Article DOI: https://doi.org/10.3201/eid2702.204443

Severe Acute Respiratory Syndrome Coronavirus 2 Outbreak in a Nightclub, Germany, 2020

Appendix

Outbreak Case Definition

A confirmed case in the outbreak was defined as any person with laboratory-confirmed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection who attended club X between February 29 and March 5, 2020; had an epidemiologic link to a case that attended club X between February 29 and March 5, 2020; or both. A probable case was defined as any person with clinical symptoms of coronavirus disease (COVID-19) (*1*) who attended club X between February 29 and March 5, 2020; had an epidemiologic link to a case that attended club X between February 29 and March 5, 2020; or both.

Laboratory confirmation of SARS-CoV-2 was defined by the detection of SARS-CoV-2 nucleic acid via PCR in a clinical specimen or by detection of SARS-CoV-2–specific IgG antibodies. Because club X was closed on March 6, 2020 until further notice, no cases linked to the outbreak were reported after March 2020.

Cases were assigned to the outbreak if they fulfilled the case definition and confirmation of positive case status was given by the person or by the local health authority of their place of residence.

Epidemiologic Outbreak Investigation

Data on the day of symptom onset was retrieved from the national infectious diseases notification database, which was collected and notified by public health officials from local public health authorities. Among all cases linked to the outbreak, dates of symptom onset were available for 64 cases.

We conducted semistructured telephone interviews with first-generation cases to gather information related to their exposure in the club, prior travel history, and characteristics of clinical symptoms. Among all first-generation cases linked to the outbreak, contact information was available for 44 cases and the study team interviewed them. We performed analysis by time, generation, symptoms, sex, and age. For analysis by time, we stratified cases by guests, staff members, and generation. For continuous variables, if not normally distributed, we calculated medians and interquartile ranges (IQR). In addition, members of the outbreak investigation team performed a site visit of club X to gain insight into the outbreak setting (Appendix Figure 1).

Virological Outbreak Investigation

SARS-CoV-2 Antibody Screening of Staff Members

For laboratory-confirmation of cases, qualitative real-time RT-PCR for SARS-CoV-2 was performed on purified RNA from swabs as described (*2*), or the Cobas SARS-CoV-2 test (Roche, https://www.roche.com), both of which target the SARS-CoV-2 E gene.

For nightclub staff members who had negative PCR tests for SARS-CoV-2 or who were not tested after the exposure, we performed SARS-CoV-2 antibody screening during June 2–24, 2020, approximately 3 months after the outbreak, by using a 2-step approach. First, we screened samples by using Anti-SARS-CoV-2 S1 IgG and IgA ELISAs (Euroimmun, https://www.euroimmun.com) according to the manufacturer's protocol. Second, we performed a plaque reduction neutralization test (PRNT), as previously described (*3*,*4*). In the PRNT, we tested all dilutions in duplicate. Only serum samples showing an optical density ratio >0.8 in the IgA or IgG ELISA were considered reactive and tested in the PRNT.

Whole-Genome Sequencing

To investigate the sequence diversity of the outbreak, we performed whole-genome sequencing (WGS) on available samples from initial diagnostic testing that had sufficient sample material. For WGS we followed two approaches. First, we performed direct sequencing of native samples with a high viral load (cycle threshold [C_t] value <25); then, for samples with lower SARS-CoV-2 concentration, we used a PCR amplicon-based sequencing approach.

For sequencing of native samples with a high viral load (C_t value <25), we used $\leq$100 ng in 5 µL of extracted RNA for library preparation by using the KAPA RNA Hyper Prep Kit

(Roche Molecular Diagnostics, https://diagnostics.roche.com) according to manufacturer's instructions. The RNA was fragmented for 6 min at 85°C. Indexed libraries were then amplified for 8–13 PCR cycles. All DNA libraries were measured by Qubit dsDNA HS Assay kit (Thermo Fisher Scientific, https://www.thermofisher.com), pooled together at equimolar ratios, and normalized.

