

Surveillance of SARS-CoV-2 in the environment and animal samples of the Huanan Seafood Market

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2 **Huanan Seafood Market**

3
4 **Running title:**

5 Prevalence of SARS-CoV-2 in Huanan Seafood Market

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40

41 **Abstract**

42 Emerging in December 2019, coronavirus disease 2019 (COVID-19) eventually
43 became a pandemic and has posed a tremendous threat to global public health.
44 However, the origins of SARS-CoV-2, the causative agent of COVID-19, remain to
45 be determined. It has reported that a certain number of the early case clusters had a
46 contact history with Huanan Seafood Market. Therefore, surveillance of
47 SARS-CoV-2 within the market is of vital importance. Herein, we presented the
48 SARS-CoV-2 detection results of 1380 samples collected from the environment and
49 the animals within the market in early 2020. By SARS-CoV-2-specific RT-qPCR, 73
50 environmental samples tested positive for SARS-CoV-2 and three live viruses were
51 successfully isolated. The viruses from the market shared nucleotide identity of
52 99.980% to 99.993% with the human isolate HCoV/Wuhan/IVDC-HB-01. In contrast,
53 no virus was detected in the animal swabs covering 18 species of animals in the
54 market. The SARS-COV-2 nucleic acids in the positive environmental samples
55 showed significant correlation of abundance of *Homo sapiens* with SARS-CoV-2. In
56 summary, this study provided convincing evidence of the prevalence of SARS-CoV-2
57 in the Huanan Seafood Market during the early stage of COVID-19 outbreak.

58

59

60 **Keywords:**

61 COVID-19, SARS-CoV-2, Huanan Seafood Market, zoonotic origin, cold chain,
62 high-throughput sequencing, virus isolation

63

64 Infections with novel human coronavirus 2019 (HCoV-19) (1, 2), named as severe
65 acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by International Committee
66 on Taxonomy of Viruses (ICTV) (3), can result in coronavirus disease 2019
67 (COVID-19), characterized by various clinical outcomes from asymptomatic
68 infections to severe pneumonia and even death (4-6). Globally, as of September 10th
69 2021, there have been 223,022,538 confirmed cases of COVID-19, including
70 4,602,882 deaths. No sign hints the pandemic has declined. (covid19.who.int).

71

72 Early human cases with COVID-19 were first reported in late December 2019 from
73 Wuhan, China with pneumonia of unknown etiology (PUE), and a majority of them
74 were found to be linked to Huanan Seafood Market (HSM) in Wuhan (6, 7), where
75 various animal meats and exotic seafoods were available for purchase. Therefore,
76 HSM has been suspected to be the source of the COVID-19 pandemic (8). However,
77 the possibility that some individuals outside the market had brought the virus into the
78 market through human-to-human transmission or cold chain could not be ruled out,
79 considering some of the early cases without epidemiological link to the market (7, 9).
80 In addition, recent studies from different countries suggest that SARS-CoV-2
81 circulation preceded the initial detection of cases by weeks. Some of the suspected
82 positive samples were detected even earlier than the first case in Wuhan, suggesting
83 that potential circulation of the virus in other regions may have been missed(10-14).

84

85 Considering the findings that SARS-CoV-2 had high similarities with a few
86 coronaviruses derived from bats in Asian countries including China, Japan, Cambodia,
87 Thailand and Laos, scientists have proposed that bats might be the original source of
88 SARS-CoV-2 (1, 15-20). However, it is yet a mystery whether another animal might
89 act as an intermediate host to have facilitated the virus spillover between bats and
90 humans (21). One of such important findings was the discovery of SARS-CoV-2
91 related coronaviruses from pangolins that harbored highly similar receptor binding
92 domain with that of SARS-CoV-2 (22-24). Despite that pangolins might be involved

93 in the ecology of coronaviruses, whether the pangolins are the intermediate hosts is
94 still fairly controversial. A recent study documented the animal species in HSM
95 between May 2017 and November 2019 and noted that no pangolins or bats were
96 traded (25). Thus far, the origins of SARS-CoV-2 (26, 27) and the role of HSM in the
97 origins and spread of SARS-CoV-2 remain poorly understood.

98

99 The HSM is located in the Jiangnan District, a downtown of Wuhan, and is
100 approximately 800 m far from Hankou Railway Station, a major railway travel hub. It
101 occupies >50,000 m², with 678 stalls located close to each other in an extremely
102 crowded condition (Fig. 1A). The market is separated into two zones, eastern and
103 western zone, with seafood and animals mainly sold in the western zone and livestock
104 meat in the eastern zone. Among the 678 stalls of the market, 10 domesticated
105 wildlife animal-selling stalls (1.5%) were identified, located in the south-western
106 corner of western zone (8/10) and the north-western corner of eastern zone (2/10),
107 respectively (Fig. 1A). According to sales records, during late December 2019,
108 animals or animal products were sold in these 10 animal stalls and animals included
109 snakes, avian species (chickens, ducks, geese, pheasants and doves), Sika deer,
110 badgers, rabbits, bamboo rats, porcupines, hedgehogs, salamanders, giant salamanders,
111 bay crocodiles and Siamese crocodiles, etc., among which snakes, salamanders and
112 crocodiles were traded as live animals.

113

114 The market was closed in the morning of January 1st, 2020 shortly after the
115 identification of the PUE. At the same time, in order to investigate the potential
116 introduction of SARS-CoV-2 into the market, Chinese Center for Disease Control and
117 Prevention (China CDC) dispatched an epidemiological team, together with experts
118 from Hubei Provincial CDC and Wuhan Municipal CDC, to the HSM to collect
119 environmental samples in the early morning of January 1st, 2020 (Fig. 1B). From
120 January 1st, 2020 to March 2nd, 2020, a total of 923 environmental samples from
121 different locations within and around this market and 457 animal samples including

122 animal bodies, stray animals and their feces were collected, with some stray animals
123 sampled until March 30th ([Extended Data Table 1](#)), which may reflect the profile of
124 SARS-CoV-2 contamination in the market during the early phase of the outbreak.
125 After the closure of the stalls in the market, the outside surface of the rolling shutter
126 doors of the stalls and the corridors was disinfected (with 1% bleach mixed with water)
127 throughout January and February 2020. The goods inside the stalls were completely
128 cleared and disinfected until early March 2020.

129

130 Out of the 923 environmental samples collected in and around the market, 73 were
131 found to be positive for SARS-CoV-2 with positive rate of 7.9% through the nucleic
132 acid test (NAT), with Cycle threshold (*C_t*) values of real-time polymerase chain
133 reaction (PCR) ranging from 23.9 to 41.7 ([Table 1](#)). Among the 828 samples inside
134 the HSM, 64 samples (7.7%) were positive. For the 14 samples from warehouses
135 related to the HSM, five tested positive. Among the 51 sewerage wells in the
136 surrounding areas, three tested positive ([Table 1](#)). Notably, one out of the 30
137 environmental samples from other markets in Wuhan tested positive.

138

139 For these 64 SARS-CoV-2 positive samples inside HSM, 87.5% (56/64) were
140 collected in the western zone of the market, in particular streets from no. 1 to 8, with
141 71.4% (40/56) positive samples identified herein ([Fig. 1A](#)). Of the 110 samples
142 collected from sewers or sewerage wells in the market, 24 samples were positive for
143 SARS-CoV-2 nucleic acid. All the four sewerage wells in the market tested positive.
144 This suggested that either contaminated sewage may have played a role in the cluster
145 of cases in the market or that the infected people in the market contaminated the
146 sewage.

