Genomic analysis of early SARS-CoV-2 strains introduced in Mexico

Blanca Taboada^{1*}, Joel Armando Vazquez-Perez^{2*}, José Esteban Muñoz Medina^{3*}, Pilar Ramos Cervantes^{4*}, Marina Escalera-Zamudio^{5*}, Celia Boukadida⁶, Alejandro Sanchez-Flores⁷, Pavel Isa¹, Edgar Mendieta Condado⁸, José Arturo Martínez-Orozco², Eduardo Becerril-Vargas², Jorge Salas-Hernández², Ricardo Grande⁷, Carolina González-Torres⁹, Francisco Javier Gaytán-Cervantes⁹, Gloria Vazquez⁷, Francisco Pulido⁷, Adnan Araiza Rodríguez⁸, Fabiola Garcés Ayala⁸, Cesar Raúl González Bonilla¹⁰, Concepción Grajales Muñiz¹¹, Víctor Hugo Borja Aburto¹², Gisela Barrera Badillo⁸, Susana López¹, Lucía Hernández Rivas⁸, Rogelio Perez-Padilla², Irma López Martínez⁸, Santiago Ávila-Ríos⁶, Guillermo Ruiz-Palacios⁴, José Ernesto Ramírez-González^{8*}, and Carlos F. Arias^{1*}

¹Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico; ²Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Ciudad de México, Mexico; ³División de Laboratorios de Vigilancia e Investigación Epidemiológica, Instituto Mexicano del Seguro Social, Ciudad de México, México; ⁴Instituto Nacional de Ciencias Médicas y Nutrición, Ciudad de México, Mexico; ⁵Department of Zoology, Oxford University, Oxford, UK; ⁶Centro de Investigación en Enfermedades Infecciosas, Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas, Ciudad de México, Mexico; ⁷Unidad Universitaria de Secuenciación Masiva y Bioinformática, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico; ⁸Instituto de Diagnóstico y Referencia Epidemiológicos, Dirección General de Epidemiología, Ciudad de México, Mexico; ⁹División de Desarrollo de la Investigación, Instituto Mexicano del Seguro Social, Ciudad de México, México; ¹⁰Coordinación de Investigación en Salud, Instituto Mexicano del Seguro Social, Ciudad de México, México; ¹¹Coordinación de Control Técnico de Insumos, Instituto Mexicano del Seguro Social, Ciudad de México, México; ¹²Dirección de Prestaciones Médicas, Instituto Mexicano del Seguro Social, Ciudad de México, Mexico.

*Blanca Taboada, Joel Armando Vazquez-Perez, José Esteban Muñoz Medina, Pilar Ramos Cervantes, and Marina Escalera-Zamudio contributed equally to this work

This work is the result of the collaboration of five institutions into one research consortium: three public health institutes and two universities. From the beginning of this work, it was agreed that the experimental leader of each institution would share the first authorship. Those were the criteria followed to assign first co-first authorship in this manuscript. The order of the other authors was randomly assigned.

+Corresponding authors: José Ernesto Ramírez-González, and Carlos F. Arias

JERG: Instituto de Diagnóstico y Referencia Epidemiológicos, Dirección General de Epidemiología, Ciudad de México, Mexico. e-mail: ernesto.ramirez@salud.gob.mx

CFA: Departamento de Genética del Desarrollo y Fisiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, Mexico. e-mail: arias@ibt.unam.mx

Running Titer: SARS-CoV-2 genome sequences in Mexico

ABSTRACT

The COVID-19 pandemic has affected most countries in the world. Studying the evolution and transmission patterns in different countries is crucial to implement effective strategies for disease control and prevention. In this work, we present the full genome sequence for 17 SARS-CoV-2 isolates corresponding to the earliest sampled cases in Mexico. Global and local phylogenomics, coupled with mutational analysis, consistently revealed that these viral sequences are distributed within 2 known lineages, the SARS-CoV-2 lineage A/G, containing mostly sequences from North America, and the lineage B/S containing mainly sequences from Europe. Based on the exposure history of the cases and on the phylogenomic analysis, we characterized fourteen independent introduction events. Additionally, three cases with no travel history were identified. We found evidence that two of these cases represent local transmission cases occurring in Mexico during mid-March 2020, denoting the earliest events described in the country. Within this Mexican cluster, we also identified an H49Y amino acid change in the spike protein. This mutation is a homoplasy occurring independently through time and space, and may function as a molecular marker to follow on any further spread of these viral variants throughout the country. Our results depict the general picture of the SARS-CoV-2 variants introduced at the beginning of the outbreak in Mexico, setting the foundation for future surveillance efforts.

IMPORTANCE

Understanding the introduction, spread and establishment of SARS-CoV-2 within distinct human populations is crucial to implement effective control strategies as well as the evolution of the pandemics. In this work, we describe that the initial virus strains introduced in Mexico came from Europe and the United States and the virus was circulating locally in the country as early as mid-March. We also found evidence for early local transmission of strains having the mutation H49Y in the Spike protein, that could be further used as a molecular marker to follow viral spread within the country and the region.

INTRODUCTION

The 2019 coronavirus disease (COVID-19), declared a pandemic by the WHO on March 11th, 2020¹, is caused by a novel betacoronavirus known as the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), detected in December of 2019 in the province of Wuhan in China¹. This is the third outbreak related to zoonotic betacoronaviruses known to occur in humans in the last two decades, after SARS (severe acute respiratory syndrome) in 2002 and MERS (Middle East respiratory syndrome) in 2012. After its emergence in China, SARS-CoV-2 spread initially to other parts of the world by people with a travel history to China, but gradually shifted to local transmissions². Viral spread was first detected in Thailand, South Korea and Japan, and by the second half of January, the first positive cases appeared in the USA and Europe (France, Italy and Spain). The current SARS-CoV-2 genome analysis in the Nextstrain site³, points out that viral transmission is now mainly community-driven^{4,5}.

In many countries, despite diagnostic efforts and initial control strategies, SARS-CoV-2 spread went on undetected until a critical number of cases requiring hospitalization and intensive care was reached, alerting the authorities in charge. As for May 4th 2020, SARS-CoV-2 has infected more than 3,578,000 people and caused around 251,000 deaths worldwide³. In Mexico, the first case of SARS-CoV-2 was detected on February 27th 2020, corresponding to a person who travelled back to Mexico from Italy, and who was in direct contact with a confirmed SARS-CoV-2 case. Soon after, additional cases were detected among travelers that returned from the USA and Europe, increasing every day. By May 4th, there were over 23,400 confirmed cases and 2,150 deaths within the country, indicating local transmission⁶. Understanding the introduction, spread and establishment of SARS-CoV-2 within distinct human populations is crucial to implement effective control strategies. In this work, we studied the early introduction dynamics of the first SARS-CoV-2 cases in Mexico. For this, we used a whole genome sequencing and phylogenomic approach. We obtained 17 full viral genome sequences, including the first case detected and sampled within the country. Phylogenomic placement showed that these viruses belong to the A2/G and B/S lineages, two of the three circulating viral lineages reported so far. Our analysis also confirms that there have been multiple independent introduction events in Mexico from travelers abroad. We also found evidence for early local transmission of strains having the mutation H49Y in the Spike protein, that could be further used as a molecular marker to follow viral spread within the country.

METHODS

Ethical statement

All clinical samples were processed at the “Instituto de Diagnóstico y Referencia Epidemiológicos” (InDRE), following official procedures⁷. All samples used for this work are considered part of the national response to COVID-19, and the data collected is directly related to disease control.

Sample collection and diagnostics

All samples used in this study were collected under the Mexican Official Norm NOM-024-SSA2-1994 for prevention and control of acute respiratory infections in the primary health attention, as part of the early diagnostics scheme for SARS-CoV-2 in public health laboratories and hospitals in Mexico City (Red Nacional de Laboratorios Estatales de Salud Pública, RNLSP; Instituto Nacional de Enfermedades Respiratorias, INER; Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, INCMNSZ; and Instituto Mexicano del Seguro Social, IMSS). Oro- and naso-pharyngeal swabs were collected and placed in virus transport medium upon collection, following InDRE official procedures⁸. A tracheal aspirate was also obtained from one patient and it was frozen at -70°C until use. Diagnosis was done using validated protocols for SARS-CoV 2, as approved by InDRE and by the World Health Organization (WHO)⁹.

Sample processing and Whole Genome Sequencing

All samples were prepared for RNA extraction, as described^{10,11}. Briefly, centrifuged and filtered supernatants were treated with Turbo DNase and RNase. Nucleic acids were then extracted using the PureLink™ Viral RNA/DNA Kit (ThermoFisher), following the manufacturer’s instructions and using linear acrylamide (Ambion) as RNA carrier. cDNA was synthesized using the SuperScript III Reverse Transcriptase System (ThermoFisher) and primer A (5'-GTTTCCCAGTAGGTCTCN9-3'). The second strand was generated by two rounds of synthesis with Sequenase 2.0, followed by 15 cycles of amplification using Phusion DNA polymerase using primer B (5'-GTTTCCCAGTAGGTCTC-3). Next, cDNA was purified using the DNA Clean & Concentrator Kit (Zymo Research) and used as input material for generating sequencing libraries, following the Nextera XT DNA Library Preparation Kit¹² (Illumina) . Finally, all samples were sequenced on the Illumina NextSeq 500 platform using a 150 cycle High Output Kit v2.5 to obtain paired end reads of 75 base pairs. Sequencing yields are reported in SI Table 1.

