) were female. Seven individuals reported international travel or contact with travelling people. Six different SARS-CoV-2 lineages (A.2, B.1, B.1.1, B.2.1, B.2.2 and B.6) were detected in our sample (**Supplementary Table 1),** according to the nomenclature proposed by Rambaut *et al* [7]. Most Brazilian SARS-CoV-2 sequences here obtained were classified as clade B.1 (95%, n = 90), and particularly within the sub-clade B.1.1 (92%, n = 87) (**Fig. 1B**). The prevalence of the sub-clade B.1.1 in our sample (92%) was much higher than that observed in other Brazilian sequences available in GISAID (36%) (**Fig. 1C**). The clade B.1.1 was the only lineage detected in the 18 individuals with no history of recent international travel, while four different lineages (B.1, B.1.1, B2.1 and B.6) were detected among the six individuals with recent history of international travel (imported cases) and their contacts. (**Supplementary Table 1**).

To investigate whether the observed high prevalence of the lineage B.1.1 in Brazil resulted from one or multiple independent viral introductions into the country, we performed a ML phylogenetic analysis of the 87 B.1.1 Brazilian sequences identified in this study, together with 3,764 SARS-CoV-2 complete genome sequences available in GISAID as of 4th June representing the current global diversity of the B.1.1 clade. Brazilian isolates were distributed throughout the phylogenetic tree, consistent with the hypothesis of multiple independent introductions (**Fig. 2**). A significant proportion of Brazilian B.1.1 sequences (65%, *n* = 74/114), however, branched in a monophyletic cluster (SH-*aLRT* = 74%) here designated as B.1.1.BR, that comprises sequences

from Brazil, other South American countries (Argentina, Chile and Uruguay), North America (Canada and USA), Australia and England. The lineage B.1.1.BR is nested within a highly supported (SH-*aLRT* = 87%) clade, here referred as B.1.1.EU/BR, containing basal sequences from Western Europe and Brazil, (**Fig. 2**). We also detected two other well-supported (SH-*aLRT* > 80%) monophyletic clades of small size (n = 2-11) mostly composed by Brazilian sequences (**Supplementary Fig. 1**).

In addition to sharing the three nucleotide mutations (G28881A, G28882A, G28883C) characteristic of the clade B.1.1, sequences of clusters B.1.1.EU/BR and B.1.1.BR harbor a non-synonymous mutation T29148C at the Nucleocapsid protein (I292T); and another non-synonymous mutation T27299C at the ORF6 (I33T) was shared only by sequences of the lineage B.1.1.BR. Mutations T29148C or T27299C were not detected in the other 7,551 B.1.1 genomes available in GISAID, supporting the hypothesis that they are synapomorphic traits of the B.1.1.EU/BR and B.1.1.BR clades, respectively. The clades B.1.1.EU/BR and B.1.1.BR were detected in different countries around the world, but the overall estimated prevalence of these clades in Brazil (6% and 44%, respectively) is much higher than that estimated in Europe, North America or Australia (**Supplementary Table 2**). Such difference could not be explained by sampling bias as those regions comprise the most densely sampled countries worldwide and is suggestive of local dissemination of those clades in Brazil. Consistent with this hypothesis, none of the individuals infected with clade B.1.1.BR from our cohort or with clade B.1.1.EU/BR from a previous cohort [21] reported international travel. The clades B.1.1.EU/BR and B.1.1.BR were not homogeneously distributed across Brazilian states (**Supplementary Table 3**). The clade B.1.1.EU/BR was highly prevalent in Minas Gerais and also detected in the Federal District, while the clade B.1.1.BR was predominant in Rio de Janeiro and also identified in some samples from the Northern, Central-Western and Northeastern Brazilian regions. Notably, none of these lineages were detected in the most populated state of Sao Paulo.

Finally, we conducted a Bayesian phylogeographic analysis to reconstruct the spatiotemporal dissemination dynamics of the B.1.1.EU/BR and B.1.1.BR lineages. Linear regression of root-to-tip genetic distance against sampling date revealed a weak temporal structure in our dataset (R^2 = 0.19) (**Supplementary Fig. 2**). Despite the low genetic diversity, analyses of geographic structure rejected the null hypothesis of a panmixed population (**Supplementary Table 4**), supporting that geographic subdivision of the B.1.1.EU/BR and B.1.1.BR sequences was greater than expected by chance. The time-scaled Bayesian tree was then reconstructed using a strict molecular clock model with a fixed substitution rate (8×10^{-4} substitutions/site/year). Bayesian reconstructions traced the origin of the B.1.1.EU/BR lineage most probably to Europe

(*Posterior state probability* [*PSP*] = 0.64) at 2nd February (95% High Posterior Density [HPD]: 7th January – 20th February) and its dissemination to Brazil at 19th February (95% HPD: 4th February – 28th February) (**Fig. 3A**). The origin of the B.1.1.BR lineage was traced with high probability to Brazil (*PSP* = 0.95) at 22th February (95% HPD: 10th February – 28th February). From Brazil, the B.1.1.BR lineage probably disseminated to neighboring South American countries (Argentina, Chile and Uruguay) and to more distant regions (Australia, USA and UK).

Discussion

Our genomic survey identified a major SARS-CoV-2 B.1.1 lineage, here designated as B.1.1.BR, that seems to be responsible for a substantial fraction of the community viral transmissions in Brazil. This lineage harbors two non-synonymous synapomorphic mutations at positions T27299C and T29148C located at the ORF6 (I33T) and the Nucleocapsid protein (I292T), respectively. Basal to this clade, we identified a group of Brazilian and European sequences composing a paraphyletic clade, designated as B.1.1.EU/BR, that only carry the synapomorphic mutation T29148C and seems to represent and evolutionary intermediate between clades B.1.1 and B.1.1.BR.

Our phylogeographic reconstruction supports that clade B.1.1.EU/BR most probably arose in Europe (*PSP* = 0.64) around 2nd February and was introduced into Brazil a couple of weeks later, where it spread and rapidly fixed the T27299C mutation, originating the clade B.1.1.BR (**Fig. 4A**). This evolutionary pattern agrees with the earlier detection of the clade B.1.1.EU/BR in Western Europe (28th February) than in Brazil (13th March) (**Fig. 3B**); but the extremely low prevalence of the clade B.1.1.EU/BR in Europe (<1% of total SARS-CoV-2 sequences) makes this transmission history a highly unlikely epidemiological scenario. Once our phylogeographic analysis also estimated Brazil as a putative ancestral state at the root of clade B.1.1.EU/BR (*PSP* = 0.35), an alternative hypothesis would be that a highly prevalent B.1.1 strain was introduced from Western Europe into Brazil before 2nd February and that synapomorphic mutations T29148C and T27299C were fixed at sequential steps during subsequent virus local spread (**Fig. 4B**). The relative high prevalence of clade B.1.1.EU/BR in some Brazilian locations makes the dissemination of this lineage from Brazil to Western Europe a quite plausible transmission scenario. Retrospective analyses of Brazilian samples obtained from individuals with severe acute respiratory disease during February might provide unique insights to resolve the origin of the clade B.1.1.EU/BR.

Brazil was traced as the source location of the clade B.1.1.BR with high probability (*PSP* = 0.95) in our phylogeographic reconstruction. The earliest B.1.1.BR sequence currently available, however, is an Argentinean sequence sampled on 1st March 2020; while the earliest detection of

the clade B.1.1.BR in Brazil occurred in two samples isolated in the Distrito Federal on 13th March 2020 (**Fig. 3B**). Of note, none of the 30 Brazilian genomes analyzed between 25th February and 12th March, including 12 B.1.1 genomes from imported cases, belong to the clade B.1.1.BR. This suggests that clade B.1.1.BR may have arisen in a Brazilian state that was not included in our dataset and/or that this lineage circulated cryptically for several weeks before being detected in symptomatic carriers. The nearly simultaneous detection of the clade B.1.1.BR in distant states from the Central-Western (Federal District, 13th March), Northern (Amapa, 17th March) and Southeastern (Rio de Janeiro, 20th March) Brazilian regions, supports the second hypothesis and further suggests a wide geographic spread of this Brazilian lineage. Our results also suggest a high geographic compartmentalization of SARS-CoV-2 genetic diversity within Brazil. Considering the three most populated and densely sampled states of Brazil, the clade B.1.1.EU/BR was only detected in Minas Gerais, the clade B.1.1.BR only in Rio the Janeiro, and none of those in Sao Paulo.