147

148 The merchants' activities were assessed against the NAT results of the environmental
149 samples. The sampling covered 19.8% (134/678) of the vendors in the market (95%
150 confidence interval (CI): 16.8-23.0%). Of the positive samples, 44 were distributed

151 among 21 vendors in the market, 19 of whom were located in the west zone and the
152 remaining two located in the east area (Fig. 1A). Some vendors sold more than one
153 product type, leading to differences in the denominators. While the results provide
154 some indication of association of cases with different products, but no significant
155 differences were observed between different vendors, including cold-chain products
156 (18.4%, 16/87, 95% CI: 10.9-28.1%), aquatic products (17.8%, 13/73, 95% CI:
157 9.8-28.5%), seafood products (11%, 6/56: 95% CI: 4-21.9%), poultry (22%, 8/37:
158 95% CI: 9.8-38.2%), livestock (14%, 5/36: 95% CI: 4.7-29.5%), wildlife products
159 (11%, 1/9: 95% CI: 0.3-48.2%) and vegetables (25%, 2/8: 95% CI: 3.2-65%)
160 (Extended Data Fig. 1). These results suggested that SARS-CoV-2 might have been
161 circulating in the market, especially the western zone, for a period of time in
162 December 2019, leading to an extensive distribution of the virus within the market,
163 which might have been facilitated by the crowded buyers and the contaminated
164 environment.

165

166 The 457 animal samples mainly collected between January 1st and March 2nd, 2020
167 included 188 individuals belonging to 18 species (with some stray animals sampled
168 until March 30th) (Table 2). The sources of the samples include unsold goods kept in
169 refrigerators and freezers in the stalls of HSM, and goods kept in warehouses and
170 refrigerators related to the HSM. Samples from stray animals in the market were also
171 collected, i.e. swab samples from 10 stray cats, 27 cat feces, one dog, one weasel, and
172 10 rats. All the 457 animal samples tested negative for SARS-CoV-2 nucleic acid,
173 suggesting that the animal infections with SARS-CoV-2 might be rare in the market.

174

175 To determine whether there was live virus in the HSM, we inoculated 27
176 SARS-CoV-2 positive environmental samples collected on January 1st, 2020 into cell
177 lines, including Vero E6 and Huh7.5 cells. Cytopathic effects (CPE) were observed 3
178 days post inoculation with sample F13 on Vero E6 cells. CPE was also observed 5
179 days post inoculation on Huh7.5 cells. We performed RT-qPCR on the supernatant of

180 the inoculated Vero E6 and Huh7.5 cells. The supernatants were found to be
181 SARS-CoV-2 positive, with *Ct* values of 15.11 (Vero E6) and 14.44 (Huh7.5) for the
182 ORF1ab gene, and 13.98 (Vero E6) and 13.59 (Huh7.5) for the N gene, respectively.
183 We further used transmission electron microscopy to observe the culture supernatant
184 and ultra-thin section of Vero E6 cells 5 days post inoculation. The electron
185 micrographs showed that virus particles were present in both the supernatant and the
186 cells. Negative-stained virus particles were generally spherical, pleomorphic and
187 60-140 nm in diameter. Spike protrusions were observed around the particles in a
188 crown (corona) shape. In the ultra-thin cultured cell sections, a group of virus
189 particles could be seen outside the cells, and sheets of virus particles could also be
190 observed inside the cells (Fig. 2). Totally, live viruses were isolated from samples F13,
191 F54, and B5, which were the only three samples with *Ct* values <30 in the NAT. F54
192 and B5 were swab samples of the ground and F13 were swab samples of the wall.
193 Notably, samples F13 and F54 were from the stalls with confirmed patients. All the
194 results of successful virus isolation and the *Ct* values of the original samples revealed
195 the existence of live SARS-CoV-2 with high titers in the environment of HSM.

196

197 We further performed high-throughput sequencing on the Miseq platform and
198 successfully obtained seven complete SARS-CoV-2 genome sequences, including
199 three sequences from three environmental samples (F13, F54, and A20), and four
200 sequences from cell supernatants of F13, F54, and B5 (Fig. 3). The genome sequences
201 of two environmental samples, F13 and F54, were found to be highly identical to the
202 reference strain HCoV/Wuhan/IVDC-HB-01 (WH01, sequence identity of 99.993%)
203 and completely identical to the human stain Wuhan-Hu-1 (GenBank: NC_045512)
204 (Fig. 3A). The genome sequence of the isolated virus from environmental sample F54
205 had two more synonymous mutations compared to HCoV/Wuhan/IVDC-HB-01, with
206 sequence identity of 99.987% (Fig. 3A). Therefore, the SARS-CoV-2 sequences from
207 environmental samples were highly similar to the clinical strains obtained during the
208 early stage of the COVID-19 outbreak.

209

210 Previously, SARS-CoV-2 has been proposed to be classified into two major
211 genotypes based on the two highly linked single nucleotide polymorphisms (SNPs): S
212 (8782T and 28144C) and L (8782C and 28144T), with S most likely to be the
213 ancestral lineage, because all of the SARS-CoV-2 related coronaviruses from bats and
214 pangolins possessed 8782T and 28144C (28). Phylogenetic analysis revealed that
215 most of the environmental strains belong to the L lineage and they fall into the basal
216 positions of the L lineage (Fig. 3B). However, the environmental sample A20 fell
217 within the S lineage in the tree (Fig. 3B, Extended Data Table 2).

218

219 To explore the potential origins of the SARS-COV-2, we conducted RNA-seq
220 analysis using 27 SARS-CoV-2 positive environmental samples collected on January
221 1st, 2020 from the HSM. We analyzed the correlation of SARS-CoV-2 and the
222 abundance of other species. The abundance of *Homo sapiens* showed the correlation
223 to SARS-CoV-2 (Figure 4), which highly suggests the SARS-CoV-2 might have
224 derived from *Homo sapiens* in the HSM. No animals were concluded, implying that
225 no animal host of SARS-CoV-2 can be deduced.

226

227 In summary, SARS-CoV-2 RNA was detected in stalls in the western zone of HSM,
228 suggesting the prevalence of SARS-CoV-2 in the market. Thus, the market might
229 have acted as an amplifier due to the high number of visitors every day, causing many
230 initially identified infection clusters in the early stage of the outbreak as indicated in
231 the Report of WHO-convened global study of origins of SARS-CoV-2 (10). In
232 addition, live SARS-CoV-2 viruses also existed in the environmental samples.
233 However, no SARS-CoV-2 was detected in the animal samples from the market.
234 Definitely, more work involving international coordination is needed to investigate
235 the real origins of SARS-CoV-2(10), especially considering the SARS-CoV-2
236 positive results of samples collected in 2019 in retrospective studies of different
237 countries (29, 30). Surveillance of wild animals using a viromic approach should be

238 enhanced to explore the natural and intermediate hosts for SARS-CoV-2 (8, 31),
239 which would help to prevent future potential pandemics caused by animal-derived
240 coronaviruses or alike.

