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1   **Genomic surveillance and phylogenetic analysis reveal multiple introductions of**

2   **SARS-CoV-2 into a global travel hub in the Middle East**

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Abstract

International travel played a significant role in the early global spread of SARS-CoV-2. Understanding transmission patterns from different regions of the world will further inform global dynamics of the pandemic. Using data from Dubai in the United Arab Emirates (UAE), a major international travel hub in the Middle East, we establish SARS-CoV-2 full genome sequences from the index and early COVID-19 patients in the UAE. The genome sequences are analyzed in the context of virus introductions, chain of transmissions, and possible links to earlier strains from other regions of the world. Phylogenetic analysis showed multiple spatiotemporal introductions of SARS-CoV-2 into the UAE from Asia, Europe, and the Middle East during the early phase of the pandemic. We also provide evidence for early community-based transmission and catalogue new mutations in SARS-CoV-2 strains in the UAE. Our findings contribute to the understanding of the global transmission network of SARS-CoV-2.

Keywords: SARS-CoV-2; COVID-19; Dubai; United Arab Emirates; whole genome sequencing; molecular phylogeny; epidemiology

Introduction

In December 2019, several cases of a new respiratory illness (now called COVID-19) were reported in the city of Wuhan (Hubei province, China) and in January 2020 it was confirmed these infections were caused by a novel coronavirus subsequently named SARS-CoV-2 [1-2]. On 12 March 2020, the ongoing SARS-CoV-2 outbreak was declared a pandemic by the World Health Organization (WHO) [3]. As of 3 June 2020, there have been 6.4 million laboratory-confirmed cases of COVID-19 and more than 380,000 deaths in 188 countries [4].

Dubai in the United Arab Emirates (UAE) is a cosmopolitan metropolis that has become a popular tourist destination and home to one of the busiest airport hubs in the world connecting the east with the west [4]. Currently, the UAE has reported 35,788 confirmed cases and 269 COVID-19-associated deaths (0.8% case fatality; 3 June 2020)

59   [5]. In view of Dubai's important tourism and travel connections, we attempted to

60   characterize the full-genome sequence of SARS-CoV-2 strains from the index and early

61   patients with COVID-19 in Dubai to gain a deeper understanding of the molecular

62   epidemiology of the outbreak in Asia, Europe, and the Middle East.

63

64   **Methods**

65   **Human Subjects and Ethics Approval**

66   Sociodemographic and clinical data was extracted from the electronic medical records

67   of the earliest 49 patients with laboratory confirmed SARS-CoV-2 from 29 January to

68   18 March 2020 using the WHO case report form. This study was approved by the Dubai

69   Scientific Research Ethics Committee - Dubai Health Authority (approval number

70   #DSREC-04/2020_02).

71   **SARS-CoV-2 Whole Genome Sequencing**

72   All 49 COVID-19 patients tested positive for SARS-CoV-2 by RT-qPCR using RNA

73   extracted from nasopharyngeal swabs following the QIAamp Viral RNA Mini or the

74   EZ1 DSP Virus Kits (Qiagen, Hilden, Germany). RNA libraries from all samples were

75   then prepared for shotgun transcriptomic sequencing using the TruSeq Stranded Total

76   RNA Library kit from Illumina (San Diego, CA, USA), following manufacturer's

77   instructions. Libraries were sequenced using the NovaSeq SP Reagent kit (2 X 150

78   cycles) from Illumina (San Diego, CA, USA). Sample L5630 underwent a target

79   enrichment approach where double stranded DNA (synthesized using the QuantiTect

80   Reverse Transcription Kit from Qiagen, Hilden, Germany) was amplified using 26

81   overlapping primer sets covering most of the SARS-CoV-2 genome [6]. PCR products

82   were then sheared by ultra-sonication (Covaris LE220-plus series, MA, USA) and

83   prepared for sequencing using the SureSelectXT Library Preparation kit (Agilent, CA,

