

1 Genomic surveillance of SARS-CoV-2 reveals community transmission of a major lineage

2 during the early pandemic phase in Brazil

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42 **Abstract**

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

55

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

58

59 **Introduction**

60 COVID-19, the disease caused by Severe Acute Respiratory Syndrome Coronavirus-2
61 (SARS-CoV-2), is leading to high rates of acute respiratory syndrome, hospitalization, and death
62 ^{1,2}. Brazil, the second most hit country in the world so far, has reported 923.189 cases and 45.241
63 deaths (last update 17th June 2020) ³. The first positive case of SARS-CoV-2 infection in Brazil
64 was reported on 26th February 2020 in an individual traveling from Europe to Sao Paulo
65 metropolitan region ⁴, and during the following two weeks, the virus was detected in all country
66 regions ⁵

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

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

89 To investigate the SARS-CoV-2 strains circulating in Brazil, we recovered 95 whole-
90 genomes collected from 10 different Brazilian states during the first two months of the COVID-
91 19 epidemic. New SARS-CoV-2 Brazilian viral sequences were combined with other Brazilian
92 and global reference sequences available in GISAID and subjected to maximum likelihood (ML)
93 and Bayesian coalescent analyses.

94 Methods

95 *Sampling and ethical aspects*

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

99 up to processing. This study was approved by the FIOCRUZ-IOC Ethics Committee
100 (68118417.6.0000.5248) and the Brazilian Ministry of Health SISGEN (A1767C3).

101 *Nucleic acid isolation and RT-qPCR*

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

114 *SARS-CoV-2 whole-genome amplification and sequencing*

115 Total RNA from positive samples presenting Ct values up to 30,0 for gene E was reverse
116 transcribed using SuperScript™ IV First Strand Synthesis System (Invitrogen). Two multiplex
117 PCR reactions using the primer scheme previously described²⁵ (Pool A = nine amplicons and Pool
118 B = eight amplicons), were performed using the Q5® High-Fidelity DNA Polymerase (NEB).
119 Amplicons were purified using Agencourt AMPure XP beads (Beckman Coulter™) and the DNA
120 quantified with Qubit 4 Fluorometer (Invitrogen) using the Qubit dsDNA HS Assay Kit
121 (Invitrogen) and sequenced using Illumina MiSeq or NextSeq (San Diego, CA, USA) and
122 Nanopore (Oxford, UK) platforms. Illumina short reads DNA libraries were generated from the
123 pooled amplicons using Nextera XT DNA Sample Preparation Kit (Illumina, San Diego, CA,
124 USA) according to the manufacturer specifications. The size distribution of these libraries was
125 evaluated using a 2100 Bioanalyzer (Agilent, Santa Clara, USA) and the samples were pair-end
126 sequenced (Micro V2, 300 cycles) on a MiSeq equipment (Illumina, San Diego, USA) in around
127 18 hours. The Nanopore library protocol is optimized for long reads (2 kb amplicons)²⁵. Library
128 preparation was conducted using Ligation Sequencing 1D (SQK-LSK109 Oxford Nanopore
129 Technologies (ONT) and Native Barcoding kit 1 to 24 (ONT), according to the manufacturer's

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

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

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

151 *SARS-CoV-2 genotyping*

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

157 *Maximum Likelihood phylogenetic analyses*

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

160 genomes (> 10% of ambiguous positions), we obtained a final dataset of 7,674 sequences. Because
161 most sequences recovered (75%) were from the United Kingdom (UK), we generate a “non-
162 redundant” global balanced dataset by removing very closely related sequences (genetic similarity
163 > 99.99%) from the UK. To achieve this aim, sequences from the UK were grouped by similarity
164 with the CD-HIT program ²⁷ and one sequence per cluster was selected. With this sampling
165 procedure, we obtained a balanced global reference B.1.1 dataset containing 3,764 sequences that
166 were aligned with the new B.1.1 Brazilian sequences generated in this study using MAFFT v7.467
167 ²⁸ and then subjected to maximum-likelihood (ML) phylogenetic analyses. The ML phylogenetic
168 tree was inferred using IQTREE v1.6.12 ²⁹, under the GTR+F+I+G4 nucleotide substitution model
169 as selected by the ModelFinder application ³⁰ and the branch support was assessed by the
170 approximate likelihood-ratio test based on a Shimodaira–Hasegawa-like procedure (SH-aLRT)
171 with 1,000 replicates. The ML tree was visualized using the FigTree v1.4
172 (<http://tree.bio.ed.ac.uk/software/figtree/>).