Our analyses support that the clade B.1.1.BR not only spread within Brazil but was also exported from Brazil to neighboring South American countries and also to more distant countries (i.e. Canada, USA, UK and Australia). The chance introduction of SARS-CoV-2 strains from Western Europe into Brazil during February and the subsequent exportation of Brazilian SARS-CoV-2 lineages to neighboring South American countries, Western Europe and North America during following weeks agrees with the high influx of tourists from those regions into Brazil during January and February (**Fig. 4C**). Our findings support that when first control measures for international travels were implemented in Brazil around the middle March, the clades B.1.1.EU/BR and B.1.1.BR were already established in the country and also spread from Brazil to other countries. Of note, no B.1.1.EU/BR or B.1.1.BR sequences were detected in Europe, North America or Oceania after 15th April, coinciding with a sharp decrease in the influx of international air travels to Brazil (**Fig. 4C**) that might have greatly reduced the chance of exportation of SARS-CoV-2 Brazilian lineages to other countries.

Our phylogeographic reconstruction suggests that the clade B.1.1.BR might have seeded secondary outbreaks in Argentina, Chile, Australia and the US, but those findings should be interpreted with caution because of the low support of local clusters. Although high-quality full genomes of SARS-CoV-2 currently available contain enough information to allow reliable phylogenetic inferences, the low genetic diversity of within-country (or regional) transmission clusters imposes a serious limitation for accurate phylogeographic reconstructions [39,40]. Indeed, the MC test supports a random phylogenetic clustering of B.1.1.EU/BR and B.1.1.BR strains from most locations, with exception of Brazil, Argentina and Europe (**Supplementary Table 4**). The

B.1.1.BR sequences sampled at different Brazilian states were also highly similar or identical, making it difficult to trace with precision the origin and within-country fluxes of this viral clade during the early epidemic phase in Brazil. Another important limitation of our study is the uneven spatial and temporal sampling scheme. Most SARS-CoV-2 sequences recovered in the present study were from the Rio de Janeiro state and might thus not represent the viral diversity in other Brazilian states. More accurate reconstructions of the origin and regional spread of the clade B.1.1.BR will require a denser sampling from Brazil and neighboring South American countries, particularly during the very early phase of the epidemic.

In summary, this study reveals the existence of a major SARS-CoV-2 B.1.1 lineage associated with community transmission in Brazil and widespread in a national scale. This major B.1.1 Brazilian lineage emerged in Brazil in February 2020, probably before the detection of the first imported SARS-CoV-2 case in the country, and reached different Brazilian regions by the middle of March 2020. Continuous efforts for widespread sequencing of SARS-CoV-2 may provide unique insight about its local dissemination in Brazil and other South American countries.

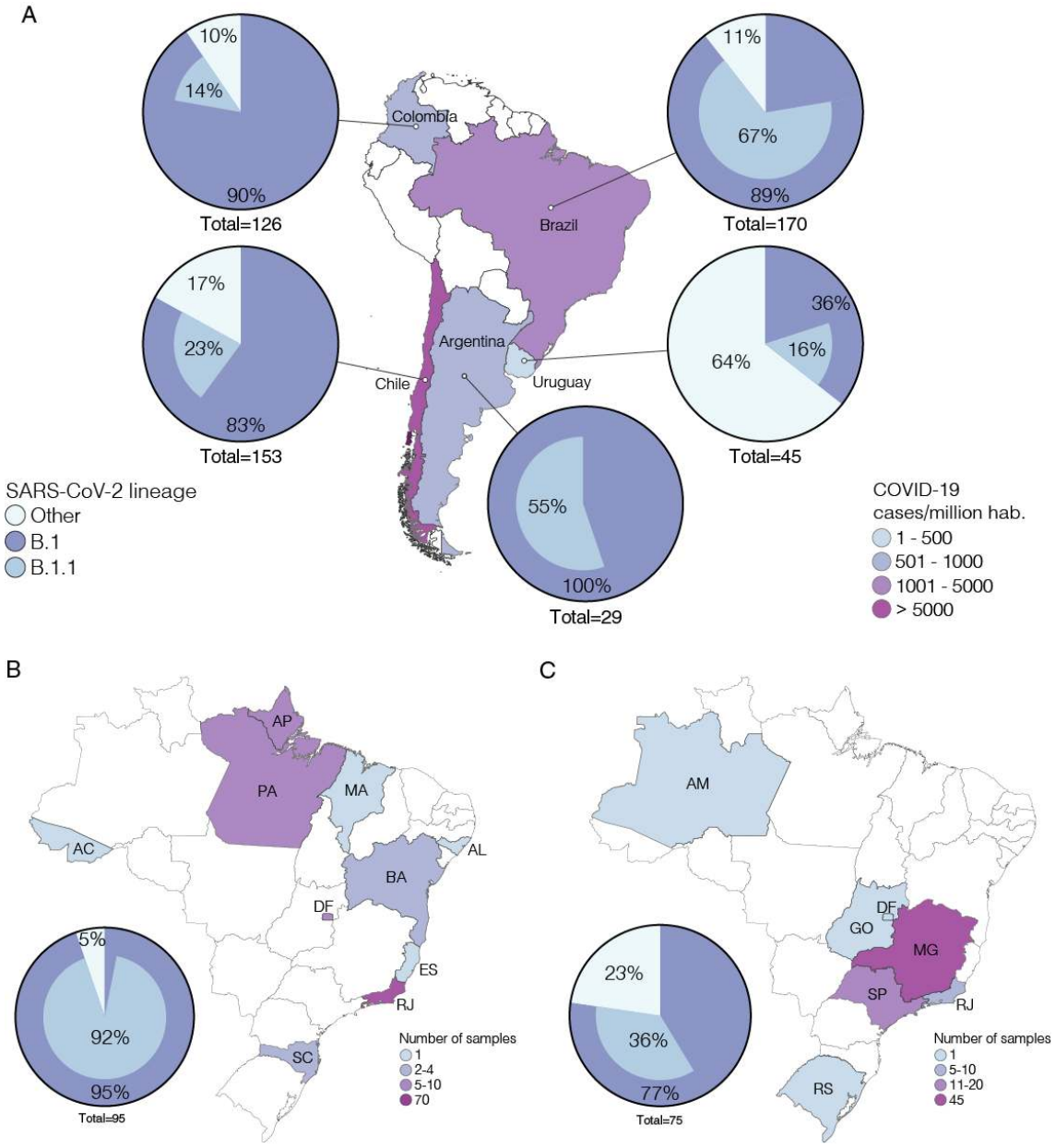


Figure 1. **Prevalence of SARS-CoV-2 clades B.1 and B.1.1 in Brazil and other South American countries.** A) Map showing the prevalence of SARS-CoV-2 clades B.1 and B.1.1 across different South American countries with more than five viral genomes available in the GISAID (https://www.gisaid.org/) database as of 4th June. Countries were colored according to the incidence of COVID-19. B and C) Brazilian maps showing the prevalence of SARS-CoV-2 clades B.1 and B.1.1 across different states considering the viral sequences generated in this study (B) or those generated by others and deposited in the GISAID database. Brazilian states are colored according to the number of SARS-CoV-2 available. Colors in pie charts correspond to the viral lineage.

