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SARS-CoV-2 Delta Variant among Asiatic Lions, India

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In May 2021, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was detected in Asiatic lions in a zoological park in India. Sequence and phylogenetic analyses showed the SARS-CoV-2 strains were the B.1.617.2 (Delta) variant. To reduce transmission of variants of concern, surveillance of SARS-CoV-2 in wild animal populations should be increased.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), in natural conditions, has shown a broad host susceptibility range (*1*). Identifying susceptible animal species, reservoirs, and cross-species transmission is a global scientific and public health concern. We found evidence of natural SARS-CoV-2 infection in Asiatic lions (*Panthera leo persica*) caused by the lineage B.1.617.2 (Delta) variant (World Health Organization nomenclature). We provide coronavirus disease (COVID-19) case information and detailed genomic characterization.

Arignar Anna Zoological Park in Chennai, India, houses 13 Asiatic lions, 9 in a lion safari and 2 each in separate moat enclosures. Beginning May 21, 2021, four of the safari lions started showing signs of loss of appetite, nasal discharge, and occasional coughing. Nasal swab, rectal swab, and fecal samples were collected from 11 lions during May 24–29, 2021, and sent to the Indian Council of Agricultural Research–National Institute of High Security Animal Diseases (Bhopal, India) for molecular investigations (Appendix Table 1, https://wwwnc.cdc.gov/EID/article/27/10/21-1500-App1.pdf).

We used the VIRALDTECT II Multiplex Real Time PCR Kit for COVID-19 (Genes2Me, https://genes2me.com) to confirm SARS-CoV-2 in 9/11 lions. The other 2 lions were sampled on June 19, 2021, and tested negative for SARS-CoV-2. We also used a World Organisation for Animal Health–recommended reverse transcription PCR (RT-PCR) method to test for canine distemper virus on samples from all 13 lions; all tested negative (*2*). Two of the infected lions died of COVID-19, one on June 3 and the other on June 16, 2021.

After we confirmed SARS-CoV-2 infection, we performed whole-genome sequencing directly from nasal swab specimens of 4 lions that initially showed symptoms by using the MinION sequencing platform (Oxford Nanopore Technologies, https://nanoporetech.com) (Appendix). We deposited sequences in GenBank (accession nos. MZ363851–4) and GISAID (https://www.gisaid.org; accession nos. EPI_ISL_2821077–80).

To elucidate the temporal dynamics of SARS-CoV-2 among the lions, we downloaded 310 complete SARS-CoV-2 genomes from GISAID (*3*) that had high coverage and were sequenced from the Tamil Nadu state of

India, where the zoological park is located, during January 1–June 11, 2021. To generate a set of representative sequences, we used a UCLUST algorithm (*4*) to select sequences that clustered at the 99.9% identity threshold. We used MAFFT version 7.475 (*5*) to align representative SARS-CoV-2 sequences from GISAID with sequences from the lions; then we constructed a phylogenetic tree by using the general time reversible plus gamma model in RAxML version 8.2.12 (*6*) (Figure).

The amino acid substitutions and deletions in the spike protein of SARS-CoV-2 in lions typically matched with the SARS-CoV-2 Delta variant (Appendix Table 2). We noted amino acid changes in the N terminal domain (NTD), including T19R, G142D, E156del, F157del, R158G; in the receptor binding motif (RBM), including L452R and T478K; and in D614G of subdomain 2. We also noted a substitution close to S1/S2 protease cleavage site at P681R and heptad repeat 1 at D950N (Appendix Figures 1, 2). In addition, the lion sequences had the K77T substitution in the NTD, which has been detected in SARS-CoV-2 genomes from 24 countries. In India, frequency of the K77T substitution generally is low (0.44%) but occurred in 27.42% (65/237) of sequences in the B.1.167.2 lineage collected in Tamil Nadu state (Appendix Table 2).

The changes in the spike protein, including E156del, F157del, and R158G, of lion sequences were not found in human SARS-CoV-2 sequences from the same geographic area, nor were changes in nonstructural protein 3 (NS3) V88I, possibly because SARS-CoV-2 sequencing is limited in the region. Furthermore, these changes in spike and NS3 were not seen in previously reported lion SARS-CoV-2 sequences, ruling out the possibility that these are host-adapted mutations (*7*) (Appendix Figure 3). Further investigations could delineate whether changes in the spike protein, namely E156del, F157del, R158G, and K77T, are escape mutants or are associated with increased transmissibility or pathogenicity.

