Spatiotemporal Evolution of SARS-CoV-2 Alpha and Delta Variants during Large Nationwide Outbreak of COVID-19, Vietnam, 2021

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We analyzed 1,303 SARS-CoV-2 whole-genome sequences from Vietnam, and found the Alpha and Delta variants were responsible for a large nationwide outbreak of COVID-19 in 2021. The Delta variant was confined to the AY.57 lineage and caused >1.7 million infections and >32,000 deaths. Viral transmission was strongly affected by nonpharmaceutical interventions.

After successfully controlling SARS-CoV-2 transmission in 2020 (*1*), Vietnam experienced a large nationwide outbreak of infection with SARS-CoV-2 in 2021. This outbreak was characterized by 2 distinct phases: January–April, with 1,632 infections and no deaths, and May–December, with 1,727,398 infections and 32,359 deaths (*2*).

Genomic surveillance has been one of the top priorities of the World Health Organization and has generated major insights into the spatiotemporal evolution of SARS-CoV-2 (*3*), which are critical for pandemic response. However, most of the studies published about genetic evolution of SARS-CoV-2 are based on datasets from high-income countries with relatively open borders (*4*–*6*), and little is known about the transmission dynamics of SARS-CoV-2 in

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DOI: https://doi.org/10.3201/eid2905.221787

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countries such as Vietnam where strict nonpharmaceutical interventions were implemented. We analyzed the spatiotemporal evolution of SARS-CoV-2 in Vietnam during 2021 and mapped patterns of viral evolution and diffusion against the public health measures implemented during the study period.

The Study

The study was conducted at the National Hospital for Tropical Diseases in Hanoi, Vietnam, and the Hospital for Tropical Diseases in Ho Chi Minh City, Vietnam. Both are tertiary referral hospitals for COVID-19 patients in northern (hospital in Hanoi) and southern (hospital in Ho Chi Minh City) Vietnam. The laboratories of the 2 hospitals were responsible for SARS-CoV-2 diagnosis and sequencing in Vietnam. We compiled detailed study methods, including public health measures (Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/29/5/22-1787-App1.pdf), and demographic features of study participants (Table).

We selected 1,365 nasopharyngeal throat swab specimens for whole-genome sequencing and obtained 1,303 complete genome sequences. We detected no recombinants. Most obtained sequences belonged to Delta variant (93.8%, n = 1,222), followed by Alpha (5.7%, n = 74), A.23.1 (0.4%, n = 5), and B.1.637 (0.2%, n = 2) variants. Of the Delta sequences, 1,212 (99.2%) were assigned to AY.57 lineage by PANGO lineage (*8*). The remaining were assigned to AY.23 and AY.79 (n = 3 each), AY.6, AY.38, AY.85, and B.1.617.2 (n = 1 each).

We temporally documented the Alpha and A.23.1 sequences during January–May 2021. We detected the first 3 Delta sequences, including 2 AY.57 and 1 B.1.617.2, in late April 2021. From June on, Delta was the only variant detected, coinciding with an upsurge in the number of infections and deaths during the 2021 outbreak (Appendix Figure 2).

Maximum-likelihood phylogenetic analysis of Alpha variant sequences showed that they were closely related to the contemporary sequences detected in the region and clustered into 4 major groups, corresponding to the sporadic community transmission clusters detected in northern, central, and southern Vietnam in early 2021 (Figure 1). This finding suggested that multiple importations and exportations of the Alpha variant into Vietnam occurred during January–May 2021.

Because of the dominance of the AY.57 lineage, and the small number of AY.57 sequences reported outside Vietnam, and especially in the nearby region (Cambodia, n = 5; Thailand, n = 5; Laos, n = 0; Singapore, n = 5), we focused our phylogeographic analysis on the 1,212 Delta AY.57/1.303 sequences obtained from Vietnam. After we removed identical, low-quality, and outlier sequences, as suggested by TempEst software, (https://tempest-solutions.com), 748 nonidentical sequences were available for analysis.

Results confirmed that AY.57 viruses were introduced into the northeastern region in early 2021 (Figure 2, https://wwwnc.cdc.gov/EID/article/29/5/22-1787-F2.htm) probably by a single introduction event (Appendix Figure 4). The estimated time to the most recent common ancestor was March 14, 2021 (95% CI February 22, 2021–April 8, 2021), shortly after the discovery of the Delta variant in November 2020. In the following months, the northeastern and Red River delta regions then acted as a source seeding the virus to neighboring and southeastern provinces, with limited viral dispersal between provinces/cities (Figure 2). During July–December 2021, the southeastern region was the main source, seeding the virus back to the northern region and the rest of Vietnam. In addition, we observed the establishment of multiple localized clusters of AY.57 lineage elsewhere in the southcentral coastal region and the Red River delta (Figure 2).

A Bayesian Skyride showed a sharp increase in genetic diversity during April–August 2021 (Figure 3, panel A), reflecting the expansion of the AY.57 lineage

Table. Demographics of study participants for spatiotemporal evolution of SARS-CoV-2 Alpha and Delta variants during large nationwide outbreak of COVID-19, Vietnam, 2021*

Characteristic	No. sequences, n = 1,303	Alpha, n = 74	AY.57, n = 1,212
Age, y			
Median (Q1–Q3)	43 (29–61)	35 (30–55)	44 (29–61)
Missing	38 (2.9)	31 (41.9)	2 (0.2)
Sex			
M	689 (52.9)	22 (29.7)	661 (54.5)
F	578 (44.4)	21 (28.4)	551 (45.5)
Unknown	36 (2.8)	31 (41.9)	
Region			
Central	120 (9.2)	24 (32.4)	94 (7.8)
Northern	504 (38.7)	44 (59.5)	450 (37.1)
Southern	679 (52.1)	6 (8.1)	668 (55.1)

*Values are no. (%) unless indicated otherwise. Characteristics of A.23.1 variant–infected cases were previously reported (7). Q, quartile.

