SARS-CoV-2 Variants in Rhode Island; May 2022 Update

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ABSTRACT

BACKGROUND: Genomic surveillance allows identification of circulating SARS-CoV-2 variants. We provide an update on the evolution of SARS-CoV-2 in Rhode Island (RI).

METHODS: All publicly available SARS-CoV-2 RI sequences were retrieved from https://www.gisaid.org. Genomic analyses were conducted to identify variants of concern (VOC), variants being monitored (VBM), or non-VOC/non-VBM, and investigate their evolution.

RESULTS: Overall, 17,340 SARS-CoV-2 RI sequences were available between 2/2020–5/2022 across five (globally recognized) major waves, including 1,462 (8%) sequences from 36 non-VOC/non-VBM until 5/2021; 10,565 (61%) sequences from 8 VBM between 5/2021–12/2021, most commonly Delta; and 5,313 (31%) sequences from the VOC Omicron from 12/2021 onwards. Genomic analyses demonstrated 71 Delta and 44 Omicron sub-lineages, with occurrence of variant-defining mutations in other variants.

CONCLUSION: Statewide SARS-CoV-2 genomic surveillance allows for continued characterization of circulating variants and monitoring of viral evolution, which inform the local health force and guide public health on mitigation efforts against COVID-19.

KEYWORDS: COVID-19, SARS-CoV-2, variants, genomic sequencing, Rhode Island

BACKGROUND

The coronavirus disease 2019 (COVID-19) pandemic, resulting from the spread of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), has caused significant global morbidity and mortality. As of May 17, 2022, the COVID-19 pandemic has resulted in at least 519,766,413 infections and 6,268,690 deaths worldwide, of which at least 81,682,634 infections and 992,415 deaths were in the United States alone.[1] However, despite the ongoing public health mitigation efforts – including travel bans, social distancing, and stay-at-home orders – and the availability of vaccines, COVID-19-related morbidity and mortality continue to rise.[2]

Since publication of its first genomic sequence,[3] SARS-CoV-2 has accumulated numerous mutations throughout its genome, with gradual predominance of those conferring selective advantage in human hosts.[4,5] These mutations are natural to the viral life cycle and, as seen in SARS-CoV-2, often result from an error-prone replication process, yielding viruses with novel mutations termed variants.[6] In SARS-CoV-2, such mutations commonly occur in the receptor binding domain (RBD) of the spike protein, which interacts with the angiotensin-converting enzyme 2 (ACE2) human cellular receptor and facilitates viral fusion and entry into host cells.[7]

Enhanced genomic surveillance efforts since the end of 2020, when the first clinically significant SARS-CoV-2 Alpha variant was recognized, have noted the continued emergence of multiple lineages and sub-lineages in different parts of the world,[8-12] some of which have shared attributes that justified close monitoring and public health actions. The United States Centers for Disease Control and Prevention (CDC) continues to define variants being monitored (VBM; associated with severe disease or increased transmission but circulating at low levels); variants of interest (VOI; associated with changes to receptor binding, reduced antibody neutralization, reduced treatment efficacy, or increased transmissibility); variants of concern (VOC; associated with significantly reduced antibody neutralization, reduced treatment/vaccine efficacy, increased transmissibility, increased disease severity, or diagnostic detection failures); and variants of high consequence (VOHC; associated with significantly reduced effectiveness of prevention measures or medical countermeasures).[13] The current CDC-defined variant classifications, as well as the related World Health Organization (WHO) nomenclature,[14] are provided in **Table 1**.

Since the first reported case of SARS-CoV-2 in China in December 2019, five major COVID-19 waves have occurred in RI, in parallel to the global trends. These waves and the associated predominant variants, discussed further below, include the (i) Wuhan-Hu-1 strain from February 2020 to July 2020 (peak incidence in April 2020),[15] (ii) D614G variant from June 2020 to February 2021 (peak incidence in November 2020),[16] (iii) Alpha variant from January 2021 to July 2021 (peak incidence in April 2021),[17] (iv) Delta variant from April 2021 to February 2022 (peak incidence in September 2021),[18] and (v) Omicron variant from November 2021 to present (peak incidence in January 2022) (**Figure 1A**; see **Appendix** for all Figures).[1,19,20]

Table 1. SARS-CoV-2 Variants of Concern and Variants Being Monitored in RI as of May 17, 2022.

Variant of Concern	Region Variant Originally Identified	Number of Total Cases in RI	Range of Sampling Dates
Omicron (B.1.1.529 and BA lineages)	South Africa	5313	Nov 30, 2021 to Apr 30, 2022

Variant Being Monitored	Region Variant Originally Identified	Number of Total Cases in RI	Range of Sampling Dates
Delta (B.1.617.2 and AY lineages)	India	7567	Apr 20, 2021 to Feb 04, 2022
Alpha (B.1.1.7 and Q lineages)	UK	1415	Jan 19, 2021 to Jul 27, 2021
Beta (B.1.351 and descendent lineages)	South Africa	7	Mar 16, 2021 to May 25, 2021
Gamma (P.1 and descendent lineages)	Brazil	286	Mar 03, 2021 to Sep 27, 2021
Epsilon (B.1.427 and B.1.429)	California, USA	156	Jan 06, 2021 to May 04, 2021
Eta (B.1.525)	New York, USA	57	Feb 03, 2021 to Apr 27, 2021
Iota (B.1.526)	New York, USA	1051	Jan 07, 2021 to Jun 28, 2021
Kappa (B.1.617.1)	India	0	—
B.1.617.3	India	0	—
Mu (B.1.621, B.1.621.1)	USA	26	May 22, 2021 to Aug 10, 2021
Zeta (P.2)	Brazil	0	—

In September 2021, we reported on SARS-CoV-2 variants in RI, describing the state's genomic surveillance program, circulating lineages, and independent mutation evolution, covering the state's first three waves.[21] Since then, similar to the rest of the world, RI has seen a significant upsurge in COVID-19-related infections, morbidity and mortality, along with an influx of new variants replacing the previously dominant strains, within the fourth and fifth waves. As of May 17, 2022, RI has seen a total of 384,187 SARS-CoV-2 infections (up by 252% from our prior report) and 3,559 associated deaths (up by 130% from our prior report), of which many were caused by the more recent Delta and Omicron variants and their sublineages.[22] In this manuscript, we provide an update on SARS-CoV-2 variants in RI, and discuss their continued potential implications on public health mitigation efforts. Such local characterization of variants can continue to guide regional public health mitigation measures, and offer educational opportunities for health providers, public health officials, and the general public.

METHODS

Collection of SARS-CoV-2 samples

Diagnostic clinical laboratories across RI have been collecting specimens from individuals who were hospitalized, reside or work at long-term care or correctional facilities, study or work at educational facilities including K–12 schools, colleges and universities, as well as the general population, and testing them for SARS-CoV-2. Selected specimens were submitted to the RI State Health Laboratory (RISHL), which coordinates SARS-CoV-2 sequencing in the state and aggregates genomic data from collaborating laboratories, including the CDC, the Broad Institute, the Kantor Laboratory, and its own internal sequencing capacity.[22] Deidentified sequences were submitted to the public database Global Initiative on Sharing All Influenza Data (GISAID).[23] The present paper continues to utilize sequences originating from RI residents that were aggregated from GISAID since the beginning of the COVID-19 pandemic.

SARS-CoV-2 sequencing and sequence analysis

At the RISHL, for specimens with low (<30) cycle thresholds (Ct), RNA was extracted, reverse transcribed and amplified, and the entire SARS-CoV-2 genome was sequenced by next generation sequencing (NGS) using the Illumina platform. Alignment and variant-designation of sequences against the Wuhan-Hu-1 reference sequence (NCBI accession number MN908947) were conducted at the Kantor laboratory with a pipeline that combines available tools for SARS-CoV-2 sequence analysis.[15,24] This pipeline is available under an open-source license from https://github.com/kantorlab/covid-pipeline. The classification of SARS-CoV-2 variants as VOC, VBM, or non-VOC/non-VBM was performed by Phylogenetic Assignment of Named Global Outbreak Lineages (Pangolin, https://cov-lineages.org/resources/pangolin.html),[24] according to the current CDC classification.[13]

Analysis of SARS-CoV-2 variant diversity

To further evaluate viral evolution within and beyond designated variants, we first characterized the diversity within the recent Delta and Omicron waves in RI, by examining their designated common sub-lineages since the beginning of the pandemic. We then identified the key mutations that distinguish those sub-lineages from the Wuhan-Hu-1 reference sequence and from their parent lineages (NCBI accession number OK091006.1 for Delta; and NCBI accession number OM570283.1 for Omicron),[25,26] as defined by Outbreak.info, a well-recognized source, which combines together data and metadata from a large number of credible data sources.[27,28]

Analysis of SARS-CoV-2 mutations

To further investigate the continued development of viral mutations beyond their designated lineages, we examined the occurrence of the five most common amino acid mutations that have been associated with the parent Delta lineage but that occurred at least once in sequences designated as Omicron, and the occurrence of the five most common amino acid mutations that have been associated with the parent Omicron lineage, but that occurred at least once in sequences designated as Delta.

