Clinical and Laboratory Standards Institutes guidelines include standardized *I. limosus* antimicrobial susceptibility testing. However, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry accurately identifies *I. limosus*. *I. limosus* displays high MICs for colistin and almost all β-lactams, except imipenem and meropenem (*9*). It has been suggested that the multidrug resistance of *I. limosus* enhances its selection in CF patients (*2*). In our case, successive treatment with drugs that were ineffective against *I. limosus* could have enabled its selection.

In conclusion, we emphasize a pathogenic role of *I. limosus* in lung transplant recipients several years after respiratory clearance of the bacteria. Chronic graft dysfunction, intensifying immunosuppression, and SARS-CoV-2 infection in this patient could have favored colonization with *I. limosus*. Characteristics of the bacterium such as colony morphotypes and multidrug resistance could delay effective therapy.

About the Author

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Genomic Analysis of Early Monkeypox Virus Outbreak Strains, Washington, USA

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DOI: https://doi.org/10.3201/eid2903.221446

We conducted a genomic analysis of monkeypox virus sequences collected early in the 2022 outbreak, during July–August , in Washington, USA. Using 109 viral genomes, we found low overall genetic diversity, multiple introductions into the state, ongoing community transmission, and potential for co-infections by multiple strains.

[1]These first authors contributed equally to this article.

The World Health Organization declared the 2022 mpox (formerly monkeypox) outbreak a public health emergency of international concern on July 23, 2022, after cases were identified in nearly 80 countries (*1*). By August 26, 2022, a total of 411 mpox cases had been confirmed in Washington, USA (*2*), and 17,432 cases had been confirmed in the United States (https://www.cdc.gov/poxvirus/monkeypox/response/2022/us-map.html).

Viral whole-genome sequencing (WGS) can augment contact tracing efforts and identify emerging variants, which potentially could affect infectivity, virulence, vaccine escape, and treatment resistance. By late August 2022, Washington had deposited more monkeypox virus (MPXV) sequences into public databases than any other state in the country. Here, we describe the Washington outbreak by using 109 MPXV genomes collected in the state.

We attempted WGS on 140 residual clinical specimens, primarily lesion swabs, that were PCR-positive for MPXV and had a cycle threshold (Ct) value <31 (range 15.9–30.4). We performed sequencing by using a hybridization probe-capture–based approach, as previously described (*3*), and probes designed by using the MPXV 2022/MA001 strain (Genbank accession no. ON563414) (Appendix, https://wwwnc.cdc.gov/EID/article/29/3/22-1446-App1.pdf). We generated consensus genomes by using Revica (https://github.com/greninger-lab/revica), a custom pipeline that performs trimming, filtering, and iterative remapping (Appendix). Sequences with <1% ambiguous bases (Ns) were deposited to GenBank under BioProject accession no. PRJNA862948 (Appendix Table). We used Augur, Auspice, and Nextclade to perform phylogenetic analysis (*4*,*5*), and we used UShER (*6*) to perform phylogenetic placement on a global tree (Appendix). This study was approved by the University of Washington Institutional Review Board STUDY00000408.

The analysis comprised a total of 109 sequences from 98 persons whose specimens were collected during July 6–August 19, 2022, primarily from King and Pierce Counties. Of the 98 patients, 90 (91.84%) were male and 1 (1.02%) female; 7 (7.14%) had unknown or undeclared sex. Median age at specimen collection was 36.0 (range 19–57) years.

We identified multiple identical genomes from different persons, suggesting ongoing community transmission (Figure, panel A). All 109 genomes fell within the predominant 2022 outbreak lineage B.1 (*7*), and sublineages included B.1.1 (n = 18), B.1.2 (n = 6), B.1.3 (n = 10), B.1.4 (n = 2), and B.1.8 (n = 2), suggesting separate MPXV introductions into the state. Among sublineages, we identified the nearest neighbor sequences from Germany (B.1.1); Connecticut, USA (B.1.2); Canada (B.1.4); Florida, USA (B.1.8); and multiple countries in Europe (B.1.3) (Appendix).

