RESEARCH ARTICLE

SARS-CoV-2 Omicron variant of concern in the Seychelles: Introduction and spread

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Abstract

Background: The emergence of the Omicron variant of concern in late 2021 led to a resurgence of SARS-CoV-2 infections globally. By September 2022, Seychelles had experienced two major surges of SARS-CoV-2 infections driven by the Omicron variant. Here, we examine the genomic epidemiology of Omicron in the Seychelles between November 2021 and September 2022.

Methods: We analysed 618 SARS-CoV-2 Omicron genomes identified in the Seychelles between November 2021 and September 2022 to infer virus introductions and local transmission patterns using

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phylogenetics and the ancestral state reconstruction approach. We then evaluated the impact of government coronavirus 2019 (COVID-19) countermeasures on the estimated number of viral introductions during the study period.

Results: The genomes classified into 43 distinct Pango lineages. The first surge in Omicron cases (beginning November 2021 and peaking in January 2022) was predominated by the BA.1.1 lineage (59%) co-circulating with 11 other Omicron lineages. In the second surge (between April and June 2022), four lineages (BA.2, BA.2.10, BA.2.65 and BA.2.9) co-circulated and these were swiftly replaced by BA.5 subvariants in July 2022, which remained predominant through to September 2022. In the latter period, sporadic detections of BA.5 subvariants BQ.1, BE and BF were observed. We estimated 109 independent Omicron importations into Seychelles over the 11-month period, most of which occurred between December 2021 and March 2022 when strict government restrictions (SI>50%) were still in force. The districts Anse Royale, and Baie St. Anne Praslin appeared to be the major dispersal points fuelling local transmission.

Conclusions: Our results suggest that the waves of Omicron infections in the Seychelles were driven by multiple lineages and multiple virus introductions. The introductions were followed by substantial local spread and successive lineage displacement that mirrored the global patterns.

Keywords
Seychelles, Omicron, SARS-CoV-2

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Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) Omicron variant of concern (VOC) was first detected in South Africa and Botswana in late November 2021 and outcompeted the Delta VOC to become the globally dominant SARS-CoV-2 variant since March 2022[1]. The genetic evolution of Omicron has resulted in the emergence of 623 lineages within it as of the 9th of January 2023, some with enhanced fitness, such as BA.2.75, BA.4, BA.5, BQ.1, BQ.1.1 and XBB.1.5[2–5]. These lineages are characterized by either reduced sensitivity to neutralization by specific monoclonal antibodies[6], or reduced sensitivity to sera of convalescent patients or vaccinated people[7,8].

By September 2022, Seychelles had experienced three waves of infections (*Extended data,* Supplementary Figure 1[9]). The highest surge of cases occurred between November 2021 and February 2022, and was caused by the Omicron variant *Extended data*, Supplementary Figure 1[9]). In a previous study, we described the genomic epidemiology of SARS-CoV-2 in Seychelles from the beginning of the pandemic until December 2021[10]. The study revealed that multiple introductions of distinct variants of concern in the country drove the wave of infections in the country. Since January 2021, observed infections in the islands were primarily due to the introduction of highly transmissible variants; Alpha, Beta and Delta[10].

To characterize the emergence and spread of Omicron in the Seychelles, we phylogenetically analyzed consensus genomes obtained from samples collected in the islands between November 2021 and September 2022 and compared them to contemporaneously sampled global sequences (n=6,366) that were similar to Omicron lineages detected in Seychelles. We used phylogeographic reconstructions to characterise the geographical origin of introduction events in the Seychelles and the subsequent dispersal of the virus between districts in Seychelles.

Methods

Ethical considerations

The study protocol was reviewed and approved by the Scientific and Ethics Review Committee (SERU) at Kenya Medical Research Institute (KEMRI), Nairobi, Kenya in July 2020 (SERU protocol #4035). Individual patient consent was not required by the committee for the use of these samples for genomic surveillance to inform public health response.

Study samples

SARS-CoV-2 positive samples identified as part of the routine coronavirus 2019 (COVID-19) surveillance in the country, as well as those from individuals who had contact with confirmed COVID-19 cases were considered for whole genomes sequencing. Samples were sequenced at Seychelles Public Health Laboratory (SPHL), Mont Fleuri, Mahe, Seychelles or KEMRI-Wellcome Trust Programme (KWTRP) in Kilifi, Kenya as part of a collaboration with WHO AFRO, Africa-CDC and SPHL to support the sequence of SARS-CoV-2 positive cases detected in the Seychelles.