For amplicon-based complete genome sequencing of samples with a lower viral load (C_t value $\geq$25) we followed 2 approaches. First, we used 108 SARS-CoV-2 whole genomes, available in early February 2020 to design 48 overlapping heminested PCR fragment primers. Fragment size ranged between 507 bp and 950 bp for first-round products and 414–877 bp for second-round products. Primer names including "i" were modified versions (Appendix Table 2). For the first-round PCR, a 25 μL reaction was performed by using the SuperScript III One-Step RT-PCR System with Platinum Taq DNA (Invitrogen, https://www.thermofisher.com) with 5 μL of RNA, 12.5 μL of 2× reaction buffer (provided with the kit), 1 μL of enzyme mixture from the kit, additional 0.4 μL of a 50 mmol magnesium sulfate solution, 400 nmol concentrations of each first-round primer, and 1 μg of bovine serum albumin (Roche). For the second-round, 50 μL reactions were carried out by using the Platinum Taq Polymerase Kit (Invitrogen), with 1 μL of the first-round PCR product, 5 μL of 10× reaction buffer provided with the kit, 2.5 mmol $MgCl_2$, 200 μM of each dNTP, 0.2 μL of Platinum Taq, and 400 nmol of each second-round primer. First-round RT-PCRs were carried out by using a thermocycling protocol with reverse transcription at 55°C for 20 min and subsequent PCR at 95°C for 3 min, followed by 45 cycles of 95°C for 15 s, 56°C for 15 s, and 72°C for 55 s, followed by a final 2-min extension step at 72°C. Second-round reactions used the same cycling protocol but without the RT step. Second, for amplicon-based WGS we used random hexamers and the SuperScript III Reverse transcription kit (Invitrogen) according to manufacturer's instructions, then amplified the SARS-CoV-2 genome by using the primer sets (V1) published by the Artic Network (dx.doi.org/10.17504/protocols.io.bdbfi2jn). A 25 μL PCR master mix was set up by using the Q5 High-Fidelity DNA Polymerase kit (New England Biolabs, https://www.neb.com) with 5 μL 5× Q5 Reaction Buffer (New England Biolabs), 13.15 μL RNase-free water, 0.5 10 mmol dNTPs, 3.6 μL of either 10 μmol primer pool 1 or 2, 2.5 μL cDNA and 0.25 μL Q5 High-Fidelity DNA Polymerase (New England Biolabs). PCR was carried out by using a thermocycling protocol with initial denaturation at 98°C for 30 sec, followed by 35 cycles of

98°C for 15 s, 65°C for 2 min 30 sec, followed by a final 2-min extension step at 72°C. PCR products were pooled and purified by using KAPA Pure Beads (Roche Molecular Diagnostics) according to manufacturer's instructions.

For DNA library preparation of the purified PCR amplicons, we used ≤5 ng DNA and the KAPA Hyper Prep Kit (Roche Molecular Diagnostics). All pooled PCR amplicons and DNA libraries were measured by Qubit dsDNA HS Assay kit (Thermo Fisher Scientific).

Sequencing was performed by using the 600-cycle MiSeq reagent v3 cartridge (Illumina, https://www.illumina.com), the 150/300-cycle NextSeq, and 100-cycle NovaSeq (Illumina) according to manufacturer's instructions (Appendix Table 3).

Bioinformatic Sequence Analysis

For each sample, data from the individual sequencing runs were mapped to a reference sequence (GISAID accession no. EPI_ISL_402125, GenBank accession no. NC_045512.2) by using bowtie2 version 2.3.5.1 and the sensitive-local option (*5*). Duplicates were removed using GATK MarkDuplicatesSpark version 4.1.4.1 (*6*), and the consensus reads were called at positions with coverage ≥3 reads by using bcftools version 1.10.2–31-gffa7016 and bcftools call–ploidy 1-mv-Oz-o (https://samtools.github.io/bcftools/bcftools.html). We included the following sequences in the phylogenetic tree: 1 sequence from each clade assigned by Pangolin; all sequences from Germany sampled before April 16, 2020 and available in GISAID on July 22, 2020; and representative sequences from GISAID clade G sampled by April 15, 2020 and available in GISAID on July 22, 2020. Sequences from each country were clustered by using CD-HIT version 4.8.1 by using a sequence identity threshold of 0.99 (*7*) and we picked 1 sequence from each cluster. Then we included 4 sequences from the U.S. and 1 from Canada that have the same additional SNP as sequences ChVir-W1248–16 and ChVir-D715-D799–17 from this outbreak. The phylogenetic tree was inferred by using RAxML-ng version 0.7.0 BETA (*8*) and an HKY substitution model, with gamma distribution rate heterogeneity among sites and invariant sites. We performed 100 bootstrap replicates and created a phylogenic tree by using baltic (*9*) (Appendix Figure 2).

Ethics Approval

The outbreak investigation was conducted within the framework of the German Infection Protection Act (*10*) as part of an outbreak response and public health practice. Mandatory

regulations were respected, and thus review by an ethics committee was not required. Support by the Robert Koch Institute was provided after official request. Participation in the questionnaire and blood specimen collection for antibody testing was voluntary, for which verbal consent was obtained. For antibody testing, additional written informed consent was obtained.

Description of the Outbreak Setting

Club X is located in a basement. The area accessible to guests is ≈150 m^2 with a height of ≈3 m (Appendix Figure 1). Ventilation of the space is ensured by a mechanical air exhaust and supply system and maintenance was performed according to the manufacturer's instructions. To avoid noise pollution in the surrounding neighborhood, windows are usually closed during events.

