patient, warrants further study. To our knowledge, no studies demonstrate the relationship between anti-GM-CSF AAb and COVID-19 vaccines. However, research showed that autoantibody such as anti–smooth muscle antibody was triggered by COVID-19 vaccine as autoimmune hepatitis (*7*). In conclusion, clinicians should be aware of possible *C. tetragattii* fungal infection in patients who have traveled to meningitis-endemic regions.

About the Author

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Metagenomic Sequencing of Monkeypox Virus, Northern Mexico

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Monkeypox virus (MPXV) has gained interest because of a multicountry outbreak of mpox (formerly monkeypox) cases with no epidemiologic link to MPXV-endemic regions. We sequenced the complete genome of MPXV isolated from a patient in northern Mexico. Phylogenetic analysis grouped the virus with isolates from Germany.

Monkeypox virus (MPXV) is a zoonotic pathogen that causes mpox (formerly monkeypox), a febrile rash disease, in humans. It has caused multiple outbreaks in the past (*1*) but recently acquired international attention because of a multicountry outbreak of mpox cases with no epidemiologic link to MPXV-endemic regions (*2*). During January–June 2022, a total of 3,413 laboratory-confirmed cases and 1 death were reported to the World Health Organization (*3*).

MPXV is a double-stranded DNA virus that belongs to the genus *Orthopoxvirus* within the Poxviridae family. A total of 3 clades have been proposed: clades 1, 2, and 3 (*4*). Genomes belonging to the recent outbreaks gather at clade 3 and create the human MPXV1 subclade.

As of July 4, 2022, Mexico had 27 confirmed mpox infections (*5*). On June 28, 2022, a 34-year-old man with HIV sought care at the Dermatology Service of the Hospital Universitario Dr. José Eleuterio González (Monterrey, Mexico). The patient had multiple 1–2-cm flesh-colored papules with ulcerated centers and elevated borders on the dorsal area of the penis and the groin area and had bilateral inguinal lymphadenopathy. He had engaged in multiple high-risk sexual encounters during the previous several months and had traveled to Mexico City and Guadalajara. We collected skin swab samples from the lesions and extracted DNA by using the High Pure PCR Template Preparation Kit

(Roche, https://www.roche.com) in a biological safety cabinet. We disposed of hazardous biological infectious waste according to NOM-087-ECOL-SSA1–2002 and Centers for Disease Control and Prevention regulations. We used quantitative PCR to detect viral DNA as described in Li et al. (*6*). The study was approved by the ethics committee of the Hospital Universitario Dr. José Eleuterio González (registration no. IF22-00006). Written informed consent was obtained from the patient.

We performed genome sequencing at the Medical Virology Research and Innovation Center (Monterrey, Mexico). We used 700 ng of DNA to prepare the library with the Ligation Sequencing Kit (Oxford Nanopore Technologies, https://nanoporetech.com), by duplicate, following the manufacturer's protocol. We loaded libraries to a FLO-MIN106 R9.4.1. on a MinION device (Oxford Nanopore Technologies). We used high-accuracy basecalling and depleted the human genome by adaptive sampling reference (GRCh38.p14, GCF_000001405.40) in MinKNOW version 22.05.5 (Oxford Nanopore Technologies).

We assembled the genome with Minimap2 version 2.17, using an MPXV reference genome (GenBank accession no. NC_063383.1), and consensus sequence was obtained with Medaka version 1.0.3 (https://pypi.org/project/medaka/1.0.3) and bcftools version 1.10.12 (https://github.com/samtools/bcftools/releases). We deposited the obtained sequence in GISAID (accession no. EPI_ISL_13607904) and GenBank (accession no. ON911481). Furthermore, raw reads were mapped against the human genome and filtered out using Minimap2 and Samtools version 1.16 (http://www.htslib.org). Remaining reads were assembled de novo with Flye version 2.9.1 (https://anaconda.org/bioconda/flye) and subsequently polished using Medaka and homopolish version 0.4 (https://anaconda.org/bioconda/homopolish/files).