A nucleotide similarity comparison of the 4 lion SARS-CoV-2 sequences against the sequences available in GISAID and phylogenetic analysis revealed that the lion sequences closely matched with a representative human SARS-CoV-2 sequence of B.1.617.2 lineage, GISAID accession no. EPI_ISL_2463770, that comprises 152 viral genome pools collected from the same geographic region during the same month that the lions' samples were collected (Figure; Appendix Figure 4). The park's management strictly adhered to COVID-19 guidelines and did not introduce any new animals to the zoo during India's widespread COVID-19 pandemic. The primary source of SARS-CoV-2 infection in the lions might have been an asymptomatic or paucisymptomatic person. Among the 9 infected lions, 7 were in the lion safari and shared a common habitat, shelter, feeding spaces, and water sources. The other 2 infected lions were on display in separate enclosures that shared a common moat. Because shared habitats offered opportunities for close physical contact, identifying genetically identical SARS-CoV-2 infections in these lions in a short period of time indicates the possibility of lion-to-lion transmission.

In conclusion, evidence of confirmed natural SARS-CoV-2 Delta variant infections in Asiatic lions in India justifies need for increased SARS-CoV-2 surveillance in wild animal species. In addition, strict biosecurity measures should be implemented for wild animals kept in captivity.

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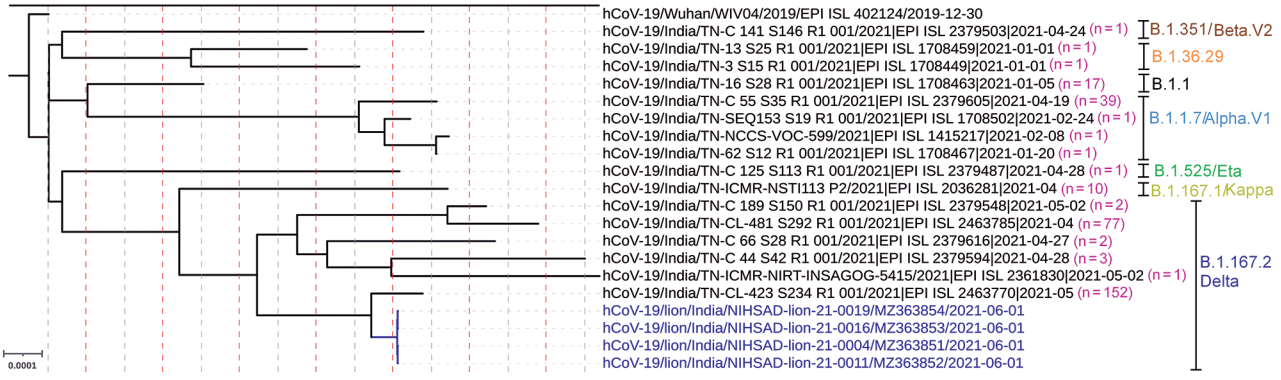

Figure. Complete genome phylogenetic analysis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detected in Asiatic lions (*Panthera leo persica*), India (blue text), and representative sequences of different clusters generated at 99.9% identity threshold from the available SARS-COV-2 sequences from Tamil Nadu, India, in the GISAID. The maximum-likelihood tree was rooted to Wuhan-Hu-1 reference sequence (GISAID accession no. EPI_ISL_402124). GenBank accession numbers are provided for the sequences from this study. Pink numbers in parentheses indicate the number of SARS-CoV-2 genome sequences clustered at 99.9% identity threshold. Other text colors represent SARS-CoV-2 variants. Scale bar indicates nucleotide substitutions per site.

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SARS-CoV-2 Variants in Immunocompromised Patient Given Antibody Monotherapy

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A 72-year-old immunocompromised man infected with severe acute respiratory syndrome coronavirus 2 received bamlanivimab monotherapy. Viral evolution was monitored in nasopharyngeal and blood samples by melting curve analysis of single-nucleotide polymorphisms and whole-genome sequencing. Rapid emergence of spike receptor binding domain mutations was found, associated with a compartmentalization of viral populations.

