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Quick Response Code:

Correspondence

Mapping the genomic landscape & diversity of COVID-19 based on >3950 clinical isolates of SARS-CoV-2: Likely origin & transmission dynamics of isolates sequenced in India

Sir,

The COVID-19 pandemic has stalled the world and catapulted the global health systems into unprecedented chaos. More than 200 countries have been affected by this pandemic, resulting in 2.54 million cases in a short period of time and >0.17 million deaths (as of April 23, 2020), with a mere 0.7 million recoveries[1]. The movement of COVID-19 hotspot from China to Europe, and now to the USA, has been partly due to the staggered restrictions in global travel and partly due to potent transmission through asymptomatic carriers[2].

India, with 21,393 cases and 681 deaths (as of April 23, 2020)[1], had the lowest figures for any country of the comparable population (0.5 deaths per million population). International travellers or their close contacts formed the majority of initially reported cases. The delayed onset of COVID-19 in India has given it an edge, which allowed it to impose severe restrictions to contain the local spread[3-5].

In our in-depth analyses of 1500+ genomes, variability among clinical isolates was shown along the timeline, leading to distinct clustering of SARS-CoV-2 across the globe (unpublished observation). It was predicted, based on the aggregation propensity of the spike protein in the Wuhan and other isolates of SARS-CoV-2, that this virus would exhibit very high transmissibility and confer survival fitness[6,7]. Genetic diversity of the virus increases with disease progression and can be utilized to model the evolution and propagation of the disease[6,] Recently, phylogenetic network analysis of 160 SARS-CoV-2 genome samples showed a parallel evolution of the virus and its evolutionary selection in their human hosts[8]. Similar whole-genome analyses of the Indian isolates and their comparison with global isolates can provide a better understanding of dominant clades within the population and unveil targets for developing specific interventions.

In the present study, machine learning-based t-SNE analysis of global clinical isolates has been utilized to segregate the clinical isolates into clusters while accommodating the outliers[9,10]. Whole-genome analysis of 3968 global isolates obtained from GISAID (Global initiative on sharing all influenza data)[11], including 25 SARS-CoV-2 genomes sequenced in India [next-genome sequencing (NGS) data submitted by the ICMR-National Institute of Virology, Pune, India] and presented in Figure 1 (Supplementary Fig. 1 (available from *http://www.ijmr.org.in/articles/2020/151/5/images/IndianJMedRes_2020_151_5_474_284485_sm5.pdf*) and Supplementary Table I (available from *http://www.ijmr.org.in/articles/2020/151/5/images/IndianJMedRes_2020_151_5_474_284485_sm6.pdf*)), was an attempt to dissect the global genome diversity and also critically evaluate the placement of Indian isolates to understand the COVID-19 pandemic in India.

The initial cases reported from India had a travel history to China, which explained its position in a Chinese cluster[5] (Fig. 2). The travel ban from China to India, in early February 2020, has prevented the large-scale spill-over directly from China to the Indian Sub-continent. However, various isolates transmitted from other South-East Asian countries might fall in the same cluster. The overlap of Indian samples majorly with European samples (Supplementary Fig. 1, Panel III) reiterated the fact that the delayed travel restriction

Supplementary material available from *http://www.ijmr.org.in/article.asp?issn=0971-5916;year=2020;volume=151;issue=5;spage=474;epage=478;aulast=Singh*