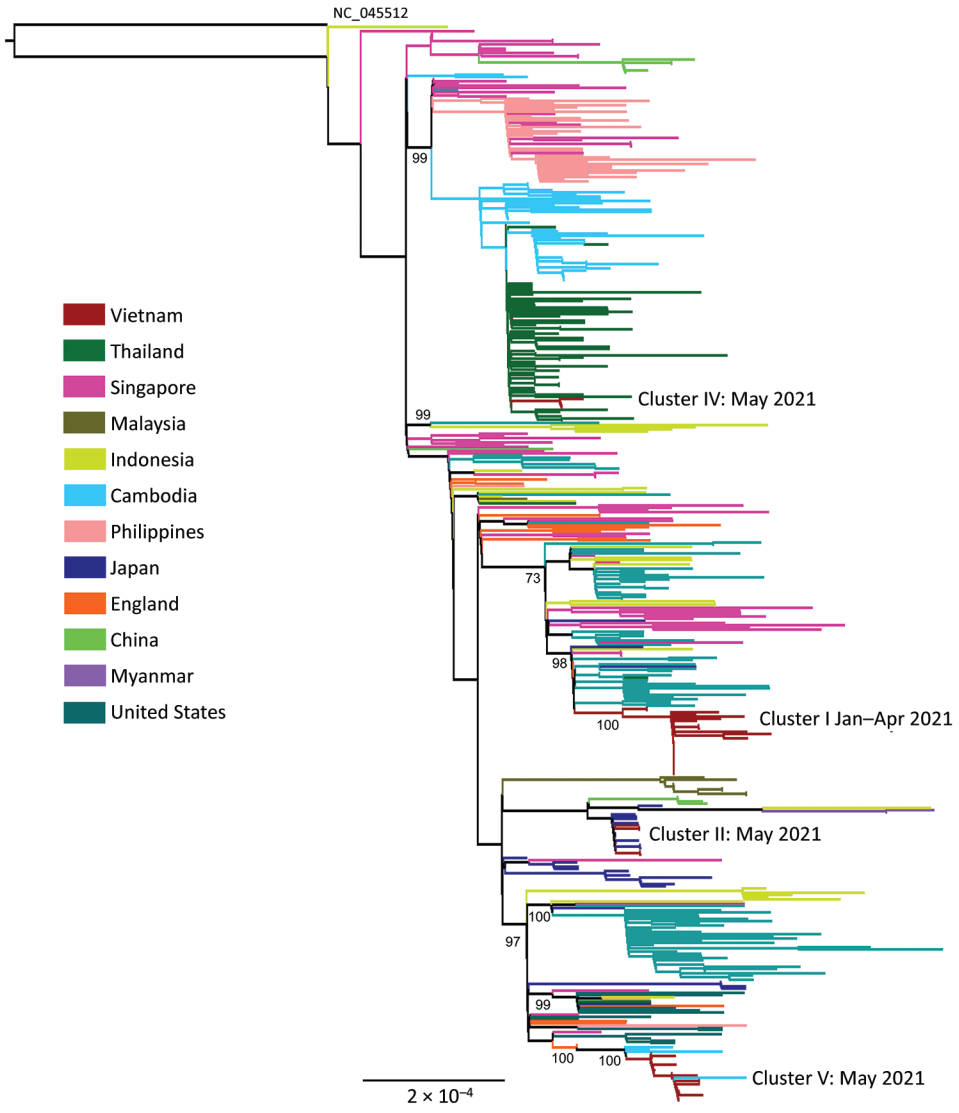

Figure 1. Maximum-likelihood tree of SARS-CoV-2 Alpha and Delta variants during large nationwide outbreak of COVID-19, Vietnam, 2021. Complete coding sequences of Alpha variant viruses circulating in Vietnam in 2021 are shown. Phylogenetic clusters were named accordingly to community clusters recorded during the study period shown in Appendix Figure 1, https://wwwnc.cdc.gov/EID/article/29/5/22-1787-App1.pdf). Numbers along branches are bootstrap values. Cluster II was linked with a traveler from Japan (Appendix Figure 1). Outgroup was the SARS-CoV-2 wild-type strain (GenBank accession no. NC_0455122).

across the country, paralleling the start of the large nationwide outbreak from May onward (Appendix Figures 1, 2). In the following months, the viral population size remained relative stable, despite some fluctuations in the number of viral sequences obtained (Figure 3, panel B), followed by a slight decrease in the genetic diversity during November. The estimated mean of the nonsynonymous to synonymous substitution (dN/dS) value was 0.86, albeit it varied across the genomes (Appendix Table 1), and the evolutionary rate of AY.57 coding sequences was 5.29×10^{-4} substitutions/site/year (95% CI 4.966×10^{-4} to 5.639×10^{-4} substitutions/site/year).

Conclusions

We showed that the Alpha and especially Delta variants were the main causes of SARS-CoV-2 outbreaks of >1.7 million infections and >32,000 deaths in Vietnam during 2021. The Alpha variant was introduced into Vietnam during early 2021 by different importation events but only caused sporadic community outbreaks until May, when a second wave associated with the Delta variant predominated from June on. The viruses of the Delta variant were confined to AY.57 lineage and were responsible for the major wave in 2021, probably by a single introduction event. Viral movement between provinces was not apparent. During the study period, nearly 200 sublineages of the Delta variant were documented worldwide (*9*), and in other countries, such as the United Kingdom and the United States, where use of nonpharmaceutical measures was relaxed, viral dispersal across the localities was more apparent (*10,11*). The rigorous containment approach applied in Vietnam

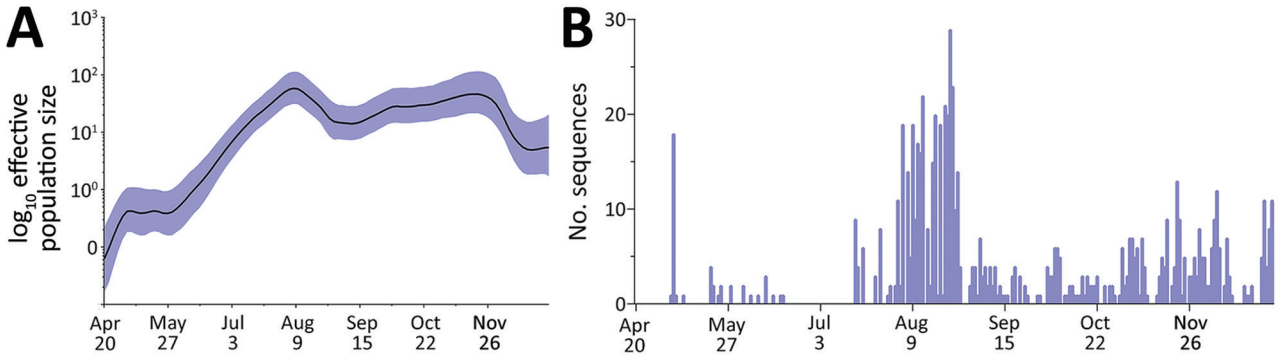

Figure 3. Genetic diversity and distribution of SARS-CoV-2 AY.57 lineage during large nationwide outbreak of COVID-19, Vietnam, 2021. A) Bayesian skyride plot illustrating the relative genetic diversity of AY.57 lineage in Vietnam during 2021. Purple shading indicates 95% highest posterior density interval. B) Distribution of the number of AY.57 sequences used for analysis over the study period.

in 2021, with limited domestic travels and tight border controls, was probably the key factor determining the localization of a single AY.57 lineage in Vietnam and its limited dispersal across the country.

The sharp increase in the relative genetic diversity of the AY.57 lineage during April–July 2021 marked the start of the nationwide outbreak in subsequent months despite in-country lockdown measures. Although nonpharmaceutical interventions were sufficient to prevent uncontrolled community transmission in 2020 (*1*), they were not sufficient after introduction of the Delta variant. This finding was probably caused by the much higher transmissibility of the Delta variant and the immunologically naive population in Vietnam at the time. During April–July 2021, only <1% of 97 million persons in Vietnam were vaccinated against SARS-CoV-2 (*12*).

Although our estimated evolutionary rate was AY.57-lineage specific, the result was within the range of previously estimated values for the Delta variant more broadly (*10*; N. Benazi, Institut Pasteur of Algeria, and S. Bounab, University of M'sila, pers. comm., email, 2023 Mar 1. This finding points to a fast evolution of the AY.57 lineage in Vietnam during the study. Although the role of population immune landscapes in shaping the evolution of SARS-CoV-2 merits further research, a recent report showed that vaccines had played a role in selective adaptation of the SARS-CoV-2 Delta variant (*13*).

The potential bias toward our referral-hospital based sampling approach represents a limitation of our study, which might have failed to comprehensively capture the genetic diversity of the pathogen. However, this limitation was probably modest given that our results are consistent with sequences uploaded to GISAID (https://www.gisaid.org) (*9*).

In conclusion, we report how rigorous public health measures in Vietnam influenced the introduction and spread of the Alpha and Delta variants during the large nationwide outbreak of COVID-19 in 2021. Genomic surveillance is critical to inform pandemic response.

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Acknowledgments

We thank Le Nguyen Minh Hoa and technicians at Department of Microbiology and Molecular, National Hospital for Tropical Diseases for collecting swab samples and initial testing of SARS-CoV-2 diagnostics; the OUCRU team for supporting whole-genome sequencing and providing data entry; data contributors and their laboratories for obtaining specimens for this study; and laboratories that submitted and shared their generated genetic sequences and metadata via GISAID, on which this research is based.