Laboratory procedures

RNA extraction and PCR. Ribonucleic acid (RNA) was extracted from nasopharyngeal/oropharyngeal (NP/OP) samples using either the QIAamp Viral RNA Mini Kit (Qiagen, Manchester, United Kingdom, Catalog Number: 52906) as recommended by the manufacturer or the UNI MEDICA - Automatic DNA Extraction System NFAST96. SARS-CoV-2 was detected using two different RT-PCR assays. The first assay was the TaqPath COVID-19 Combo Kit (ThermoFisher, UK, Catalog Number; A47814), which targeted the N gene, S gene and ORF1ab gene, and included an internal control (MS2 phage) provided by the kit. The second assay used was the Seegene Allplex™ SARS-CoV-2 Assay (Seegene, Korea, Catalog number: RV10248X), which targeted the RdRP, E, S, and N genes and included both the kit positive control and the kit internal control. Both RT-PCR assays were run on the Applied Biosystems™ 7500 Real-Time PCR System (Applied Biosystems, Warrington, United Kingdom). The thermal cycling conditions for the Taq TaqPath COVID-19 Combo Kit (ThermoFisher, UK, Catalog Number; A47814) were as follows: reverse transcription at 50°C for 5 minutes, activation at 95°C for 20 seconds, followed by 40 cycles of 95°C for 3 seconds and 58°C for 45 seconds. The thermal cycling conditions for the Seegene Allplex™ SARS-CoV-2 Assay were as follows: initial incubation at 50°C for 20 minutes and 95°C for 15 minutes followed by 45 cycles of 94°C for 10 seconds, 60°C for 30 seconds, and 72°C for 10 seconds.

Sequencing and genome assembly. Samples were considered for whole genome sequencing based on PCR cycle threshold (Ct) scores below 34 for samples sequenced at KWTRP lab and below 30 for samples sequenced at SPHL. Sequencing was carried out using Oxford Nanopore Technologies (ONT) GridION (n=469) and Illumina iseq100 (n=149). Sequencing by ONT was carried out as previously described[1]. Briefly, reverse transcription was done using LunaScript RT SuperMix kit as recommended by the manufacturer (New England Biolabs, UK, Catalog number: E3010) and the cDNA amplified using Q5® Hot Start High-Fidelity 2x kit (New England Biolabs, UK, Catalog number: M0494) with V4.1 primers (Eurofins Genomics, Germany). Sequencing libraries were generated using LSK-109 ligation kit (Oxford Nanopore Technologies Ltd, UK, Catalog number: SQK-LSK109) and the ONT Native Barcoding Expansion kit 1-96 (New England Biolabs, UK, Catalog number: EXP-NBD196) for sequencing on ONT GridION platform. Sequencing by Illumina was carried out at SPHL. Reverse transcription was done using LunaScript RT SuperMix kit (New England Biolabs, UK, Catalog number: E3010) and the thermal cycler conditions were set as follows; annealing at 65°C for 3 minutes followed by 25°C for 5 minutes, 50°C for 10 minutes, 80°C for 5 minutes and hold at 4°C. cDNA was amplified using Q5® Hot Start High-Fidelity 2x kit (New England Biolabs, UK, Catalog number: M0494) with V3 primers. PCR conditions were set as follows; initial denaturation for 30 minutes at 98°C, followed by 35 cycles of denaturation for 15 seconds at 98°C, and 5 minutes of annealing and extension at 63°C and hold at 4°C. DNA tagmentation was done using the COVIDSeq Library preparation kit Library kit (Illumina, USA,) with an incubation at 55°C for 5 minutes and hold at 10°C. The tagmented DNA was then amplified using Illumina IDT-ILM Nextera DNA UD Indexes 384 – Nextera XT (Illumina, USA, Catalog number: FC-131-1002). PCR was carried out using the following conditions: initialization for 3 minutes at 72°C, initial denaturation for 3 minutes at 98°C, followed by 7 cycles of denaturation for 20 seconds at 98 °C, annealing for 30 seconds at 65°C, extension for 1 minute at 72°C, and a final extension step for 3 minutes at 72°C and hold at 10°C. The library was then denatured and sequenced on iseq100.

Genome assembly. The sequence reads were assembled using ARTIC bioinformatics pipeline for ONT using the 4.1 primer scheme and DRAGEN COVID lineage application for the Illumina using the V3 primer scheme.

Lineage assignment. Sequence quality checks and mutation calling were performed using NextClade v2.11.0. Lineage analysis with Pangolin v4.0.6. The sequences were aligned using Nextalign version 1.4.1 (accessed on 12 June 2022).

Global contextual sequences. The global contextual sequences were retrieved from GISAID using the inclusion criteria: (i) presence of the full sample collection date (year–month–day), (ii) sample collected between November 20, 2021, and September 30, 2022, (iii) Omicron lineages detected in the Seychelles, and (iii) had >95% coverage (less than 5% Ns). All the sequences and associated metadata in this dataset are published in GISAID EpiCoV database under the GISAID EPI_SET identifier: 10.55876/gis8.230119zw or EPI_SET_ID : EPI_SET_230119zw, showing the contributor of each sequence with details of accession number, virus name, collection date, originating lab, submitting lab and the list of authors.

Phylogenetic analyses. The global sequence dataset and the sequences from the Seychelles were aligned to the reference SARS-CoV-2 genome (accession NC_045512) using Nextalign version 1.11.0 (accessed on 06 June 2022). A phylogenetic tree was inferred using IQTREE version 2.1.3 (accessed on 06 June 2022). IQTREE initiates tree reconstruction after assessment and selection of the best model of nucleotide substitution for the alignment. The ML tree topology was then time calibrated using TreeTime version 0.8.1[11]. The resulting trees were visualized using the Bioconductor “ggTree version 2.2.4” package[12] in R v4.1.1[13].