Bioinformatic analysis

Data quality control and processing

Read quality control was carried out using FAST-QC¹³ under the default parameters. Adapter sequences and low-quality bases were removed using Fastp v0.19¹⁴. Low complexity reads, those with a length shorter than 40 bases, and duplicates were excluded using CD-HIT-DUP v.4.6.8¹⁵. Off-target reads were then filtered out using Bowtie2 v2.3.4.3 with the default parameters against the human genome version GRCh38.p13, and the SILVA database¹⁶ as a reference to filter out human DNA and ribosomal sequences.

Viral genome assembly

The reads obtained were used as input to assemble viral genomes using the Wuhan-Hu-1 reference genome sequence (MN908947). For this, the reads obtained for each sample were mapped against the reference using Bowtie2 v2.3.4.3. Aligned reads were then used for *de novo* assembly using SPAdes v¹⁷.

Consensus genome sequences were generated using the majority threshold criterion. Only sequences with a coverage above 80% and a mean depth of $\geq 8X$ were considered for the analyses (SI Table 1).

Phylogenetic analyses

Data collation

From 4698 complete SARS-CoV-2 genomes deposited in the Global Initiative on Sharing All Influenza Data (GISAID) platform on the morning of April 7th 2020, a total of 3014 sequences genomes ($>29,000$ nt and only high coverage) were downloaded to generate a local database. As the collection dates of the Mexican samples range from late February to March, we filtered sequences collected between 02/01/2020 to 03/31/2020 for our database (Table 1). From these, unique sequences were extracted and those identical were collapsed, leaving 2633. We then included the 17 consensus viral genomes determined in this study and the Wuhan-Hu-1 reference genome sequence, yielding a total of 2651 sequences. We aligned the whole genome nt dataset using MUSCLE v3.8¹⁸ under default parameters, and then used the *getorf*s script from the EMBOSS suite¹⁹ to extract complete ORFs (Open Reading Frames) above 300 nt (Orf1a, Orf1b, Spike, M, Orf3a, Orf7a, Orf8 and N), that were then individually re-aligned as described¹⁸. Finally, to exclude UTRs and non-coding intergenic regions from the

phylogenomic analyses, individual ORFs were concatenated to generate an additional 28,320 nt-long whole genome (WG) alignment.

Data subsampling and tree inference

The individual and concatenated alignments were then reversed to nucleotides and used for estimating maximum likelihood (ML) trees using RAxML v8²⁰ under the following parameters: -T 2 -f a -x 390 -m GTRGAMMA -p 580 -N 100. All trees were rooted on Wuhan-Hu-1 reference genome sequence²¹. Given that the SARS-CoV-2 virus shows a low degree of genetic variation²², clade definition must be based on consensus branching patterns within different trees and by shared nucleotide substitution patterns²³, in addition to bootstrap support values. Based on these criteria, the position of the Mexican sequences was determined within the whole genome (WG) and individual ORF1a, ORF1b and S trees (SI Table 2), and was then confirmed on the global phylogeny available in Nextstrain²⁴.

To visualize details on the phylogenetic relatedness of the Mexican sequences, we then subsampled the previous large-scale WG alignment in a phylogenetically-informed manner, as based on the position of the Mexican strains within the large-scale trees and by selecting sequences using pairwise genetic distances^{25,26}. Briefly, all Mexican sequences were retained together with their immediate ancestors and descendants, and 184 sequences were further selected based on the minor pairwise genetic distance in relation to the Mexican genomes under a threshold of 99.5% (SI Table 3). The subsampled WG alignment was scanned for recombinant sequences using the GARD algorithm²⁷ in the Datamonkey server^{28,29}. A total of 201 sequences, including the Wuhan-Hu-1 reference genome, were used to re-estimate subsampled trees, as described above.

Global phylogenetic analysis and shared nucleotide substitution patterns²³ were used to confirm the position of the Mexican sequences based on the consensus clustering patterns observed within the large-scale WG tree, the subsampled WG tree, and the individual large-scale ORF trees, using a bootstrap value >50 for branch support, when possible (SI Table 2). In general, we observed consistency within the global tree and our large-scale and subsampled trees, as depicted by a conserved general structure at an internal branch level. Finally, analysis of the phylogenetic relationship between the Mexican and other viral sequences to identify groups of introduction events (IE) and local transmissions (LT) was done based on the following local definition, in which and IE or LT must include: i) one or more

Mexican sequences, *ii*) a minimum of one closest related sister sequence(s), *iii*) and/or the immediate common ancestor^{30,31}.

Mutation identification

Snippy³² was used to identify all mutations unique to the Mexican genomes, as compared to the reference genome sequence of isolate Wuhan-Hu-1. The large-scale WG alignment in nucleotides (including UTR and intergenic regions) was used as input for SI Table 4, while the large-scale WG concatenated ORF alignment was used as input for SI Table 5. The frequency and distribution for nucleotide and amino acid changes were determined using a normalized Sequence Logo, implemented by JalView³³. Non-conservative amino acid changes were determined based on amino acid properties. Finally, the list of amino acid changes obtained was compared to the list of known sites scored to be evolving under pervasive, episodic or directional positive selection, as tested under several *dN/dS* models^{34,35}.

RESULTS AND DISCUSSION

Multiple introduction events of SARS-CoV-2 variants from two different lineages

A total of 17 full viral genome sequences were obtained from selected Mexican samples representing the earliest sampled cases detected in the country (Figure 1). From the epidemiological data associated with the Mexican samples, 15 of the cases corresponded to introduction events from travelers returning from abroad that entered the country through Mexico City airport, with 5 of them then relocating to other places within the country using local transportation (either aerial or terrestrial). Two additional cases reported no travel history (Table 1). Global phylogenetic analysis²³ confirmed that 8 Mexican strains (samples 8, 17, 19, 24, 27, 28, 30, 31) grouped within the SARS-CoV-2 lineage B (also called lineage S, composed by sequences predominantly from the Americas). The remaining 9 Mexican strains (samples 2, 5, 6, 7, 13, 16, 22, 32, and 33) grouped within lineage A2 and subclade A2a (also called lineage G, composed by sequences predominantly from Europe) (Figure 2)³⁶. Both the A2/G and B/S lineages are contemporary, as the estimated date of emergence for lineage B/S is 12/29/2019 (CI: 12/20/2019, 01/03/2020), while for lineage A2/G is 01/18/2020 (CI 01/02/2020, 01/19/2020)²³. The collection dates for the Mexican samples that fall within lineage B1 range from 03/04/2020 to 03/15/2020, while those that fall within lineage A2

range from 02/27/2020 to 03/15/2020, including the strain corresponding to the first reported case in México³⁷ REF (sample 33). This suggests an initial co-circulation of both the A2/G and B/S lineages in Mexico (Figure 2). Viruses belonging to the third lineage reported, V, were not identified in this study.

Consensus clustering patterns observed within the large-scale and subsampled trees (SI Table 2), showed eight well-supported independent introduction events (IE1, IE3, IE5, IE7, IE9, IE10, IE12 and IE13; Figure 2). For IE2, IE4, IE6 and IE8, we were unable to determine their origins and the immediate phylogenetic relatedness of the Mexican sequences, due to low support values and inconsistent clustering patterns across trees (SI Table 2). The current resolution of phylogenomic analyses is limited by the low diversity of the SARS-CoV-2 virus. Thus, for the characterized Mexican viral strains, we could only determine with confidence the geographical origins at a regional level, rather than at country-level resolution. Altogether, these observations suggest that the virus variants identified in this study were more closely related to virus strains circulating at the time in the USA and Europe rather than in China or South East Asia.

Evidence for early local transmission

Sequences 27 and 31 correspond to two individuals that shared travel history to Vail, CO, USA, and that were in direct contact with case 28 in the return flight to Mexico. Nonetheless, the distribution of sequence 28 in an independent group (IE12) in relation to sequences 27 and 31 (Figure 2, SI Table 2), suggests that there were at least two different viral variants co-circulating within this specific location in the USA. On the other hand, sequences 8, 27, 30 and 31 grouped together, representing a local transmission cluster (LT11) (Figure 2). This observation is supported by phylogenetic consistency within our local trees and the global tree²³, and a high support value (bs: 100) observed for LT11 in all cases (S1 Table 2, Figure 2). Case 8 corresponds to an individual with no epidemiological relationship or contact with cases 27, 30 and 31, and with no travel history. Case 30 had travel history to Madrid, Spain, but no direct exposure with cases 27 and 31. For case 30, the possibility of this person acquiring the virus whilst being abroad cannot be ruled out. However, our analysis shows evidence that this person may have contracted the virus whilst already being in Mexico. Thus, LT11 strongly supports the occurrence of at least one independent local transmission event in Mexico City (case 8), as early as the second week of March.

Genetic variation within the Mexican viral genomes

When compared to the Wuhan-Hu-1 reference genome, the Mexican sequences displayed between 4 and 10 nucleotide substitutions, and between 1 and 5 amino acid changes. This is consistent with the reported rate of evolution of $\sim 8 \times 10^{-4}$ nucleotide substitutions per site per year, equivalent to ~ 2 substitutions per month^{38,39}. Collectively, 46 nucleotide substitutions and 20 amino acid residues were identified within the Mexican viral genomes (SI Table 4 and 5). As expected, the majority of these variants were not conserved through the genomes, and only 15 nucleotide changes and 6 amino acid changes were shared by more than one sequence. These results exclude sequences 6, 13 and 16, which showed a considerably lower coverage and depth when compared to the remaining 14 high-quality viral genomes obtained. Thus, most of the variability observed could be explained by errors introduced during reverse transcription, PCR amplification, sequencing or assembly (SI Table 1 and 4).