Genomic surveillance was supported by the Wellcome Trust (222574/Z/21/Z). L.V.T. and G.T. are supported by the Wellcome Trust of Great Britain (204904/Z/16/Z and 106680/B/14/Z, respectively).

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Article DOI: https://doi.org/10.3201/eid2905.221787

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Appendix

Vietnam COVID-19 Containment Approach in 2021

After successfully controlling community transmission during the first 8 months in 2020, Vietnam cautiously reopened its borders, allowing some repatriation flights, while steadily lifting in-country travel restrictions (*1*). However, following the emergence of new SARS-CoV-2 variants Alpha and Delta variants in late, Vietnam suspended all inbound flights from countries reporting the detection of community transmission associated with these two variants. Additionally, all travelers entering Vietnam were subjected to 14-day isolation, and testing on the day of arrival and on day 14 of quarantine. Yet, after 28 days of no community transmission, on 18th January 2021, two clusters of SARS-CoV-2 infection with epidemiologic links were detected in two neighboring provinces in the north of Vietnam, Hai Duong and Quang Ninh (*2*). This was followed by the detection of several other community transmission clusters of unclear origin in several provinces across the country in subsequent months before the start of the 2021 major outbreak (Appendix Figure 1). At the national level, the responses were initially targeted e.g., lockdown at community/province/city level where a community transmission cluster was detected. However, in response to the escalation of the outbreak starting in July 2021 (*3*), Vietnam suspended all arriving international flights in May 2021, and re-applied in-country travel restriction (Appendix Figure 1) until 10th October 2021 when nearly 70% of the eligible population had received at least 1 dose of vaccine (*3*). In parallel, under the zero COVID-19 policy, Vietnam implemented the meticulous contact tracing and mass testing of the cases and their contacts conducted by provincial Centers for Diseases Control (CDC). This had enabled

accurate identification of cases of community transmission origin alongside the demographic data for analysis (*4*).

Nasopharyngeal Swabs and Sample Selection for Sequencing

The laboratories of National Hospital for Tropical Diseases (NHTD) in Hanoi, and the Hospital for Tropical Diseases (HTD) in Ho Chi Minh City, Vietnam were responsible for SARS-CoV-2 diagnosis and sequencing in Vietnam. Therefore, nasopharyngeal swab (NPS) samples submitted to NHTD and HTD laboratories for testing and sequencing were either from provincial CDC or from inpatients being treated at these two hospitals.

To increase the chance of successfully obtaining the virus genome, we first applied a preselection criterion based on the cycle threshold (Ct) value of the tested samples generated by the Lightmix Modular SARS-CoV-2 RdRp/E gene assay (Tib Molbiol, Berlin, Germany) (*5*). This assay could detect both Alpha and Delta variants without compromising the sensitivity (*6*). Accordingly, at NHTD, only NPS samples with a cycle threshold (Ct) value ≤30 for the RdRp gene were eligible, while at HTD, a sample with Ct value of ≤25 for the E gene was included. Additionally, because of the availability of the resources, between January and June 2021 when community transmission remained limited, our approach focused on new community clusters detected through contact tracing under zero-COVID strategy. Between July and December 2021 during which community transmission was escalating, the selection of samples for sequencing was carried out by using WHO recommendations (*7*). Epidemiologic data, and data on infections and deaths were retrieved from e-hospital record or provided by the National Institute of Hygiene and Epidemiology and the Vietnamese Ministry of Health. Here, we focused our analysis on sequences obtained cases of locally acquired infection, and generated by NHTD or HTD laboratories, of which we had accurate sampling date and demographic data.

Our study formed part of the national COVID-19 response and was approved by the local Institutional Review Board (approval no. 2221/QĐ-BVBNĐ at the Hospital for Tropical Diseases in Ho Chi Minh City and no. 17–2022/HĐĐĐ -NĐTW at the National Hospital for Tropical Diseases in Hanoi) and Oxford Tropical Research Ethics Committee (approval no. 557–21). Since only deidentified nasopharyngeal swabs (NPS) were used, the need for individual informed consent was waived.

RNA Extraction, Whole-Genome Sequencing, and Sequence Assembly

Total RNA was extracted from NPS by using QIAamp viral RNA mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions, and finally eluted in 50µl of elution buffer (provided with the extraction kit). Whole genome amplification was performed on the extracted RNAby using either the long pooled amplicons protocol, developed by the University of Sydney (*8,9*), or the ARTIC V3 protocol (*10*) on an Illumina MiSeq platform as previously described. Library preparation was carried out by using the Nextera XT Library preparation kit (Illumina, USA), followed by library quantification by using KAPA Library Quant Kit (Kapa Biosystems, Wilmington, MA, USA), according to the manufacturer's instructions. The prepared library was sequenced by using iSeq reagent kit V2 (300 cycles) on a Miseq platform (Illumina). For each run, tested samples were multiplexed and differentiated by double indexes by using IDT-ILMN Nextera DNA UD indexes (IDT).

Sequence assembly of the obtained sequencing data was carried out by using a reference-based mapping approach available in CLC genomics workbench (v.21.0.4) and Geneious 8.1.5 (Biomatters, San Francisco, CA, USA). This method involved mapping of sequencing output of individual samples to a reference genome (WuHan-Hu-1: NC_045512, Alpha: EPI_ISl_905782, Delta: EPI_ISL_1942165), followed by manual editing of the obtained consensus to ensure the accuracy of the results, as described previously (*8*). The consensus sequences generated in this study were submitted to the National Center for Biotechnology Information under the assigned accession numbers ON458864-ON459533, ON459545-ON459608, ON755375-ON755859, OQ415286- OQ415315, and OP647358-OP647411, and GISAID with assigned numbers: EPI_ISL_10079201-EPI_ISL_10079251, EPI_ISL_2455221-EPI_ISL_2458062, EPI_ISL_4503976-EPI_ISL_4504960, EPI_ISL_4748289-EPI_ISL_4748342, EPI_ISL_4942690-EPI_ISL_4942691, EPI_ISL_4969141-EPI_ISL_4969175, EPI_ISL_5098167-EPI_ISL_5098169, EPI_ISL_5458630-EPI_ISL_5458977, EPI_ISL_6773765-EPI_ISL_6773813, EPI_ISL_7368716-EPI_ISL_7368772, EPI_ISL_7648835-EPI_ISL_7648869, EPI_ISL_7650414-EPI_ISL_7650455, EPI_ISL_5945255- EPI_ISL_5945295, EPI_ISL_6388348- EPI_ISL_6388497, EPI_ISL_ 7195776- EPI_ISL_7196062, EPI_ISL_7204787- EPI_ISL_7204791, EPI_ISL_1273214, EPI_ISL_16828666 –EPI_ISL_16828699, EPI_ISL_17016415-EPI_ISL_17016441,

EPI_ISL_17027400-EPI_ISL_17027401, EPI_ISL_17016442-EPI_ISL_17016449, EPI_ISL_4503984.

Classifications of SARS-CoV-2 Variants

SARS-CoV-2 variant classification of the obtained consensuses was determined by using PANGO lineage (*11*) with pangolin v4.1.2 and pangolin-data v1.13 (*12*). The analysis was carried out by using the Ultrafast Sample Placement on Existing Trees option to assign the lineage based on the nearest lineage on existing global tree. Sequence alignment was carried out by using the tool available on Nextclade (*13*) and Minimap2 (*14*). Recombination detection was inferred by using a combination of Freyja v 1.3.10 (*15*) and sc2rf (*16*).