173 *Analysis of temporal signal and phylogeographic structure*

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

184 *Bayesian phylogeographic analyses*

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

191 coalescent prior ³⁵. Viral migrations across time were reconstructed using a reversible discrete
192 phylogeographic model ³⁶ with a CTMC rate reference prior ³⁷. Two MCMC chains were run for
193 100 million generations and then combined to ensure stationarity and good mixing. Stationarity
194 (constant mean and variance of trace plots) and good mixing (Effective Sample Size >200) for all
195 parameter estimates were assessed using TRACER v1.7 ³⁸. The maximum clade credibility (MCC)
196 tree was summarized with TreeAnnotator v1.10 and visualized using the FigTree v1.4 program.

197 **Results**

198 In this study, we analyzed 95 viral whole-genomes (>99% coverage) obtained from
199 individuals with confirmed SARS-CoV-2 infection, who underwent testing and genomic
200 sequencing at the Laboratory of Respiratory Viruses and Measles, Oswaldo Cruz Institute (IOC)-
201 FIOCRUZ, in Rio de Janeiro, and the Evandro Chagas Institute, in Para, Brazil ²⁵. Samples were
202 collected between 29th February and 28th April 2020 from individuals that reside in 10 different
203 Brazilian states from the Southeastern (Rio de Janeiro and Espírito Santo), Central-Western
204 (Distrito Federal), Northern (Acre, Amapá and Para), Northeastern (Alagoas, Bahia and
205 Maranhão) and Southern (Santa Catarina) regions. (**Supplementary Table 1**). The median age of
206 patients with COVID-19 illness was 42-year-old (range 0 to 85 years) and 54 (57%) were female.
207 Seven individuals reported international travel or contact with travelling people. Six different
208 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
209 (**Supplementary Table 1**), according to the nomenclature proposed by Rambaut *et al* ⁷. Most
210 Brazilian SARS-CoV-2 sequences here obtained were classified as clade B.1 (95%, n = 90), and
211 particularly within the sub-clade B.1.1 (92%, n = 87) (**Fig. 1B**). The prevalence of the sub-clade
212 B.1.1 in our sample (92%) was much higher than that observed in other Brazilian sequences
213 available in GISAID (36%) (**Fig. 1C**). The clade B.1.1 was the only lineage detected in the 18
214 individuals with no history of recent international travel, while four different lineages (B.1, B.1.1,
215 B.2.1 and B.6) were detected among the six individuals with recent history of international travel
216 (imported cases) and their contacts. (**Supplementary Table 1**).

217 To investigate whether the observed high prevalence of the lineage B.1.1 in Brazil resulted
218 from one or multiple independent viral introductions into the country, we performed a ML
219 phylogenetic analysis of the 87 B.1.1 Brazilian sequences identified in this study, together with
220 3,764 SARS-CoV-2 complete genome sequences available in GISAID as of 4th June representing
221 the current global diversity of the B.1.1 clade. Brazilian isolates were distributed throughout the
222 phylogenetic tree, consistent with the hypothesis of multiple independent introductions (**Fig. 2**). A
223 significant proportion of Brazilian B.1.1 sequences (65%, n = 74/114), however, branched in a
224 monophyletic cluster (SH-aLRT = 74%) here designated as B.1.1.BR, that comprises sequences

225 from Brazil, other South American countries (Argentina, Chile and Uruguay), North America
226 (Canada and USA), Australia and England. The lineage B.1.1.BR is nested within a highly
227 supported (SH-*aLRT* = 87%) clade, here referred as B.1.1.EU/BR, containing basal sequences
228 from Western Europe and Brazil, (Fig. 2). We also detected two other well-supported (SH-*aLRT*
229 > 80%) monophyletic clades of small size ($n = 2-11$) mostly composed by Brazilian sequences
230 (**Supplementary Fig. 1**).

231 In addition to sharing the three nucleotide mutations (G28881A, G28882A, G28883C)
232 characteristic of the clade B.1.1, sequences of clusters B.1.1.EU/BR and B.1.1.BR harbor a non-
233 synonymous mutation T29148C at the Nucleocapsid protein (I292T); and another non-
234 synonymous mutation T27299C at the ORF6 (I33T) was shared only by sequences of the lineage
235 B.1.1.BR. Mutations T29148C or T27299C were not detected in the other 7,551 B.1.1 genomes
236 available in GISAID, supporting the hypothesis that they are synapomorphic traits of the
237 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
238 detected in different countries around the world, but the overall estimated prevalence of these
239 clades in Brazil (6% and 44%, respectively) is much higher than that estimated in Europe, North
240 America or Australia (**Supplementary Table 2**). Such difference could not be explained by
241 sampling bias as those regions comprise the most densely sampled countries worldwide and is
242 suggestive of local dissemination of those clades in Brazil. Consistent with this hypothesis, none
243 of the individuals infected with clade B.1.1.BR from our cohort or with clade B.1.1.EU/BR from
244 a previous cohort²¹ reported international travel. The clades B.1.1.EU/BR and B.1.1.BR were not
245 homogeneously distributed across Brazilian states (**Supplementary Table 3**). The clade
246 B.1.1.EU/BR was highly prevalent in Minas Gerais and also detected in the Federal District, while
247 the clade B.1.1.BR was predominant in Rio de Janeiro and also identified in some samples from
248 the Northern, Central-Western and Northeastern Brazilian regions. Notably, none of these lineages
249 were detected in the most populated state of Sao Paulo.

