



SARS-COV-2 genomic monitoring in the state of São Paulo unveils two emerging AY.43 sublineages

Alex Ranieri Jerônimo Lima¹ | Gabriela Ribeiro¹ | Vincent Louis Viala¹ |
Loyze Paola Oliveira de Lima¹ | Antonio Jorge Martins¹ |
Claudia Renata dos Santos Barros¹ | Elaine Cristina Marqueze¹ |
Jardelina de Souza Todao Bernardino¹ | Debora Botequio Moretti¹ |
Evandra Strazza Rodrigues² | Elaine Vieira Santos² | Ricardo Augusto Brassaloti³ |
Raquel de Lello Rocha Campos Cassano³ | Pilar Drummond Sampaio Corrêa Mariani⁴ |
Luan Gaspar Clemente³ | Patricia Akemi Assato⁵ | Felipe Allan da Silva da Costa⁵ |
Mirele Daiana Poletti⁶ | Jessika Cristina Chagas Lesbon⁶ | Elisangela Chicaroni Mattos⁶ |
Cecilia Artico Banho⁷ | Lívia Sacchetto⁷ | Marília Mazzi Moraes⁷ | Melissa Palmieri⁸ |
Maiara Martininghi⁹ | Luiz Artur Vieira Caldeira⁹ | Fabiana Erica Vilanova da Silva⁹ |
Rejane Maria Tommasini Grotto^{5,10} | Jayme A. Souza-Neto⁵ | Marta Giovanetti^{11,12} |
Luiz Carlos Junior Alcantara^{11,12} | Maurício Lacerda Nogueira⁷  | Heidge Fukumasu⁶ |
Luiz Lehmann Coutinho³ | Simone Kashima²  | Raul Machado Neto¹ |
Dimas Tadeu Covas^{1,2} | Svetoslav Nanev Slavov² | Sandra Coccuzzo Sampaio¹ |
Maria Carolina Elias¹

¹Center of Scientific Development, Butantan Institute, São Paulo, Brazil

²Blood Center of Ribeirão Preto, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

³Centro de Genômica Funcional da ESALQ, University of São Paulo, Piracicaba, São Paulo, Brazil

⁴NGS Soluções Genômicas, Piracicaba, São Paulo, Brazil

⁵Department of Bioprocesses and Biotechnology, School of Agricultural Sciences, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil

⁶Department of Veterinary Medicine, School of Animal Science and Food Engineering, University of São Paulo, Pirassununga, São Paulo, Brazil

⁷Medicine School of São José do Rio Preto (FAMERP), São José do Rio Preto, São Paulo, Brazil

⁸Coordenação de Vigilância em Saúde—Secretaria Municipal da Saúde, São Paulo, Brazil

⁹Secretaria Municipal da Saúde, São Paulo, Brazil

¹⁰Molecular Biology Laboratory, Applied Biotechnology Laboratory, Clinical Hospital of the Botucatu Medical School, Botucatu, São Paulo, Brazil

¹¹Laboratório de Genética Celular e Molecular, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

¹²Laboratório de Flavivírus, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil

Correspondence

Svetoslav Nanev Slavov, Blood Center of Ribeirão Preto, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, São Paulo 05503-000, Brazil.
Email: svetoslav.slavov@hemocentro.fmrp.usp.br

Abstract

Delta VOC is highly diverse with more than 120 sublineages already described as of November 30, 2021. In this study, through active monitoring of circulating severe acute

Alex Ranieri Jerônimo Lima and Gabriela Ribeiro authors contributed equally to this study.

Sandra Coccuzzo Sampaio and Maria Carolina Elias, Center of Scientific Development, Butantan Institute, São Paulo 05503-000, Brazil. Email: sandra.coccuzzo@butantan.gov.br and carolina.eliasabbaga@butantan.gov.br

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respiratory syndrome coronavirus-2 (SARS-CoV-2) variants in the state of São Paulo, southeast Brazil, we identified two emerging sublineages from the ancestral AY.43 strain which were classified as AY.43.1 and AY.43.2. These sublineages were defined by the following characteristic nonsynonymous mutations ORF1ab:A4133V and ORF3a:T14I for the AY.43.1 and ORF1ab:G1155C for the AY.43.2 and our analysis reveals that they might have a likely-Brazilian origin. Much is still unknown regarding their dissemination in the state of São Paulo and Brazil as well as their potential impact on the ongoing vaccination process. However, the results obtained in this study reinforce the importance of genomic surveillance activity for timely identification of emerging SARS-CoV-2 variants which can impact the ongoing SARS-CoV-2 vaccination and public health policies.

