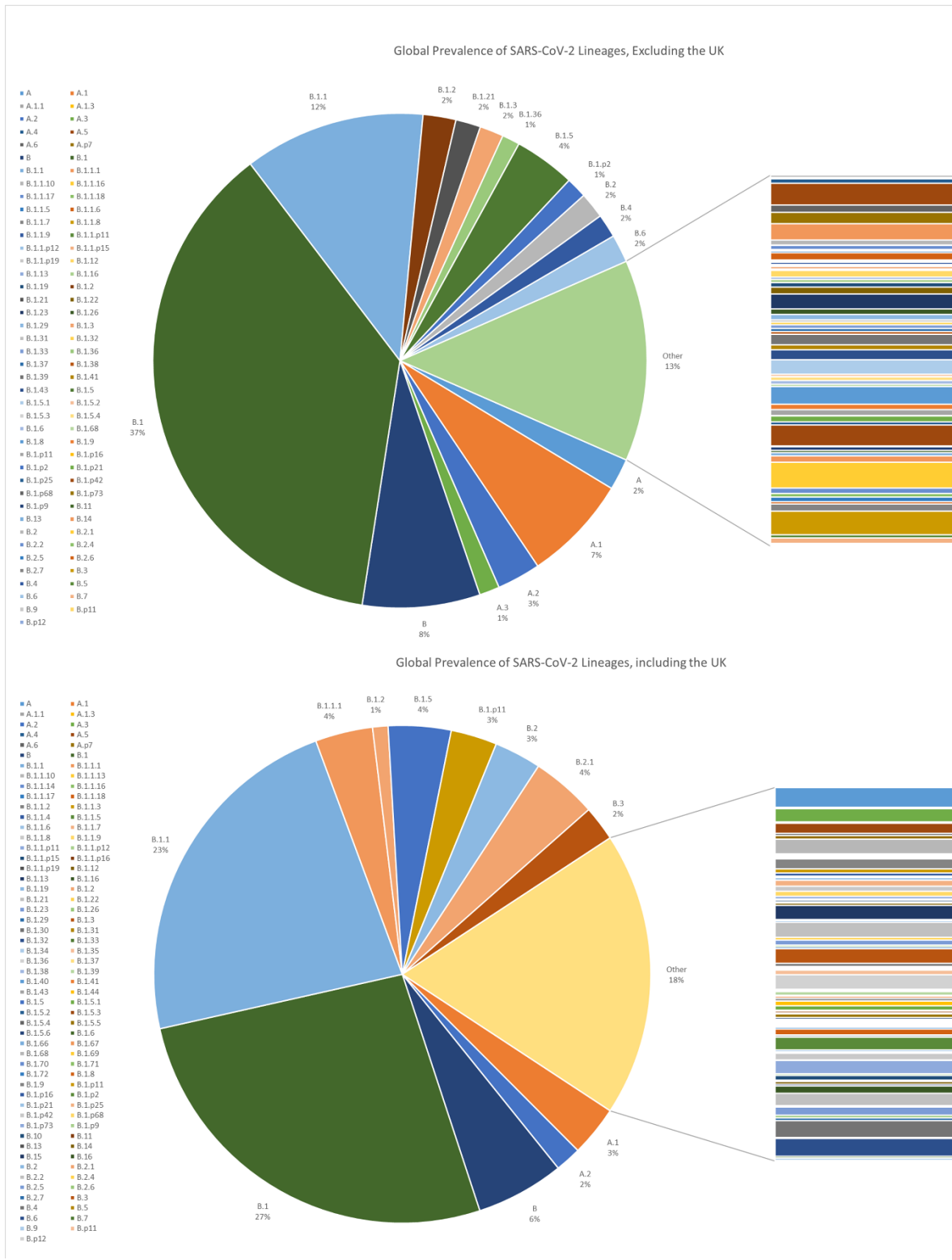


Supplemental Figure 1: Details of the samples included in the cohort of retrospectively tested respiratory samples. The sample type used (A), sex of each patient (B), ages of patients (C), the prevalence of virus species in those with a diagnosed viral infection (D) and the number of samples collected each week (E).



Supplemental Figure 2: The proportion of SARS-CoV-2 lineages detected globally between January and June 2020

ARTIC amplicon sequencing protocol for SARS-CoV-2

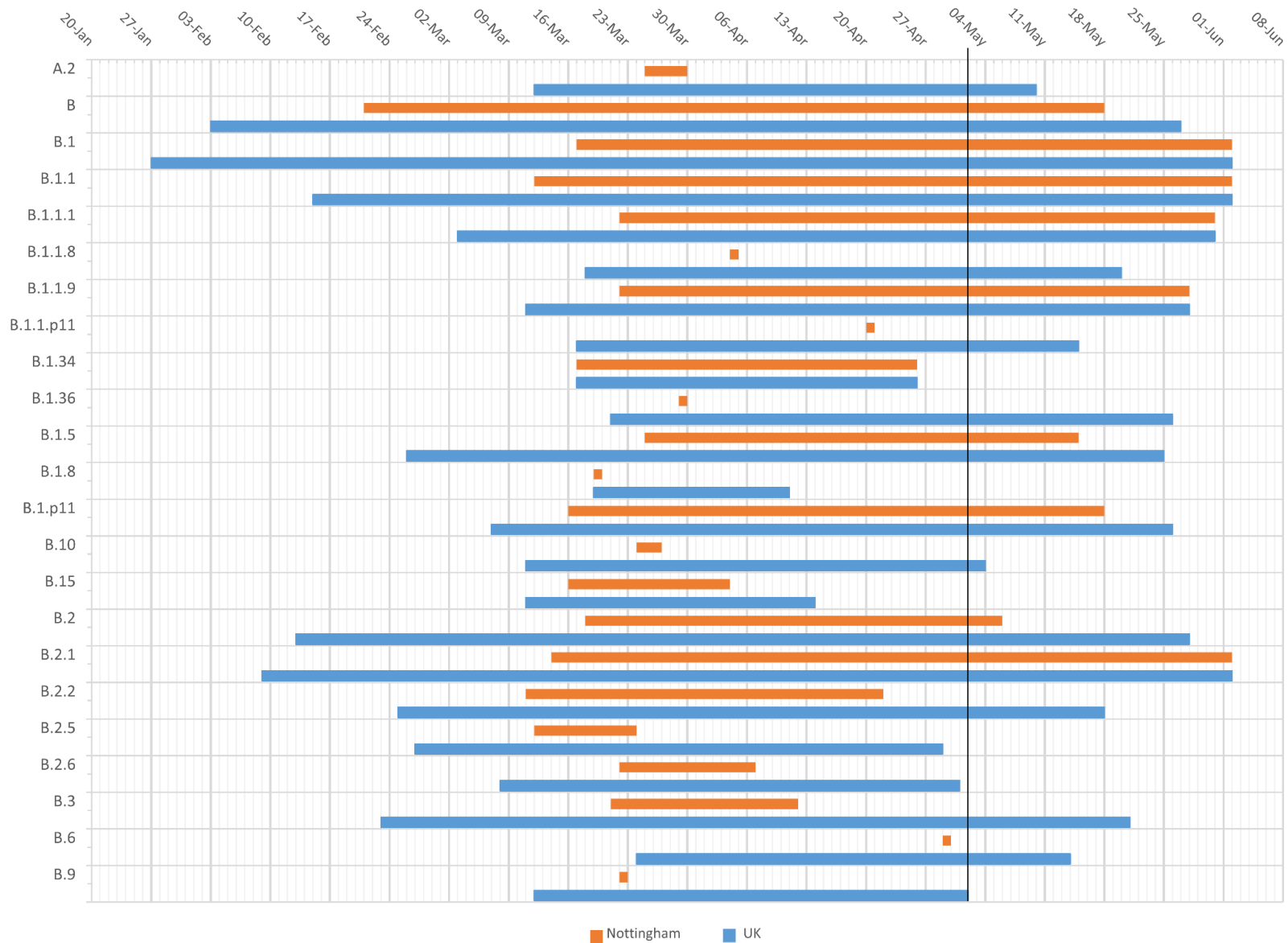
Sequencing libraries were prepared from cDNA using the ARTIC amplicon sequencing protocol v2 (<https://www.protocols.io/view/ncov-2019-sequencing-protocol-bbmuik6w>) with minor modifications.

ARTIC V3 primers (<https://artic.network/resources/ncov/ncov-amplicon-v3.pdf>) were used in multiplex PCR reactions to generate 98 overlapping amplicons of ~ 400 bp that tile across the SARS-CoV-2 genome. The ARTIC V3 primers consist of two multiplex primer pools that were used to set up two PCR reactions for each sample; Pool 1 containing odd numbered amplicons and Pool 2 containing even numbered amplicons. Q5 Hot Start High-Fidelity 2X Master Mix (NEB; M0494L) was used for amplification and optimization experiments determined that a touchdown PCR approach gave the most even coverage of amplicons across the genome. Thermal cycling conditions included an initial heat activation step at 98 °C for 30 s, followed by 25 cycles of denaturation at 98 °C for 15 s and annealing/extension at 65 °C for 5 min, with the annealing/extension temperature being decreased by 0.1 °C each cycle, followed by 10 cycles of denaturation at 98 °C for 15 s and annealing/extension at 62.5 °C for 5 min. For each sample, the two amplicon pools were combined and then purified using AMPure XP beads (Beckman Coulter; A63882) at a 1:1 ratio. The bead pellets were washed twice with 70% ethanol, dried and then eluted in Buffer EB (Qiagen; 19086). The eluate was quantified using the Qubit 4 Fluorometer (Thermo Fisher Scientific) and the Qubit 1X dsDNA HS Assay Kit (Thermo Fisher Scientific; Q33230).