Overall, we observed low genetic diversity and a median of 1 aa (range 0–7 aa) mutation (substitutions or deletions) across the genome relative to the

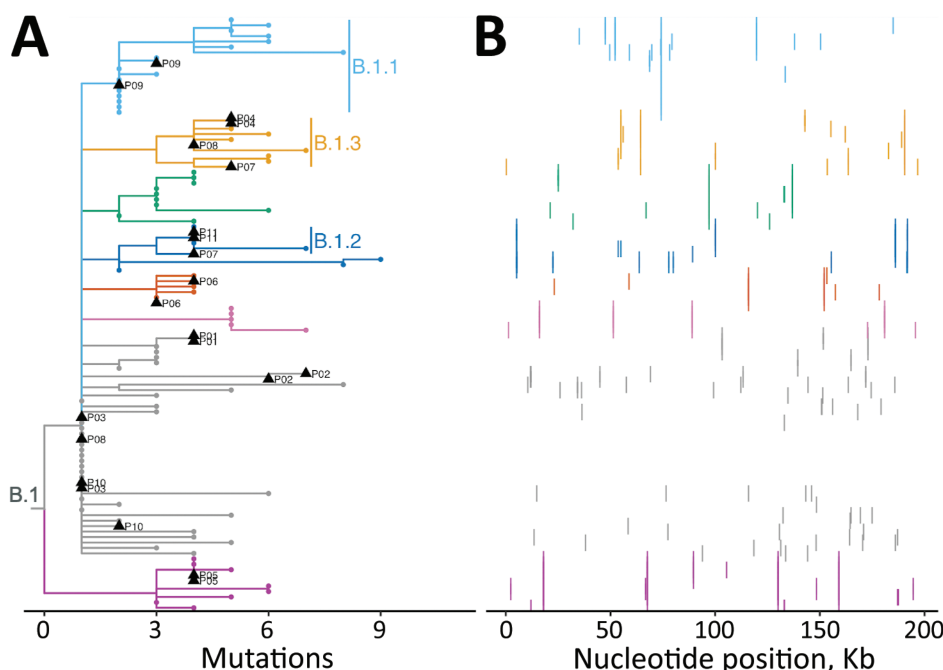

Figure. Phylogenomic analysis of 109 early monkeypox virus outbreak strains, Washington, USA. A) Phylogenetic tree showing that all Washington sequences fall within the major outbreak lineage B.1. The many identical sequences suggest community transmission; distinct sublineages suggest multiple MPXV introductions into the state. Black triangles indicate sequences from multiple swabs from the same patient, which were available for 11 persons, patients P01–P11. Clades with ≥5 sequences were assigned a color for tips and branches, and have text labels for the major sublineages, B.1.1, B.1.2, and B.1.3. All other tips and smaller clades are indicated in gray. B) Single nucleotide polymorphisms from each sample in panel A arrayed across the MPXV genome. Colors correspond to lineage coloring in panel A. MPXV, monkeypox virus.

B.1 ancestor (genome MPXV_USA_2022_MA001; Genbank accession no. ON563414). We identified 138 unique SNPs across the genome in the 109 sequences (Figure, panel B), producing 66 unique mutations (amino acid substitutions or deletions) in 51 genes. Of these, 5 unique aa substitutions (S553N, A1232V, D1546N, D1604N, and S1633L) occurred in surface glycoprotein OPG210, and 3 (E306K, D441Y, and E553K) in OPG189, which encodes one of several ankyrin-repeat proteins (Appendix Figure 1). We noted an abundance of G to A and C to T nucleotide substitutions (Appendix Figure 2), indicative of apolipoprotein B mRNA editing catalytic polypeptide-like3 activity consistent with other reports (*8*). We did not identify any substitutions or deletions in OPG057, a membrane glycoprotein homologous to F13L in vaccinia virus and the putative target of the therapeutic antiviral tecovirimat currently used to treat mpox (*9*).