241

242

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248 **Author contributions**

249 The study was designed by W.J.L, G.F.G. and G-Z.W. Samples were collected by
250 W.J.L., W.L, Z.J., X.H., J.W., F.W., G.W., K.Q., R.G., J.Z., M.L. and W.X.
251 Experiment and data analysis were performed by W.J.L., P.L., Z.J., X.H., L.L., W.S.,
252 Y.T., S.Z., X.Z., J.W., F.W., G.W., Y.G., Z.X., Y.Z., J.S., Jing Z., W.Z., W-T.Z., B.Y.,
253 J.S., M.Y., W-M.Z., Y.B., K.C., D.W., W.T., J.H. and W-B.X. The manuscript was
254 written by W.J.L., P.L., W.S., Y.T., G.W., G.F.G. and G-Z.W.

255

256 **Competing interest declaration**

257 No competing interest exists.

258

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260 References

- 261 1. W. Tan *et al.*, A Novel Coronavirus Genome Identified in a Cluster of Pneumonia Cases –
262 Wuhan, China 2019–2020. *China CDC Wkly* **2**, 61–62 (2020).
- 263 2. S. Jiang *et al.*, A distinct name is needed for the new coronavirus. *Lancet* **395**, 949 (2020).
- 264 3. V. Coronaviridae Study Group of the International Committee on Taxonomy of, The species
265 Severe acute respiratory syndrome–related coronavirus: classifying 2019–nCoV and naming
266 it SARS–CoV–2. *Nat Microbiol* **5**, 536–544 (2020).
- 267 4. W. J. Liu, G. Wu, Convincing the confidence to conquer COVID–19: From epidemiological
268 intervention to laboratory investigation. *Biosaf Health* **2**, 185–186 (2020).
- 269 5. N. Zhu *et al.*, A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl*
270 *J Med* **382**, 727–733 (2020).
- 271 6. C. Wang, P. W. Horby, F. G. Hayden, G. F. Gao, A novel coronavirus outbreak of global
272 health concern. *Lancet* **395**, 470–473 (2020).
- 273 7. Q. Li *et al.*, Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus–Infected
274 Pneumonia. *N Engl J Med* **382**, 1199–1207 (2020).
- 275 8. P. Daszak, K. J. Olival, H. Li, A strategy to prevent future epidemics similar to the
276 2019–nCoV outbreak. *Biosaf Health* **2**, 6–8 (2020).
- 277 9. N. Chen *et al.*, Epidemiological and clinical characteristics of 99 cases of 2019 novel
278 coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet* **395**, 507–513 (2020).
- 279 10. Anonymous, WHO–convened global study of origins of SARS–CoV–2: China Part.
280 [https://www.who.int/publications/i/item/who-convened-global-study-of-origins-of-
281 -sars-cov-2-china-part](https://www.who.int/publications/i/item/who-convened-global-study-of-origins-of-sars-cov-2-china-part) (2021).
- 282 11. A. Eskild, L. Morkrid, S. B. Mortensen, T. M. Leegaard, Prevalence of antibodies against
283 SARS–CoV–2 among pregnant women in Norway during the period December 2019 through
284 December 2020. *Epidemiol Infect* 10.1017/S0950268822000073, 1–9 (2022).
- 285 12. G. Apolone *et al.*, Unexpected detection of SARS–CoV–2 antibodies in the prepandemic
286 period in Italy. *Tumori* **107**, 446–451 (2021).
- 287 13. F. Carrat *et al.*, Evidence of early circulation of SARS–CoV–2 in France: findings from the
288 population–based "CONSTANCES" cohort. *Eur J Epidemiol* **36**, 219–222 (2021).
- 289 14. J. C. Chandler *et al.*, SARS–CoV–2 exposure in wild white–tailed deer (*Odocoileus*
290 *virginianus*). *Proc Natl Acad Sci U S A* **118** (2021).
- 291 15. P. Zhou *et al.*, A pneumonia outbreak associated with a new coronavirus of probable bat
292 origin. *Nature* **579**, 270–273 (2020).
- 293 16. S. Murakami *et al.*, Detection and Characterization of Bat Sarbecovirus Phylogenetically Related
294 to SARS–CoV–2, Japan. *Emerg Infect Dis* **26**, 3025–3029 (2020).
- 295 17. S. Wacharapluesadee *et al.*, Evidence for SARS–CoV–2 related coronaviruses circulating in
296 bats and pangolins in Southeast Asia. *Nat Commun* **12**, 972 (2021).
- 297 18. H. Zhou *et al.*, A Novel Bat Coronavirus Closely Related to SARS–CoV–2 Contains Natural
298 Insertions at the S1/S2 Cleavage Site of the Spike Protein. *Curr Biol* **30**, 2196–2203 e2193
299 (2020).
- 300 19. H. Zhou *et al.*, Identification of novel bat coronaviruses sheds light on the evolutionary origins
301 of SARS–CoV–2 and related viruses. *Cell* **184**, 4380–4391 e4314 (2021).
- 302 20. J. Li, S. Lai, G. F. Gao, W. Shi, The emergence, genomic diversity and global spread

303 of SARS-CoV-2. *Nature* **600**, 408–418 (2021).

304 21. R. Lu *et al.*, Genomic characterisation and epidemiology of 2019 novel coronavirus:
305 implications for virus origins and receptor binding. *Lancet* **395**, 565–574 (2020).

306 22. T. T. Lam *et al.*, Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins.
307 *Nature* **583**, 282–285 (2020).

308 23. K. Xiao *et al.*, Isolation of SARS-CoV-2-related coronavirus from Malayan pangolins. *Nature*
309 **583**, 286–289 (2020).

310 24. S. Niu *et al.*, Molecular basis of cross-species ACE2 interactions with SARS-CoV-2-like
311 viruses of pangolin origin. *EMBO J* **40**, e107786 (2021).

312 25. X. Xiao, C. Newman, C. D. Buesching, D. W. Macdonald, Z. M. Zhou, Animal sales
313 from Wuhan wet markets immediately prior to the COVID-19 pandemic. *Sci Rep* **11**, 11898
314 (2021).

315 26. Q. Wang *et al.*, Tracing the origins of SARS-CoV-2: lessons learned from the past. *Cell*
316 *Res* **31**, 1139–1141 (2021).

317 27. Y. Tong *et al.*, The origins of viruses: discovery takes time, international resources, and
318 cooperation. *Lancet* **398**, 1401–1402 (2021).

319 28. X. Tang *et al.*, On the origin and continuing evolution of SARS-CoV-2. *Nat Sci Rev* **7**,
320 2 (2020).

321 29. G. Fongaro *et al.*, The presence of SARS-CoV-2 RNA in human sewage in Santa Catarina,
322 Brazil, November 2019. *Sci Total Environ* **778**, 146198 (2021).

323 30. G. Chavarria-Miró *et al.*, Sentinel surveillance of SARS-CoV-2 in wastewater anticipates
324 the occurrence of COVID-19 cases. *medRxiv*
325 [medrxiv.org/content/10.1101/2020.06.13.20129627v1](https://doi.org/10.1101/2020.06.13.20129627v1) (2020).

326 31. H. Li *et al.*, Human-animal interactions and bat coronavirus spillover potential among rural
327 residents in Southern China. *Biosaf Health* **1**, 84–90 (2019).

328 32. D. E. Wood, J. Lu, B. Langmead, Improved metagenomic analysis with Kraken 2. *Genome*
329 *Biol* **20**, 257 (2019).