We used Nextstrain's mpox bioinformatic processing workflow (*7*) for our initial analysis. Subsequently, we downloaded 399 sequences from GenBank that belonged to clade hMPXV1 from outbreaks in 2017–2019 and 2022 (the available sequences as of July 14, 2022).

We masked and aligned sequences in MAFFT version 7.505 (https://mafft.cbrc.jp/alignment/software). We inferred a maximum-likelihood phylogenetic tree with IQ-TREE version 2.0.3 (http://www.iqtree.org) using the Hasegawa-Kishino-Yano plus base frequencies plus invariant sites substitution model with 1,000 replicates for SH-like approximate likelihood ratio test and 1,000 replicates for ultrafast bootstrap.

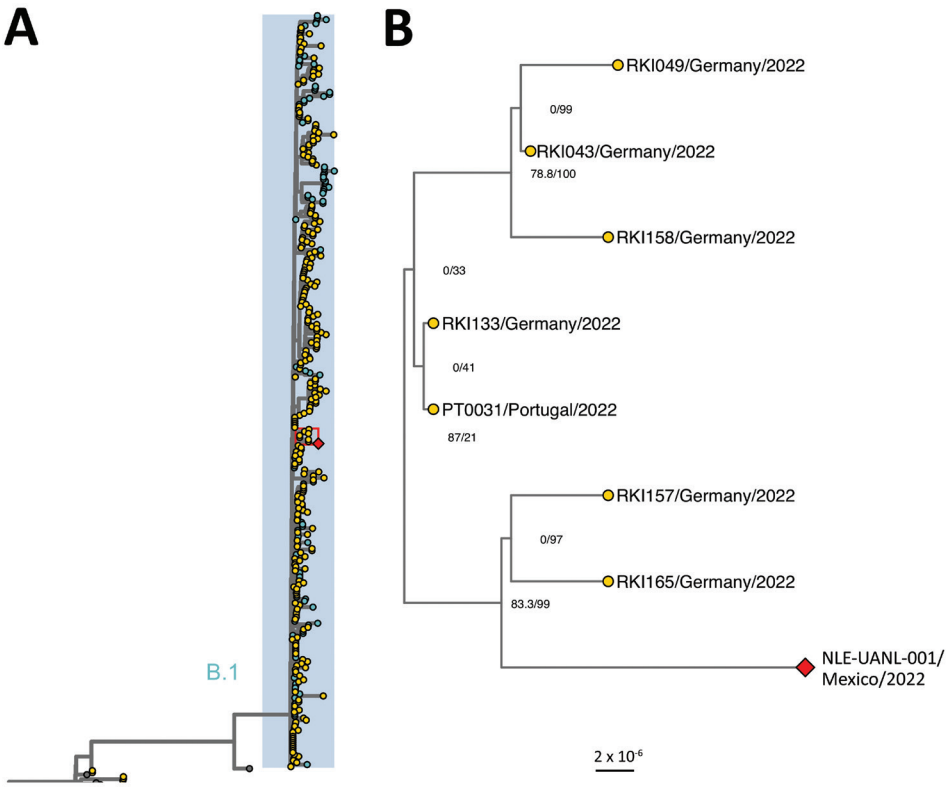

Figure. Phylogenetic relationship of monkeypox virus genomes, 2017–2022. A) Maximum-likelihood phylogenetic tree with 400 whole-genome sequences of monkeypox virus made in IQTree (http://www.iqtree.org). Highlighted genomes in blue belong to the proposed B.1 lineage. B) Magnification of area in red box in panel A showing clade indicating the Mexico isolate and related sequences. Node numbers indicate SH-like approximate likelihood ratio test and ultrafast bootstrap values. The red diamond corresponds to the sequence from this study and yellow circles to sequences from Europe. The list of accession numbers of the sequences is shown in the Appendix Table (https://wwwnc.cdc.gov/EID/article/29/2/22-1199-App1.pdf). The tree was annotated in ggtree (https://bioconductor.org/packages/release/bioc/html/ggtree.html). Scale bar indicates substitutions per site.