Clinical Symptoms of Cases

Among a total of 74 cases linked to the outbreak, dates of symptom onset were available for 64 cases. Of those, 44 cases could be interviewed on clinical symptoms during their COVID-19 infection. All 44 cases reported having ≥1 symptom. The most common symptoms experienced were dysgeusia (65%), cough (61%), headache (58%), and dysosmia (58%) (Appendix Table 4).

References

1. European Centers for Disease Control. Case definition for coronavirus disease 2019 (COVID-19), as of 29 May 2020 [cited 2020 Oct 21]. https://www.ecdc.europa.eu/en/covid-19/surveillance/case-definition

2. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25. PubMed https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

3. Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature. 2020;581:465–9. PubMed https://doi.org/10.1038/s41586-020-2196-x

4. Buchholz U, Müller MA, Nitsche A, Sanewski A, Wevering N, Bauer-Balci T, et al. Contact investigation of a case of human novel coronavirus infection treated in a German hospital, October-November 2012. Euro Surveill. 2013;18:20406. PubMed

5. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nat Methods. 2012;9:357–9. PubMed https://doi.org/10.1038/nmeth.1923

6. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20:1297–303. PubMed https://doi.org/10.1101/gr.107524.110

7. Li W, Godzik A. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics. 2006;22:1658–9. PubMed https://doi.org/10.1093/bioinformatics/btl158

8. Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A. RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics. 2019;35:4453–5. PubMed https://doi.org/10.1093/bioinformatics/btz305

9. baltic - Python library for parsing phylogenetic trees [cited 2020 Sep 8]. https://github.com/evogytis/baltic

10. Federal Ministry for Justice and Consumer Protection. Law on the Prevention and Control of Infectious Diseases in Humans [in German] [cited 2020 11 27]. https://www.gesetze-im-internet.de/ifsg/index.html

Appendix Table 1. Mapping statistics and single nucleotide polymorphisms of severe acute respiratory syndrome coronavirus 2 from an outbreak associated with a nightclub, Berlin, Germany, March 2020*

ID	Coverage depth†	Genome coverage, %	Ambiguous positions*	SNPs relative to the majority*	Sampling date, 2020
ChVir-D712–1	971.6 (3–6,636)	100	None	None	Mar 7
ChVir-D666–2	85.8 (4–307)	100	None	None	Mar 7
ChVir-D718–3	482.9 (3–6,898)	100	None	None	Mar 7
ChVir-D672–4	214 (3–487)	100	None	None	Mar 7
ChVir-D658–5	58.9 (3–206)	100	None	None	Mar 8
ChVir-D665–6	2,342.1 (3–5,966)	100	None	None	Mar 6
ChVir-D667–7	294.9 (3–773)	100	None	None	Mar 7
ChVir-D671–8	41.3 (2–107)	100	None	None	Mar 7
ChVir-D670–9	13.3 (0–51)	99.8	None	None	Mar 7
ChVir-D710–10	2,273.3 (34–4,992)	100	Position 20469 R, 2,144 reads have A, 1,573 reads have G. Confirmed by Sanger sequencing. 3rd codon position, synonymous.	None	Mar 7
ChVir-D711–11	2,924.1 (0–6,965)	99.8	Position 16570 K, 7,031 reads have G, 156 reads have T, but Sanger suggests T. 1st codon position, non-synonymous, C>G; Position 21515 R, 856 reads have A, 864 reads have G, 2nd codon position, non-synonymous, N > S; Position 25419 R; 4,657 reads have A, 6,708 reads have G, 3rd codon position, synonymous	Position 29780 A>G, 3′ UTR	Mar 7
ChVir-D717-D761–12	3,645.9 (3–5,927)	100	Position 14801 R (reference has A), 93 reads have G, 122 reads have A. 2nd codon position, D>G change.	None	Mar 7
ChVir-W1191–13	96.6 (3–427)	100	None	None	Mar 8
ChVir-D679–14	52.6 (3–225)	100	None	None	Mar 4
ChVir-D929–15	3,791.3 (0–427)	98	Position 545 K, 4,591 reads have G, 7,074 have T confirmed by Sanger, 1st codon position, non-synonymous, G>C	None	Mar 5
ChVir-W1248–16	132.2 (0–422)	99	None	Position 29254 G>T, 3rd codon position, synonymous	Mar 7
ChVir-D715-D799–17	4,553.4 (3–6646)	100	None	Position 29254 G>T, 3rd codon position, synonymous	Mar 6

*Positions are given relative to the reference genome, EPI_ISL402125 (Wuhan-1).
†Coverage depth indicates the mean number of reads covering each position in the genome. Numbers in parentheses show the minimum and maximum number of reads.