Phylogenetic analysis

To provide a continued snapshot of the phylogenetic spectrum of the available RI SARS-CoV-2 sequences from the start of the COVID-19 pandemic, we created a maximum likelihood tree using RAxML.[29] This phylogenetic tree includes (i) the earliest and latest RI non-VOC/non-VBM sequences since the start of the pandemic; (ii) the earliest and latest Delta sequences detected in RI; (iii) the earliest and latest five common Delta AY lineage sequences detected in RI; (iv) the earliest and latest Omicron sequences detected in RI; (v) the earliest and latest Omicron BA lineage sequences detected in RI; (vi) the earliest and latest VBM sequences detected in RI (besides Delta, which is included above); (vii) one reference sequence for each of the VOC/VBM (Alpha B.1.1.7, GISAID accession number EPI_ISL_683466; Beta B.1.351, EPI_ISL_678615; Gamma P.1, EPI_ISL_792683; Delta B.1.617.2, EPI_ISL_1663516; Epsilon B.1.427, EPI_ISL_730092; EtaB.1.525, EPI_ISL_1035819; Iota B.1.526.1, EPI_ISL_801973; Kappa B.1.617.1, EPI_ISL_1372093; Mu B.1.621, EPI_ISL_4369031; Omicron BA.1, EPI_ISL_12327737; and Omicron BA.2, EPI_ISL_8212418)[23]; and (viii) the original SARS-CoV-2 sequence from Wuhan.[15] The number of sequences included was limited to allow for a reasonable tree resolution.

RESULTS

In our previous report we included genomic surveillance efforts since the first COVID-19 case on 2/28/20 until the end of the B.1.1.7 (Alpha) variant wave on 7/27/21 in RI.[21] Since then, there has been a marked increase in genomic surveillance of SARS-CoV-2 within the state, particularly due to the recent uptick in Omicron cases (**Figure 1A**). As of mid-May 2022, the number of SARS-CoV-2 specimens successfully sequenced from RI residents has increased by over 4-fold to 17,340 sequences (**Figure 1B**).

Table 1 lists cases identified as VOCs and VBMs at the time of this writing, according to the current CDC definitions, which have been modified since our last report. Of the 17,340 RI sequences available on GISAID, 5,313 (31%) are VOC, 10,565 (61%) are VBM, and 1,462 (8%) are non-VOC/non-VBM. The VOC (Omicron) and eight (Alpha, Beta, Gamma, Epsilon, Eta, Iota, Delta, and Mu) of the eleven defined VBMs were detected in RI. The earliest reported variant (then VOC; currently VBM) in RI was Iota (B.1.526 lineage), first detected on 1/7/21. The most frequently detected VBM has been Delta (B.1.617.2 and its AY lineages; see below), first sampled on 4/20/21. Other common VBM lineages include Alpha (B.1.1.7 and its Q sub-lineages), Iota (B.1.526), and Gamma (P.1 and its sub-lineages), first detected on 1/19/21, 1/7/21, and 3/3/21, respectively. Omicron (B.1.1.529 and its BA sub-lineages; see below), the only current VOC, was first detected on 11/30/2021.

Phylogenetic analysis of SARS-CoV-2 variants in RI demonstrated expected clustering of local variants with reference sequences, as well as the continued viral evolution over time, with earlier sequences in the COVID-19 pandemic being closer to the root (left in the figure) of the tree and more recent sequences (right of the tree) being more distal to the Wuhan-Hu-1 strain (**Figure 2**). Similarly, within variants, more recent sequences have evolved further as compared to reference and earlier sequences, indicating continued development of new mutations over time.

Multiple VOC, VBM, and non-VOC/non-VBM lineages have been circulating in RI. Non-VOC/non-VBM lineages predominated till December 2020, followed by the appearance of VBM lineages in January 2021 and their predominance till December 2021, and finally by the appearance of VOC, which overtook the landscape to become the predominant variant in RI thereafter (**Figure 3A**).

Figure 3B provides further breakdown of the non-VOC/non-VBM lineages in RI. Around the end of 2020, as SARS-CoV-2 genomic surveillance intensified, multiple non-VOC/non-VBM lineages were observed. However, only few have made up substantial numbers at any one time. Until October 2020, B.1 dominated the non-VOC/non-VBM cases. Beginning in November 2020, an increase in prevalence of lineages like B.1.2, B.1.375, and B.1.517 was observed. By the end of May 2021, the non-VOC/non-VBM variants were overtaken by the VBMs and their prevalence remains low to this day.

Figure 3C provides further breakdown of the VOC/VBM lineages in RI. Around the start of 2021, RI, like the rest of the world, saw the emergence of several VBMs, mostly Iota and Alpha, which rose in numbers and quickly dominated the variant landscape till June 2021. In July 2021, Delta (then VOC; currently VBM) supplanted the other VBMs and became the sole variant in RI, until the emergence of VOC Omicron in November 2021 and its subsequent, and current, predominance.

As has been observed with all SARS-CoV-2 variants, further analysis of both the VBM Delta and the VOC Omicron sequences revealed continued accumulation of mutations, with subsequent evolution into sub-lineages. Among the 71 sub-lineages of Delta seen in RI, AY.103 (n=1266), AY.3 (n=1206), AY.44 (n=914), AY.25.1 (n=787), and AY.25 (n=744) were noted to comprise the majority of the sequences

designated as Delta at any time during the Delta wave between July and December of 2021 (**Figure 3D**).

Similarly, among the 44 sub-lineages of Omicron seen in RI, BA.1.1 (n=2,464), BA.2 (n=1,171), BA.1 (n=474), BA.1.15 (n=319) and BA.1.17.2 (n=169) were noted to comprise the majority of the sequences designated as Omicron at any time during the Omicron wave between December 2021 and May 2022 (**Figure 3E**).

These sub-lineages represent continued viral evolution and are differentiated from their parent variants and from the Wuhan-Hu-1 strain by various mutations seen throughout their genomes (**Figure 4**). The original Delta sequence (B.1.617.2) differs from the Wuhan-Hu-1 sequence by 29 amino acid mutations, and the most common Delta AY sub-lineages outlined above have additional 1-4 mutations, which further define them (**Figure 4A**).

The original Omicron sequence (B.1.1.529) differs from the Wuhan-Hu-1 sequence by 33 amino acid mutations, and the most common Omicron BA sub-lineages outlined above have additional 20–24 mutations which further define them (**Figure 4B**). The extent of this large number of mutations, as compared to Delta, can also be seen on the tree in **Figure 2** indicating more evolved phylogenetic branches of the Omicron lineages.

Further exploration of the mutation development in the most common recent VOC (Omicron) and VBM (Delta) variants demonstrated that mutations that define each variant are not necessarily characteristic of that specific variant only, indicating continued viral evolution beyond conventional variant definitions. For instance, five of the amino acid mutations that define Delta and five of the amino acid mutations that define Omicron, are the same mutations. Of the remaining unique 24 Delta amino acid mutations, nine mutations were noted in at least one RI sequence designated as Omicron. **Figure 5A** demonstrates the frequencies of the five most common Delta mutations/deletions, that were noted in 26 RI Omicron sequences, and which decrease over time. Of the remaining unique 28 Omicron amino acid mutations, eleven mutations were noted in at least one RI sequence designated as Delta. **Figure 5B** demonstrates the frequencies of the five most common Omicron mutations, that were noted in 108 RI Delta sequences, and which generally increase over time, as the Omicron predominance continues.