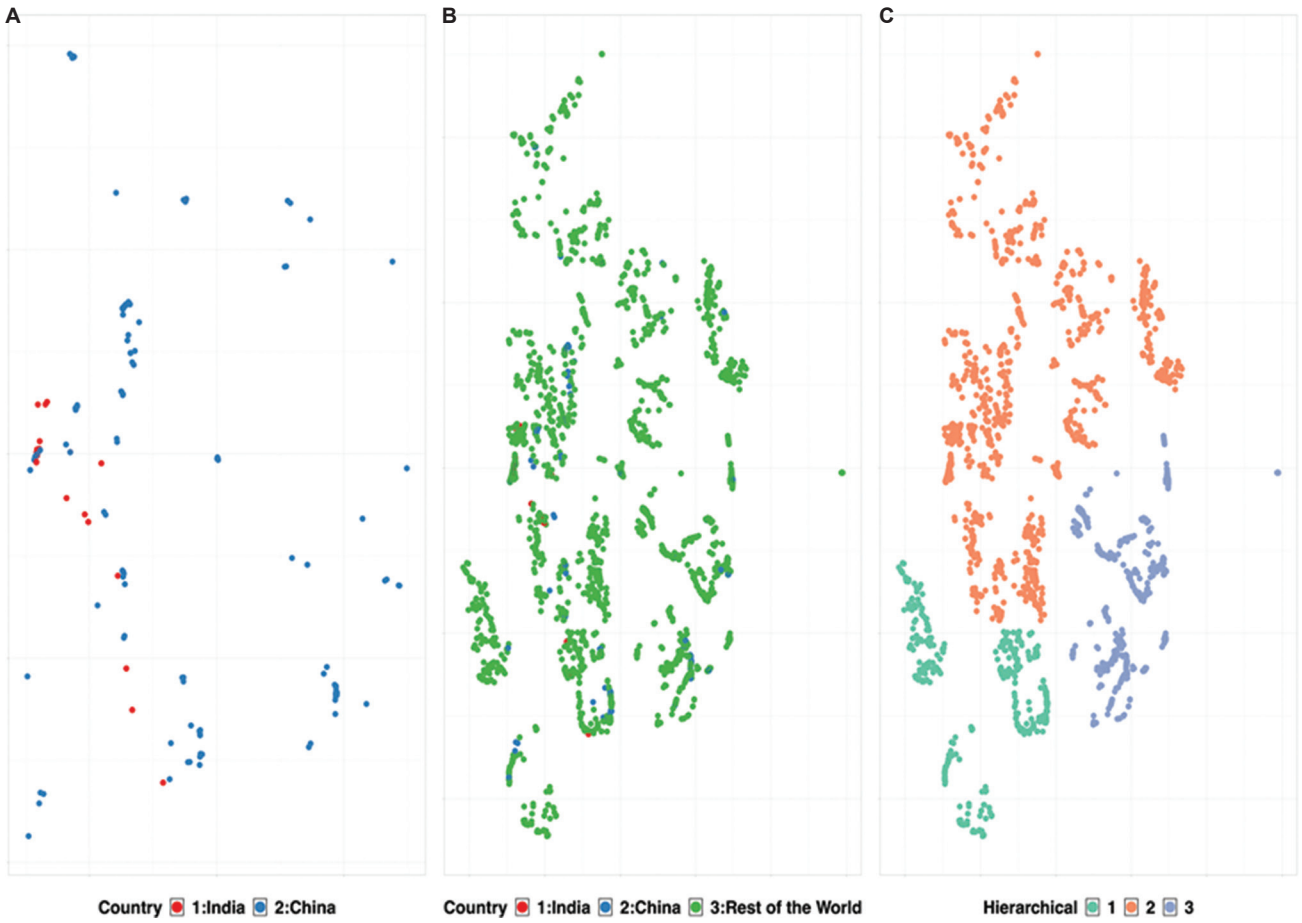

Fig. 1. Whole-genome-based t-SNE clustering of 3968 clinical isolates. (**A**) Comparative genome-based clustering of Indian isolates (red) with Chinease isolates (blue). (**B**) Comapartive genome-based clustering of Indian isolates (red) and Chinese isolates (blue) with rest of the world (green). (**C**) Diversity in clinical isolates showing three distinct clustering using hierarchical clustering on the t-SNE clusters. (t-SNE: *https://github.com/jdonaldson/rtsne*).

from the European hotspot regions affected not just India but also many countries.

Hierarchical-based clustering further yielded exciting outcomes on the inter-continent transmission of COVID-19. The segregation of SARS-CoV-2 genomes into three clades indicates the emergence of evolutionary diversity (Fig. 1C). The heterogeneity of these clusters, grouped along with Chinese counterparts, validates a global spill-over event originating from Wuhan[5,12]. Hierarchical cluster 2 in Supplementary Figure 1 Panel II (coloured by the continents) indicates the introduction of SARS-CoV-2 in India from the European, other Asian and North American nations (Supplementary Fig. 1). Detailed comparative analysis of Indian isolates with respect to other countries showed its close relationship with samples from China, USA, Canada, Spain and Kuwait, suggestive of exposure to COVID-19 due to travel history from these nations (Fig. 2). However, limited genome sequences from India make it difficult to differentiate and ascertain global transmission and transmission within the country.

The conservation of an amino acid in any protein sequence denotes its functional importance[13,14] as it undergoes fewer amino acid replacements or is more likely to substitute amino acids with similar biochemical properties. The amino acid conservation is inversely proportionate to the evolutionary rate. This is a valuable gauge of the evolutionary divergence and the analogous genomic regions. Sequence similarity between the open reading frames (ORFs) of Indian isolates and the initial sample collected in Wuhan unravels conservation in five ORFs corresponding to envelope protein, membrane glycoprotein, ORF6, ORF7b and ORF10 proteins (Fig. 3A). On the contrary, a number of mutations were observed in ORF1a, ORF1b, spike protein (surface