Barcoded sequencing library were prepared using on a one-pot native barcoding method derived from the Oxford Nanopore Native Barcoding Ligation Sequencing protocol and using the Native Barcoding Expansion 1-12 and 13-24 kits (Oxford Nanopore Technologies; EXP-NBD104 and EXP-NBD114). Amplicon pools were normalized to 5 ng/μL and 50 ng of each were used in EndPrep reactions using the NEBNext Ultra II End Repair/dA-Tailing Module (NEB; E7546L). Reactions were incubated at 20 °C for 10 min, then 65 °C for 10 min. EndPrep reactions (5 ng of each) were added directly to 10 μL barcode adapter ligation reactions containing 1.25 μL of native barcode (NB01 to NB24) and 5 μL of Ultra II Ligation Master Mix and 0.15 μL of Ligation Enhancer from the NEBNext Ultra II Ligation Module (NEB; E7595). Ligations were incubated at 20 °C for 20 min then at 65 °C for 10 min. Barcoded samples were pooled and purified with 0.4 x the total pool volume of AMPure XP beads. AMPure XP bead pellets were washed twice by resuspending in 250 μL of SFB (Oxford Nanopore Technologies; EXP-SFB001) and once by adding 200 μL of 70 % ethanol. Bead pellets were dried at room temperature for 5-10 min then resuspended in Buffer EB.

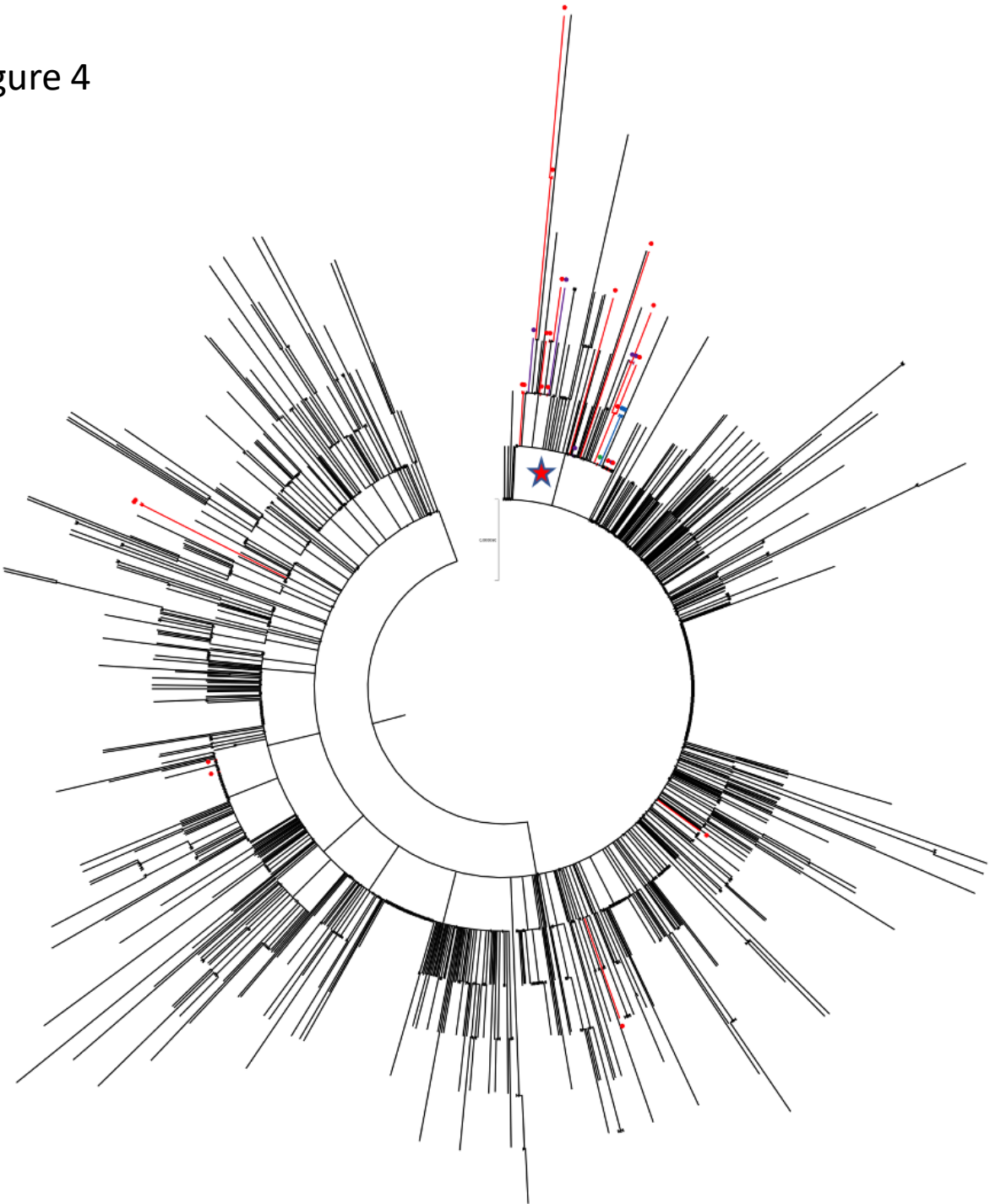
Library preparation was completed by ligating the AMII adapter from the Native Barcoding Expansion Kits, using the NEBNext Quick Ligation Module (NEB; E6056L). The ligation reaction was incubated at room temperature for 20 min then purified using a 1:1 ratio of AMPure XP beads. AMPure XP bead pellets were washed twice by resuspending in in 250 μL of SFB (Oxford Nanopore Technologies; EXP-SFB001) and eluted in EB (Oxford Nanopore Technologies; EXP-AUX001). Finished libraries were quantified using the Qubit 4 Fluorometer (Thermo Fisher Scientific) and the Qubit 1X dsDNA HS Assay Kit (Thermo Fisher Scientific; Q33230). 20 ng of library was loaded onto a MinION flow cell (Oxford Nanopore Technologies; FLO-MIN106 R9.4.1) and sequenced on the GridION X5 Mk1.

RAMPART (<https://artic.network/ncov-2019/ncov2019-using-rampart.html>) was used for real-time data analysis.



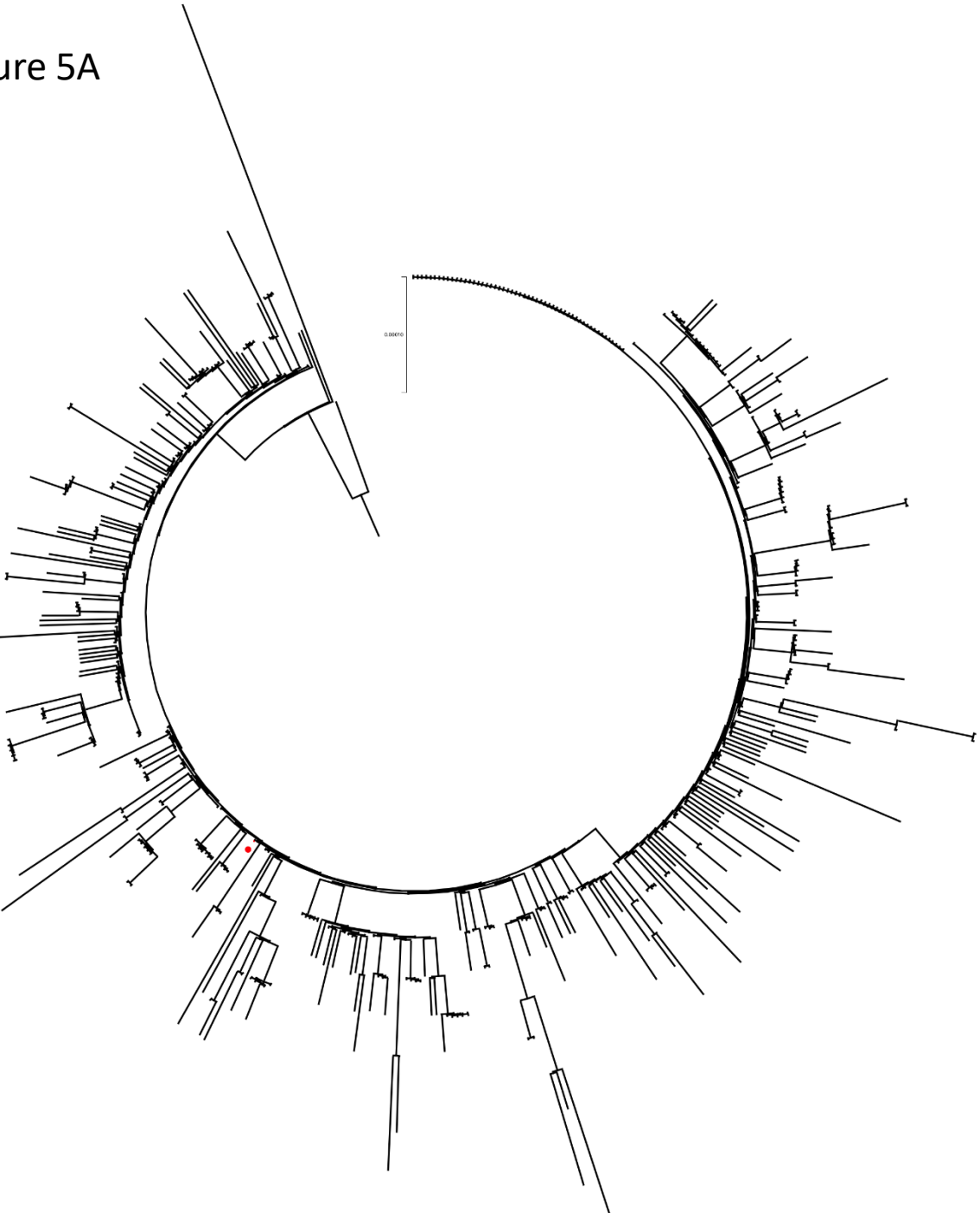
Supplementary Figure 3: The date in which each lineage was first and last detected in both Nottingham and the UK. The black line marks the 2nd of May, one month prior to the latest available data at the time of writing (2nd of June). Lineages which were last detected before this date are considered to be ‘Unobserved’.