84   USA). This library was sequenced using the Illumina MiSeq Micro Reagent Kit, V2 (2

85   X 150 cycles).

86   **SARS-CoV-2 Genome Assembly**

87   High quality (>Q30) sequencing reads were trimmed and then aligned to the reference

88   SARS-CoV-2 genome from Wuhan, China (GenBank accession number:

89   NC_045512.2) using a custom-made bioinformatics pipeline (Fig. S1). Assembled

90   genomes with at least 20X average coverage across most nucleotide positions (56-

91   29,797) were used for subsequent phylogenetic analysis (Table S1). A total of 25 viral

92   genomes (24 by shotgun and 1 by target enrichment) met this inclusion criterion and

93   were submitted to the Global Initiative on Sharing All Influenza Data (GISAID)

94   database under accession IDs: EPI_ISL_435119-435142 (Table S2).

95   **Phylogenetic Analysis**

96   We downloaded 157 global non-UAE sequences (Table S2) with largely complete

97   genomes (nucleotide positions 56-29,797) submitted to GISAID EpiCoV

98   (https://www.epicov.org/) between December 2019 and 04 March 2020 [7]. All 182

99   sequences, including the 25 UAE sequences generated in this study, were analyzed

100   using Nexstrain [8], which consists of Augur v6.4.3 pipeline for multiple sequence

101   alignment (MAFFT v7.455 [9]) and phylogenetic tree construction (IQtree v1.6.12

102   [10]). Tree topology was assessed using the fast bootstrapping function with 1000

103   replicates. Tree visualization and annotations were performed in FigTree v1.4.4 [11] for

104   Figure 1 and in auspice v2.13.0 tool [8] for Figure 2. SARS-CoV-2 clades annotations

105   were performed in auspice v2.13.0 and cross-checked with nextstrain.org as of 12 May

106   2020.

5

107

Results

Patient Cohort and SARS-CoV-2 Whole Genome Sequencing

110 The 49 patients included in this study were the earliest confirmed cases in the UAE. The

111 time period of 29 January to 18 March 2020 was specifically selected to focus on early

112 SARS-CoV-2 viral introductions into the UAE. The first index patient in the UAE was

113 reported on the 29 January 2020. Subsequently, Emirates airlines suspended flights to

114 and from 30 global destinations from 18 March 2020 and Dubai airport was closed to

115 passenger flights on 25 March 2020; hence, patients after 18 March 2020 were expected

116 to be more likely a result of community transmission as opposed to imported infections.

117 The index patient in the UAE was a female Chinese tourist (aged 63 years) travelling

118 from Wuhan with other family members to visit her son in Dubai. The Chinese family

119 arrived in Dubai on 16 January 2020 and tested positive on the 29 January 2020 (Table

120 1). Over the next seven weeks, there were multiple new cases among tourists and

121 residents with travel history (44.9% had travel history from Europe) (Table 1). Nearly

122 two-thirds (63.3%) of patients were male and 61.2% were aged between 20 and 44

123 years reflecting the young age structure of the UAE population [4]. Majority of patients

124 (88%) were asymptomatic or had mild symptoms and only four required intensive care

125 with invasive ventilation.

126 SARS-CoV-2 whole genome sequencing was performed on all 49 COVID-19

127 patient samples. Only genomes with almost complete coverage (n=25, Methods) were

128 used for phylogenetic analysis. The 25 genomes were obtained from cases with disease

129 onset in late January (n=1), early February (n=1), late February (n=6), early March

130 (n=8), and late March (n=9). Of those, approximately two-thirds were male and aged

131   between 10 and 40 years (Table 1).

Phylogenetic Analysis

133   To understand early viral transmission in Dubai in the global context, we performed

134   phylogenetic analysis on the 25 novel viral genomes we sequenced from early patients

135   in the UAE (Table 1) in this study (Methods) along with 157 largely complete SARS-

136   CoV-2 genomes deposited in GISAID from different countries between December 2019

137   and early March 2020 [7] (Table S1).

138   Consistent with multiple independent introductions, the UAE SARS-CoV-2

139   isolates were distributed across the phylogenetic tree (Figure 1). The majority (76%)

140   clustered with clades A2a (48%) and A3 (28%) which are largely composed of isolates

141   from COVID-19 patients in Europe and Iran, respectively. This clearly suggests that the

142   major introductions into the UAE during the early phase of the pandemic originated

143   from Europe and the Middle East/Iran.