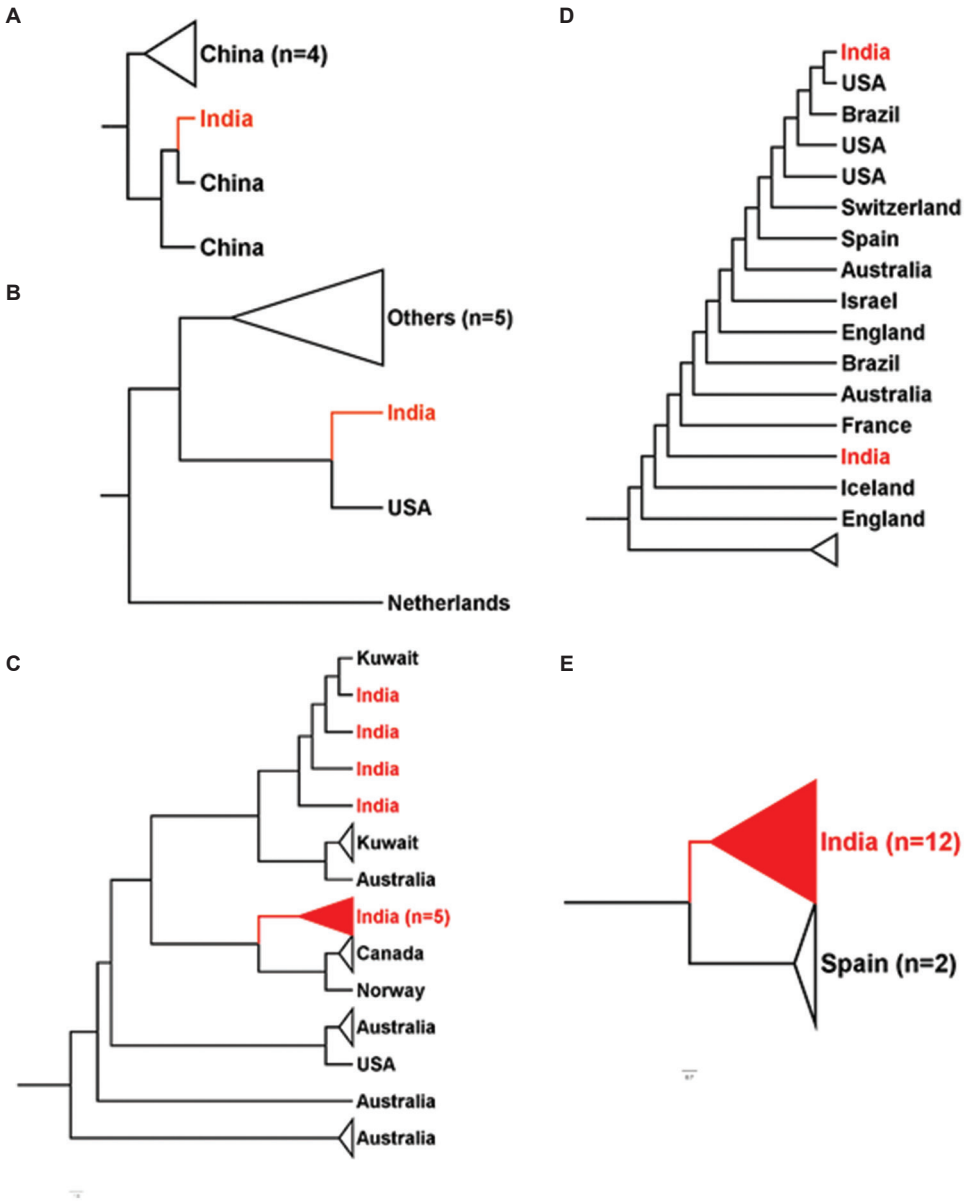

Fig. 2. Position of various Indian isolates with other nations. (**A-E**) Clustering of SARS-CoV-2 genome sequences from India (red) with other nations around the globe. Indian samples clustered with samples from different nations – China, Kuwait, Canada, USA and Spain in whole-genome-based clustering. Figures were generated using FigTree v1.4.4 (*http://tree.bio.ed.ac.uk/software/figtree/*).

glycoprotein), ORF3a, ORF7a, ORF8 and nucleocapsid phosphoprotein (Supplementary Fig. 2 (available from *http://www.ijmr.org.in/articles/2020/151/5/images/IndianJMedRes_2020_151_5_474_284485_sm7.pdf*) and Supplementary Table II (available from *http://www.ijmr.org.in/articles/2020/151/5/images/IndianJMedRes_2020_151_5_474_284485_sm8.pdf*)). Mean similarity calculated for these ORFs revealed that ORF1a in the Indian isolates was less conserved (more mutated) compared to global isolates (Fig. 3A and Supplementary Table III (available from *http://www.ijmr.org.in/articles/2020/151/5/images/IndianJMedRes_2020_151_5_474_284485_sm9.pdf*)). In all other ORFs, a relatively higher conservation was observed among Indian isolates compared to Wuhan strain. When compared with global isolates, Indian isolates have higher entropy for changes in ORF 1a and ORF 1b (Supplementary Fig. 3 (available from *http://www.ijmr.org.in/articles/2020/151/5/images/IndianJMedRes_2020_151_5_474_284485_sm10.pdf*)). Further, qualitative analysis of mutations in non-conserved ORFs showed that each type of amino acid had undergone mutation in the Indian isolates (Fig. 3B).