A 72-year-old immunocompromised man in France who had chronic lymphocytic leukemia associated with hypogammaglobinemia for 4 years experienced diarrhea, asthenia, fever, and cough associated with coronavirus disease (COVID-19). Although he had received 1 injection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) mRNA vaccine (BNT162b2; Pfizer/BioNTech, https://www.pfizer.com) 20 days earlier, we confirmed a diagnosis of COVID-19 by using a semiquantitative SARS-CoV-2 reverse transcription PCR (RT-PCR) viral load assay. This assay showed a cycle threshold (C_t) value of 27 for a nasopharyngeal swab specimen. His most recent monoclonal antibody (mAb) chemotherapy treatment (venetoclax and rituximab) had been conducted 17 days earlier. Because of his immunocompromised status, treatment with bamlanivimab (LY-CoV555), a neutralizing IgG1 mAb, was initiated at day 0, 4 days after onset of symptoms (Table). The patient received an infusion of 700 mg in a single dose and was discharged.

Analysis of samples showed a high viral load in a nasopharyngeal swab specimen (C_t 20) and a blood sample (C_t 37) (Table). Three days after the mAb infusion, the patient's symptoms worsened, and he was hospitalized in the Infectious Diseases Department at Grenoble Hospital (Grenoble, France) on day 6. The condition of the patient had deteriorated; he had an additional need for oxygen, which resulted in a convalescent-phase plasma transfusion on day 10.

After this treatment, the condition of the patient continued to deteriorate, and he was transferred to

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SARS-CoV-2 Delta Variant among Asiatic Lions, India

Appendix

Supplementary Methods

SARS-CoV-2 Whole-Genome Sequencing on the Oxford Nanopore MinION platform

We performed whole-genome sequencing of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) directly from nasal swabs of 4 Asiatic lions (*Panthera leo persica*) using the MinION (Oxford Nanopore Technologies, https://nanoporetech.com) sequencing platform. The lions tested included Jeya, a 3-year-old female; Shankar, an 18-year-old male; Niranjana, a 2-year-old female; and Pradeep, a 2-year-old male.

In brief, we performed tiling PCR spanning the whole genome of SARS-CoV-2 by using the Artic (https://artic.network) network primers. We performed cleaning and quantification of PCR products and used 100 ng of each sample to create a barcoded sequencing library by using the PCR Barcoding Kit (Oxford Nanopore). We used an Oxford Nanopore Technologies MinION with a R9.4.1 flow cell for sequencing, which yielded a total of 300 MB of data. To assemble the whole genome, we used guppy version 5.0.7 (Oxford Nanopore Technologies) for base calling and demultiplexing; and then we used Porechop (https://github.com/rrwick/Porechop) for adaptor removal. We mapped the readings to the SARS-CoV-2 reference genome (GenBank accession no. NC 045512) by using Minimap2 version 2.17 (r941) (*1*), and the called variations by using Nanopolish version 0.13.2 (https://github.com/jts/nanopolish) (*2*). After 2 rounds of Nanopolish, we generated 4 complete genomes.

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Appendix Table 1. Characteristics and results of quantitative reverse transcription PCR on samples collected from 11 Asiatic lions, Arignar Anna Zoological Park, Chennai, India*

Lion identity	Age, y/sex	Sample type	C_t value			Results
			E gene	RdRp	N	
Jeya	3/F	Nasal swab	20.16	19.65	19.97	Positive
		Rectal swab	19.23	18.27	19.75	Positive
Padmnabhan	12/M	Nasal swab	18.90	18.10	18.50	Positive
		Rectal swab	27.41	26.75	27.79	Positive
Kavitha	18/F	Nasal swab	23.79	22.87	23.89	Positive
		Rectal swab	32.19	31.44	31.90	Positive
		Fecal sample	27.56	27.20	27.72	Positive
Shankar	18/M	Nasal swab	19.26	18.66	18.82	Positive
		Rectal swab	30.22	29.41	29.45	Positive
Neela	9/F	Nasal swab	22.71	23.22	22.57	Positive
		Rectal swab	25.09	24.74	24.46	Positive
		Fecal sample	–	–	–	Negative
Niranjana	2/F	Nasal Swab	19.68	19.04	19.17	Positive
		Rectal swab	38.61	38.14	33.69	Negative
		Fecal sample	–	–	–	Negative
Pradeep	2/M	Nasal swab	17.59	16.85	17.35	Positive
		Rectal swab	29.05	28.60	29.02	Positive
		Fecal sample	–	–	–	Negative
Vishnu	4/M	Fecal sample	24.34	23.80	24.72	Positive
Jeya@Bhuvana	12/F	Fecal sample	28.11	27.39	28.49	Positive
Veera	10/M	Fecal sample	–	–	–	Negative
Shiva	12/M	Nasal swab	–	–	–	Negative
		Throat swab	–	–	–	Negative
		Trachea sample	–	–	–	Negative
		Lymph node sample	–	–	–	Negative
		Lung sample	–	–	–	Negative