Consistent with our phylogenomic analysis, all Mexican sequences belonging to lineage A2 showed two clade-specific nucleotide substitutions, C241T and A23403G. A23403G results in the D614G amino acid change in the Spike protein (Table 2). All lineage A2a sequences (including the Mexican isolates) had the additional nucleotide substitution C14408T, resulting in the amino acid change P314L in Orf1b²³. Sequences within lineage B had the nucleotide substitution T28144C rendering the L84S amino acid change in the Orf8 protein. Similarly, lineage B1 sequences also showed the nucleotide substitution C18060T (Table 2). No evidence for recombination was found within any of the alignments, in agreement with previous observations²⁸. Taken together, our results suggest the Mexican viral sequences display the genetic changes according to their phylogenetic placement.

H49Y amino acid change in Mexican sequences within LT1

We further identified within all Mexican sequences grouping with the local transmission cluster (LT11), an H to Y amino acid change in position 49 of the Spike protein, corresponding to the C21707T nucleotide change in the whole genome and nucleotide alignment numbering. H49Y represents a non-conservative mutation located within the N-terminal domain (NTD) of the S glycoprotein trimer⁴⁰. No evidence of episodic or directional positive selection was found for this site, as tested under several *dN/dS* models^{34,35}.

Mapping this nucleotide change onto the Nextstrain global tree²³ with dates ranging from December 2019 to April 2020, revealed that this mutation represents a homoplasy that has occurred with a frequency of 0.4% (20/4533) within the genomes displayed in the global tree as of May 6th 2020. This

change first occurred in a single cluster of 14 sequences mainly from China (representative sequence: Jiangsu/JS02/2020 EPI_ISL_411952). The estimated date of emergence for this cluster that eventually died off (stopped circulating and had no more descendants) was of 01/12/2020 (CI:01/08/2020-01/16/2020). This mutation was detected again in the Mexican sequences in LT11 (Table 1, Figure 2). Since then, the C21707T/H49Y mutation has also appeared independently in three strains from Australia (EPI_ISL_426898, EPI_ISL_430632 and EPI_ISL_430633), two strains from the UK (EPI_ISL_433757 and EPI_ISL_432818), one strain from Taiwan (EPI_ISL_417518) and two strains from the USA (EPI_ISL_408010 and EPI_ISL_430050), with collection dates ranging from 01/29/2020 to 04/05/2020 (sequences deposited on GISAID platform by May 10th 2020). Of notice, all the sequences displaying the H49Y mutation belong to different viral lineages (either, A2, B1 or others), confirming non-phylogenetic correlation (e.g. founder effect), but rather an independent occurrence. It would be important to follow up the genomic surveillance of homoplasie mutations that keep emerging independently through time and space, either to determine if these are sequencing errors⁴¹, or to know if these are real homoplasies that may be associated with changes in biological properties or the virus, such as increased virulence as has been shown for other viruses²⁵. In addition, using H49Y as a molecular marker could be used to follow up any further circulation and spread of these viral variants in Mexico.

Data Availability

The generated sequences SARS-CoV-2 from Mexico can be found in GISAID and in Nextstrain²⁴. The corresponding GISAID accession numbers are listed in Table 1.

ACKNOWLEDGEMENTS

This work was partially supported by grant “Epidemiología genómica de los virus SARS-CoV-2 circulantes en México” from the National Council for Science and Technology (CONACyT)-Mexico to CFA, and by grants from the Mexican Government (Comisión de Equidad y Género de las Legislaturas LX-LXI y Comisión de Igualdad de Género de la Legislatura LXII de la H. Cámara de Diputados de la República Mexicana) to SAR and CB. MEZ is supported by a Leverhulme Trust ECR Fellowship (ECF-2019-542). We thank the “Unidad de Secuenciación Masiva y Bioinformática” of the “Laboratorio Nacional de Apoyo Tecnológico a las Ciencias Genómicas” (CONACyT #260481) for their support in sequencing services. We thank all the staff of the Technological Development and Molecular Research Unit, Virology

312 Department, and Sample Control and Services Department at InDRE for their technical assistance. The
313 findings and conclusions in this report are only the responsibility of the authors and do not necessarily of
314 the institutions involved.

315

FIGURE LEGENDS

Figure 1. Epidemiological positioning of the SARS-CoV-2 samples from Mexico

Epidemiological curve for the early SARS-CoV-2 epidemic in Mexico, dating from late February until early April. The rise in cumulative cases is shown in red, whilst the daily incidence is shown with orange bars. Dates of collection for the samples used in this study are indicated with the black arrows. One arrow might indicate the collection of more than two samples (dates shown in Table 1).

Figure 2. Phylogenomic positioning of the SARS-CoV-2 strains from Mexico.

RAxML tree estimated from the reduced whole genome alignment built using the concatenated main viral ORFs, and rooted using the Wuhan-Hu-01 strain. Sequences corresponding to the viral isolates from Mexico sequenced in this work are shown in red, while the region of origin for the identified closest related immediate ancestors and/or sister isolates is indicated in black. Support values for the branches of interest are indicated with numbers next to the branches. The clusters identified in this work representing introduction events (IE1-IE10 and IE12 and IE13) and the local transmission (LT11) are shown next to the strain names.

REFERENCES

1. Zhou, P. *et al.* A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* **579**, 270–273 (2020).
2. Pinotti, F. *et al.* Lessons learnt from 288 COVID-19 international cases: importations over time, effect of interventions, underdetection of imported cases. *medRxiv* 2020.02.24.20027326 (2020).
3. auspice. <https://nextstrain.org/narratives/ncov/sit-rep/2020-01-23>.
4. Li, Q. *et al.* Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. *N. Engl. J. Med.* **382**, 1199–1207 (2020).
5. Liu, J. *et al.* Community Transmission of Severe Acute Respiratory Syndrome Coronavirus 2, Shenzhen, China, 2020. *Emerg. Infect. Dis.* **26**, (2020).
6. [No title].
https://www.gob.mx/cms/uploads/attachment/file/547271/Comunicado_Tecnico_Diario_COVID-19_2020.04.18.pdf.
7. de Salud, S. Sistema Nacional de Vigilancia Epidemiológica. *gob.mx*
<http://www.gob.mx/salud/acciones-y-programas/sistema-nacional-de-vigilancia-epidemiologica>.
8. http://www.censida.salud.gob.mx/descargas/biblioteca/documentos/manual_para_la_toma_envio_y_recepcion_de_muestras.pdf.
9. https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2.
10. Taboada, B. *et al.* Is There Still Room for Novel Viral Pathogens in Pediatric Respiratory Tract Infections? *PLoS One* **9**, e113570 (2014).
11. Taboada, B. *et al.* The Geographic Structure of Viruses in the Cuatro Ciénegas Basin, a Unique Oasis in Northern Mexico, Reveals a Highly Diverse Population on a Small Geographic Scale. *Appl. Environ. Microbiol.* **84**, (2018).
12. Nextera XT DNA Sample Prep Kit Documentation.
https://support.illumina.com/sequencing/sequencing_kits/nextera_xt_dna_kit/documentation.html.
13. Babraham Bioinformatics - FastQC A Quality Control tool for High Throughput Sequence Data.
<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
14. Chen, S., Zhou, Y., Chen, Y. & Gu, J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**, i884–i890 (2018).
15. Fu, L., Niu, B., Zhu, Z., Wu, S. & Li, W. CD-HIT: accelerated for clustering the next-generation

- sequencing data. *Bioinformatics* **28**, 3150–3152 (2012).
16. Quast, C. *et al.* The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res.* **41**, D590–6 (2013).
17. Bankevich, A. *et al.* SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* **19**, 455–477 (2012).
18. Edgar, R. C. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* **5**, 1–19 (2004).
19. Rice, P., Longden, I. & Bleasby, A. EMBOSS: The European Molecular Biology Open Software Suite. *Trends Genet.* **16**, 276–277 (2000).
20. Stamatakis, A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313 (2014).
21. Wu, F. *et al.* A new coronavirus associated with human respiratory disease in China. *Nature* **579**, 265–269 (2020).
22. Pond, S. Genomic diversity and divergence of SARS-CoV-2/COVID-19. *Observable* <https://observablehq.com/@spond/current-state-of-sars-cov-2-evolution> (2020).
23. <https://nextstrain.org/ncov/global?dmax=2020-04-08>.
24. <https://nextstrain.org/ncov/global>.
25. Escalera-Zamudio, M. *et al.* Parallel Evolution in the Emergence of Highly Pathogenic Avian Influenza A Viruses. *bioRxiv* 370015 (2019) doi:10.1101/370015.
26. Hassan, A. S., Pybus, O. G., Sanders, E. J., Albert, J. & Esbjörnsson, J. Defining HIV-1 transmission clusters based on sequence data. *AIDS* **31**, 1211–1222 (2017).
27. Kosakovsky Pond, S. L., Posada, D., Gravenor, M. B., Woelk, C. H. & Frost, S. D. W. GARD: a genetic algorithm for recombination detection. *Bioinformatics* **22**, 3096–3098 (2006).
28. Robertson, D. I. *et al.* nCoV's relationship to bat coronaviruses & recombination signals (no snakes) - no evidence the 2019-nCoV lineage is recombinant. *Virological* <http://virological.org/t/ncovs-relationship-to-bat-coronaviruses-recombination-signals-no-snakes-no-evidence-the-2019-ncov-lineage-is-recombinant/331> (2020).
29. Datamonkey Adaptive Evolution Server. <http://datamonkey.org/>.
30. Shiino, T. *et al.* Molecular Evolutionary Analysis of the Influenza A(H1N1)pdm, May–September, 2009: Temporal and Spatial Spreading Profile of the Viruses in Japan. *PLoS One* **5**, e11057 (2010).