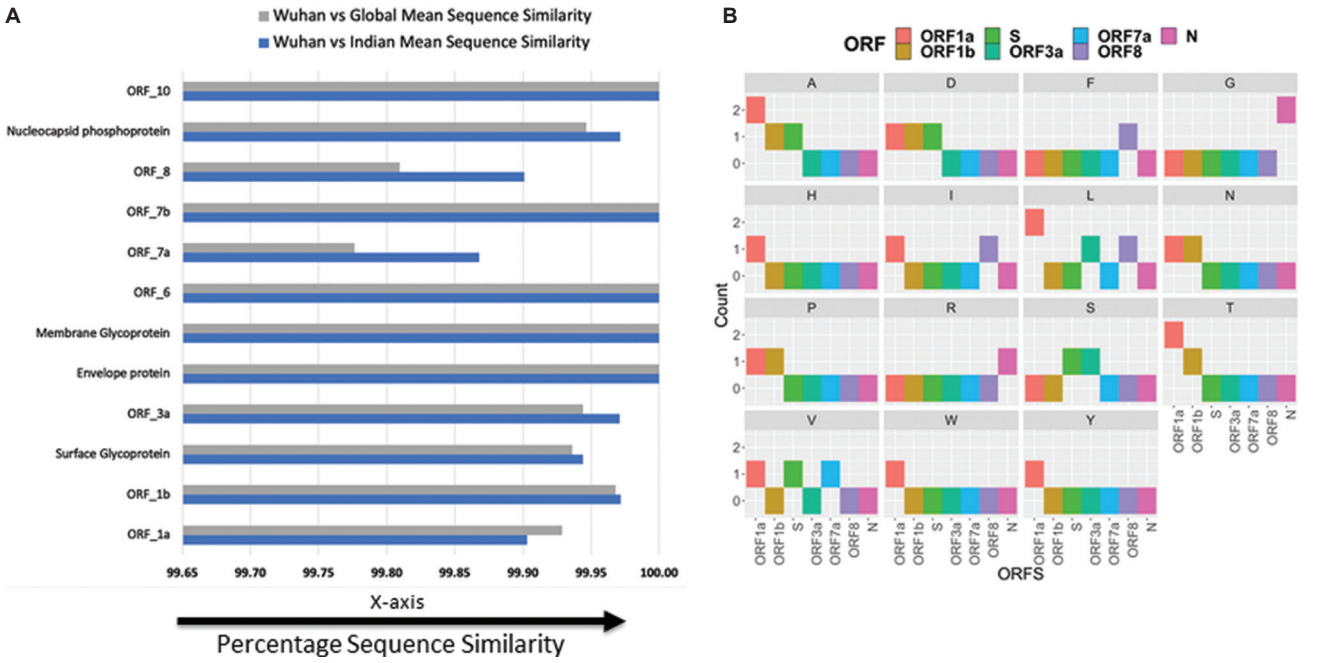

Fig. 3. Sequence similarity and mutation analysis of open reading frames. (**A**) Comparison of mean sequence similarity for open reading frames between Indian and global isolates with Wuhan strain. (**B**) Qualitative analysis on type of mutations occurring in non-conserved open reading frames (ORFs) of Indian isolates compared to Wuhan strain.

These mutations could be a major contributing factor for the separation of Indian isolates into three distinct clusters. Higher sampling rate driven by NGS of the Indian isolates would help in better understanding of actual variability in SARS-CoV-2 and assist both in identifying better diagnostic markers and in developing specific interventions in terms of vaccine candidates and drug targets.

Evolutionary divergence, corroborated by epidemiological data, is a valuable tool to implement appropriate measures against this pandemic. The population density of India and the presence of functionally distinct isolates in the Indian population raise concerns and warrant an urgent need for higher sampling rate for better assessment of the evolution of SARS-CoV-2 in India. The situation is further confounded by the fact that many of these Indian isolates submitted in databanks include those of Indians living in Iran, Italian tourists visiting India, and also contains samples cultured *in vitro*.

In conclusion, a whole-genome diversity analysis of 3968 global clinical isolates, including 25 isolates sequenced in India, of SARS-CoV-2 was done. The variations in different open reading frames (ORFs) of SARS-CoV-2, which drives the formation of distinct Indian clusters and functional heterogeneity, were highlighted. Five ORFs corresponding to envelope protein, membrane glycoprotein, ORF6, ORF7b and ORF10 were found to be highly conserved, while a number of mutations were observed in ORF1a, ORF1b, spike protein, ORF3a, ORF7a, ORF8 and nucleocapsid phosphoprotein. Generating diverse genomic datasets will provide insight into the propagation dynamics of COVID-19, leading to a better understanding of pathogenesis and evolution of SARS-CoV-2, which will eventually lead to better intervention methods.