Appendix Table 2. Oligonucleotide used for amplification and sequencing of SARS-CoV-2 genome fragments

Primer ID	Sequence (5′→3′)	Use
SARS2_1_F	CAACTTTCGATCTCTTGTAGATCTG	1st round
SARS2_1_Fnest	GTCACTCGGCTGCATGCTTAGTG	2nd round
SARS2_1_R	GTTATCGACATAGCGAGTGTATGC	1st round and 2nd round
SARS2_2_F	GGAGCTGGTGGCCATAGTTACG	1st round
SARS2_2_Fnest	CATTTGACTTAGGCGACGAGC	2nd round
SARS2_2_R	TCCAAAGGCAATAGTGCGACC	1st round and 2nd round
SARS2_3_F	AAGTAGGACCTGAGCATAGTCTTG	1st round
SARS2_3_Fnest	CTTGCCGAATACCATAATGAATCTG	2nd round
SARS2_3_R	GTCTCTAAGAAACTCTACACCTTCCT	1st round and 2nd round
SARS2_4_F	ATCTAGTTGTAATGGCCTACATTACAG	1st round
SARS2_4_iF	TTCAGTTGAYTTCGCAGTGGC	1st round
SARS2_4_Fnest	GGCTAACTAACATCTTTGGCACTG	2nd round
SARS2_4_R	CGAACTCATTTACTTCTGTACCGAG	1st round and 2nd round
SARS2_5_F	TGCCCTTGCACCTAATATGATGG	1st round
SARS2_5_Fnest	ATAGAAGTGCAAGGTTACAAGAGTG	2nd round
SARS2_5_R	TGTTTAGCAAGATTGTGTCCGCT	1st round and 2nd round
SARS2_6_F	AGGAGGTGTTGCAGGAGCCT	1st round
SARS2_6_Fnest	ATAAGGCTACTAACAATGCCATGC	2nd round
SARS2_6_R	CTTTGCCTCCTCTACAGTGTAACC	1st round and 2nd round
SARS2_7_F	CAGATTCTGCCACTCTTGTTAGTG	1st round
SARS2_7_Fnest	CACTCTTGTTAGTGACATTGACATCAC	2nd round
SARS2_7_iFnest	TGGCACTACTGAAATGCTAGCGA	1st round
SARS2_7_R	AAGGTGATAACTTCACCATCTAGGTG	1st round and 2nd round
SARS2_8_F	CACCTGATGCTGTTACAGCGT	1st round
SARS2_8_iF	GATCTCTCAAAGTGCCAGCTACAG	1st round
SARS2_8_Fnest	ACCATCTCACTTGCTGGTTCCT	2nd round
SARS2_8_R	AAAGTGTGCCCATGTACATAACAGC	1st round and 2nd round
SARS2_9_F	CTGCTCTACAAGATGCTTATTACAG	1st round
SARS2_9_Fnest	AAGACAGTAGGTGAGTTAGGTGATG	2nd round
SARS2_9_R	TCTCTTGAAGCAGGTTTCTTATAACC	1st round and 2nd round
SARS2_10_F	GTACAGAAATTGACCCTAAGTTGGACA	1st round
SARS2_10_Fnest	CCATATCCAAACGCAAGCTTCG	2nd round
SARS2_10_R	AACAGTATTCTTTGCTATAGTAGTCGG	1st round and 2nd round
SARS2_11_F	GCTACTCATGGTTTAGCTGCTG	1st round
SARS2_11_Fnest	GCTGCTGTTAATAGTGTCCCTTG	2nd round
SARS2_11_R	TACATTCTAACCATAGCTGAAATCGG	1st round and 2nd round
SARS2_12_F	TGAACTCTACTAATGTCACTATTGCAAC	1st round
SARS2_12_Fnest	GCTTTTGGCTTAGTTGCAGAGT	2nd round
SARS2_12_R	TGCAACTTCCGCACTATCACC	1st round and 2nd round
SARS2_13_F	GAGCTAATAACACTAAAGKTTCATTGC	1st round
SARS2_13_Fnest	AGCGTCTGTTTACTACAGTCAGC	2nd round
SARS2_13_R	GCGCACTACAGTCAATACAAGC	1st round and 2nd round
SARS2_14_F	GCAAGGGTTTGTTGATTCAGATGTAG	1st round
SARS2_14_Fnest	ATCTGACATAGAAGTTACTGGCGATAG	2nd round
SARS2_14_R	CTGATGTTGCAAAGTCAGTGTACTC	1st round and 2nd round
SARS2_15_F	TGCTGCAGTCATAACAAGAGAAG	1st round
SARS2_15_iF	AAGCTTGCCCATTGATTGCTGC	1st round
SARS2_15_Fnest	GCCTGGCACGATATTACGCA	2nd round
SARS2_15_R	AAAGGTGTGAACATAACCATCCACTG	1st round and 2nd round
SARS2_16_F	GCTTTTGGTGAATACAGTYATGTAGTTG	1st round
SARS2_16_Fnest	CATTCAYTGTACTCTGTTTAACACCAG	2nd round
SARS2_16_R	CTTATACTTAGGTGTCTTAGGATTGGC	1st round and 2nd round
SARS2_17_F	CACCTCTGAAGACATGCTTAACC	1st round
SARS2_17_Fnest	ACAGGCTGGTAATGTTCAACTCAG	2nd round
SARS2_17_R	GAACAAAGACCATTGAGTACTCTGGAC	1st round and 2nd round
SARS2_18_F	AGGACCTCTTTCTGCTCAAACTG	1st round
SARS2_18_iF	TTCTGCTCAAACTGGAATTGCCG	1st round
SARS2_18_Fnest	TGCAAAATGGTATGAATGGACGTAC	2nd round
SARS2_18_iFnest	ACTGCAAAATGGTATGAATGGACGTAC	2nd round
SARS2_18_R	CCAAGAGTCAGTCTAAAGTAGCG	1st round and 2nd round
SARS2_19_F	GAGTATTGCCCTATTTTCTTCATAACTG	1st round
SARS2_19_Fnest	TTCTTCATAACTGGTAATACACTTCAGTG	2nd round
SARS2_19_R	TCTAAGCATAGTGAAAAGCATTGTCTG	1st round and 2nd round
SARS2_20_F	TGAATGTGGCTAAATCTGAATTTGACC	1st round
SARS2_20_Fnest	CAGCCATGCAACGTAAGTTGG	2nd round
SARS2_20_R	CTTGTAGACGTACTGTGGCAGC	1st round and 2nd round
SARS2_21_F	GCACTGATGACAATGCGTTAGC	1st round
SARS2_21_Fnest	CTTGCACTGTTATCCGATTTACAGG	2nd round