31. Baillie, G. J. *et al.* Evolutionary Dynamics of Local Pandemic H1N1/2009 Influenza Virus Lineages Revealed by Whole-Genome Analysis. *J. Virol.* **86**, 11–18 (2012).
32. Seemann, T. tseemann/snippy. *GitHub* <https://github.com/tseemann/snippy>.
33. Waterhouse, A. M., Procter, J. B., Martin, D. M. A., Clamp, M. & Barton, G. J. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics* **25**, 1189–1191 (2009).
34. Pond, S. Natural selection analysis of SARS-CoV-2/COVID-19. *Observable* <https://observablehq.com/@spond/natural-selection-analysis-of-sars-cov-2-covid-19> (2020).
35. Dashboard | SARS-CoV-2 Admin. <http://covid19.datamonkey.org/2020/04/01/covid19-analysis>.
36. Rambaut, A. *et al.* A dynamic nomenclature proposal for SARS-CoV-2 to assist genomic epidemiology. *bioRxiv* 2020.04.17.046086 (2020) doi:10.1101/2020.04.17.046086.
37. Garcés-Ayala, F. *et al.* Full Genome Sequence of the first SARS-CoV-2 detected in Mexico. *Arch. Virol.* (2020). *In press*.
38. Bedford, T. *et al.* Cryptic transmission of SARS-CoV-2 in Washington State. *medRxiv* 2020.04.02.20051417 (2020).
39. Rambaut, A. *et al.* Phylodynamic Analysis | 176 genomes | 6 Mar 2020. *Virological* <http://virological.org/t/phylodynamic-analysis-176-genomes-6-mar-2020/356> (2020).
40. Gui, M. *et al.* Cryo-electron microscopy structures of the SARS-CoV spike glycoprotein reveal a prerequisite conformational state for receptor binding. *Cell Res.* **27**, 119–129 (2016).
41. <http://virological.org/t/issues-with-sars-cov-2-sequencing-data/473>.

Figure 1

Figure 2

Table 1. List of viral genomes derived from Mexican samples.

Sample ID	Virus name	Accession GISAID ID	Location Country City	Age	Sex M/F	EH Place	Collection date	Arrival to Mexico	Port of Entry
2	Mexico/CDMX-INER_01	EPI_ISL_424345	Mexico_CDMX	42	M	EH_Spain	12/03/20	12/03/20	Mexico City
5	Mexico/CDMX-INER_02	EPI_ISL_424348	Mexico_CDMX	55	F	EH_Spain_France	13/03/20	12/03/20	Mexico City
6	Mexico/CDMX-INER_03	EPI_ISL_424625	Mexico_CDMX	29	M	EH_Spain	13/03/20	11/03/20	Mexico City
7	Mexico/CDMX-INER_04	EPI_ISL_424626	Mexico_CDMX	25	F	EH_Egypt_Barcelona_Spain	15/03/20	15/03/20	Mexico City
8	Mexico/CDMX-INER_05	EPI_ISL_424627	Mexico_CDMX	38	M	EH_None	15/03/20	N/A	N/A
13	Mexico/Chihuahua-IMSS_01	EPI_ISL_424731	Mexico_Chihuahua	21	M	EH_UK_France_USA	13/03/20	12/03/20	Mexico City
16	Mexico/Chiapas-InDRE_02	EPI_ISL_424666	Mexico_Chiapas	18	F	EH_Italy	29/02/20	25/02/20	Mexico City
17	Mexico/EdoMex-InDRE_03	EPI_ISL_424667	Mexico_Edomex	71	M	EH_Italy	04/03/20	21/02/20	Mexico City
19	Mexico/Queretaro-InDRE_04	EPI_ISL_424670	Mexico_Queretaro	43	M	EH_Spain_Holand_USA	10/03/20	06/03/20	Mexico City
22	Mexico/Puebla-InDRE_05	EPI_ISL_424672	Mexico_Puebla	31	M	EH_Spain_France	11/03/20	09/03/20	Mexico City
24	Mexico/CDMX-InDRE_06	EPI_ISL_424673	Mexico_CDMX	63	F	EH_Denver_USA	12/03/20	06/03/20	Mexico City
27	Mexico/CDMX-INCMNSZ_01	EPI_ISL_426361	México_CDMX	70	F	EH_Vail_USA	12/03/20	08/03/20	Mexico City
28	Mexico/CDMX-INCMNSZ_02	EPI_ISL_426362	México_CDMX	70	M	EH_Vail_USA	12/03/20	08/03/20	Mexico City
30	Mexico/CDMX-INCMNSZ_03	EPI_ISL_426363	México_CDMX	59	M	EH_Madrid_Spain	08/03/20	12/03/20	Mexico City
31	Mexico/CDMX-INCMNSZ_04	EPI_ISL_426364	México_CDMX	71	M	EH_Vail_USA_Germany	10/03/20	08/03/20	Mexico City
32	Mexico/CDMX-INCMNSZ_05	EPI_ISL_426365	México_CDMX	32	M	EH_None	12/03/20	N/A	N/A
33	Mexico/CDMX-InDRE_01	EPI_ISL_412972	México_CDMX	35	M	EH_Italy	27/02/20	N/A	Mexico City

Table 2. Nucleotide and amino acid changes in the Mexican samples compared to the reference strain Wuhan-Hu-1.

		nt change/position in WG nt aln ¹ vs MN908947				
		241	14408	18060	23403	28144
MN908947		C	C	C	A	U
7_Mexico/CDMX-INNER_04		U	U	.	G	.
22_Mexico/Puebla-InDRE_05		U	U	.	G	.
2_Mexico/CDMX-INNER_01		U	U	.	G	.
32_Mexico/CDMX-INCMNSZ_05		U	U	.	G	.
5_Mexico/CDMX-INNER_02		U	U	.	G	.
6_Mexico/CDMX-INNER_03		U	U	.	N	.
13_Mexico/Chihuahua-IMSS_01		U	U	.	G	.
33_Mexico/CDMX-InDRE_01		U	U	.	G	.
16_Mexico/Chiapas-InDRE_02		U	N	.	N	N
27_Mexico/CDMX-INCMNSZ_01		.	.	U	.	C
30_Mexico/CDMX-INCMNSZ_03		.	.	U	.	C
31_Mexico/CDMX-INCMNSZ_04		.	.	U	.	C
8_Mexico/CDMX-INNER_05		.	.	U	.	C
24_Mexico/CDMX-InDRE_06		.	.	U	.	C
28_Mexico/CDMX-INCMNSZ_02		.	.	.	.	C
19_Mexico/Queretaro-InDRE_04		.	.	.	.	C
17_Mexico/EdoMex-InDRE_03		.	.	.	.	C
Lineage defining mutations ²		A2/G	A2a	B1	A2/G	B/S

		aa change/position in WG ORF concatenated aln ¹ vs MN908947												
Genome Region		Orf1a (1-4405)				Orf1b (4406-7000)			S (7001-8282)		Orf3 (8505-8779)	Orf7a (8780-8900)	Orf8 (8901-9021)	N (9022-9440)
Position in WG concatenated CDS aln ¹		224	892	3071	3606	4619	5696	5732	7058	7623	8700	8817	8984	9225
MN908947		T	P	F	L	P	S	P	H	D	G	G	L	G
7_Mexico/CDMX-INNER_04		.	L	.	.	L	.	.	.	G	.	.	.	.
22_Mexico/Puebla-InDRE_05		.	.	.	.	L	L	.	.	G	.	V	.	.
2_Mexico/CDMX-INNER_01		.	.	.	.	L	.	.	.	G	.	.	.	.
32_Mexico/CDMX-INCMNSZ_05		.	.	.	.	L	.	.	.	G	.	.	.	.
5_Mexico/CDMX-INNER_02		.	.	.	.	L	.	.	.	G	.	.	.	.
6_Mexico/CDMX-INNER_03		.	X	.	.	L	.	.	.	X	.	X	.	X
13_Mexico/Chihuahua-IMSS_01		.	.	X	.	L	.	.	.	G	.	X	.	R
33_Mexico/CDMX-InDRE_01		.	.	.	.	L	.	.	.	G	.	.	.	R
16_Mexico/Chiapas-InDRE_02		.	.	.	.	X	.	.	.	X	.	X	X	R
27_Mexico/CDMX-INCMNSZ_01		.	.	.	.	.	.	L	Y	.	.	.	S	.
30_Mexico/CDMX-INCMNSZ_03		.	.	.	.	.	.	L	Y	.	.	.	S	.
31_Mexico/CDMX-INCMNSZ_04		.	.	.	.	.	.	L	Y	.	.	.	S	.
8_Mexico/CDMX-INNER_05		.	.	.	.	.	.	L	Y	.	.	.	S	.
24_Mexico/CDMX-InDRE_06		I	.	.	F	.	.	L	.	.	.	.	S	.
28_Mexico/CDMX-INCMNSZ_02		.	.	Y	.	.	.	.	.	.	V	.	S	.
19_Mexico/Queretaro-InDRE_04		.	.	.	.	.	.	.	.	.	.	.	S	.
17_Mexico/EdoMex-InDRE_03		.	.	.	.	.	.	.	.	.	.	.	S	.
Lineage defining mutations/positions ¹						A2a (P314L)			H49Y	A2/G (D614G)			B/S (L84S)	
Non-conservative amino-acid change ²														
Changes observed ²		#	*	§	WS	WS	†	WS			§	‡	WS	WS
Frequency ³		T99	P99	F98 Y2	L83 F15	L58 P42	S99	P87 L12	H99	Y58 D41	G98 V2	G99	L82 S17	G83 R16
Evidence for positive selection ⁴														

¹ General lineage defining mutations are shown in blue and orange, as defined in <https://nextstrain.org/ncov/global> and in GISAID preliminary analysis summary update 2020-04-07 1500UTC. No changes observed in M CDS (8283-8504).