144   Supporting its European origin, all individuals with the A2a clade isolates were

145   mostly European and/or with recent travel history to a European country, mainly to Italy

146   (n=4), Germany (n=3), United Kingdom (n=2), Spain (n=1), and Norway (n=1) (Table

147   1 and Figure 2). Onset of symptoms reported in this group was within or after the

148   second week of March (Table 1) suggesting that the viral infections in this group could

149   have occurred during late February to early March. Of note, a SARS-CoV-2 isolate

150   submitted from Mexico (GISAID ID: EPI_ISL_412972) was 100% identical to that

151   from an Italian expatriate working in the UAE (L0881), while another submitted in

152   Germany (GISAID ID: EPI_ISL_412912) differed by a single mutation (Figure 1). All

153   three individuals had a recent travel history to Italy and overlapping infection time

154   frames (late February – early March). Within this group, isolates from patients L1758,

155   L0484, and L2185 were identical (Figure 2) suggesting a possible common direct

156   source of transmission.

157   Isolates in the A3 clade were obtained from five individuals with travel history

158   to Iran (L2409, L6627, L0904, L0184, and L4682), one Indian resident (L0231), and

159   one Indian tourist (L0068) (Figure 2). Onset of symptoms for the five individuals with

160   travel history in this group was reported to be around 21-24 February (Table 1). Patient

161   L0231 had no travel history and reported symptom onset on 7 March suggesting a

162   possible community-based transmission event. Interestingly, all but one isolate obtained

163   from patient L4682 – the only patient in this group with severe clinical presentation –

164   shared a common ancestral strain identical to that obtained from patient L2409. The

165   SARS-CoV-2 isolate from L4682 had two unique missense variants in the ORF1ab

166   gene (Table S3) which might be worth investigating for any possible biological

167   effect(s). Consistent with its Iranian origin, a SARS-CoV-2 sequence submitted by the

168   University of Sydney (GISAID ID: EPI_ISL_412975) on 28 February 2020 differed by

169   only two mutations from that of L2409, and both this Iranian male tourist and the

170   Australian male had a recent travel history to Iran. We speculate that individuals with

171   travel history to Iran around this time frame (L8386, L6867, and L3280), for whom a

172   full viral genome sequence could not be obtained, were also very likely to cluster within

173   the A3 clade.

174   Only one viral strain obtained from L5630, a family member of the early

175   Chinese index patient, belonged to the B2 clade. Although we did not obtain full viral

176   genome sequences from the other members of that Chinese family, we expect that all

177   had a similar strain to L5630. Interestingly, our data do not suggest any transmission of

178   this clade at least among the earliest patients (Figure 2) included in this study which is

179 consistent with the reported early detection and isolation of this family. This finding

180 also supports the notion of secondary source(s) for the ongoing local transmission.

181 The remaining five isolates did not belong to A2a, A3, B2, or any of the clades

182 on nextrain.org as of 12 May 2020, suggesting earlier introduction(s). Those isolates

183 were obtained from four Asians, two residents (L4280, L6599) and two tourists (L4184,

184 L9766), and one Czech resident (L1014) working as an airline cabin crew with travel

185 history to Austria (Table 1). Consistent with the Asian predominance among this patient

186 group and the fewer (1 or 2) mutations for most of their isolates (4 out of 5) relative to

187 the Wuhan reference genome (Figure 2), several early viral strains submitted in Asia

188 clustered very closely to this group (Figure 1). L4280 was the first sequenced patient

189 without travel history and became infected after transporting a work colleague, L0826,

190 to hospital. Patient L0826 reported symptoms onset on 22 January suggesting that

191 community-based transmission started in the UAE in early-to-mid January. L6599 was

192 an Indian expatriate living with three other Filipino and Sri Lankan expatriates (L3715,

193 L2771, L8480) (Table 1). All four individuals had no documented recent travel history

194 suggesting local transmission, and although full viral genome sequences could only be

195 obtained from one patient L6599, it is very likely that all have related isolates.

