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SHORT COMMUNICATION

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# Introduction of SARS-CoV-2 C.37 (WHO VOI lambda) in the Sao Paulo State, Southeast Brazil

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## Abstract

The Lambda variants of interest (VOI) (C37/GR/452Q.V1/21G) was initially reported in Lima, Peru but has gained rapid dissemination through other Latin

  

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American countries. Nevertheless, the dissemination and molecular epidemiology of the Lambda VOI in Brazil is unknown apart from a single case report. In this respect, we characterized the circulation of the SARS-CoV-2 Lambda VOI (C37/GR/452Q.V1/21G) in Sao Paulo State, Brazil. From March to June 2021, we identified seven Lambda isolates in a set of approximately 8000 newly sequenced genomes of the Network for Pandemic Alert of Emerging SARS-CoV-2 variants from Sao Paulo State. Interestingly, in three of the positive patients, the Lambda VOI infection was probably related to a contact transmission. These individuals were fully vaccinated to COVID-19 and presented mild symptoms. The remaining positive for Lambda VOI individuals showed different levels of COVID-19 symptoms and one of them needed hospitalization (score 5, WHO). In our study, we present a low level of Lambda VOI circulation in the Sao Paulo State. This reinforces the essential role of molecular surveillance for the effective SARS-CoV-2 pandemic response, especially in regard to circulating variants.

**KEYWORDS**
Lambda variant, molecular epidemiology, SARS-CoV-2 variants, variants of interest

## 1 | INTRODUCTION

Currently, the State of Sao Paulo, located in southeastern Brazil harbors the highest number of reported COVID-19 cases in the country (4.4 million until October 3, 2021). This high incidence (9515/100 000 inhabitants) has been linked to the circulation of several variants of concern (VOCs) and interest (VOIs) with an almost total predominance of the Gamma VOC (P.1/GR 5-1Y.V3/20J).[1] Despite the high frequency of Gamma VOC in the national and State scenario, the prompt detection of other circulating VOCs (Alpha, Beta, and Delta) and VOIs (Zeta and Lambda) is challenging and highly necessary, especially due to the ongoing vaccination process. In this respect, until now, 66% of the population in the Sao Paulo State have received their first vaccine application and about 26% are fully vaccinated according to the Health Surveillance Agency of the State (https://www.saopaulo.sp.gov.br/). In early 2021, Sao Paulo implemented the Network for Pandemic Alert of Emerging SARS-CoV-2 Variants aiming to characterize the SARS-CoV-2 circulating variants. This Network contributes to the Brazilian SARS-CoV-2 genomic surveillance by random sequencing between 0.2% and 12.9% of positive cases per epidemiological week and nowadays the Sao Paulo State is the Brazilian Federal Unit, which provides the largest quantity of SARS-CoV-2 complete genomes deposited in GISAID. In view of this scenario, we described the circulation of an underestimated SARS-CoV-2 Lambda VOI (C.37/GR/452Q.V1/21G lineage) in the Sao Paulo State, providing a primary overview of its transmission dynamics. This VOI has been largely disseminated in many South American countries, including Chile, Peru, Argentina, Ecuador, and Colombia[2–6] but there is a unique report considering Brazil.[7] In total, we detected seven Lambda VOI isolates in a total of approximately 8000 sequenced SARS-CoV-2 genomes and therefore we describe their molecular epidemiology and mutational profile in the Sao Paulo State.

## 2 | MATERIALS AND METHODS

### 2.1 | Samples

Between 8th and 31th epidemiological week (2021), a total of 17,849 genomes were obtained from positive RT-PCR SARS-CoV-2 samples and randomly selected between 7% and 10% from each epidemiological week for SARS-CoV-2 variant screening. All of the samples belonged to the Laboratory Platform for Coronavirus Diagnosis, established by the Butantan Institute. From the total number of sequenced cases, seven Lambda VOI sequences were retrieved and analyzed.

### 2.2 | RT-PCR and sequencing for SARS-CoV-2-RNA

SARS-CoV-2-RNA detection was performed using Gene Finder™ COVID-19 Plus RealAmp kit (OSang Healthcare Co., Ltd.) targeting viral RdRp, E, and N genes. All Lambda samples showed a cycle threshold ($C_t$) value range between 18 and 23 for all tested SARS-CoV-2 genes. SARS-CoV-2 genomic libraries were generated using the COVIDSeq kit (Illumina) following the manufacturer's specifications. The normalized sample libraries were sequenced through the Illumina MiSeq platform using the MiSeq Reagent v.2 Kit (2 × 300 cycles) (Illumina). The obtained sequences were of high quality, mean read number of 462 050, a mean depth of 1656 and 99.8% coverage.