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

259 (*Posterior state probability [PSP] = 0.64*) at 2nd February (95% High Posterior Density [HPD]: 7th
260 January – 20th February) and its dissemination to Brazil at 19th February (95% HPD: 4th February
261 – 28th February) (**Fig. 3A**). The origin of the B.1.1.BR lineage was traced with high probability to
262 Brazil (*PSP = 0.95*) at 22th February (95% HPD: 10th February – 28th February). From Brazil, the
263 B.1.1.BR lineage probably disseminated to neighboring South American countries (Argentina,
264 Chile and Uruguay) and to more distant regions (Australia, USA and UK).

265 **Discussion**

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

274 Our phylogeographic reconstruction supports that clade B.1.1.EU/BR most probably arose
275 in Europe (*PSP = 0.64*) around 2nd February and was introduced into Brazil a couple of weeks
276 later, where it spread and rapidly fixed the T27299C mutation, originating the clade B.1.1.BR (**Fig.**
277 **4A**). This evolutionary pattern agrees with the earlier detection of the clade B.1.1.EU/BR in
278 Western Europe (28th February) than in Brazil (13th March) (**Fig. 3B**); but the extremely low
279 prevalence of the clade B.1.1.EU/BR in Europe (<1% of total SARS-CoV-2 sequences) makes this
280 transmission history a highly unlikely epidemiological scenario. Once our phylogeographic
281 analysis also estimated Brazil as a putative ancestral state at the root of clade B.1.1.EU/BR (*PSP*
282 = 0.35), an alternative hypothesis would be that a highly prevalent B.1.1 strain was introduced
283 from Western Europe into Brazil before 2nd February and that synapomorphic mutations T29148C
284 and T27299C were fixed at sequential steps during subsequent virus local spread (**Fig. 4B**). The
285 relative high prevalence of clade B.1.1.EU/BR in some Brazilian locations makes the
286 dissemination of this lineage from Brazil to Western Europe a quite plausible transmission
287 scenario. Retrospective analyses of Brazilian samples obtained from individuals with severe acute
288 respiratory disease during February might provide unique insights to resolve the origin of the clade
289 B.1.1.EU/BR.

290 Brazil was traced as the source location of the clade B.1.1.BR with high probability (*PSP*
291 = 0.95) in our phylogeographic reconstruction. The earliest B.1.1.BR sequence currently available,
292 however, is an Argentinean sequence sampled on 1st March 2020; while the earliest detection of

