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Draft genome of Orthopoxvirus monkeypox virus hMpxV/Uganda/MUWRP-H1922SL/2025 detected in Uganda

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ABSTRACT Mpox virus from Clade I exhibits high virulence and poses significant mortality risks, especially among children. We isolated a strain of the mpox virus (hMpxV/Uganda/MUWRP-H1922SL/2025) from an adult woman in Uganda. Using phylogenetic tree analysis and PoxServer evaluation, we determined that this strain belongs to Clade/Lineage Ib.

KEYWORDS mpox, *Poxviridae*, *Orthopoxvirus*

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Mpox virus, previously known as monkeypox virus (MPXV), is a double-stranded DNA virus in the genus *Orthopoxvirus* and family *Poxviridae* (1). MPXV is divided into two clades: the virulent Central African Clade I and the less virulent West African Clade II (2). Transmission occurs by contact with wild mammals and person-to-person spread via respiratory secretions, skin contact, and contaminated fomites. Symptoms include fever, papular and vesiculopustular lesions, and lymphadenopathy (2). Severe infection can result in pneumonitis, encephalitis, keratitis, bacterial superinfection, and sometimes death (3). This study reports the genome of the mpox virus isolated from the skin lesion of an adult female at General Military Hospital Bombo (latitude:0.586389; longitude:32.536111) in Uganda.

A sample collected from a skin lesion was put in viral transport medium. DNA extraction from the sample was done using QIAamp MinElute Virus Spin Kit (50) (Qiagen, Cat No. 57704). An in-house real-time RT-PCR assay was used as previously described (4) to confirm the sample as positive for MPXV with a Ct value of 18. RT-PCR amplification was performed according to the SISPA Long-Fragment Superscript IV Reverse Transcriptase Protocol. The library was prepared according to the Rapid Sequencing DNA V14 Barcoding (SQK-RBK114.24 or SQK-RBK114.96) MinION Protocol and purified with AMPure XP beads to remove unwanted fragments. The final library was quantified using the Qubit dsDNA High-Sensitivity Assay, Qubit 4 fluorometer (Thermo Fisher Scientific, Waltham, United States). Sequencing of the prepared library was done on R10 MinION flow cells (FLO-MIN114, Oxford Nanopore Technologies, England) using MinION Mk1D sequencer with MinKNOW 25.03.9 software for 24 h. Base calling during sequencing was conducted using Dorado v1.1.1 (5). The sequencing produced 6,422,894 reads with an N50 value of 604 bp. Sequence read quality filtering was performed using fastp v0.23.2 to remove adapter sequences and short reads (6). An in-house tool (Virnano v001) built using Minimap2 v2.30-r1287 (7), Racon v1.4.13 (8), and Medaka v1.7.3 was used to map raw reads to the reference PQ178862 (9) and generate a consensus assembly. This assembly produced a consensus sequence with a coverage of 99.64× and an average depth of 90.26 reads. Clade assignment was done by matching consensus to related sequences in PoxSurver in GISAID (https://gisaid.org/). Contemporary sequences (2024–2025) from both clades I and II were retrieved from GISAID for comparison. Multiple

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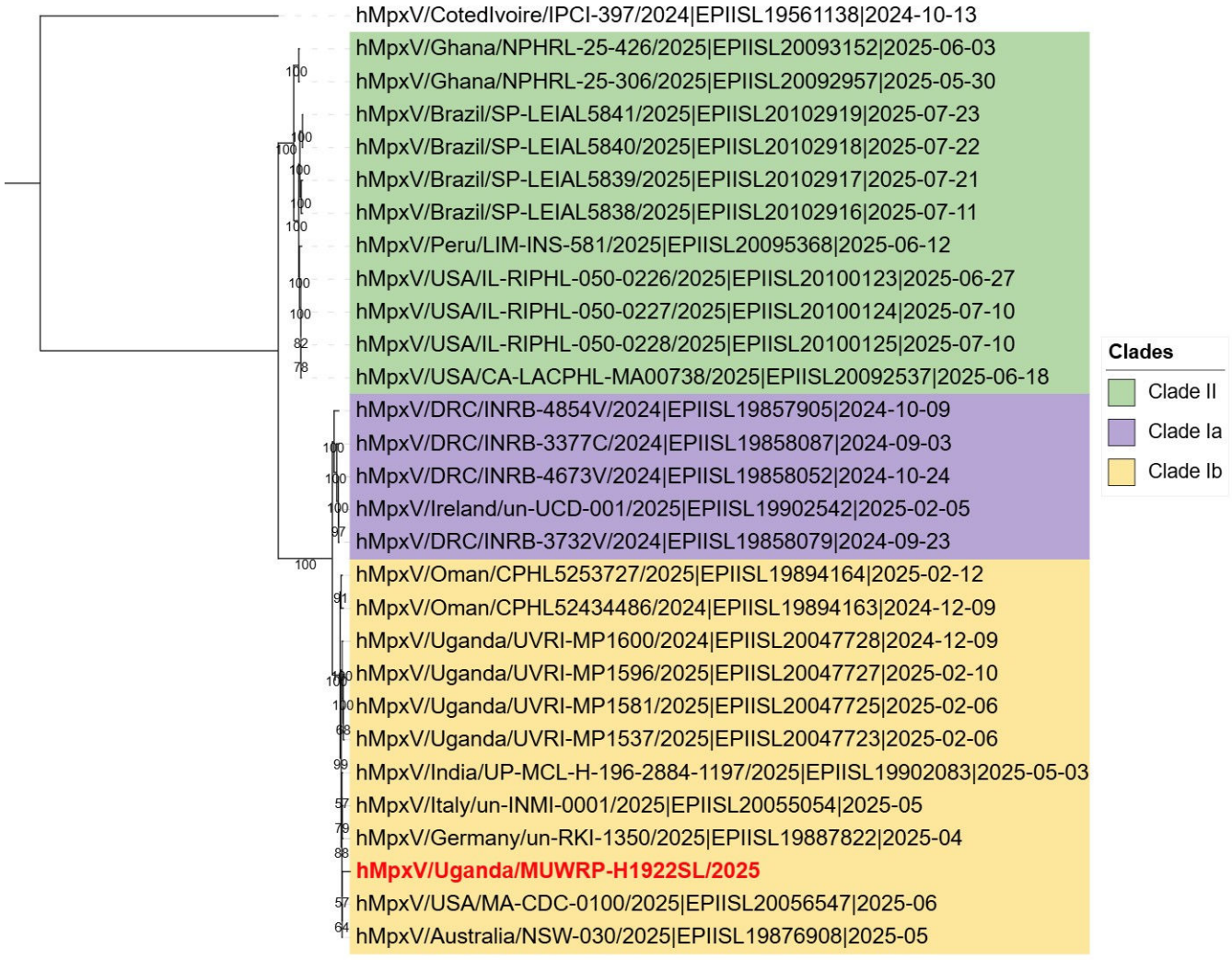