Adding both sequencing runs, we obtained 1.77 million reads with an estimated N50 of 17.2 kb after a 20-h run. The MPXV genome sequence obtained by reference assembly was composed of 55,512 mapped reads with a breadth of 197,414 bp, 751× average depth, and 1,212× maximum depth. The sequence obtained by de novo assembly was composed of 58,259 mapped reads with a breadth 197,875 bp, 752× average depth, and 1,215× maximum depth.

Phylogenetic analyses revealed that the obtained sequence clusters within the newly proposed B.1 lineage from clade 3, where sequences from the multicountry mpox outbreak are located (Figure, panel A; Appendix Figure 1, panel A, https://wwwnc.cdc.gov/EID/article/29/2/22-1199-App1.pdf). Sequences from Europe (specifically Portugal and Germany) dominate the tree because they make up most of the available sequences. The Mexico sequence groups in a well-supported clade with sequences from Germany (Figure, panel B; Appendix Figure 1, panel B), although we could not establish an epidemiologic relationship. The sequence is grouped in the same clade in both approaches, independent of the assembly method (Appendix Figure 2). The inferred date of the most recent common ancestor of the clade is May 26, 2022 (95% CI May 26, 2022–June 2, 2022). Sequences from this clade share the mutation G120241A, causing a nonsynonymous substitution in OPG137, D214N. OPG137 encodes for a virion membrane assembly protein, which is involved in the crescent membrane and immature virion formation (*8*). Aspartic acid is an acidic amino acid and asparagine is neutral. The effect of this mutation has yet to be determined.

In conclusion, we sequenced the whole genome of MXPV from a patient with mpox in northern Mexico. Subsequent sequencing will unravel how the virus is transmitting and adapting throughout the population. According to our results, this patient likely had close contact with an infected person who traveled from Europe.

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Metagenomic Sequencing of Monkeypox Virus Case, Northern Mexico