Sequences from multiple swabs from the same person at the same time point had a median pairwise nucleotide difference of 1 (range 0–10 for 11 sample pairs) outside of labile tandem repeat regions (*10*). We observed even greater similarity in protein sequences with 0 (range 0–6) median pairwise aa differences. Among sample pairs from 3 patients, patient 06 (P06) had 1 aa difference, P07 had 6 aa differences, and P08 had 2 aa differences. Relative to the B.1 ancestral strain MA001, one of the P06 pair featured a V195I mutation in OPG079. One of the P08 pair had synonymous mutations in OPG073 and OPG083, and an OPG003:R84K substitution. Finally, differences in repeat samples from P07 suggest possible co-infection with strains from the B.1.2 and B.1.3 lineages, consistent with the patient's clinical history indicating multiple sexual partners. Relative to the MA001 B.1 reference strain, one of the P07 samples had synonymous mutations in OPG083 and OPG189, OPG180:D325N, and OPG016:R84K. The other of the P07 pair shared none of those SNPs, but had OPG015:V261A, OPG109:I66V, and the B.1.2-defining OPG210:D1604N. These mutations remained after re-extracting and re-sequencing the original specimens and, compared with interhost variation, suggest the possibility of co-infection with different MPXV strains (Appendix Table).

Overall, our data showed ongoing community MPXV transmission in Washington. The limited MPXV genetic diversity makes it challenging to use WGS data for contact tracing. However, continued genomic surveillance will be crucial for tracking viral evolution and identifying mutations associated with vaccine escape or antiviral treatment resistance.

This article was preprinted at https://doi.org/10.1101/2022.09.19.22280115.

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Article DOI: https://doi.org/10.3201/eid2903.221446

Genomic Analysis of Early Monkeypox Virus Outbreak Strains, Washington, United States

Appendix

Additional Methods

Sequencing Approach

DNA was extracted by using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche, https://www.roche.com), and sequencing libraries were prepared by using the DNAPrep Kit (Illumina, https://www.illumina.com) and a custom xGEN NGS Hybridization Capture DNA panel (Integrated DNA Technologies, https://www.idtdna.com) based on MXPV 2022/MA001 strain sequence (GenBank accession no. ON563414.2). Libraries were sequenced on Nextseq 2000 or NovaSeq 6000 (Illumina) instruments by using 2 × 150-bp kits targeting ≥1 million reads per sample.

Bioinformatic Analysis

Paired-end raw reads were adaptor- and quality-trimmed with Trimmomatic version 0.39 (www.usadellab.org/cms/?page=trimmomatic). Unpaired reads and reads shorter than 120-bp were discarded. Trimmed reads were aligned to the West Africa MPXV reference strain (GenBank accession no. NC_063383.1) by using bbmap version 38.96 (https://github.com/BioInfoTools/BBMap), and duplicate reads were discarded. Ambiguously mapped reads were randomly assigned to one of the top-scoring sites to give the inverted terminal repeats regions even coverage. The consensus genome was generated by 3 iterations of consensus calling using Samtools mpileup version 1.15 (https://www.htslib.org/doc/samtools-mpileup.html) and iVar consensus version 1.3.1(https://andersen-lab.github.io/ivar/html/manualpage.html) with a minimum base quality of 15, a minimum frequency threshold of 0.6, and a minimum depth of 5, then remapped reads to the most recent

consensus. After each iteration, any leading or trailing ambiguous bases (Ns) were removed by using Revica (https://github.com/greninger-lab/revica).

Phylogenetic Placement on Global MPXV Tree

We used the UShER tool (https://github.com/bpt26/USHER_ANALYSES) to place our sequences on a global tree of all available MPXV genomes at the time of writing via the web interface (https://genome.ucsc.edu/cgi-bin/hgPhyloPlace) with default settings. We visualized the resulting subtrees using Nextstrain/Auspice (Appendix Figure 3).

