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## SARS-COV-2 VARIANT CIRCULATION IN BRUNEI DARUSSALAM - NOVEMBER 2021 TO JULY 2022.

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### ABSTRACT

This is a report of 8-months progression of circulating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants in Brunei Darussalam from November 2021. Whole-genome sequencing (WGS) was performed on nearly 3,000 random local COVID-19 patients in samples with a cycle-threshold ( $C_T$ ) of less than 25. Phylogenetic analysis revealed that the Delta variant, particularly AY.24.1, was the predominant variant towards the end of 2021. The same variant was then completely superseded by the Omicron variants in mid-January 2022. During the early wave of Omicron variants, BA.2.3.19 and its paternal BA.2.3 were found to be the prominent sublineage until it was replaced by BA.5 lineages four months later, mainly BA.5.2 and BF.5. Further analysis revealed that AY.24.1 and BA.2.3.19 that was once a prevalent variant are predominantly observed in Brunei. Both variants can be differentiated by the presence of a particular mutation in the *S* gene that encodes the viral spike protein that is essential for host cell interaction. Brunei is continuously monitoring the circulating SARS-CoV-2 variants as part of its genomic surveillance following the World Health Organization (WHO) guidance for surveillance.

**Keywords:** Brunei, Next-generation sequencing, SARS-CoV-2, SARS-CoV-2 variants, Whole-genome sequencing.

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**Keywords:** Brunei, Next-generation sequencing, SARS-CoV-2, SARS-CoV-2 variants, Whole-genome sequencing.

## INTRODUCTION

In December 2019, a novel coronavirus disease called COVID-19 was first reported in Wuhan, China, and it was announced a global pandemic by the World Health Organization (WHO) in March 2020 as the number of cases increased worldwide at an alarming rate.<sup>1,2</sup>

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The virus responsible for this outbreak is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the complete viral genome sequence isolated from the index patient, of which became the *de-facto* SARS-CoV-2 reference genome, has now been published.<sup>2</sup>

Brunei recorded its first COVID-19 case in March 2020 and this started the first COVID-19 wave but strict control measures enforced by the Ministry of Health successfully limited the outbreak to just two months.<sup>3</sup> In

May 2021, WHO declared the Delta variant originating from India as 'Variant of Concern' and the same variant was found responsible for the second wave of COVID-19 in Brunei three months after the declaration.<sup>4</sup> Since then, local COVID-19 cases have fluctuated and the Department of Laboratory Services, Ministry of Health has set up a Microbial Genomics Services to monitor circulations of local SARS-CoV-2 variants using next-generation sequencing (NGS) technology. In this paper, we report our observations on the SARS-CoV-2 variant circulation in Brunei from November 2021 to July 2022.

## METHODS

**RNA extraction and detection.** SARS-CoV-2 RNA was extracted from nasopharyngeal swab sample of COVID-19 patients using Beijing Genomic Institute (BGI) MGIEasy Nucleic Acid Extraction Kit with MGISP-960 platform (BGI, China) following the manufacturer's protocol. For the viral RNA detection, reverse transcriptase-polymerase chain reaction (RT-PCR) was performed using BGI real-time RT-PCR kit for detecting 2019-nCoV (BGI, China). The primers target the viral *ORF1ab* gene, and the RT-PCR was run for 40 cycles.

### Sample selection

Random COVID-19 samples with a cycle-threshold ( $C_T$ ) value of  $< 25$  from various swabbing centres in Brunei were selected for whole-genome sequencing (WGS). A total of 2,920 samples were sequenced from the period of November 2021 to July 2022.

### Sequencing and analysis

NGS libraries were produced from the extracted viral RNA using MGI ATOplex RNA Universal Library Prep Module and sequenced with MGI DNBSEQ-G50 platform (MGI Tech Co, China) which utilises PCR-free nanoarrays of DNA nanoballs (DNBs). Sequencing was conducted according to the manufacturer's protocol for paired-end 150-bp reads. ATO-

Plex SARS-CoV-2 analysis tool called iGenome Virus Detector (iGVD) was used to process the raw NGS reads and assemble consensus SARS-CoV-2 genomes in a FASTA file format. The assembled genomes are then uploaded to the Nextclade webpage<sup>a</sup> (version 2.4.2) for phylogenetic analysis against the SARS-CoV-2 reference genome from Wuhan, China, and the SARS-CoV-2 variants are organised according to the Nextclade clade system.<sup>5</sup> Lastly, using Cov-Lineages<sup>b</sup>, the spread of the locally detected SARS-CoV-2 variants can be monitored by submitting their exact sublineages.<sup>6</sup>

### Data availability

All assembled SARS-CoV-2 genomes, in a form of FASTA file, were uploaded into Global Initiative on Sharing Avian Influenza Data (GISAID) repository<sup>c</sup>, once a genomic sequence database for influenza viruses now also used for SARS-CoV-2.<sup>7</sup>

## RESULTS

### SARS-CoV-2 variant analysis

From November 2021 to July 2022, two different variant shifts were observed in Brunei (Figure 1). The Delta variants were predominant in late 2021 until the Omicron variants were detected in early December 2021, of which they continue to be prevalent as of July 2022. In the span of six months where the Omicron variants became predominant, there was a shift in Omicron clades from 21L to 22B in mid-May 2022.