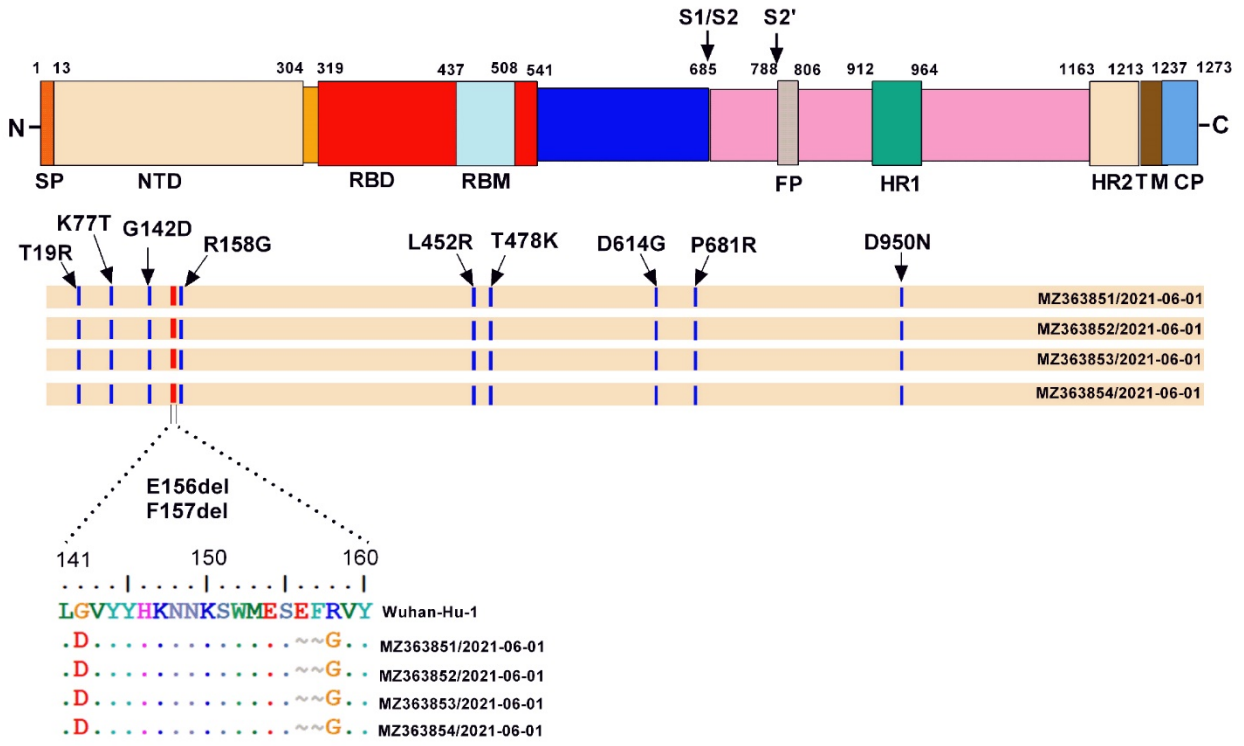
*C_t, cycle threshold; E, envelop gene; N, nucleocapsid; RdRP, RNA-dependant RNA polymerase; –, not detected.