Appendix

Appendix Table. NCBI Genbank dataset used in this study

Accession number	Country	Lineage	Collection date	Reference
KJ642615.1	Nigeria	NA	1978	Nakazawa,Y., 2014
KJ642617.1	Nigeria	NA	1971	Nakazawa,Y., 2014
MG693723.1	Nigeria	A	2017–12–31	Faye,O. 2017
MG693724.1	Nigeria	A	2017	Faye,O. 2017
MK783027.1	Nigeria	A	2017	Yinka-Ogunleye,A. 2019
MK783028.1	Nigeria	A	2017–11–09	Yinka-Ogunleye,A. 2019
MK783029.1	Nigeria	A	2017–12–06	Yinka-Ogunleye,A. 2019
MK783030.1	Nigeria	A	2017–11–30	Yinka-Ogunleye,A. 2019
MK783031.1	Nigeria	A	2017–11–09	Yinka-Ogunleye,A. 2019
MK783032.1	Nigeria	A	2017–11	Yinka-Ogunleye,A. 2019
MK783033.1	Nigeria	A	2017	Yinka-Ogunleye,A. 2019
MN648051.1	Israel	A.1	2018–10–04	Cohen Gihon,I. 2019
MT250197.1	Singapore	A.1	2019	Yong,SEF. 2020
MT903337.1	Nigeria	A	2018	Mauldin,M.R. 2020
MT903338.1	Nigeria	A	2018	Mauldin,M.R. 2020
MT903339.1	Nigeria	A	2018	Mauldin,M.R. 2020
MT903340.1	Nigeria	A	2018	Mauldin,M.R. 2020
MT903341.1	Nigeria	A.1	2018–08–14	Mauldin,M.R. 2020
MT903342.1	Singapore	A.1	2019–04–30	Mauldin,M.R. 2020
MT903343.1	UK	A.1	2018	Mauldin,M.R. 2020
MT903344.1	UK	A.1	2018	Mauldin,M.R. 2020
MT903345.1	UK	A.1	2018	Mauldin,M.R. 2020
NC_063383.1	Nigeria	A	2018–08–01	Mauldin,M.R. 2022
ON563414.3	USA	B.1	2022–05	Gigante,C.M. 2022
ON568298.1	Germany	B.1	2022–05–19	Antwerpen,M.H. 2022
ON585029.1	Portugal	B.1	2022–05–04	Isidro,J. 2022
ON585030.1	Portugal	B.1	2022–05–15	Isidro,J. 2022
ON585031.1	Portugal	B.1	2022–05–15	Isidro,J. 2022
ON585032.1	Portugal	B.1	2022–05–17	Isidro,J. 2022
ON585033.1	Portugal	B.1	2022–05–15	Isidro,J. 2022
ON585034.1	Portugal	B.1	2022–05–15	Isidro,J. 2022
ON585035.1	Portugal	B.1	2022–05–15	Isidro,J. 2022
ON585036.1	Portugal	B.1	2022–05–15	Isidro,J. 2022
ON585037.1	Portugal	B.1	2022–05–15	Isidro,J. 2022
ON585038.1	Portugal	B.1	2022–05–15	Isidro,J. 2022
ON595760.2	Switzerland	B.1	2022–05–19	Laubscher,F. 2022
ON602722.2	France	B.1	2022–05	Croville,G. 2022
ON609725.2	Slovenia	B.1	2022–05–23	Zakotnik,S. 2022
ON614676.1	Italy	B.1	2022–05–18	Gruber,C.E.M. 2022
ON615424.1	Netherlands	B.1	2022–05	Oude Munnink,B.B. 2022
ON619835.2	UK	B.1	2022–05	Groves,N. 2022
ON619836.2	UK	B.1	2022–05	Groves,N. 2022
ON619837.2	UK	B.1	2022–05	Groves,N. 2022
ON619838.2	UK	B.1	2022–05	Groves,N. 2022
ON622712.1	Belgium	B.1	2022–05–19	Vanmechelen,B. 2022
ON622713.1	Belgium	B.1	2022–05–22	Wawina-Bokalanga,T. 2022
ON622718.1	Spain	B.1	2022–05	Martinez-Puchol,S. 2022
ON622720.1	Switzerland	B.1	2022–05–24	Laubscher,F. 2022
ON622722.2	France	B.1	2022–05–22	2022
ON627808.1	USA	B.1	2022–05–20	Young,E.L. 2022
ON631241.1	Slovenia	B.1	2022–05–24	Zakotnik,S. 2022
ON631963.1	Australia	B.1	2022–05	Hammerschlag,Y. 2022
ON637938.1	Germany	B.1	2022–05	Brinkmann,A. 2022
ON637939.1	Germany	B.1	2022–05	Brinkmann,A. 2022