DISCUSSION

Following our previous report on SARS-CoV-2 variants in RI published in September 2021, as the Delta variant was emerging globally, this paper presents current data on SARS-CoV-2 variants in RI as of mid-May 2022. We demonstrate enhanced genomic surveillance efforts in RI since our first SARS-CoV-2 infection on February 28, 2020, similar to other states and countries across the world.[30–33] Through these efforts, we have noted the emergence and disappearance of multiple statewide SARS-CoV-2 variants, representing expected viral evolution. Some variants predominated in more substantial waves, likely driven by epidemiological, immunological, or clinical factors that provide them with selective advantage to dominate over others variants. Such changes in variant landscape are concerning and highlight the need for continued local characterization of SARS-CoV-2 variants in order to capture the tremendous clinical and public health impact of COVID-19. At the same time, they increase awareness of the overall burden of the pandemic, and guide current and future public health mitigation efforts.

SARS-CoV-2 variants accumulate mutations through an error-prone, intra-host replication process,[6] resulting in the multitude of lineages and sub-lineages that are observed in RI and elsewhere. The significance of the lineages and sub-lineages that rapidly appear is unclear and should not be over interpreted. Such diversity represents continued viral evolution and, owing to the enhanced genomic surveillance efforts, has been unprecedently witnessed in near-real time throughout the pandemic, in RI and elsewhere. Even at the time of this writing, newer Omicron sub-lineages continue to occur (e.g. by mid-June 2022, BA.4 and BA.5 were identified in 8 and 10 RI patients, respectively, and BA.2.12.1 was the most common recently sequenced Omicron sub-lineage).[2] We should acknowledge and recognize this process as we continue to characterize the evolution of SARS-CoV-2, and monitor the linkage of viral evolution to clinical and epidemiological data.

Lineages and sub-lineages that end up predominating, even if temporarily, usually have some selective advantage. As discussed here, the VBM Delta, responsible for the fourth COVID-19 wave in RI, has about 30 mutations, and the VOC Omicron, responsible for the fifth wave, has about 50 mutations that distinguish them from the Wuhan-Hu-1 strain. Although few mutations are common across lineages, both Delta and Omicron have mutations in the open reading frame (ORF) 1a/1b (thought to encode a polyprotein involved in viral processing and immune evasion),[34] and in the spike (S) protein (crucial for viral interaction with host ACE2 receptor and entry into host cells).[35] The sub-lineages of these variants rapidly accumulate additional mutations in the ORFs and S protein domains,[36,37] possibly enabling increased infectivity,[38,39] breakthrough infections,[40] immune evasion,[41,42] and enhanced morbidity and mortality.[43]

The mutations that define the predominant variant in each wave are not necessarily exclusive to that variant and may be observed in other variants at varying frequencies over time. Omicron's genome, for instance, while drastically different from the Wuhan-Hu-1 strain, contains mutations that are also seen in some RI Delta sequences; and vice versa for Delta's genome. Though this currently only occurs in a minority of sequences, rising proportions of such variant-defining mutations in other variants justifies increased

awareness, as they highlight the potential convergence of mutations from multiple variants known to have clinical or epidemiological advantages into novel 'super-variants'.[44,45] The cross-occurrence of 'Delta mutations' in Omicron and of 'Omicron mutations' in Delta, such as those noted in the ORF and S protein domains of RI sequences, suggest the need for increased and careful genomic surveillance for tracking viral genomic evolution and mitigating the public health impact of SARS-CoV-2.

Genomic sequencing and surveillance of variants is, however, challenging and not without limitations. The nomenclature and SARS-CoV-2 lineage delineation is constantly evolving and undergoes relatively frequent modifications, including re-definition of VOC and VBM. As a result, variant classifications have changed even since our recent paper, and continue to change with the emergence of novel variants, which can impact genomic analyses and interpretations. This can be further complicated with changing selection pressures like vaccines and treatment options, which impact viral genomic evolution. Additionally, the generated sequences may not necessarily reflect the actual variant landscape of SARS-CoV-2, as not all COVID-19 infections are sampled and sequenced. Finally, delays in the sequencing process also create lags between generated results and actual spread of the evolving virus, delaying, at times needed, real time clinical and public health decisions.

In conclusion, SARS-CoV-2 has continued to evolve since its emergence, with and without epidemiologic impact, despite substantial scientific advances and mitigation efforts. Most recently, the VOC Omicron has become the predominant lineage in RI, the United States, and globally, attributed to its increased transmissibility compared to previous SARS-CoV-2 variants. While Omicron's case burden has declined substantially from peak levels, it continues to persist in RI despite the public health mitigation efforts and high vaccination rates (>80% fully vaccinated). The impact of continued SARS-CoV-2 evolution on public health remains to be determined as we travel within the spectrum of "end games" that may constitute the resolution of the COVID-19 pandemic.[46] As such, enhanced genomic surveillance, which allowed for the quick identification of Delta and Omicron waves, and local and global vaccination efforts must be pursued to return to pre-pandemic lives.

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Disclaimer

The views expressed herein are those of the authors and do not necessarily reflect the views of the Rhode Island Department of Health.

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Figure 1A. SARS-CoV-2 waves and cases in RI.

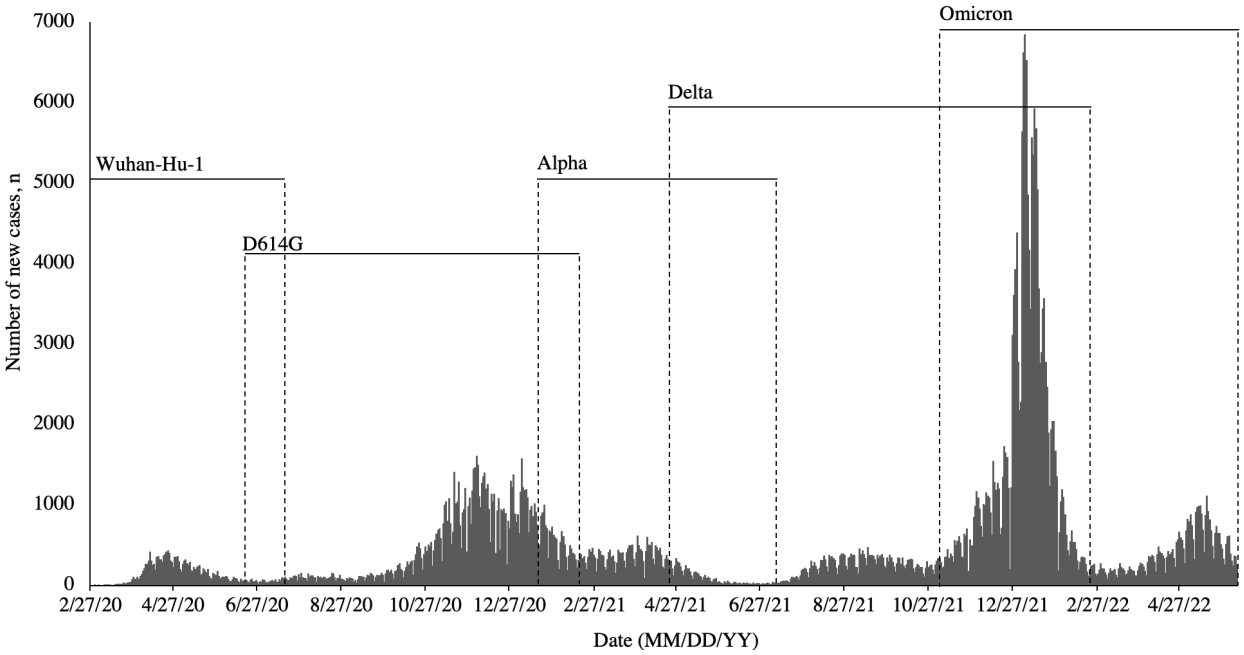

Figure 1A. Burden of SARS-CoV-2 in RI. Bar graph highlighting the number of new cases of SARS-CoV-2 (Y axis) in RI over time (X axis), since the beginning of the pandemic. Horizontal solid lines and vertical dash lines indicate the time periods of the five major waves of COVID-19, including overlaps, and black text above the horizontal lines shows the predominant variant responsible for each wave. Source for the numbers of new cases: RI Department of Health SARS-CoV-2 Variant Information (https://ri-department-of-health-covid-19-data-rihealth.hub.arcgis.com/).

Figure 1B. Cumulative number of available SARS-CoV-2 sequences collected from infected individuals in RI.