²

Non-conservative amino-acid changes shown in red

Cluster defining homoplasic mutation of the Mexican sequences are shown in black.

Widespread mutations are indicated under WS. Unique changes to the Mexican sequences representing singletons are excluded (extended info available in SI Table 5).

Shared with USA/WA_UW153/2020|EPI_ISL_416691|2020_03_13

* Shared with England/SHEF_C0707/2020|EPI_ISL_420270|2020_03_22

§ Shared with 45 sequences, mostly from Australia and Spain.

† Shared with 5 sequences from Australia, Portugal, Spain and USA.

‡ Shared with Portugal/PT0024/2020|EPI_ISL_418009|2020_03_15

§ Shared with USA/WA_UW304/2020|EPI_ISL_418872|2020_03_23

³ Frequency of a given aa in percentage observed in the aln. Only changes present in > 1% of the sequences in the aln are shown.

⁴ Sites with a dN/dS >1 are shown in green, as compared to the list of sites determined under diverse dN/dS models described in <http://covid19.datamonkey.org/2020/04/01/covid19-analysis/>

Supplementary Table 1. Sequencing results.

Sample ID	Virus_name	Sample Type [#]	Raw valid reads	On-target reads (%)	Genome coverage	Depth	Mean Depth [*]
2	Mexico/CDMX-INER_01	NPS	13,949,246	12,406 (0.09)	99.17	30	30
5	Mexico/CDMX-INER_02	NPS	26,958,293	28,208 (0.10)	98.56	69	70
6	Mexico/CDMX-INER_03	NPS	11,143,321	3,970 (0.04)	84.25	9	11
7	Mexico/CDMX-INER_04	NPS	10,372,811	21,080 (0.20)	99.82	51	51
8	Mexico/CDMX-INER_05	NPS	2,274,643	11,648 (0.51)	99.73	28	28
13	Mexico/Chihuahua-IMSS_01	NPS	12,785,238	7,331 (0.06)	90.98	18	20
16	Mexico/Chiapas-InDRE_02	OPS	4,322,780	1,739 (0.04)	83.06	4	8
17	Mexico/EdoMex-InDRE_03	NAS	8,173,886	85,170 (1.04)	99.90	200	200
19	Mexico/Queretaro-InDRE_04	OPS	9,394,551	60,545 (0.64)	99.91	145	145
22	Mexico/Puebla-InDRE_05	OPS	5,030,534	39,210 (0.78)	99.90	93	93
24	Mexico/CDMX-InDRE_06	OPS	3,890,600	109,000 (2.81)	99.92	256	256
27	Mexico/CDMX-INCMNSZ_01	NPS	14,770,367	79,364 (0.54)	99.88	164	164
28	Mexico/CDMX-INCMNSZ_02	NPS	2,898,415	21,985 (0.76)	99.90	53	53
30	Mexico/CDMX-INCMNSZ_03	NPS	3,956,195	23,550 (0.60)	99.91	57	57
31	Mexico/CDMX-INCMNSZ_04	NPS	4,581,562	97,388 (2.13)	99.92	228	228
32	Mexico/CDMX-INCMNSZ_05	NPS	2,648,330	7,813 (0.30)	99.34	19	19
33	Mexico/CDMX-InDRE_01	NPS	2,662,304	1,000,109 (37.57)	100.00	3983	3983

[#] Abbreviation: NPS=nasopharyngeal swab, OP= oropharyngeal swab, NAS=nasopharyngeal aspirate

^{*} In relationship to the ratio of unambiguous bases to the reference sequence length (using at least 5 unique reads and 90% agreement during base calling)

Supplementary Table 2. Clustering patterns for the Mexican samples within the reduced and large-scale WG and individual ORF ML trees.

reduced -WG ¹	large-scale WG ¹	large-scale Orf1a ¹	large-scale Orf1b ¹	large-scale S ¹
<p>IE1-bs 78 22_Mexico/Puebla-InDRE_05/2020 EPI_ISL_424672 Mexico_Puebla 31 M EH_Spain_France 3_11_2020 Portugal/PT0024/2020 EPI_ISL_418009 PRT_Portugal Age_29 Sex_F D0_NA EH_NA 3_15_2020 Basal-bs 96: Spain/Madrid_H11_40/2020 EPI_ISL_417967 ESP_Madrid Age_44 Sex_F D0_NA EH_NA 3_12_2020</p> <p>IE2-bs 8 32_Mexico/CDMX-INCMNSZ_05/2020 EPI_ISL_426365 Mexico_CDMX 70 F EH_Vail_USA 3_12_2020 Australia/VIC254/2020 EPI_ISL_419949 AUS_Victoria Age_67 Sex_M D0_NA EH_NA 2020_03_21 Basal-bs 0: Australia/VIC164/2020 EPI_ISL_419861 AUS_Victoria Age_37 Sex_NA D0_NA EH_NA 2020_03_18</p> <p>IE3-bs 0 5_Mexico/CDMX-INER_02/2020 EPI_ISL_424348 Mexico_CDMX 55 F EH_Spain_France 3_13_2020 Spain/Madrid_H12_1905/2020 EPI_ISL_421175 ESP_Madrid Age_25 Sex_F D0_NA EH_NA 2020_03_29 Basal-bs: None</p> <p>IE4-bs 56 2_Mexico/CDMX-INER_01/2020 EPI_ISL_424345 Mexico_CDMX 42 M EH_Spain 3_12_2020 Hungary/2/2020 EPI_ISL_418183 HUN_Baranya Age_26 Sex_F D0_H EH_NA 2020_03_17 Basal-bs 0: None</p> <p>IE5-bs 68 Wales/PHWC_25551/2020 EPI_ISL_421004 GBR_Wales Age_73 Sex_M D0_NA EH_NA 2020_03_23 16_Mexico/Chiapas-InDRE_02/2020 EPI_ISL_424666 Mexico_Chiapas 18 F EH_Italy 2_29_2020 Basal-bs 0: None</p> <p>IE6-bs 0 33_Mexico/CDMX-InDRE_01/2020 EPI_ISL_412972 Mexico_CDMX 35 M EH_Italy 2_27_2020 Basal-bs 1: England/20122119502/2020 EPI_ISL_418681 GBR_England Age_31 Sex_F D0_NA EH_NA 2020_03_17</p> <p>IE7-bs 84 6_Mexico/CDMX-INER_03/2020 EPI_ISL_424625 Mexico_CDMX 29 M EH_Spain 3_13_2020 England/20108034006/2020 EPI_ISL_417258 GBR_England Age_74 Sex_F D0_NA EH_NA 2020_03_06</p> <p>IE8-bs 42 Italy/TE4959/2020 EPI_ISL_418259 ITA_Abruzzo Age_76 Sex_M D0_NA EH_NA 2020_03_14 13_Mexico/Chihuahua-IMSS_01/2020 EPI_ISL_424731 Mexico_Chihuahua 21 M EH_UK_France_USA 3_13_2020 Basal-bs 0: None</p> <p>IE9-bs 91 7_Mexico/CDMX-INER_04/2020 EPI_ISL_424626 Mexico_CDMX 25 F EH_Egypt_Barcelona_Spain 3_15_2020 England/SHEF_C0707/2020 EPI_ISL_420270 GBR_England Age_73 Sex_F D0_NA EH_NA 2020_03_22 Basal-bs: None</p> <p>IE10-bs 99 Australia/VIC110/2020 EPI_ISL_419716 AUS_Victoria Age_62 Sex_F D0_NA EH_NA 2020_03_18 24_Mexico/CDMX-InDRE_06/2020 EPI_ISL_424673 Mexico_CDMX 63 F EH_Denver_USA 3_12_2020</p> <p>LT11-bs 100 CLUSTER 31_Mexico/CDMX-INCMNSZ_04/2020 EPI_ISL_426364 Mexico_CDMX 70 M EH_Vail_USA 3_12_2020 8_Mexico/CDMX-INER_05/2020 EPI_ISL_424627 Mexico_CDMX 38 M EH_None 3_15_2020 27_Mexico/CDMX-INCMNSZ_01/2020 EPI_ISL_426361 Mexico_CDMX 32 M EH_None 3_12_2020 30_Mexico/CDMX-INCMNSZ_03/2020 EPI_ISL_426363 Mexico_CDMX 38 M EH_None 3_12_2020 Basal-bs: None</p> <p>IE12-bs 2 28_Mexico/CDMX-INCMNSZ_02/2020 EPI_ISL_426362 Mexico_CDMX 71 M EH_Vail_USA_Germany 3_10_2020 Basal-bs 0: Spain/CastillaLeon201372/2020 EPI_ISL_418249 ESP_Castilla_y_Leon Age_25 Sex_F D0_NA EH_NA 2020_03_03</p> <p>IE13 -bs 87 Chile/Talca_2/2020 EPI_ISL_414578 CHL_Talca Age_33 Sex_F D0_NA EH_NA 2020_03_04 Chile/Talca_1/2020 EPI_ISL_414577 CHL_Talca Age_33 Sex_M D0_R EH_Asia_Europe 2020_03_02 17_Mexico/EdoMex-InDRE_03/2020 EPI_ISL_424667 Mexico_Edomex 71 M EH_Italy 3_4_2020 Basal-bs 2: 19_Mexico/Queretaro-InDRE_04/2020 EPI_ISL_424670 Mexico_Queretaro 43 M EH_Spain_Holand_USA 13_10_2020</p>	<p>IE1-bs 6 YES</p> <p>IE2-bs 27 YES</p> <p>IE3-bs 0 YES</p> <p>IE4-bs 61 NO</p> <p>IE5-bs 19 YES</p> <p>IE6-bs 1 NO</p> <p>IE7-bs 67 YES</p> <p>IE8-bs 18 YES</p> <p>IE9-bs 79 YES</p> <p>IE10-bs 0 NO</p> <p>IE11-bs 98 CLUSTER YES</p> <p>IE12-bs 0 YES</p> <p>IE13-bs 0 YES</p>	<p>IE1-bs 0 NO</p> <p>IE2-bs 0 NO</p> <p>IE3-bs 0 NO</p> <p>IE4-bs 0 NO</p> <p>IE5-bs 0 NO</p> <p>IE6-bs 0 NO-POLYTOMY</p> <p>IE7-bs 0 YES</p> <p>IE8-bs 0 NO</p> <p>IE9-bs 0 YES</p> <p>IE10-bs 2 YES</p> <p>C11.1-bs 0 BROKEN CLUSTER</p> <p>IE12-bs 0 YES</p> <p>IE13-bs 0 YES</p>	<p>IE1-bs 0 YES</p> <p>IE2-bs 0 NO</p> <p>IE3-bs 0 YES-POLYTOMY</p> <p>IE4-bs 0 NO-POLYTOMY</p> <p>IE5-bs 0 NO</p> <p>IE6-bs 0 NO-POLYTOMY</p> <p>IE7-bs 27 YES</p> <p>IE8-bs 0 NO-POLYTOMY</p> <p>IE9-bs 0 NO-POLYTOMY</p> <p>IE10-bs 0 YES-POLYTOMY</p> <p>C11.1-bs 0 BROKEN CLUSTER</p> <p>IE12-bs 0 YES-POLYTOMY</p> <p>IE13-bs 33 YES</p>	<p>IE1-bs 0 NO-POLYTOMY</p> <p>IE2-bs 0 NO</p> <p>IE3-bs 0 NO</p> <p>IE4-bs 0 NO</p> <p>IE5-bs 0 NO-POLYTOMY</p> <p>IE6-bs 0 NO-POLYTOMY</p> <p>IE7-bs 0 NO</p> <p>IE8-bs 0 NO-POLYTOMY</p> <p>IE9-bs 0 NO</p> <p>IE10-bs 0 YES-POLYTOMY</p> <p>IE11-bs 89 CLUSTER YES</p> <p>IE12-bs 0 NO</p> <p>IE13-bs 0 NO</p> <p>NO</p>
NO-POLYTOMY-BROKEN CLUSTER WITH 19	NO-POLYTOMY-BROKEN CLUSTER WITH 19	NO-POLYTOMY-BROKEN CLUSTER WITH 19	NO-POLYTOMY-BROKEN CLUSTER WITH 19	NO