Supplementary Figure 4
Lineage B

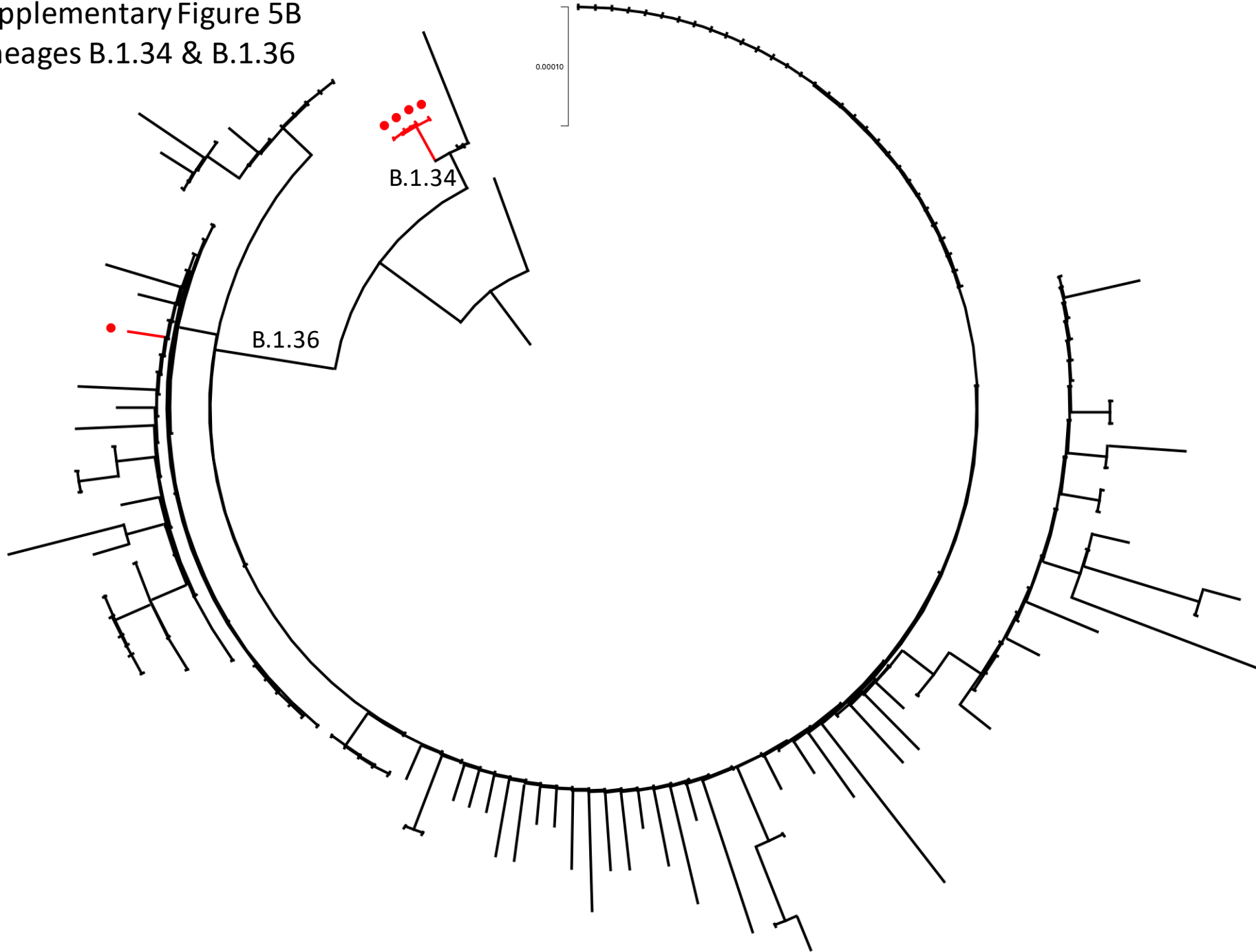


Supplementary figure 4: Phylogenetic relationships of SARS-CoV-2 sequences from lineage B based on their entire genome (29,412nt). Nottingham lineage B sequences (Coloured) were compared alongside other sequences of the same lineage. The tree was rooted on a Wuhan sequence sampled on 2020-01-05. The region marked with a red star represents the sub-tree shown in figure 4, which contains the majority of Nottingham-derived, lineage B sequences.