KEYWORDS

AY.43, Delta VOC, emerging sublineages, genomic surveillance, SARS-CoV-2

1 | INTRODUCTION

Currently in Brazil have been applied more than 381 million doses of anti-severe acute respiratory syndrome coronavirus-2 (anti-SARS-CoV-2) vaccines and the individuals who have been fully vaccinated are bordering 140 million, which makes Brazil one of the most vaccinated nations in the world (<https://www.gov.br/saude/pt-br/vacinacao>). In Brazil, on the background of this solid process of vaccination, the first cases of Delta variant of concern (VOC) introductions were reported.¹ Despite the full substitution of the pre-existing Gamma VOC in Brazil with the Delta VOC, no exponential growth of the new cases has been observed, most probably due to the ongoing vaccination. Delta VOC is highly diversified, and more than 120 sublineages have been classified within the Pango lineages (<https://cov-lineages.org/>) with the continuous description of novel lineages and sublineages.

The molecular surveillance of the SARS-CoV-2 variants is of crucial importance for tracking the genomic profile of this virus and the accumulation of mutations which on one hand can alter the viral functions in terms of infectivity and transmissibility and on the other might be important for the emergence of novel variants, lineages, and sublineages which can exert significant pressure on the healthcare system of a given country.²

In this study, we identified two emerging Brazilian sublineages belonging to the ancestral AY.43 lineage, which were named AY.43.1 and AY.43.2. Most of these sequences originated from the city of São Paulo and the genome monitoring was performed by the Butantan Network for Pandemic Alert of SARS-CoV-2 Variants (Butantan Network) of the São Paulo State.

2 | MATERIALS AND METHODS

From October 8 to October 30, 2021, nasopharyngeal swab samples were collected by Butantan Network from inhabitants suspected to be infected with SARS-CoV-2 from all 17 Health Divisions of the São

Paulo State. Viral ribonucleic acid (RNA) was isolated from 100 μ l of nasopharyngeal swab suspension using the Extracta Kit RNA viral (Loccus) in 96-well plate automated extractor (Extracta, Loccus) following the manufacturer's instructions. All samples were tested for SARS-CoV-2 by targeting viral RdRp, E, and N genes using the real-time polymerase chain reaction (PCR) assay Gene FinderTM COVID19 Plus RealAmp Kit (Osang Healthcare Co. Ltd.), and positive samples with C_t below 30 were selected for whole genome sequencing. The RNA from positive samples was re-extracted from the original nasopharyngeal swab suspension for sequencing. The SARS-CoV-2 sequencing libraries were prepared using the COVID-Seq Kit (Illumina Inc.), which amplifies the whole SARS-CoV-2 genome using the ARTIC v3 tiling PCR primer panel.³ Paired-end libraries were sequenced on Illumina's MiSeq (V2 kit, 2150 cycles) or NextSeq. 2000 (P2 kit, 2100 cycles) platform.

To obtain the final SARS-CoV-2 genome sequences, the following bioinformatics approaches were performed. The raw sequencing data obtained were submitted to quality control analysis using the FastaQC⁴ software version 0.11.8. To select the sequences with the best quality score (>30), quality filtering was performed using Trimmomatic⁵ version 0.3.9. We mapped the quality-filtered sequences against the SARS-CoV-2 reference (Genbank RefSeq NC_045512.2) using BWA⁶ and used SAMtools⁷ for indexing the mapping results. The mapped files were submitted for improvement using Pilon⁸ to correct possible deletions and insertions caused by the mapping process. The quality-filtered sequences were subjected to a remapping against the genome improved by Pilon. Finally, we use bcftools⁹ for variant calling and seqtk¹⁰ for the assembly of the consensus SARS-CoV-2 genomes. Positions covered by fewer than 10 reads ($DP < 10$) and bases with a quality score lower than 30 were considered as an assembly gap and thus converted into Ns. Coverage values for each genome were calculated using SAMtools v1.12. We assessed the consensus genome sequence quality using Nextclade v0.8.1.¹¹