330 33. F. P. Breitwieser, S. L. Salzberg, Pavian: interactive analysis of metagenomics data for
331 microbiome studies and pathogen identification. *Bioinformatics* **36**, 1303–1304 (2020).

332

333 **Figure legends**

334 **Figure 1. The distribution of the positive environmental samples in Huanan**
335 **Seafood Market.**

336 A. As the place of the early cluster of COVID-19 patients, the Huanan Seafood
337 Market is separated into eastern and western zones with the Xinhua Rd. between them.
338 To detect for the presence of SARS-CoV-2 RNA, reverse transcription, quantitative
339 polymerase chain reaction (RT-qPCR) was performed. The locations of the positive
340 samples were marked in the map of the market within orange, while the location of
341 the samples that the live viruses were isolated from were labeled with red. The map
342 also shows locations of stalls where domesticated wildlife products were sold. B.
343 Timeline of environmental and animal samples collected within and around Huanan
344 Seafood Market.

345

346 **Figure 2. The SARS-CoV-2 virus isolation from environmental samples of**
347 **Huanan Seafood Market.**

348 The electron micrographs of the SARS-CoV-2 viruses isolated from the
349 environmental samples in Huanan Seafood Market. To determine whether
350 SARS-CoV-2 particles could be visualized from the cell supernatant and lysate, we
351 used transmission electron microscopy to observe the culture supernatant and
352 ultra-thin section cells based from both VeroE6 and Huh7.5 cells. The electron
353 micrographs showed that virus particles were present in both the supernatant (A, B)
354 and the cells (C, D). Negative-stained virus particles were generally spherical,
355 pleomorphic and 60-140 nm in diameter. Spike protrusions were observed around the
356 particles in a crown (corona) shape (A, B). In ultra-thin cultured cell sections, a group
357 of virus particles can be seen outside the cell (C), and sheets of virus particles can also
358 be observed inside the cells (D).

359 **Figure 3. The genomic analysis of SARS-CoV-2 sequenced from Huanan Seafood**
360 **Market.**

361

362 **Figure 4. Clustering of environmental samples in Huanan Seafood Market.**
363 A, Correlation of SARS-COV-2 with other species in SARS-COV-2 positive cases.
364 The abundance of SARS-COV-2 and other species was used for analysis. Both
365 Pearson's and Spearman's correlation values were plotted. B, Correlation of the
366 abundance of SARS-COV-2 and *Homo sapiens*.
367

368 **Tables**369 Table 1. Information of the positive environmental samples from Huanan Seafood
370 Market.

No.	Sample type	Sampling date	PCR	<i>C_t</i>	PCR target	NGS
E61	Ground	2020/1/1	+	36.04	ORF1ab/N	NA
A101	Surface of the door	2020/1/1	+	36.82	ORF1ab/N	NA
A14	Surface of packing bag for hairtail	2020/1/1	+	36.42	ORF1ab/N	NA
A15	Surface of the door	2020/1/1	+	35.51	ORF1ab/N	NA
A18	Shoe covers and soles	2020/1/1	+	33.79	ORF1ab/N	NA
A2	Ground	2020/1/1	+	35.07	ORF1ab/N	+
A20	Gloves	2020/1/1	+	32.48	ORF1ab/N	+
A33	Garbage truck	2020/1/1	+	34.46	ORF1ab/N	NA
A55	Ground	2020/1/1	+	34.84	ORF1ab/N	+
A61	Ground	2020/1/1	+	32.04	ORF1ab/N	NA
A63	Ground	2020/1/1	+	34.43	ORF1ab/N	+
A87	Surface of the door	2020/1/1	+	36.94	ORF1ab/N	NA
A88	Ground	2020/1/1	+	36.69	ORF1ab/N	NA
A90	Ground	2020/1/1	+	33.14	ORF1ab/N	NA
A96	Ground	2020/1/1	+	33.97	ORF1ab/N	NA
B17	Scale	2020/1/1	+	34.16	ORF1ab/N	NA
B5*	Ground	2020/1/1	+	29.32	ORF1ab/N	+
D32	Surface of a cart	2020/1/1	+	33.72	ORF1ab/N	NA
E48	Blood on the ground in front of the door	2020/1/1	+	35.93	ORF1ab/N	+
E7	Styrofoam desk in front of the door	2020/1/1	+	36.44	ORF1ab/N	+
F100	Ground	2020/1/1	+	34.72	ORF1ab/N	+
F13*	Surface of the wall	2020/1/1	+	23.85	ORF1ab/N	+
F33	Roller shutter	2020/1/1	+	34.13	ORF1ab/N	NA
F46	Ground	2020/1/1	+	31.8	ORF1ab/N	+
F54*	Ground	2020/1/1	+	25.8	ORF1ab/N	+
F98	Ground	2020/1/1	+	34	ORF1ab/N	+
G93	Sewage	2020/1/1	+	33.23	ORF1ab/N	NA
Q37	Inner surface of the freezer	2020/1/12	-	\	ORF1ab/N	+
Q61	Cart1	2020/1/12	-	\	ORF1ab/N	+
Q64	Cart2	2020/1/12	+	+	ORF1ab/N	+
Q68	Ground	2020/1/12	+	+	ORF1ab/N	+
Q69	Feather removal machine	2020/1/12	+	+	ORF1ab/N	+
Q70	Iron container in inner room	2020/1/12	-	\	ORF1ab/N	+
1-27-33	Water drain	2020/1/27	+	36	ORF1ab	NA
1-27-37	Water drain	2020/1/27	+	35	ORF1ab	NA
1-29-4	Water drain	2020/1/29	+	36	ORF1ab	NA
1-29-8	Water drain	2020/1/29	+	37	ORF1ab	NA
8-25-D	Ground inside the stalls	2020/2/3	+	35.9	ORF1ab	+
8-25-M1	Ground inside the stalls	2020/2/3	+	36.5	ORF1ab	+
WS-1-1	West sewage well 1	2020/2/5	+	36.00	ORF1ab	NA
WS-1-2	West sewage well 2	2020/2/5	+	38.00	ORF1ab	NA
WS-1-3	West sewage well 3	2020/2/5	+	34.01	ORF1ab	NA
WS-1-4	West sewage well 4	2020/2/5	+	38.00	ORF1ab	NA
WS-2-1	West sewage well 1	2020/2/5	+	37.30	ORF1ab	NA
WS-2-2	West sewage well 2	2020/2/5	+	37.65	ORF1ab	NA
WS-2-3	West sewage well 2	2020/2/5	+	36.72	ORF1ab	NA
WS-3-2	West sewage well 3	2020/2/5	+	37.60	ORF1ab	NA
WS-4-2	West sewage well 4	2020/2/5	+	37.10	ORF1ab	NA
zong-1	Water drain	2020/2/9	+	34.94	ORF1ab	NA
w-6-29-33	Water drain	2020/2/15	+	37.97	ORF1ab	NA
w-zong-1	Water drain	2020/2/15	+	36.54	ORF1ab	NA
EWS-2#-2	East sewage well 2	2020/2/15	+	35.32	ORF1ab	NA
EWS-3#-2	West sewage well 3	2020/2/15	+	36.05	ORF1ab	NA
WWS-1#	West sewage well 1	2020/2/15	+	34.44	ORF1ab	NA
WWS-1#-2	West sewage well 1	2020/2/15	+	33.63	ORF1ab	NA