### 2.3 | Serology

The serological testing for the presence of anti-SARS-CoV-2 neutralizing antibodies was performed using the Liaison® SARS-CoV-2 TrimericS IgG kit (DiaSorin) and Elecys® Anti-SARS-CoV-2 Spike and Nucleocapsid (Roche) following the manufacturer's instructions. The serological results obtained by both tests have been shown to correlate positively with the titer of neutralizing antibodies.[8]

### 2.4 | Bioinformatic and phylogenetic analysis

The raw sequence data were submitted to quality control analysis using the FastaQC[9] software v. 0.11.8. Trimming was performed using Trimmomatic[10] v. 0.3.9 to select the best quality sequences. We mapped the trimmed sequences against the SARS-CoV-2 reference (Genbank refseq NC_045512.2) using BWA[11] software and samtools[12] for read indexing. The mapped files were submitted to refinement with the software Pilon[13] to obtain the indels and insertions in the most correct way possible. Finally, we used bcftools[14] for variant calling and seqtk[15] for the creation of consensus genomes.

For performing phylogenetic analysis, we used a dataset containing 630 Lambda VOI genomes obtained from GISAID up to September 26, 2021. Only genomes >29 000 bp and <1% of ambiguities were retrieved, low-quality genomes (>10% of ambiguous positions) were excluded. Sequence alignment was performed using MAFFT v7.475[16] and manually curated to remove artifacts using Aliview.[17] Maximum likelihood (ML) phylogenetic trees were estimated using IQ-TREE v.2,[18] applying the ML algorithm with statistical support of ultrafast bootstrap with 1000 replicates. We inferred time-scaled trees and rooted these with least-squares criteria and the evolutionary rate of $>1.1 \times 10^{-3}$ substitutions/site/year estimated by Duchene et al.[19] using the TreeTime v.1 software.[20]

## 3 | RESULTS

Lambda VOI comprised 0.04% (7/17, 849 genomes) of the total number of genomes sequences obtained from March to June 2021 by the Butantan Network for Pandemic Alert of SARS-CoV-2 Variants. Lambda VOI was first detected in the eighth epidemiological week, 2021 followed by six more cases in Week 22 and one in the 31st epidemiological week. This VOI emerged in distinct regions of the state (coastal and inner regions). The performed phylogenetic analysis revealed that the obtained in this study SARS-CoV-2 genome sequences formed two independent clusters and grouped together with isolates from other countries mainly from South America. This indicated two possible Lambda VOI introduction events in the Sao Paulo State that may have additionally contributed to the dissemination of this VOI in the region (Figure 1 and Table S1).

Epidemiological investigation of the Lambda VOI infected individuals demonstrated that in three of them (SP 82131, SP 83842, and SP 85093) contact transmission was observed and they were fully vaccinated against SARS-CoV-2 (Figure 1). These individuals reported no travel abroad. The SARS-CoV-2 infection was acquired approximately 2 weeks after the application of the second vaccination dose and the symptoms persisted for an average of 9–14 days. All of the patients presented mild symptoms (score 2, WHO) [21] and did not need hospitalization. The infection was probably related to high viral load as deduced by the low mean cycle threshold values ($C_t$) obtained by the diagnostic RT-PCR for the SARS-CoV-2 genes E, N, and RdRP, which were 19.0, 20.4, and 20.5, respectively. In two of the patients, the serological results demonstrated positive values for SARS-CoV-2 neutralizing antibodies (nucleocapsid, anti-spike RBD antibodies, and trimeric IgG) with the serological test performed after 30 days upon Lambda VOI RT-PCR confirmation.

Nevertheless, one Lambda positive patient (SP-RP 89551) was not vaccinated, needed hospitalization, and presented COVID-19 severity with a score of 5 (WHO).[21] We also characterized the mutational profile of this VOI, which harbored the following mutations: N gene: P13L, R203K, G204R, G214C; ORF1a: T1246I, P2287S, F2387V, L3201P, T3355I, G3278S, Del.3675-3677; ORF1b: P314L; and the Spike gene: G75V, T76I, L452Q, F490S, D614G, T859N. Additional nonsynonymous mutations in nucleocapsid protein (N)—P365L and T366I—and in ORF1a (S944L and H1113T) were observed in two of the vaccinated individuals (SP 83842 and SP 85093).