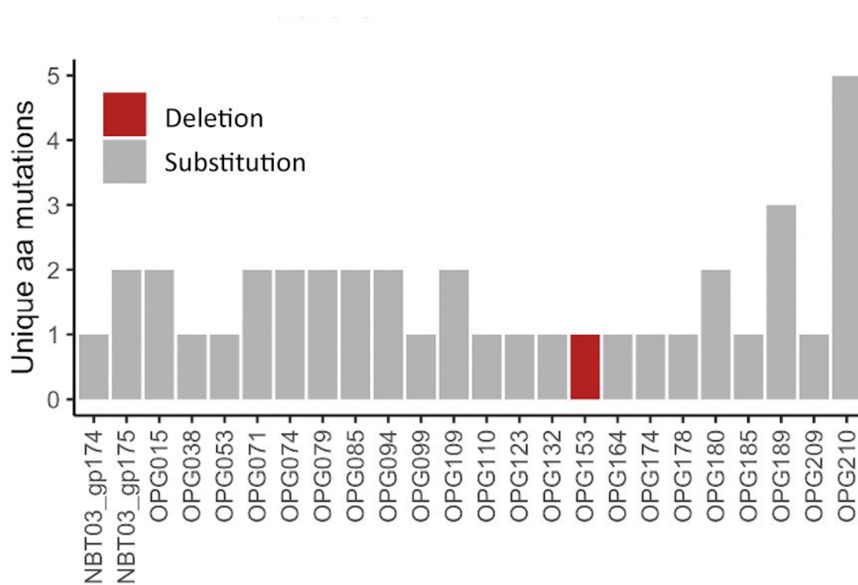
Appendix Table. Sequencing data obtained during genomic analysis of early monkeypox virus outbreak strains, Washington State, United States*

Isolate Name	GenBank accession no.	SRA accession no.	Patient no.†	Lineage
MpxV/human/USA/WA-UW-083698/2022	OP442945.1	SRR21524973	P01	B.1
MpxV/human/USA/WA-UW-088793/2022	OP442947.1	SRR21524979	P01	B.1
MpxV/human/USA/WA-UW-083781/2022	OP392544.1	SRR21524974	P02	B.1
MpxV/human/USA/WA-UW-085171/2022	OP392546.1	SRR21524961	P02	B.1
MpxV/human/USA/WA-UW-087006/2022	OP257260.1	SRR21236084	P03	B.1
MpxV/human/USA/WA-UW-088092/2022	OP392551.1	SRR21524968	P03	B.1
MpxV/human/USA/WA-UW-086040/2022	OP257258.1	SRR21236086	P04	B.1.3
MpxV/human/USA/WA-UW-082770/2022	OP392540.1	SRR21524969	P04	B.1.3
MpxV/human/USA/WA-UW-083953/2022	OP328307.1	SRR21236131	P05	B.1
MpxV/human/USA/WA-UW-087336/2022	OP392550.1	SRR21524987	P05	B.1
MpxV/human/USA/WA-UW-087301/2022	OP310047.1	SRR21236139	P06	B.1
MpxV/human/USA/WA-UW-084148/2022	OP392545.1	SRR21524986	P06	B.1
MpxV/human/USA/WA-UW-074949/2022	OP184762.1	SRR20973038 **SRR21616210**	P07	B.1.3
MpxV/human/USA/WA-UW-074988/2022	OP184765.1	SRR20973035 **SRR21616209**	P07	B.1.2
MpxV/human/USA/WA-UW-073669/2022	OP123049.1	SRR20736989	P08	B.1.3
MpxV/human/USA/WA-UW-076854/2022	OP123050.1	SRR20736988	P08	B.1
MpxV/human/USA/WA-UW-076773/2022	OP184763.1	SRR20973037	P09	B.1.1
MpxV/human/USA/WA-UW-074932/2022	OP184764.1	SRR20973036	P09	B.1.1
MpxV/human/USA/WA-UW-071121/2022	OP123047.1	SRR20736991	P10	B.1
MpxV/human/USA/WA-UW-076861/2022	OP123048.1	SRR20736990	P10	B.1
MpxV/human/USA/WA-UW-087424/2022	OP257248.1	SRR21236101	P11	B.1.2
MpxV/human/USA/WA-UW-085462/2022	OP392547.1	SRR21524972	P11	B.1.2
MpxV/human/USA/WA-UW-073909/2022	OP055800.1	SRR20653196	NA	B.1.1
MpxV/human/USA/WA-UW-076724/2022	OP055804.1	SRR20653192	NA	B.1
MpxV/human/USA/WA-UW-079141/2022	OP055806.1	SRR20653190	NA	B.1
MpxV/human/USA/WA-UW-074372/2022	OP055807.1	SRR20653189	NA	B.1
MpxV/human/USA/WA-UW-075986/2022	OP055808.1	SRR20653188	NA	B.1
MpxV/human/USA/WA-UW-076082/2022	OP055809.1	SRR20653187	NA	B.1
MpxV/human/USA/WA-UW-073974/2022	OP123040.1	SRR20731575	NA	B.1
MpxV/human/USA/WA-UW-070134/2022	OP123041.1	SRR20731574	NA	B.1.1
MpxV/human/USA/WA-UW-077622/2022	OP123042.1	SRR20731573	NA	B.1
MpxV/human/USA/WA-UW-077836/2022	OP123043.1	SRR20731572	NA	B.1
MpxV/human/USA/WA-UW-071246/2022	OP123044.1	SRR20736996	NA	B.1
MpxV/human/USA/WA-UW-076225/2022	OP123045.1	SRR20736995	NA	B.1.2
MpxV/human/USA/WA-UW-078870/2022	OP123046.1	SRR20736992	NA	B.1.1
MpxV/human/USA/WA-UW-070499/2022	OP169336.1	SRR20913445	NA	B.1
MpxV/human/USA/WA-UW-074224/2022	OP169340.1	SRR20913438	NA	B.1
MpxV/human/USA/WA-UW-074184/2022	OP169341.1	SRR20913437	NA	B.1.1
MpxV/human/USA/WA-UW-071966/2022	OP169344.1	SRR20913433	NA	B.1.1
MpxV/human/USA/WA-UW-075687/2022	OP169345.1	SRR20913443	NA	B.1.3
MpxV/human/USA/WA-UW-072839/2022	OP169346.1	SRR20913442	NA	B.1.1
MpxV/human/USA/WA-UW-079401/2022	OP184760.1	SRR20973040	NA	B.1
MpxV/human/USA/WA-UW-078603/2022	OP184761.1	SRR20973039	NA	B.1
MpxV/human/USA/WA-UW-089015/2022	OP257243.1	SRR21236108	NA	B.1.2
MpxV/human/USA/WA-UW-080268/2022	OP257244.1	SRR21236106	NA	B.1
MpxV/human/USA/WA-UW-084909/2022	OP257245.1	SRR21236104	NA	B.1
MpxV/human/USA/WA-UW-083578/2022	OP257246.1	SRR21236103	NA	B.1
MpxV/human/USA/WA-UW-088432/2022	OP257247.1	SRR21236102	NA	B.1