Two different Delta clades were observed in late 2021 which were 21I and 21J. The former was found to be the prominent clade and includes lineages such as AY.24 and AY.75. Further investigation revealed that the 21I clade was largely dominated by the sublineage AY.24.1. The 21J clade encompasses various lineages, but the most prevalent was AY.23 which was predominant-

<sup>a</sup> <https://clades.nextstrain.org/>

<sup>b</sup> <https://cov-lineages.org/>

<sup>c</sup> <https://gisaid.org/>

ly found in Singapore and Indonesia. The Delta variants were superseded by the Omicron variants around mid-February 2022.

The first reported Omicron variant case in Brunei belonged to the BA.1 lineage under the 21K clade. However, it was the 21L clade, specifically the BA.2 lineage, that became the predominant clade. There were several BA.2 sub-lineages detected, e.g., BA.2.2 and BA.2.4, but majority were either BA.2.3.19 or its paternal BA.2.3. Four months later, two new Omicron clades were observed, namely 22A and 22B, that includes BA.4 and BA.5 lineages, respectively. As of July 2022, 22B became the prominent clade and under this clade, the prevalent sublineages were divided between BA.5.2.1 and BF.5.

#### Brunei-specific SARS-CoV-2 sublineages

Further research on the AY.24.1 and BA.2.3.19 sublineages under the 21I and 21L clades, respectively, revealed that both were predominantly observed in Brunei. The two sublineages differ from their paternal lineage by set of mutations (Table I). The Delta variant AY.24 was prevalent in Indonesia and its sub-lineage AY.24.1 has acquired several mutations, including M1237I substitution in the *S* gene and G34W and D144Y substitutions in the *N* gene. In contrast, the Omicron variant BA.2.3 has been detected in various countries

and it branches out into several further sublineages. The local BA.2.3.19 can be differentiated from its paternal lineage by the presence of two mutations which are K417T and C2239G substitutions in the *S* and *ORF1a* genes, respectively.

## DISCUSSION

The observed changes in the SARS-CoV-2 variant in Brunei over an 8-month period starting from November 2021 could potentially account for the fluctuating number of local COVID-19 cases <sup>4</sup>. During the period when the Delta variant was dominant in late 2021, the number of COVID-19 cases was relatively low as the peak of the wave had passed. On 17<sup>th</sup> of December 2021, the first Omicron case, belonging to the BA.1 lineage under the 21K clade, was detected. It is interesting to note that a significant increase in COVID-19 cases was observed approximately one month later. In addition, this spike in cases was attributed not by the BA.1 lineage but by the BA.2 lineage under the 21L clade. This delay could be attributed to the stringent COVID-19 control measures that were in place during that time.

Brunei detected its first Omicron case nearly a month later after WHO announced that it become the 'Variant of Concern' on the 26<sup>th</sup> of November 2021.<sup>8</sup> In

**Table I: Set of mutations that distinguish SARs-COV-2 Variants AY.24.1 and BA.2.3.19 from its paternal lineage.**

SARs-COV-2 Variant	Nucleotide substitution	Amino acid substitution	Gene	Gene function
AY.24.1	G25273C	M1237I	<i>S</i>	Encodes for viral spike protein
	G28373T	G32W	<i>N</i>	Encodes for viral nucleocapsid
	G23703T	D144Y		
	G1820A	G519S	<i>ORF1a</i>	Encodes a polyprotein responsible for RNA synthesis
	C6354T	S2030L		
	A6458G	N2065D		
BA.2.3.19	A9650G	M3129V		
	T6980G	C2239G	<i>ORF1a</i>	Encodes a polyprotein responsible for RNA synthesis
	A22812C	K417T	<i>S</i>	Encodes for viral spike protein