196 In aggregate, we identified 70 variants relative to the reference GenBank SARS-

197 CoV-2 sequence NC_045512.2. The majority of these variants were missense (n=41)

198 with the most frequent nucleotide change being C>T (n=33), and more than half (38/70)

199 were localized in the ORF1ab gene (Table S3). Notably, 17 out of the 70 variants were

200 novel as they were not identified in the Chinese National Center for Bioinformation

201 Database (https://bigd.big.ac.cn/ncov/variation/annotation; last accessed April 29,

202 2020). Of those, 12 were coding missense variants distributed across the ORF1ab (n=8),

203 S (n=1), ORF7a (n=1), and the N (n=2) genes.

204

205 **Discussion**

206 Our findings suggest multiple independent spatiotemporal introductions of SARS-CoV-

207 2 into the UAE where the majority of introductions (76%) were from Iran and Europe

208 during two different time frames (mid-late February and early March, respectively).

209 Although we show evidence for possible local transmission within the Middle

210 Eastern/Iranian isolates, it will be important to sequence further isolates at subsequent

211 dates to determine whether these introductions succeeded in seeding more clustering

212 and whether such clustering was affected by proactive and vigilant public health

213 measures, such as transitioning to online learning for schools and universities,

214 implementing work-from-home protocols across all sectors, and nationwide disinfection

215 campaigns.

216   Six isolates (22%) did not cluster with the European or Iranian groups and

217 represented earlier introductions which did not appear to seed larger clusters in our

218 sampled cohort. However, additional sequencing is needed to determine the extent of

219 community transmission, especially given that our data strongly suggest that the earliest

220 patient (early to mid-January) in the UAE could have been a secondary infection from

221 one of those introductions.

222   The new SARS-CoV-2 mutations identified in the UAE warrant further

223 investigation to explore whether they influence viral characteristics, especially

224 pathogenicity, or provide important information for vaccine development. One of the

225 major strengths of the study was the non-biased representative sample of early cases,

226 including the index family cluster, in Dubai from the only central testing lab, along with

227 detailed demographic and clinical information. Limitations included the inability to

228 conduct full whole genome sequencing on more samples most likely due to low viral

229　load issues, although we were able to deduce the origin of transmission in most of those

230　individuals based on travel history. Regardless, this study contributes important

231　molecular epidemiological data that can be used to further understand the global

232　transmission network of SARS-CoV-2.

233

About the Author

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235　Dr. Abou Tayoun is an associate professor in the Department of Genetics, Mohammed

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238　are focused around clinical and population genomics in general, and specifically in the

239　Middle East.

240

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278

279 **Figure Legends**

280 **Figure 1.** Phylogenetic relationships of SARS-CoV-2 isolates from early patients in

281 Dubai and early global strains. A maximum likelihood phylogeny of 182 SARS-CoV-2

282 genomes (157 obtained from GISAID as of early Match and 25 genomes in this study).

283 Bootstrap support values >70% supporting major branches are shown. The 3 non-UAE

284 isolates in clades A2a and A3 namely, Mexico/CDMX-InDRE_01,

285 Germany/Baden/Wuerttemberg-1, and Australia/NSW05 are the GISAID ID:

286 EPI_ISL_412972, GISAID ID: EPI_ISL_412912, and GISAID ID: EPI_ISL_412975,

287 respectively, referred to in the main text. Scale bar represents number of nucleotide

288 substitutions per site. UAE = United Arab Emirates.

289

290 **Figure 2.** Relationship of early SARS-CoV-2 isolates in the UAE based on

291 phylogenetic analysis and patients' travel history. A maximum likelihood phylogenetic

292 tree of all 25 UAE SARS-CoV-2 sequences, generated in this study, is shown. The two

293 Wuhan genomes (Wuhan-Hu-1/2019, GISAID ID: EPI_ISL_402125 and

294 Wuhan/WH01/2019, GISAID ID: EPI_ISL_406798) were used as reference genomes

295 (blue filled circles). UAE viral strains (black filled circles) were labeled with sample ID

296 and Nationality (in brackets). Bootstrap support values >70% supporting major

297 branches are shown. Branch lengths mark divergence from the reference Wuhan SARS-