Estimation of virus importation and exportation into the Seychelles. The global time-calibrated tree was used to estimate the number of viral transmission events between the Seychelles and the rest of the world as described previously[10,14]. Briefly, The ML tree was transformed into a dated phylogenetic tree, mapping the location of sampled sequences to the external tips of the trees. TreeTime’s mugration model was used to infer the most likely location for internal nodes in the trees. We then counted the number of state changes from the root to the external tips. The state changes are counted when an internal node transitions from one country to a different country in the resulting child-node or tip(s). The timing of transition events is then recorded which serves as the estimated import or export event. The transmission events were visualised using ggalluvial[15] and tidyverse[16] packages in R v4.1.1[13].

Results

A total of 618 SARS-CoV-2 genomes, designated as the Omicron VOC were generated in this study. The generated genomes were from samples collected from all the 26 administrative districts and one Island group (Outer Islands group) in the Seychelles between November 27th 2021 and September 30th 2022 (*Extended data,* Supplementary Table 1[9]). The median age of the individuals with sequenced samples was 32 years (range 0 – 100 years), 54.9% of whom were female (n = 339).

SARS-COV-2 Omicron variant in the Seychelles

The 618 Seychelles genomes were classified into 43 Pango lineages (Table 1). Omicron was first detected in the Seychelles in

Table 1. Omicron lineages detected in the Seychelles between 29 November 2021 and September 30th, 2022.

Clade*	Lineage**	Count	% of VOC
21K (Omicron)	BA.1	14	2.3
	BA.1.1	361	59
	BA.1.1.6	1	0.2
	BA.1.1.7	1	0.2
	BA.1.10	1	0.2
	BA.1.14	1	0.2
	BA.1.15	1	0.2
	BA.1.17	2	0.3
	BA.1.17.2	9	1.5
	BA.1.18	10	1.6
	BA.1.19	1	0.2
	BA.1.20	3	0.5
	BA.1.21	2	0.3
	BA.1.7	1	0.2
	BA.1.9	2	0.3
21L (Omicron)	BA.2	50	8.2
	BA.2.10	8	1.3
	BA.2.2.1	1	0.2
	BA.2.37	2	0.3
	BA.2.65	10	1.6
	BA.2.69	1	0.2
	BA.2.76	1	0.2
	BA.2.9	47	7.7
	BA.2.9.2	1	0.2
	BA.2.9.5	7	1.1

Clade*	Lineage**	Count	% of VOC
22A (Omicron)	BA.4	3	0.5
	BA.4.1	11	1.8
22B (Omicron)	BA.5.1	7	1.1
	BA.5.1.23	1	0.2
	BA.5.2	13	2.1
	BA.5.2.1	11	1.8
	BA.5.2.12	1	0.2
	BA.5.2.20	3	0.5
	BA.5.2.26	3	0.5
	BA.5.2.8	2	0.3
	BA.5.3	1	0.2
	BE.1	3	0.5
	BE.1.1	3	0.5
	BE.1.1.1	1	0.2
	BE.4	1	0.2
	BF.3	1	0.2
22C (Omicron)	BA.2.12.1	3	0.5
22D (Omicron)	BA.2.75.2	1	0.2
	BA.2.75.6	1	0.2
22E (Omicron)	BQ.1	4	0.7
Recombinant	XAH	6	1
Total		**618**	**100**

* Classification based on NextClade (https://clades.nextstrain.org)

** Classification based Pangolin (https://github.com/cov-lineages/pangolin)

samples collected in Beau Vallon District on the 29th November 2021 (Figure 1). After its detection in the country, the Omicron variant co-circulated with Delta (lineage AY.122) until February 2022, after which it was the only variant detected in all sequenced samples (Figure 1). Omicron BA.1.1 lineage dominated, accounting for 59% of all Omicron sequences analyzed, followed by BA.2 (9%) and BA.2.9 (7%). The remaining 40 lineages accounted for ~24% of all the Omicron sequences analysed (Table 1). BA.1.1 was the prevalent lineage in the country for the first five months of the Omicron wave (December 2021 to April 4, 2022), and was the most common variant among samples collected during the upsurge of cases in late 2021 and early 2022 (Figure 1). During the same period, lineages BA.1.9, BA.1, BA.1.17, BA.1.18. BA.1.20, BA.1.14, BA.1.17.2 and BA.2 were also seen, albeit in lower numbers. Other prevalent lineages include BA.2 and BA.2.9 which accounted for 67% of the samples collected between March and May 2022, BA.5.2 (July–September), and BQ.1 (September).

Lineage BA.1.1 was the most geographically widespread lineage in our dataset, identified in all the 26 different districts/three main islands and one island group (Figure 1). Other notable lineages that had widespread distribution include BA.1 and BA.2. From our dataset, we also detected a recombinant lineage - XAH.