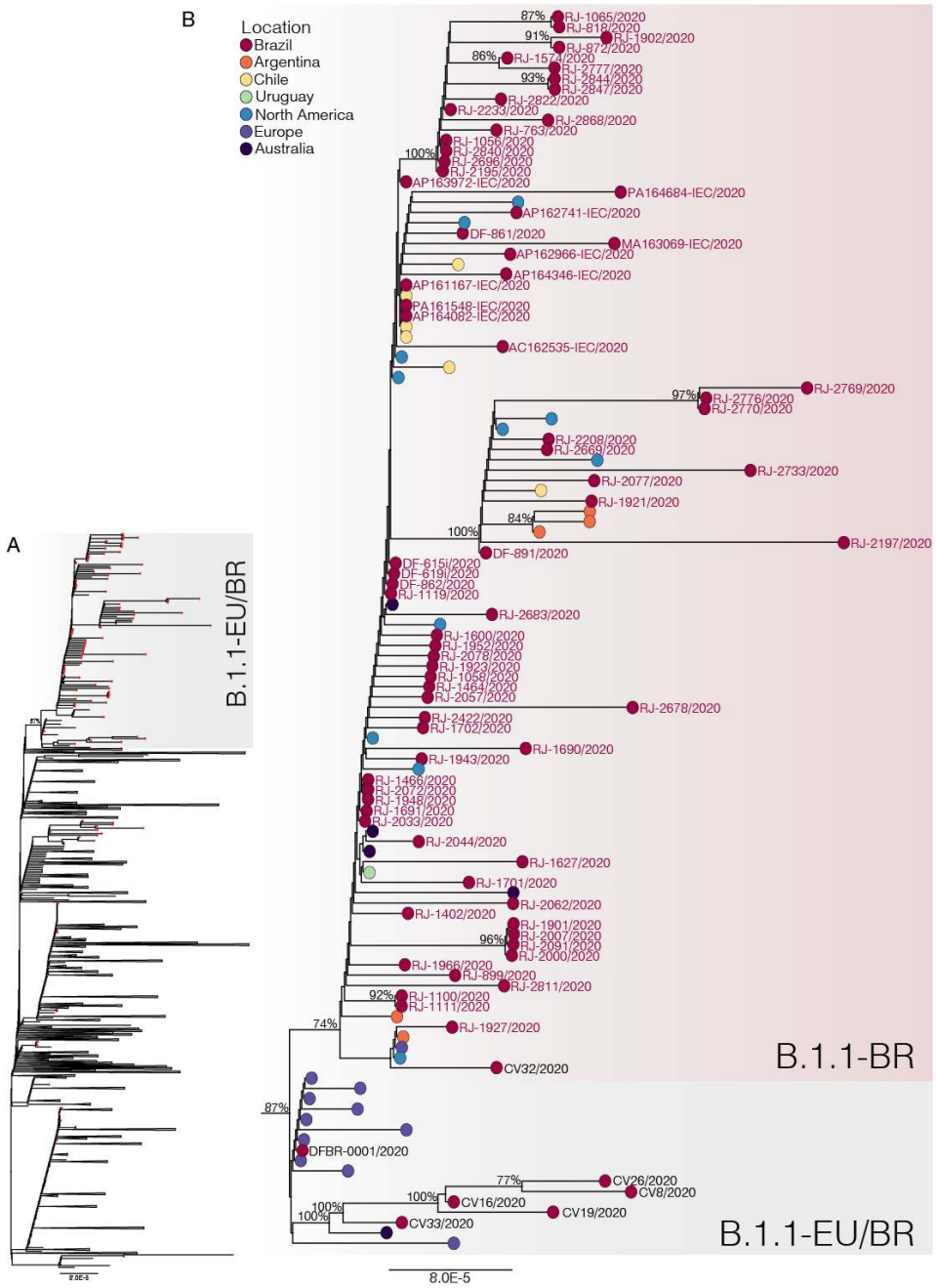


Figure 2. **Phylogenetic relationships of SARS-CoV-2 B.1.1 Brazilian and global strains.** A) ML phylogenetic tree of 87 B.1.1 Brazilian genomes obtained in this survey (red circles) along with 3,764 B.1.1 worldwide reference sequences from GISAID database. B) Zoomed view of the clusters B.1.1.EU/BR and B.1.1.BR. Names of Brazilian SARS-CoV-2 genomes generated in this study (red tips) or deposited in GISAID databank (black tips) are shown. Tip circles are colored according to the sampling location. Only node supports (aLRT) above 70% are shown. Shaded boxes highlight the position of clusters B.1.1.EU/BR and B.1.1.BR. Tree was rooted on midpoint and branch lengths are drawn to scale with the bars at the bottom indicating nucleotide substitutions per site.

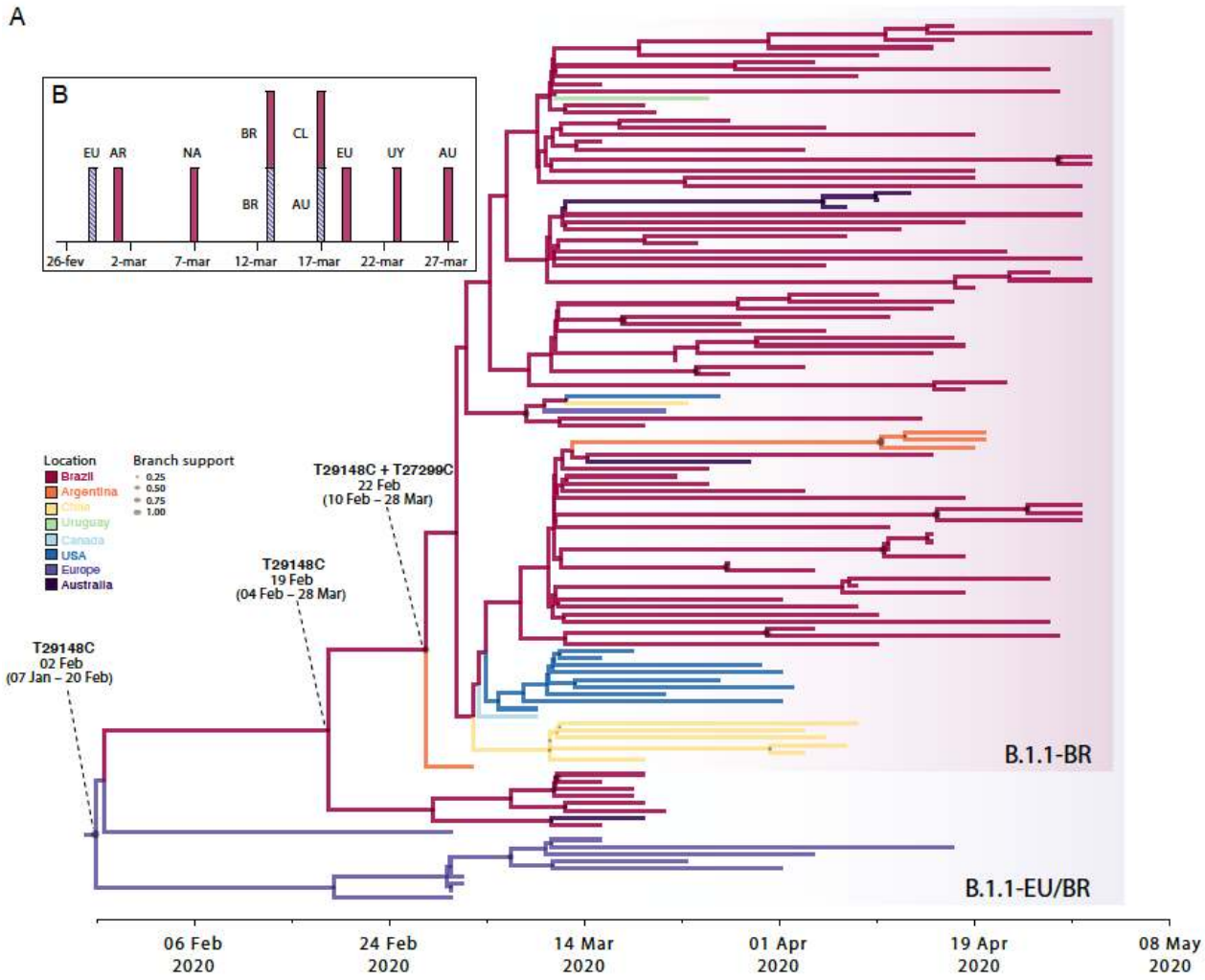


Figure 3. Spatiotemporal dissemination of the SARS-CoV-2 clades B.1.1.EU/BR and B.1.1.BR. A) Time-scaled Bayesian phylogeographic MCC tree of the major B.1.1 lineages circulating in Brazil. Branches are colored according to the most probable location state of their descendent nodes as indicated at the legend. Circles size at internal nodes is proportional to the corresponding posterior probability support as indicated at the legend. The inferred T_{MRCA} (based on the median of the posterior heights) and nucleotide substitutions fixed at ancestral key nodes are shown. Shaded boxes highlight the position of the clades B.1.1.EU/BR and B.1.1.BR. The tree is automatically rooted under the assumption of a strict molecular clock and all horizontal branch lengths are drawn to a scale of years. B) Timeline of the earliest detection of clades B.1.1.EU/BR (blue bars) and B.1.1.BR (red bars) in Europe (EU), North America (NA), Australia (AU), Argentina (AR), Brazil (BR), Chile (CL) and Uruguay (UY).