WWS-1#-3	West sewage well 1	2020/2/15	+	33.58	ORF1ab	NA
WWS-2#-2	West sewage well 2	2020/2/15	+	37.44	ORF1ab	NA
WWS-2#-3	West sewage well 2	2020/2/15	+	36.88	ORF1ab	NA
wws-1#-0	West sewage well 1	2020/2/15	+	33.75	ORF1ab	NA
W-8-25-D1	Ground inside the stalls	2020/2/15	+	36.77	ORF1ab	NA
W-8-25-D2	Ground inside the stalls	2020/2/15	+	33.91	ORF1ab	NA
W-8-25-L	Container	2020/2/15	+	34.58	ORF1ab	NA
W-8-25-L2	Container	2020/2/15	+	37.16	ORF1ab	NA
C8	Wall inside the stalls	2020/2/20	+	32.59/-/37.34	RDRP/N/E	NA
SJ-D	Storehouse ground	2020/2/22	+	39.39/39.25/35.48	RDRP/N/E	NA
SJ-CS	Storehouse weight scale	2020/2/22	+	40.21/40.1/36.37	RDRP/N/E	NA
SJ-L3	Storehouse wire fence	2020/2/22	+	41.77/41.62/37.61	RDRP/N/E	NA
RLC-4	Storehouse bag surfaces	2020/2/22	+	41.71/41.53/37.31	RDRP/N/E	NA
RLC-3	Storehouse bag surfaces	2020/2/22	+	36.18/36.05/32.36	RDRP/N/E	NA

371 Note: Four positive samples were not included in this table. One sample was collected from other
372 market in Wuhan and 3 collected from sewerage wells in surrounding areas.
373

374 Table 2. The animal samples collected in the Huanan Seafood Market.

Species	Animal number	Sample number	RT-PCR positive number	Remarks
Rabbit/Hares	52	104	0	
Stray cat	27	80 ^a	0	Including faeces
Snake	40	80	0	
Hedgehog	16	67	0	
Muntjac	6	18	0	
Dog	7	17	0	Including one stray dog
Badger	6	16	0	
Bamboo rat	6	15	0	
Rat	10	12	0	Captured around the market
Pig	NA ^c	6 ^b	0	
Chicken	5	5	0	
Chinese giant salamander	3	5	0	
Crocodile	2	4	0	
Wild boar	2	4	0	
Soft-shelled turtle	2	3	0	
Weasel	1	2	0	Captured around the market
Fish	2	2	0	
Sheep	1	1	0	
Others	NA ^c	16	0	
Rabbit/Hares	52	104	0	
Total	188	457	0	

375 ^a Six of the cats were from the Huanan Seafood Market.376 ^b Other markets.377 ^c Not applicable.

378 **Extended data methods**

379 **Sample collection**

380 Huanan Seafood Market (HSM) was closed on early morning of January 1st 2020
381 and at the same time, China CDC began collecting environmental and animal samples.
382 Staff from China CDC entered the market about 30 times before the market's final
383 clean-up on 2 March 2020, with some stray animals sampled outside the market until
384 March 30th. Environmental samples in the HSM were collected to represent
385 exhaustively as possible, from a wide diversity of surfaces, animals and products
386 (Table 1 and 2) according to different sampling principles, as described in detail in the
387 Joint Report of WHO-convened Global Study of Origins of SARS-CoV-2: China Part
388 (10).

389 The principles and ranges of in-market sampling covered: (1) environmental
390 samples from stalls related to early cases; (2) environmental samples from doors and
391 floors of all stalls in the blocks where the early cases were located; (3) environmental
392 samples in the east wing of the market were collected according to blocks; (4)
393 transport carts, trash cans and similar objects; (5) environmental samples from stalls
394 that sold livestock, poultry, farmed wildlife (also called “domesticated wildlife” or
395 “domesticated wildlife products” in this report); (6) samples of sewage and silt from
396 drainage channels and sewerage wells; (7) stray cats, rats and other potential vector
397 animals in the market; (8) animal products and other commodity samples kept in the
398 cold storages and refrigerators in the market; (9) the market's ventilation and
399 air-conditioning system; and (10) public toilets, public activity rooms and other places
400 where people gathered in the market.

401 The investigators used full personal protective equipment during the sampling in
402 the market. Commercial products of swabs and virus preservation solution were used
403 for the sampling (Disposable Virus Sampling Tube, V5-S-25, Shen Zhen Zi Jian
404 Biotechnology Co., Ltd., Shenzhen, China). For environmental samples, sampling
405 swabs were applied to smear the floors, walls or surfaces of objects and then
406 preserved them in virus preservation solution.

407 For animal samples, depending on the type of animal and whether it was alive or
408 frozen, pharyngeal, anal, body surface and body cavity swabs or tissue samples were
409 collected for nucleic acid testing (NAT). Generally, for alive animal and frozen full
410 bodies, three samples, including pharyngeal, anal, and body surface swabs were
411 collected for each animal individuals. And for animal bodies after “bai tiao” disposing
412 (remaining parts of poultry or livestock after removal of hair and viscera), the body
413 cavity swabs were collected.

414 Sewage (silt) samples were collected by the use of virus sampling swabs to probe
415 into the silt at the bottom of drainage channels in the market. Sewage and silt samples
416 were preserved in virus preservation solution. For the sewage well, a container was
417 used to take a silt-water mixture from a location near the bottom of the well, and an
418 appropriate amount of sample was collected by using virus sampling swabs and then
419 preserved in virus preservation solution.

420 **Nucleic acid extraction and SARS-CoV-2 real-time PCR assay**

421 A virus nucleic acid extraction kit (Xi'an Tianlong) was used to extract viral nucleic
422 acid from samples using an automated nucleic acid extraction instrument according to
423 the manufacturer’s instructions. Real-time (RT) PCR was performed on extracted
424 nucleic acid samples with a SARS-CoV-2 nucleic acid assay kit. The reagent brands
425 include BioGerm (40/38, cycle number/cut-off value, the same as below), DAAN
426 (45/40), and BGI (40/38).

427 **Virus isolations**

428 Samples positive for SARS-CoV-2 were cultured in Vero E6 and Huh7.5 cells. The
429 cell lines were inoculated with positive samples and three blind passages were
430 performed for each sample. The culture supernatant and cell pellet of each passage
431 were harvested for RT PCR. The morphology of viral particles in the cell sections and
432 the supernatant were observed by transmission electron microscope (TEM).

433 **Metagenomic sequencing**

434 Metagenomic sequencing was conducted at Wuhan BGI. Nucleic acid was extracted
435 using Qiagen's viral RNA microextraction kit and human nucleic acid was removed

436 using an enrichment kit to improve the sensitivity of viral RNA detection. Extracted
437 RNA was reverse transcribed into cDNA and segmented into 150-200 bp by enzyme
438 digestion. After repair, fitting, purification, PCR amplification and purification,
439 sample concentration was assayed and SE50+10 sequencing performed by
440 DNBSEQ-T7, and an average output of more than 200 million reads was obtained.
441 Sequencing data were compared with those in a SARS-CoV-2 database to determine
442 whether the samples contained coronavirus sequences.