SARS-CoV-2 Omicron genetic diversity in the Seychelles

To understand the global context for Omicron variants in the Seychelles, we carried out phylogenetic analysis of the sequences collected from the Seychelles coupled with a background of 6,366 globally sampled contemporaneous sequences. Time-stamped phylogeny of these data showed that the Seychelles viruses were interspersed as clusters (> 2 sequences) or singletons within the contemporaneous global samples, suggesting that multiple independent viral introductions have occurred through time followed by local transmission (Figure 2). The Seychelles monophyletic clusters observed on global trees contained sequences from different geographical districts indicative of extensive within-country spread of the virus (Figure 2). Notably, the Seychelles XAH cluster (six sequences) composed of sequences only sampled in Desroches island on a single day (3rd April 2022) pointing towards local transmission (Figure 2). A sub-analysis of the XAH recombinant lineage linked to a possible local transmission in Desrosches Island revealed that these genomes differed minimally (zero to five nucleotide differences). Our analysis shows that the XAH lineages carried a C8772T nucleotide change that was rare outside the Seychelles in XAH viruses reported elsewhere around the globe. The Seychelles sequences lacked five nucleotide changes that were common in viruses outside the Seychelles. This could imply that the XAH virus in the Seychelles emerged locally. The XAH genomes were screened for potential recombination breakpoints using the sc2rf tool. Breakpoints were detected between positions 24504 and 26059 of the genome. The sc2rf tool confirmed the XAH lineage in circulation was a recombinant of BA.2 lineage as the major parent and BA.1.1 lineage as the minor parent in all the six XAH cases (Figure 3). This hypothesis is conceivable since lineages BA.1.1 and BA.2 co-circulated when the XAH lineage was detected.

Estimating the number of viral introductions into the Seychelles

To infer the number of viral imports and exports between the Seychelles and the rest of the world, and within the Seychelles, we used ancestral location state reconstruction on a time-stamped phylogeny. The regression of root-to-tip genetic distance against sampling dates revealed a strong temporal signal (rate = 7.90 x10^{-4}, r^2 = 0.46,) in our final dataset after excluding outlier sequences (n=302). Through this approach, we estimated at least 109 virus introductions into the country during the study period, mainly originating from Europe (n=68), Asia (n=22) and Africa (n=10) (Figure 4). The number of imports and exports were high between December 2021 and March 2022 despite strict government restrictions (SI>50%) (Figure 4), though during this time the country was open to incoming air travel/tourists. The high number of imports into the Seychelles occurred when the surge of cases driven by

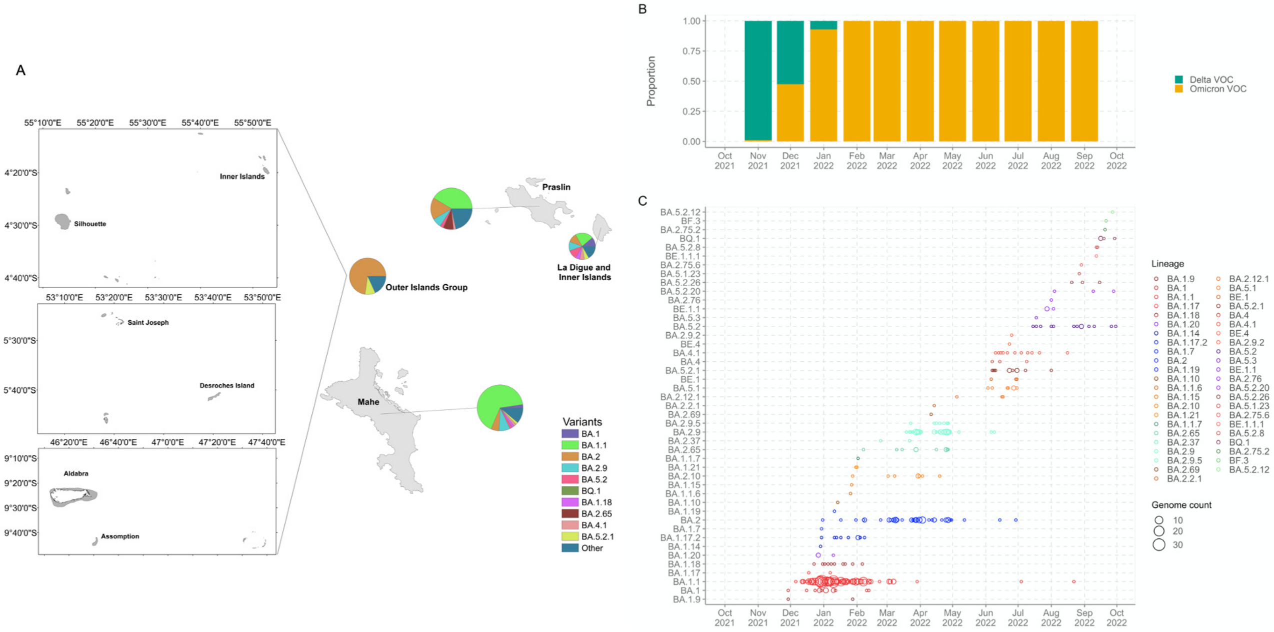

Figure 1. A) Map of the Seychelles showing the distribution of the Omicron lineage in the three main islands (Mahe, Praslin and La Digue and inner islands) and a selection from the Outer Islands group. Inset maps show a selection of islands belonging to the Outer Islands group. The size of the pie chart is proportional to the number of genomes per island or island group. **B**) Temporal distribution of the lineages identified in the Seychelles between November 2021 and September 2022. **C**) Temporal occurrence of the Omicron lineages between November 2021 and September 2022.

