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1 **An 81 base-pair deletion in SARS-CoV-2 ORF7a identified from sentinel surveillance in**
2 **Arizona (Jan-Mar 2020)**

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23 On January 26 2020, the first Coronavirus Disease 2019 (COVID-19) case was reported in
24 Arizona of an individual with travel history (3rd case in the US) (1). Here, we report on early
25 SARS-CoV-2 sentinel surveillance in Tempe, Arizona (USA). Genomic characterization
26 identified an isolate encoding a 27 amino acid in-frame deletion in accessory protein ORF7a,
27 the ortholog of SARS-CoV immune antagonist ORF7a/X4.

28 In anticipation of COVID-19 spreading in the state of Arizona, we initiated a surveillance effort
29 for local emergence of SARS-CoV-2 starting January 24, 2020. We leveraged an ongoing
30 influenza surveillance project at Arizona State University (ASU) Health Services in Tempe,
31 Arizona. Individuals presenting with respiratory symptoms (ILI) were tested for influenza A and
32 B virus (Alere BinaxNOW). Subsequently, we tested influenza-negative nasopharyngeal (NP)
33 swabs for SARS-CoV-2. We extracted total nucleic acid using the bioMérieux eMAG automated
34 platform and performed real-time RT-PCR (qRT-PCR) assays specific for SARS-CoV-2 N and E
35 genes (2, 3). Out of 382 NP swabs collected from January 24, 2020 to March 25, 2020, we
36 detected SARS-CoV-2 in 5 swabs in the week of March 16 to 19 (**Figure 1**). This corresponds
37 to prevalence of 1.31%. Given the estimated 1 – 14-day incubation period for COVID-19, it is
38 possible that the spike in cases might be related to university spring-break holiday travel (March
39 8 – 15) as previously seen in other outbreaks (4, 5).

40 To understand the evolutionary relationships and characterize the SARS-CoV-2 genomes, we
41 performed next-generation sequencing (Illumina NextSeq, 2x76) directly on specimen RNA,
42 thereby avoiding cell culture passage and potentially associated mutations. This generated an
43 NGS dataset of 20.7 to 22.7 million paired-end reads per sample. We mapped quality-filtered
44 reads to a reference SARS-CoV-2 genome (MN908947) using BBMap (version 39.64) to
45 generate three full-length genomes: AZ-ASU2922 (376x coverage), AZ-ASU2923 (50x) and AZ-
46 ASU2936 (879x) (Geneious prime version 2020.0.5). We aligned a total of 222 SARS-CoV-2
47 genome sequences comprising at least 5 representative sequences from phylogenetic lineages

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48 defined by Rambaut *et al.* (6), ranging from January 5 to March 31, 2020 from 25 different
49 countries. We performed phylogenetic reconstruction with BEAST (version 1.10.4, strict
50 molecular clock, HKY + Γ nucleotide substitution, exponential growth for coalescent model) (7-
51 10). The ASU sequences were phylogenetically distinct indicating that they were independent
52 transmissions (**Figure 2A**).

53 Similar to SARS-CoV, the SARS-CoV-2 genome encodes multiple open reading frames in the 3'
54 region. We found that the SARS-CoV-2 AZ-ASU2923 genome has an 81 base-pair deletion in
55 the ORF7a gene resulting in a 27 amino-acid in-frame deletion (**Figure 2B**). The SARS-CoV
56 ORF7a ortholog is a viral antagonist of host restriction factor BST-2/Tetherin and induces
57 apoptosis (11-14). Based on the SARS-CoV ORF7a structure (15), the 27-aa deletion in SARS-
58 CoV-2 ORF7a maps to the putative signal peptide (partial) and first two beta strands. To
59 validate the deletion, we performed RT-PCR using primers spanning the region and verified by
60 Sanger sequencing the amplicons (**Figure 2C and Supplementary Figure 1**).

61 Collectively, although global NGS efforts indicate that SARS-CoV-2 genomes are relatively
62 stable, dynamic mutations can be selected in symptomatic individuals.