Accession number	Country	Lineage	Collection date	Reference
ON644344.1	Italy	B.1	2022–05–25	Licastro,D. 2022
ON645312.1	UK	B.1	2022–05–11	Alcolea-Medina,A. 2022
ON649708.1	Portugal	B.1	2022–05–19	Isidro,J. 2022
ON649709.1	Portugal	B.1	2022–05–18	Isidro,J. 2022
ON649710.1	Portugal	B.1	2022–05–18	Isidro,J. 2022
ON649711.1	Portugal	B.1	2022–05–18	Isidro,J. 2022
ON649712.1	Portugal	B.1	2022–05–19	Isidro,J. 2022
ON649713.1	Portugal	B.1	2022–05–19	Isidro,J. 2022
ON649714.1	Portugal	B.1	2022–05–20	Isidro,J. 2022
ON649715.1	Portugal	B.1	2022–05–20	Isidro,J. 2022
ON649716.1	Portugal	B.1	2022–05–23	Isidro,J. 2022
ON649717.1	Portugal	B.1	2022–05–21	Isidro,J. 2022
ON649718.1	Portugal	B.1	2022–05–20	Isidro,J. 2022
ON649719.1	Portugal	B.1	2022–05–23	Isidro,J. 2022
ON649720.1	Portugal	B.1	2022–05–19	Isidro,J. 2022
ON649721.1	Portugal	B.1	2022–05–19	Isidro,J. 2022
ON649722.1	Portugal	B.1	2022–05–19	Isidro,J. 2022
ON649723.1	Portugal	B.1	2022–05–20	Isidro,J. 2022
ON649724.1	Portugal	B.1	2022–05–23	Isidro,J. 2022
ON649725.1	Portugal	B.1	2022–05–23	Isidro,J. 2022
ON649879.1	Israel	B.1	2022–05–20	Israeli,O. 2022
ON674051.1	USA	A.2	2022–05	Gigante,C.M. 2022
ON675438.1	USA	A.2	2022–05	Gigante,C.M. 2022
ON676703.1	USA	B.1	2022–05	Gigante,C.M. 2022
ON676704.1	USA	B.1	2022–05	Gigante,C.M. 2022
ON676705.1	USA	B.1	2022–05	Gigante,C.M. 2022
ON676706.1	USA	B.1	2022–05	Gigante,C.M. 2022
ON676707.1	USA	A.2	2021–07	Gigante,C.M. 2022
ON676708.1	USA	A.1.1	2021–11	Gigante,C.M. 2022
ON682263.3	Germany	B.1	2022–05–04	Brinkmann,A. 2022
ON682264.3	Germany	B.1	2022–05–31	Brinkmann,A. 2022
ON682265.3	Germany	B.1	2022–05–15	Brinkmann,A. 2022
ON682266.2	Germany	B.1	2022–05–04	Brinkmann,A. 2022
ON682267.2	Germany	B.1	2022–05–01	Brinkmann,A. 2022
ON682268.2	Germany	B.1	2022–05–04	Brinkmann,A. 2022
ON682269.3	Germany	B.1	2022–05–01	Brinkmann,A. 2022
ON682270.2	Germany	B.1	2022–05–01	Brinkmann,A. 2022
ON694329.1	Germany	B.1	2022–05–15	Brinkmann,A. 2022
ON694330.1	Germany	B.1	2022–05–31	Brinkmann,A. 2022
ON694331.1	Germany	B.1	2022–05–18	Brinkmann,A. 2022
ON694332.1	Germany	B.1	2022–05–31	Brinkmann,A. 2022
ON694333.1	Germany	B.1	2022–05–15	Brinkmann,A. 2022
ON694334.1	Germany	B.1	2022–05–31	Brinkmann,A. 2022
ON694335.1	Germany	B.1	2022–05–23	Brinkmann,A. 2022
ON694336.1	Germany	B.1	2022–05–01	Brinkmann,A. 2022
ON694337.1	Germany	B.1	2022–05–31	Brinkmann,A. 2022
ON694338.1	Germany	B.1	2022–05–15	Brinkmann,A. 2022
ON694339.1	Germany	B.1	2022–05–18	Brinkmann,A. 2022
ON694340.1	Germany	B.1	2022–05–31	Brinkmann,A. 2022
ON694341.1	Germany	B.1	2022–05–04	Brinkmann,A. 2022
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ON720848.1	Spain	B.1	2022–05–27	Buenestado-Serrano,S. 2022
ON720849.1	Spain	B.1	2022–05–27	Buenestado-Serrano,S. 2022
ON736420.1	Canada	B.1	2022–05–31	Croxen,M. 2022
ON745215.1	Italy	B.1	2022–05–19	Giombini,E. 2022
ON745225.1	Spain	B.1	2022–05–27	Buenestado-Serrano,S. 2022
ON751962.1	Brazil	B.1	2022–06–07	Claro,I.M. 2022
ON754984.1	Slovenia	B.1	2022–06–01	Zakotnik,S. 2022
ON754985.1	Slovenia	B.1	2022–06–01	Zakotnik,S. 2022
ON754986.1	Slovenia	B.1	2022–06–01	Zakotnik,S. 2022
ON754987.1	Slovenia	B.1	2022–05–23	Zakotnik,S. 2022
ON754989.1	Canada	B.1	2022–06–06	Croxen,M. 2022
ON755039.1	France	B.1	2022–05–19	Jarjaval,F. 2022
ON755040.1	France	B.1	2022–05–20	Jarjaval,F. 2022
ON755231.1	Germany	B.1	2022–06–01	Brinkmann,A. 2022
ON755232.1	Germany	B.1	2022–06–01	Brinkmann,A. 2022
ON755233.1	Germany	B.1	2022–06–01	Brinkmann,A. 2022
ON755234.1	Germany	B.1	2022–06–01	Brinkmann,A. 2022
ON755235.1	Germany	B.1	2022–06–01	Brinkmann,A. 2022