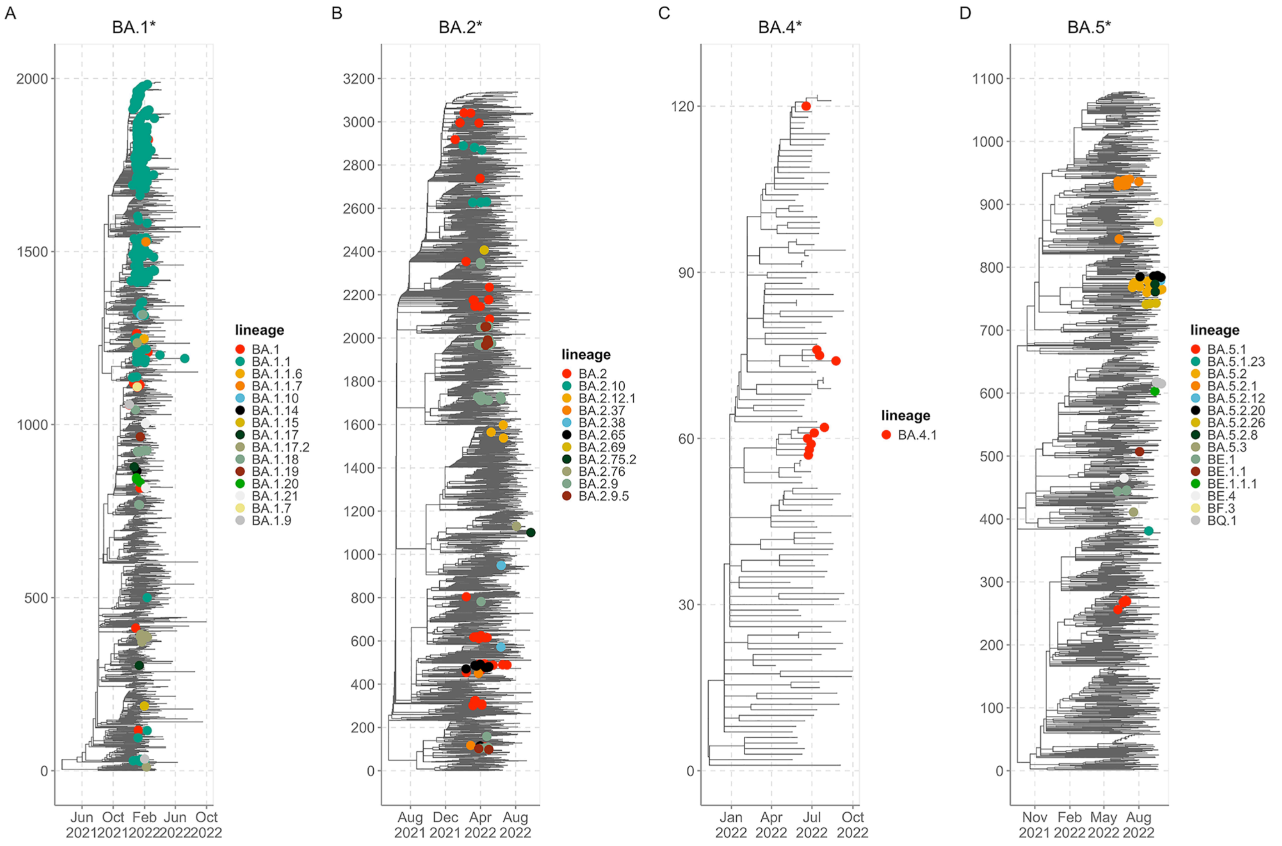

Figure 2. Global phylogenetic context of Omicron variants observed in the Seychelles. A) Phylogeny of BA.1 lineages. **B**) Phylogeny of BA.2 lineages. **C**) Phylogeny of BA.4 lineages. **D**) Phylogeny of BA.5 lineages. Seychelles genomes are identified by circular tip labels and the distinct colours identify the different Omicron lineages.

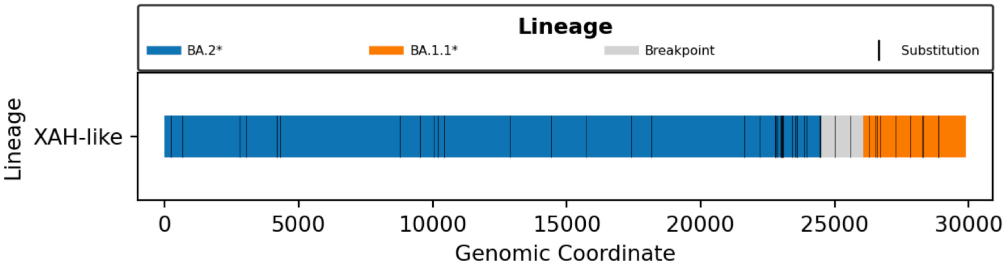

Figure 3. Recombination plot of XAH genomes showing the putative parent lineages and coordinates of breakpoints on the genome. This figure was created using ncov-recombinant.