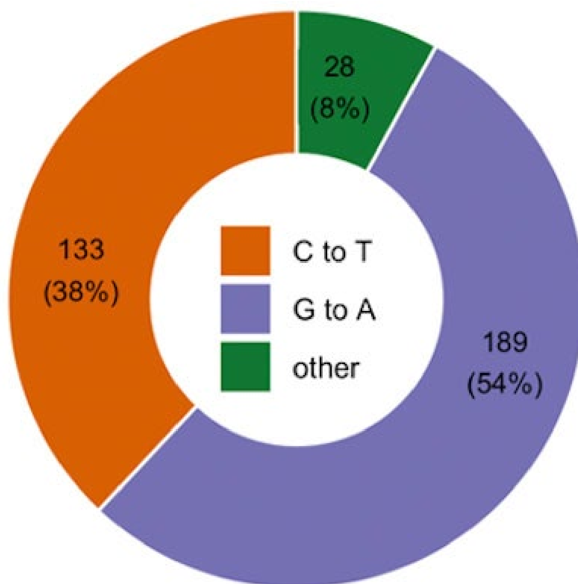
Isolate Name	GenBank accession no.	SRA accession no.	Patient no.†	Lineage
MpxV/human/USA/WA-UW-081786/2022	OP257249.1	SRR21236100	NA	B.1
MpxV/human/USA/WA-UW-087527/2022	OP257250.1	SRR21236099	NA	B.1
MpxV/human/USA/WA-UW-081272/2022	OP257251.1	SRR21236096	NA	B.1.1
MpxV/human/USA/WA-UW-085937/2022	OP257252.1	SRR21236095	NA	B.1
MpxV/human/USA/WA-UW-088081/2022	OP257253.1	SRR21236094	NA	B.1
MpxV/human/USA/WA-UW-080706/2022	OP257257.1	SRR21236088	NA	B.1.1
MpxV/human/USA/WA-UW-085684/2022	OP257259.1	SRR21236085	NA	B.1
MpxV/human/USA/WA-UW-084325/2022	OP257261.1	SRR21236083	NA	B.1
MpxV/human/USA/WA-UW-087619/2022	OP257262.1	SRR21236082	NA	B.1.3
MpxV/human/USA/WA-UW-089987/2022	OP257263.1	SRR21236081	NA	B.1
MpxV/human/USA/WA-UW-080976/2022	OP257264.1	SRR21236080	NA	B.1
MpxV/human/USA/WA-UW-089183/2022	OP257265.1	SRR21236078	NA	B.1
MpxV/human/USA/WA-UW-083179/2022	OP257266.1	SRR21236077	NA	B.1
MpxV/human/USA/WA-UW-084449/2022	OP257267.1	SRR21236142	NA	B.1
MpxV/human/USA/WA-UW-080617/2022	OP310039.1	SRR21236109	NA	B.1
MpxV/human/USA/WA-UW-088656/2022	OP310040.1	SRR21236098	NA	B.1
MpxV/human/USA/WA-UW-080185/2022	OP310041.1	SRR21236087	NA	B.1.1
MpxV/human/USA/WA-UW-089266/2022	OP310042.1	SRR21236076	NA	B.1.1
MpxV/human/USA/WA-UW-086841/2022	OP310043.1	SRR21236075	NA	B.1
MpxV/human/USA/WA-UW-083888/2022	OP310044.1	SRR21236074	NA	B.1
MpxV/human/USA/WA-UW-083914/2022	OP310046.1	SRR21236140	NA	B.1.2
MpxV/human/USA/WA-UW-088871/2022	OP310048.1	SRR21236138	NA	B.1
MpxV/human/USA/WA-UW-082029/2022	OP310051.1	SRR21236135	NA	B.1
MpxV/human/USA/WA-UW-081935/2022	OP310052.1	SRR21236134	NA	B.1
MpxV/human/USA/WA-UW-082670/2022	OP310053.1	SRR21236133	NA	B.1
MpxV/human/USA/WA-UW-088213/2022	OP310054.1	SRR21236132	NA	B.1.1
MpxV/human/USA/WA-UW-086360/2022	OP310055.1	SRR21236130	NA	B.1.1
MpxV/human/USA/WA-UW-089437/2022	OP310056.1	SRR21236129	NA	B.1
MpxV/human/USA/WA-UW-089714/2022	OP310057.1	SRR21236128	NA	B.1
MpxV/human/USA/WA-UW-087349/2022	OP310058.1	SRR21236127	NA	B.1.1
