Mutant	Mutant		Genomic	Nucleotide	Amino acid	Time	Variant	Genomes	
(major)	(minor)	Gene	Position	change	change	(days)	Frequency	mapped	First location
μı		ORF1ab	2416	U>C		0	98.4%	0	China, Asia
µ2		ORF1ab	19524	U>C		0	99.0%	18	China, Asia
μ_3		S	23929	U>C		0	98.9%	0	China, Asia
μ_4		ORF1ab	15933	U>C		0	98.8%	0	China, Asia
μ_5		ORF8	27944	U>C		0	97.0%	0	China, Asia
μ_6		ORF1ab	6286	U>C		0	95.6%	0	China, Asia
μ ₇		S	22444	U>C		0	98.7%	0	China, Asia
α1		ORF1ab	18060	U>C		0	97.3%	1114	China, Asia
	α _{1a}	Ν	28657	C>U		63	1.0%	3	France, Europe
	a _{1b}	ORF1ab	9477	U>A	F>Y	63	0.7%	3	France, Europe
	a _{1c}	Ν	28863	C>U	S>L	63	0.7%	7	France, Europe
	α _{1d}	ORF3a	25979	G>U	G>V	63	0.7%	451	France, Europe
α ₂		ORF1ab	8782	U>C		0	94.9%	51	China, Asia
α3		ORF8	28144	C>U	S>L	0	94.9%	1281	China, Asia
	α _{3a}	ORF1ab	1606	U>C		43	0.9%	578	United Kingdom, Europe
	a _{3b}	ORF1ab	11083	G>U	L>F	24	7.5%	417	China, Asia
	a3c	Ν	28311	C>U	P>L	64	1.4%	4	South Korea, Asia
	a _{3d}	ORF1ab	13730	C>U	A>V	33	1.4%	5	China, Asia
	a _{3e}	ORF1ab	6312	C>A	T>K	71	1.2%	767	Taiwan, Asia
	α _{3f}	ORF3a	26144	G>U	G>V	28	3.0%	160	China, Asia
	α _{3α}	ORF1ab	14805	C>U		54	3.7%	511	United Kingdom, Europe
	α _{3h}	ORF1ab	17247	U>C		64	1.0%	682	Switzerland, Europe
	α _{3i}	ORF1ab	2558	C>U	P>S	54	1.0%	44	United Kingdom, Europe
	α _{3i}	ORF1ab	2480	A>G	I>V	54	1.0%	648	United Kingdom, Europe
βı	-,	ORF1ab	3037	C>U		31	87.2%	45	China, Asia
β ₂		S	23403	A>G	D>G	31	87.2%	15	China, Asia
β3		ORF1ab	14408	C>U	P>L	41	87.1%	4450	Saudi Arabia, Middle East
	β _{3a}	ORF1ab	20268	A>G		64	6.0%	2388	Italy, Europe
	β _{3b}	Ν	28854	C>U	S>L	29	4.5%	1782	China, Asia
	β _{3c}	ORF1ab	15324	C>U		29	2.2%	1463	China, Asia
	β _{3d}	ORF3a	25429	G>U	V>L	77	1.1%	719	United Kingdom, Europe
	β _{3e}	Ν	28836	C>U	S>L	74	0.8%	3	Switzerland, Europe
	β _{3f}	ORF1ab	13862	C>U	T>I	74	0.8%	85	Switzerland, Europe
	β3α	ORF1ab	10798	C>A		86	0.6%	435	United Kingdom, Europe
V 1	5	ORF3a	25563	G>U	Q>H	41	24.4%	1671	Saudi Arabia, Middle East
	¥1a	ORF1ab	18877	C>U		41	4.2%	1201	Saudi Arabia, Middle East
	Y _{1b}	М	26735	C>U		41	2.7%	1784	Saudi Arabia, Middle East
δı		ORF1ab	1059	C>U	T>I	54	17.6%	8284	Singapore, Asia
	δ _{1a}	S	24368	G>U	D>Y	75	0.7%	466	Sweden, Europe
	δ _{1b}	ORF8	27964	C>U	S>L	76	2.9%	1152	USA, North America
	δ1c	ORF1ab	11916	C>U	S>L	72	1.9%	807	USA, North America
	δ _{1d}	ORF1ab	18998	C>U	A>V	72	0.7%	458	USA, North America
	δ _{1e}	ORF1ab	10319	C>U	L>F	76	1.2%	799	USA, North America
ζ1	10	ORF1ab	445	U>C		179	4.4%	18	Netherlands, Europe
ζ2		М	26801	C>G		82	4.3%	7	Canada, North America
ζ3		S	22227	C>U	A>V	84	4.5%	1	Spain, Europe
ζ4		Ν	28932	C>U	A>V	96	4.4%	5	Portugal, Europe
ζ5		ORF10	29645	G>U	V>L	78	4.4%	2	Denmark, Europe
ζ6		ORF1ab	21255	G>C		80	4.4%	1557	USA, North America
ζ7		S	21614	C>U	L>F	79	2.5%	1442	United Kingdom, Europe

Supplementary Table 1. SARS-CoV-2 variants in the 68KG dataset.