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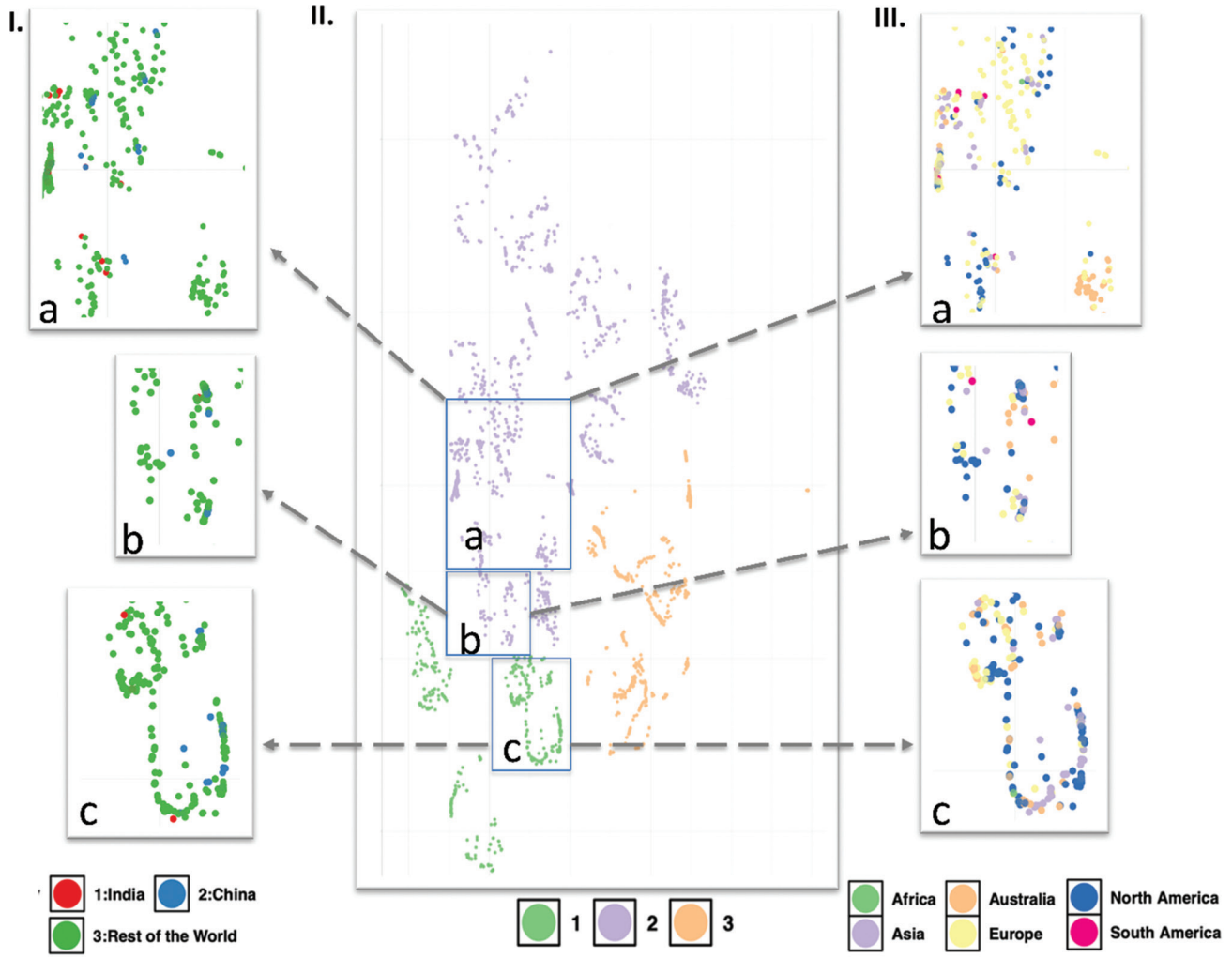
SUPPLEMENTARY INFORMATION

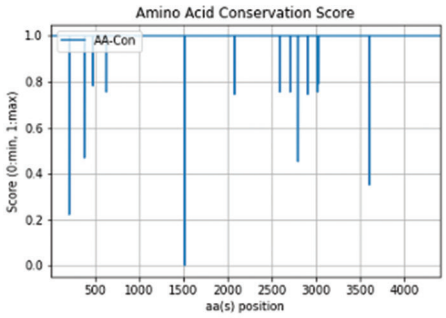
Mapping the genomic landscape and diversity of COVID-19 based on >3950 clinical isolates of SARS-CoV-2: Likely origin and transmission dynamics of isolates sequenced in India