443 **Identification of the species abundances**

444 The species abundances were obtained from the RNA-seq datasets of the
445 environmental samples. The raw sequenced reads were mapped to the NCBI
446 Nucleotide database using the kraken2 software (confidence 0.05) (32). The matrix of
447 species was obtained by using the pavian algorithm (33). The correlation value
448 between SARS-COV-2 and other species were examined by the Pearson's and
449 Spearman's correlation coefficient method.

450

451 **Extended data figure legends/tables.**

452

453 **Extended Data Figure S1. Positive environmental samples associated with**
454 **different products in the Huanan Seafood Market.**

455 Dots represent the percentage of positive environmental samples associated with each
456 product. Bars represent 95% confidence intervals for the binomials in the text above.

457 Note that the CI for some products (e.g. vegetables, farmed wildlife) have broad error
458 bars that are likely due to the low number of vendors for these categories in the
459 market. Nine of the 10 vendors selling farmed wildlife have been sampled.

460

461

462 **Extended Data Table 1. Overview of environmental sample sampling and testing in the**
463 **Huanan Seafood Market.**

	Number of samples	Number of positive samples by RT-PCR	Number of isolated viruses
Huanan Seafood Market	718	40	3
Warehouses related to the Huanan Seafood Market	14	5	
Other markets in Wuhan*	30	1	
Drainage system in the Huanan Seafood Market	110	24	
Sewerage wells in surrounding areas	51	3	
Total	923	73	3

464 *The other markets were Dongxihu market and Huanggang Center market.

465

466

467 **Extended Data Table 2. Summary of number of reads mapped to positions 8782 and 28144**
468 **in different samples.**

Position	8782				28144			
	A	T	C	G	A	T	C	G
A101	0	0	0	0	0	0	0	0
A14	0	0	0	0	0	0	0	0
A15	0	0	0	0	0	0	0	0
A18	0	0	0	0	0	0	0	0
A20	7	359	1	0	1	5	1596	5
A2	0	0	0	0	0	0	0	0
A33	0	0	2	0	0	0	1	0
A55	0	0	0	0	0	0	0	0
A61	0	0	2	0	0	0	1	0
A63	0	0	0	0	0	0	0	0
A87	0	0	0	0	0	1	0	0

A88	0	0	0	0	0	2	0	0
A90	0	0	0	0	0	0	0	0
A96	0	0	0	0	0	0	0	0
B17	0	0	0	0	0	0	0	0
B5	0	0	86	0	0	85	0	0
D32	0	0	0	0	0	0	0	0
E48	0	0	0	0	0	0	0	0
E61	0	0	0	0	0	0	0	0
E7	0	0	0	0	0	0	0	0
F100	0	0	0	0	0	0	0	0
F13	5	10	1874	3	0	1669	0	0
F33	0	0	0	0	0	0	0	0
F46	0	0	1	0	0	0	0	0
F54	1	1	106	0	1	39	0	0
F98	0	0	20	0	0	3	0	0
G93	0	0	0	0	0	0	0	0

469

Figures

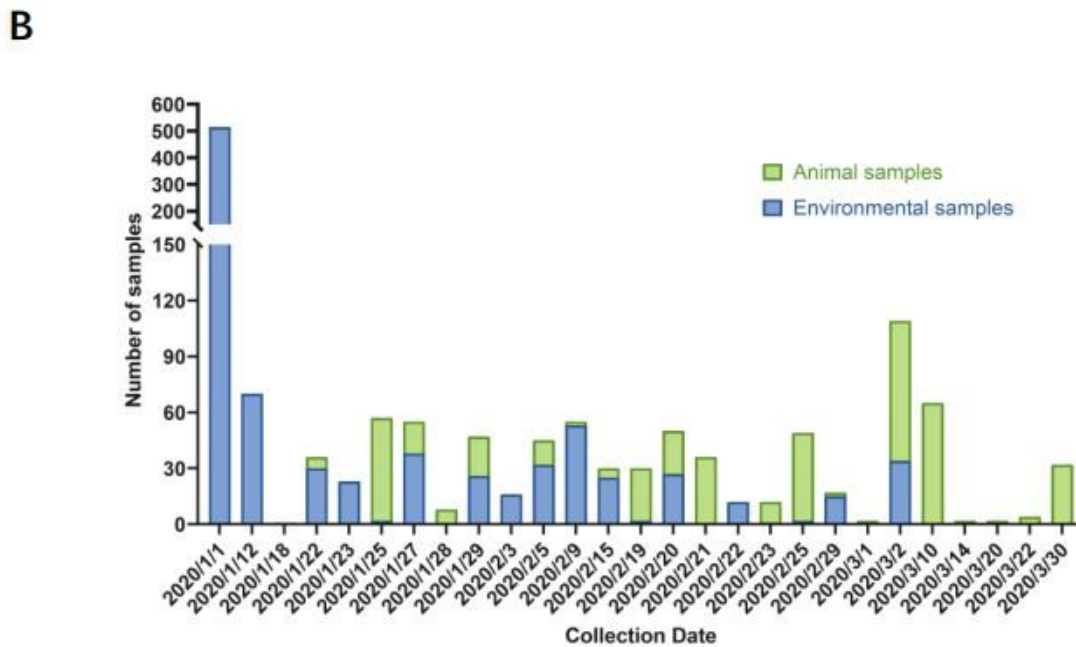
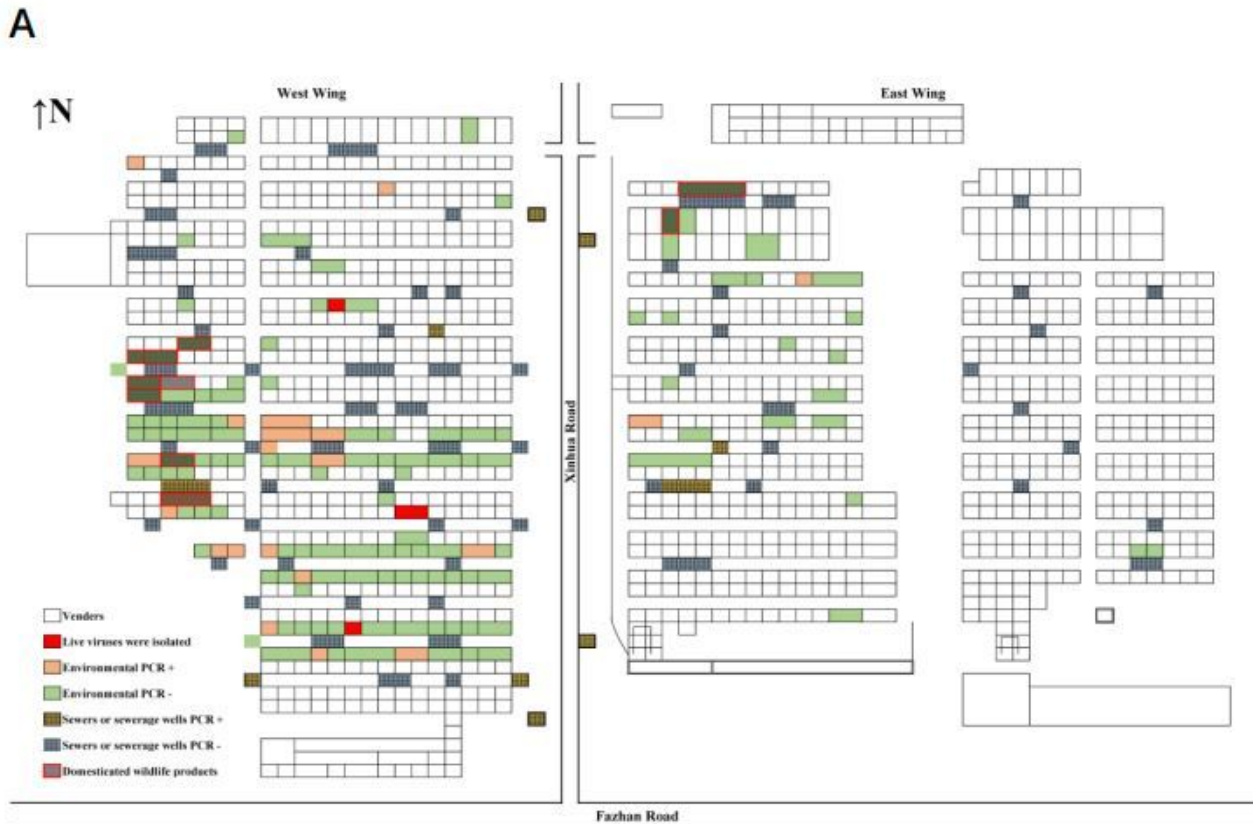