Appendix Table 2. Amino acid substitutions and functional roles identified in different proteins encoded by severe acute respiratory syndrome coronavirus 2 detected in lions, India*

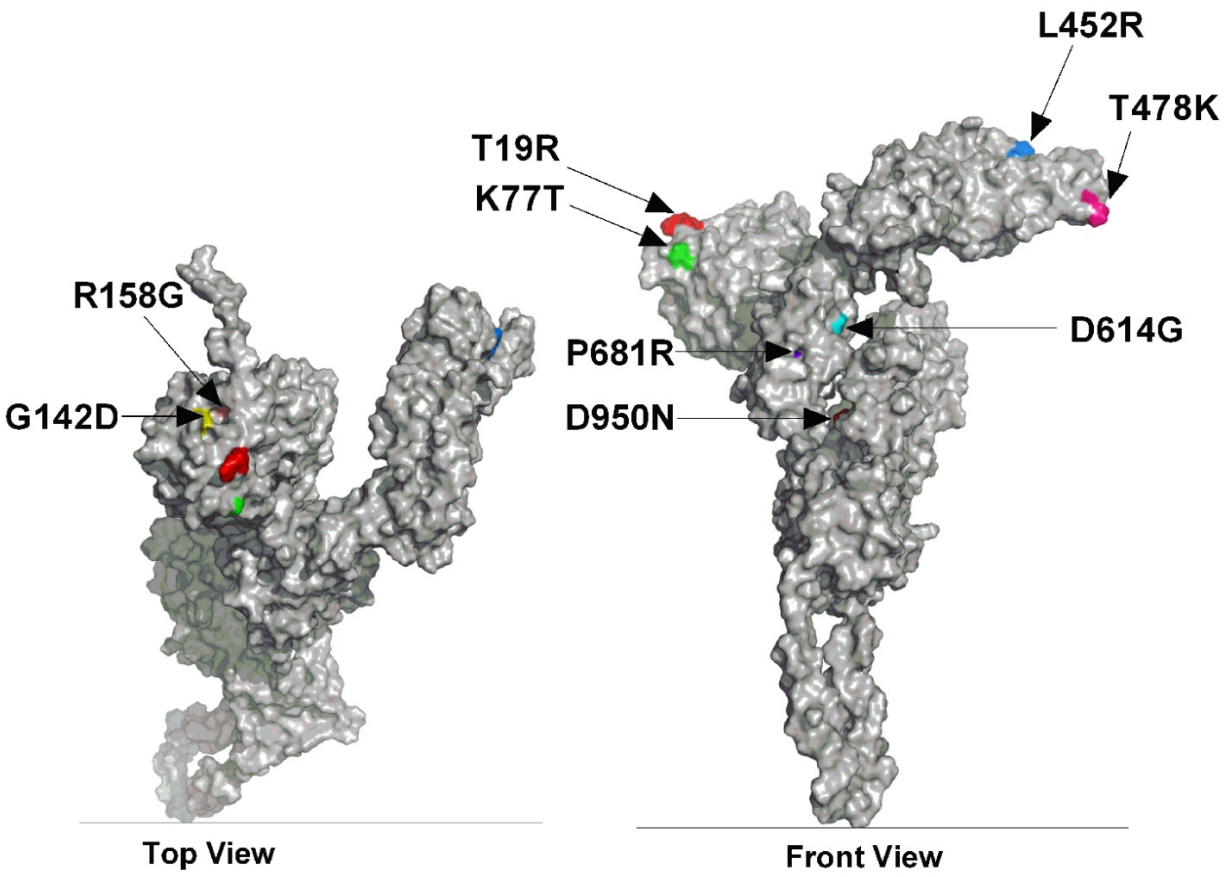
Amino acid substitutions	No. times reported (%)†	No. of countries‡	Mo. collected§	Accession no.¶	Functional roles of substitutions
M_I82T	51,777 (2.75)	99	Apr	hCoV-19/Indonesia/GO-NIHRD-PME20208/2020	–
N_D377Y	72,190 (3.83)	106	Feb	hCoV-19/USA/OH-ODH-SC1040172/2020	–
N_D63G	36,273 (1.93)	62	Apr	hCoV-19/Indonesia/GO-NIHRD-PME20208/2020	Antigenic drift; viral oligomerization interfaces
N_R203M	40,703 (2.16)	73	Mar	hCoV-19/Spain/NC-IBV-001370/2020	–
NS3_S26L	42,135 (2.24)	83	Mar	hCoV-19/USA/NY-NYCPHL-000016/2020	–
NS3_V88I	71 (0.00)	14	Mar	hCoV-19/Austria/CeMM0388/2020 hCoV-19/India/PB-ICMR-148040/2020 EPI_ISL_1165106 (B.1.36.8, Punjab, JUL-2020)	Viral oligomerization interfaces
NS7a_T120I	40,866 (2.17)	77	Feb	hCoV-19/Scotland/CVR01/2020	–
NS7a_V82A	39,371 (2.09)	66	Apr	hCoV-19/Indonesia/GO-NIHRD-PME20208/2020	Antigenic drift
NSP12_P323L	1,802,848 (95.76)	182	Oct	hCoV-19/Italy/MAR-UnivPM30_45476/2020	–
NSP15_K259R	4,692 (0.25)	54	Jul	hCoV-19/USA/CA-IGI-0320/2020	–
NSP2_P129L	13,166 (0.70)	91	Mar	hCoV-19/Japan/PG-1597/2020	–
NSP3_P822L	14,468 (0.77)	77	Feb	hCoV-19/USA/CA-CDPH018/2020	Host cell protein/RNA interaction; viral oligomerization interfaces
NSP4_D217N	2,516 (0.13	55	Mar	hCoV-19/England/BIRM-61F00/2020	–