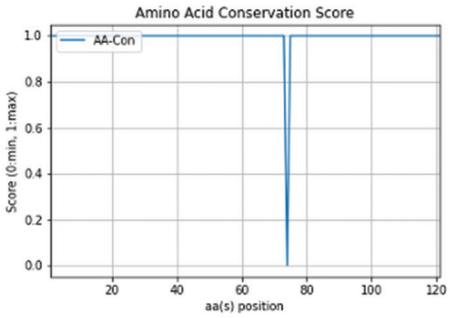
Supplementary Fig. 1. Position of Indian genome sequences in sub-cluster a, b, and c with respect to other global genome sequences. Panel I shows presence of Indian isolates in three distinct sub-clusters with respect to Chinese isolates and remaining global isolates. Panel II highlights the placement of Indian clusters in two of the three hierarchical clusters (a, b, and c) obtained from t-SNE whole genome clustering of 3968 sequences. Panel III displays the prevalence of samples from various continents. Continent codes; Africa (Green), Australia (Orange), North America (Blue), Asia (Light purple), Europe (Yellow) and South America (Pink).

Supplementary Table I. Details of Indian samples along with their origin and placement in Hierarchical clusters. Genomic sequences were retrieved from GISAID (*https://www.gisaid.org*)

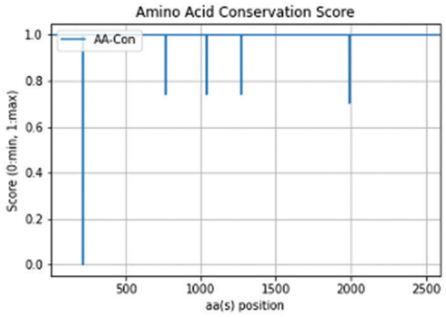
Accession ID	Contact History	Gender	Age	Virus name	Location	Collection date	Hierarchical Cluster ID
EPI_ISL_413523	Travel history to China	Male	23	hCoV-19/India/1-31/2020	Asia/India/Kerala	2020-1-31	2
EPI_ISL_420543	Italian tourist	Female	73	hCoV-19/India/763/2020	Asia/India	2020-3-3	2
EPI_ISL_420544	Vero CCL81 isolate P1	NA	NA	hCoV-19/India/2020763/2020	Asia/India	2020	1
EPI_ISL_420545	Italian tourist	Female	77	hCoV-19/India/770/2020	Asia/India	2020-3-3	2
EPI_ISL_420546	Vero CCL81 isolate P1	NA	NA	hCoV-19/India/2020770/2020	Asia/India	2020	1
EPI_ISL_420547	Italian tourist	Female	70	hCoV-19/India/772/2020	Asia/India	2020-3-3	2
EPI_ISL_420548	Vero CCL81 isolate P1	NA	NA	hCoV-19/India/2020772/2020	Asia/India	2020	2
EPI_ISL_420549	Italian tourist	Female	65	hCoV-19/India/773/2020	Asia/India	2020-3-3	2
EPI_ISL_420550	Vero CCL81 isolate P1	NA	NA	hCoV-19/India/2020773/2020	Asia/India	2020	2
EPI_ISL_420551	Indian contact of Italian tourist	Male	59	hCoV-19/India/777/2020	Asia/India	2020-3-3	2
EPI_ISL_420552	Vero CCL81 isolate P1	NA	NA	hCoV-19/India/2020777/2020	Asia/India	2020	2
EPI_ISL_420553	Italian tourist	Male	66	hCoV-19/India/781/2020	Asia/India	2020-3-3	2
EPI_ISL_420554	Vero CCL81 isolate P1	NA	NA	hCoV-19/India/2020781/2020	Asia/India	2020	2
EPI_ISL_420555	Indian contact of Indian Patient having travel history to Italy	Female	37	hCoV-19/India/c32/2020	Asia/India	2020-3-3	2
EPI_ISL_420556	Vero CCL81 isolate P1	NA		hCoV-19/India/2020c32/2020	Asia/India	2020	1
EPI_ISL_421662	Indian citizen sampled at Iran	Male	68	hCoV-19/India/1073/2020	Asia/India	2020-3-10	2
EPI_ISL_421663	Indian citizen sampled at Iran	Male	45	hCoV-19/India/1093/2020	Asia/India	2020-3-10	2
EPI_ISL_421664	Indian citizen sampled at Iran	Male	72	hCoV-19/India/1100/2020	Asia/India	2020-3-10	2
EPI_ISL_421665	Indian citizen sampled at Iran	Male	43	hCoV-19/India/1104/2020	Asia/India	2020-3-10	1
EPI_ISL_421666	Indian citizen sampled at Iran	Female	54	hCoV-19/India/1111/2020	Asia/India	2020-3-10	2
EPI_ISL_421667	Indian citizen sampled at Iran	Male	66	hCoV-19/India/1115/2020	Asia/India	2020-3-10	2
EPI_ISL_421669	Indian citizen sampled at Iran	Female	70	hCoV-19/India/1616/2020	Asia/India	2020-3-12	2
EPI_ISL_421670	Indian citizen sampled at Iran	Female	50	hCoV-19/India/1617/2020	Asia/India	2020-3-12	2
EPI_ISL_421671	Indian citizen sampled at Iran	Female	55	hCoV-19/India/1621/2020	Asia/India	2020-3-12	2
EPI_ISL_421672	Indian citizen sampled at Iran	Male	59	hCoV-19/India/1644/2020	Asia/India	2020-3-12	2