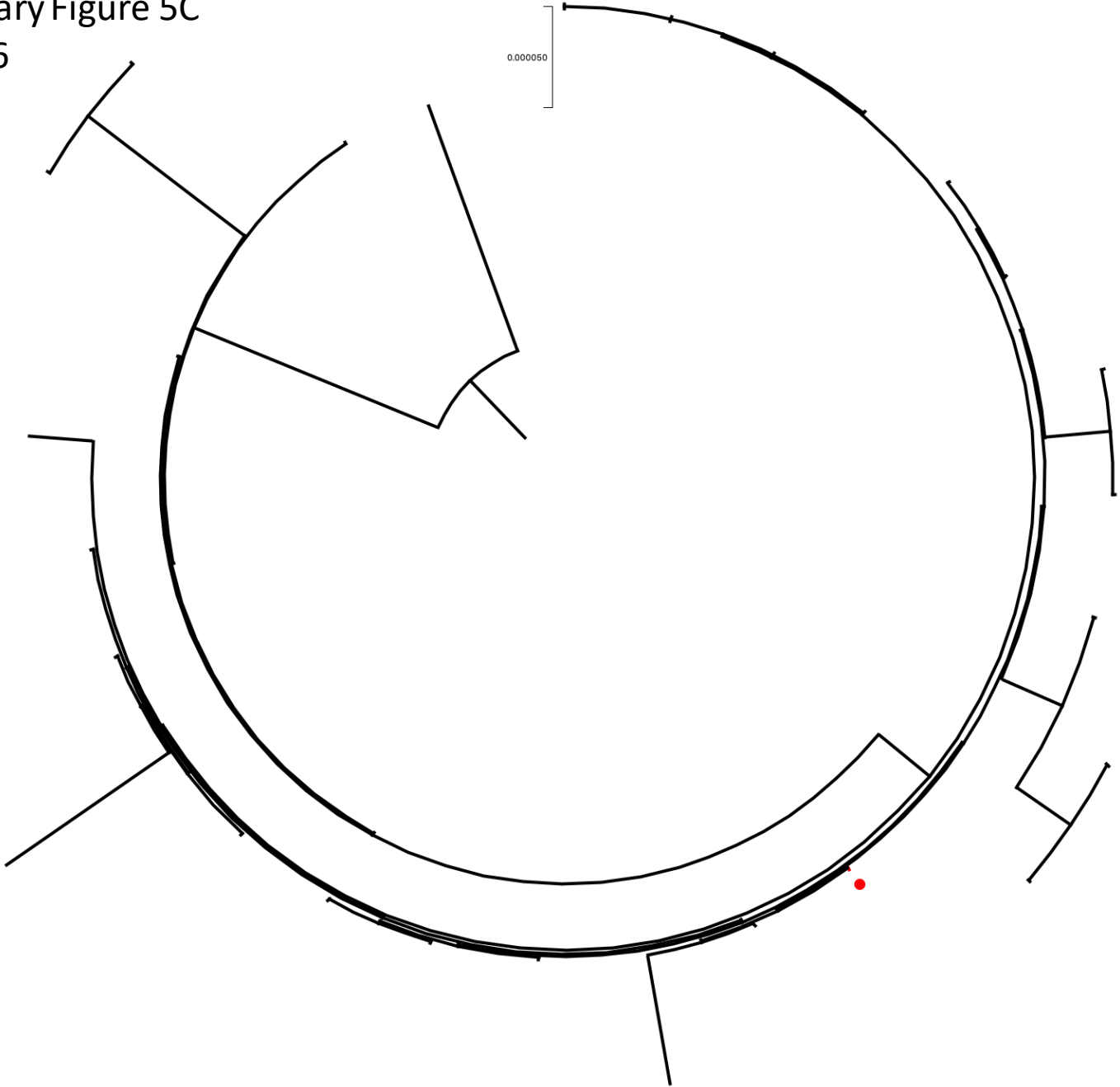
Supplementary Figure 5A
Lineage B.1.5



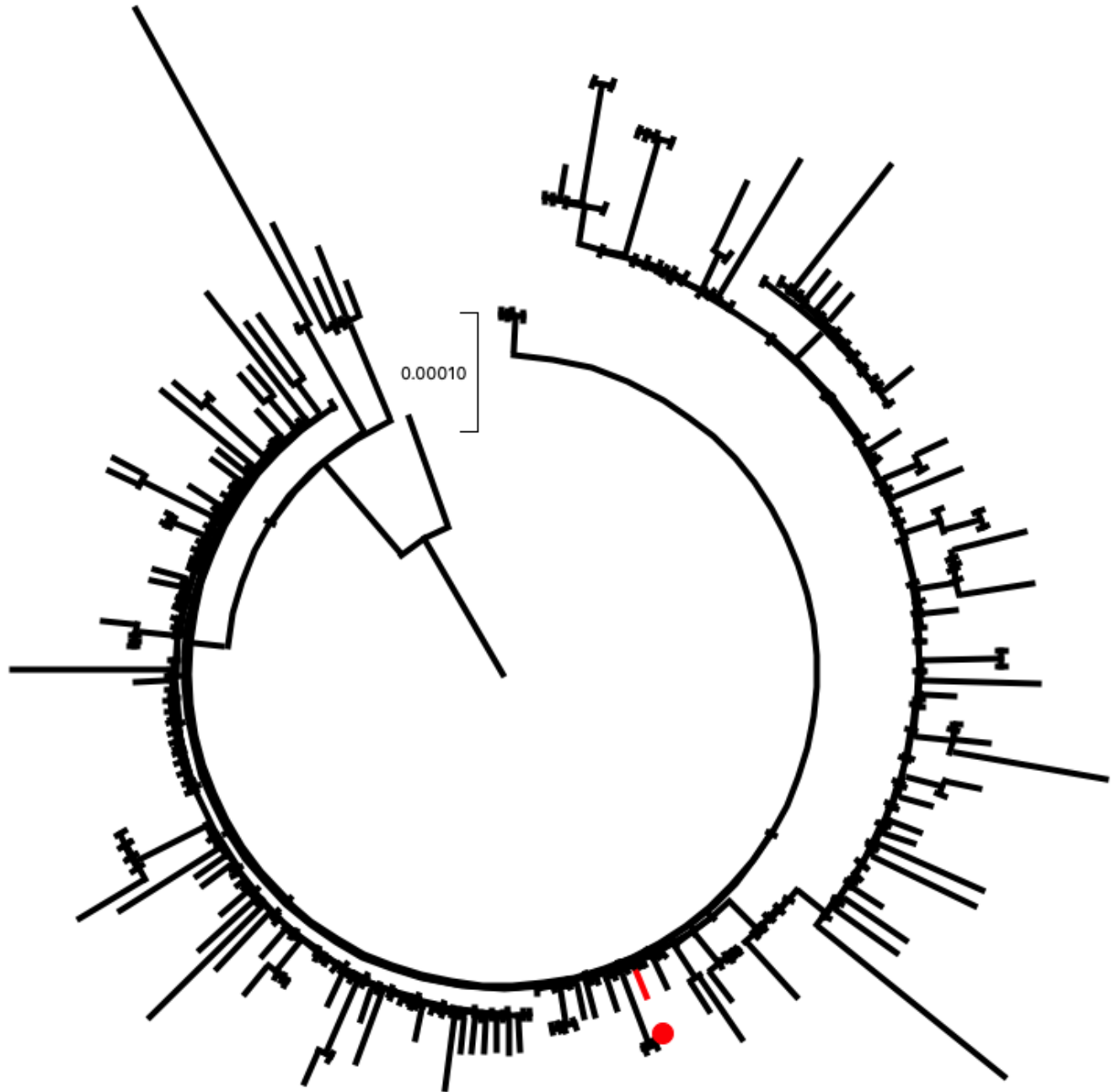
Supplementary Figure 5B
Lineages B.1.34 & B.1.36