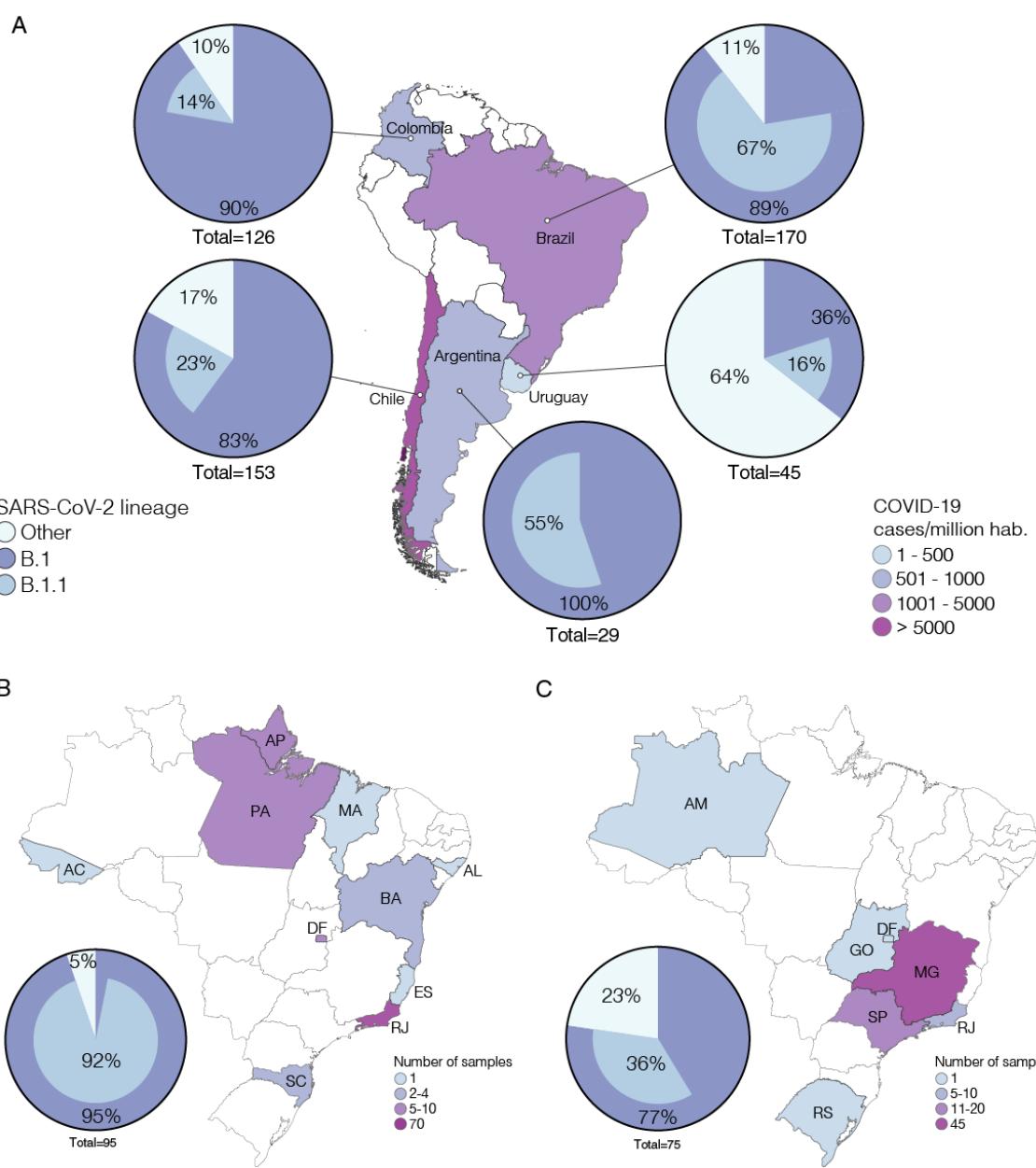
293 the clade B.1.1.BR in Brazil occurred in two samples isolated in the Distrito Federal on 13th March
294 2020 (**Fig. 3B**). Of note, none of the 30 Brazilian genomes analyzed between 25th February and
295 12th March, including 12 B.1.1 genomes from imported cases, belong to the clade B.1.1.BR. This
296 suggests that clade B.1.1.BR may have arisen in a Brazilian state that was not included in our
297 dataset and/or that this lineage circulated cryptically for several weeks before being detected in
298 symptomatic carriers. The nearly simultaneous detection of the clade B.1.1.BR in distant states
299 from the Central-Western (Federal District, 13th March), Northern (Amapa, 17th March) and
300 Southeastern (Rio de Janeiro, 20th March) Brazilian regions, supports the second hypothesis and
301 further suggests a wide geographic spread of this Brazilian lineage. Our results also suggest a high
302 geographic compartmentalization of SARS-CoV-2 genetic diversity within Brazil. Considering
303 the three most populated and densely sampled states of Brazil, the clade B.1.1.EU/BR was only
304 detected in Minas Gerais, the clade B.1.1.BR only in Rio the Janeiro, and none of those in Sao
305 Paulo.