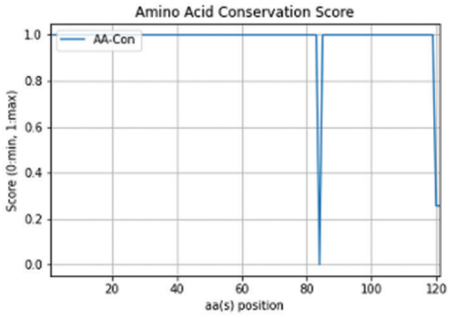
ORF_1a



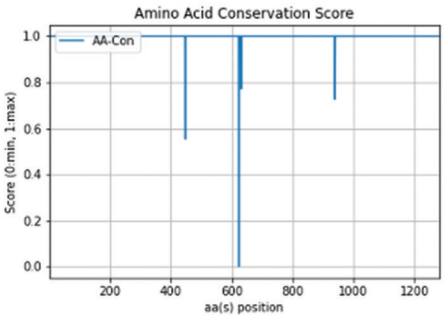
ORF_7a



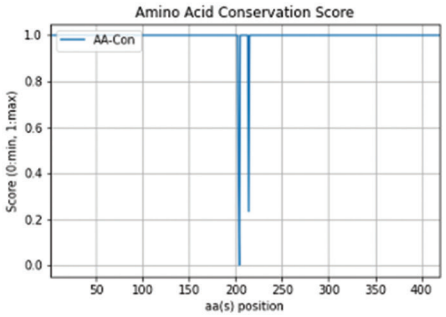
ORF_1b



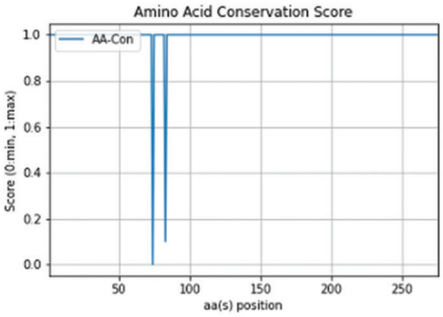
ORF_8



Surface Glycoprotein



Nucleocapsid



ORF_3a

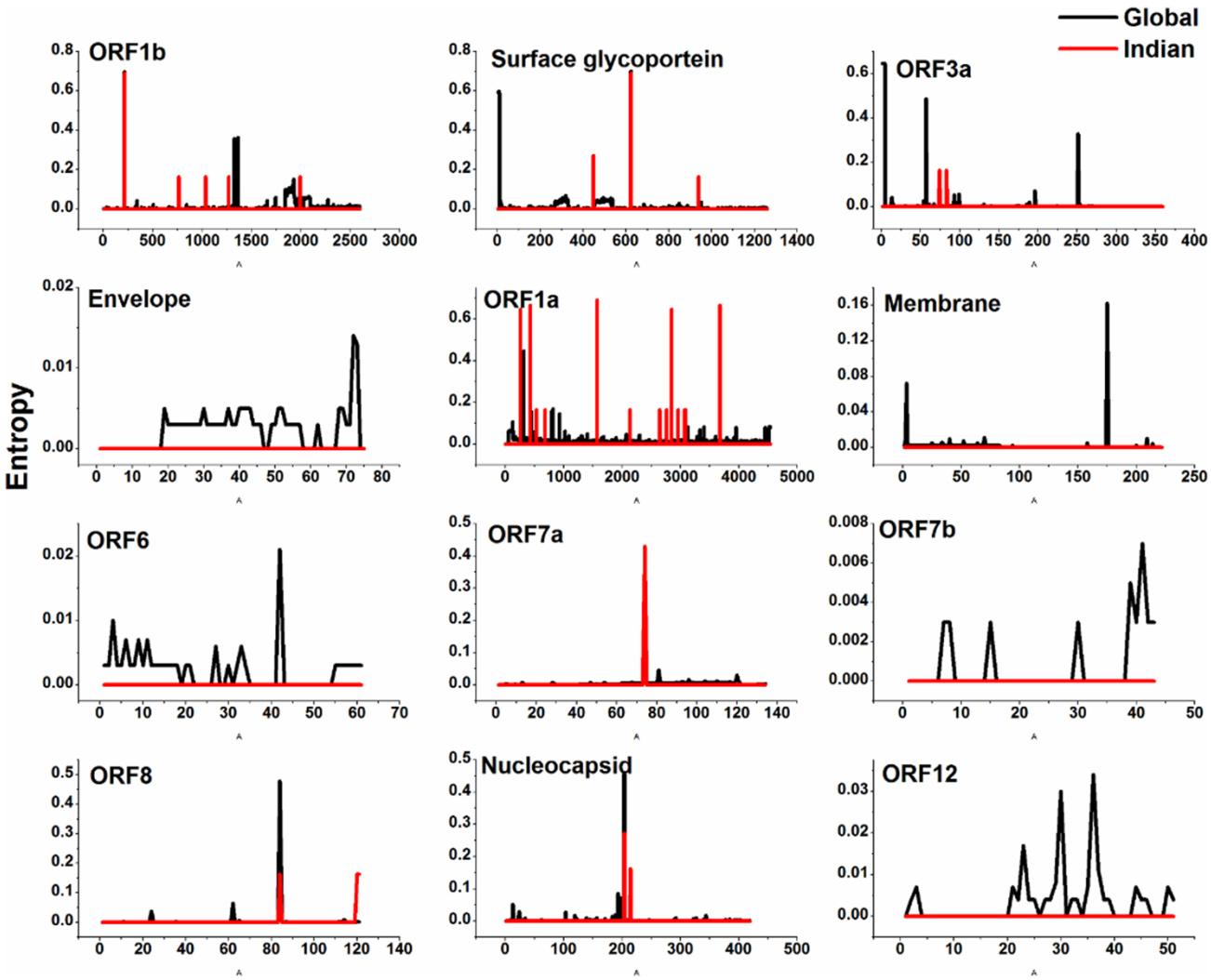
Supplementary Fig. 2. Comparison of all Indian SARS-CoV-2 genomes with Wuhan strain (first collected sample) shows variation in ORF1a and 1b protein, surface glycoprotein, ORF3a protein, ORF7a protein, ORF8 and nucleocapsid protein.

Supplementary Table II. Specific high frequency (>=10%) mutations in individual ORFs in Indian isolates compared with reference strain (Wuhan_IPBCAMS-WH-01_2019_EPI_ISL_402123). The sequences with less than 25% gaps were selected for all the studies. Genomic sequences were retrieved from GISAID (*https://www.gisaid.org*)

ORF	Position	Amino acid in reference	Mutated amino acid	Mutation
ORF1a	207	Arginine (R)	Cysteine	R->C
ORF1a	378	Valine (V)	Isoleucine (I)	V->I
ORF1a	1515	Serine (S)	Phenylalanine (F)	S->F
ORF1a	2796	Methionine (M)	Isoleucine (I)	M->I
ORF1a	3606	Leucine (L)	Phenylalanine (F)	L->F
ORF1b	314	Proline (P)	Leucine (L)	P->L
S	614	Aspartic acid (D)	Glycine (G)	D->G
ORF7a	74	Valine (V)	Phenylalanine (F)	V->F

