

1 *Communication*

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3 **SARS-CoV-2 Delta variant of concern in Brazil - multiple introductions, communitary transmission, and early**
4 **signs of local evolution**

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48 **Abstract:** The dissemination of the Delta VOC in Brazil is still unclear, despite the frequent reports
49 of isolated cases from different Brazilian states. In this report we characterize the dissemination of
50 the Delta VOC in Brazil and where the introductions of this lineage fall within the global Delta
51 phylogeny. We also examined the mutational profile of the largest clade within the Brazilian Delta
52 VOCs, with a focus on samples which were obtained in the State of São Paulo, and especially in the
53 city of São Paulo, the largest metropolis of South America, and a national and international trans-
54 portation hub.

55 **Keywords:** SARS-CoV-2; Delta VOC; viral phylogeny

56

57 1. Introduction

58 Brazil has been a continuous source of new Covid-19 variants since 2020 [1] espe-
59 cially with the emergence of P.1 (Gamma), a variant of concern (VOC) which has spread
60 throughout the whole country since January 2021, accounting for more than 90% of the
61 cases weekly [2]. At the same time, Brazil is a world exporter and importer of
62 SARS-CoV-2 lineages [1]. This is especially alarming considering the Delta VOC, which
63 emerged in India in early September, 2020 and led to a massive death toll locally. The
64 Delta VOC is now spread worldwide, contributing to a relevant rise in hospitalizations
65 and deaths. As an illustrative example of its impact, in the UK it surpassed the previously
66 dominant VOC, the B.1.1.7 (Alpha) lineage (source: outbreak.info). The dissemination of
67 the Delta VOC in Brazil, first detected by Lamarca et al. [3], is still unclear, despite the
68 frequent reports of isolated cases from different Brazilian states like São Paulo (SP), Rio
69 de Janeiro (RJ), Minas Gerais (MG), Rio Grande do Sul (RS), Tocantins (TO), Paraná (PR),
70 Goiás (GO), and Maranhão (MA). In this report we characterize the dissemination of the
71 Delta VOC in Brazil and where the introductions of this lineage fall within the global
72 Delta phylogeny. We also performed characterization of the mutational profile of the
73 largest clade within the Brazilian Delta VOCs, with a focus on samples which were ob-
74 tained in the State of São Paulo and especially in the city of São Paulo, the largest me-
75 tropolis of South America, and a national and international transportation hub.

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77 2. Materials and Methods

78 From all SARS-CoV-2 positive samples belonging to the Laboratory Platform for
79 Coronavirus Diagnosis, established by the Butantan Institute, we randomly select and
80 sequence around 7-10% from each epidemiological week (epiweek). Viral genotyping
81 was performed only on samples with cycle threshold (Ct) values up to 35. Briefly, RNA
82 extraction was performed with the Extracta kit AN viral (Loccus) in an automated ex-

83 tractor (Extracta 32, Loccus) following the manufacturer's guidelines. SARS-CoV-2 mo-
84 lecular diagnosis was carried out using the GeneFinder™ COVID19 Plus RealAmp kit
85 (Osang Healthcare Co. Ltd.) in all laboratories comprising the diagnostic platform, which
86 reduces variations related to Ct values.

87 SARS-CoV-2 genomic libraries were generated using the COVIDSeq kit (Illumina,
88 San Diego, CA) following the manufacturer's specifications. The normalized sample li-
89 braries were sequenced on a Illumina MiSeq instrument (Kit v2, 2x300) (Illumina, San
90 Diego, CA, USA). The obtained Delta sequences were of high quality, with mean read
91 number above 200,000, mean depth above 800, and coverage above 99%. Refer-
92 ence-assembly following a modified pipeline after the COVIDSeq protocol
93 (<https://artic.network/ncov-2019>) was used. Assembled genomes were deposited in
94 GISAID (accessions provided in Suppl. Mat. Table S1).

