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Supplementary Materials for

Cryptic transmission of SARS-CoV-2 in Washington state

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MDAR Reproducibility Checklist (.pdf)
Data S1 (.pdf)

Materials and Methods

Specimen collection

Specimens analyzed in this manuscript were obtained in a collaboration between the Washington State Department of Health, the Seattle Flu Study and the University of Washington Laboratory Medicine Department of Virology (UW Virology).

Specimens were collected by the Washington State Department of Health following CDC criteria. Specimens from UW Virology were obtained as part of clinical testing for SARS-CoV-2. Nasopharyngeal/oropharyngeal swabs were received from local healthcare providers and from the Washington State Department of Health to perform qualitative detection of SARS-CoV-2 RNA by a one-step real-time RT-PCR assay. Sequencing was performed on all samples with a positive or inconclusive RT-PCR assay result. The Seattle Flu Study (SFS) was established in the 2018-2019 flu season to combine clinical and innovative community sampling methods to measure how influenza, RSV, and other respiratory pathogens enter and circulate with the Seattle metropolitan region (*27*). Samples screened for COVID-19 were collected as part of routine clinical testing, and residual samples were utilized for this study. Samples were additionally collected as part of prospective community enrollment of individuals with acute respiratory illness. Detailed protocols for sample collection are described in Chu et al. (*28*).

Diagnostics and sequencing

Extracted nucleic acids (Magna Pure, 96 Roche) were screened for SARS-CoV-2 in a multiplexed Taqman assay with primer/probe sets targeting SARS-CoV-2 Orf1B (FAM) and human RNaseP (VIC) in duplicate (Life Technologies assay ID APGZJKF and A30064) in 384 well plates. Samples that are positive for SARS-CoV-2 are retested using the CDC-designed rtRT-PCR assays acquired directly from IDT (2019 nCoV Kit lot# 0000500389, 2019-nCoV_N positive control lot# 0000500326) according to CDC instructions with the exception that a 384-well plate and ViiA7 thermocycler was used.

SARS-CoV-2 genome sequencing was conducted using a targeted enrichment approach. RNA from positive specimens was converted to cDNA using random hexamers and reverse transcriptase (Superscript IV, Thermo) and a sequencing library was constructed using the Illumina TruSeq RNA Library Prep for Enrichment kit (Illumina). The sequencing library was enriched for SARS-CoV-2 using the Twist Respiratory Virus Research Panel (Twist). Libraries

were sequenced on a MiSeq or NextSeq instruments. The resulting reads were assembled against the SARS-CoV-2 reference genome Wuhan-Hu-1/2019 (Genbank accession MN908947) using the bioinformatics pipeline https://github.com/seattleflu/assembly. Consensus sequences were deposited to Genbank and GISAID.

For UW Virology samples, sequencing was performed as described previously (*29*). Libraries were sequenced on Illumina MiSeq or NextSeq instruments using 1x185 or 1x75 runs respectively. Consensus sequences were assembled using a custom bioinformatics pipeline (https://github.com/proychou/hCoV19) adapted for SARS-CoV-2 from previous work (*30*, *31*). Briefly raw reads were trimmed to remove adapters and low quality regions using BBDuk and a k-mer based filter was used to pull out reads matching the reference sequence NC_045512. Filtered reads were *de novo* assembled using SPAdes (*32*) and contigs were ordered against the reference using BWA-MEM (*33*). Gaps were filled by remapping reads against the assembled scaffold and a consensus sequence was called from this alignment using a custom script in R/Bioconductor. Consensus sequences were annotated using Prokka (*34*) and deposited to Genbank (MT152824, MT419827-MT419859, MT598633-MT598642, MT605815-MT605817, MT627212-MT627318, MT627608-MT627638, MT632500, MT632502, MT632506, MT632518, MT632541-MT632624, MT632837, MT632951, MT632990), GISAID (accessions in Data Table S1), and NCBI SRA (Bioproject PRJNA610428).

Strains USA/WA-S31/2020 and USA/WA-S242/2020 had site 17747 masked to 'N' in our standard pipeline due to 99% and 96% respectively of reads coming from the forward strand. However, USA/WA-S31/2020 had 1483 and USA/WA-S242/2020 had 1497 reads all with 'T'. Given the importance of this site to determining whether a virus falls into the Washington State outbreak clade, we updated both strains to call 'T' at site 17747.

We examined the date of sample collection and other available metadata to remove possible duplicates from sequencing. For the present analysis, we restricted the dataset to samples collected on or before 15 March 2020. The final dataset of 455 SARS-CoV-2 genomes from Washington State represent a convenience sample from the underlying outbreak. All Washington State sequences used in the paper are available here https://github.com/blab/ncov-cryptic-transmission.

Phylogenetics

SARS-CoV-2 genomes from the global COVID-19 pandemic were downloaded from GISAID (*9*, *10*) and processed using the Nextstrain (*25*) bioinformatics pipeline *Augur* to align genomes via MAFFT v7.4 (*35*), build maximum likelihood phylogeny via IQTREE v1.6 (*36*) and reconstruct nucleotide and amino acid changes on the ML tree. Branch locations were estimated using the maximum-likelihood discrete traits model in *Augur*. The resulting tree was visualized in the Nextstrain web application *Auspice* to view resulting inferences. Workflows to reproduce phylogenetic trees shown in Figure 1 and Figure 2 are available from https://github.com/blab/ncov-cryptic-transmission.