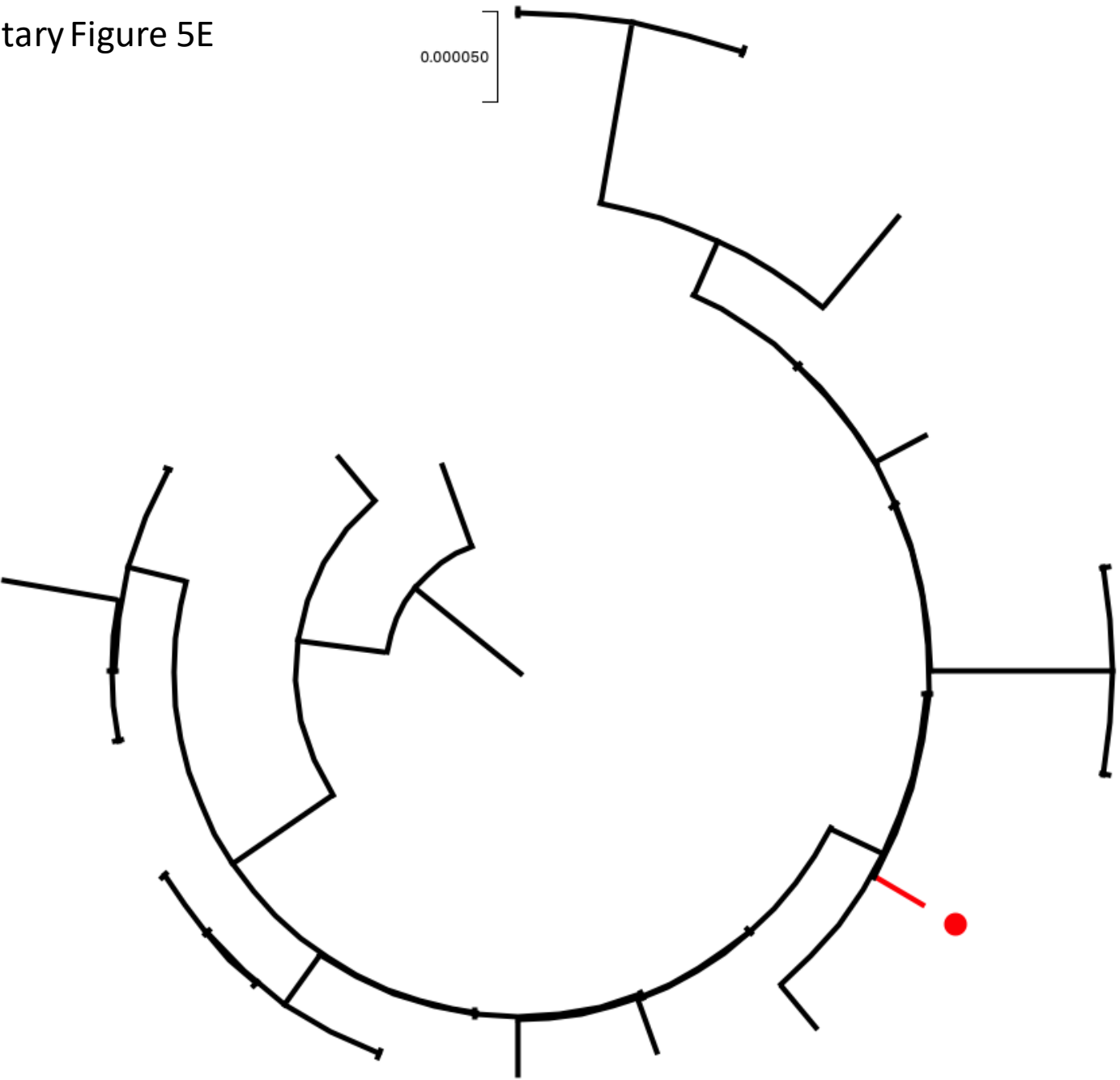
Supplementary Figure 5C
Lineage B.2.6



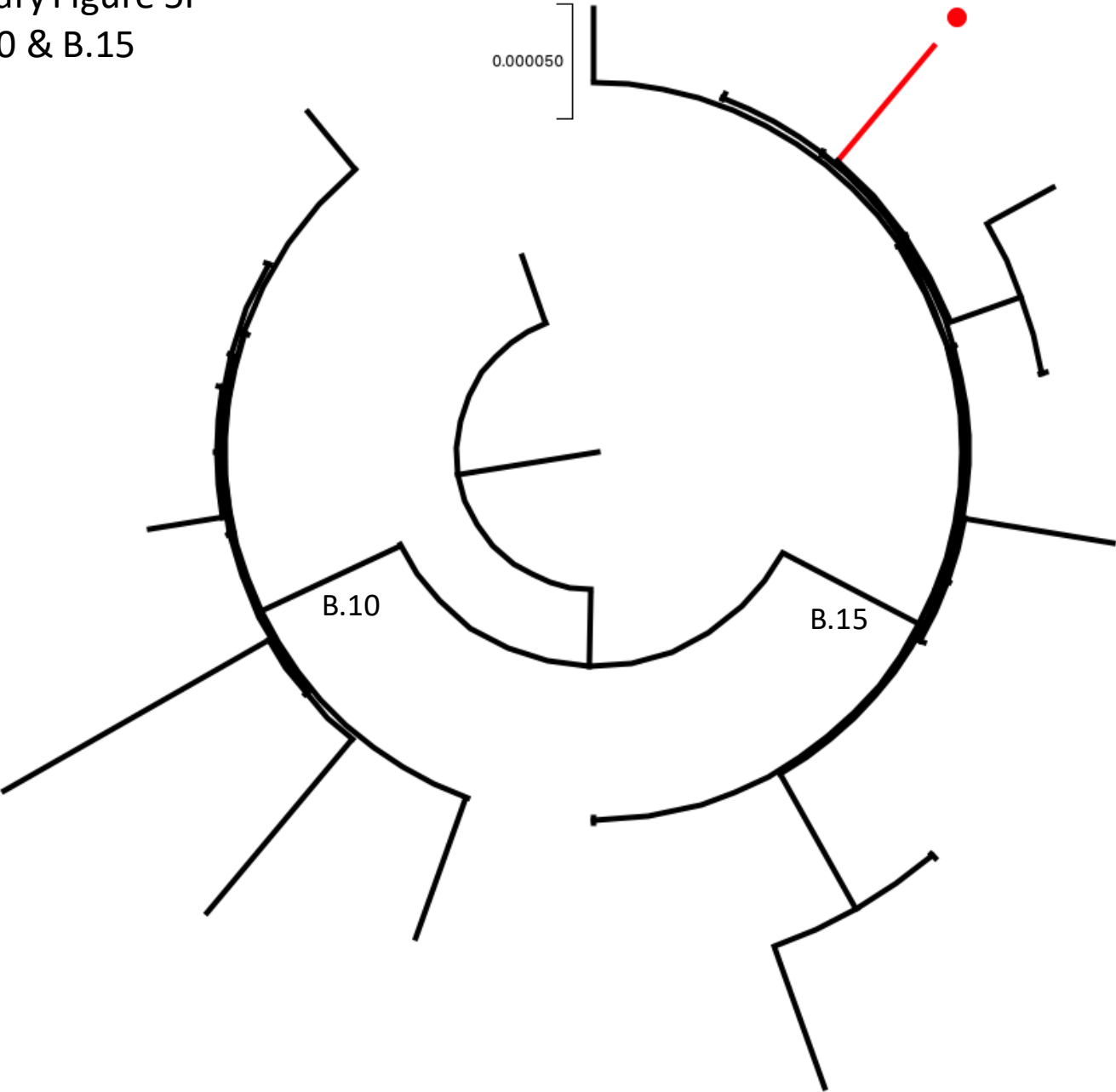
Supplementary Figure 5D
Lineage B.6



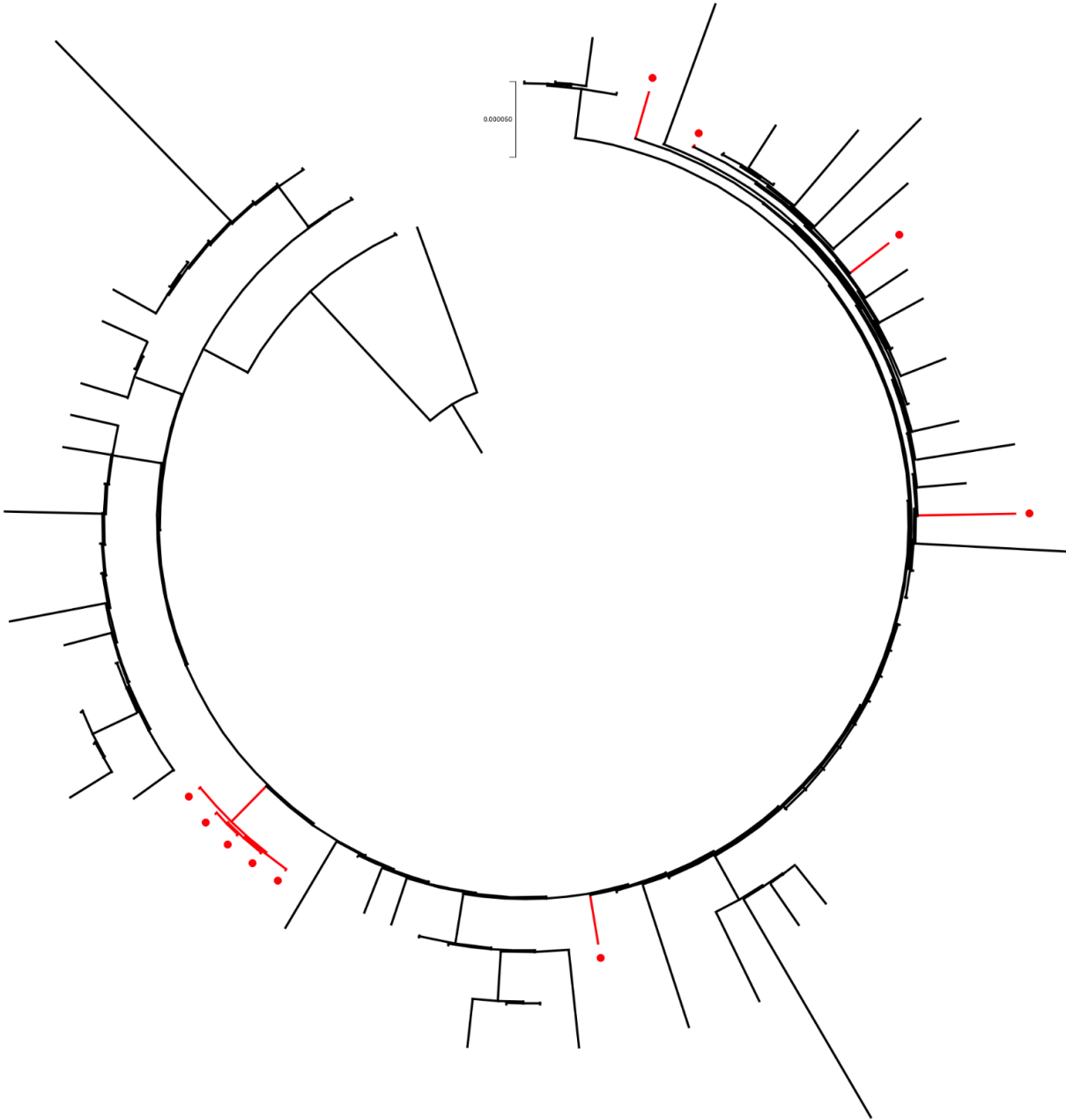
Supplementary Figure 5E
Lineage B.9



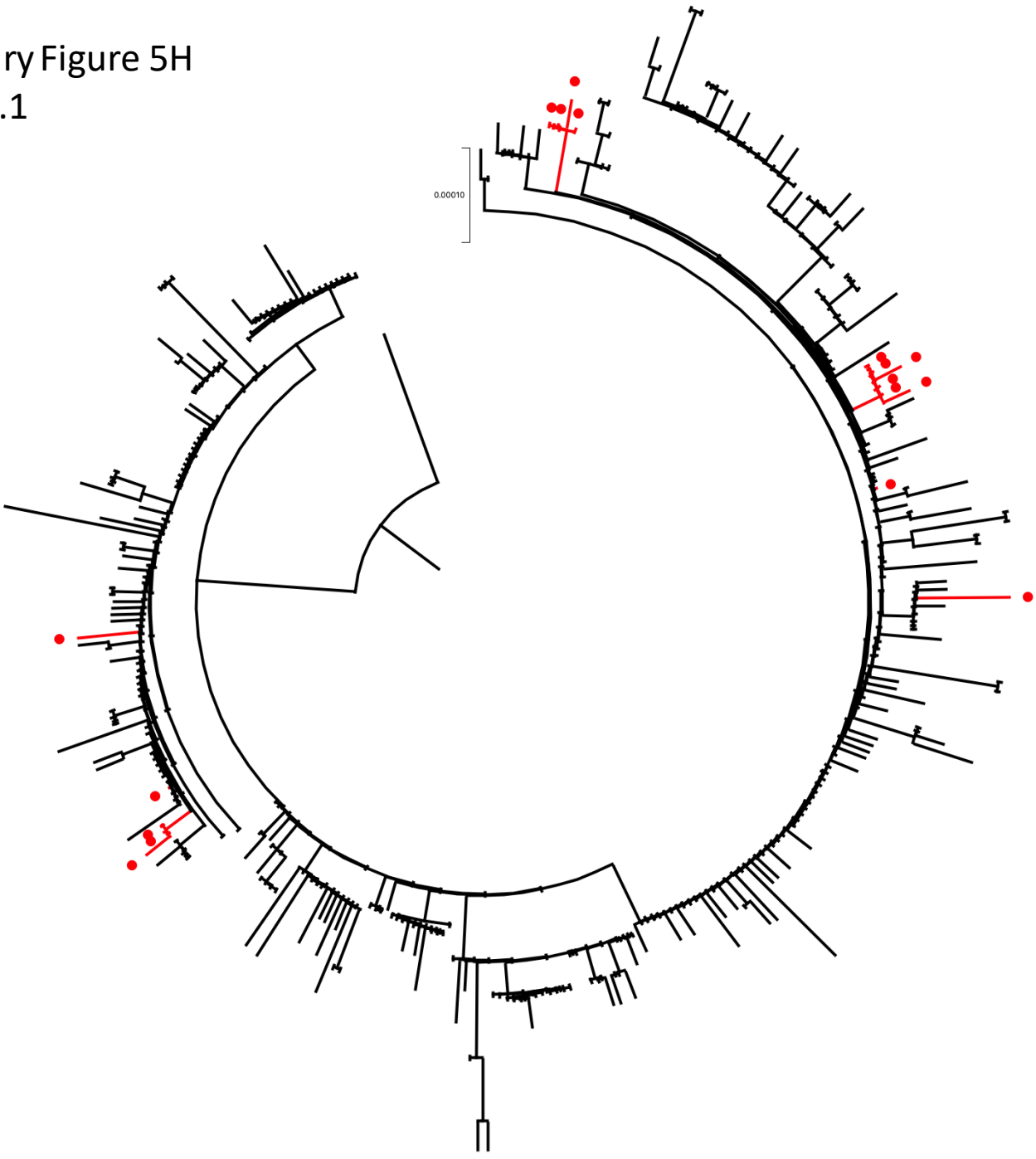
Supplementary Figure 5F
Lineages B.10 & B.15