FIG 1 Phylogenetic tree showing the mpox virus clade with strain hMpxV/Uganda/MUWRP-H1922SL/2025 from Uganda clusters.

sequence alignment was done with Mafft v7.526 (10), and a phylogenetic tree was constructed using IQtree v2.0.7 (11). The tree was annotated in ITOL (12). Default parameters for software were used, except where otherwise noted.

The partial genome was 195,619 bp in length and had a guanine-cytosine (GC) content of 33.06% and 179 predicted genes. PoxServer classified the clade/lineage of this genome as Ib. The genome matches closely to strain hMpxV/DRC/005/1978 in PoxServer. Phylogenetic tree analysis grouped this strain alongside clade Ib sequences from various geographic regions (Fig. 1). This information is crucial for ongoing surveillance of the mpox virus and its evolution and spread across the East and Central Africa region.

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DATA AVAILABILITY

The genome sequence has been deposited in GenBank and can be retrieved using accession number PX711335. The raw sequence reads are available in the Sequence Read Archive (SRA) under BioProject PRJNA1331542 and can be retrieved with accession SRR35645093.

ETHICS APPROVAL

The study was approved by Makerere University School of Public Health Higher Degrees and Research Ethics Committee (HDREC087).

REFERENCES

1. Ulaeto D, Agafonov A, Burchfield J, Carter L, Happi C, Jakob R, Krpelanova E, Kuppalli K, Lefkowitz EJ, Mauldin MR, de Oliveira T, Onoja B, Otieno J, Rambaut A, Subissi L, Yinka-Ogunleye A, Lewis RF. 2023. New nomenclature for mpox (monkeypox) and monkeypox virus clades.

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Lancet Infect Dis 23:273–275. https://doi.org/10.1016/S1473-3099(23)00055-5
2. Pastula DM, Tyler KL. 2022. An overview of monkeypox virus and its neuroinvasive potential. Ann Neurol 92:527–531. https://doi.org/10.1002/ana.26473
3. Thornhill JP, Barkati S, Walmsley S, Rockstroh J, Antinori A, Harrison LB, Palich R, Nori A, Reeves I, Habibi MS, et al. 2022. Monkeypox virus infection in humans across 16 Countries - April-June 2022. N Engl J Med 387:679–691. https://doi.org/10.1056/NEJMoa2207323
4. Mucker EM, Freyn AW, Bixler SL, Cizmeci D, Atyeo C, Earl PL, Natarajan H, Santos G, Frey TR, Levin RH, et al. 2024. Comparison of protection against mpox following mRNA or modified vaccinia Ankara vaccination in nonhuman primates. Cell 187:5540–5553. https://doi.org/10.1016/j.cell.2024.08.043
5. Oxford Nanopore Technologies. 2025. Dorado, a high-performance basecaller. Available from: https://github.com/nanoporetech/dorado
6. Chen S, Zhou Y, Chen Y, Gu J. 2018. Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884–i890. https://doi.org/10.1093/bioinformatics/bty560
7. Li H. 2018. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics 34:3094–3100. https://doi.org/10.1093/bioinformatics/bty191
8. Vaser R, Sović I, Nagarajan N, Šikić M. 2017. Fast and accurate de novo genome assembly from long uncorrected reads. Genome Res 27:737–746. https://doi.org/10.1101/gr.214270.116
9. Langat SK, Gathii K, Limbaso K, Roba A, Ndia M, Mutai B, Pilarowski G, Ochieng M, Juma B, Onyango C, Nyunja A, Okunga E, Ofula V, Oluniyi P, Chepkorir E, Lutomiah J, Herman-Roloff A, Lucchi N, Limo H, Langat D, Khamadi S, Kiiru J, Amoth P, Waitumbi J, Songok E. 2025. Complete genome of an mpox clade 1b virus from Kenya. Edited by S. Roux. Microbiol Resour Announc 14:e0005025. https://doi.org/10.1128/mra.00050-25
10. Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30:3059–3066. https://doi.org/10.1093/nar/gkf436
11. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R. 2020. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Edited by E. Teeling. Mol Biol Evol 37:1530–1534. https://doi.org/10.1093/molbev/msaa015
12. Letunic I, Bork P. 2024. Interactive Tree of Life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. Nucleic Acids Res 52:W78–W82. https://doi.org/10.1093/nar/gkae268

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