Primer ID	Sequence (5′→3′)	Use
SARS2_21_R	AGACGGGCTGCACTTACACC	1st round and 2nd round
SARS2_22_F	CAAATACCTACAACTTGTGCTAATGACC	1st round
SARS2_22_iF	TGCCGTTGCCACATAGATCATC	1st round
SARS2_22_Fnest	GGTTATGGCTGTAGTTGTGATCAAC	2nd round
SARS2_22_R	GCAGTTAAAGCCCTGGTCAAGGT	1st round and 2nd round
SARS2_22_iR	CCGAAATCATACCAGTTACCATTGAG	1st round and 2nd round
SARS2_23_F	TACGCCAACTTAGGTGAACGTG	1st round
SARS2_23_Fnest	TGATGCCATGCGAAATGCTG	2nd round
SARS2_23_R	CTGATAGCAGCATTACCATCCTG	1st round and 2nd round
SARS2_24_F	ACTAGATAAACGCACTACGTGCT	1st round
SARS2_24_iF	TAAGGAATTACTTGTGTATGCTGCTG	1st round
SARS2_24_Fnest	TAGCTGCACTTACTAACAATGTTGC	2nd round
SARS2_24_iFnest	TTCTATGACTTTGCTGTGTCTAAGG	2nd round
SARS2_24_R	GAGCAAGAACAAGTGAGGCCAT	1st round and 2nd round
SARS2_25_F	AATAGCCGCCACTAGAGGAG	1st round
SARS2_25_Fnest	GATTATCCTAAATGTGATAGAGCCATGC	2nd round
SARS2_25_R	CTATAGCTAAAGACACGAACCGTTC	1st round and 2nd round
SARS2_26_F	GCAAAATGTTGGACTGAGACTGACC	1st round
SARS2_26_Fnest	CTCAACATACAATGCTAGTTAAACAGG	2nd round
SARS2_26_R	TGAGTCTTTCAGTACAGGTGTTAGC	1st round and 2nd round
SARS2_27_F	TCAACTTTACTTAGGAGGTATGAGCT	1st round
SARS2_27_Fnest	CACCCATTAGTTTTCCATTGTGTGC	2nd round
SARS2_27_R	AAAGACATACTGTTCTAATGTTGAATTCAC	1st round and 2nd round
SARS2_28_F	AAGTATTCTACACTCCAGGGACCAC	1st round
SARS2_28_iF	GAGCACTATGTTAGAATTACTGGCT	1st round
SARS2_28_Fnest	TACTACCCTTCTGCTCGCATAG	2nd round
SARS2_28_R	GAGCCCTGTGATGAATCAACAGT	1st round and 2nd round
SARS2_29_F	CAGGCCACAAATAGGCGTGG	1st round
SARS2_29_Fnest	CTTACACGTAACCCTGCTTGGAG	2nd round
SARS2_29_R	TCTCCAGGCGGTGGTTTAGC	1st round and 2nd round
SARS2_30_F	CCCGCGAAGAAGCTATAAGAC	1st round
SARS2_30_Fnest	CATGGATTGGCTTCGATGTCG	2nd round
SARS2_30_R	GGTTACCAATGTCGTGAAGAACTGG	1st round and 2nd round
SARS2_31_F	CCATGATCTGTATTGTCAAGTCCATG	1st round
SARS2_31_Fnest	TCTAGCTGTCCACGAGTGCT	2nd round
SARS2_31_R	CCACAAGCTAAAGCCAGCTGA	1st round and 2nd round
SARS2_32_F	GATTTGACACTAGAGTGCTATCTAACC	1st round
SARS2_32_Fnest	TAGAGTGCTATCTAACCTTAACTTGC	2nd round
SARS2_32_R	CAGTGAGTGGTGCACAAATCGT	1st round and 2nd round
SARS2_33_F	GCGCAACATTAAACCAGTACCAG	1st round
SARS2_33_Fnest	ACATTGCTGCTAATACTGTGATCTG	2nd round
SARS2_33_R	CCTTAGAAACTACAGATAAATCTTGGGA	1st round and 2nd round
SARS2_34_F	TGGTTTACATCTACTGATTGGACTAGC	1st round
SARS2_34_Fnest	ATAACAGATGCGCAAACAGGTTC	2nd round
SARS2_34_R	TTATCTTTATAGCCACGGAACCTCC	1st round and 2nd round
SARS2_35_F	TTTGATTGGTGATTGTGCAACTGTAC	1st round
SARS2_35_Fnest	GGATCTCATTATTAGTGATATGTACGACC	2nd round
SARS2_35_R	TGGGTCTTCGAATCTAAAGTAGTACC	1st round and 2nd round
SARS2_36_F	CTCAGTTTTACATTCAACTCAGGACT	1st round
SARS2_36_Fnest	TAACCCTGTCCTACCATTTAATGATGG	2nd round
SARS2_36_R	GGTCAAGTGCACAGTCTACAGC	1st round and 2nd round
SARS2_37_F	AGTGCGTGATCTCCCTCAGG	1st round
SARS2_37_Fnest	AGGTTGGACAGCTGGTGCTG	2nd round
SARS2_37_R	AAGGTGTGCTACCGGCCTG	1st round and 2nd round
SARS2_37_iR	GTCCACAAACAGTTGCTGGTGC	1st round and 2nd round
SARS2_38_F	GAAGTCAGACAAATCGCTCCAG	1st round
SARS2_38_Fnest	CCAGATGATTTTACAGGCTGCG	2nd round
SARS2_38_R	ACTAGCGCATATRCCTGCACC	1st round and 2nd round
SARS2_39_F	CAGGAACAAATACTTCTAACCAGGTTG	1st round
SARS2_39_Fnest	GAAGTCCCTGTTGCTATTCATGC	2nd round
SARS2_39_R	TAACAGTGCAGAAGTGTATTGAGC	1st round and 2nd round
SARS2_40_F	AGATCCATCAAAACCAAGCAAGAG	1st round
SARS2_40_Fnest	GACACTTGCAGATGCTGGCT	2nd round
SARS2_40_R	CCATGAGGTGCTGACTGAGG	1st round and 2nd round
SARS2_41_F	AGGCTGAAGTGCAAATTGATAGGT	1st round
SARS2_41_Fnest	TAGAGCTGCAGAAATCAGAGC	2nd round
SARS2_41_R	GACTCCTTTGAGCACTGGCT	1st round and 2nd round
SARS2_42_F	CTCATCGATCTCCAAGAACTTGG	1st round
SARS2_42_Fnest	GCTTGATTGCCATAGTAATGGTGAC	2nd round