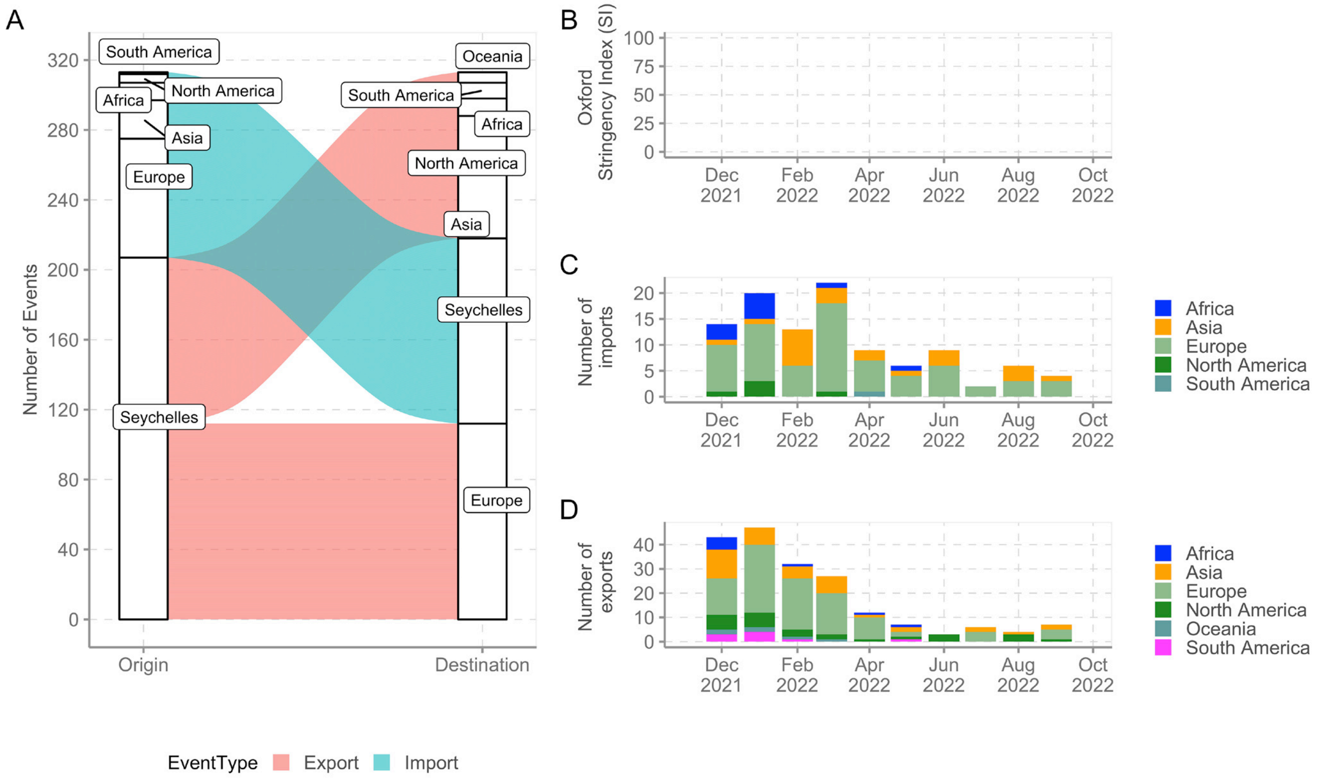

Figure 4. Virus importations and exportations from the Seychelles. A) Alluvium plots showing the estimated number of importations into and exportations from the Seychelles. **B**) The Seychelles government intervention levels as measured by the Oxford stringency index (SI)[17]. **C**) Number of importation events into the Seychelles by month. **D**) Number of exportation events from the Seychelles by month.

BA.1 and BA.2 were observed in Europe Asia and Africa. Our phylogeographic analysis established that Anse Royale, Baie St. Anne Praslin, Perseverance, Anse Aux Pins, and Anse Etoile districts had a significant role in the viral circulation and were the major sources of viral dispersal events to other surrounding districts within this island country (*Extended data*, Supplementary Figure 2[9]).

Discussion

This study provides a description of the introductions and spread of the Omicron variant in Seychelles based on phylogenetic and phylodynamic analyses. We counted 109 independent introductions of the Omicron variant into the Seychelles between November 2021 and September 2022 likely originating from Europe, Asia, and Africa. From our dataset, we show that the rapid surge of COVID-19 cases that began in November 2021 and peaked in January 2022 was mainly driven by BA.1.1 lineage, together with other co-circulating lineages such as variants BA.1.9, BA.1, BA.1.17, BA.1.18, BA.1.20, BA.1.14, BA.1.7, BA.1.17.2, BA.1.10, BA.1.19 and BA.2. The rapid surge of cases is attributed to the high infectivity properties of the Omicron variant[18], leading to a high percentage of infections in the country within a short period of time. The BA.5-like lineages were first detected in July 2022 and remained dominant throughout the surveillance period, with sporadic detections of BQ.1-like, BE-like, and BF-like lineages occurring in

September and October of 2022. At the time of writing this report (January 2023), Omicron BQ.1-like lineages are on the rise at the global scale[1], and possess mutations that are thought to offer increased immune-escape properties[4]. The detection of BQ.1 (n=4) in the Seychelles in samples collected in September 2022 demonstrates the value of routine surveillance to provide early warning signals of possible outbreaks and it calls for enhanced genomic surveillance to monitor the extent of the spread of this lineage in the country.