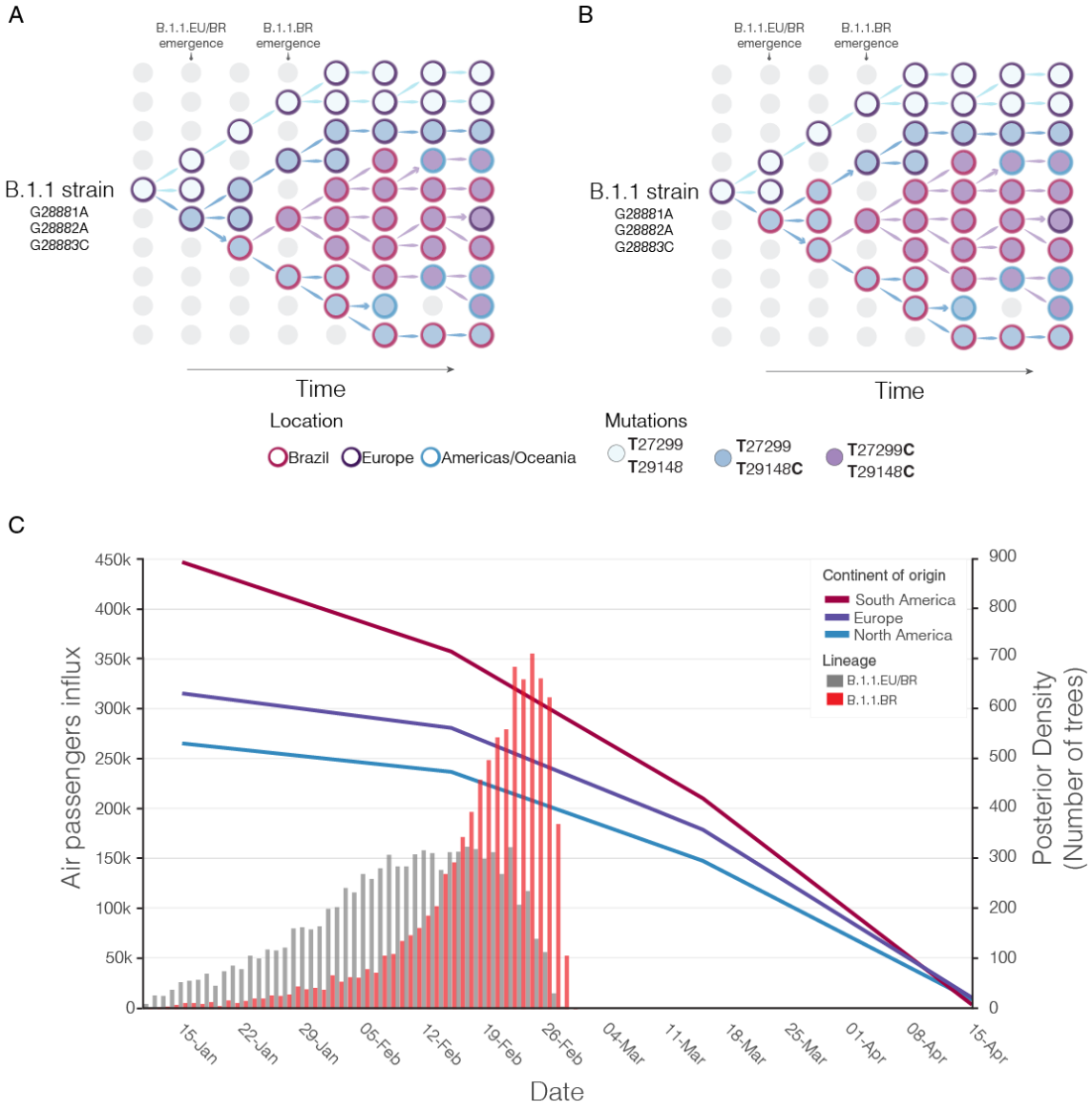


Figure 4. **Putative origin and transmission history of the SARS-CoV-2 clades B.1.1.EU/BR and B.1.1.BR.** A) Diagrams showing two alternative scenarios for the origin and dissemination of clades B.1.1.EU/BR and B.1.1.BR. The left panel depicts the hypothetical scenario where a B.1.1.EU/BR strain carrying the mutation T29148C was introduced into Brazil from Europe and after a period of local transmission in Brazil arose the B.1.1.BR variant carrying the mutation T27299C, which dispersed all over the country and from Brazil to other countries in the Americas and Oceania. The right panel depicts the hypothetical scenario where a B.1.1 strain was introduced from Europe to Brazil and mutations T29148C and T27299C arose at sequential steps during local transmission. According to this second scenario, Brazil was the epicenter of dissemination of both clades B.1.1.EU/BR and B.1.1.BR to other countries in Europe, the Americas and Oceania. B) Graphic showing the monthly number of international air passengers from South America, North America and Europe that arrived in Brazil during 2020 (available at: https://www.anac.gov.br)

(left hand axis) along with probability density of T_{MRCA} estimates for clades B.1.1.EU/BR (gray) and B.1.1.BR (red).

References

1 Zhu, N. *et al.* A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* **382**, 727-733, doi:10.1056/NEJMoa2001017 (2020).

2 Coronaviridae Study Group of the International Committee on Taxonomy of, V. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol* **5**, 536-544, doi:10.1038/s41564-020-0695-z (2020).

3 Dong, E., Du, H. & Gardner, L. An interactive web-based dashboard to track COVID-19 in real time. *Lancet Infect Dis* **20**, 533-534, doi:10.1016/S1473-3099(20)30120-1 (2020).

4 Brazilian Ministry of Health. Brasil confirma primeiro caso da doença – COVID-19. (2020).

5 Brazilian Ministry of Health, S. V. S. Doença pelo Coronavirus 2019. **06** (2020). <https://portalarquivos.saude.gov.br/images/pdf/2020/April/03/BE6-Boletim-Especial-do-COE.pdf>.

6 Hadfield, J. *et al.* Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics* **34**, 4121-4123, doi:10.1093/bioinformatics/bty407 (2018).

7 Rambaut, A. *et al.* A dynamic nomenclature proposal for SARS-CoV-2 to assist genomic epidemiology. *bioRxiv*, doi:10.1101/2020.04.17.046086 (2020).

8 Shu, Y. & McCauley, J. GISAID: Global initiative on sharing all influenza data - from vision to reality. *Euro Surveill* **22**, doi:10.2807/1560-7917.ES.2017.22.13.30494 (2017).

9 Rockett, R. J. *et al.* Revealing COVID-19 Transmission by SARS-CoV-2 Genome Sequencing and Agent Based Modelling. *bioRxiv*, doi:10.1101/2020.04.19.048751 (2020).

10 Seemann, T. *et al.* Tracking the COVID-19 pandemic in Australia using genomics. *medRxiv*, doi:10.1101/2020.05.12.20099929 (2020).

11 Dellicour, S. *et al.* A phylodynamic workflow to rapidly gain insights into the dispersal history and dynamics of SARS-CoV-2 lineages. *bioRxiv*, doi:10.1101/2020.05.05.078758 (2020).

12 Bluhm, A. *et al.* SARS-CoV-2 Transmission Chains from Genetic Data: A Danish Case Study. *bioRxiv*, doi:10.1101/2020.05.29.123612 (2020).

13 Gámbaro, F. *et al.* Introductions and early spread of SARS-CoV-2 in France. *bioRxiv*, doi:10.1101/2020.04.24.059576 (2020).

14 Gudbjartsson, D. F. *et al.* Spread of SARS-CoV-2 in the Icelandic Population. *N Engl J Med*, doi:10.1056/NEJMoa2006100 (2020).

15 Miller, D. *et al.* Full genome viral sequences inform patterns of SARS-CoV-2 spread into and within Israel. *medRxiv*, doi:10.1101/2020.05.21.20104521 (2020).

16 Oude Munnink, B. B. *et al.* Rapid SARS-CoV-2 whole genome sequencing for informed public health decision making in the Netherlands. *bioRxiv*, doi:10.1101/2020.04.21.050633 (2020).

17 Díez-Fuertes, F. *et al.* Phylodynamics of SARS-CoV-2 transmission in Spain. *bioRxiv*, doi:10.1101/2020.04.20.050039 (2020).

18 Worobey, M. *et al.* The emergence of SARS-CoV-2 in Europe and the US. *bioRxiv*, doi:10.1101/2020.05.21.109322 (2020).

19 Gonzalez-Reiche, A. S. *et al.* Introductions and early spread of SARS-CoV-2 in the New York City area. *Science*, doi:10.1126/science.abc1917 (2020).

20 Deng, X. *et al.* Genomic surveillance reveals multiple introductions of SARS-CoV-2 into Northern California. *Science*, doi:10.1126/science.abb9263 (2020).