Phylogenetic analysis was performed using the Nextstrain v3.0.3 SARS-CoV-2 workflow.¹¹ In brief, this workflow aligns the input

sequences using nextalign and then reconstructs the phylogenetic trees using the IQTree v.2.¹² Subsequently, TreeTime¹³ is used to reroot the resulting tree, resolve polytomies, prune sequences, infer internal node dates and label them. To reconstruct the phylogeny of the AY.43 hypothetical sublineages we used 1616 SARS-CoV-2 sequences generated from the Butantan Network distributed between the epidemiological Weeks 41–43 (GISAID accession ID in File S1) as input for the Nextstrain workflow. To use as a background for our analysis, a representative global data set was retrieved from GISAID (3711 sequences, downloaded from nextregions global on November 10, 2021—GISAID accession ID in File S2) (<https://www.gisaid.org/>).

3 | RESULTS

In the performed phylogenetic analysis we observed a large cluster of the AY.43 lineage containing 492 sequences, the majority of which ($n = 352$, 71.5%) were from the Butantan Network, present inside the

Brazilian clade (Figure 1). The AY.43 sublineage can be distinguished from other AY sublineages of Delta VOC by the presence of mutations N:Q9L and ORF9b:S6C (<https://www.pango.network/summary-of-designated-ay-lineages/>). We observed two subdivisions of the AY.43 cluster based on the presence of the nonsynonymous mutations, labeling them as AY.43.1 (ORF1ab: A4133V and ORF3a:T14I) and AY.43.2 (ORF1ab:G1155C). The subcluster of AY.43.1 was composed of 100 strains (Figure 1—purple box), the majority of which were obtained from the city of São Paulo (46.0%). The AY.43.2 subcluster was composed of 99 strains (Figure 1—blue box), of which 97 of them were sequenced by Butantan Network with most sequences from the city of São Paulo (53%).

The rest of the AY.43 sequences from Butantan Network ($n = 155$) were mainly obtained from the city of São Paulo (60.0%) and represented the highest number of sequences composing the AY.43 sublineage cluster. The AY.43 sequences, including the newly identified sublineages, were distributed in several cities of the São Paulo State with higher concentrations in the city of São Paulo (Figure 2).