Figure 1

The distribution of the positive environmental samples in Huanan Seafood Market. A. As the place of the early cluster of COVID-19 patients, the Huanan Seafood Market is separated into eastern and western zones with the Xinhua Rd. between them. To detect for the presence of SARS-CoV-2 RNA, reverse

transcription, quantitative polymerase chain reaction (RT-qPCR) was performed. The locations of the positive samples were marked in the map of the market within orange, while the location of the samples that the live viruses were isolated from were labeled with red. The map also shows locations of stalls where domesticated wildlife products were sold. B. Timeline of environmental and animal samples collected within and around Huanan Seafood Market

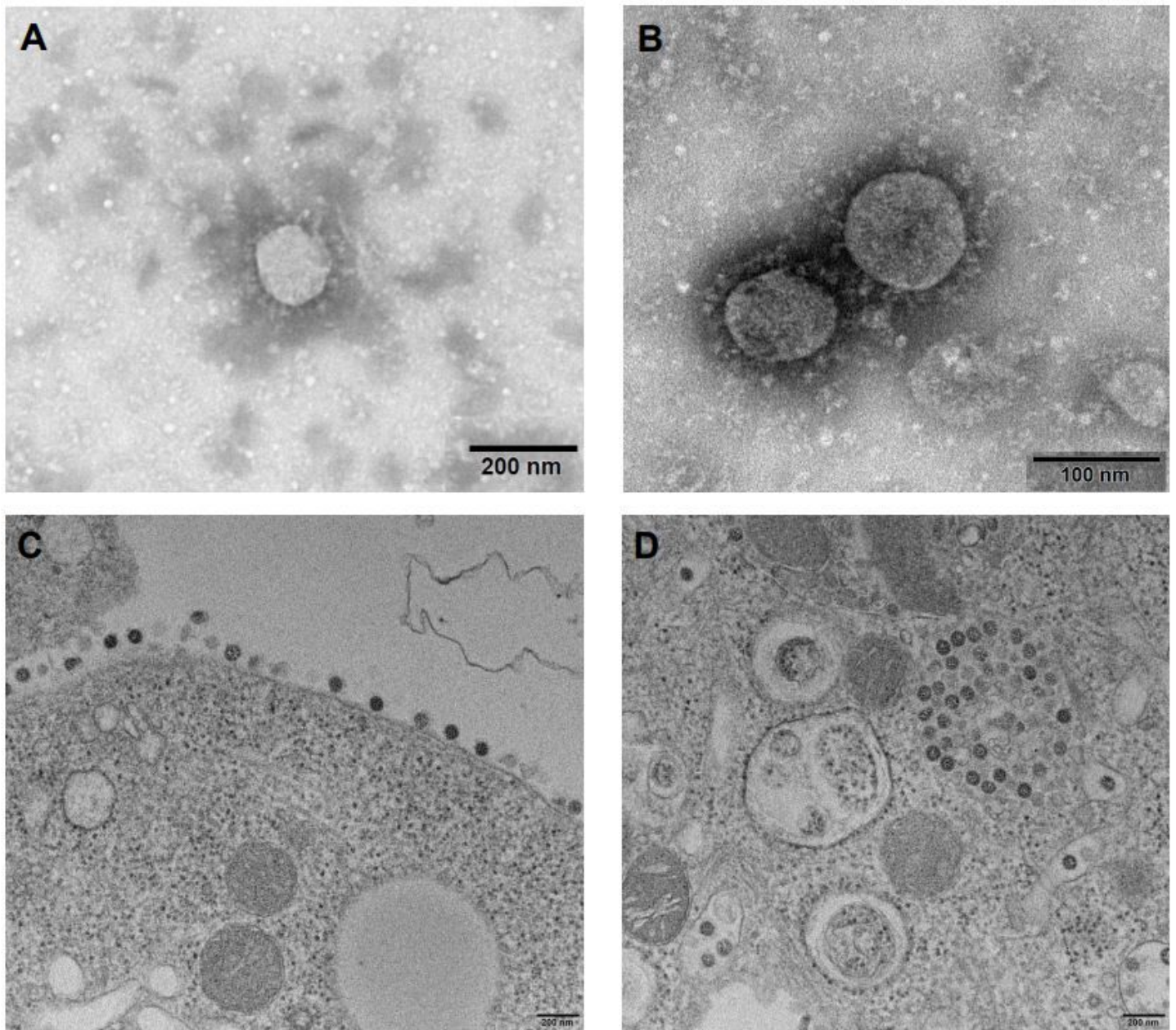


Figure 2

The SARS-CoV-2 virus isolation from environmental samples of Huanan Seafood Market. The electron micrographs of the SARS-CoV-2 viruses isolated from the environmental samples in Huanan Seafood Market. To determine whether SARS-CoV-2 particles could be visualized from the cell supernatant and

lysate, we used transmission electron microscopy to observe the culture supernatant and ultra-thin section cells based from both VeroE6 and Huh7.5 cells. The electron micrographs showed that virus particles were present in both the supernatant (A, B) and the cells (C, D). Negative-stained virus particles were generally spherical, pleomorphic and 60-140 nm in diameter. Spike protrusions were observed around the particles in a crown (corona) shape (A, B). In ultra-thin cultured cell sections, a group of virus particles can be seen outside the cell (C), and sheets of virus particles can also be observed inside the cells (D).