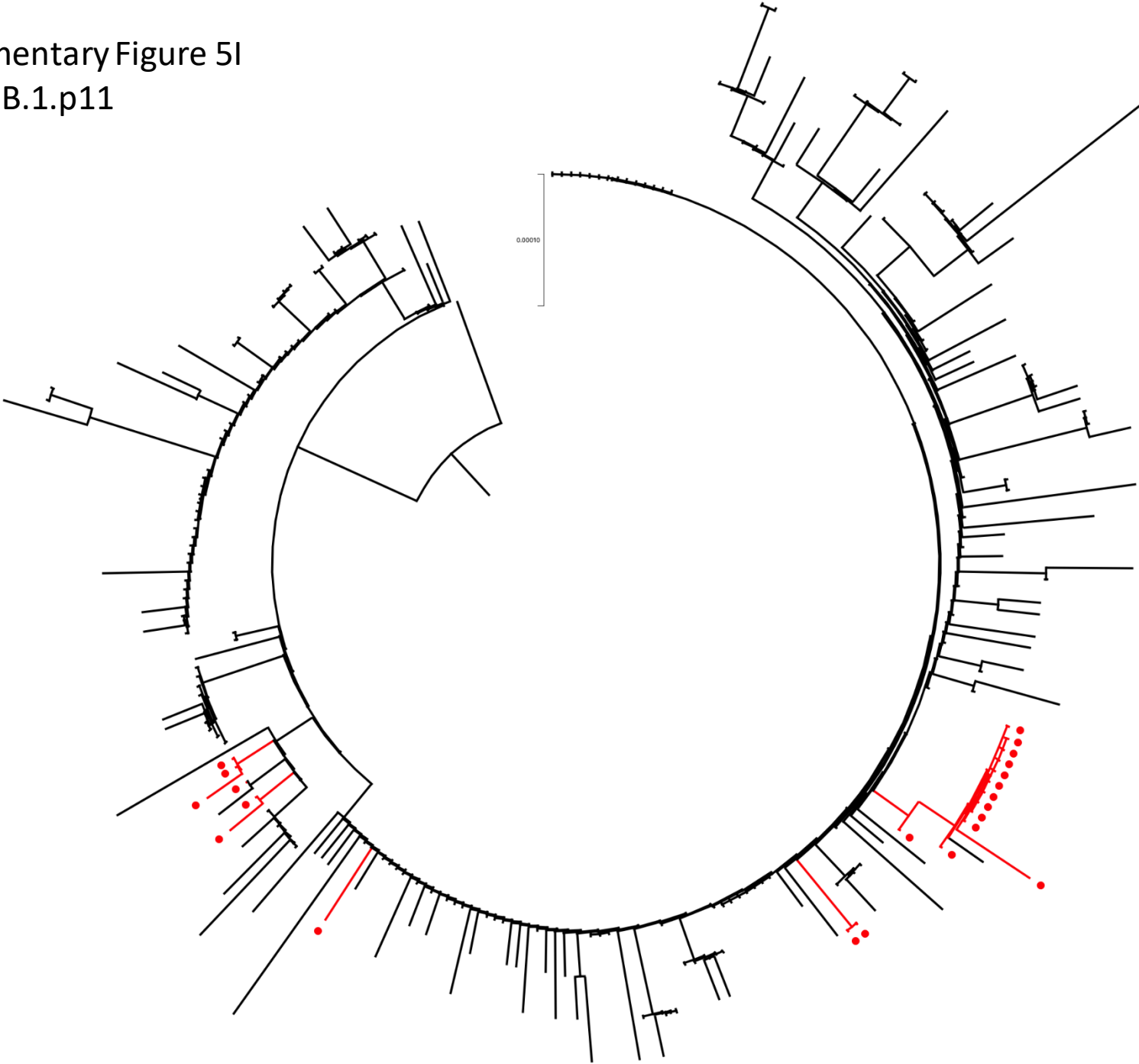
Supplementary Figure 5G
Lineage B.2.2



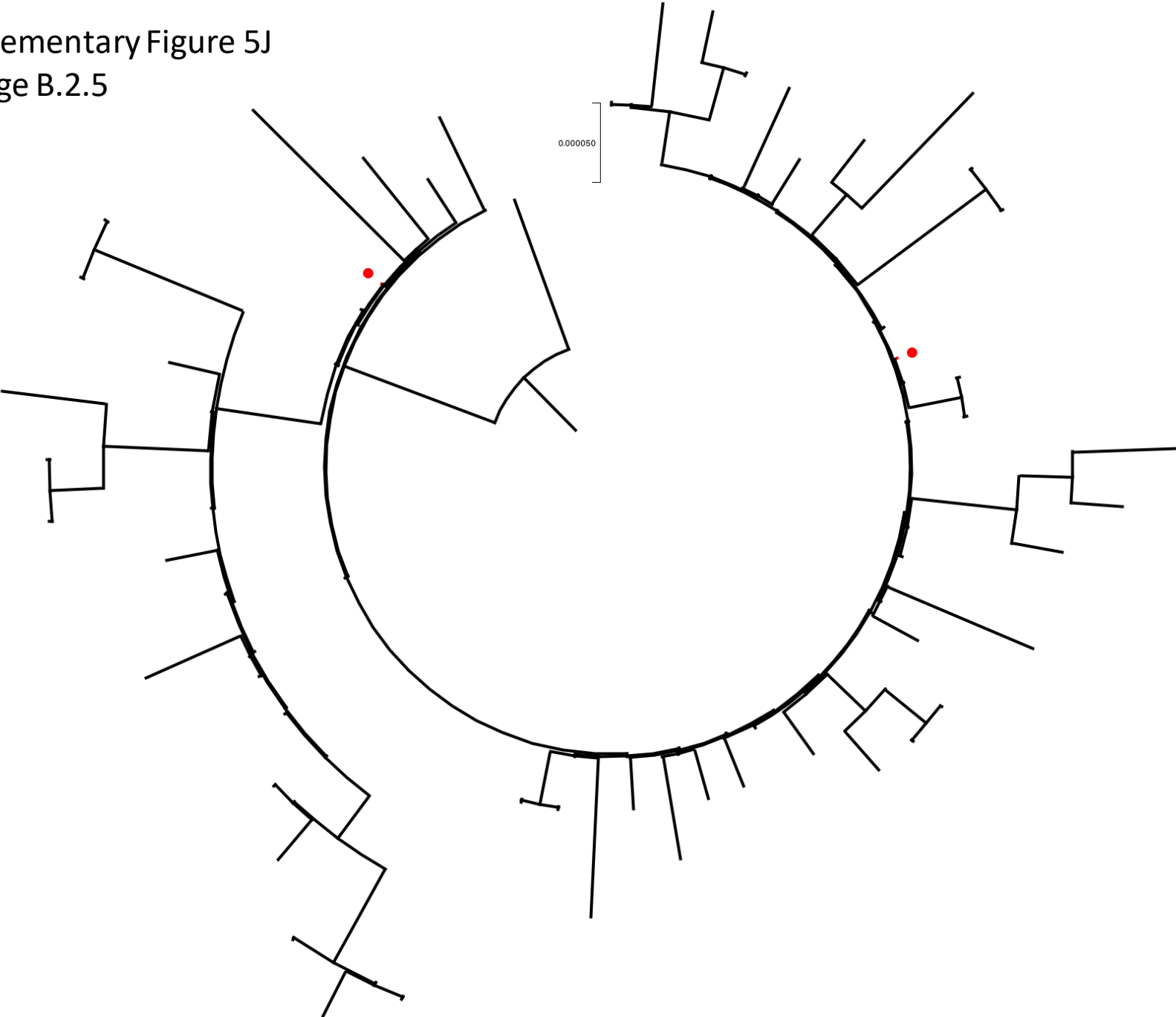
Supplementary Figure 5H
Lineage B.1.1.1



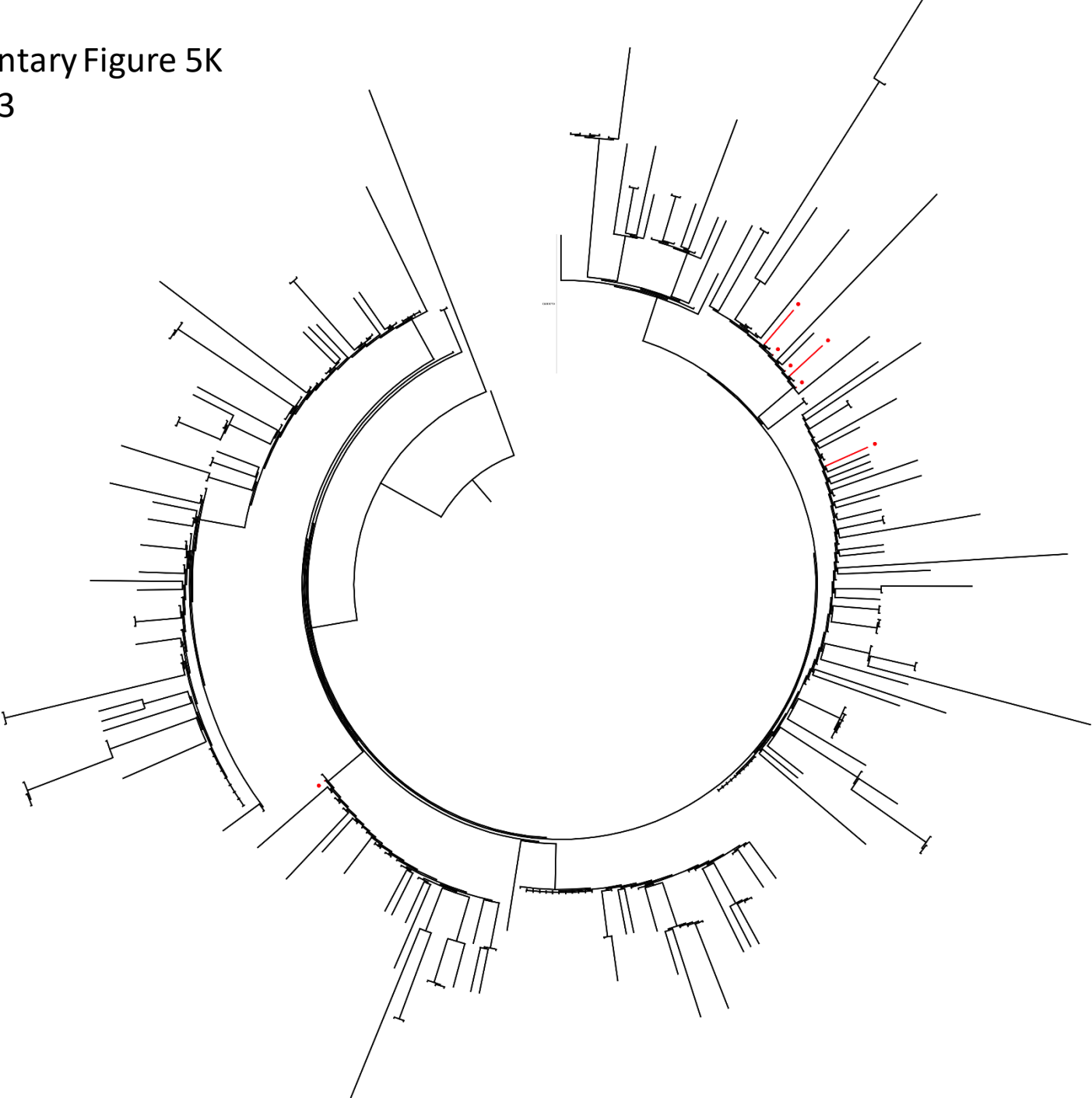
Supplementary Figure 5I
Lineage B.1.p11



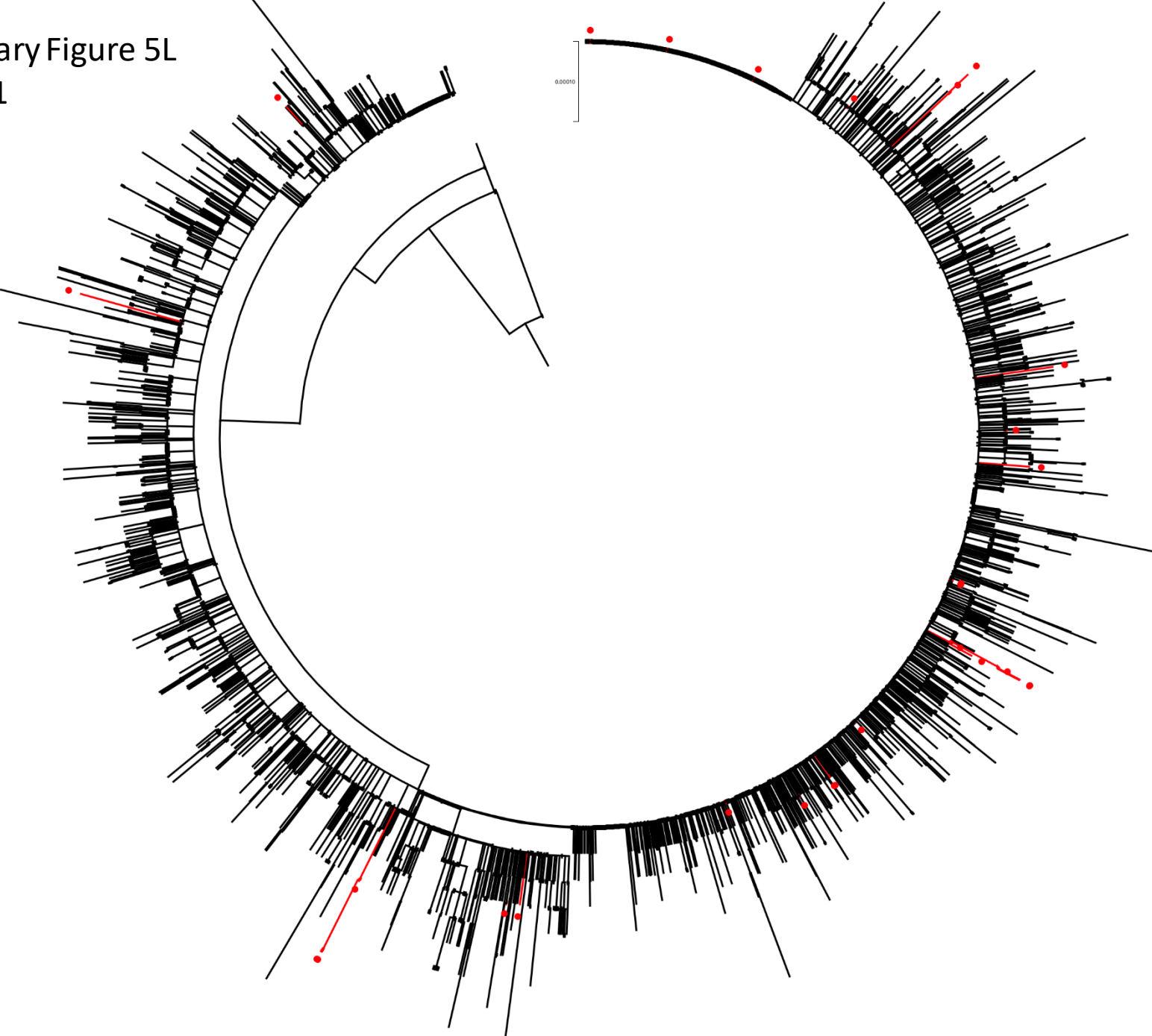
Supplementary Figure 5J
Lineage B.2.5



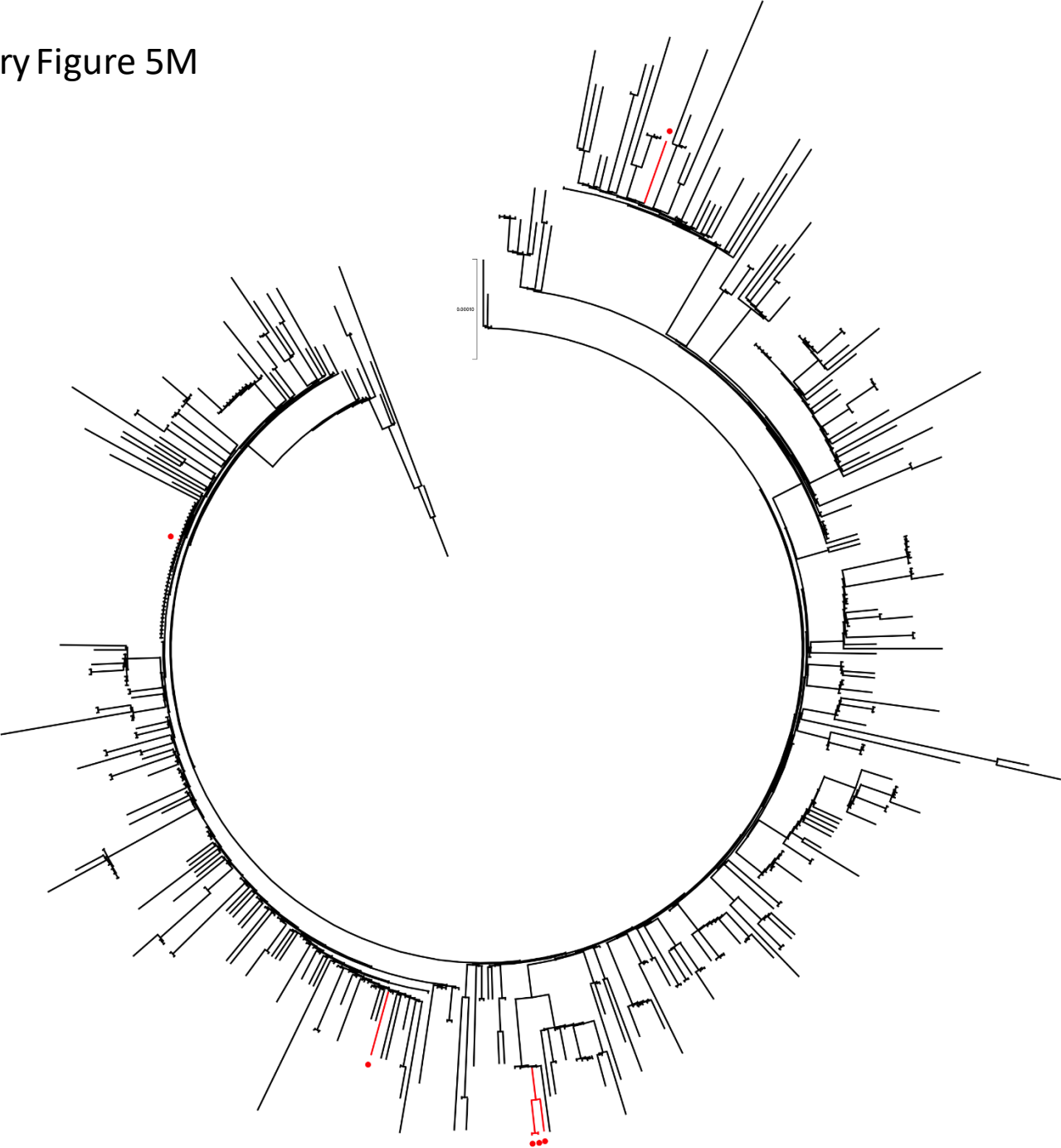
Supplementary Figure 5K
Lineage B.3



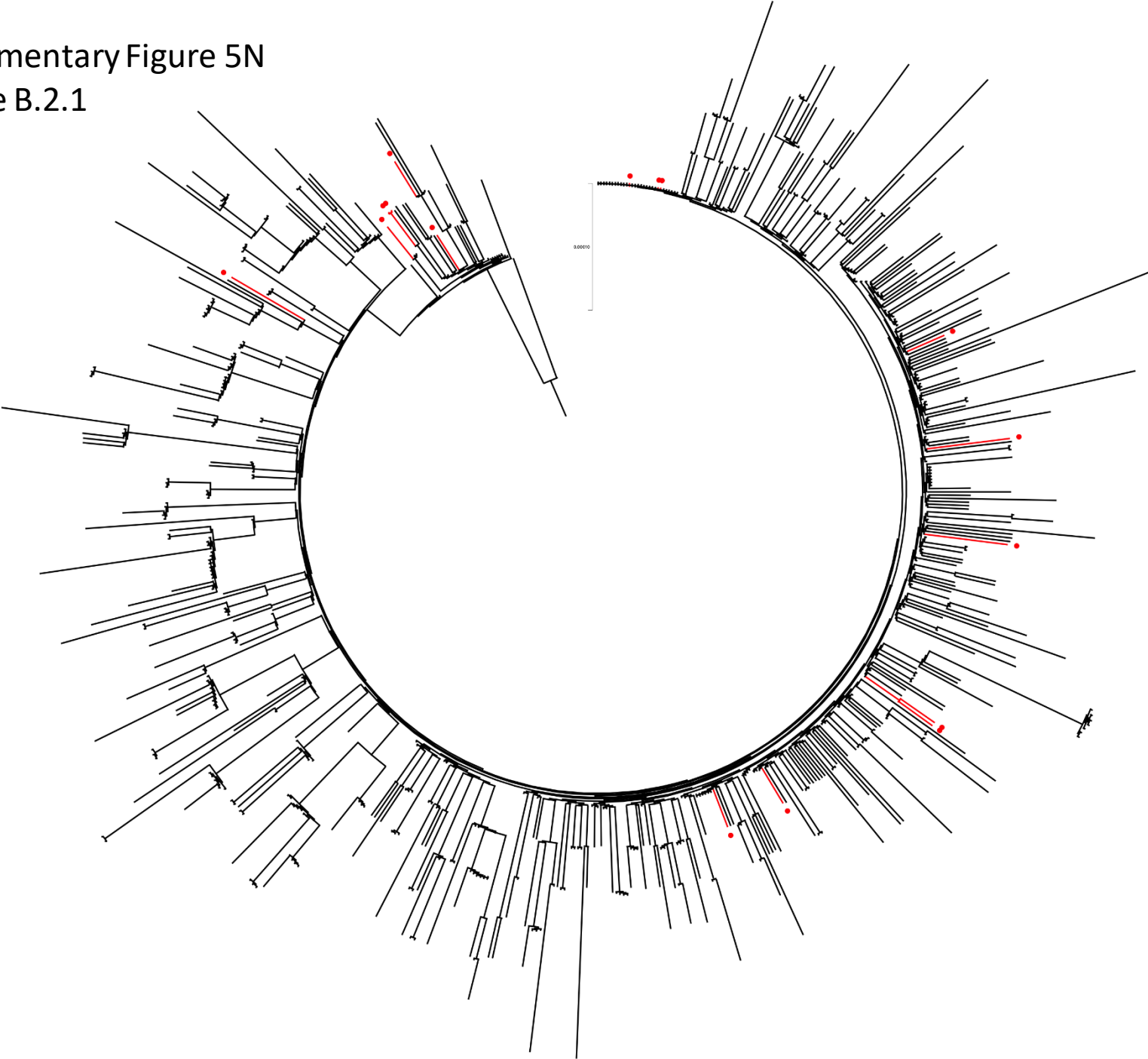