¹Consistency for clustering patterns observed within >2 trees and/or bootstrap support values >50 are indicated in red. YES/NO indicates conserved clustering patterns.

Supplementary Table 3. List of sequences and corresponding metadata used for the reduced alignments.

Virus name	Accession_ID	Location_Country_City	Age	Sex	MF	OO_S/DNA	EH_Country	Collection_date
AustraliaNSW5/2020	EPI_ISL_412975	AUS New_South_Wales	Age_43	Sex_M	DO_R		EH_Aus	2_28_2020
AustraliaNSW14/2020	EPI_ISL_413800	AUS New_South_Wales	Age_41	Sex_F	DO_R		EH_Aus	3_3_2020
AustraliaNSW27/2020	EPI_ISL_417390	AUS New_South_Wales	Age_72	Sex_M	DO_NA		EH_Aus	3_8_2020
AustraliaQLD09/10/2020	EPI_ISL_417331	AUS Queensland	Age_63	Sex_F	DO_NA		EH_Aus	3_11_2020
AustraliaVIC10/2020	EPI_ISL_419716	AUS_Victoria	Age_82	Sex_F	DO_NA		EH_Aus	3_18_2020
AustraliaVIC12/20/2020	EPI_ISL_419731	AUS_Victoria	Age_89	Sex_M	DO_NA		EH_Aus	3_20_2020
AustraliaVIC12/28/2020	EPI_ISL_419730	AUS_Victoria	Age_77	Sex_M	DO_NA		EH_Aus	3_20_2020
AustraliaVIC13/20/2020	EPI_ISL_419823	AUS_Victoria	Age_74	Sex_M	DO_NA		EH_Aus	3_21_2020
AustraliaVIC14/20/2020	EPI_ISL_419827	AUS_Victoria	Age_81	Sex_M	DO_NA		EH_Aus	3_22_2020
AustraliaVIC13/28/2020	EPI_ISL_419834	AUS_Victoria	Age_79	Sex_NA	DO_NA		EH_Aus	2_23_2020
AustraliaVIC14/20/2020	EPI_ISL_419851	AUS_Victoria	Age_39	Sex_M	DO_NA		EH_Aus	3_17_2020
AustraliaVIC15/20/2020	EPI_ISL_419860	AUS_Victoria	Age_28	Sex_M	DO_NA		EH_Aus	3_18_2020
AustraliaVIC16/20/2020	EPI_ISL_419861	AUS_Victoria	Age_37	Sex_NA	DO_NA		EH_Aus	3_18_2020
AustraliaVIC17/20/2020	EPI_ISL_419874	AUS_Victoria	Age_66	Sex_M	DO_NA		EH_Aus	3_19_2020
AustraliaVIC19/20/2020	EPI_ISL_419890	AUS_Victoria	Age_31	Sex_M	DO_NA		EH_Aus	3_19_2020
AustraliaVIC20/2020	EPI_ISL_419741	AUS_Victoria	Age_NA	Sex_NA	DO_NA		EH_Aus	3_8_2020
AustraliaVIC20/20/2020	EPI_ISL_419896	AUS_Victoria	Age_59	Sex_M	DO_NA		EH_Aus	3_18_2020
AustraliaVIC21/30/2020	EPI_ISL_419908	AUS_Victoria	Age_24	Sex_M	DO_NA		EH_Aus	3_20_2020
AustraliaVIC22/20/2020	EPI_ISL_419917	AUS_Victoria	Age_74	Sex_M	DO_NA		EH_Aus	3_20_2020
AustraliaVIC22/20/2020	EPI_ISL_419921	AUS_Victoria	Age_67	Sex_M	DO_NA		EH_Aus	3_20_2020
AustraliaVIC22/20/2020	EPI_ISL_419923	AUS_Victoria	Age_67	Sex_NA	DO_NA		EH_Aus	3_20_2020
AustraliaVIC24/20/2020	EPI_ISL_419938	AUS_Victoria	Age_59	Sex_M	DO_NA		EH_Aus	3_21_2020
AustraliaVIC24/20/2020	EPI_ISL_419944	AUS_Victoria	Age_58	Sex_M	DO_NA		EH_Aus	3_21_2020
AustraliaVIC24/20/2020	EPI_ISL_419949	AUS_Victoria	Age_67	Sex_M	DO_NA		EH_Aus	3_21_2020
AustraliaVIC25/20/2020	EPI_ISL_420036	AUS_Victoria	Age_61	Sex_F	DO_NA		EH_Aus	3_21_2020
AustraliaVIC27/20/2020	EPI_ISL_419967	AUS_Victoria	Age_80	Sex_F	DO_NA		EH_Aus	3_22_2020
AustraliaVIC27/20/2020	EPI_ISL_419971	AUS_Victoria	Age_40	Sex_M	DO_NA		EH_Aus	3_22_2020
AustraliaVIC29/20/2020	EPI_ISL_419984	AUS_Victoria	Age_48	Sex_F	DO_NA		EH_Aus	3_23_2020
AustraliaVIC29/20/2020	EPI_ISL_419990	AUS_Victoria	Age_74	Sex_F	DO_NA		EH_Aus	3_23_2020
AustraliaVIC30/20/2020	EPI_ISL_419991	AUS_Victoria	Age_35	Sex_NA	DO_NA		EH_Aus	3_23_2020
AustraliaVIC30/20/2020	EPI_ISL_419995	AUS_Victoria	Age_89	Sex_F	DO_NA		EH_Aus	3_23_2020
AustraliaVIC30/20/2020	EPI_ISL_419772	AUS_Victoria	Age_42	Sex_M	DO_NA		EH_Aus	3_13_2020
AustraliaVIC38/2020	EPI_ISL_419782	AUS_Victoria	Age_65	Sex_M	DO_NA		EH_Aus	3_13_2020
AustraliaVIC39/2020	EPI_ISL_419783	AUS_Victoria	Age_62	Sex_F	DO_NA		EH_Aus	3_13_2020
AustraliaVIC7/20/2020	EPI_ISL_419791	AUS_Victoria	Age_37	Sex_M	DO_NA		EH_Aus	3_14_2020
AustraliaVIC32/2020	EPI_ISL_419794	AUS_Victoria	Age_43	Sex_M	DO_NA		EH_Aus	3_14_2020
AustriaCeMM001/2020	EPI_ISL_419854	AUT	Age_27	Sex_F	DO_NA		EH_Aus	2_26_2020
AustriaCeMM002/2020	EPI_ISL_419865	AUT_Vienna	Age_73	Sex_M	DO_NA		EH_Aus	2_26_2020
AustriaCeMM009/2020	EPI_ISL_419862	AUT_Vienna	Age_72	Sex_D	DO_NA		EH_Aus	2_14_2020
AustriaCeMM020/2020	EPI_ISL_419873	AUT	Age_37	Sex_F	DO_NA		EH_Aus	3_22_2020
BelarusChV202/2/2020	EPI_ISL_419892	BLR	Age_NA	Sex_NA	DO_NA		EH_Aus	03_2020
BelgiumBL_032511/2020	EPI_ISL_420438	BEL_Herselt	Age_85	Sex_M	DO_NA		EH_Aus	3_25_2020
BelgiumDME_032413/2020	EPI_ISL_420392	BEL_Linter	Age_76	Sex_M	DO_NA		EH_Aus	3_24_2020
BelgiumDMM_032518/2020	EPI_ISL_420437	BEL_Gentwegen	Age_38	Sex_M	DO_NA		EH_Aus	3_25_2020
BelgiumDRV_032517/2020	EPI_ISL_420414	BEL_Wanze	Age_29	Sex_M	DO_NA		EH_Aus	3_25_2020
BelgiumDV_032417/2020	EPI_ISL_420374	BEL_Biersel	Age_49	Sex_F	DO_NA		EH_Aus	3_24_2020
BelgiumDVC_032516/2020	EPI_ISL_420403	BEL_Geraardsbergen	Age_56	Sex_M	DO_NA		EH_Aus	3_25_2020
BelgiumJUC_032516/2020	EPI_ISL_420422	BEL_Tiet_Winge	Age_91	Sex_M	DO_NA		EH_Aus	3_25_2020
BelgiumML_032418/2020	EPI_ISL_420387	BEL_Sint_Amandus_Berchem	Age_51	Sex_F	DO_NA		EH_Aus	3_24_2020
BelgiumRUS_030257/2020	EPI_ISL_418986	BEL_Hoegaarden	Age_18	Sex_M	DO_NA		EH_Aus	3_2_2020
BelgiumUGent_11/2020	EPI_ISL_419259	BEL_Ghent	Age_64	Sex_M	DO_NA		EH_Aus	3_24_2020
BelgiumLGS_10/28/2020	EPI_ISL_421202	BEL_Liese	Age_45	Sex_F	DO_NA		EH_Aus	3_30_2020
BelgiumVAG_03/01/2020	EPI_ISL_415155	BEL_Huldenberg	Age_14	Sex_F	DO_NA		EH_Aus	3_1_2020
BelgiumVHV_03/24/18/2020	EPI_ISL_420375	BEL_Tenurne	Age_38	Sex_F	DO_NA		EH_Aus	3_24_2020
BelgiumVLM_03/01/2020	EPI_ISL_415153	BEL_Huldenberg	Age_48	Sex_F	DO_NA		EH_Aus	3_3_2020
BelgiumVPE_13/06/20/2020	EPI_ISL_418605	BEL_Denize	Age_28	Sex_M	DO_NA		EH_Aus	3_6_2020
BrazilES_2/25/2020	EPI_ISL_415128	BRA_Espirito_Santo	Age_37	Sex_F	DO_NA		EH_Aus	2_28_2020
CanadaBC_407583/2020	EPI_ISL_418824	CAN_British_Columbia	Age_59	Sex_F	DO_NA		EH_Aus	3_3_2020
CanadaON_P163458/20/2020	EPI_ISL_418368	CAN_Ontario	Age_34	Sex_F	DO_NA		EH_Aus	3_12_2020
CanadaON_P167590/2020	EPI_ISL_418373	CAN_Ontario	Age_70	Sex_M	DO_NA		EH_Aus	3_14_2020