Mutant	Mutant		Genomic	Nucleotide	Amino acid	Time	Variant	Genomes
(major)	(minor)	Gene	Position	change	change	(days)	Frequency	mapped
ε ₁		Ν	28881	G>A	R>K	54	41.7%	5
ε2		Ν	28882	G>A	R>K	54	41.6%	0
£ 3		Ν	28883	G>C	G>R	54	41.6%	13394
	ε _{3a}	ORF1ab	313	C>U		64	2.4%	1630
	ε _{3b}	ORF1ab	19839	U>C		64	2.9%	1227
	ε _{3c}	М	27046	C>U	T>M	69	0.8%	548
	٤ _{3d}	ORF1ab	10097	G>A	G>S	69	3.2%	11
	ε _{3e}	S	23731	C>U		69	3.2%	425
	ε _{3f}	Ν	28580	G>U	D>Y	69	1.0%	678
	ε _{3α}	ORF1ab	13536	C>U		69	1.6%	23
	ε _{3h}	ORF1ab	4002	C>U	T>I	69	1.6%	1066
	ε _{3i}	ORF1ab	10265	G>A	G>S	63	1.4%	879
	ε _{3i}	S	21575	C>U	L>F	54	1.0%	248
	ε _{3k}	S	21637	C>U		111	1.3%	873
	£31	ORF8	28169	A>G		103	1.3%	0
	٤ _{3m}	ORF1ab	16968	G>U		114	1.0%	702
nı	0111	ORF1ab	1163	A>U	I>F	86	9.6%	339
.1.	n _{1a}	ORF1ab	14202	G>U		159	1.1%	7
	η _{1b}	ORF1ab	19542	G>U	M>I	81	1.2%	23
	η _{1c}	S	22388	C>U		90	1.2%	21
	η _{1d}	Ν	29466	C>U	A>V	91	1.2%	4
	ηıe	ORF1ab	19718	C>U	T>I	73	1.5%	23
	η _{1f}	ORF3a	26060	C>U	T>I	92	1.2%	7
	η_{1g}	Ν	29227	G>U		55	1.2%	24
	ηıh	ORF1ab	3256	U>C		167	1.1%	0
	η _{1i}	ORF1ab	5622	C>U	P>L	67	1.2%	775
η₂		ORF1ab	18555	C>U		51	8.0%	25
η₃		ORF1ab	16647	G>U		84	8.0%	8
η 4		ORF1ab	7540	U>C		86	7.9%	0
η₅		S	23401	G>A		86	7.9%	1
η 6		S	22992	G>A	S>N	86	8.5%	4583
	η_{6a}	S	22480	C>U		66	1.3%	878
V ₁		ORF1ab	17858	A>G	Y>C	59	2.6%	61
V ₂		ORF1ab	17747	C>U	P>L	59	2.5%	1677

Supplementary Table 1. SARS-CoV-2 variants in the 68KG dataset (continued).

<u>Note</u>.- Genomic locations correspond to those of the NCBI genome (GenBank ID: NC_04551.2). Amino acid changes are shown for nonsynonymous variants.



Supplementary Figure 1. Flowchart summarizing the procedure to generate two curated multiple sequence alignments used in this study (29KG and 68KG) from the SARS-CoV-2 genomes available in the GISAID database. Abbreviations are as follows: GISAID (Global Initiative on Sharing Avian Influenza Data [https://www.gisaid.org]); NCBI (National Center for Biotechnology Information [https://www.ncbi.nlm.nih.gov]).



Supplementary Figure 2. Flowchart summarizing the steps performed to construct the mutation order tree from the curated SARS-CoV-2 alignments (i.e., 29KG and 68KG). Abbreviations are as follows: GISAID (Global Initiative on Sharing Avian Influenza Data); SCITE (Single Cell Inference of Tumor Evolution); FPR (False Positive Rates); FNR (False Negative Rates); COI (Co-Occurrence Index).



Supplementary Figure 3. A comparison of mutation-based and phylogeny-based classifications of 29KG genomes. Phylogeny-based classification is obtained by using the Pangolin service (v2.0.3; <u>https://pangolin.cog-uk.io/)</u>. Only the terminal variants are shown in mutation-based fingerprints for convenience. Each cell's value is the number of genomes that belong to the corresponding mutation-based and phylogeny-based groups. All phylogenetic-based groups with fewer than 20 genomes are excluded. Cells with fewer than five genomes matching have been left empty to make the comparison more straightforward and allow for sequencing and estimation errors.



Supplementary Figure 4. An example of sequence classification (ENA Accession MT675945) based on the 84 signature mutations (<u>http://sars2evo.datamonkey.org/</u>; "Classify your Sequence" option). (**a**) Input window to provide identifiers of sequences to be classified (e.g., MT675945). (**b**) The input sequence is classified into a mutational fingerprint. A list of mutations that are appeared in the input sequence is shown in the output window. (**c**) A waterfall phylogeny shows the input sequence's location in the phylogeny, which appears after clicking the closet signature matched mutation in panel b.