21 Xavier, J. *et al.* The ongoing COVID-19 epidemic in Minas Gerais, Brazil: insights from epidemiological data and SARS-CoV-2 whole genome sequencing. *medRxiv*, doi:10.1101/2020.05.05.20091611 (2020).

22 Jesus, J. G. *et al.* Importation and early local transmission of COVID-19 in Brazil, 2020. *Rev Inst Med Trop Sao Paulo* **62**, e30, doi:10.1590/s1678-9946202062030 (2020).

23 Nascimento, A. A., Corado, A. L. G., Nascimento, F. O., Costa, A. K. A. , Duarte, D. C. G., Jesus, M. S., Luz, S. L. B., Gonçalves, L. M. F., Costa, C. F., Delatorre, E., Naveca, F. G. Genomic and phylogenetic characterization of an imported case of SARS-CoV-2 in Amazonas State, Brazil. *Mem Inst Oswaldo Cruz* **Fast track**, doi:10.1590/0074-02760200310 (2020).

24 Corman, V. M. *et al.* Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill* **25**, doi:10.2807/1560-7917.ES.2020.25.3.2000045 (2020).

25 Resende, P. C. *et al.* SARS-CoV-2 genomes recovered by long amplicon tiling multiplex approach using nanopore sequencing and applicable to other sequencing platforms. *bioRxiv*, doi:10.1101/2020.04.30.069039 (2020).

26 Charif D., L. J. R. SeqinR 1.0-2: A Contributed Package to the R Project for Statistical Computing Devoted to Biological Sequences Retrieval and Analysis. *Structural Approaches to Sequence Evolution. Biological and Medical Physics, Biomedical Engineering. Springer, Berlin, Heidelberg* **In: Bastolla U., Porto M., Roman H.E., Vendruscolo M. (eds)**, doi:10.1007/978-3-540-35306-5_10 (2007).

27 Li, W. & Godzik, A. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* **22**, 1658-1659, doi:10.1093/bioinformatics/btl158 (2006).

28 Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* **30**, 772-780, doi:10.1093/molbev/mst010 (2013).

29 Nguyen, L. T., Schmidt, H. A., von Haeseler, A. & Minh, B. Q. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* **32**, 268-274, doi:10.1093/molbev/msu300 (2015).

30 Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A. & Jermiin, L. S. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods* **14**, 587-589, doi:10.1038/nmeth.4285 (2017).

31 Rambaut, A., Lam, T. T., Max Carvalho, L. & Pybus, O. G. Exploring the temporal structure of heterochronous sequences using TempEst (formerly Path-O-Gen). *Virus Evol* **2**, vew007, doi:10.1093/ve/vew007 (2016).

32 Parker, J., Rambaut, A. & Pybus, O. G. Correlating viral phenotypes with phylogeny: accounting for phylogenetic uncertainty. *Infect Genet Evol* **8**, 239-246, doi:10.1016/j.meegid.2007.08.001 (2008).

33 Suchard, M. A. *et al.* Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evol* **4**, vey016, doi:10.1093/ve/vey016 (2018).

34 Ayres, D. L. *et al.* BEAGLE 3: Improved Performance, Scaling, and Usability for a High-Performance Computing Library for Statistical Phylogenetics. *Syst Biol* **68**, 1052-1061, doi:10.1093/sysbio/syz020 (2019).

35 Drummond, A. J., Rambaut, A., Shapiro, B. & Pybus, O. G. Bayesian coalescent inference of past population dynamics from molecular sequences. *Mol Biol Evol* **22**, 1185-1192, doi:10.1093/molbev/msi103 (2005).

36 Lemey, P., Rambaut, A., Drummond, A. J. & Suchard, M. A. Bayesian phylogeography finds its roots. *PLoS Comput Biol* **5**, e1000520, doi:10.1371/journal.pcbi.1000520 (2009).

37 Ferreira, M. A. R. & Suchard, M. A. Bayesian analysis of elapsed times in continuous-time Markov chains. *Canadian Journal of Statistics* **36**, 355-368, doi:10.1002/cjs.5550360302 (2008).

38 Rambaut, A., Drummond, A. J., Xie, D., Baele, G. & Suchard, M. A. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Syst Biol* **67**, 901-904, doi:10.1093/sysbio/syy032 (2018).

39 Mavian, C., Marini, S., Prosperi, M. & Salemi, M. A Snapshot of SARS-CoV-2 Genome Availability up to April 2020 and its Implications: Data Analysis. *JMIR Public Health Surveill* **6**, e19170, doi:10.2196/19170 (2020).

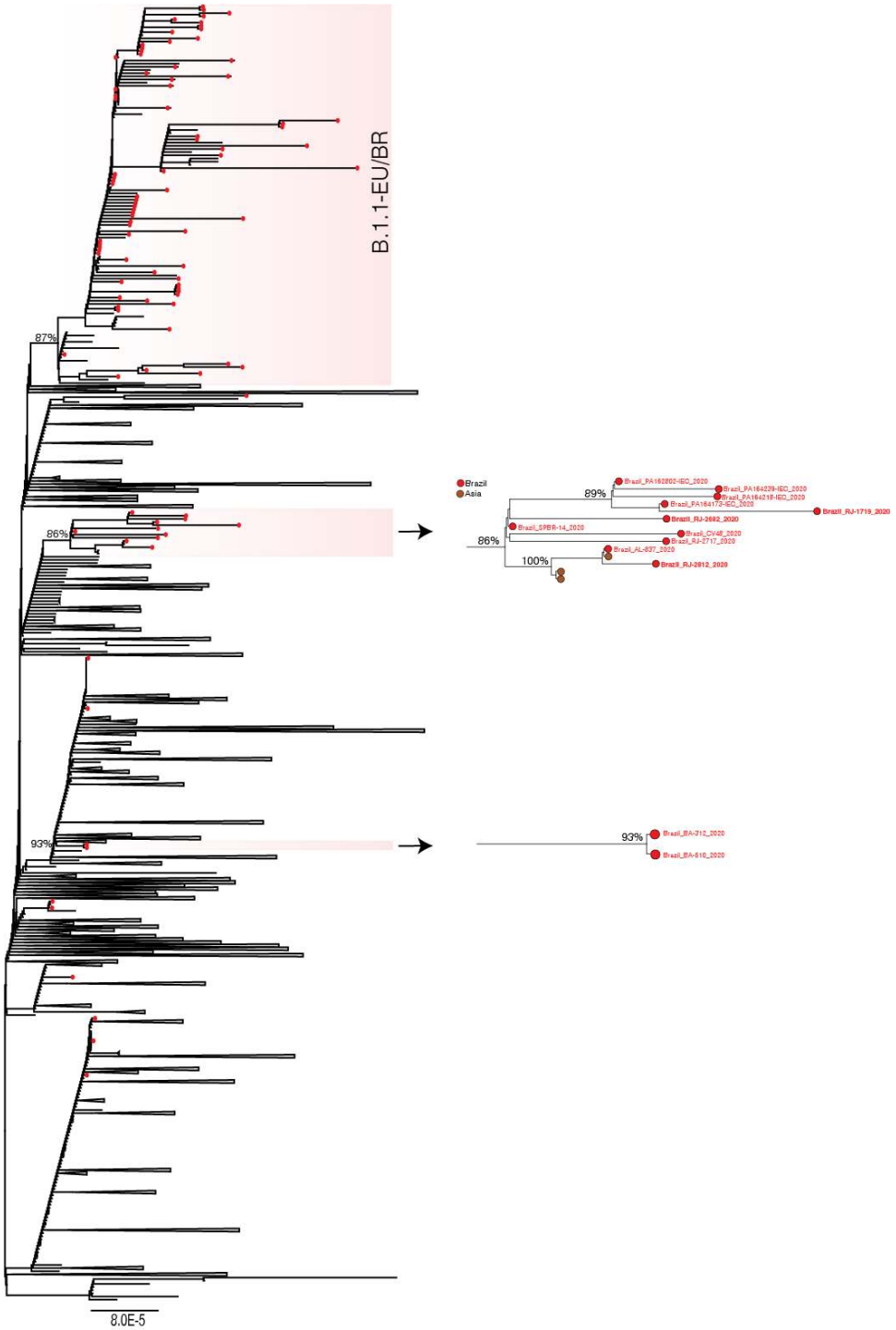
40 Villabona-Arenas, C. J., Hanage, W. P. & Tully, D. C. Phylogenetic interpretation during outbreaks requires caution. *Nat Microbiol*, doi:10.1038/s41564-020-0738-5 (2020).