CanadaON_P168580/2020	EPI_ISL_418330	CAN_Ontario	Age_40	Sex_F	DO_NA		EH_Aus	3_5_2020
Chileantiago_04/17/2020	EPI_ISL_415681	CHL_Santiago	Age_17	Sex_M	DO_NA		EH_Aus	3_18_2020
ChileTalia_1/2020	EPI_ISL_414377	CHL_Talia	Age_33	Sex_M	DO_R		EH_Asia_Europe	3_2_2020
ChileTalia_2/2020	EPI_ISL_414378	CHL_Talia	Age_33	Sex_F	DO_NA		EH_Aus	3_4_2020
DenmarkSSI_02/22/2020	EPI_ISL_416143	DNK_Copenhagen	Age_54	Sex_M	DO_NA		EH_Aus	2_28_2020
DenmarkSSI_04/22/2020	EPI_ISL_416153	DNK_Copenhagen	Age_49	Sex_M	DO_NA		EH_Aus	3_2_2020
England2010604/12/2020	EPI_ISL_414008	GBR_England	Age_42	Sex_F	DO_NA		EH_Aus	2_7_2020
England2010605/15/2020	EPI_ISL_414009	GBR_England	Age_43	Sex_F	DO_NA		EH_Aus	2_25_2020
England2010609/27/2020	EPI_ISL_414012	GBR_England	Age_54	Sex_F	DO_NA		EH_Aus	2_27_2020
England2010610/30/2020	EPI_ISL_417238	GBR_England	Age_80	Sex_M	DO_NA		EH_Aus	3_3_2020
England2010803/4/2020	EPI_ISL_417258	GBR_England	Age_74	Sex_F	DO_NA		EH_Aus	3_6_2020
England2010805/7/2020	EPI_ISL_417268	GBR_England	Age_59	Sex_M	DO_NA		EH_Aus	3_8_2020
England2012207/4/2020	EPI_ISL_418676	GBR_England	Age_89	Sex_M	DO_NA		EH_Aus	3_10_2020
England2012211/5/2020	EPI_ISL_418681	GBR_England	Age_31	Sex_F	DO_NA		EH_Aus	3_12_2020
England2012404/9/2020	EPI_ISL_418723	GBR_England	Age_78	Sex_F	DO_NA		EH_Aus	3_16_2020
England2012803/20/2020	EPI_ISL_420477	GBR_England	Age_56	Sex_F	DO_NA		EH_Aus	3_18_2020
England2013804/7/2020	EPI_ISL_420907	GBR_England	Age_89	Sex_F	DO_NA		EH_Aus	3_27_2020
England2013804/8/2020	EPI_ISL_420998	GBR_England	Age_61	Sex_M	DO_NA		EH_Aus	3_28_2020
England2013805/30/2020	EPI_ISL_420715	GBR_England	Age_86	Sex_F	DO_NA		EH_Aus	3_28_2020
England2013805/6/2020	EPI_ISL_420716	GBR_England	Age_88	Sex_M	DO_NA		EH_Aus	3_26_2020
England2013805/7/2020	EPI_ISL_420717	GBR_England	Age_88	Sex_M	DO_NA		EH_Aus	3_26_2020
England2013806/20/2020	EPI_ISL_420731	GBR_England	Age_64	Sex_F	DO_NA		EH_Aus	3_28_2020
England2013806/27/2020	EPI_ISL_420742	GBR_England	Age_93	Sex_F	DO_NA		EH_Aus	3_28_2020
England2013806/27/2020	EPI_ISL_418740	GBR_England	Age_45	Sex_M	DO_NA		EH_Aus	3_28_2020
England2013806/27/2020	EPI_ISL_418313	GBR_England	Age_93	Sex_M	DO_NA		EH_Aus	3_24_2020
England2013806/27/2020	EPI_ISL_420255	GBR_England	Age_31	Sex_M	DO_NA		EH_Aus	3_25_2020
England2013806/27/2020	EPI_ISL_420260	GBR_England	Age_53	Sex_F	DO_NA		EH_Aus	3_25_2020
England2013806/27/2020	EPI_ISL_420247	GBR_England	Age_45	Sex_F	DO_NA		EH_Aus	3_25_2020
England2013806/27/2020	EPI_ISL_420243	GBR_England	Age_85	Sex_F	DO_NA		EH_Aus	3_25_2020
England2013806/27/2020	EPI_ISL_420270	GBR_England	Age_73	Sex_F	DO_NA		EH_Aus	3_22_2020
England2013806/27/2020	EPI_ISL_420258	GBR_England	Age_54	Sex_F	DO_NA		EH_Aus	3_28_2020
England2013806/27/2020	EPI_ISL_414344	GBR_England	Age_27	Sex_F	DO_NA		EH_Aus	3_4_2020
England2013806/27/2020	EPI_ISL_414351	GBR_England	Age_26	Sex_M	DO_NA		EH_Aus	3_3_2020
FinlandFIN003020/2020	EPI_ISL_413603	FIN_Helsinki	Age_55	Sex_F	DO_NA		EH_Aus	2_27_2020
FranceGE158/2020	EPI_ISL_414600	FRA_Grand_Est	Age_33	Sex_M	DO_H		EH_Aus	2_26_2020
FranceGE158/2020	EPI_ISL_414623	FRA_Grand_Est	Age_36	Sex_M	DO_NA		EH_Aus	2_25_2020
GeorgiaTL_4/6/2020	EPI_ISL_415643	GEO_Tbilisi	Age_NA	Sex_M	DO_NA		EH_Aus	3_10_2020
GeorgiaTL_5/3/2020	EPI_ISL_415640	GEO_Tbilisi	Age_NA	Sex_M	DO_NA		EH_Aus	3_11_2020
GeorgiaTL_5/4/2020	EPI_ISL_415641	GEO_Tbilisi	Age_NA	Sex_F	DO_NA		EH_Aus	3_27_2020
GermanyBayP43/2020	EPI_ISL_414521	DEU_Bavaria	Age_38	Sex_F	DO_H		EH_Aus	3_2_2020
GermanyNRW_01/2020	EPI_ISL_413488	DEU_North_Rhine_Westphalia	Age_NA	Sex_NA	DO_NA		EH_Aus	2_28_2020
GermanyNRW_04/2020	EPI_ISL_414499	DEU_North_Rhine_Westphalia	Age_NA	Sex_NA	DO_NA		EH_Aus	2_28_2020
GermanyNRW_09/2020	EPI_ISL_414509	DEU_North_Rhine_Westphalia	Age_NA	Sex_NA	DO_NA		EH_Aus	2_28_2020
GermanyNRW_15/2020	EPI_ISL_419532	DEU_Düsseldorf	Age_NA	Sex_NA	DO_NA		EH_Aus	3_11_2020
Greece16/2020	EPI_ISL_418265	GRC_Athens	Age_NA	Sex_NA	DO_NA		EH_Aus	3_18_2020
Hungary2/2020	EPI_ISL_414687	HUN_Budapest	Age_50	Sex_M	DO_NA		EH_Aus	2_25_2020
Hungary2/2020	EPI_ISL_417187	HUN_Budapest	Age_51	Sex_F	DO_NA		EH_Aus	2_8_2020
Hong_KongHKP02_01/02/2020	EPI_ISL_417193	HKG_Hong_Kong	Age_22	Sex_M	DO_NA		EH_Aus	2_9_2020
Hong_KongHKP02_04/02/2020	EPI_ISL_419515	HKG_Hong_Kong	Age_86	Sex_F	DO_NA		EH_Aus	2_10_2020
Hungary2/2020	EPI_ISL_418183	HUN_Budapest	Age_26	Sex_F	DO_H		EH_Aus	3_17_2020
Iceland15/2020	EPI_ISL_417711	ISL_Reykjavik	Age_40	Sex_M	DO_NA		EH_Aus	3_13_2020
Iceland18/2020	EPI_ISL_417709	ISL_Reykjavik	Age_71	Sex_F	DO_NA		EH_Aus	3_13_2020
Iceland25/2020	EPI_ISL_417581	ISL_Reykjavik	Age_17	Sex_F	DO_NA		EH_Aus	3_17_2020
Iceland31/30/2020	EPI_ISL_417542	ISL_Reykjavik	Age_20	Sex_F	DO_NA		EH_Aus	3_18_2020
Iceland31/4/2020	EPI_ISL_417643	ISL_Reykjavik	Age_22	Sex_M	DO_NA		EH_Aus	3_18_2020
Iceland33/4/2020	EPI_ISL_417663	ISL_Reykjavik	Age_68	Sex_F	DO_NA		EH_Aus	3_14_2020
Iceland33/5/2020	EPI_ISL_417664	ISL_Reykjavik	Age_50	Sex_F	DO_NA		EH_Aus	3_15_2020
Iceland38/2020	EPI_ISL_417672	ISL_Reykjavik	Age_38	Sex_M	DO_NA		EH_Aus	3_16_2020
ItalyIMM3/2020	EPI_ISL_417921	ITA_Rome	Age_32	Sex_M	DO_NA		EH_Aus	3_1_2020
ItalyTE495/2020	EPI_ISL_418259	ITA_Abruzzo	Age_76	Sex_M	DO_NA		EH_Aus	3_14_2020
JapanDP0236/2020	EPI_ISL_416585	JPN_Diamond_Princess	Age_NA	Sex_NA	DO_A		EH_Aus	2_16_2020
JapanDP0457/2020	EPI_ISL_416601	JPN_Diamond_Princess	Age_NA	Sex_NA	DO_A		EH_Aus	2_16_2020
JapanDP0588/2020	EPI_ISL_416610	JPN_Diamond_Princess	Age_NA	Sex_NA	DO_A		EH_Aus	2_17_2020
JapanDP0699/2020	EPI_ISL_416617	JPN_Diamond_Princess	Age_NA	Sex_NA	DO_A		EH_Aus	2_17_2020
JapanDP0743/2020	EPI_ISL_416621	JPN_Diamond_Princess	Age_NA	Sex_NA	DO_A		EH_Aus	2_17_2020
JapanDP0880/2020	EPI_ISL_416633	JPN_Diamond_Princess	Age_NA	Sex_NA	DO_A		EH_Aus	2_17_202