Maximum-Likelihood Framework to Study Genetic Relatedness of Alpha Variant Sequences

To explore the phylogenetic relationship of Vietnamese Alpha variant sequences obtained as part of the present study, we used a dataset consisting of the complete coding region (29,408 bp) of the obtained sequences and Alpha variant sequences randomly selected from those produced from the region and beyond (The U.S. and the UK) during the study period submitted GISIAD. We applied maximum likelihood (ML) method by using the TIM2 nt substitution model with invariant for Alpha variant as suggested by IQ tree (*17*). Support for individual nodes was assessed by using a bootstrap procedure with 1,000 replicates. To assess the placement of the Vietnamese variants in the context of global sequences, we used the phylogenetic framework incorporated in NextClade by using default setting (*13*), i.e., taking into account representatives of global sequences submitted to GISAID (Date of accession: 27 October 2022). Phylogenetic clusters were manually inspected by naked eyes.

Maximum-Likelihood Phylogenetics, Phylogeography, and Phylodynamics Analysis of Delta Variant Sequences

In addition to applying PANGO lineage tool to classify the viral lineages of the Delta variant sequences as detailed above, we used maximum likelihood method and NextClade based phylogenetic analysis frameworks to assess the relatedness of the Delta sequences at national and global scale, respectively. For the former, we applied UNREST+F0+R4 as suggested by IQ tree (*17*). Support for individual nodes was assessed by using a bootstrap procedure with 1,000 replicates. For the latter, we used default setting as outline above.

To study the phylogeographics and phylodynamics of AY.57 lineage, the main lineage detected in Vientam in 2021, we applied Bayesian phylogenetic interference in BEAST v1.10.4 (*18*). We first excluded identical sequences and sequences of low quality (e.g., internal gaps). We then used TempEst 1.5 to assess the temporal signal of the dataset (*19*). Subsequently, we excluded the sequences not conforming to a linear evolutionary pattern as suggested by TempEst. For phylogeographic analysis, we divided Vietnam into eight major geographic regions according to key economic zones (Northeast, Northwest, Red River Delta, North Central Coast, South Central Coast, Highland, Southeast and Mekong Delta) (*20*). Small sample sizes from individual provinces precluded phylogeographic analyses at a finer spatial scale. We used a Bayesian Markov chain Monte Carlo framework (available in BEAST) with 1 billion steps and sampling every 100,000 steps by using the general time reversible (GTR) nucleotide substitution model with invariant (as suggested by IQ-TREE to be the best-fit model) under an uncorrelated relaxed clock model (*21*), and a Bayesian skygride coalescent tree prior (10 groups) (*22*). We assessed convergence by using Tracer version 1.5 (*23*). We selected a burn-in threshold of 10% and accepted effective sample size values above 200. Maximum-clade credibility (MCC) tree was then summarized with TreeAnnotator (available in the BEAST package) and visualized in Figtree version 1.4.2 (*24*).

To assess the effective population size trajectory, we applied Bayesian Skyride model by using the above framework. Finally, we used codon-based method (HyPhy) available in MEGA5 to measure the selection pressure on coding sequences of the pathogen genome, by estimating the ratio of nonsynonymous to synonymous substitution (dN/dS) at gene-wide level (*25*). The Hyphy used the joint maximum-likelihood reconstructions of ancestral states under a Muse-Gaut model of codon substitution.

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Appendix Table 1. Breakdown dN/dS ratios for specific coding regions

Gene	CDS	ORF1a	ORF1b	S	ORF3a	E	M	ORF6	ORF7a	ORF7b	ORF8	N
dN/dS	0.86	0.69	1.45	0.59	1.44	1.14	0.27	0.86	1.3	0.82	0.37	0.53

Appendix Table 2. GISAID numbers and geographic locations of sequences used for analysis in the present study

GISAID number	Location
EPI_ISL_2405168	China
EPI_ISL_2405175	China
EPI_ISL_16405160	China
EPI_ISL_9910213	China
EPI_ISL_9910219	China
EPI_ISL_1121993	China
EPI_ISL_9910211	China
EPI_ISL_2432955	China
EPI_ISL_2432957	China
EPI_ISL_11799982	China
EPI_ISL_2432956	China
EPI_ISL_2405171	China
EPI_ISL_1098602	Cambodia
EPI_ISL_1098604	Cambodia
EPI_ISL_1098606	Cambodia
EPI_ISL_1098608	Cambodia
EPI_ISL_1532810	Cambodia
EPI_ISL_1532811	Cambodia
EPI_ISL_1532815	Cambodia
EPI_ISL_1534538	Cambodia
EPI_ISL_1534543	Cambodia
EPI_ISL_1711976	Cambodia
EPI_ISL_1711977	Cambodia
EPI_ISL_1711983	Cambodia
EPI_ISL_1711997	Cambodia
EPI_ISL_1818952	Cambodia
EPI_ISL_1818965	Cambodia
EPI_ISL_1969674	Cambodia
EPI_ISL_1969689	Cambodia
EPI_ISL_2106235	Cambodia
EPI_ISL_2106238	Cambodia
EPI_ISL_2106241	Cambodia
EPI_ISL_2106249	Cambodia
EPI_ISL_2106272	Cambodia
EPI_ISL_2106275	Cambodia
EPI_ISL_2106280	Cambodia
EPI_ISL_2106282	Cambodia
EPI_ISL_2231565	Cambodia
EPI_ISL_2231569	Cambodia
EPI_ISL_2231572	Cambodia
EPI_ISL_2231574	Cambodia
EPI_ISL_2231576	Cambodia
EPI_ISL_2231583	Cambodia
EPI_ISL_2231585	Cambodia
EPI_ISL_2231588	Cambodia
EPI_ISL_2343208	Cambodia
EPI_ISL_2343220	Cambodia
EPI_ISL_2343225	Cambodia
EPI_ISL_2343230	Cambodia
EPI_ISL_2343236	Cambodia
EPI_ISL_2343241	Cambodia
EPI_ISL_2343242	Cambodia

GISAID number	Location
EPI_ISL_2343249	Cambodia
EPI_ISL_2343251	Cambodia
EPI_ISL_2406462	Cambodia
EPI_ISL_2406465	Cambodia
EPI_ISL_2406469	Cambodia
EPI_ISL_2406479	Cambodia
EPI_ISL_2547286	Cambodia
EPI_ISL_4489759	Cambodia
EPI_ISL_4503196	Cambodia
EPI_ISL_1118931	Indonesia
EPI_ISL_1169048	Indonesia
EPI_ISL_1169049	Indonesia
EPI_ISL_1415427	Indonesia
EPI_ISL_1415898	Indonesia
EPI_ISL_1416191	Indonesia
EPI_ISL_1824606	Indonesia
EPI_ISL_2233088	Indonesia
EPI_ISL_2258213	Indonesia
EPI_ISL_2262257	Indonesia
EPI_ISL_2262261	Indonesia
EPI_ISL_2382408	Indonesia
EPI_ISL_2500465	Indonesia
EPI_ISL_2500467	Indonesia
EPI_ISL_2500468	Indonesia
EPI_ISL_2617521	Indonesia
EPI_ISL_2617531	Indonesia
EPI_ISL_2617533	Indonesia
EPI_ISL_2631491	Indonesia
EPI_ISL_2854698	Indonesia
EPI_ISL_2854714	Indonesia
EPI_ISL_2931788	Indonesia
EPI_ISL_2987647	Indonesia
EPI_ISL_3070877	Indonesia
EPI_ISL_3070893	Indonesia
EPI_ISL_3070899	Indonesia
EPI_ISL_3278297	Indonesia
EPI_ISL_5328539	Indonesia
EPI_ISL_7550108	Indonesia
EPI_ISL_1787318	Malaysia
EPI_ISL_1787319	Malaysia
EPI_ISL_2342546	Malaysia
EPI_ISL_3246353	Malaysia
EPI_ISL_3246393	Malaysia
EPI_ISL_3246395	Malaysia
EPI_ISL_3246401	Malaysia
EPI_ISL_3356277	Malaysia
EPI_ISL_3356278	Malaysia
EPI_ISL_934424	Malaysia
EPI_ISL_2592630	Myanmar
EPI_ISL_2595725	Myanmar
EPI_ISL_953385	Singapore
EPI_ISL_1524793	Singapore
EPI_ISL_1543968	Singapore
EPI_ISL_1524785	Singapore
EPI_ISL_1652099	Singapore
EPI_ISL_1620138	Singapore
EPI_ISL_1524779	Singapore
EPI_ISL_1524778	Singapore
EPI_ISL_1367549	Singapore
EPI_ISL_1489719	Singapore
EPI_ISL_1543965	Singapore
EPI_ISL_1173251	Singapore
EPI_ISL_825082	Singapore
EPI_ISL_1897641	Singapore
EPI_ISL_1719883	Singapore