Accession number	Country	Lineage	Collection date	Reference
ON755236.1	Germany	B.1	2022–06–01	Brinkmann,A. 2022
ON755237.1	Germany	B.1	2022–06–01	Brinkmann,A. 2022
ON755238.1	Germany	B.1	2022–06–15	Brinkmann,A. 2022
ON755239.1	Germany	B.1	2022–06–15	Brinkmann,A. 2022
ON755240.1	Germany	B.1	2022–06–15	Brinkmann,A. 2022
ON755241.1	Germany	B.1	2022–06–01	Brinkmann,A. 2022
ON755242.1	Germany	B.1	2022–06–01	Brinkmann,A. 2022
ON755243.1	Germany	B.1	2022–06–15	Brinkmann,A. 2022
ON755244.1	Germany	B.1	2022–06–15	Brinkmann,A. 2022
ON755245.1	Germany	B.1	2022–06–01	Brinkmann,A. 2022
ON755246.1	Germany	B.1	2022–06–01	Brinkmann,A. 2022
ON755247.1	Germany	B.1	2022–06–15	Brinkmann,A. 2022
ON755248.1	Germany	B.1	2022–06–01	Brinkmann,A. 2022
ON755249.1	Germany	B.1	2022–06–15	Brinkmann,A. 2022
ON755250.1	Germany	B.1	2022–06–01	Brinkmann,A. 2022
ON755251.1	Germany	B.1	2022–06–15	Brinkmann,A. 2022
ON755252.1	Germany	B.1	2022–06–15	Brinkmann,A. 2022
ON755253.1	Germany	B.1	2022–06–01	Brinkmann,A. 2022
ON755254.1	Germany	B.1	2022–06–15	Brinkmann,A. 2022
ON755255.1	Germany	B.1	2022–06–15	Brinkmann,A. 2022
ON755256.1	Germany	B.1	2022–06–15	Brinkmann,A. 2022
OL504741.1	UK	A.1	2019–12	Atkinson,B. 2022
OL504742.1	UK	A.1	2019–12	Atkinson,B. 2022
OL504743.1	UK	A.1	2019–12	Atkinson,B. 2022
ON780016.1	Italy	B.1	2022–05–19	Rueca,M. 2022
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ON782054.1	Spain	B.1	2022–05–31	Alcoba-Florez,J. 2022
ON782055.1	Spain	B.1	2022–05–31	Alcoba-Florez,J. 2022
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ON792322.1	Switzerland	B.1	2022–06–02	Kufner,V. 2022
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ON803435.1	Canada	B.1	2022–05–20	Knox,N. 2022
ON803436.1	Canada	B.1	2022–05–20	Knox,N. 2022
ON803437.1	Canada	B.1	2022–05–20	Knox,N. 2022
ON803438.1	Canada	B.1	2022–05–20	Knox,N. 2022
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ON808416.1	UK	B.1	2022–05–15	Filipe,A. 2022