The 455 focal genomes from Washington State were placed on a background of 494 publicly available genomes from GISAID collected between December 2019 and 15 March 2020. These 494 genomes were subsampled from an available 10,484 global genomes to attempt to equitably subsample through space and through time while enriching for genomes that are similar to Washington State focal samples. This was done by subsampling to 60 genomes per continent-level geographic region per month. Within each of these bins, genomes that are closer in genetic distance to focal genomes are preferentially selected. This approach ensures spatiotemporal diversity of background samples while preferentially including genomes that are likely to be phylogenetically informative for the focal sample. Subsampling algorithms are included in the linked workflow. We only analyzed genomes with at least 27000 bases (90% coverage) and only analyzed genomes with completely specified date information.

Additionally, 384 SARS-CoV-2 aligned genomes from the Washington State outbreak clade were analyzed in BEAST (*37*) to estimate time of common ancestor and rate of epidemic growth. This analysis used an exponential growth coalescent model in which effective population size and rate of exponential growth are estimated. We assumed a HKY85 nucleotide substitution model (*38*) with gamma distributed rate variation and a strict molecular clock with an informed lognormal prior with mean 6×10^{-4} and standard deviation of 5×10^{-5}. This prior was chosen based on the global clock analysis described below. Full analysis details, including BEAST XML, are available at https://github.com/blab/ncov-cryptic-transmission.

To establish an informed prior on the rate of SARS-CoV-2 molecular evolution, we performed an additional BEAST (*37*) analysis in which we subsampled 309 SARS-CoV-2 viruses sampled globally and collected between December 2019 and July 2020. This sampling gives enough temporal range to more accurate assess rate of evolution. We assumed a HKY85 nucleotide

substitution model (*38*) with gamma distributed rate variation and a strict molecular clock with an CTMC rate reference prior. This analysis produced a posterior estimate of the rate of SARS-CoV-2 molecular evolution of 6.0×10^{-4} subs per site per year (95% HPD 5.3 to 6.7×10^{-4} subs per site per year).

Sequential Monte Carlo

Proportion of positive specimens was estimated following a sequential Monte Carlo procedure. We recorded the vector $(n_0, \ldots, n_{75})$ = **n** of daily totals for specimens collected between 1 January and 15 March (shown in **Fig. 3A**). We also recorded the vector **k** of daily positive specimens (shown in **Fig. 3B**). We sought to estimate the vector **p** of daily proportion positive. We chose a process model of daily change to **p** as a standard diffusion process with $p_t = N(p_{t-1}, \sigma)$, where the volatility parameter $\sigma = 0.01$. We chose an observation model as a standard binomial probability $k_t = B(n_t, p_t)$. We estimated **p** using a sequential Monte Caro procedure via a bootstrap filter with 2000 particles and a reweighting (*39*, *40*)

Supplementary Text

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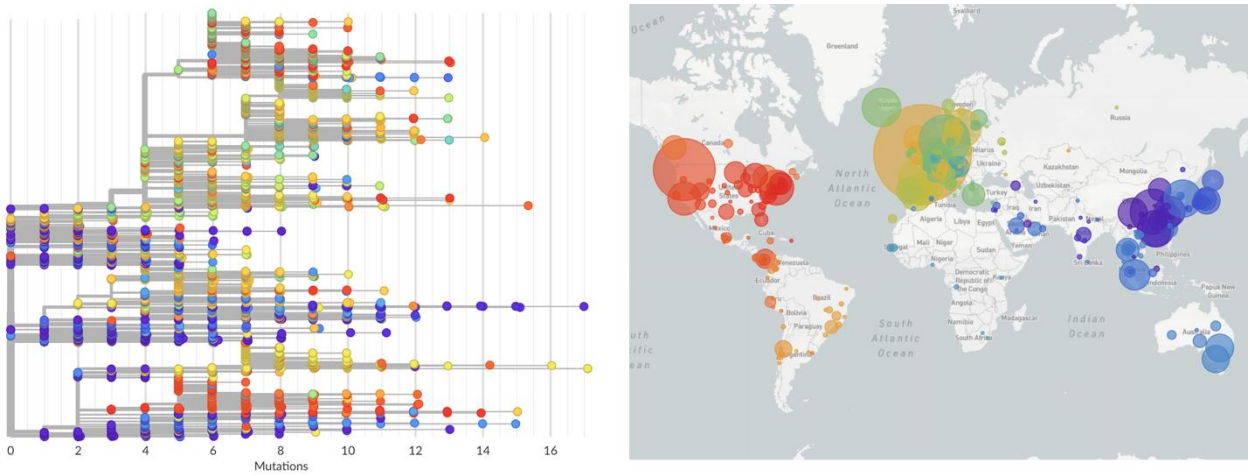
Supplementary Figures



Supplementary Figure S1. Maximum-likelihood phylogeny of 6827 SARS-CoV-2 viruses sampled globally and collected between 24 December 2019 and 14 March 2020. Tips are colored based on location shown in the map on the right-hand side, branch lengths are proportional to number of mutations along a branch and the x-axis is labeled with the number of substitutions relative to the root of the phylogeny, here equivalent to basal Wuhan outbreak viruses. An interactive version of this figure is available at https://nextstrain.org/groups/blab/ncov/early-pandemic allowing zooming to specific clades of interest. This analysis represents a non-subsampled dataset of viruses present in GISAID as of 4 August 2020.

Data S1. GISAID EpiCoV acknowledgments table. This table lists consensus SARS-CoV-2 genomes used in this manuscript's analysis including strain name and GISAID EpiCoV accession. This includes SARS-CoV-2 genomes generated as part of the present study as well as background genomes shared by research groups from all over the world.

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