Amino acid substitutions	No. times reported (%)†	No. of countries‡	Mo. collected§	Accession no.¶	Functional roles of substitutions
NSP4_F375S	1,136 (0.06)	33	Jun	hCoV-19/USA/WA-UW-10769/2020	–
NSP6_H11Q	3,639 (0.19)	48	Apr	hCoV-19/Italy/LOM-Pavia-41147/2020	–
Spike_D614G	1,839,357 (97.70)	185	Oct	hCoV-19/Italy/MAR-UnivPM30_45476/2020	Antigenic drift; virulence and host change; ligand binding; viral oligomerization interfaces
Spike_D950N	36,277 (1.93)	70	Mar	hCoV-19/Iran/K1r-108/2020	Viral oligomerization interfaces
Spike_E156G	32,810 (1.74)	62	Mar	hCoV-19/Panama/328688/2020	–
Spike_F157del	33,063 (1.76)	63	Jul	hCoV-19/USA/TX-HMH-MCoV-40913/2020	–
Spike_G142D	25,756 (1.37)	61	Mar	hCoV-19/England/BRIS-124CD4/2020	–
Spike_K77T	777 (0.04)	24	Dec	hCoV-19/Switzerland/ZH-ETHZ-431373/2020	–
Spike_L452R	101,257 (5.38)	108	Mar	hCoV-19/Denmark/ALAB-HH65/2020	Host and other changes; antigenic drift; antibody recognition sites
Spike_P681R	45,877 (2.44)	91	Apr	hCoV-19/Indonesia/GO-NIHRD-PME20208/2020	Increased rate of membrane fusion, internalization, and thus better transmissibility
Spike_R158del	33,071 (1.76)	63	Jul	hCoV-19/USA/TX-HMH-MCoV-40913/2020	Antibody recognition sites
Spike_T19R	320,114 (1.07)	64	Apr	hCoV-19/Indonesia/GO-NIHRD-PME20208/2020	Removes a potential N-glycosylation site that might also affect antigenic and other properties of this strain
Spike_T478K	56,250 (2.99)	79	Apr	hCoV-19/Indonesia/GO-NIHRD-PME20208/2020	Host and other changes; antigenic drift; host surface receptor binding; antibody recognition sites; viral oligomerization interfaces