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

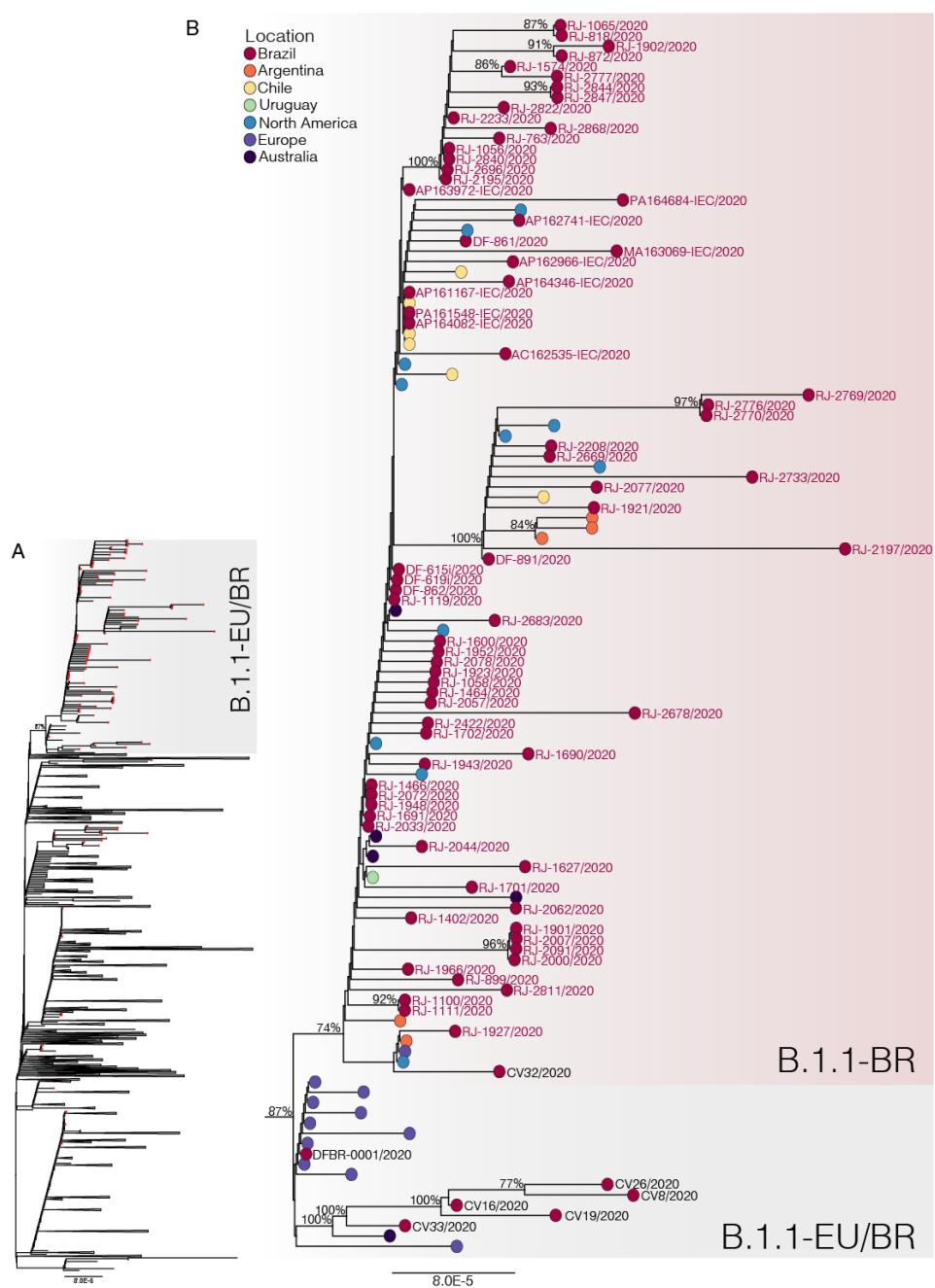
319 Our phylogeographic reconstruction suggests that the clade B.1.1.BR might have seeded
320 secondary outbreaks in Argentina, Chile, Australia and the US, but those findings should be
321 interpreted with caution because of the low support of local clusters. Although high-quality full
322 genomes of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