298 CoV-2 genome (GenBank accession number: NC_045512.2) in mutations numbers,

299 while branch color represents travel history. Travel history to Europe for patients

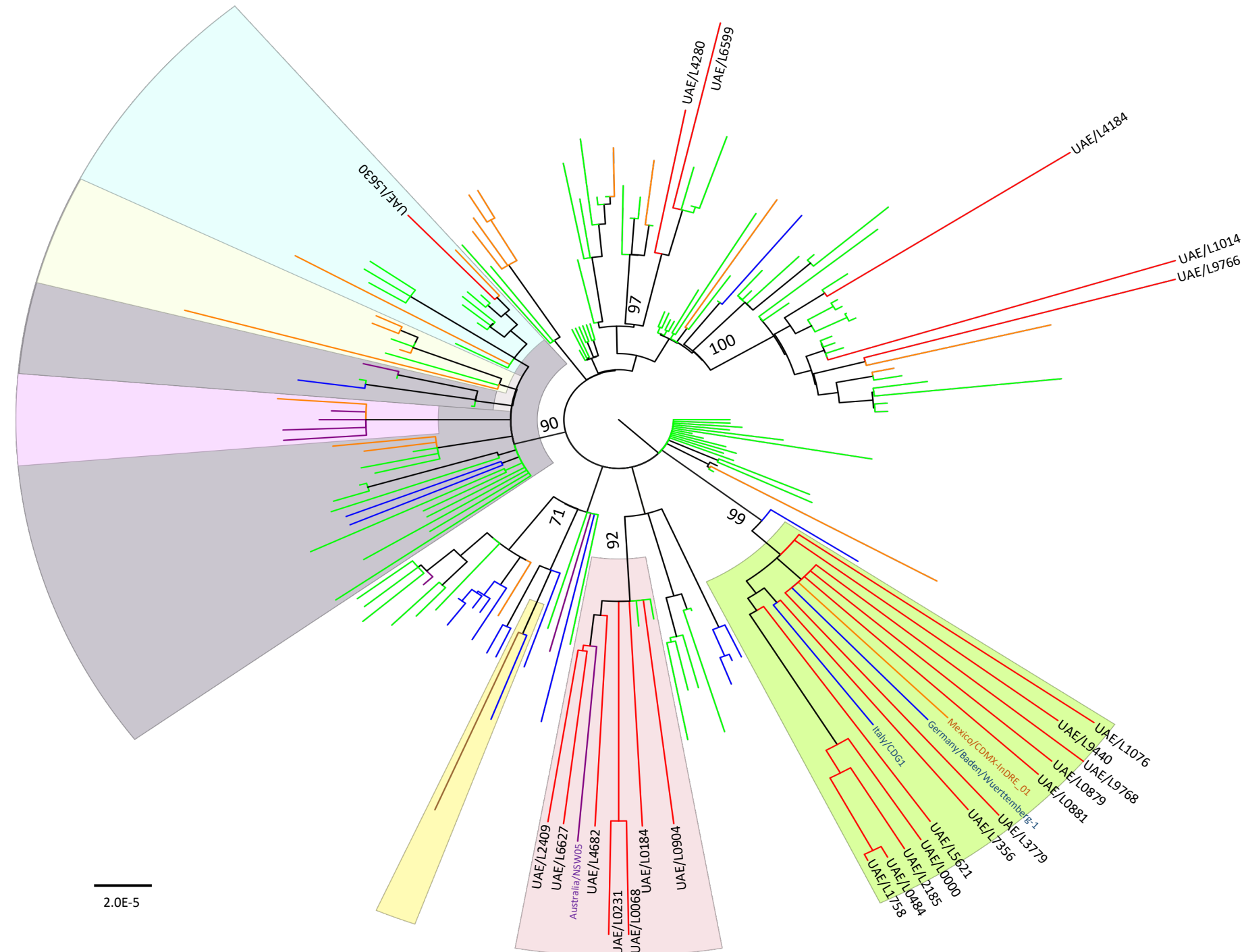300 UAE/L1758, UAE/L0484, UAE/L2185, and UAE/L0881 is obscured by vertical lines.