Our ancestral state reconstruction analysis identified Anse Royale, Baie St. Anne Praslin, Perseverance, Anse Aux Pins, and Anse Etoile districts as the major contributors of viral exchanges to other locations within the Seychelles. These districts have the largest populations in the Seychelles[19], but then again, this observation could be influenced by a high number of genomes representation from these regions. The variations in the number of sequences from different districts do affect the migration rate estimates and the ancestral reconstructions. Despite these potential biases, our phylogeographic reconstruction likely reveals general patterns of viral spread across the country.

This study reveals the high genomic diversity of Omicron lineages circulating in the Seychelles, as indicated by the multiple clusters on the global phylogenetic tree. The Seychelles virus diversity appears to be nested within the global viral diversity as a result of the global transmission of the virus facilitated by unrestricted movement, increased connectivity, and social mixing after relaxation of COVID-19 countermeasures in the country.

This study had some limitations. First, only a small proportion of confirmed cases were sequenced, we only prioritized samples with a Ct value of <34.0 for samples sequenced at KWTRP and <30.0 for samples sequenced at SPHL. Second, we acknowledge that we did not include other data types in trying to infer virus importations, such as travel history data. Incorporating travel data in phylogeography analysis could help address representation of under sampled locations[20]. Third, the import/export inferences can be influenced by sampling biases of the global dataset used in the analysis.

Overall, our results suggests that the Omicron wave in the Seychelles was driven by multiple viral introductions followed by wide scale, spread within the country. Observation of a high number of imports when the government COVID-19 restrictions were strict (SI>50) and low when the government restriction were relaxed (SI<50) supports suggestions by other studies[21], to focus control strategies that will reduce local transmission rather than international travel/border closure. The detection of a recombinant Omicron virus in Desrosches Island is of interest as it shows the potential of insular communities producing recombinant strains which may have an impact on the global community, e.g. chikungunya outbreak in 2005 in the Reunion Islands. Furthermore, SARS-CoV-2 has shown the capacity to diversify at an accelerated rate leading to new variants/lineages. Therefore, as the pandemic progresses and new variants/lineages and recombinants continue to emerge around the globe, it is essential to monitor their global transmission and trace international arrivals into the country in order to develop effective control strategies.

Data availability

The genome sequences reported here are available on GISAID (accession number: EPI_SET_230119bd https://doi.org/10.55876/gis8.230119bd)

Underlying data

Harvard Dataverse: Replication Data for: SARS-CoV-2 Omicron Variant of Concern in Seychelles: Introduction and Spread. https://doi.org/10.7910/DVN/YA4LXD[9]

This project contains the following underlying data and script used in the analyses:

- data.zip (Contains all data files used in the analysis: 5 csv and 4 nexus files)
- JMwita_Omicron_Seychelles_Data_Codebook.pdf (Describes data in the data.zip and script-1.zip)
- JMwita_Omicron_Seychelles_Data_Readme.txt (Describes data in the data.zip and script-1.zip)
- scripts-1.zip (Contains R and python scripts used to analyze the data)

NCBI Genbank: Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome, Accession number NC_045512, https://identifiers.org/nucleotide:NC_045512

Extended data

Harvard Dataverse: Replication Data for: SARS-CoV-2 Omicron Variant of Concern in Seychelles: Introduction and Spread. https://doi.org/10.7910/DVN/YA4LXD[9].

This project contains the following extended data:

- SuppFig1_09012022.pdf (Epidemic curve for Seychelles showing the daily positive case numbers)
- SuppFig2_09012022.pdf (Number of viral exchange between the 27 administrative district in Seychelles)
- Supplementary_Table1.docx (Number of Omicron genomes per administrative district)
- Supplementary_Table2.docx (Describe the demographic distribution of sequenced Omicron cases)
- gisaid_supplemental_table_epi_set_230119zw.pdf (Contain link to the global sequence dataset used files that were used in the analysis and were downloaded from GISAID.

Acknowledgments

We thank the Seychelles Ministry of Health, Public Health Laboratory, Africa-CDC, WHO/AFRO, WHO-Kenya Office, WHO-Seychelles Office for facilitating sharing of SARS-CoV-2 samples that were sequenced in this study. We gratefully acknowledge the Authors from the Originating laboratories responsible for obtaining the specimens and the Submitting laboratories where genetic sequence data were generated and shared via the GISAID Initiative[22], on which this research is based. Submitting and the Originating laboratories of the GISAID data used in this study are listed in the gisaid_supplemental_table_epi_set_230119zw.pdf (see *Extended data*[9]).

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Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 30 August 2023

https://doi.org/10.21956/wellcomeopenres.20965.r64997

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Steven C. Holland
Arizona State University, Tempe, Arizona, USA

The report SARS-CoV-2 Omicron variant of concern in the Seychelles by Marobe et al. Overall the article is well written, generally well presented, and draws well-reasoned conclusions. The greatest strength of this article is its use of a semi-isolated region (Seychelle island system) to analyze viral transmission events using genomic epidemiology practices. The semi-isolated nature of the island system provides enough data to analyze and create a clearer picture of transmission, opposed to another location that would have too many transmissions and introductions. There are however, some areas that I would like to see addressed before final approval.

Major concern:
The analysis provided by the reviewer is very interesting, as the origin of viral import events matches well with visitor/tourist origin. I think explicitly mentioning this fact in the article would strengthen the article.