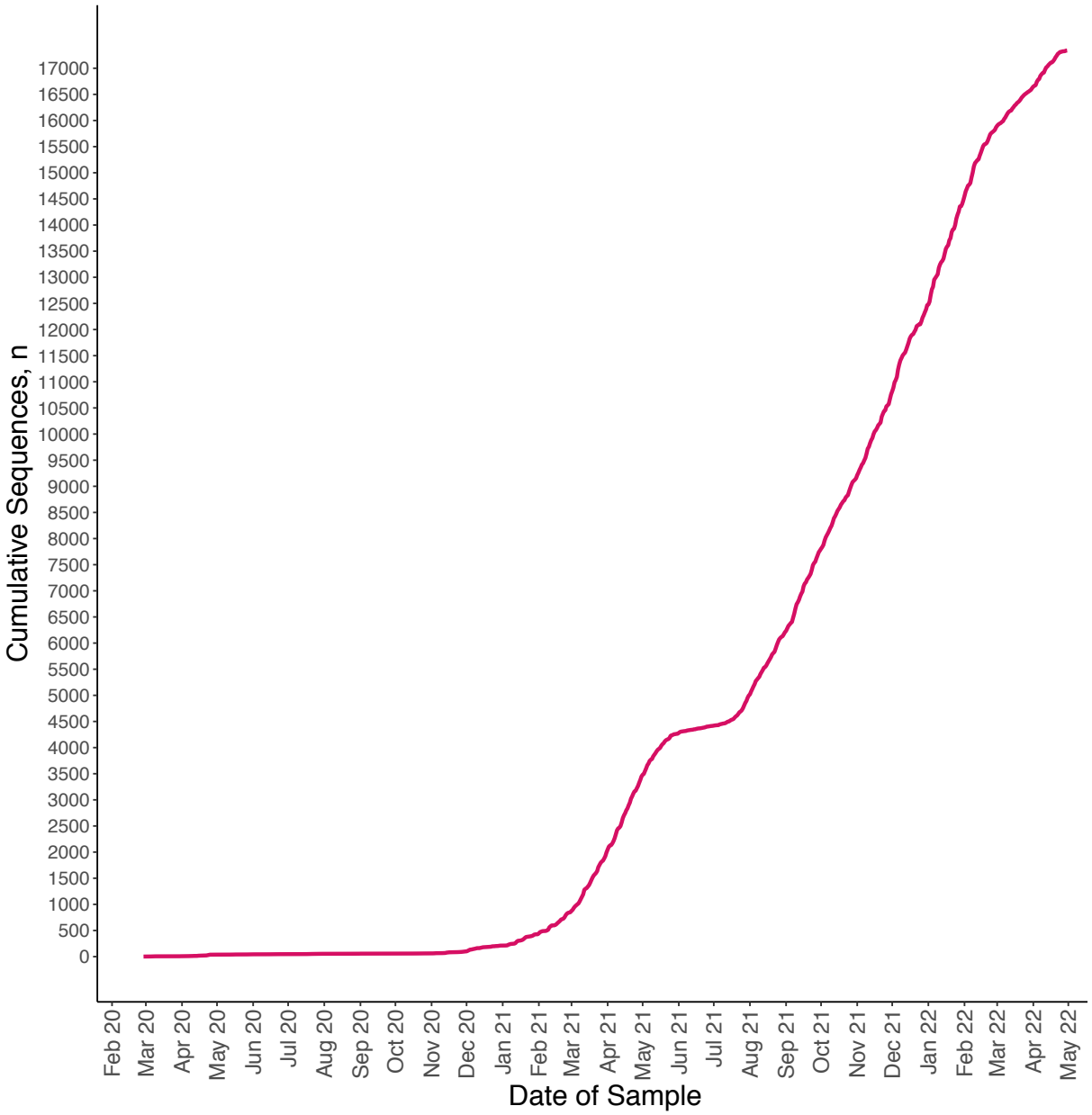

FIGURE 1B. Burden of SARS-CoV-2 in RI. Line graph highlighting the cumulative number of SARS-CoV-2 sequences in RI (Y axis) according to the dates on which the samples were collected (X axis).

Figure 2. Phylogenetic tree of the spectrum of available RI SARS-CoV-2 sequences since the beginning of COVID-19 pandemic

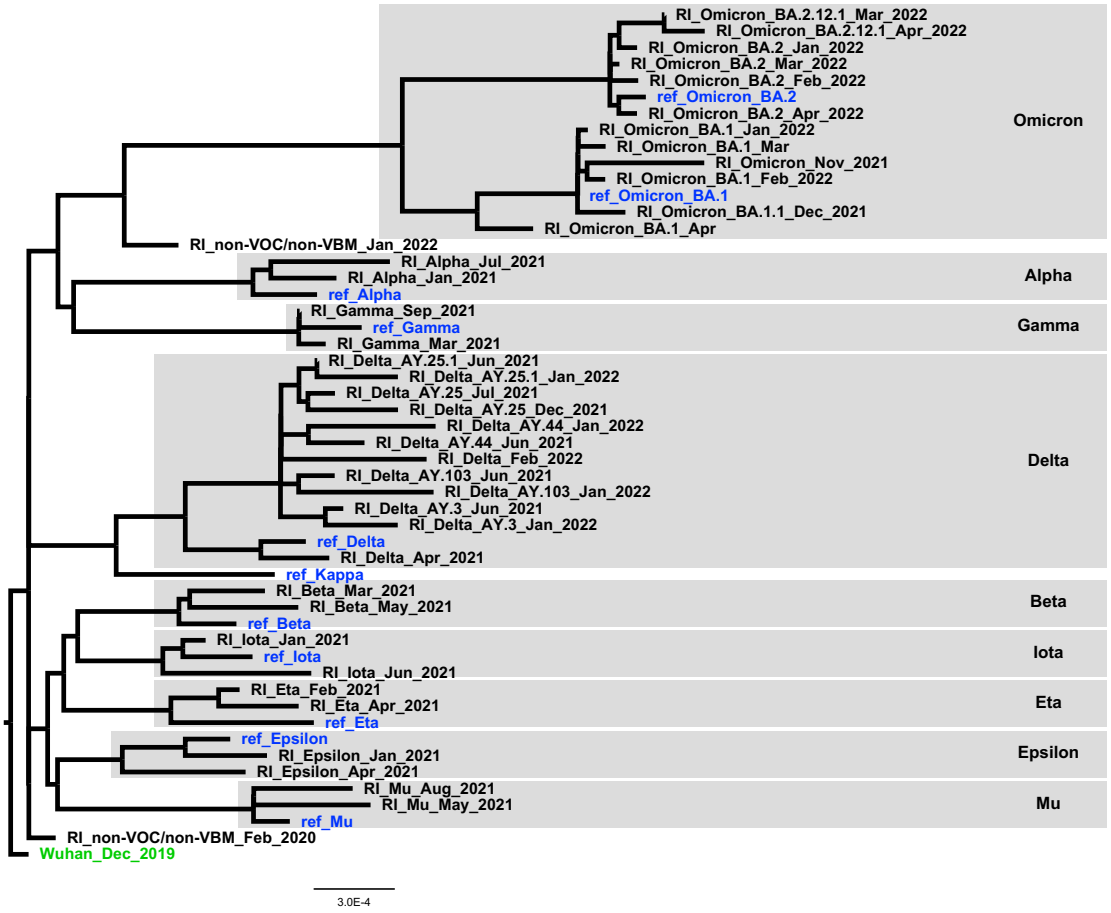

Figure 2. The phylogenetic tree shows the RI non-VOC/non-VBM, VOC, and VBM sequences (in black), reference sequences for the VOC/VBM (in blue), and the Wuhan-Hu-1 sequence (in green; used as a root). See Methods for further details on sequence selection. Rhode Island sequences start with 'RI' followed by WHO-defined variant name, Pango-defined variant sub-lineage name when applicable, and month/year of sampling. VOC and VBM clusters are highlighted in gray, with the VOC/VBM they belong to indicated to the right. The tree scale is shown at the bottom of the tree.