95 In order to contextualize the evolution of Brazilian Delta genomes internationally,
96 we assembled a global and clade-representative vetted dataset of 4.000 sequences avail-
97 able from GISAID, spanning a plethora of lineages (including Delta). Subsequently we
98 blasted (Blastn) each Brazilian Delta genome separately and collected only the 1,000
99 closest genomes of each Brazilian sample, using the whole set of more than 170,000 Delta
100 genomes available on GISAID (as of July 19th, 2019) as the Blast database. Duplicate en-
101 tries were then removed. Finally, we included eventual Brazilian Deltas that had been left
102 out of either of those two data sets (either the global representative, or the
103 Blastn-derived). A total of 11,742 genomes were included for phylogenetic inference, in-
104 cluding 98 Brazilian Delta VOC sequences, out of which 13 were obtained in this study
105 from the city of São Paulo, spanning epiweeks 25 to 27. Additionally, a larger set of Delta
106 VOC genomes from São Paulo state was established up to epiweek 29 to refine Delta's
107 growth curve as well as to show this variant has been disseminated along the State.

108 A maximum-likelihood (ML) tree was obtained [4], and only the Delta clade plus its
109 neighbor clade (B.1.6171) were kept for subsequent analyses, for a total of 8,214 samples.
110 Tip-dating was employed to assess divergence times [5]. Branches with approximate
111 likelihood support (aLRT) lower than 0.8 were collapsed. Protein-changing substitutions
112 were assessed by checking individual gene alignments
113 (<https://github.com/neherlab/nextalign>).

114 3. Results and Discussion

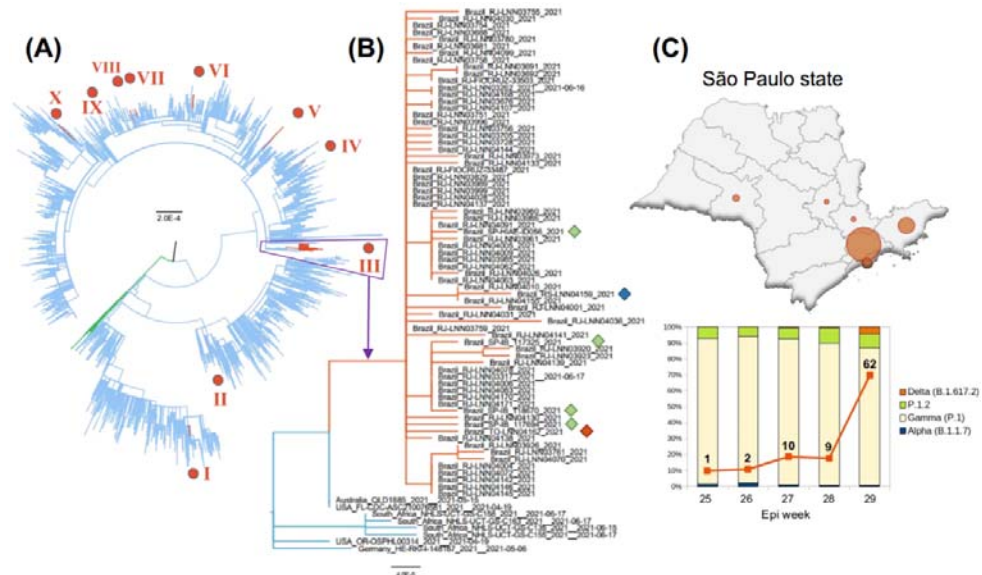
115 According to our analyses, at least 10 independent introductions have occurred until
116 performing the present study in Brazil (clades I-X in Fig. 1). Some of them were imports
117 related to either Australia (clades III and V; Figs. 1 and 2) or USA (clade III), while others
118 are more related to UK samples (clades VI and VIII; Fig. 2 - also clades VII and IX, not
119 shown). Others clustered within a large polytomy with numerous international samples
120 hence precluding accurate source inference (e.g., clade I; Fig. 2). Four clades included
121 three or more samples (branch support ≥ 0.8): clade I (3 from Parana State), clade III (71
122 tips, mostly from Rio de Janeiro, but also 4 obtained from São Paulo, 1 from Rio Grande
123 do Sul, and 1 from Tocantins), clade VI (6 from the State of Maranhão), and clade VIII
124 containing three samples from Goiás State. We also mention a cluster of three SP and
125 two PR sequences in cluster V, but these do not form an exclusive clade, being also
126 closely related to Australian samples.