GISAID number	Location
EPI_ISL_833375	Singapore
EPI_ISL_1652101	Singapore
EPI_ISL_1098836	Singapore
EPI_ISL_857471	Singapore
EPI_ISL_1524782	Singapore
EPI_ISL_1312383	Singapore
EPI_ISL_857470	Singapore
EPI_ISL_2349770	Singapore
EPI_ISL_1098835	Singapore
EPI_ISL_1719879	Singapore
EPI_ISL_981009	Singapore
EPI_ISL_1524777	Singapore
EPI_ISL_803963	Singapore
EPI_ISL_1719878	Singapore
EPI_ISL_1519405	Singapore
EPI_ISL_1489722	Singapore
EPI_ISL_1543964	Singapore
EPI_ISL_1524781	Singapore
EPI_ISL_1719881	Singapore
EPI_ISL_1816928	Singapore
EPI_ISL_1704825	Singapore
EPI_ISL_1652102	Singapore
EPI_ISL_1315627	Singapore
EPI_ISL_995295	Singapore
EPI_ISL_1081939	Singapore
EPI_ISL_1652097	Singapore
EPI_ISL_1719880	Singapore
EPI_ISL_1519398	Singapore
EPI_ISL_1524784	Singapore
EPI_ISL_981012	Singapore
EPI_ISL_1816930	Singapore
EPI_ISL_1652098	Singapore
EPI_ISL_1312382	Singapore
EPI_ISL_2349794	Singapore
EPI_ISL_1367555	Singapore
EPI_ISL_1568432	Singapore
EPI_ISL_1620134	Singapore
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EPI_ISL_2349771	Singapore
EPI_ISL_1897640	Singapore
EPI_ISL_1367558	Singapore
EPI_ISL_1897638	Singapore
EPI_ISL_1749430	Singapore
EPI_ISL_1524783	Singapore
EPI_ISL_1543970	Singapore
EPI_ISL_1749431	Singapore
EPI_ISL_1489726	Singapore
EPI_ISL_1620140	Singapore
EPI_ISL_1620139	Singapore
EPI_ISL_1524795	Singapore
EPI_ISL_1295938	Singapore
EPI_ISL_2349832	Singapore
EPI_ISL_2508722	Singapore
EPI_ISL_2508714	Singapore
EPI_ISL_953387	Singapore
EPI_ISL_1620135	Singapore
EPI_ISL_1489723	Singapore
EPI_ISL_4115388	Japan
EPI_ISL_4115394	Japan
EPI_ISL_4115327	Japan
EPI_ISL_4115385	Japan
EPI_ISL_2335681	Japan
EPI_ISL_4115342	Japan
EPI_ISL_4115345	Japan
EPI_ISL_4115348	Japan

GISAID number	Location
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EPI_ISL_10993952	Japan
EPI_ISL_10993953	Japan
EPI_ISL_10993954	Japan
EPI_ISL_10993955	Japan
EPI_ISL_10993957	Japan
EPI_ISL_825387	Japan
EPI_ISL_825391	Japan
EPI_ISL_825392	Japan
EPI_ISL_1931071	Japan
EPI_ISL_1929159	Japan
EPI_ISL_1929390	Japan
EPI_ISL_1927819	Japan
EPI_ISL_1931135	Japan
EPI_ISL_1927824	Japan
EPI_ISL_3196853	Japan
EPI_ISL_1929315	Japan
EPI_ISL_11823586	Japan
EPI_ISL_3196812	Japan
EPI_ISL_1941905	Japan
EPI_ISL_1929186	Japan
EPI_ISL_1927816	Japan
EPI_ISL_1927037	Japan
EPI_ISL_2328029	Japan
EPI_ISL_1933713	Japan
EPI_ISL_1932608	Japan
EPI_ISL_1927180	Japan
EPI_ISL_3196934	Japan
EPI_ISL_3827196	Thailand
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EPI_ISL_2433456	Thailand
EPI_ISL_2433401	Thailand
EPI_ISL_984304	Thailand
EPI_ISL_5030241	Thailand
EPI_ISL_2350995	Thailand
EPI_ISL_2350939	Thailand
EPI_ISL_2433387	Thailand
EPI_ISL_2433362	Thailand
EPI_ISL_4255541	Thailand
EPI_ISL_2433447	Thailand
EPI_ISL_2351073	Thailand
EPI_ISL_3826630	Thailand
EPI_ISL_2350954	Thailand
EPI_ISL_2351100	Thailand
EPI_ISL_3826563	Thailand
EPI_ISL_3827396	Thailand
EPI_ISL_2351155	Thailand
EPI_ISL_4255170	Thailand
EPI_ISL_2351062	Thailand
EPI_ISL_2351101	Thailand
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EPI_ISL_2350956	Thailand
EPI_ISL_3827237	Thailand
EPI_ISL_3827251	Thailand
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EPI_ISL_2433548	Thailand
EPI_ISL_3826668	Thailand
EPI_ISL_2350928	Thailand
EPI_ISL_3826650	Thailand
EPI_ISL_3826648	Thailand
EPI_ISL_3826620	Thailand
EPI_ISL_3826641	Thailand

GISAID number	Location
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EPI_ISL_2351065	Thailand
EPI_ISL_3826704	Thailand
EPI_ISL_3826720	Thailand
EPI_ISL_3827380	Thailand
EPI_ISL_3827207	Thailand
EPI_ISL_3827347	Thailand
EPI_ISL_3826524	Thailand
EPI_ISL_2678115	Thailand
EPI_ISL_3827373	Thailand
EPI_ISL_3826579	Thailand
EPI_ISL_2350960	Thailand
EPI_ISL_2351177	Thailand
EPI_ISL_877766	Thailand
EPI_ISL_3826661	Thailand
EPI_ISL_3892089	Thailand
EPI_ISL_3827254	Thailand
EPI_ISL_3826531	Thailand
EPI_ISL_2433539	Thailand
EPI_ISL_2351160	Thailand
EPI_ISL_3826664	Thailand
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EPI_ISL_2433465	Thailand
EPI_ISL_3826721	Thailand
EPI_ISL_2351047	Thailand
EPI_ISL_2433503	Thailand
EPI_ISL_3827178	Thailand
EPI_ISL_1205541	England
EPI_ISL_1205533	England
EPI_ISL_1205524	England
EPI_ISL_1205518	England
EPI_ISL_1205562	England
EPI_ISL_1205566	England
EPI_ISL_1205517	England
EPI_ISL_2739810	England
EPI_ISL_2739841	England
EPI_ISL_2740023	England
EPI_ISL_2739779	England
EPI_ISL_2739793	England
EPI_ISL_2739796	England
EPI_ISL_2753420	England
EPI_ISL_1638023	England
EPI_ISL_5566664	Philippines
EPI_ISL_5567559	Philippines
EPI_ISL_2188177	Philippines
EPI_ISL_2859726	Philippines
EPI_ISL_4741371	Philippines
EPI_ISL_2188745	Philippines
EPI_ISL_2188045	Philippines
EPI_ISL_2859714	Philippines
EPI_ISL_2859778	Philippines
EPI_ISL_5567366	Philippines
EPI_ISL_5566756	Philippines
EPI_ISL_2859720	Philippines
EPI_ISL_2155994	Philippines
EPI_ISL_2188916	Philippines
EPI_ISL_2156243	Philippines
EPI_ISL_2859269	Philippines
EPI_ISL_5567999	Philippines
EPI_ISL_4741345	Philippines
EPI_ISL_5566428	Philippines
EPI_ISL_5566378	Philippines
EPI_ISL_5568163	Philippines
EPI_ISL_5568544	Philippines