Figure 3A. SARS-CoV-2 sequences in RI categorized by VOC, VBM, or non-VOC/non-VBM

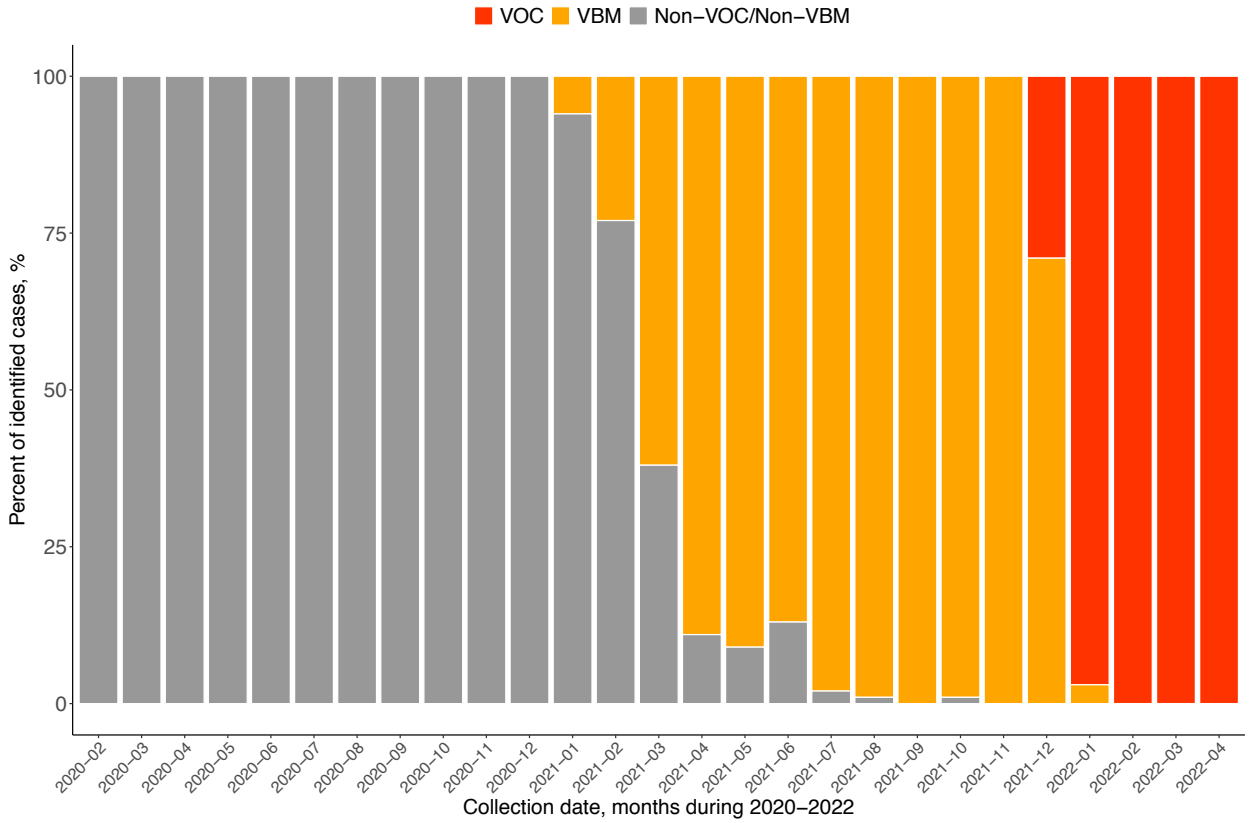

Figure 3A. Stacked bar graphs demonstrating the proportions and diversity of SARS-CoV-2 variants in RI. Stacked bar graph demonstrating the proportion of RI SARS-CoV-2 sequences (Y axis), presented as non-VOC/non-VBM (gray), VBM (yellow), and VOC (red), according to the month and year they were detected (X axis).

Figure 3B. Non-VOC/non-VBM SARS-CoV-2 lineages in RI.

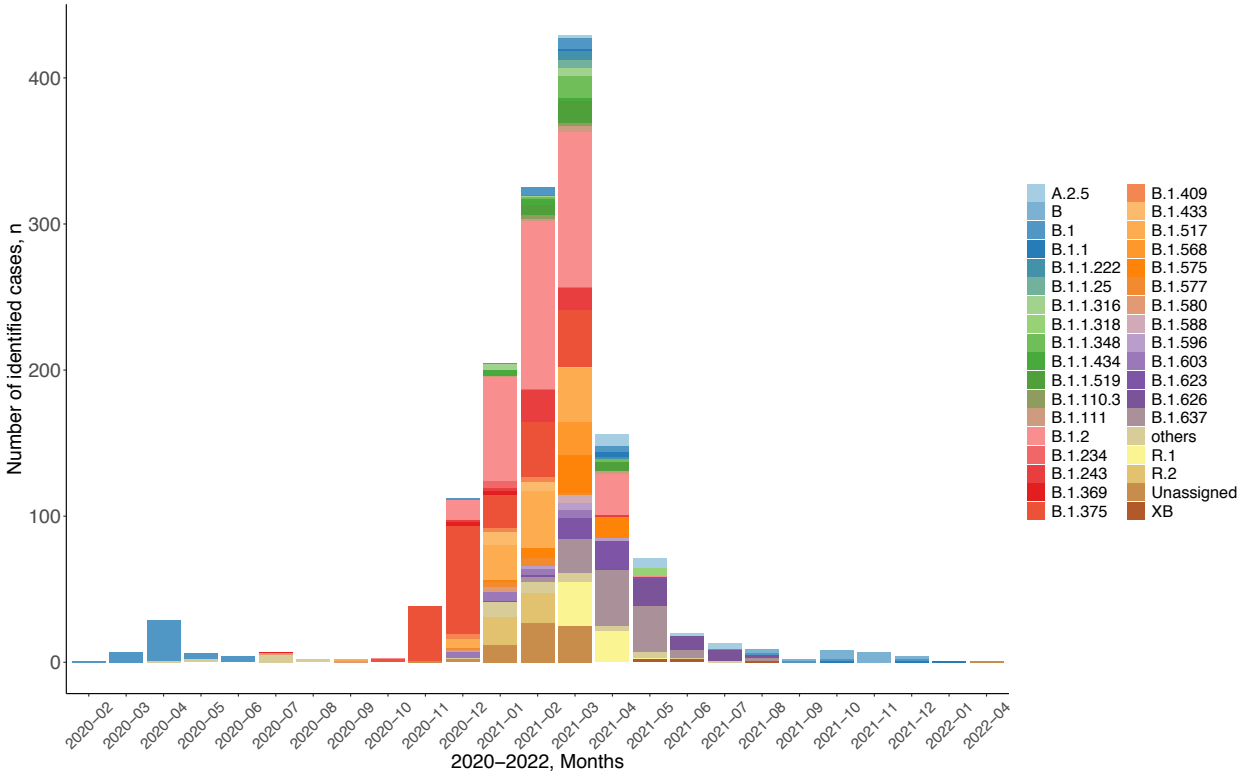

Figure 3B. Stacked bar graphs demonstrating the proportions and diversity of SARS-CoV-2 variants in RI. Stacked bar graph demonstrating the breakdown of the frequency of RI non-VOC/non-VBM lineages presented in Figure 3A. Lineages are distinguished by color, as shown in the figure legend.

Figure 3C. Proportions of VOC, VBM, and non-VOC/non-VBM over time in RI.