Major:
The initial introduction of Omicron lineages was problematic for amplicon generating primers in the ARTIC (and other) workflows. Were these issues present when creating the genome sequences used in the study? If regions of the resulting genomes are ambiguous, were they penalized in the alignments/phylogenies, or treated as wildcards?

Addressing this issue in the limitations is suggested. This limitation would also be present in many GISAID submissions around that timeframe.

Major:
Figure 4 is missing data. Also, provide a citation for Oxford Stringincy Index within the main text body at first mention. Is citation #17 correct? The provided link does not mention the stringency index methodology, just data that could be used to make that determination, possibly this (https://ourworldindata.org/covid-international-domestic-travel)? I guess simply: how was SI calculated?

Minor:
A little confusing that the primer design scheme (i.e. ARTIC) for sequencing wasn't provide when first mentioned during library building. It is later mentioned/linked in the genome assembly, but earlier mention would be helpful. Also, mention ARTIC and DRAGEN pipeline version numbers, should links deprecate in the future.

Minor:
Some purported recombinant lineages have been erroneously published by other groups due to sequencing technicalities. I am not suggesting errors on the part of the authors, but I would suggest uploading sequencing data for XAH recombinant lineage to the Sequencing Read Archive (SRA), or other database, if permissible. This would allow others to review the data if they have concerns.

Minor:
Difficulty in reading some figure 1. Text size, weight, and color into account and try to improve contrast

Minor:
Figure 3 legend: List ncov-recombinant version.
Minor:
"(" needed before "Extended data, Supplementary Figure 1)"

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbiology, molecular genetics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 18 July 2023

https://doi.org/10.21956/wellcomeopenres.20965.r60421

? **Jordan Douglas**
The University of Auckland, Auckland, New Zealand

Morobe and coauthors have analysed the spread of Omicron in Seychelles using phylogenetic methods. They estimated the number and source of introductions into the country. This manuscript is very well-written and succinct, yet also thorough in its methodology. I very much enjoyed reviewing this paper. However, I do have some concerns which I am sure can be addressed in a revised version. These concerns are mostly related to the discussion and figures.

Major concern: The methodologies being used are not cited properly. For example: NextClade, Pangolin, IQTREE, ARTIC, nov-recombinant, and many others. This manuscript provides URLs to the various webpages of the methodologies/applications used, however in most cases the actual methodology articles are completely lacking from the references section.

Major concern: There are massive sampling biases on GISAID, for example a disproportionately high number of their submissions are from the UK even though they have just a small % of the world's population. This kind of sampling bias can have quite profound effects on phylogeographical models (eg. see De Maio 2015, Plos genetics). My question is did the sampling protocol employed here try to correct for these biases, for example by downsampling from overrepresented parts of the world? The discussed caveat “Third, the import/export inferences can be influenced by sampling biases of the global dataset used in the analysis.” could do with more detail around the potential effect of sampling bias.

Following from this issue, I was initially sceptical about the estimate that 68 of the 109 introductions were from Europe, given that Seychelles is in Africa. But after visiting the Seychelles tourism website https://www.nbs.gov.sc/statistics/tourism, I see now that in the year 2022, Europe was number 1 ranked by visitor arrivals, followed by Asia and then Africa. These numbers are quite consistent with what was reported in the manuscript (68 from Europe, 22 Asia, 10 Africa). I think the migration data corroborates your results very nicely, and the similarity should be discussed. It will also address my concern above about potential effects of sampling bias.

Major concern: This is far from the first study on COVID phylodynamics (see Attwood 2022, Nature reviews), especially those which count introductions from other parts of the world. And yet, there is no discussion on how these methods/results compare with prior studies, particularly nations with similar geography/population density, eg. low density islands/archipelagos, or nearby nations in Africa. How did the number/ratio of introductions to exports compare with other studies? How did the cluster sizes compare?

Figure 1A: The font is too small, making the figure quite hard to read. This figure is quite important because most readers won’t be familiar with Seychelles. Moreover, the major districts (especially those named in the main text: Anse Royale, Baie St. Anne Praslin etc), should be pointed out on this map, rather than just in the extended data.

Figure 1C: This figure is important and tells a very interesting story, but the image itself is quite hard to read as everything is too small and there are too many dashed lines. It’s hard on the eye. The colour coding doesn’t really help either because there are too many colours. I think the image would be a lot clearer if the y-axis labels were moved to the right so that the label of each lineage was at the x-position of the first case. This would also mean that the horizontal dashed lines can be dropped, making it easier on the eye.
Figure 2: the years on the x-axis are too crammed, especially on fig 2A. You could drop the first 2 digits eg. ‘21 and ‘22 to make more room. I’m also confused why there is a ‘*’ next to each variant name at the top of the figure

Figure 4B: why is there no dataset on this plot?

Minor issues:

- Page 5: “*Briefly, The ML tree was transformed*” -> “The” should be lowercase
- “SI” is used in the final paragraph but its meaning (stringency index) is only defined in a figure caption. The acronym should be defined in the main text, not just the caption
- “*chikungunya outbreak in 2005 in the Reunion Islands*” -> is there a reference, perhaps a public health statement, which documents this outbreak?

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Phylogenetics, phylodynamics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have

significant reservations, as outlined above.