Primer ID	Sequence (5′→3′)	Use
SARS2_42_R	TGAGTACAGCTGGTAATAGTCTGAAG	1st round and 2nd round
SARS2_43_F	TTTGCTGGAAATGCCGTTCCA	1st round
SARS2_43_Fnest	CTTTGCTGGCATACTAATTGTTACG	2nd round
SARS2_43_R	TGTAGAAGACAAATCCATGTAAGGAATAG	1st round and 2nd round
SARS2_44_F	CTTCTAGAGTTCCTGATCTTCTGG	1st round
SARS2_44_Fnest	CCATGGCAGATTCCAACGGTAC	2nd round
SARS2_44_R	GCTATAGTAACCTGAAAGTCAACGAG	1st round and 2nd round
SARS2_45_F	CAGTCGCTACAGGATTGGCA	1st round
SARS2_45_Fnest	CACAGACCATTCCAGTAGCAGTG	2nd round
SARS2_45_R	GACACGGGTCATCAACTACATATGG	1st round and 2nd round
SARS2_46_F	TTAGGAATCATCACAACTGTAGCTG	1st round
SARS2_46_Fnest	TAGCTGCATTTCACCAAGAATGTAG	2nd round
SARS2_46_R	TGGTAGCTCTTCGGTAGTAGCC	1st round and 2nd round
SARS2_46_iR	GAAGTTGTAGCACGATTGCAGC	1st round and 2nd round
SARS2_47_F	TAACCAGAATGGAGAACGCAGTG	1st round
SARS2_47_Fnest	GGTTCACCGCTCTCACTCAAC	2nd round
SARS2_47_R	CGGCCAATGTTTGTAATCAGTTCC	1st round and 2nd round
SARS2_48_F	CTGCTGAGGCTTCTAAGAAGC	1st round
SARS2_48_iF	GCTTGACAGATTGAACCAGCTTG	1st round
SARS2_48_Fnest	TGGCAGACGTGGTCCAGAAC	2nd round
SARS2_48_R	CTCCTRAGAAGCTATTAAAATCACATGG	1st round and 2nd round