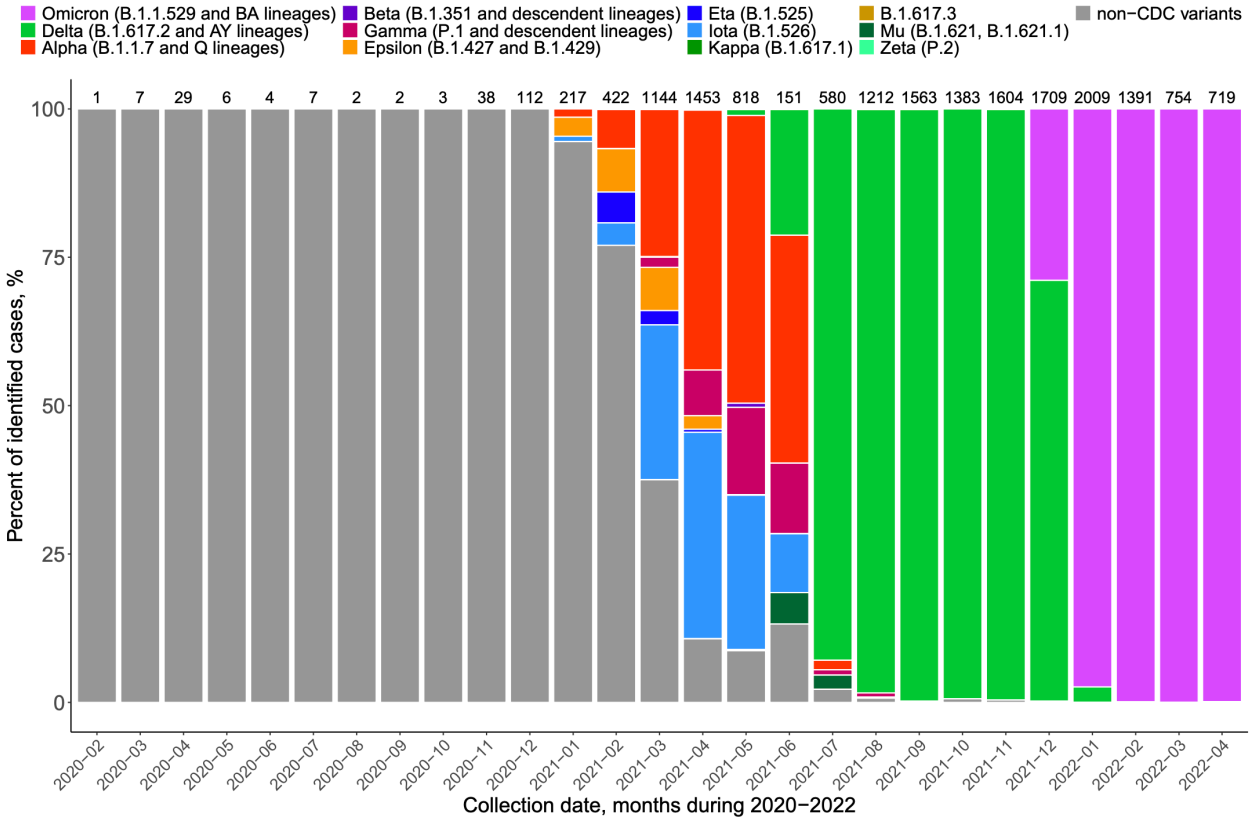

Figure 3C. Stacked bar graphs demonstrating the proportions and diversity of SARS-CoV-2 variants in RI. Stacked bar graph demonstrating the breakdown of the proportions of RI VOC and VBM that were presented in Figure 3A. VOC and VBM lineage proportions are distinguished by color, as shown in the figure legend. Non-VOC/non-VBM proportions are gray. Sequences numbers are provided at the top of the bars.

Figure 3D. SARS-CoV-2 Delta sub-lineages in RI.

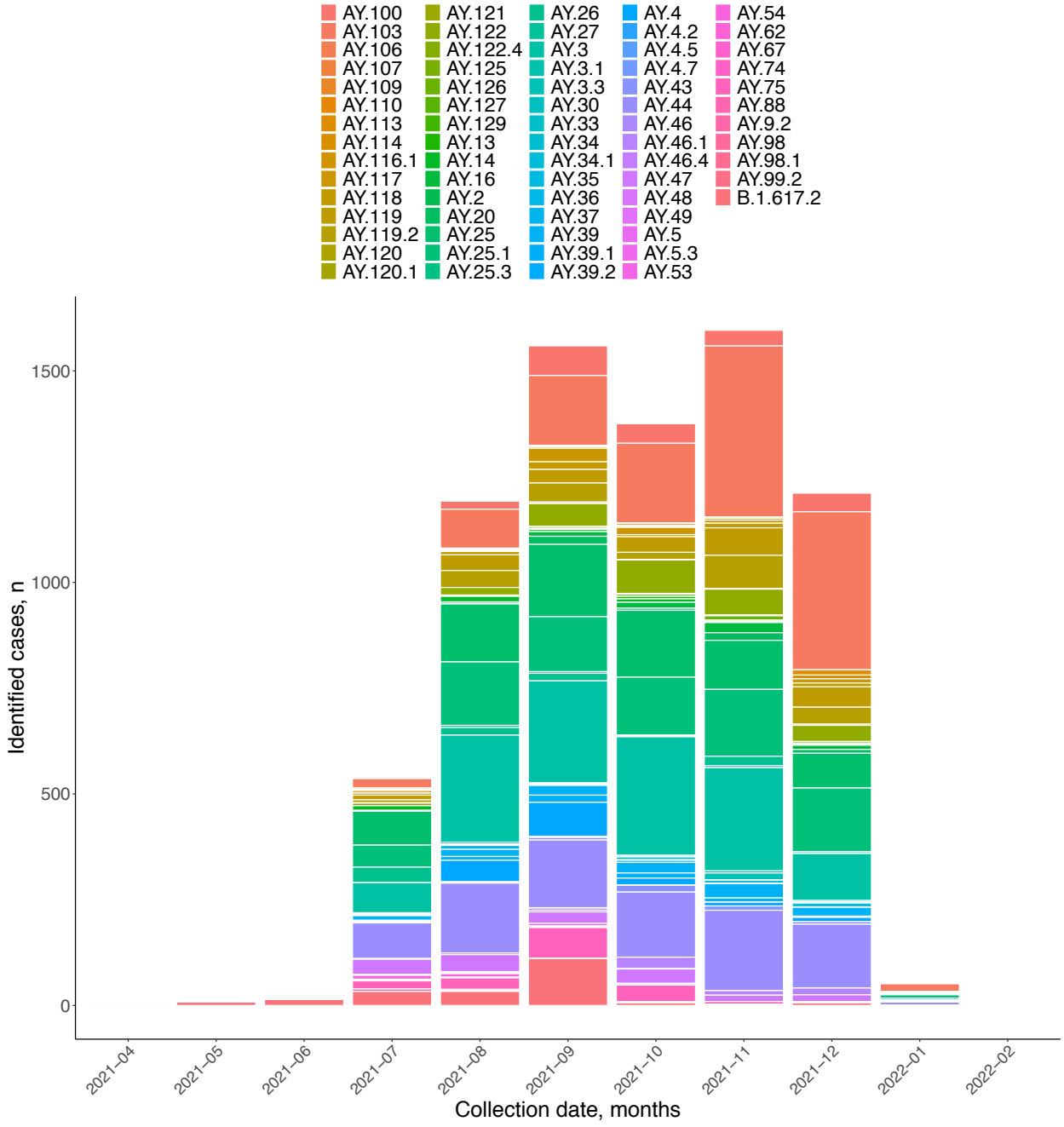

Figure 3D. Stacked bar graphs demonstrating the proportions and diversity of SARS-CoV-2 variants in RI. Stacked bar graph demonstrating the breakdown of the frequency of RI VBM Delta that was presented in Figure 3C. The AY sub-lineages are distinguished by color, as shown in the figure legend.

Figure 3E. SARS-CoV-2 Omicron lineages in RI.