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

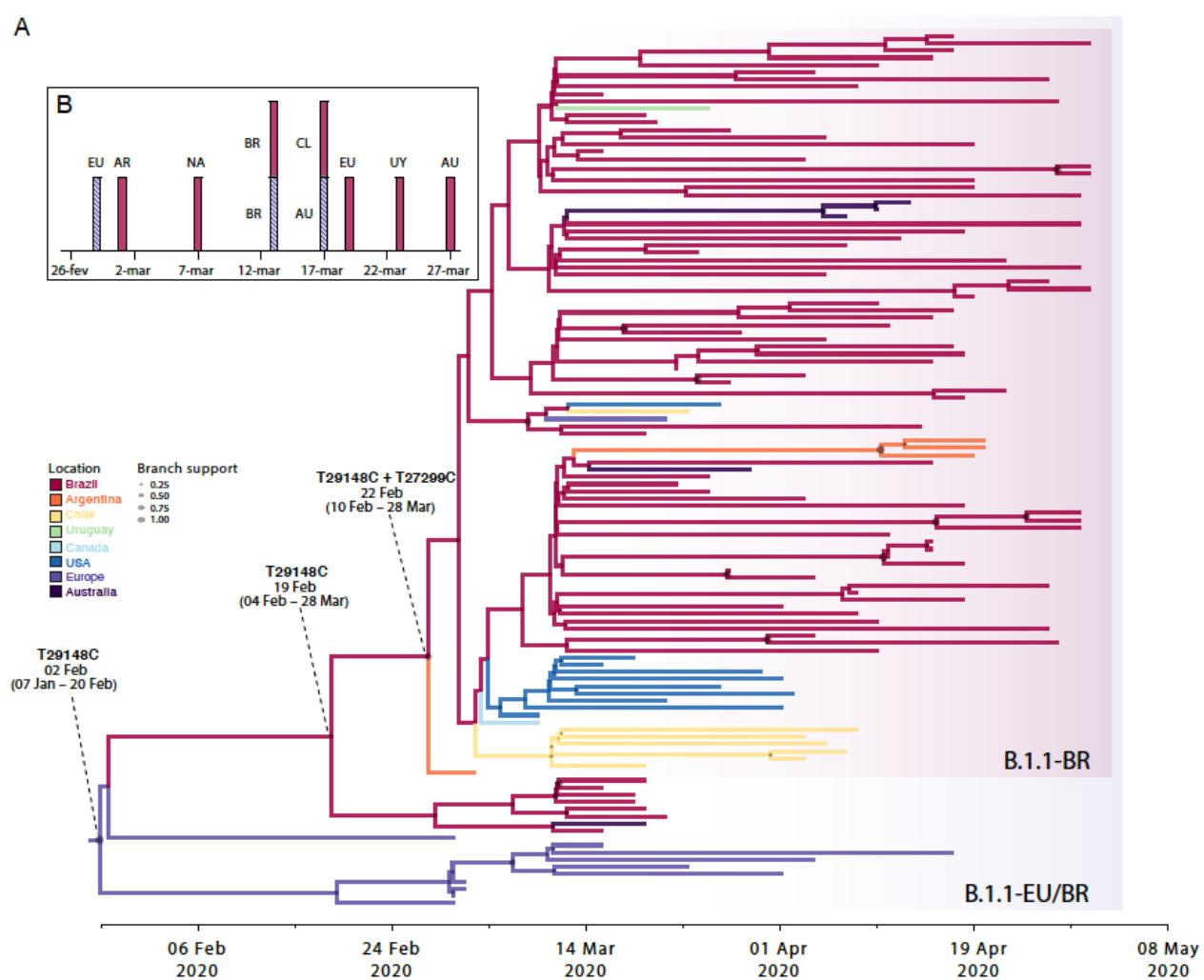
335 In summary, this study reveals the existence of a major SARS-CoV-2 B.1.1 lineage
336 associated with community transmission in Brazil and widespread in a national scale. This major
337 B.1.1 Brazilian lineage emerged in Brazil in February 2020, probably before the detection of the
338 first imported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