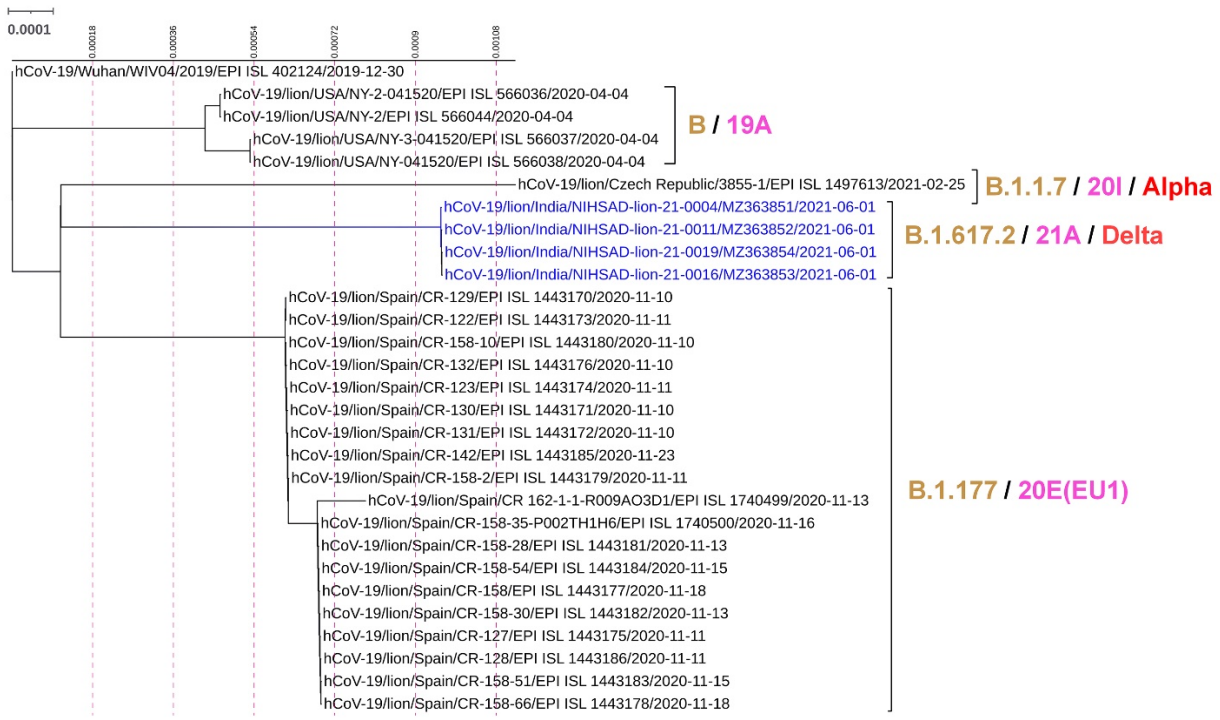
*Reference sequence Wuhan-Hu-1 (GISAID accession no. EPI_ISL_402124) was used for comparison. –, no role identified.
†Percentage of sequences with this gene showing the same mutation.
‡Number of countries reporting the same mutations.
§Month first strain with this mutation was collected during 2020.
¶Accession number of the first strain to show the mutation.