## 4 | DISCUSSION

In this report, we provide more detailed information about the Lambda VOI circulation in Sao Paulo State, which was detected as part of a wide program for SARS-CoV-2 genomic surveillance in this part of Brazil. The mutation profile of this VOI[2] was also characterized. In the first place, the performed genomic surveillance for the period of 8th to 31st epidemiological week demonstrated a very low circulation of the Lambda VOI in the State of Sao Paulo, despite the intensive presence of this VOI in the neighboring countries, and principally Peru, where it accounts almost approximately 97% of the identified genomes by April 2021.[22] We believe that the limited Lambda circulation in the Sao Paulo state might be due to the already established expressive presence of the Gamma VOC by the studied period, which did not permit effective Lambda VOI dissemination. This might be a reason that only sporadic Lambda VOC introductions have been reported in Brazil,[23] which did not lead to dissemination with important epidemiologic significance similar to our case. The performed phylogenetic study classified the strains obtained in this study in two different clusters showing two potential introductions of this VOI in Sao Paulo state. The majority of the basal isolates was originating from South America and this was expected, as Lambda VOI is circulating extensively in countries, such as Peru, Argentina, and Colombia, which border Brazil.[24] There is scarce information about the circulation of the Lambda VOI in Brazil except from one study that characterizes the first imported case in the Rio Grande do Sul State (South Brazil)[7] and a recent one describing several Lambda