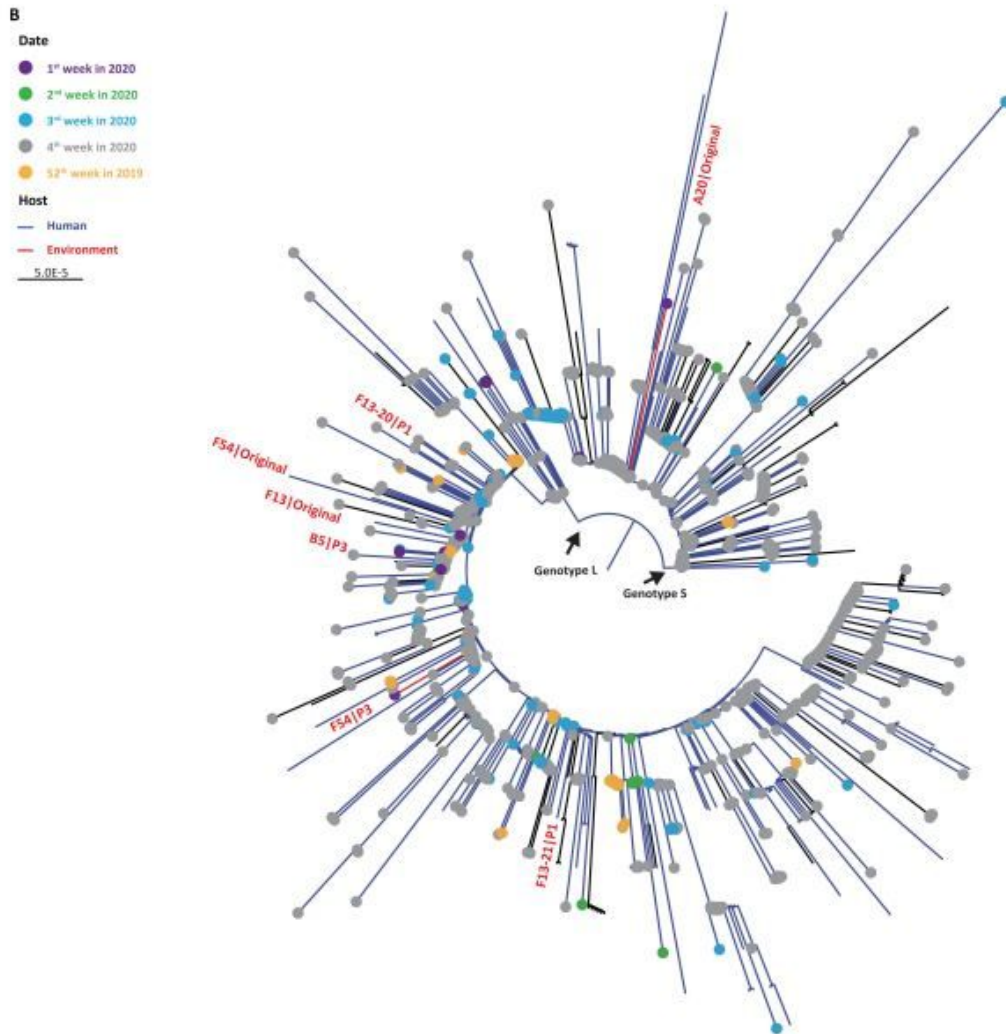
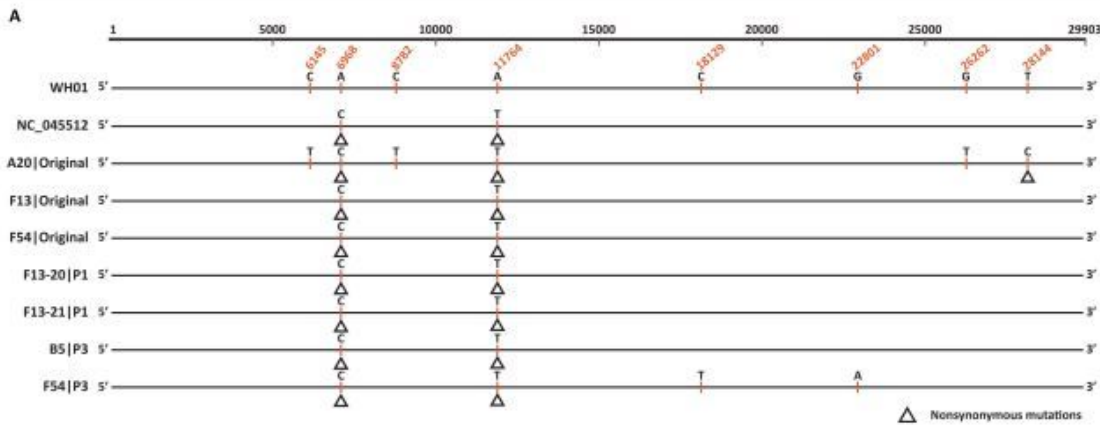


Figure 3

The genomic analysis of SARS-CoV-2 sequenced from Huanan Seafood Market.

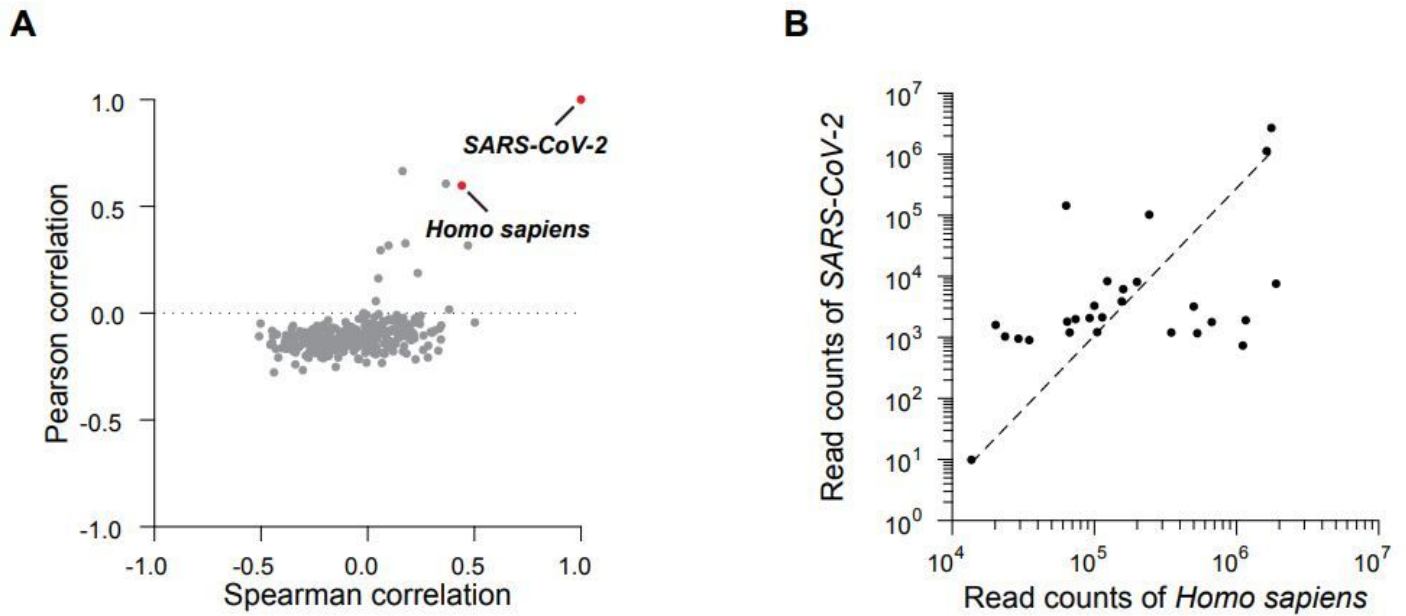


Figure 4

Clustering of environmental samples in Huanan Seafood Market. A, Correlation of SARS-COV-2 with other species in SARS-COV-2 positive cases. The abundance of SARS-COV-2 and other species was used for analysis. Both Pearson's and Spearman's correlation values were plotted. B, Correlation of the abundance of SARS-COV-2 and *Homo sapiens*.