301 UAE = United Arab Emirates.

302

303 **Author Contributions**
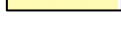
304 AAA, AT, NN, and TL conceived the study and drafted the protocol. All authors

305 provided critical input into the protocol. AAA, AAK, and HK coordinated the ethical

306 approval and sample retrieval. RV and ZD conducted the RT-qPCR analysis. AT, SR,

307 and DH performed the whole genome sequencing and phylogenetic analysis. HK
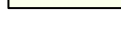
308 performed data extraction from the medical records and TL completed data analysis for

309 the manuscript. AT and TL drafted the manuscript, AAA and NN refined it before

310 AAK, AS, DH HA, HK, MU, QH, RH, RHA, RV, SR, and ZD, provided comments and

311 feedback on the first draft. All authors read and approved the final version of the

312 manuscript.

313

Financial Support and Conflict of Interest Disclosure

Table 1. Sociodemographic and clinical characteristics of the index and early patients (n=49) with laboratory-confirmed SARS-CoV2 in Dubai, United Arab Emirates, 29 January-18 March 2020

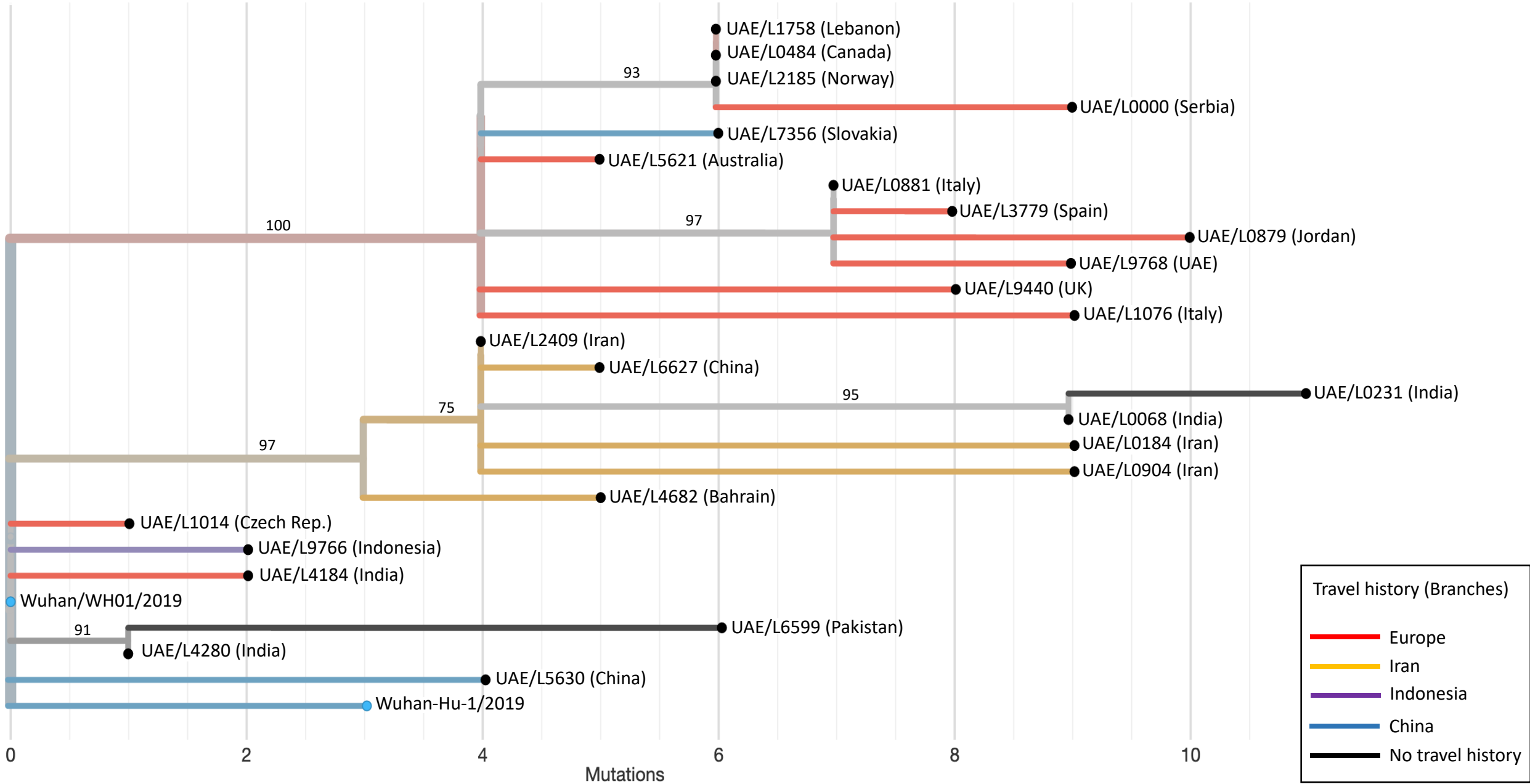| Study ID	Age (years)	Sex	Nationality	Resident /Tourist	Travel History	Symptom Onset Date 2020‡	Severity	Self-Reported History Fever/Cough	ICU Admission/ Ventilator	Full Genome Sequence	GISAID Accession ID