127 We focus on the largest clade (III) embracing Rio de Janeiro, São Paulo, Tocantins
128 and Rio Grande do Sul samples (Fig. 1), which was also the clade with largest internal
129 branch across Brazilian samples, denoting early signs of local evolution within the coun-
130 try. This clade encompasses 71 genomes (mostly from RJ), further showing its importance
131 in terms of surveillance concern. The mutational profile revealed three mutations exclu-

132 sive to this clade, ORF1a: T4087I (ORF1a), and the remaining two being synonymous.
133 The T4087I mutation is related to a change of a hydrophilic amino acid (Thr) to hydro-
134 phobic (Ile). ORF1a does not embrace any structural proteins, but it may be possible that
135 such a mutation affects the replication rate (hence viral load), which can in turn impact
136 infection rates; more studies are needed to explore such putative effects of this substitu-
137 tion.

138

139



140 **Figure 1.** Aspects of Delta evolution in Brazil. (A) ML tree with 8,214 samples showing phylogeny
141 of this variant (blue), with lineage B.1.617.1 (green) as outgroup (other lineages basal relative to the
142 above are not shown). Brazilian Delta samples (epiweeks 25-27) are indicated in red with roman
143 numerals. All branches < 0.8 support were collapsed. (B) Clade III, the largest clade, with samples
144 from RJ, SP (green diamonds), RS (blue), and TO (red). (C) top: SP state health departments with
145 presence of Delta samples proportional to circle size (epiweeks 25-29); bottom: variation in Delta
146 frequency in SP from epiweeks 25-29 (absolute and relative to lineages most commonly found).

147

148 The first reported cases of Delta VOC infections in Brazil are associated with a cargo
149 ship departed from Malaysia on March 27th, and with connection in South Africa that
150 had arrived in Brazil by May 14th, carrying over 20 crewmen, six of which positive for
151 Delta. Initially communitary transmission had been ruled out, but our results indicate
152 otherwise (clade VI, Fig. 2). Regarding the largest clade with communitary transmission
153 (clade III, Fig. 1), its most recent common ancestor (MRCA) is from early June 2021 ac-
154 cording to dating inference, suggesting a recent expansion unrelated to MA cases.

155 We detected at least four independent communitary transmission chains state-wise
156 (one in RJ, one in GO, another in MA, and also in PR; Figs. 1 and 2). At the same time,
157 some samples from the same state belong to different clades (e.g., SP samples in clades III
158 and V; RJ in clades III and VI; PR in clades I and V), evincing the multiple introductions
159 mentioned above.