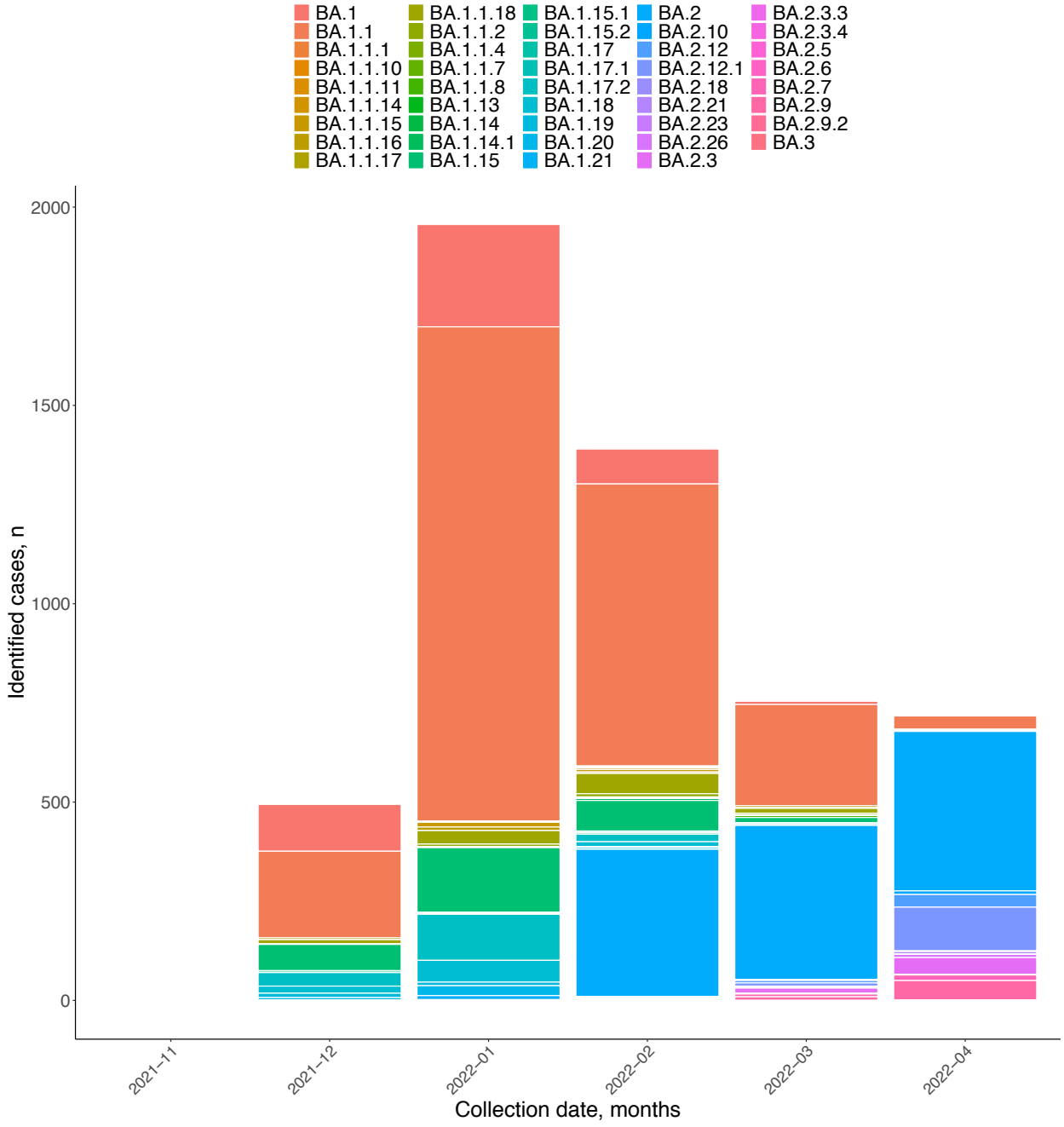

Figure 3E. Stacked bar graphs demonstrating the proportions and diversity of SARS-CoV-2 variants in RI. Stacked bar graph demonstrating the breakdown of the frequency of RI VOC Omicron that was presented in Figure 3C. The BA sub-lineages are distinguished by color, as shown in the figure legend.

Figure 4A. Mutations seen in the dominant Delta sub-lineages in RI.

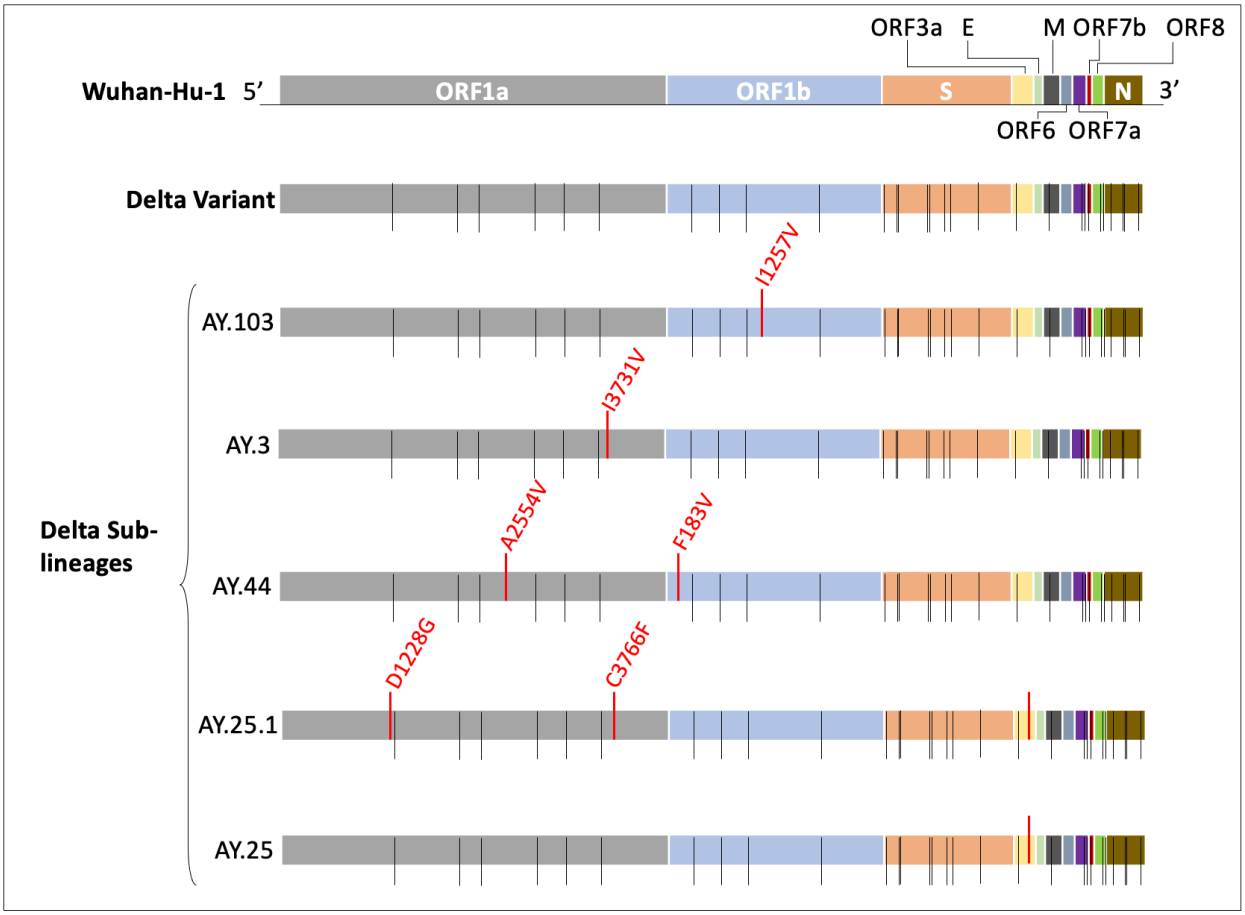

Figure 4A. Genomic comparison of the parent Delta and Omicron lineages against their respective sub-lineages and the Wuhan-Hu-1 strain. This schematic figure demonstrates genomic differences between the Delta core sequence and its sub-lineages detected in RI (lines 2-7), and the Wuhan-Hu-1 strain (top line). Black vertical lines in each sequence indicate point amino acid mutations that differentiate the core Delta sequence (B.1.617.2; second sequence in the figure) as well as its five most commonly observed sub-lineages in RI (lines 3-7), from Wuhan-Hu-1. Red vertical lines indicate amino acid mutations that further differentiate the AY sub-lineages from the core Delta sequence. Red text further indicates the point amino acid mutations that are unique to only that specific AY sub-lineage in RI.

Figure 4B. Mutations seen in the dominant Omicron sub-lineages in RI.