GISAID number	Location
EPI_ISL_2859679	Philippines
EPI_ISL_4712193	Philippines
EPI_ISL_5566890	Philippines
EPI_ISL_2860194	Philippines
EPI_ISL_5934900	Philippines
EPI_ISL_4712464	Philippines
EPI_ISL_5568013	Philippines
EPI_ISL_5561549	Philippines
EPI_ISL_2189162	Philippines
EPI_ISL_4459456	Philippines
EPI_ISL_4347131	United States
EPI_ISL_4347672	United States
EPI_ISL_4347113	United States
EPI_ISL_4347286	United States
EPI_ISL_4347568	United States
EPI_ISL_4347140	United States
EPI_ISL_4347237	United States
EPI_ISL_4347529	United States
EPI_ISL_4347059	United States
EPI_ISL_4347152	United States
EPI_ISL_4347070	United States
EPI_ISL_4347309	United States
EPI_ISL_4347048	United States
EPI_ISL_4347456	United States
EPI_ISL_4347366	United States
EPI_ISL_4347315	United States
EPI_ISL_4347145	United States
EPI_ISL_4347143	United States
EPI_ISL_4347259	United States
EPI_ISL_4347543	United States
EPI_ISL_4347360	United States
EPI_ISL_4347359	United States
EPI_ISL_4347696	United States
EPI_ISL_4347032	United States
EPI_ISL_4347604	United States
EPI_ISL_4347531	United States
EPI_ISL_4347495	United States
EPI_ISL_4347094	United States
EPI_ISL_4347686	United States
EPI_ISL_4347580	United States
EPI_ISL_4347448	United States
EPI_ISL_4347213	United States
EPI_ISL_4347003	United States
EPI_ISL_4347076	United States
EPI_ISL_4347093	United States
EPI_ISL_4347717	United States
EPI_ISL_4347412	United States
EPI_ISL_4347024	United States
EPI_ISL_4347169	United States
EPI_ISL_4347648	United States
EPI_ISL_4347373	United States
EPI_ISL_4347379	United States
EPI_ISL_4347633	United States
EPI_ISL_4347147	United States
EPI_ISL_4347216	United States
United States	United States
EPI_ISL_4347046	United States
EPI_ISL_4347316	United States
EPI_ISL_4347010	United States
EPI_ISL_4347036	United States
EPI_ISL_4347674	United States
EPI_ISL_4347303	United States
EPI_ISL_4347603	United States
EPI_ISL_4347385	United States
EPI_ISL_4347424	United States