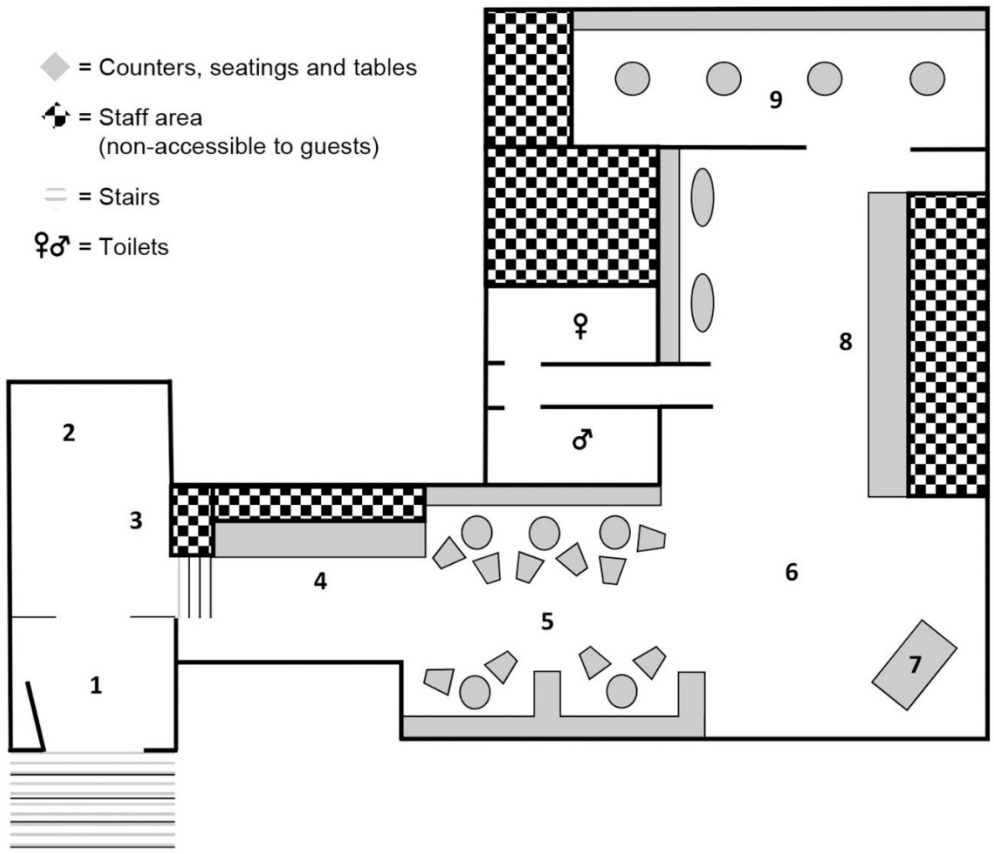
Appendix Table 3. Coverage depth and number of reads mapped against the reference (GenBank accession no. NC_045512.2) for each sequencing run*