Supplementary Table 5. Amino acid changes within the Mexican samples compared to the reference strain Wuhan-Hu-1.

	Genomic region in WG concatenated ORF a1n ¹ , site/aa change compared to MN908947																																
	Orf1a (1-4405)								Orf1b (4406-7000)								S (7001-8282)								Orf3 (8505-8779)			Orf7a (8780-8900)		Orf8 (8901-9021)		N (9022-9440)	
	224	889	892	1649	2193	2244	3071	3606	4619	5190	5207	5696	5732	5769	6068	6103	7058	7582	7623	7739	8208	8268	8571	8700	8771	8817	8885	8984	9224	9225			
MN908947	T	L	P	A	S	I	F	L	P	E	N	S	P	Y	P	A	H	T	D	S	D	D	K	G	P	G	A	L	R	G			
7_Mexico/CDMX-INER_04	.	.	L	.	T	.	.	.	P	.	.	L	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.		
22_Mexico/Puebla-InDRE_05	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	V	.	.	.	.	.		
2_Mexico/CDMX-INER_01	.	.	.	.	X	.	.	.	L	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.		
32_Mexico/CDMX-INCMNSZ_05	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.		
5_Mexico/CDMX-INER_02	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.		
6_Mexico/CDMX-INER_03	.	X	X	L	X	X	.	.	L	.	D	.	.	.	X	G	.	.	G	.	E	.	.	.	A	X	X	.	.	X	X		
13_Mexico/Chihuahua-IMSS_01	.	F	.	.	X	F	X	.	L	.	.	.	.	.	A	.	.	.	G	C	.	.	E	.	.	X	.	.	K	K	R		
33_Mexico/CDMX-InDRE_01	.	.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	K	R	R	
16_Mexico/Chiapas-InDRE_02	.	.	.	.	X	X	.	.	L	.	.	.	.	.	.	.	.	X	X	.	.	.	X	.	X	X	.	X	.	K	R	R	
27_Mexico/CDMX-INCMNSZ_01	.	.	.	.	.	.	.	.	X	K	.	.	.	L	C	.	Y	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	
30_Mexico/CDMX-INCMNSZ_03	.	.	.	.	.	.	.	.	.	.	.	.	L	C	.	.	Y	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	
31_Mexico/CDMX-INCMNSZ_04	.	.	.	.	.	.	.	.	.	.	.	.	L	C	.	.	Y	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	
8_Mexico/CDMX-INER_05	.	.	.	.	.	.	.	.	.	.	.	.	L	C	.	.	Y	.	.	.	.	.	.	.	.	.	S	.	.	.	.	.	
24_Mexico/CDMX-InDRE_06	I	.	.	.	.	.	.	F	.	.	.	.	L	C	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	
28_Mexico/CDMX-INCMNSZ_02	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	V	.	.	.	S	.	.	.	.	
19_Mexico/Queretaro-InDRE_04	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	
17_Mexico/EdoMex-InDRE_03	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	S	.	.	.	.	
Frequency ²	T99	L99	P99	A99	S99	I99	F98 Y2	L83 F15	L58 P42	E99	N99	S99	P87 L12	Y86 C13	P99	A99	H99	T99	Y58 D41	S99	D99	D99	K99	G98 V2	P99	G99	A99	L82 S18	R83 K16	G83 R16			
Non-conservative amino-acid change ²	#	.	.	.	NS	.	§	WS NS	WS	.	.	†	WS	WS	.	.	.	.	WS	.	NS	§	§	.	‡	.	.	WS	WS	WS			
Changes observed ²	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Lineage defining mutations ²	.	.	.	.	.	.	.	.	A2a (P314L)	.	.	.	.	.	.	.	.	.	A2/G (D614G)	.	.	.	.	.	.	.	.	B/S (L84S)	.	.	.	.	
Evidence for positive selection ⁴	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	

¹ General lineage defining mutations are shown in blue and orange, as defined in <https://nextstrain.org/ncov/global> and in GISAID preliminary analysis summary update 2020-04-08 1500UTC. No changes observed in M CDS (8283-8504).

² Non-conservative amino-acid changes shown in red.

Cluster defining homoplasic mutation of the Mexican sequences are shown in black.

Widespread mutations are indicated under WS. Mutations also in Nexstrain are marked as NS. Unique changes to the Mexican sequences representing singletons are shown in gray.

* Shared with USA/WA_UW153/2020|EPI_ISL_416691|2020_03_13

* Shared with England/SHEF_C0707/2020|EPI_ISL_420270|2020_03_22

§ Shared with 45 sequences, mostly from Spain.

† Shared with 5 sequences from Australia, Portugal, Spain and USA.

‡ Shared with Portugal/PT0024/2020|EPI_ISL_418009|2020_03_15

* USA/WA_UW304/2020|EPI_ISL_418872|2020_03_23

³ Frequency of a given aa in percentage observed in the a1n. Only changes present in > 1% of the sequences in the a1n are shown.

⁴ Sites with a dN/dS > 1 are shown in green, as compared to the list of sites determined under diverse dN/dS models described in <http://covid19.datamoney.org/2020/04/01/covid19-analysis/>