GISAID number	Location
EPI_ISL_4347540	United States
EPI_ISL_4347219	United States
EPI_ISL_4347150	United States
EPI_ISL_4347535	United States
EPI_ISL_4347618	United States
EPI_ISL_4347009	United States
EPI_ISL_4347320	United States
EPI_ISL_4347698	United States
EPI_ISL_4347720	United States
EPI_ISL_4347342	United States
EPI_ISL_4347409	United States
EPI_ISL_4347054	United States
EPI_ISL_4347533	United States
EPI_ISL_4347467	United States
EPI_ISL_4347683	United States
EPI_ISL_4347042	United States
EPI_ISL_4347560	United States
EPI_ISL_4347498	United States
EPI_ISL_4347020	United States
EPI_ISL_4347158	United States
EPI_ISL_4347189	United States
EPI_ISL_4347487	United States
EPI_ISL_4347575	United States
EPI_ISL_4347361	United States
EPI_ISL_4347031	United States
EPI_ISL_4347423	United States
EPI_ISL_4347428	United States
EPI_ISL_4347116	United States
EPI_ISL_4347501	United States
EPI_ISL_4347168	United States
EPI_ISL_4347205	United States
EPI_ISL_4347545	United States
EPI_ISL_4347202	United States
EPI_ISL_4347253	United States
EPI_ISL_4347630	United States
EPI_ISL_4347561	United States
EPI_ISL_4347386	United States
EPI_ISL_4347453	United States
EPI_ISL_4347635	United States
EPI_ISL_4347333	United States
EPI_ISL_4347466	United States
EPI_ISL_4347268	United States
EPI_ISL_4347124	United States
EPI_ISL_4347589	United States
EPI_ISL_4347159	United States
EPI_ISL_4347430	United States
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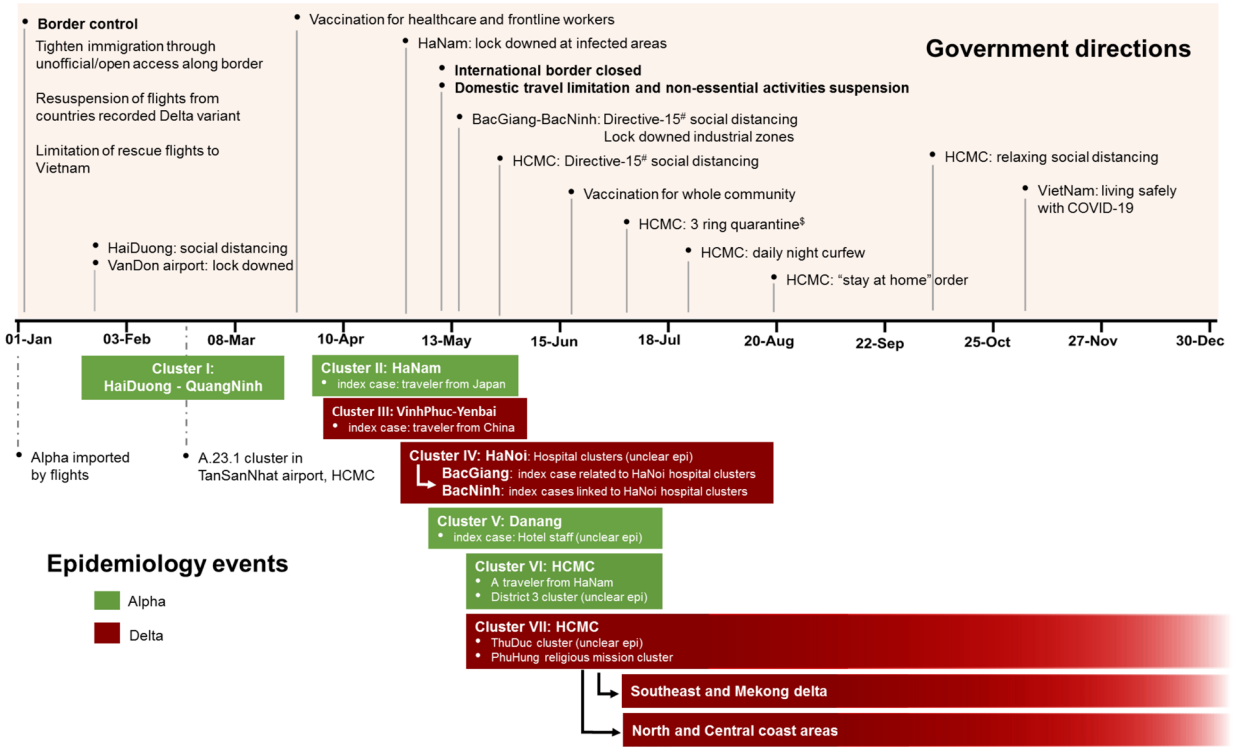
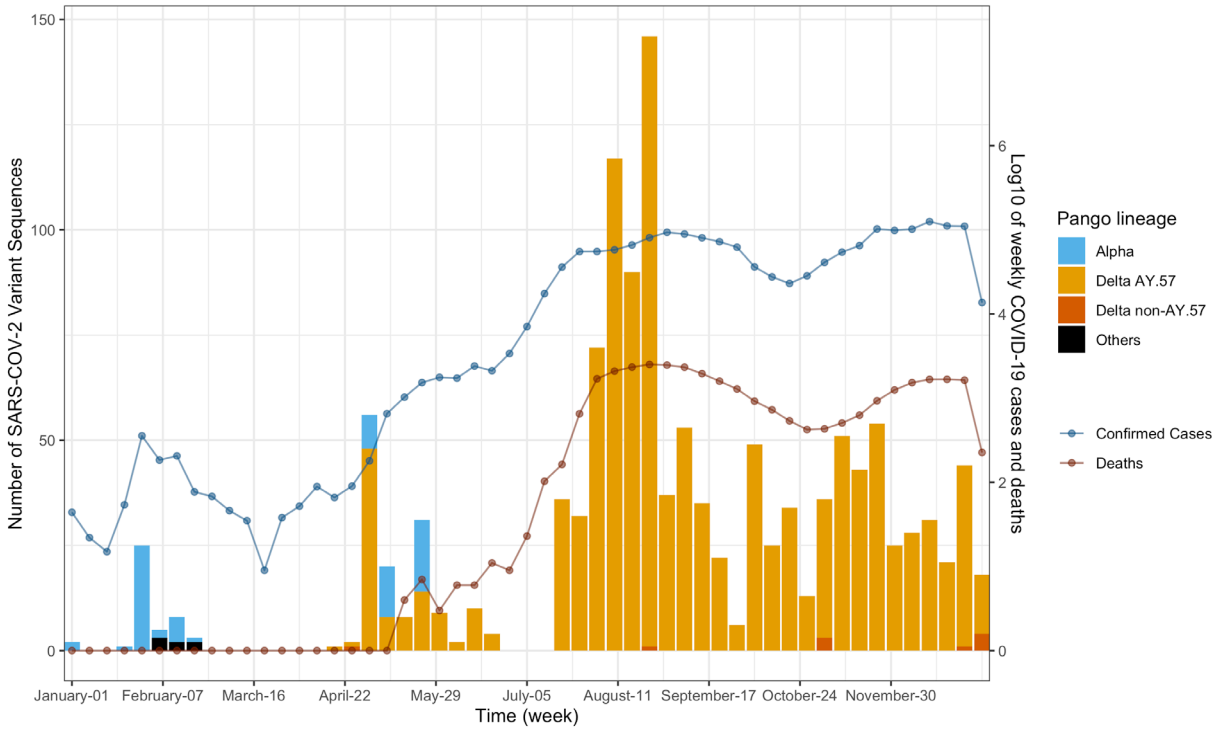
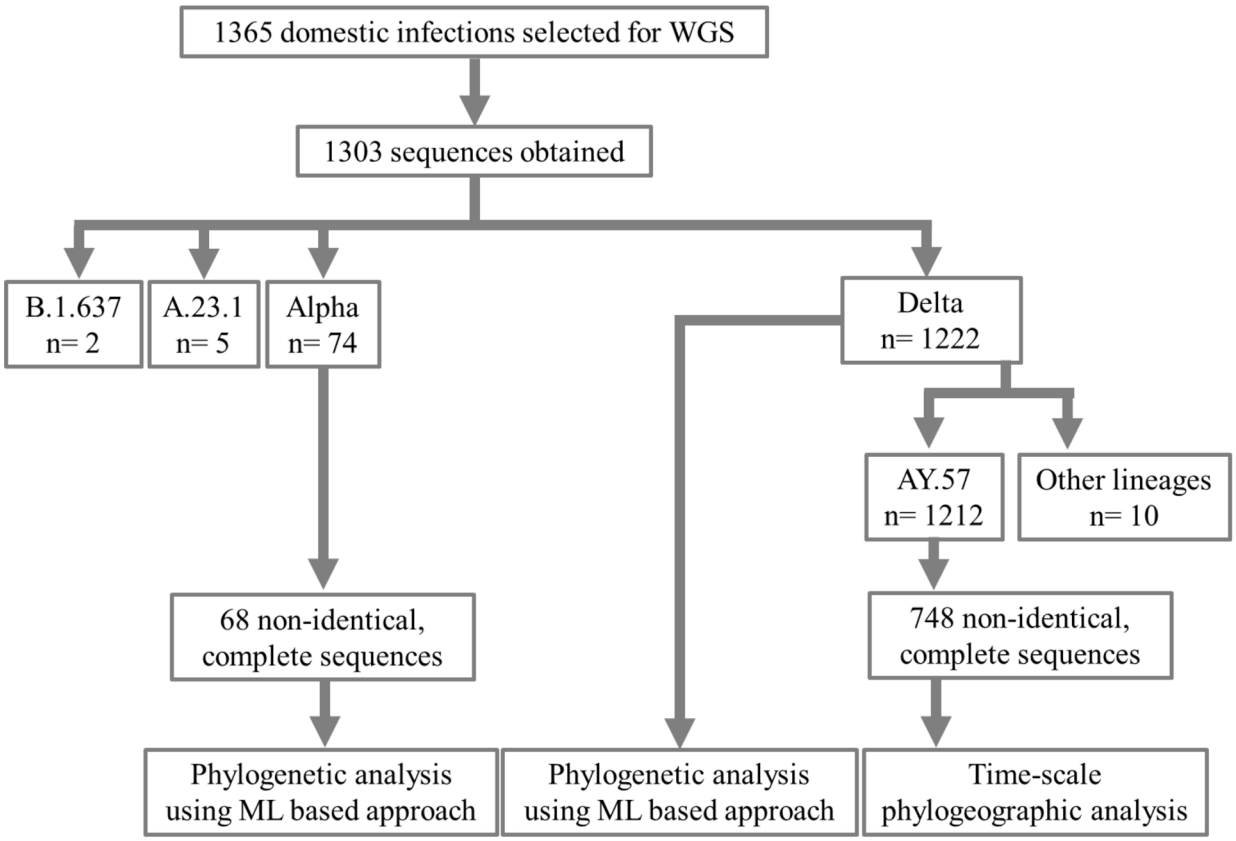
EPI_ISL_4347459	United States
EPI_ISL_4347328	United States