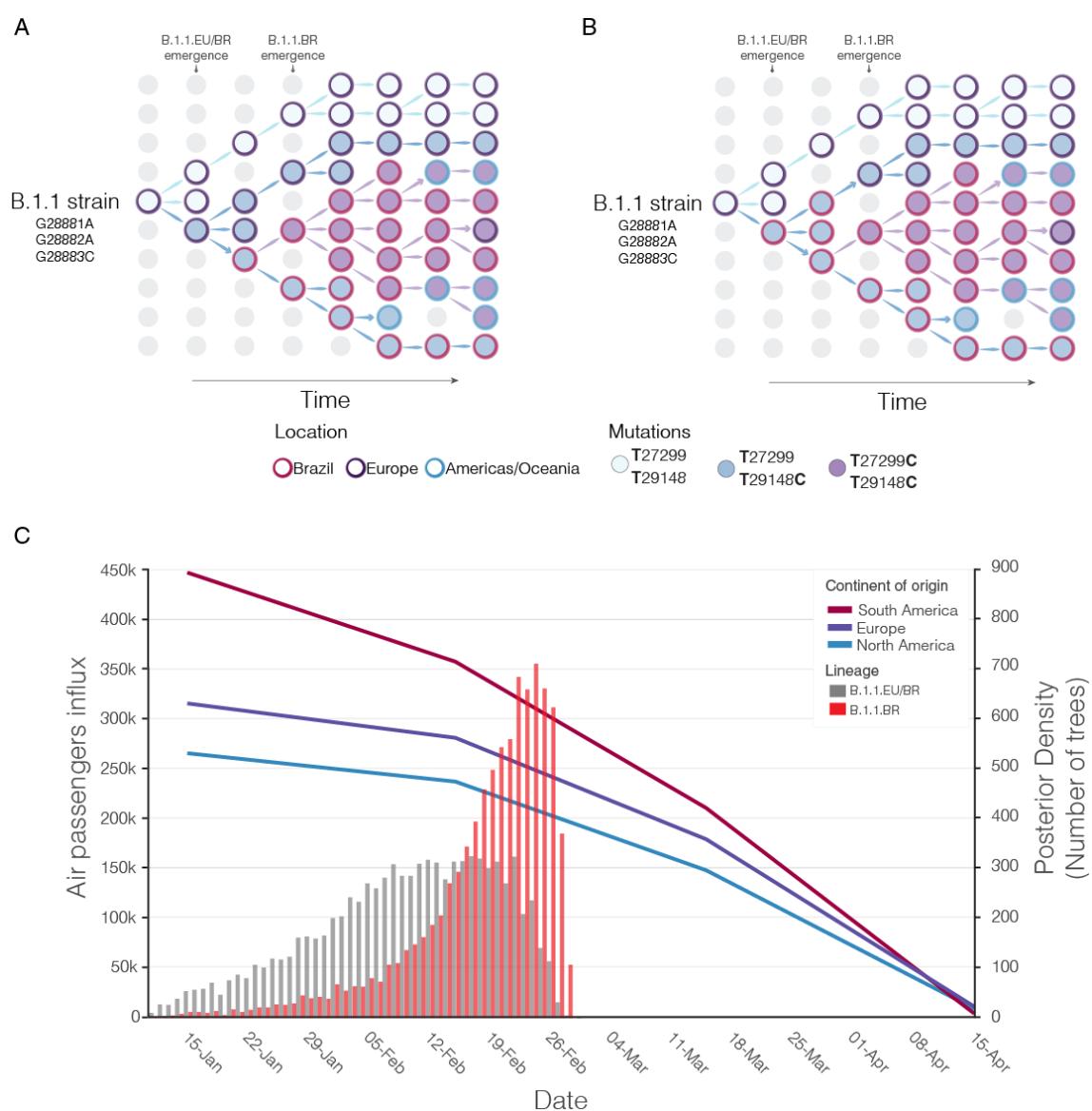
343 **Figure 1. Prevalence of SARS-CoV-2 clades B.1 and B.1.1 in Brazil and other South**
344 **American countries.** A) Map showing the prevalence of SARS-CoV-2 clades B.1 and B.1.1
345 across different South American countries with more than five viral genomes available in the
346 GISAID (<https://www.gisaid.org/>)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



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



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



374
375 **Figure 4. Putative origin and transmission history of the SARS-CoV-2 clades B.1.1.EU/BR**
376 **and B.1.1.BR.** A) Diagrams showing two alternative scenarios for the origin and dissemination of
377 clades B.1.1.EU/BR and B.1.1.BR. The left panel depicts the hypothetical scenario where a
378 B.1.1.EU/BR strain carrying the mutation T29148C was introduced into Brazil from Europe and
379 after a period of local transmission in Brazil arose the B.1.1.BR variant carrying the mutation
380 T27299C, which dispersed all over the country and from Brazil to other countries in the Americas
381 and Oceania. The right panel depicts the hypothetical scenario where a B.1.1 strain was introduced
382 from Europe to Brazil and mutations T29148C and T27299C arose at sequential steps during local
383 transmission. According to this second scenario, Brazil was the epicenter of dissemination of both
384 clades B.1.1.EU/BR and B.1.1.BR to other countries in Europe, the Americas and Oceania. B)
385 Graphic showing the monthly number of international air passengers from South America, North
386 America and Europe that arrived in Brazil during 2020 (available at: <https://www.anac.gov.br>)