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161

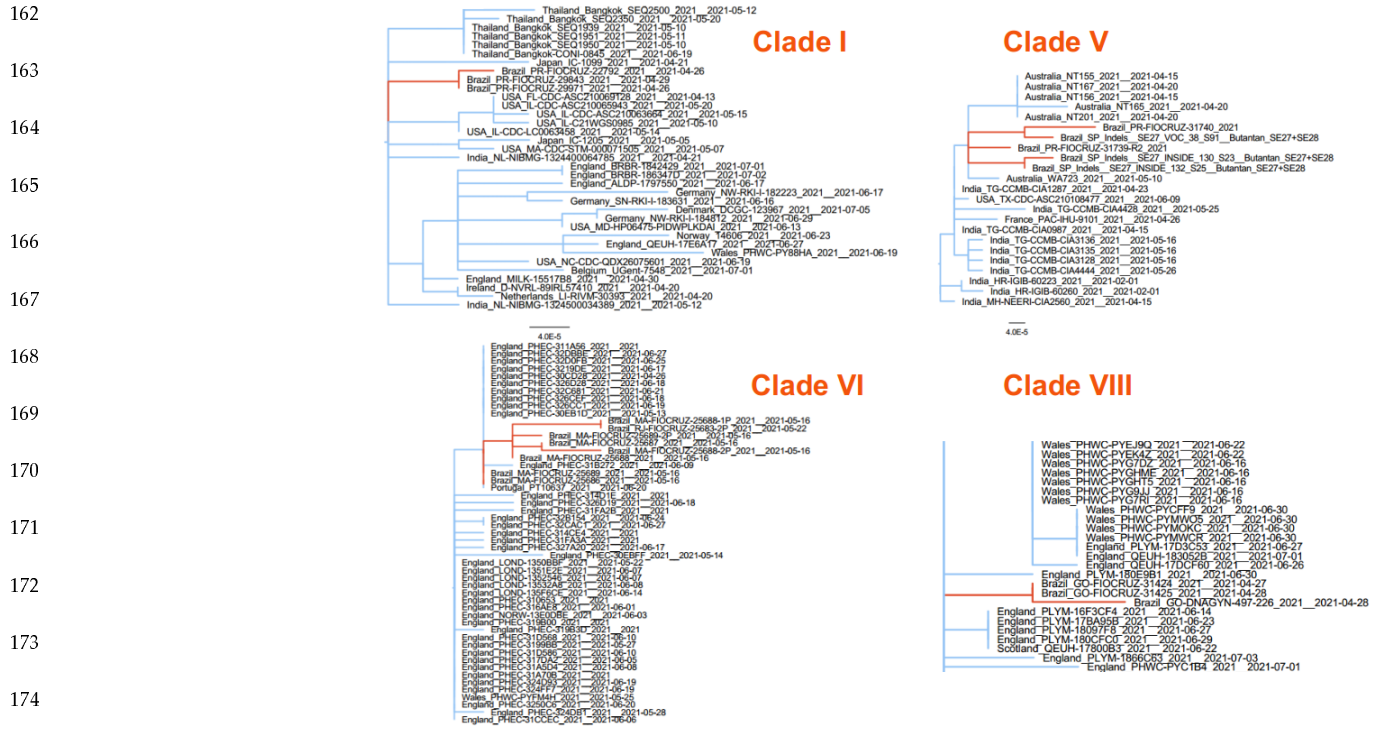


Figure 2. Clades encompassing at least three Brazilian Delta genomes.

192 Sao Paulo, the largest city of the American continents, has samples mainly from its
193 northern districts (Suppl. Mat. Table S1). The first collected sample in the city of Sao
194 Paulo (from late June) is from the southeastern region of the Great São Paulo though,
195 where it probably arrived via the Taubate Regional Health Department from RJ. Pres-
196 ently (epiweek #29), 84 samples have been found in six distinct Regional Health De-
197 partments along Sao Paulo state (Fig. 1C), indicating the Delta VOC is gaining momen-
198 tum within the state, raising concern regarding its potential impact on hospitalizations,
199 deaths, and vaccine escape (as already observed in many countries).

201 4. Conclusions

202 The SARS-CoV-2 Delta variant (B.1.617.2) is now disseminated worldwide. Besides
203 the UK (where it surpassed the previously dominant Alpha VOC), a more comparable
204 scenario to Brazil (in populational, geographic, economic and cultural terms) is Mexico,
205 where the Gamma variant had been pervasive, yet the Delta VOC has been able surpass it
206 in distribution by mid-June 2021 (source: outbreak.info). The increase in Brazilian cases
207 reported here may serve as an alert, especially given the SARS-CoV-2 impact on the
208 country's health care system, which has been severely affected by both COVID-19 hos-
209 pitalizations and deaths. In support of this is the fact that Brazil is a world leader of
210 COVID-19 morbidity and mortality (554, 626 deaths by July, 30th, surpassed only by the
211 USA). In our study, we expanded on the dataset used by the report by Lamarca et al. [3],
212 allowing a more in-depth assessment of the origin and spread of this VOC in Brazil.

213 Our analyses showed at least 10 different Delta VOC introductions and its spread
214 over eight Brazilian states in the first semester of 2021, which was not unexpected given
215 the currently limited contact tracing surveillance protocols in Brazil and the vast national
216 territory. Four apparently unrelated transmission chains (here defined as a supported
217 clades encompassing at least three samples) were identified (in the states of São Paulo,
218 Paraná, Goiás and Maranhão), indicating that the presence and transmission of the Delta
219 VOC has already been established in Brazil. Finally, the locally fixed mutation (ORF1ab:
220 T4087I) which changes a hydrophilic amino acid for a hydrophobic one, was also ob-
221 served in the report by Lamarca et al. [3], raising the concern aimed at this lineage.

222 Even though preliminary analyses indicate that SARS-CoV-2 vaccines used nation-
223 wide may offer protection against the Delta VOC [6], the introduction of any VOC in
224 a country demands significant attention. In this respect, activities related to evaluation of
225 Delta VOC evolution and dissemination with the use of continuous SARS-CoV-2 molec-
226 ular vigilance in Brazil must be widely implemented thus reducing the possible impact of
227 this VOC on both healthcare system and vaccination process.

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229
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243 the Declaration of Helsinki, and approved by the Institutional Ethics Committee of the Faculty of
244 Medicine of Ribeirão Preto, University of Sao Paulo (CAAE: 50367721.7.1001.5440).

245 **Informed Consent Statement:** Patient consent was waived once the diagnostic test had already
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254

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