63

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69 Database. A complete acknowledgements table is available at
70 https://www.dropbox.com/s/aiybuatgxjunuga/GISAID_CoV2020_Acknowledgements.xlsx?dl=0 .
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74

75 **Data availability**

76 Sequence data has been deposited to NCBI GenBank and GISAID: SARS-CoV-2 AZ-ASU2922
77 (MT339039, EPI_ISL_424668), SARS-CoV-2 AZ-ASU2923 (MT339040, EPI_ISL_424669) and
78 SARS-CoV-2 AZ-ASU2936 (MT339041, EPI_ISL_424671).

79

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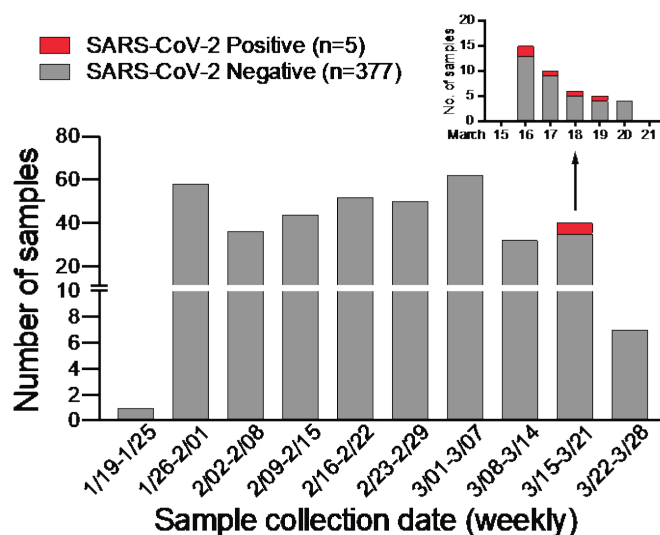
130

131

132 **Figure legends**



133

134 **Figure 1: SARS-CoV-2 surveillance in Tempe, AZ from January to March 2020.**
135 Weekly distribution of NP specimens collected by ASU Health Services tested for SARS-CoV-2
136 by qRT-PCR assays. Inset shows SARS-CoV-2 positive NP specimens collected from the week
137 of March 15 – 21, 2020.

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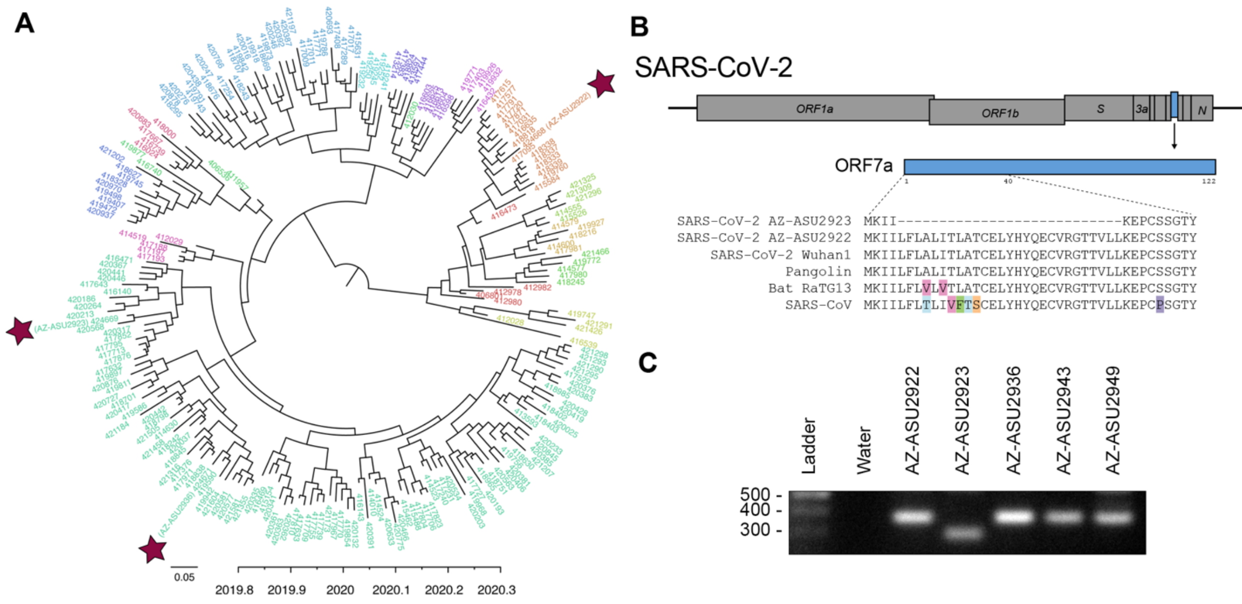