L8156	63	Female	China	Tourist	Wuhan, China	09 Jan	Asymp/Mild	No/Yes	No/No	No	-
L8497	38	Female	China	Tourist	Wuhan, China	24 Jan	Asymp/Mild	Yes/No	No/No	No	-
L5630*	9	Male	China	Tourist	Wuhan, China	28 Jan	Asymp/Mild	No/No	No/No	Yes	EPI_ISL_435137
L8205	36	Male	China	Resident	Wuhan, China	28 Jan	Asymp/Mild	No/No	No/No	No	-
L0826	42	Male	Philippines	Resident	None/Contact with positive case	22 Jan	Severe/Critical	Yes/Yes	Yes/Yes	No	-
L4280	36	Male	India	Resident	None/Contact with positive case	08 Feb	Asymp/Mild	No/No	No/No	Yes	EPI_ISL_435134
L3715	34	Male	Philippines	Resident	None/Contact with positive case	16 Feb	Asymp/Mild	No/No	No/No	No	-
L2771	25	Male	Sri Lanka	Resident	None/Contact with positive case	16 Feb	Asymp/Mild	No/No	No/No	No	-
L8480	21	Male	Sri Lanka	Resident	None/Contact with positive case	16 Feb	Asymp/Mild	No/No	No/No	No	-
L8386	70	Male	Iran	Tourist	Iran	17 Feb	Severe/Critical	No/Yes	Yes/Yes	No	-
L6599	41	Male	Pakistan	Resident	None/Contact with positive case	21 Feb	Asymp/Mild	No/No	No/No	Yes	EPI_ISL_435138
L0904	35	Male	Iran	Tourist	Iran	21 Feb	Asymp/Mild	Yes/Yes	No/No	Yes	EPI_ISL_435126
L6867	60	Female	Iran	Tourist	Iran	22 Feb	Asymp/Mild	No/Yes	No/No	No	-
L2409	59	Male	Iran	Tourist	Iran	23 Feb	Asymp/Mild	Yes/No	No/No	Yes	EPI_ISL_435131