<sup>4</sup> <https://covid19.who.int/region/wpro/country/bn>

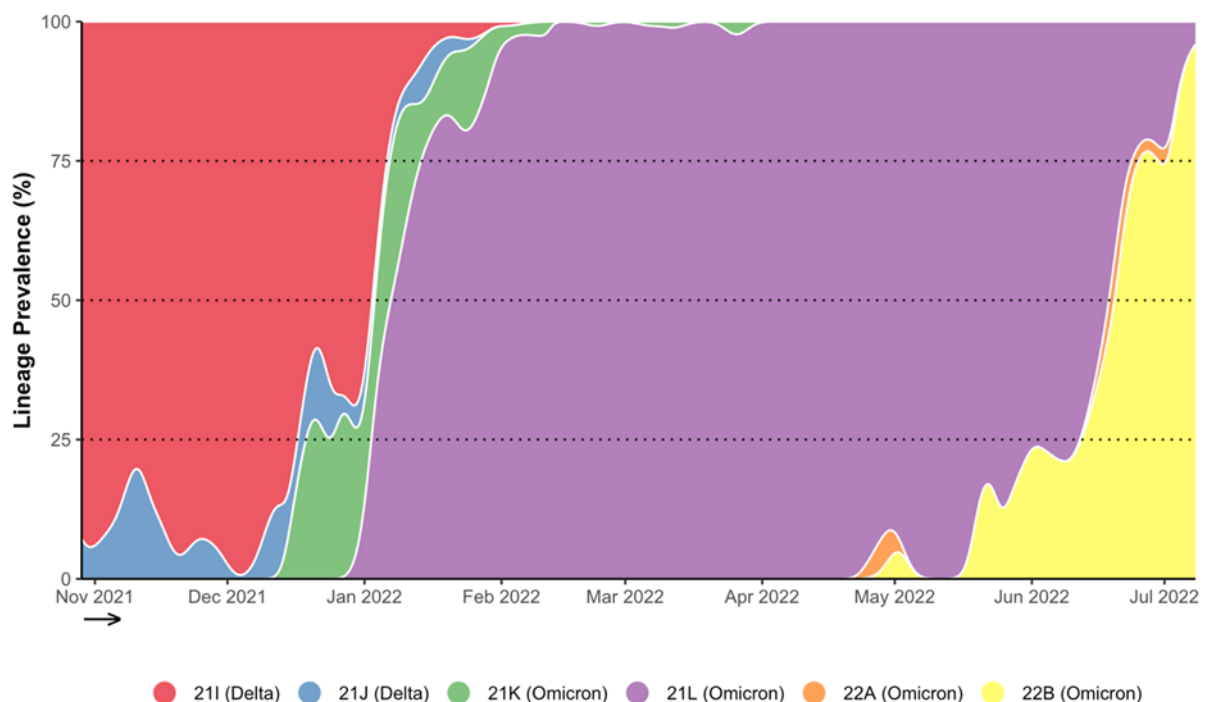


comparison to the neighbouring countries, the arrival of the Omicron variant in Brunei was relatively delayed, as Singapore and Malaysia detected it in early December.<sup>9,10</sup> This delay in detecting Omicron in Brunei can be mainly attributed to the stringent border control measures implemented in the country. Only a limited number of individuals, who are carefully selected, are permitted to travel outside of Brunei, aiming to mitigate the risk of importing new variants and controlling the spread of COVID-19 within the country.

Towards the end of May 2022, new lineages of Omicron, namely BA.4 and BA.5 under the 22A and 22B clade, respectively, were identified in Brunei. This discovery coincided with a subsequent spike in COVID-19 cases a few weeks later, primarily attributed to the BA.5 lineage. As COVID-19 control measures and border controls were gradually eased, the prominence of the BA.5 lineage became more pronounced, particularly after the middle of June. Similarly, Singapore experienced a surge of COVID-19 cases around June 2022, attributed to the BA.5 lineage.<sup>11</sup>

One major limitation of this study was the small sample size of COVID-19 samples selected on a daily basis, often consisting of about 40 samples. This limitation was primarily due to the constrained sequencing capacity available, which affected the ability to accurately represent the prevalence of different lineages. This limitation becomes evident when observing Figure 1, particularly in early May 2022, where there were days with no detected samples of the BA.4 and BA.5 lineages until later in the middle of May. While it is possible that more of these variants were present on these particular days, the low sample number can introduce a potential bias or skew the results, making it difficult to draw definitive conclusions about the absence or prevalence of certain lineages during those periods. In addition, there were occasional instances of contaminants found in the samples, resulting in the need for repeat sequencing. Moreover, technical issues with the sequencing machine periodically caused delays in detecting the variants.

It is known that over time viruses



**Figure 1: The progression of circulating SARS-CoV-2 variants in Brunei from November 2021 and early July 2022 organised according to the Nextstrain clade system.**



acquire new mutations that can be beneficial for their survival, SARS-CoV-2 included, and their effect ranges from increasing the transmissibility rate to evading the immune response.<sup>12-15</sup> It is evident that the mutations acquired by AY.24.1 and BA.2.3.19 are advantageous as they were once a predominant variant. These mutations could be those located in the *S* gene, namely M1237I and K417T, found in AY.24.1 and BA.2.3.19, respectively. The gene encodes the viral surface spike protein that not only binds to the angiotensin-converting enzyme 2 (ACE2) receptor on host cells, but also a crucial target of neutralising antibodies.<sup>14,16</sup> Mutations such as M1237I and K417T in the spike protein can alter the antigenicity of the virus which enables them to escape the immune response.<sup>14-18</sup>

Two Omicron sub-lineages, BA.5.2.1 and BF.5, under the same 22B clade were equally prevalent as of July 2022. It is unsure whether these sublineages will continue to be equally predominant or one will become predominant over the other. Moreover, a new Omicron sub-lineage, BA.2.75, has recently been reported and it may take over the BA.5 lineages.<sup>19</sup>

## CONCLUSION

In conclusion, Brunei Darussalam is continuously monitoring the circulating SARS-CoV-2 variant as well as the presence of evolving novel variant.

## CONFLICT OF INTEREST

All authors hereby declare that they have no conflicts of interest.

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