Supplementary Figure 5L
Lineage B.1.1



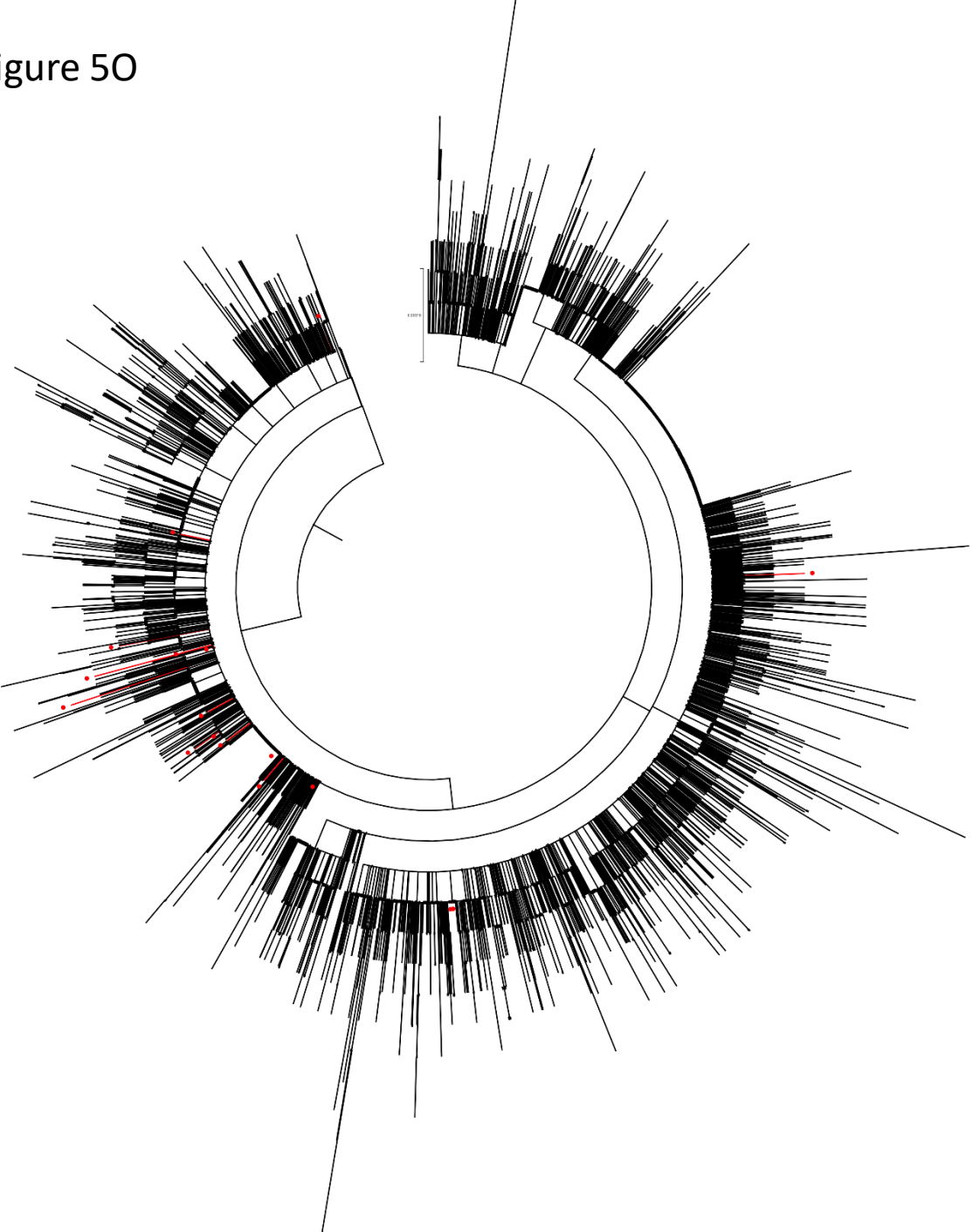
Supplementary Figure 5M
Lineage B.2



Supplementary Figure 5N
Lineage B.2.1



Supplementary Figure 50
Lineage B.1



Supplementary figure 5: Phylogenetic relationships of SARS-CoV-2 sequences from lineages B.1.5 (A), B.1.34/B.1.36 (B), B.2.6 (C), B.6 (D), B.9 (E), B.10/B.15 (F), B.2.2 (G), B.1.1.1 (H), B.1.p11 (I), B.2.5 (J), B.3 (K), B.1.1 (L), B.2 (M), B.2.1 (N) and B.1 (O) based on their entire genome (29,412nt). Nottingham sequences (marked with red circles and branches) were compared alongside other sequences of the same lineage. The tree was rooted on a Wuhan sequence sampled on 2020-01-05. For clarity branch labels have been removed. Branch lengths are drawn to a scale of nucleotide substitutions per site.

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