L0184	64	Female	Iran	Tourist	Iran	23 Feb	Asymp/Mild	Yes/Yes	No/No	Yes	EPI_ISL_435121
L4682	64	Male	Bahrain	Tourist	Iran	24 Feb	Severe/Critical	Yes/No	Yes/Yes	Yes	EPI_ISL_435135
L6627	29	Male	China	Resident	Iran	24 Feb	Asymp/Mild	Yes/No	No/No	Yes	EPI_ISL_435139
L1802	38	Male	Dominica	Resident	Iran and Pakistan	28 Feb	Moderate	Yes/Yes	No/No	No	-
L7100	44	Female	Iran	Resident	Iran	29 Feb	Asymp/Mild	Yes/No	No/No	No	-
L5752	38	Female	Italy	Tourist	Italy	04 Mar	Asymp/Mild	No/No	No/No	No	-
L8477	36	Female	India	Resident	US/contact with positive case	05 Mar	Asymp/Mild	Yes/No	No/No	No	-
L5970	70	Female	Italy	Tourist	Milan, Italy	05 Mar	Severe/Critical	Yes/No	Yes/Yes	No	-
L3280	3	Male	Saudi Arabia	Tourist	Iran	07 Mar	Asymp/Mild	No/No	No/No	No	-
L7356	23	Male	Slovakia	Resident	China and Asia	07 Mar	Asymp/Mild	No/No	No/No	Yes	EPI_ISL_435140
L0509	36	Male	Kuwait	Resident	Austria and Germany	07 Mar	Asymp/Mild	Yes/No	No/No	No	-
L0231	54	Male	India	Resident	None	07 Mar	Moderate	Yes/Yes	No/No	Yes	EPI_ISL_435122
L9766	28	Female	Indonesia	Tourist	Indonesia	07 Mar	Asymp/Mild	Yes/Yes	No/No	Yes	EPI_ISL_435142
L4184	28	Female	India	Tourist	Ireland and France	08 Mar	Asymp/Mild	Yes/No	No/No	Yes	EPI_ISL_435133
L0484	28	Male	Canada	Resident	Germany	09 Mar	Asymp/Mild	Yes/Yes	No/No	Yes	EPI_ISL_435123
L1758	30	Male	Lebanon	Resident	Germany	10 Mar	Asymp/Mild	No/No	No/No	Yes	EPI_ISL_435129
L5621	58	Male	Australia	Tourist	Italy	10 Mar	Asymp/Mild	Yes/No	No/No	Yes	EPI_ISL_435136
L9440	35	Male	UK	Resident	Italy	10 Mar	Asymp/Mild	No/No	No/No	Yes	EPI_ISL_435141
L0860	46	Male	France	Resident	US through France	10 Mar	Asymp/Mild	No/Yes	No/No	No	-
L2185	46	Male	Norway	Tourist	Norway	12 Mar	Asymp/Mild	No/No	No/No	Yes	EPI_ISL_435130
L9928	42	Female	Spain	Resident	Spain	13 Mar	Asymp/Mild	Yes/No	No/No	No	-
L0068	33	Male	India	Tourist	India	13 Mar	Asymp/Mild	Yes/No	No/No	Yes	EPI_ISL_435120

L1014	27	Female	Czech Rep.	Resident	Austria	13 Mar	Asymp/Mild	Yes/Yes	No/No	Yes	EPI_ISL_435127
L1076	30	Female	Italy	Resident	Italy	14 Mar	Asymp/Mild	No/No	No/No	Yes	EPI_ISL_435128
L0000	27	Female	Serbia	Resident	UK/contact with positive case	14 Mar	Asymp/Mild	No/No	No/No	Yes	EPI_ISL_435119
L3779	32	Female	Spain	Resident	Spain	14 Mar	Asymp/Mild	Yes/Yes	No/No	Yes	EPI_ISL_435132
L7355	20	Male	UAE	Resident	UK	14 Mar	Asymp/Mild	No/Yes	No/No	No	-
L0549	24	Female	Lebanon	Resident	None/contact with positive case	15 Mar	Asymp/Mild	No/No	No/No	No	-
L9768	19	Male	UAE	Resident	UK	18 Mar	Asymp/Mild	No/Yes	No/No	Yes	EPI_ISL_435143
L4519	54	Male	Iran	Resident	Iran	NR	NR	NR	NR	No	-
L9333	26	Male	Italy	Resident	Italy	NR	NR	NR	NR	No	-
L9756	53	Male	Italy	Resident	Italy	NR	NR	NR	NR	No	-
L0879	NR	Female	Jordan	Resident	Germany	NR	NR	NR	NR	Yes	EPI_ISL_435124
L0880	NR	Female	Germany	Resident	Germany	NR	NR	NR	NR	No	-
L0881	NR	Male	Italy	Resident	Italy	NR	NR	NR	NR	Yes	EPI_ISL_435125

*There was a second grandchild in the Chinese family cluster from Wuhan who remained negative throughout all testing. ‡Self-reported onset of symptoms extracted from medical records; NR, Not Reported; UK, United Kingdom; UAE, United Arab Emirates; US, United States.