138

139 **Figure 2: Evolutionary and genomic characterization of SARS-CoV-2 genomes.**

140 (**A**) Bayesian maximum clade credibility (MCC) polar phylogeny of 222 full-length SARS-CoV-2
141 genomes. The 3 new genomes reported in this study are indicated by red stars. Sequenced
142 were aligned in Geneious prime (version 2020.0.3) using the MAFFT v7.450 plugin, and
143 trimmed the 5' and 3' UTR (< 300 nts each). We initiated two independent runs of 500M
144 sampling every 50K steps and used Tracer v1.7.1 (16) to check for convergence and that all
145 ESS values for our statistics were > 200, LogCombiner (7) to combine the models with a 10%
146 burn-in and TreeAnnotator (7) to produce a MCC tree. We used FigTree v1.4.4 (17) to edit the
147 tree and color the tips based on lineages (6), and pangolin (18) to identify the lineages of our 3
148 new sequences based on the established nomenclature (6). The nomenclature consists of two
149 main lineages, A and B, and includes "sub-lineages" (A.1, B.2. *etc.*) up to four levels deep (e.g.
150 A.1.1, B.2.1) (6). For visualization purposes, we grouped all viruses that were not directly
151 assigned to "A" or "B" into their first sub-lineage level and colored tip labels by lineage. B.1
152 lineage: AZ-ASU2923 and AZ-ASU2936; A.1 lineage AZ-ASU2922. (**B**) ORF7a amino acid
153 alignment of SARS-CoV-2 and related genomes. GenBank and GISAID accession numbers:
154 SARS-CoV-2 AZ-ASU2922 (MT339039, EPI_ISL_424668), SARS-CoV-2 AZ-ASU2923

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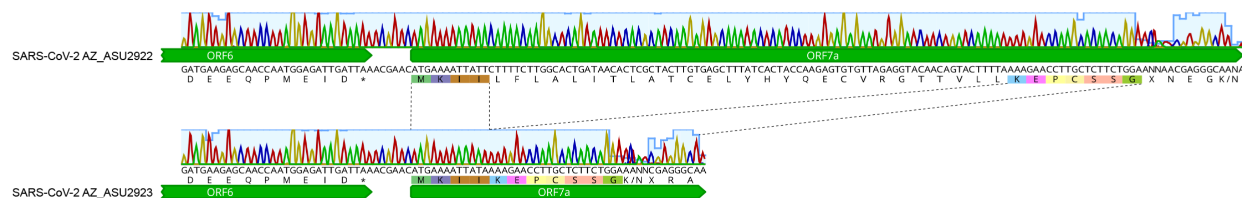
155 (MT339040, EPI_ISL_424669), SARS-CoV-2 AZ-ASU2936 (MT339041, EPI_ISL_424671),
156 SARS-CoV-2 Wuhan1 (MN908947.3), Pangolin (EPI_ISL_410721), Bat-RaTG13
157 (MN996532.1), SARS-CoV (AY278741.1). The 81-bp (27 amino acid) deletion observed in
158 SARS-CoV-2 AZ_ASU2923 ORF7a was not present in the 6,290 SARS-CoV-2 sequences
159 available from GISAID as of April 12, 2020. (**C**) We performed molecular validation by RT-PCR
160 on specimen total nucleic acid extracts with primers flanking the ORF7a N-terminus region. The
161 expected size of amplicons with intact ORF7a region is 377bp, the expected size of an amplicon
162 with the NGS-identified 81bp deletion is 296bp. Primers: SARS2-27144F 5'-
163 ACAGACCATTCCAGTAGCAGTG-3', SARS2-27520r 5'-TGCCCTCGTATGTTCCAGAAG-3'.

164

165 

166 **Supplementary Figure 1.** Sanger sequencing chromatograms verification of 81-bp deletion in
167 SARS-CoV-2 AZ-ASU2923 in the ORF7a region.