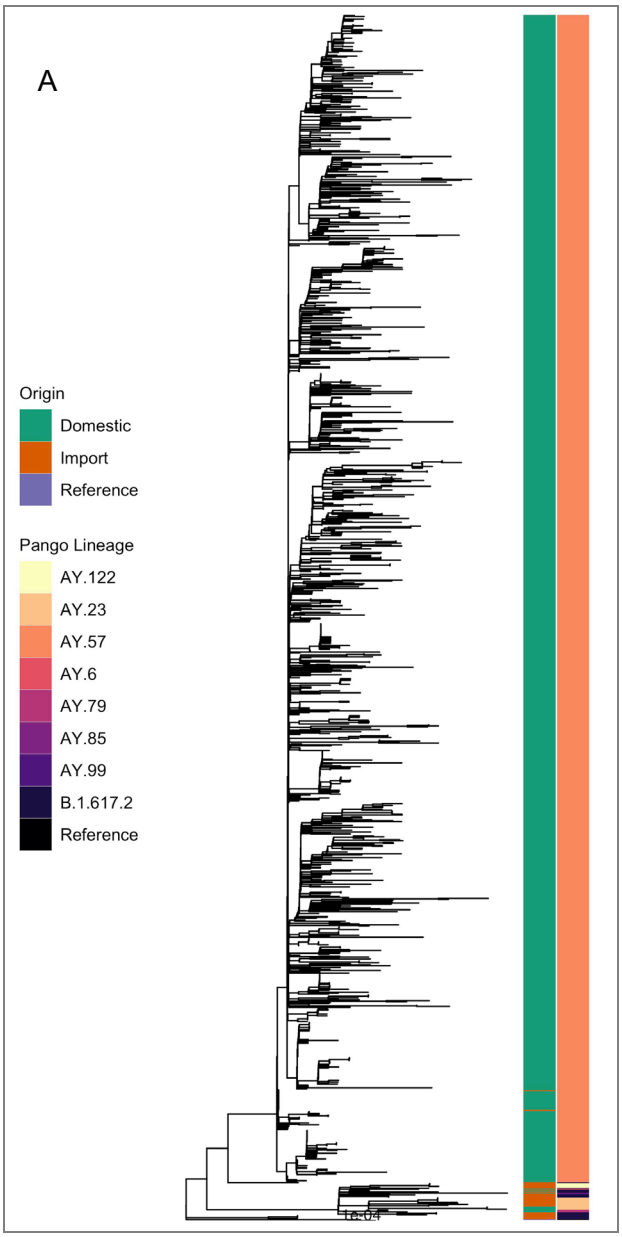
Appendix Figure 1. Government directions and COVID-19 epidemiology events in Vietnam in 2021. #Directive-15: Suspension of non-essential services/businesses and mass gatherings, applying physical distance of 2 m when contact with others, banning the gatherings of 20 persons or more in one place and 10 persons or more outside workplaces and limitation of movements. $3 ring quarantine: apply Directive-16 whole city (bans gatherings of two more persons in public and asks persons to only leave homes for emergencies, food, medicine, work in factories, and businesses that involve essential goods and services), lockdown at residential areas with covid-19 case report, home quarantine for F1. HCMC: Ho Chi Minh city.

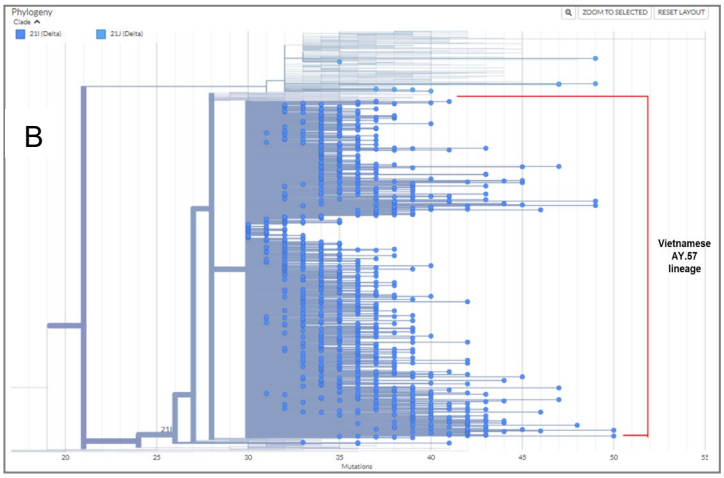


Appendix Figure 2. Line chart showing the number of reported COVID-19 cases and deaths during the 2021 outbreak of COVID-10 in Vietnam alongside the monthly number of SARS-CoV-2 variants (bar chart) detected among cases of community transmission between January and December 2021. Delta variant non-AY.57 lineage includes B.1.617.2 (n = 1), AY.23 (n = 3), AY.79 (n = 3), AY.85 (n = 1), AY.6 (n = 1), and AY.38 (n = 1). Others include lineages B.1.637 (n = 2) and A.23.1 (n = 5).

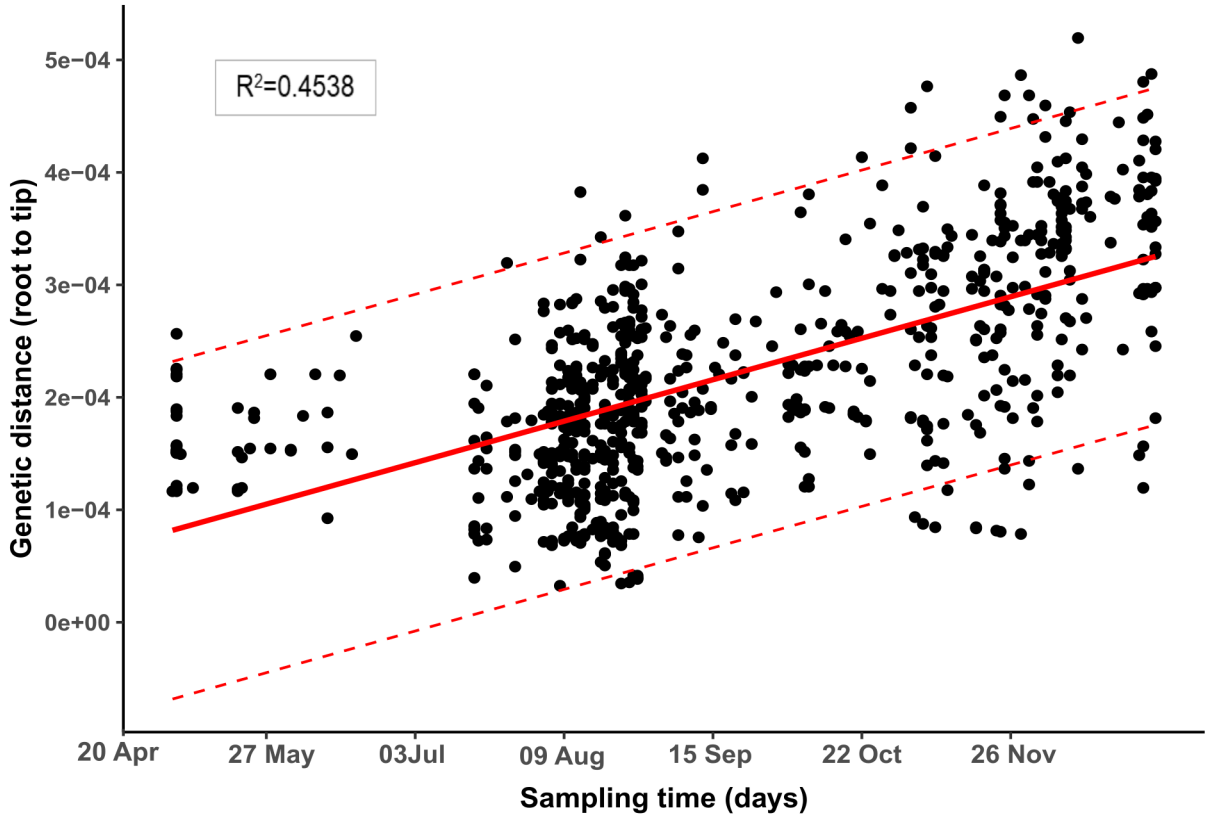


Appendix Figure 3. Workflow of the study.





Appendix Figure 4. A) Reconstructed ML tree depicting the relationship between Delta variants detected in Vietnam, and **B)** NexClade Based phylogenetic analysis illustrating the placement of the Vietnamese sequences among global sequences submitted GISAID.



Appendix Figure 5. Root to tip regression of AY.57 coding sequences using for the evolution analysis. The solid line indicates the regression line; the dotted lines represent upper and lower limits of 95% confidence interval. Outliers were excluded from subsequence spatiotemporal evolutionary analysis. TempEst analysis indicated a positive correlation between genetic divergence and sampling time; the retrieved R^2 value of 0.4538 (F = 438.4117, p value <0.001), suggesting a moderate temporal signal in the included sequences (https://doi.org/10.1093/molbev/msr121).