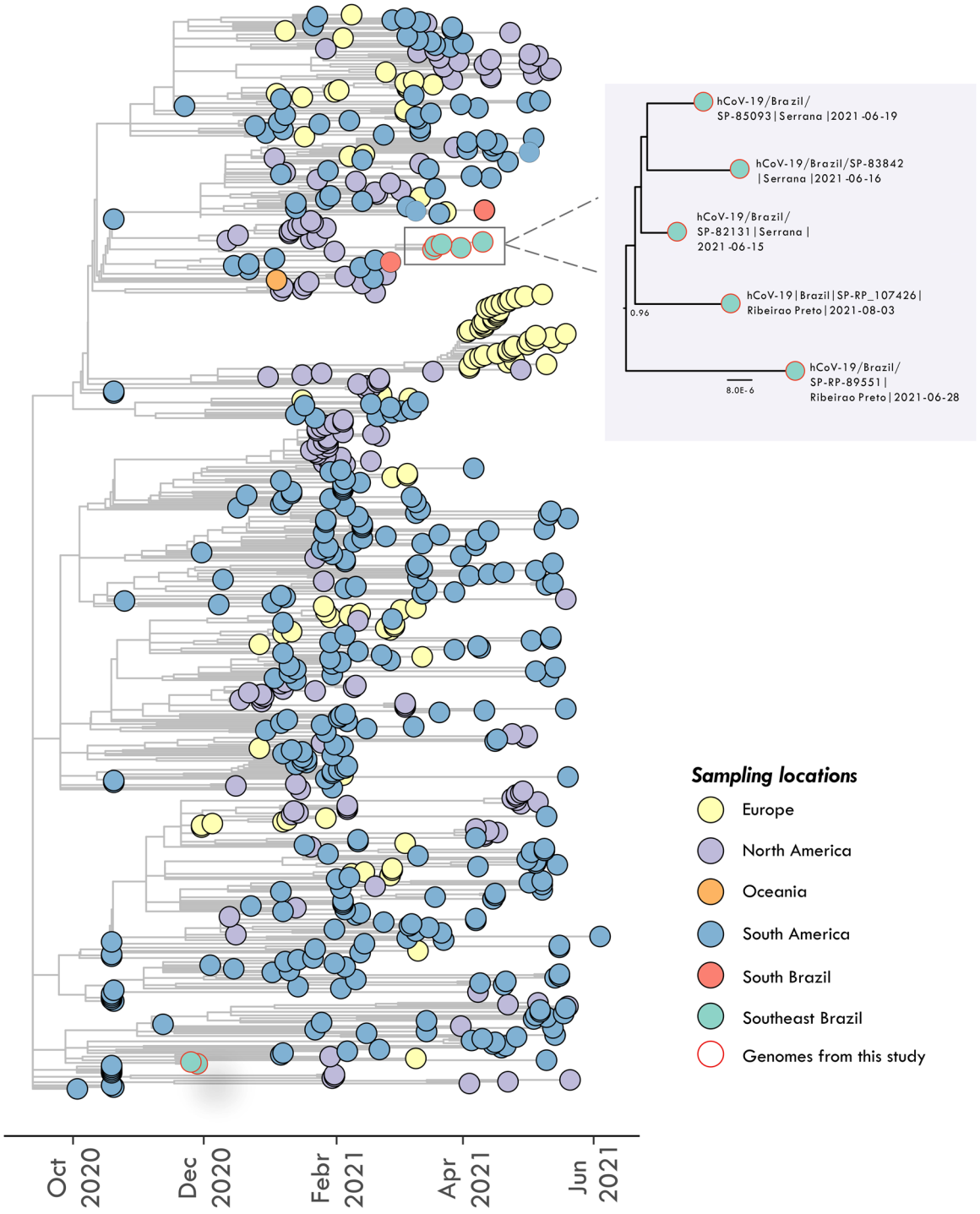


**FIGURE 1** Genomic characterization of Lambda SARS-CoV-2 variants of interest (VOI) circulating in Sao Paulo state, Brazil. Approximate maximum likelihood (ML) phylogenetic time tree, including the seven new Lambda SARS-CoV-2 isolates obtained in this study as well as 630 reference Lambda SARS-CoV-2 complete genomes obtained from GISAID (https://www.gisaid.org) until September 2021. We also represented on the right side, zoom of the sequences obtained from the inner São Paulo State with the bootstrap support of this branch

introductions in Brazil.[23] Lambda VOI detection in the Sao Paulo State was only possible due to the massive ongoing large-scale SARS-CoV-2 genomic surveillance effort in the State, allowing a nearly real-time investigation and identification of the circulating lineages. The surveillance of the Lambda VOI in Brazil is important due to the large territory of the country, which borders Latin American countries where the Lambda VOI is a dominant lineage.

The mutational profile described in our study revealed the presence of the mutations of interest L452Q, F490S, and D614G (spike region), which can contribute to higher transmissibility as demonstrated in a

recent study.[25] Of particular interest is the L452Q mutation, which has been related to increased viral transmission[26] that may contribute to the higher morbidity of this VOI in the countries in which it initially emerged.

Detailed epidemiological information of the positive for Lambda VOI patients demonstrated that three of them have been fully vaccinated against COVID-19. In addition, these individuals worked together and on the phylogenetic tree, the samples clustered with high bootstrap support (Figure 1). On one hand, the presence of the mutation L452Q[26] and D614G[27] was related to high replication and antiviral immunity fitness of the Lambda VOI, which can be explained by the reduced neutralizing activity of the vaccine-induced antibodies to this VOI.[28] In addition, Lambda VOI has demonstrated higher vaccine escape compared to the Delta VOC, which also demonstrates high rates of infectivity.[29] While Lambda VOI infection in the immunized individuals might be regarded as a vaccine breakthrough, we cannot rule out a protective effect of the vaccine, as the three patients showed mild symptoms. Although it seems reasonable that mRNA vaccines/convalescent serum might protect against symptomatic Lambda infection[30] more studies are necessary to evaluate the vaccine impact on the SARS-CoV-2 VOIs.

The Lambda strains evaluated in this study, although in low frequency, demonstrate the importance of the directed SARS-CoV-2 genomic surveillance for characterizing the circulating variants in a given region. This approach is important as it demonstrates that Lambda VOI did not reach worrisome levels of circulation. The extensive genomic surveillance of SARS-CoV-2 like in our case may further improve the accelerated discovery of SARS-CoV-2 variants, especially in the background of an ongoing vaccination process, which may exert selective pressure on this virus related to the emergence of novel variants with unpredictable behavior.

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## CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

## AUTHOR CONTRIBUTIONS

E.R.S., E.V.S., M.E., L.P.O.L., R.A.B., P.D.S.C.M., R.L.R.C.C., J.P.K., D.S., P.A.A., F.A.S.C., M.D.P., J.C.C.L., E.C.M., C.A.B., and L.S. designed and performed the experiments. R.B.S., J.S.L.P., V.L.V., V.F., S.N.S., M.C.E., S.C.S., S.K. analyzed the data and wrote the article. M.B., P.M.M.G., N.N.F., R.T.C., and D.T.C. evaluated the clinical/epidemiological data and reviewed the article. G.R.M., A.J.M., C.R.S.B and E.C.M. evaluated the epidemiological survey. M.G., L.C.J.A., L.L.C, R.M.T.G., J.A.S.N, H.F., M.L.N., M.C.E., and S.N.S. edited and reviewed the article. M.C.E., S.C.S., S.K., and D.T.C. supervised this study. All the authors agreed on the submission of the final manuscript.

## ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee of the Faculty of Medicine of Ribeirão Preto (Process CAAE: 50367721.7.1001.5440).

## DATA AVAILABILITY STATEMENT

The Lambda VOI sequences generated in this study were deposited under the following Gisaid accession numbers: EPI_ISL_2928137, EPI_ISL_4681968, EPI_ISL_4684266, EPI_ISL_4685838, EPI_ISL_4687192, EPI_ISL_1966094, EPI_ISL_1445272.

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## SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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