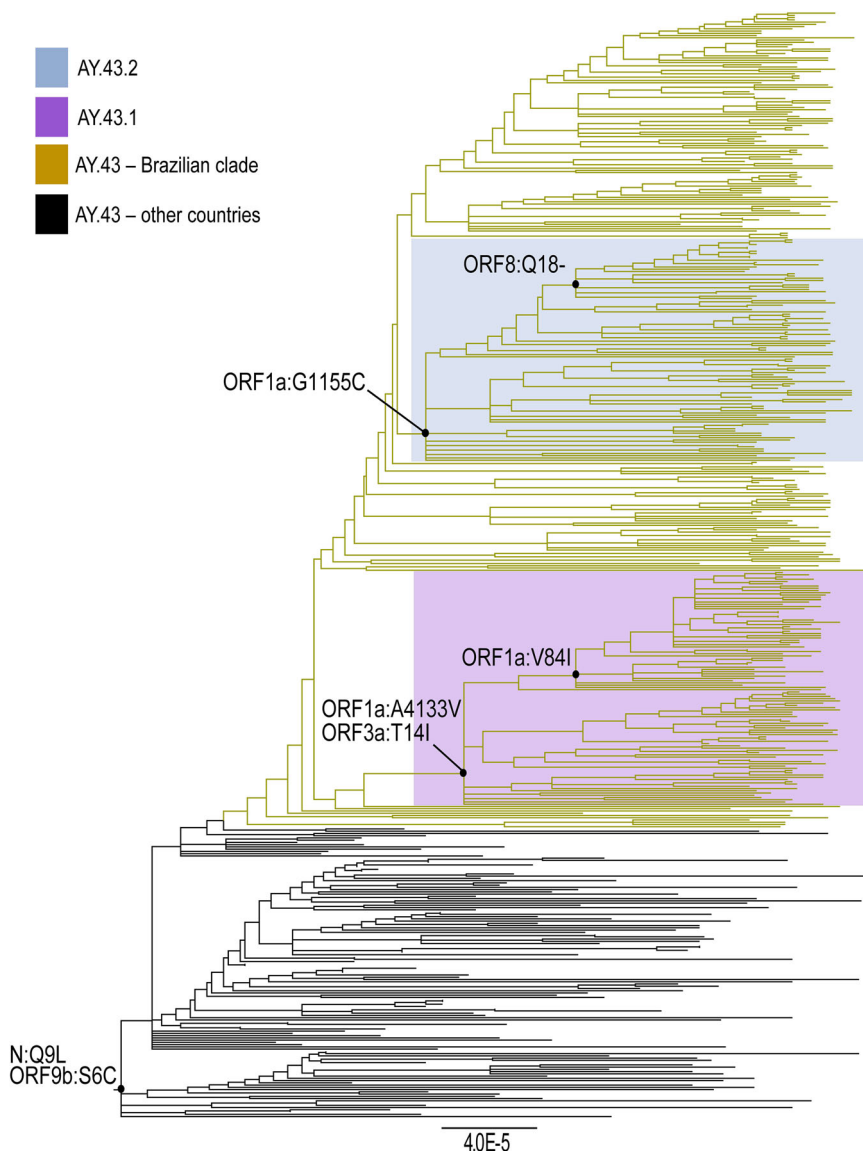


FIGURE 1 Phylogenetic analysis of the AY.43 lineage clade containing 492 genomes. The newly identified sublineages are highlighted with boxes: AY.43.1 (purple box) and AY.43.2 (blue box), and their characterizing mutations are represented on the newly formed branches. The original tree from which the AY.43 cluster was initially can be observed in Figure S1

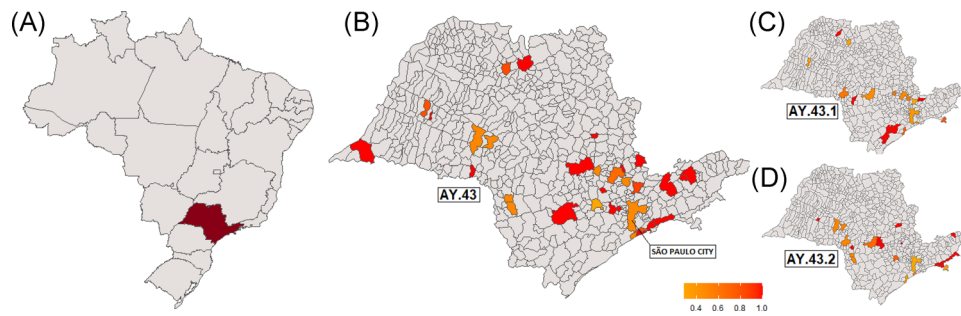


FIGURE 2 Dissemination of the AY.43 lineage and the newly identified sublineages in the state of São Paulo (A). Map of Brazil with emphasis on the state of São Paulo (B). Dissemination of the AY.43 lineage (C). Dissemination of the AY.43.1 sublineage and (D). Dissemination of the AY.43.2 sublineage. Bar: percentage of the sequenced genomes

A whole phylogenetic interactive tree, from which the AY.43 cluster was initially observed, can be accessed at the following link: https://nextstrain.org/fetch/repositorio.butantan.gov.br/bitstream/butantan/3990/1/ncov_EpiWeek_forty-one-to-forty-three.json

4 | DISCUSSION

In this study, we provide a report regarding the characterization of two novel AY.43 sublineages with a likely Brazilian origin, characterized by a specific mutational profile. Considering the number of mutations (localization in ORF1a or ORF3a) we proposed the identification of two emerging sublineages designed as AY.43.1 and AY.43.2. They were additionally recognized as such by the official Pango designation committee (<https://github.com/cov-lineages/pango-designation/issues/319>) and were released on pangoleARN release v1.2.96. The obtained data shows the importance of the SARS-CoV-2 genomic surveillance for the identification of emerging lineages. This is particularly important because SARS-CoV-2 emerging lineages can exert an enormous impact on the public health systems due to increased infectivity and transmission.²

In the newly characterized sublineages, we defined the mutations A4133V and G1155C in the ORF1a and the T14I mutation in the ORF3a, which were nonsynonymous. To our knowledge, the impact of these mutations is still unknown, except for the T14I, which shows deleterious effects on the viral proteins.¹⁴ A nonsynonymous mutation in ORF3a, which is a conserved protein involved in viral replication and release,¹⁵ may affect viral functions in addition to the mutational constellation defined for the Delta VOC.