MpxV/human/USA/WA-UW-088764/2022	OP310061.1	SRR21236124	NA	B.1.1
MpxV/human/USA/WA-UW-089836/2022	OP310062.1	SRR21236123	NA	B.1
MpxV/human/USA/WA-UW-082810/2022	OP310063.1	SRR21236122	NA	B.1.1
MpxV/human/USA/WA-UW-082497/2022	OP310064.1	SRR21236121	NA	B.1
MpxV/human/USA/WA-UW-088973/2022	OP310065.1	SRR21236119	NA	B.1.3
MpxV/human/USA/WA-UW-089152/2022	OP310066.1	SRR21236118	NA	B.1
MpxV/human/USA/WA-UW-082625/2022	OP310067.1	SRR21236116	NA	B.1
MpxV/human/USA/WA-UW-088225/2022	OP310068.1	SRR21236105	NA	B.1
MpxV/human/USA/WA-UW-088152/2022	OP310069.1	SRR21236079	NA	B.1
MpxV/human/USA/WA-UW-083083/2022	OP328308.1	SRR21236115	NA	B.1
MpxV/human/USA/WA-UW-089142/2022	OP328309.1	SRR21236114	NA	B.1
MpxV/human/USA/WA-UW-088725/2022	OP328310.1	SRR21236112	NA	B.1
MpxV/human/USA/WA-UW-088258/2022	OP328311.1	SRR21236111	NA	B.1
MpxV/human/USA/WA-UW-087564/2022	OP328312.1	SRR21236110	NA	B.1
MpxV/human/USA/WA-UW-080100/2022	OP392535.1	SRR21524985	NA	B.1
MpxV/human/USA/WA-UW-080292/2022	OP392536.1	SRR21524966	NA	B.1
MpxV/human/USA/WA-UW-080834/2022	OP392537.1	SRR21524964	NA	B.1.3
MpxV/human/USA/WA-UW-081469/2022	OP442944.1	SRR21524980	NA	B.1.3
MpxV/human/USA/WA-UW-081714/2022	OP392538.1	SRR21524978	NA	B.1
MpxV/human/USA/WA-UW-082215/2022	OP392539.1	SRR21524967	NA	B.1.3
MpxV/human/USA/WA-UW-082488/2022	OP442941.1	SRR21236117	NA	B.1.8
MpxV/human/USA/WA-UW-082880/2022	OP392541.1	SRR21524977	NA	B.1
MpxV/human/USA/WA-UW-083506/2022	OP392542.1	SRR21524981	NA	B.1
MpxV/human/USA/WA-UW-083584/2022	OP392543.1	SRR21524963	NA	B.1
MpxV/human/USA/WA-UW-084331/2022	OP442942.1	SRR21236107	NA	B.1.4
MpxV/human/USA/WA-UW-085393/2022	OP442946.1	SRR21524975	NA	B.1
MpxV/human/USA/WA-UW-086026/2022	OP442943.1	SRR21236097	NA	B.1.8
MpxV/human/USA/WA-UW-087094/2022	OP392548.1	SRR21524960	NA	B.1
MpxV/human/USA/WA-UW-087104/2022	OP392549.1	SRR21524976	NA	B.1.1
MpxV/human/USA/WA-UW-088325/2022	OP392552.1	SRR21524971	NA	B.1
MpxV/human/USA/WA-UW-088960/2022	OP392553.1	SRR21524982	NA	B.1.4