Acknowledgements

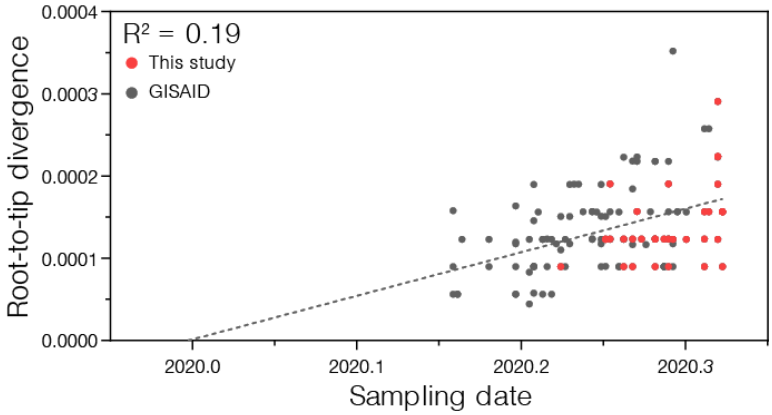
The authors wish to thank all the health care workers and scientists, who have worked hard to deal with this pandemic threat, the GISAID team and all the submitters of the database. GISAID acknowledgment tables containing sequences used in this study are in **Supplementary Tables 5** (South American SARS-CoV-2 genomes) and **Supplementary Table 6** (Global SARS-CoV 2 genomes B.1.1 lineage). Locally, we acknowledge the Respiratory Viruses Genomic Surveillance Network of the General Laboratory Coordination (CGLab) of the Brazilian Ministry of Health (MoH), Brazilian Central Laboratory States (LACENs), and local surveillance teams for the partnership in the viral surveillance in Brazil.

Funding support: CGLab/MoH (General Laboratories Coordination of Brazilian Ministry of Health) and CVSLR/FIOCRUZ (Coordination of Health Surveillance and Reference Laboratories of Oswaldo Cruz Foundation).

Supplementary Material



Supplementary Figure 1. Phylogenetic relationships of SARS-CoV-2 B.1.1 Brazilian and global strains. ML phylogenetic tree of 87 B.1.1 Brazilian genomes obtained in this survey (red circles) along with 3,764 B.1.1 worldwide reference sequences from GISAID database. Shaded box highlights the position of major Brazilian clusters B.1.1.EU/BR and B.1.1.BR, and a close view of each cluster is showed. Names of Brazilian SARS-CoV-2 genomes generated in this study (red tips) are shown. Only node supports (SH-*aLRT*) above 70% are shown. Tree was rooted on midpoint and branch lengths are drawn to scale with the bars at the bottom indicating nucleotide substitutions per site.



Supplementary Figure 2. Linear regression analysis between the sampling date of each viral sequence and the root-to-tip divergence (genetic distance of that sequence to the tree root) of a ML phylogeny of the SARS-CoV-2 clades B.1.1.EU/BR and B.1.1.BR. SARS-CoV-2 genomes generated in this study (red) were combined with other genomes available on the GISAID database (gray).

Supplementary Table 1. Clinical and epidemiological data associated with SARS-CoV-2 genomes obtained in this study.

Virus name	Accession number GISAID	Clinical Specimen	Pangolin lineage*	Town	Collection date	Onset symptoms	Travel history	Gender	Age	Clinical outcome
hCoV-19/Brazil/ES-225/2020	EPI_ISL_415128	NPS	B.2.1	Vila Velha	29/02/2020	22/02/2020	Italy	F	37y	inpatient
hCoV-19/Brazil/BA-312/2020	EPI_ISL_415105	NPS	B.1.1	Feira de Santana	04/03/2020	26/02/2020	Italy	F	34y	inpatient
hCoV-19/Brazil/RJ-314/2020	EPI_ISL_414045	NPS	B.1	Rio de Janeiro	04/03/2020	02/03/2020	Italy	F	52y	outpatient
hCoV-19/Brazil/RJ-352/2020	EPI_ISL_427299	NPS	A.2	Niteroi	05/03/2020	unknown	unknown	M	27y	unknown
hCoV-19/Brazil/RJ-477/2020	EPI_ISL_427300	NPS	B.1.1	Rio de Janeiro	11/03/2020	10/03/2020	Europe	M	48y	outpatient
hCoV-19/Brazil/RJ-477i/2020	EPI_ISL_427301	Vero E6	B.1.1	Rio de Janeiro	11/03/2020	10/03/2020	Europe	M	48y	outpatient
hCoV-19/Brazil/BA-510/2020	EPI_ISL_427293	NPS	B.1.1	Feira de Santana	06/03/2020	03/03/2020	unknown	F	41y	unknown
hCoV-19/Brazil/DF-615i/2020	EPI_ISL_427294	Vero E6	B.1.1.BR	Brasilia	13/03/2020	unknown	unknown	M	unknown	unknown
hCoV-19/Brazil/DF-619i/2020	EPI_ISL_427295	Vero E6	B.1.1.BR	Brasilia	13/03/2020	unknown	unknown	M	unknown	unknown
hCoV-19/Brazil/RJ-763/2020	EPI_ISL_427302	NPS	B.1.1.BR	Petropolis	20/03/2020	19/03/2020	no	F	47y	outpatient
hCoV-19/Brazil/SC-766/2020	EPI_ISL_427305	NPS	B.6	Joinvile	10/03/2020	08/03/2020	Europe and Africa	M	57y	unknown
hCoV-19/Brazil/SC-769/2020	EPI_ISL_427306	NPS	B.1.1	Florianopolis	10/03/2020	04/02/2020	Europe	F	59y	outpatient
hCoV-19/Brazil/RJ-818/2020	EPI_ISL_427303	NPS	B.1.1.BR	Rio de Janeiro	25/03/2020	22/03/2020	no	F	54y	outpatient
hCoV-19/Brazil/AL-837/2020	EPI_ISL_427292	NPS	B.1.1	Maceio	18/03/2020	15/03/2020	unknown	M	65y	unknown
hCoV-19/Brazil/DF-861/2020	EPI_ISL_427296	NPS	B.1.1.BR	Brasilia	23/03/2020	unknown	unknown	M	55y	unknown
hCoV-19/Brazil/DF-862/2020	EPI_ISL_427297	NPS	B.1.1.BR	Brasilia	23/03/2020	unknown	unknown	F	25y	unknown

hCoV-19/Brazil/RJ-872/2020	EPI_ISL_427304	NPS	B.1.1	Rio de Janeiro	26/03/2020	unknown	unknown	F	60y	unknown
hCoV-19/Brazil/DF-891/2020	EPI_ISL_427298	NPS	B.1.1.BR	Brasilia	22/03/2020	16/03/2020	unknown	F	61y	Deceased
hCoV-19/Brazil/RJ-1056/2020	EPI_ISL_456072	NPS	B.1.1.BR	Rio de Janeiro	01/04/2020	30/03/2020	no	F	31y	outpatient
hCoV-19/Brazil/RJ-1058/2020	EPI_ISL_456073	NPS	B.1.1.BR	Rio de Janeiro	01/04/2020	25/03/2020	no	M	27y	outpatient
hCoV-19/Brazil/RJ-1065/2020	EPI_ISL_456074	NPS	B.1.1.BR	Rio de Janeiro	01/04/2020	asymptomatic	no	F	85y	outpatient
hCoV-19/Brazil/RJ-1402/2020	EPI_ISL_456079	NPS	B.1.1.BR	Rio de Janeiro	03/04/2020	30/03/2020	no	F	55y	outpatient
hCoV-19/Brazil/RJ-1464/2020	EPI_ISL_456080	NPS	B.1.1.BR	Petropolis	06/04/2020	unknown	no	M	30y	outpatient
hCoV-19/Brazil/RJ-1466/2020	EPI_ISL_456081	NPS	B.1.1.BR	Rio de Janeiro	06/04/2020	03/04/2020	no	F	54y	outpatient
hCoV-19/Brazil/RJ-1701/2020	EPI_ISL_456086	NPS	B.1.1.BR	Rio de Janeiro	08/04/2020	05/04/2020	no	M	60y	outpatient
hCoV-19/Brazil/RJ-1702/2020	EPI_ISL_456087	NPS	B.1.1.BR	Rio de Janeiro	08/04/2020	04/04/2020	no	M	40y	outpatient
hCoV-19/Brazil/RJ-1902/2020	EPI_ISL_456090	NPS	B.1.1.BR	Rio de Janeiro	09/04/2020	06/04/2020	no	F	39y	outpatient
hCoV-19/Brazil/RJ-1921/2020	EPI_ISL_456091	NPS	B.1.1.BR	Rio de Janeiro	09/04/2020	06/04/2020	no	F	70y	outpatient
hCoV-19/Brazil/RJ-1923/2020	EPI_ISL_456092	NPS	B.1.1.BR	Rio de Janeiro	10/04/2020	08/04/2020	no	M	5m	unknown
hCoV-19/Brazil/RJ-1927/2020	EPI_ISL_456093	NPS	B.1.1.BR	Rio de Janeiro	12/04/2020	10/04/2020	no	M	46y	outpatient
hCoV-19/Brazil/RJ-1948/2020	EPI_ISL_456095	NPS	B.1.1.BR	Rio de Janeiro	13/04/2020	12/04/2020	no	F	45y	inpatient
hCoV-19/Brazil/RJ-1952/2020	EPI_ISL_456096	NPS	B.1.1.BR	Rio de Janeiro	13/04/2020	11/04/2020	no	M	29y	outpatient
hCoV-19/Brazil/RJ-2057/2020	EPI_ISL_456102	NPS	B.1.1.BR	Rio de Janeiro	16/04/2020	11/04/2020	no	M	29y	outpatient
hCoV-19/Brazil/RJ-899/2020	EPI_ISL_456071	NPS	B.1.1	Rio de Janeiro	30/03/2020	24/03/2020	no	M	42y	outpatient
hCoV-19/Brazil/RJ-1100/2020	EPI_ISL_456075	NPS	B.1.1.BR	Belford Roxo	02/04/2020	30/01/2020	unknown	M	30y	outpatient