ID	Total reads mapped against SARS-CoV-2	Mean coverage depth (range)
ChVir-D712–1	102,968	971.6 (3–6,636)
ChVir-D666–2	21,192	85.8 (4–307)
ChVir-D718–3	482,720	482.9 (3–6,898)
ChVir-D672–4	52,215	214 (3–487)
ChVir-D658–5	14,485	58.9 (3–206)
ChVir-D665–6	569,265	2,342.1 (3–5,966)
ChVir-D667–7	71,902	294.9 (3–773)
ChVir-D671–8	10,051	41.3 (2–107)
ChVir-D670–9	2,561	13.3 (0–51)
ChVir-D710–10	212,223	2,273.3 (34–4,992)
ChVir-D711–11	301,653	2,924.1 (0–6,965)
ChVir-D717-D761–12	333,782	3,645.9 (3–5,927)
ChVir-W1191–13	9,892	96.6 (3–427)
ChVir-D679–14	15,850	52.6 (3–225)
ChVir-D929–15	447,129	3,791.3 (0–427)
ChVir-W1248–16	12,763	132.2 (0–422)
ChVir-D715-D799–17	444,374	4,553.4 (3–6,646)

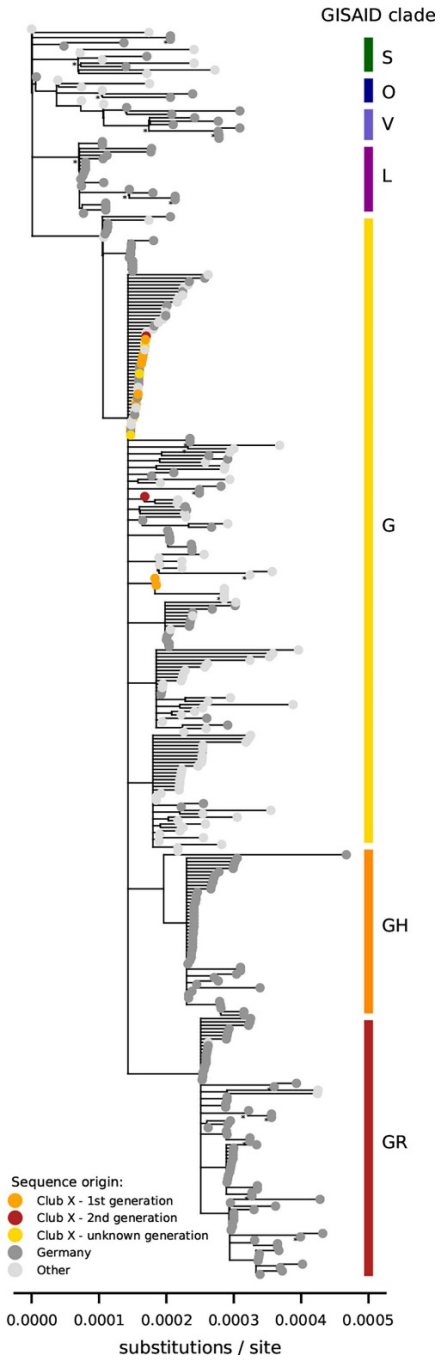
*Coverage depth indicates the mean number of reads covering each base. Minimum and maximum number of reads are given in parentheses.

Appendix Table 4. Demographics and clinical symptoms of cases in a coronavirus disease outbreak in a nightclub, Berlin, Germany, March 2020

Demographics	No. (%)
No. total cases	74 (100)
Median age, y	30
Sex	
F	37 (50)
M	37 (50)
Clinical symptoms (no. queried)	
Dysgeusia (n = 43)	28 (65)
Cough (n = 44)	27 (61)
Headache (n = 43)	25 (58)
Dysosmia (n = 43)	25 (58)
Shivering, shaking (n = 43)	21 (49)
Myalgia (n = 43)	20 (47)
Rhinitis (n = 44)	20 (45)
Fever (n = 42)	18 (43)
Sore throat (n = 42)	12 (29)
Vertigo (n = 39)	5 (13)
Nausea (n = 42)	4 (9)



Appendix Figure 1. Illustration of the floorplan of nightclub involved in a coronavirus disease outbreak, Berlin, Germany, March 2020. Numerals represent the following: 1) entry area; 2) coat check area; 3) cashier; 4) bar counter 1; 5) lounge; 6) dance floor; 7) DJ booth; 8) bar counter 2; and 9) smoking lounge. This figure is not true to scale.



Appendix Figure 2. Maximum likelihood phylogenetic tree showing the positions of the sequences associated with a coronavirus disease outbreak in a nightclub, Berlin, Germany, March 2020. Orange circles indicate cases in the nightclub outbreak. Blue circles indicate available sequences from Germany sampled before April 15, 2020. Gray circles indicate a subset of sequences from additional countries. The x-axis shows substitutions per site. Asterisks indicate nodes with bootstrap support >70. Nodes with bootstrap support <5 are shown as polytomies. To view the sequences from club X in a wider context of currently unpublished sequences from Germany, see https://civnb.info/sequences.