*Sequence data are available in GenBank in the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov); raw reads have been deposited to the NCBI sequence read archive (SRA) under BioProject no. PRJNA862948. Bold text indicates resequencing was performed to confirm coinfection and these reads were deposited in separate SRRs.

†Patient numbers are indicated for sequences obtained from the same individual and correspond to tip labels on the phylogenetic tree in Figure, panel A.

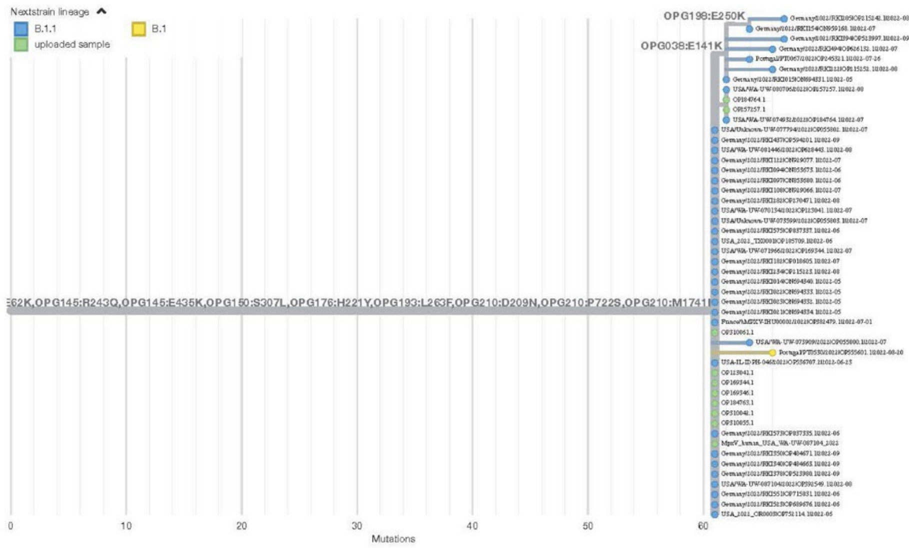


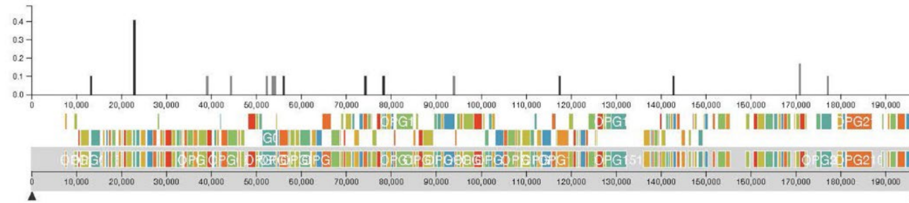
Appendix Figure 1. Unique amino acid (aa) mutations found during phylogenetic analysis of 109 genomes from early monkeypox virus outbreak, Washington, United States. After excluding genes with mutations (amino acid substitutions or deletions) in a single sample, most genes contained 1–2 mutations, except OPG189 and OPG210.