We additionally observed other subdivisions within the AY.43 clade: one in the AY.43.1 clade, presenting a nonsynonymous mutation at ORF1a:V84I (Figure 1—purple box); and another at the AY.43.2 clade, presenting a deletion at ORF8:Q18-. However, at the moment those subdivisions were characterized by a limited number of sequences and did not show sufficient support to be suggestive as novel sublineages of AY.43. Based on the performed analysis the newly classified AY.43.1 and AY.43.2 sublineages probably might have a Brazilian origin. Further studies are necessary to investigate their dissemination within Brazilian regions, but preliminary results

show that the majority of AY.43.1 and AY.43.2 sequences originated from the city of São Paulo.

In conclusion, we show that SARS-CoV-2 genomic monitoring is crucial for the prompt characterization of SARS-CoV-2 novel lineages and sublineages. By this approach, we can timely detect the presence of novel SARS-CoV-2 variants and implement strategies for preventing their dissemination which can have further implications on the ongoing SARS-CoV-2 vaccination and public health policies.

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ETHICS STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee of the Faculty of Medicine of Ribeirão Preto, University of São Paulo (CAAE: 50367721.7.1001.5440).

AUTHOR CONTRIBUTIONS

Alex Ranieri Jerônimo Lima, Gabriela Ribeiro, Vincent Louis Viala, Loyze Paola Oliveira de Lima, Antonio Jorge Martins, Claudia Renata dos Santos Barros, Elaine Cristina Marqueze, Jardelina de Souza Todao Bernardino, Debora Botequiu Moretti, Evandra Strazza Rodrigues, Elaine Vieira Santos, Ricardo Augusto Brassaloti, Raquel de Lello Rocha Campos Cassano, Pilar Drummond Sampaio Corrêa Mariani, Luan Gaspar Clemente, Patricia Akemi Assato, Felipe Allan da Silva da Costa, Mirele Daiana Poleti, Jessika Cristina Chagas Lesbon, Elaine Cristina Marqueze, Cecilia Artico Banho, Lívia Sacchetto, Marília Mazzi Moraes, Melissa Palmieri, Maiara Martininghi, Luiz Artur Vieira Caldeira, Fabiana Erica Vilanova da Silva, Rejane Maria Tommasini Grotto, and Jayme A. Souza-Neto designed and performed the experiments. Alex Ranieri Jerônimo Lima, Gabriela Ribeiro, Svetoslav Nanev Slavov, Maria Carolina Elias, Marta Giovanetti, Luiz Carlos Junior Alcantara, Sandra Coccuzzo Sampaio, and Simone Kashima analyzed the data and

wrote the article. Maiara Martininghi, Luiz Artur Vieira Caldeira, and Fabiana Erica Vilanova da Silva evaluated the clinical/epidemiological data and reviewed the article. Alex Ranieri Jerônimo Lima, Gabriela Ribeiro, Svetoslav Nanev Slavov, Maria Carolina Elias, and Marta Giovanetti edited and reviewed the article. Maurício Lacerda Nogueira, Heidge Fukumasu, Luiz Lehmann Coutinho, Simone Kashima, Raul Machado Neto, Dimas Tadeu Covas, Svetoslav Nanev Slavov, Sandra Coccuzzo Sampaio, and Maria Carolina Elias supervised this study. All the authors agreed on the submission of the final manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in GISAID at <https://www.gisaid.org/>

ORCID

Maurício Lacerda Nogueira  <http://orcid.org/0000-0003-1102-2419>

Simone Kashima  <http://orcid.org/0000-0002-1487-0141>

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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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