hCoV-19/Brazil/RJ-1111/2020	EPI_ISL_456076	NPS	B.1.1.BR	Rio de Janeiro	25/03/2020	22/03/2020	unknown	M	59y	unknown
hCoV-19/Brazil/RJ-1119/2020	EPI_ISL_456077	NPS	B.1.1.BR	Rio de Janeiro	25/05/2020	20/03/2020	unknown	M	55y	unknown
hCoV-19/Brazil/RJ-1555/2020	EPI_ISL_467344	NPS	B.2.2	Petropolis	02/04/2020	27/03/2020	unknown	M	31y	unknown
hCoV-19/Brazil/RJ-1574/2020	EPI_ISL_467345	NPS	B.1.1.BR	Rio de Janeiro	02/04/2020	25/03/2020	unknown	M	82y	unknown
hCoV-19/Brazil/RJ-1595/2020	EPI_ISL_467346	NPS	B.2.2	Rio de Janeiro	02/04/2020	28/03/2020	unknown	F	83y	unknown
hCoV-19/Brazil/RJ-1600/2020	EPI_ISL_456082	NPS	B.1.1.BR	Teresopolis	02/04/2020	24/03/2020	unknown	M	68y	unknown
hCoV-19/Brazil/RJ-1627/2020	EPI_ISL_456083	NPS	B.1.1.BR	Nova Iguaçu	03/04/2020	30/03/2020	unknown	F	44y	unknown
hCoV-19/Brazil/RJ-1690/2020	EPI_ISL_456084	NPS	B.1.1.BR	Rio de Janeiro	08/04/2020	07/04/2020	unknown	F	42y	outpatient
hCoV-19/Brazil/RJ-1691/2020	EPI_ISL_456085	NPS	B.1.1.BR	Rio de Janeiro	08/04/2020	06/04/2020	unknown	M	42y	outpatient
hCoV-19/Brazil/RJ-1719/2020	EPI_ISL_456088	NPS	B.1.1	Itaborai	06/04/2020	05/04/2020	unknown	F	36y	unknown
hCoV-19/Brazil/RJ-1901/2020	EPI_ISL_456089	NPS	B.1.1.BR	Rio de Janeiro	09/04/2020	06/04/2020	unknown	F	59y	outpatient
hCoV-19/Brazil/RJ-1943/2020	EPI_ISL_456094	NPS	B.1.1.BR	Rio de Janeiro	13/04/2020	11/04/2020	unknown	F	38y	outpatient
hCoV-19/Brazil/RJ-1966/2020	EPI_ISL_456097	NPS	B.1.1.BR	Rio de Janeiro	13/04/2020	unknown	unknown	M	unknown	outpatient
hCoV-19/Brazil/RJ-2000/2020	EPI_ISL_456098	NPS	B.1.1.BR	Rio de Janeiro	13/04/2020	13/04/2020	unknown	F	29y	unknown
hCoV-19/Brazil/RJ-2007/2020	EPI_ISL_456099	NPS	B.1.1.BR	Rio de Janeiro	13/04/2020	10/04/2020	unknown	F	58y	unknown
hCoV-19/Brazil/RJ-2033/2020	EPI_ISL_456100	NPS	B.1.1.BR	Rio de Janeiro	15/04/2020	09/04/2020	unknown	F	37y	unknown
hCoV-19/Brazil/RJ-2044/2020	EPI_ISL_456101	NPS	B.1.1.BR	Duque de Caxias	15/04/2020	14/04/2020	unknown	F	28y	unknown
hCoV-19/Brazil/RJ-2062/2020	EPI_ISL_456103	NPS	B.1.1	Rio de Janeiro	16/04/2020	13/04/2020	unknown	F	49y	unknown
hCoV-19/Brazil/RJ-2072/2020	EPI_ISL_456104	NPS	B.1.1.BR	Rio de Janeiro	16/04/2020	12/04/2020	unknown	F	51y	unknown

hCoV-19/Brazil/RJ-2077/2020	EPI_ISL_456105	NPS	B.1.1.BR	Rio de Janeiro	16/04/2020	unknown	unknown	F	38y	unknown
hCoV-19/Brazil/RJ-2078/2020	EPI_ISL_456106	NPS	B.1.1.BR	Rio de Janeiro	16/04/2020	14/04/2020	unknown	F	34y	unknown
hCoV-19/Brazil/RJ-2091/2020	EPI_ISL_467347	NPS	B.1.1.BR	Rio de Janeiro	16/04/2020	12/04/2020	unknown	F	51y	unknown
hCoV-19/Brazil/RJ-2195/2020	EPI_ISL_467348	NPS	B.1.1.BR	Rio de Janeiro	17/04/2020	14/04/2020	unknown	M	45y	unknown
hCoV-19/Brazil/RJ-2197/2020	EPI_ISL_467349	NPS	B.1.1.BR	Rio de Janeiro	17/04/2020	14/04/2020	unknown	M	32y	unknown
hCoV-19/Brazil/RJ-2208/2020	EPI_ISL_467350	NPS	B.1.1.BR	Rio de Janeiro	17/04/2020	15/04/2020	unknown	M	43y	unknown
hCoV-19/Brazil/RJ-2233/2020	EPI_ISL_467351	NPS	B.1.1.BR	Rio de Janeiro	17/04/2020	13/04/2020	unknown	F	30y	unknown
hCoV-19/Brazil/RJ-2422/2020	EPI_ISL_467352	NPS	B.1.1.BR	Queimados	20/04/2020	18/04/2020	unknown	F	27y	inpatient
hCoV-19/Brazil/RJ-2669/2020	EPI_ISL_467353	NPS	B.1.1.BR	Niteroi	24/04/2020	21/04/2020	unknown	F	25y	outpatient
hCoV-19/Brazil/RJ-2676/2020	EPI_ISL_467354	NPS	B.1.5	Rio de Janeiro	24/04/2020	23/04/2020	unknown	M	48y	unknown
hCoV-19/Brazil/RJ-2678/2020	EPI_ISL_467355	NPS	B.1.1.BR	Rio de Janeiro	24/04/2020	23/04/2020	unknown	M	29y	unknown
hCoV-19/Brazil/RJ-2682/2020	EPI_ISL_467356	NPS	B.1.1	Rio de Janeiro	24/04/2020	22/04/2020	unknown	F	29y	unknown
hCoV-19/Brazil/RJ-2683/2020	EPI_ISL_467357	NPS	B.1.1.BR	Rio de Janeiro	24/04/2020	20/04/2020	unknown	F	31y	unknown
hCoV-19/Brazil/RJ-2696/2020	EPI_ISL_467358	NPS	B.1.1.BR	Rio de Janeiro	24/04/2020	19/04/2020	unknown	F	65y	unknown
hCoV-19/Brazil/RJ-2717/2020	EPI_ISL_467359	NPS	B.1.1	Rio de Janeiro	24/04/2020	21/04/2020	unknown	F	36y	unknown
hCoV-19/Brazil/RJ-2733/2020	EPI_ISL_467360	NPS	B.1.1.BR	Duque de Caxias	25/04/2020	22/04/2020	unknown	F	49y	unknown
hCoV-19/Brazil/RJ-2769/2020	EPI_ISL_467361	NPS	B.1.1.BR	Rio de Janeiro	27/04/2020	22/04/2020	unknown	F	30y	unknown
hCoV-19/Brazil/RJ-2770/2020	EPI_ISL_467362	NPS	B.1.1.BR	Rio de Janeiro	27/04/2020	22/04/2020	unknown	F	30y	unknown
hCoV-19/Brazil/RJ-2776/2020	EPI_ISL_467363	NPS	B.1.1.BR	Rio de Janeiro	27/04/2020	21/04/2020	unknown	M	64y	unknown