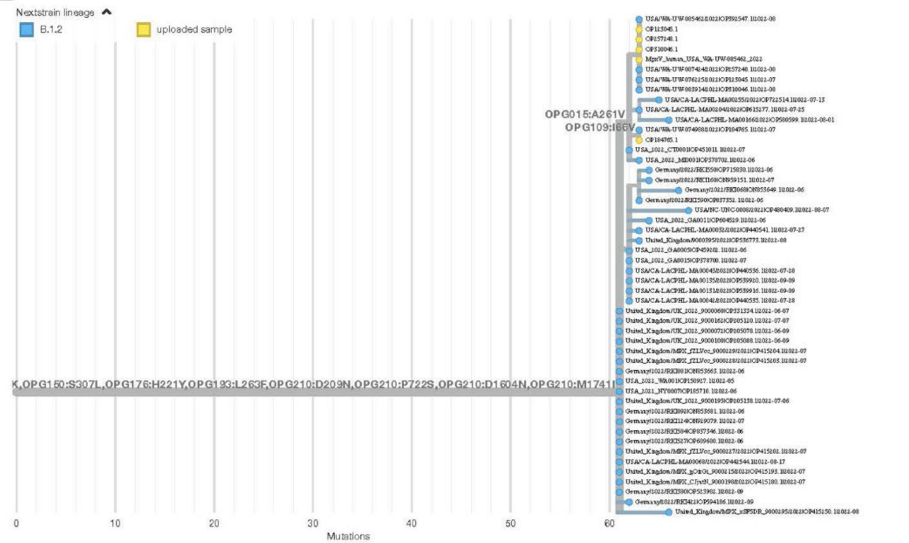
Appendix Figure 2. Distribution of mutations found during phylogenetic analysis of 109 genomes from early monkeypox virus outbreak, Washington, United States. We noted an abundance of G to A and C to T mutations, indicating likely apolipoprotein B mRNA editing catalytic polypeptide-like3 involvement, as reported by C.M. Gigante, et al., https://doi.org/10.1126/science.add4153.

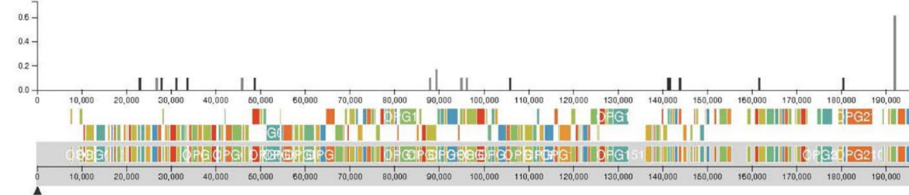
A





B





C

Nextstrain lineage

B.1.3

uploaded sample

OPG055:A142V,OPG204:M37I,OPG016:S12L

OPG016:R84K

OPG074:R665C

OPG145:E435K,OPG150:S307L,OPG176:H221Y,OPG193:L263F,OPG210:D209N,OPG210:P722S,OPG210:M1741I

OPG071:D218N

OPG016:R84K

OPG180:D325N

Mutations

D

Nextstrain lineage

B.1.4

uploaded sample

OPG105:D1137N

OPG022:E63K

OPG003:N264D

OPG040:D162N,OPG107:R96K

OPG042:W377*

OPG145:E435K,OPG150:S307L,OPG176:H221Y,OPG193:L263F,OPG210:D209N,OPG210:P722S,OPG210:M1741I

OPG107:M112T

Mutations

E

Appendix Figure 3. UShER subtrees for sequences from phylogenetic analysis of early monkeypox virus outbreak, Washington, United States. The trees show mpox lineages from 109 genomes from Washington and nearest neighbor sequences from a global phylogeny. A) B.1.1; B) B.1.2; C) B.1.3; D) B.1.4; E) B.1.8. Subtrees were visualized using Auspice (https://www.auspice.us).