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ON838178.1	Slovenia	B.1	2022–06	Zakotnik,S. 2022
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ON838940.1	Spain	B.1	2022–05–20	de la Hoz-Sanchez,B. 2022
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Accession number	Country	Lineage	Collection date	Reference
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ON853682.1	Germany	B.1	2022–06	Brinkmann,A. 2022
ON872184.1	Ireland	B.1	2022–05–30	Fletcher,N. 2022
ON880413.1	Brazil	B.1	2022–06–14	Coletti,T.M. 2022
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ON880530.1	Canada	B.1	2022–05–29	Duggan,A. 2022
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ON880534.1	Canada	B.1	2022–05–30	Duggan,A. 2022
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ON880548.1	Canada	B.1	2022–06–03	Duggan,A. 2022
ON880549.1	Canada	B.1	2022–06–03	Duggan,A. 2022
ON911481.2	Mexico	B.1	2022–06–28	Galan-Huerta,K.A. 2022
ON918656.1	Taiwan	B.1	2022–06	Lin,J.-H. 2022
ON927243.1	Spain	B.1	2022–06–21	Palomino-Cabrera,R. 2022
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ON929083.1	Germany	B.1	2022–07	Brinkmann,A. 2022
ON929084.1	Germany	B.1	2022–07	Brinkmann,A. 2022
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ON929089.1	Germany	B.1	2022–07	Brinkmann,A. 2022
ON929090.1	Germany	B.1	2022–07	Brinkmann,A. 2022
ON929091.1	Germany	B.1	2022–07	Brinkmann,A. 2022
ON950045.1	Belgium	B.1	2022–05–27	De Baetselier,I. 2022
ON954773.1	USA	B.1	2022–05	Gigante,C.M. 2022
ON959131.1	USA	B.1	2022–05	Gigante,C.M. 2022
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ON959135.1	USA	B.1	2022–05	Gigante,C.M. 2022
ON959136.1	USA	B.1	2022–06	Gigante,C.M. 2022