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

389

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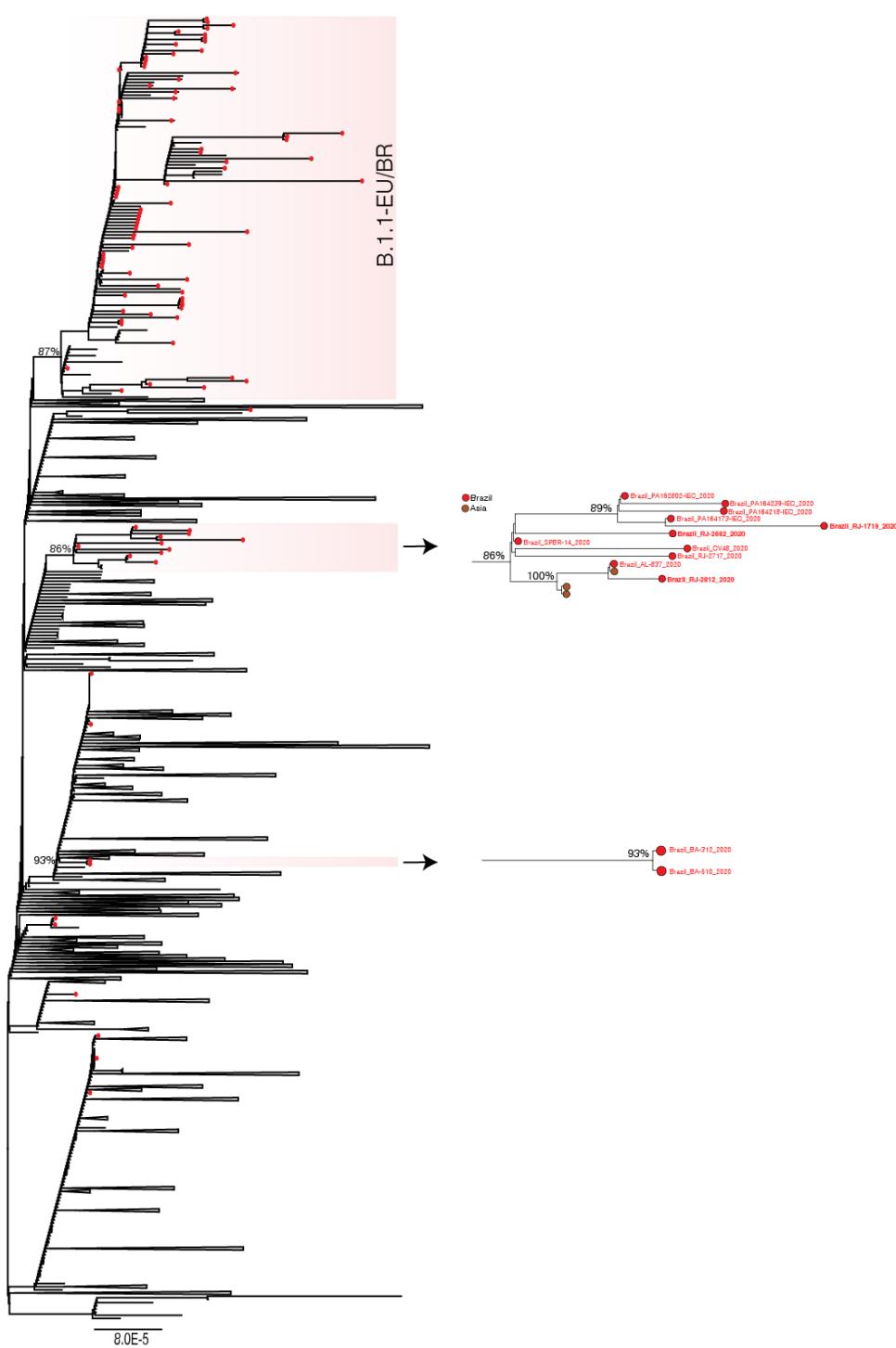
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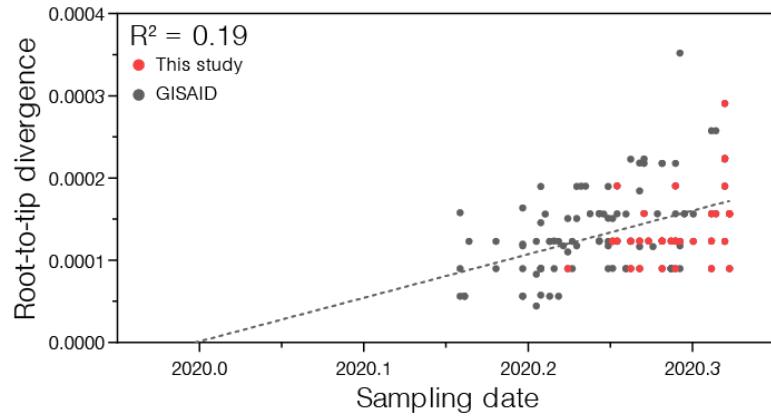
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Supplementary Material



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

1 **Supplementary Table 2.** Prevalence of SARS-CoV-2 lineages B.1.1.EU/BR and B.1.1.BR across
2 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%)	-

3
4 **Supplementary Table 3.** Prevalence of SARS-CoV-2 lineages B.1.1.EU/BR and B.1.1.BR across
5 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%)

6 * Sequences CV34 and CV45 harbor the substitution T29148C, but displayed an ambiguous
7 nucleotide at position 27299, hence assigned to lineage B.1.1.EU/BR.
8
9

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

Metric	Data			Null hypothesis			<i>p</i> value
	mean	lower 95% CI	upper 95% CI	mea n	lower 95% CI	upper 95% CI	
	AI	4.15	3.13	5.15	6.79	6.28	7.29
PS	30.74	29.00	32.00	37.9 9	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

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

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

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