hCoV-19/Brazil/RJ-2777/2020	EPI_ISL_467364	NPS	B.1.1.BR	Nova Iguaçu	25/04/2020	25/04/2020	unknown	F	34y	unknown
hCoV-19/Brazil/RJ-2811/2020	EPI_ISL_467365	NPS	B.1.1.BR	Rio de Janeiro	27/04/2020	25/04/2020	unknown	M	44y	unknown
hCoV-19/Brazil/RJ-2812/2020	EPI_ISL_467366	NPS	B.1.1	Rio de Janeiro	27/04/2020	20/04/2020	unknown	M	38y	unknown
hCoV-19/Brazil/RJ-2822/2020	EPI_ISL_467367	NPS	B.1.1.BR	Niteroi	27/04/2020	23/04/2020	unknown	M	62y	inpatient
hCoV-19/Brazil/RJ-2840/2020	EPI_ISL_467368	NPS	B.1.1.BR	Rio de Janeiro	28/04/2020	25/04/2020	unknown	M	36y	unknown
hCoV-19/Brazil/RJ-2844/2020	EPI_ISL_467369	NPS	B.1.1.BR	Rio de Janeiro	28/04/2020	24/04/2020	unknown	F	29y	outpatient
hCoV-19/Brazil/RJ-2847/2020	EPI_ISL_467370	NPS	B.1.1.BR	Rio de Janeiro	28/04/2020	22/04/2020	unknown	F	35y	unknown
hCoV-19/Brazil/RJ-2868/2020	EPI_ISL_467371	NPS	B.1.1.BR	Rio de Janeiro	28/04/2020	25/04/2020	unknown	F	49y	unknown
hCoV-19/Brazil/AP-161167-IEC/2020	EPI_ISL_450873	Sputum	B.1.1.BR	Macapa	17/03/2020	12/03/2020	No	F	36y	unknown
hCoV-19/Brazil/PA-161548-IEC/2020	EPI_ISL_450874	NPS	B.1.1.BR	Maraba	20/03/2020	16/03/2020	Sao Paulo	F	28y	unknown
hCoV-19/Brazil/AP-162741-IEC/2020	EPI_ISL_458138	Sputum	B.1.1.BR	Macapa	03/04/2020	02/04/2020	unknown	F	37y	unknown
hCoV-19/Brazil/AC-162535-IEC/2020	EPI_ISL_458139	NPS	B.1.1.BR	Rio Branco	18/03/2020	15/03/2020	No	M	81y	unknown
hCoV-19/Brazil/PA-162802-IEC/2020	EPI_ISL_458140	NPS	B.1.1	Belem	07/04/2020	06/04/2020	No	M	63y	inpatient
hCoV-19/Brazil/PA-164239-IEC/2020	EPI_ISL_458141	NPS	B.1.1	Ananindeua	26/04/2020	24/04/2020	No	M	38y	outpatient
hCoV-19/Brazil/AP-162966-IEC/2020	EPI_ISL_458142	NPS	B.1.1.BR	Macapa	05/04/2020	05/04/2020	No	F	37y	unknown
hCoV-19/Brazil/AP-164082-IEC/2020	EPI_ISL_458143	Sputum	B.1.1.BR	Macapa	15/04/2020	13/04/2020	unknown	M	44y	unknown
hCoV-19/Brazil/AP-163972-IEC/2020	EPI_ISL_458144	Sputum	B.1.1.BR	Macapa	15/04/2020	12/04/2020	unknown	M	44y	unknown
hCoV-19/Brazil/AP-164346-IEC/2020	EPI_ISL_458145	Sputum	B.1.1.BR	Macapa	20/04/2020	28/04/2020	unknown	F	62y	unknown

hCoV-19/Brazil/PA-164173-IEC/2020	EPI_ISL_458146	NPS	B.1.1	Ananindeua	23/04/2020	20/04/2020	unknown	F	38y	unknown
hCoV-19/Brazil/PA-164218-IEC/2020	EPI_ISL_458147	NPS	B.1.1	Belem	24/04/2020	unknown	unknown	M	45y	unknown
hCoV-19/Brazil/PA-164684-IEC/2020	EPI_ISL_458148	NPS	B.1.1.BR	Belem	27/04/2020	26/04/2020	no	M	38y	unknown
hCoV-19/Brazil/MA-163069-IEC/2020	EPI_ISL_458149	NPS	B.1.1.BR	Sao Luis	06/04/2020	unknown	unknown	F	40y	unknown

NPS, Nasopharyngeal swab

Supplementary Table 2. Prevalence of SARS-CoV-2 lineages B.1.1.EU/BR and B.1.1.BR across countries.

Region	Country	Total SARS-Cov-2	Lineage B.1.1.EU/BR	Lineage B.1.1.BR
South America	Brazil	170	10 (6%)	74 (44%)
	Argentina	29	-	4 (14%)
	Chile	153	-	7 (5%)
	Uruguay	45	-	1 (2%)
North America	Canada	227	-	1 (<1%)
	US	7,605	-	10 (<1%)
Oceania	Australia	1,899	1 (<1%)	4 (<1%)
Europe	United Kingdom	18,391	4 (<1%)	1 (<1%)
	Switzerland	325	4 (1%)	-
	Netherlands	840	2 (<1%)	-

Supplementary Table 3. Prevalence of SARS-CoV-2 lineages B.1.1.EU/BR and B.1.1.BR across Brazilian states.

State	Total SARS-Cov-2	Lineage B.1.1.EU/BR	Lineage B.1.1.BR
Rio de Janeiro	77	-	59 (76%)
Minas Gerais	45	9 (20%)*	-
Sao Paulo	18	-	-
Distrito Federal	7	1 (14%)	5 (71%)
Amapa	6	-	6 (100%)
Para	6	-	2 (33%)
Others	11	-	2 (18%)
Total	**170**	**10 (6%)**	**74 (44%)**

* Sequences CV34 and CV45 harbor the substitution T29148C, but displayed an ambiguous nucleotide at position 27299, hence assigned to lineage B.1.1.EU/BR.

Supplementary Table 4. Phylogeny-trait association tests to assess phylogeographic structure of the dataset

Metric	Data			Null hypothesis			*p* value
	mean	lower 95% CI	upper 95% CI	mean	lower 95% CI	upper 95% CI	
AI	4.15	3.13	5.15	6.79	6.28	7.29	**0.000**
PS	30.74	29.00	32.00	37.99	37.07	38.47	**0.000**
MC (Argentina)	2.98	3.00	3.00	1.03	1.00	1.07	**0.001**
MC (Australia)	1.06	1.00	2.00	1.05	1.00	1.14	1.000
MC (Brazil)	9.41	6.00	15.00	6.18	5.34	7.10	**0.007**
MC (Canada)	1.00	1.00	1.00	1.00	1.00	1.00	1.000
MC (Chile)	1.13	1.00	2.00	1.11	1.02	1.28	1.000
MC (Europe)	2.88	2.00	4.00	1.26	1.09	1.82	**0.001**
MC (USA)	1.25	1.00	2.00	1.22	1.06	1.70	1.000
MC (Uruguay)	1.00	1.00	1.00	1.00	1.00	1.00	1.000

AI - Association index; PS - Parsimony score; MC - Monophyletic clade

Supplementary Table 5. GISAID acknowledgment table of South America SARS-CoV-2 genomes.

Supplementary Table 6. GISAID acknowledgment table of Global SARS-CoV-2 genomes B.1.1 lineage.