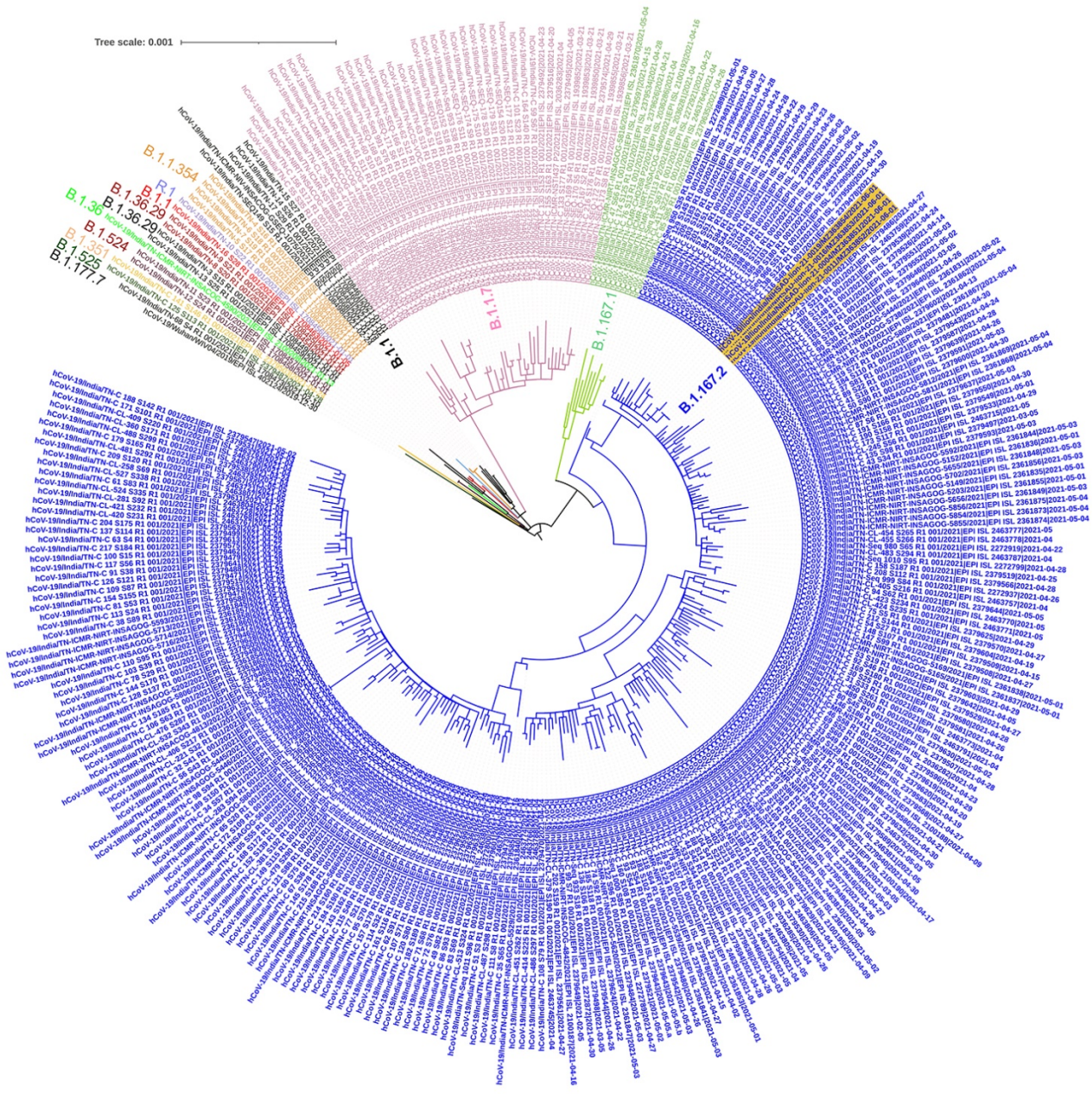
Appendix Figure 1. Comparison of amino acid changes detected in the spike protein (SP) of SARS-CoV-2 detected in Asiatic lions (*Panthera leo persica*), India. Wuhan-Hu-1 (GISAID accession no. EPI_ISL_402124) was used as the reference sequence. CT, cytoplasmic tail; FP, fusion peptide; HR, heptad repeat; NTD, N terminal domain; RBD, receptor binding domain; RBM, receptor binding motif; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TM, transmembrane domain.



Appendix Figure 2. Mapping of amino acid substitutions noted on the structural model of the spike protein of SARS-CoV-2 detected in Asiatic lions (*Panthera leo persica*), India. Model represents spike protein of SARS-CoV-2 from Asiatic lion (GenBank accession no. MZ363851) from the top (left) and the front (right). Model was built by using I-TASSER (Yang Zhang Lab, https://zhanglab.ccmb.med.umich.edu/I-TASSER) and the PDB:6acc template. Red indicates T19R; green indicates K77T; and blue indicates L452R in both views; all other areas of interest are labeled with arrows. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.



Appendix Figure 3. Maximum likelihood tree showing the phylogenetic relationship among SARS-CoV-2 detected in Asiatic lions (*Panthera leo persica*), India. Blue text indicates SARS-COV-2 sequences from this study. Comparison sequences were selected from available lion and tiger sequences in the GISAID. Scale bar indicates nucleotide substitutions per site. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.



Appendix Figure 4. The phylogenetic analysis of SARS-CoV-2 detected in Asiatic lions (*Panthera leo persica*) and all available SARS-COV-2 sequences from Tamil Nadu, India. Colors represent different PANGO lineages; sequences in gold highlighting represent SARS-CoV-2 from lions in this study. Scale bar indicates nucleotide substitutions per site. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.