Supplementary Table III. Comparison of mean sequence similarity for ORFs between Indian and global isolates with Wuhan strain

Column 1	Column 2	Wuhan vs Indian		Wuhan vs Global	
ORF	Name	Indian Mean Similarity	Indian GMean Similarity	Global Mean Similarity	Global GMean Similarity
ORF5	ORF_1a	99.90	99.90	99.93	99.93
ORF1	ORF_1b	99.97	99.97	99.97	99.97
ORF2	Surface Glycoprotein	99.94	99.94	99.94	99.94
ORF3	ORF_3a	99.97	99.97	99.94	99.94
ORF4	Envelope protein	100.00	100.00	100.00	100.00
ORF6	Membrane Glycoprotein	100.00	100.00	100.00	100.00
ORF7	ORF_6	100.00	100.00	100.00	100.00
ORF8	ORF_7a	99.87	99.87	99.78	99.78
ORF9	ORF_7b	100.00	100.00	100.00	100.00
ORF10	ORF_8	99.90	99.90	99.81	99.81
ORF11	Nucleocapsid phosphoprotein	99.97	99.97	99.95	99.95
ORF12	ORF_10	100.00	100.00	100.00	100.00



Supplementary Fig. 3. Differential entropy plots for Indian vs global isolates. Mutational entropy of each amino acid position in all ORFs calculated for Indian and global isolates with respect to reference strain (Wuhan_IPBCAMS-WH-01_2019_EPI_ISL_402123). In global isolates, Indian samples have not been included to highlight differential entropy. Genomic sequences were retrieved from GISAID (*https://www.gisaid.org*).