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ON959149.1	Germany	B.1	2022–07	Brinkmann,A. 2022
ON959150.1	Germany	B.1	2022–07	Brinkmann,A. 2022
ON959151.1	Germany	B.1	2022–07	Brinkmann,A. 2022
ON959152.1	Germany	B.1	2022–07	Brinkmann,A. 2022
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ON959154.1	Germany	B.1	2022–07	Brinkmann,A. 2022
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ON959157.1	Germany	B.1	2022–07	Brinkmann,A. 2022
ON959158.1	Germany	B.1	2022–07	Brinkmann,A. 2022
ON959159.1	Germany	B.1	2022–07	Brinkmann,A. 2022
ON959160.1	Germany	B.1	2022–07	Brinkmann,A. 2022
ON959161.1	Germany	B.1	2022–07	Brinkmann,A. 2022
ON959162.1	Germany	B.1	2022–07	Brinkmann,A. 2022
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ON959165.1	Germany	B.1	2022–07	Brinkmann,A. 2022
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Accession number	Country	Lineage	Collection date	Reference
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ON983167.1	Canada	B.1	2022–06–16	Duggan,A. 2022
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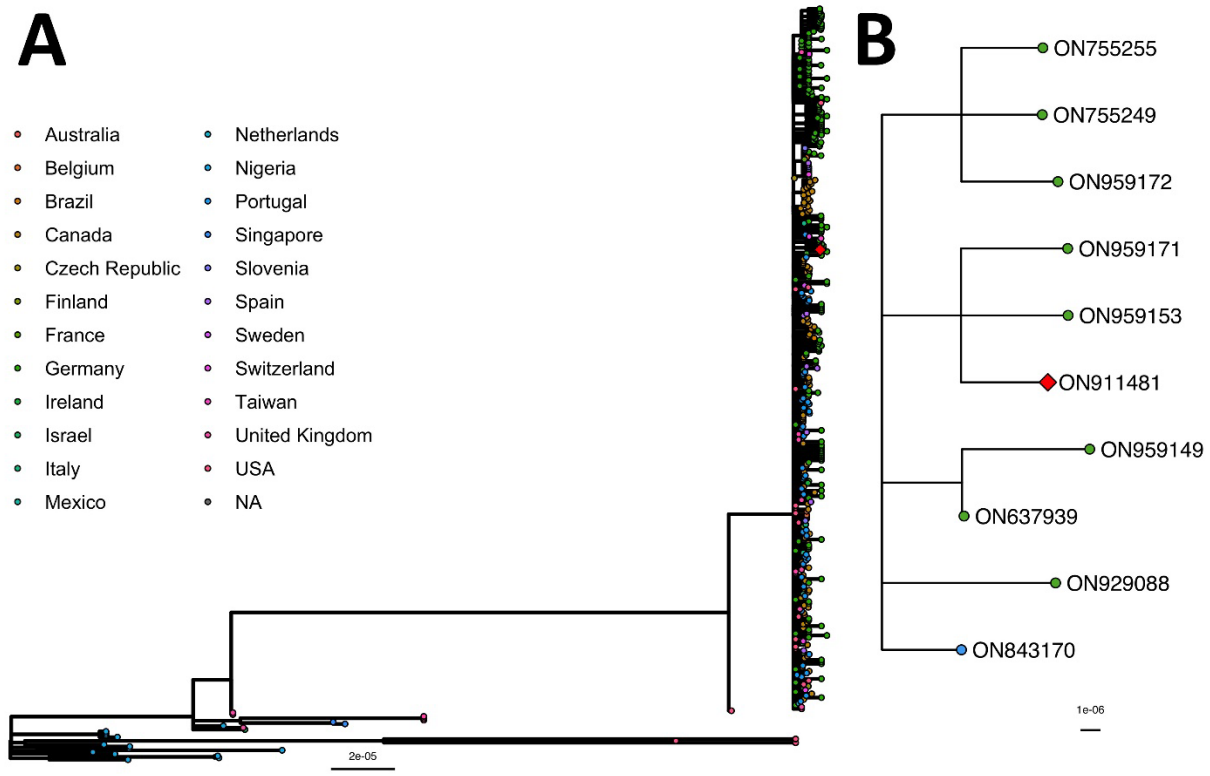
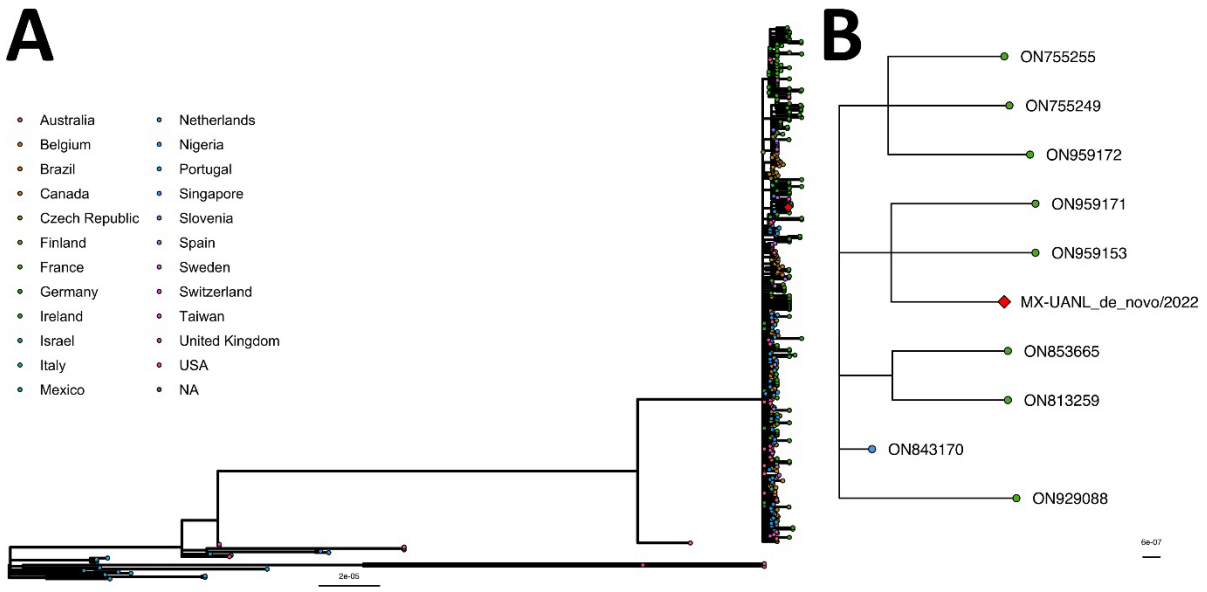
OX009124.1	Sweden	B.1	2022–05–22	Pettke,A. 2022



Appendix Figure 1. A) Phylogenetic relationship of hMPXV1 obtained from Nextstrain. B) Clade indicating the Mexican isolate and related sequences. The red diamond corresponds to the sequence from this study.



Appendix Figure 2. A) Phylogenetic relationship of hMPXV1 obtained from Nextstrain with de-novo assembly. B) Clade indicating the Mexican isolate and related sequences. The red diamond corresponds to the sequence from this study.