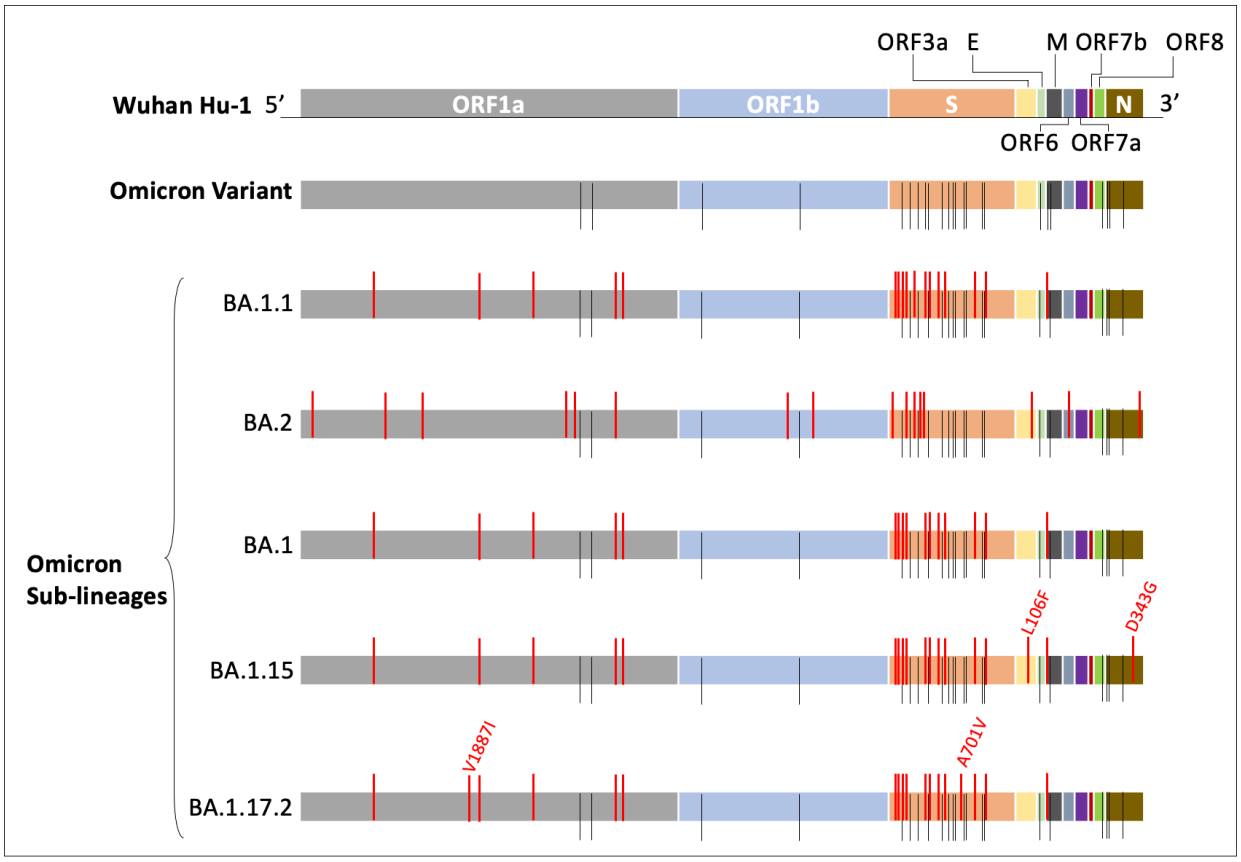

Figure 4B. Genomic comparison of the parent Delta and Omicron lineages against their respective sub-lineages and the Wuhan-Hu-1 strain. This schematic figure demonstrates genomic differences between the Omicron core sequences and its sub-lineages detected in RI (lines 2-7), and the Wuhan-Hu-1 strain (top line). Black vertical lines in each sequence indicate point amino acid mutations/deletions that differentiate the core Omicron sequence, as well as its five most commonly observed sub-lineages in RI (lines 3-7), from Wuhan-Hu-1. Red vertical lines indicate amino acid mutations that further differentiate the BA sub-lineages from the core Omicron sequence. Red text further indicates the point amino acid mutations or deletions that are unique to only that specific BA sub-lineage in RI.

Figure 5A. Most common mutations unique to Delta, occurring in Omicron sequences over time in RI.

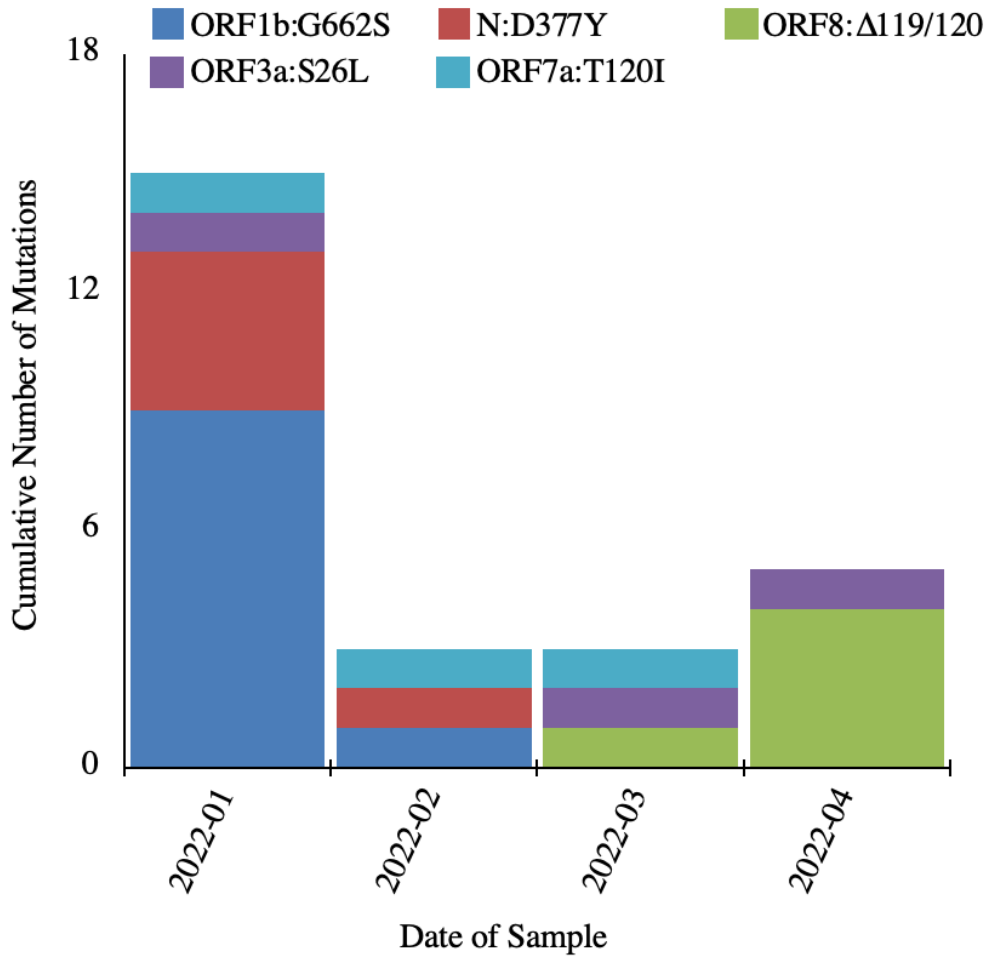

Figure 5A. Stacked bar graphs demonstrating the cumulative number of SARS-CoV-2 amino acid mutations that are associated with a VBM or VOC, but that occur outside of their associated VBM or VOC lineage. Stacked bar graph demonstrating the cumulative number (Y axis) of the five most common amino acid mutations/deletions that have been associated with the Delta variant but that were observed in RI sequences designated as Omicron, over time (X axis). The mutations are distinguished by color, as shown in the figure legend. N, Nucleocapsid; ORF, open reading frame.

Figure 5B. Most common mutations unique to Omicron, occurring in Delta sequences over time in RI.

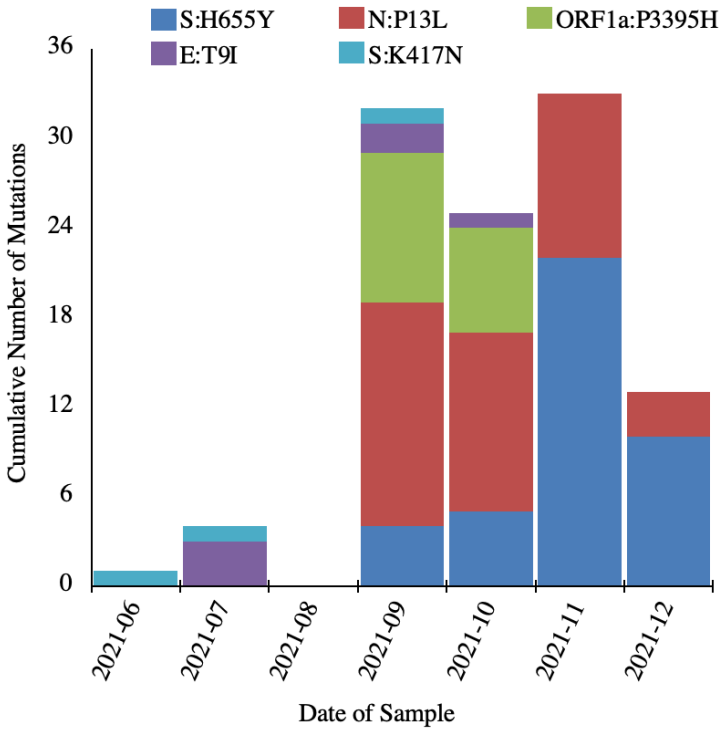

Figure 5B. Stacked bar graphs demonstrating the cumulative number of SARS-CoV-2 amino acid mutations that are associated with a VBM or VOC, but that occur outside of their associated VBM or VOC lineage. Stacked bar graph demonstrating the cumulative number (Y axis) of the five most common amino acid mutations that have been associated with the Omicron variant but that were observed in RI sequences designated as Delta, over time (X axis). The mutations are distinguished by color, as shown in the figure legend. E, envelope; N, nucleocapsid; ORF, open reading frame; S, Spike.