

## Genetic Features of 84 Genomes of Monkeypox Virus in Recent Circulation — Beijing Municipality, China, 2023

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### ABSTRACT

The first indigenous incidence of Mpox (previously known as monkeypox) within Chinese mainland was documented in May 2023, with subsequent local and imported cases identified. A comprehensive understanding of the Mpox virus's (MPXV) characteristics within Beijing remains incomplete. In this study, 84 MPXV genomes from 82 local incidents and two imported instances, detected between May and July 2023, were analyzed. All MPXV strains fell within lineage C.1 of the West African clade, displaying limited genetic heterogeneity, encompassing 76–87 nucleotide substitutions and holding nucleotide identities between 99.996% and 100%. Phylogenetic exploration indicated that all genomes exhibited high homology to those presently prevalent in neighboring East Asian and Southeast Asian regions. Forty-six distinct haplotypes were identified among the strains, with 36.90% of genomes corresponding to four common haplotypes, suggesting repeated cross-regional introductions and restrained distribution via recurrent local transmission. These findings elucidate the genetic diversity and phylogenesis of MPXVs during their nascent transmission within Beijing and provide vital information to enhance future Mpox containment strategies.

### INTRODUCTION

Monkeypox (Mpox), an *Orthopoxvirus*-induced zoonotic illness, was first reported as having infected a human in the Democratic Republic of Congo in 1970 (1). Over the ensuing decades, instances of endemic circulation have been reported only infrequently in both West and Central Africa (2). In May 2022, a case of Mpox was imported into the United Kingdom by a traveler visiting from Nigeria (3). Subsequently, a hitherto unseen outbreak has unfolded across multiple countries that hitherto had not reported cases, including nations in Europe and North America. This

led to a declaration by the World Health Organization (WHO) in July 2022 that an international public health emergency was in effect (3–5). As reported in August 2023, this worldwide outbreak incurred 89,000 confirmed cases across 114 countries, resulting in 157 fatalities (6).

Prior to 2023, the Chinese mainland reported only one imported case of Mpox, likely due to stringent border control, quarantine, and isolation policies implemented during the coronavirus disease 2019 (COVID-19) pandemic (7–8). In late May 2023, the first recorded local Mpox infection occurred in Beijing (9). Since that time, diagnoses have increased, spreading across Beijing and other provinces throughout China. Despite this, the genetic characteristics of MPXV in China remain largely unexplored. This study aims to detail the genetic diversity of MPXV in recent Beijing infections.

### MATERIALS AND METHODS

#### Participants and Sample Collection

This study enrolled all individuals in Beijing who received a positive result for MPXV via nucleic acid testing from May 31, 2023 to July 31, 2023. Samples of vesicular or pustular fluid, skin lesion swabs, and oropharyngeal swabs were acquired from confirmed symptomatic cases of Mpox. For asymptomatic individuals, rectal or perianal swabs and oropharyngeal swabs were obtained. The protocols for sample collection adhered to the Technical Guidelines for Mpox Prevention and Control (2022 edition), as issued by the National Health Commission of China.

#### Polymerase Chain Reaction Testing for MPXV

Viral DNA was extracted using the QIAamp MinElute Virus Kit (Qiagen, Dusseldorf, Germany) and detected using the MPXV polymerase chain reaction (PCR) kit (Kinghawk Co. Ltd., Beijing,

China), which targets F3L, using the ABI 7500 system (Applied Biosystems, Carlsbad, CA, US) following the manufacturers' protocols.

### Next-Generation Sequencing

The amplicon sequencing method was used to generate MPXV genomes. Viral DNA was amplified by multiplex PCR. PCR products were purified using AMPure XP Beads (Beckman, Brea, CA, US) and quantified by Qubit 3.0 fluorometry (Thermo Fisher Scientific, Waltham, MA, US). Next-generation sequencing libraries were prepared with the Nextera DNA Library Prep Kit and sequenced using the Illumina Nextseq 2000 system (Illumina, San Diego, CA, US).

### Analysis of Phylogenetics and Haplotypes

The sequence processes, including reads trimming, mapping, consensus sequence extraction, and variant calling for each sample were conducted using CLC Genomics Workbench (version 22.0, Qiagen, Hilden, Germany). Our study employed the complete MPXV genome of the current outbreak (NC\_063383), which has a genome size of 197,209 bp, as a reference genome. The alignment was accomplished via the MAFFT server, while a phylogenetic tree was constructed using the neighbor-joining method with a bootstrap value of 500 (10). MEGA (version 11) was used to calculate the nucleotide identity, and the phylogenetic tree was visualized through the ChiPlot server. For the phylogenetic analysis, 141 representative MPXV genomes from the public domain were chosen and compared with the genomes acquired in our study. The clade was determined by Nextclade (11). Haplotypes were determined by using DnaSP 6, and the haplotype frequencies in populations were calculated using Arlequin 3.5.2.2 with default parameters (12). The network was visualized using the PopART (version 1.7) (13).

## RESULTS

We conducted a comprehensive investigation on the transmission pathways of MPXV in Beijing by employing next-generation sequencing techniques on samples gathered from all identifiable Mpox incidences throughout the study duration. An aggregate of 84 high-grade MPXV genome sequences derived from 84 instances were incorporated into the analysis. The enrolled cases were exclusively male, presenting a median age bracket of 32 years (interquartile range

extending from 28 to 35 years). A minimal fraction of the cases (2.38%) contracted the infection abroad, juxtaposed against the significant majority (97.62%) who were recognized as locally-infected individuals.

A phylogenetic tree, based on the neighbor-joining method, was utilized to evaluate the relatedness of the MPXV genomes in Beijing to those globally circulating. This analysis incorporated the 84 locally sequenced genomes from this study and 141 other publicly available genomes. The 84 Beijing genomes were all found to belong to the MPXV C.1 lineage (Figure 1), with nucleotide identities ranging from 99.996% to 100%. These genomes formed a cluster in the phylogenetic tree, signifying a few related MPXV introductions. Upon closer inspection, it was clear that the Beijing case genomes were markedly homologous to those identified in other regions of China and neighboring East Asia and Southeast Asia.

Phylogenetic analysis reveals constrained diversity within the MPXV genome in Beijing. However, epidemiological investigations suggest multiple unique inflection events rather than a single source, and not all cases appear directly linked. The analysis of haplotypes identifies a high variation with 46 distinct haplotypes found within the non-ITR variable and central regions (14) (Figure 2). Notably, a substantial proportion of genomes, 36.90% or 31 genomes coalesced around four key haplotypes: Hap 12, Hap 13, Hap 16, and Hap 7. Despite this, no intermediate forms between these four haplotypes have been detected. Interestingly, another distinct cluster, Hap 11, was identified with six individual genomes. These findings hint at frequent trans-regional importation and proliferated dissemination through multiple local transmission chains.

Of the 84 MPXV genomes examined in this study, only two were identified as cases of imported infections. The first imported case was detected in Beijing upon a patient's return from Thailand in the latter part of April and confirmed positive on June 2; this case is thought to be the first local instance in Beijing (9). Interestingly, the haplotype network revealed that the first imported case and subsequent local case were identified as Hap 1, which was not the source of later cases, which derived from other related yet independent infections. Similarly, another imported infection identified on June 16 as Hap 39 possessed a notably distinct genome compared to the others. This particular imported MPXV exhibited 76 nucleotide substitutions and 34 amino acid substitutions relative to the reference genome (accession number NC\_063383.1), including changes such as C33332T (OPG050:R26Q), G37974A

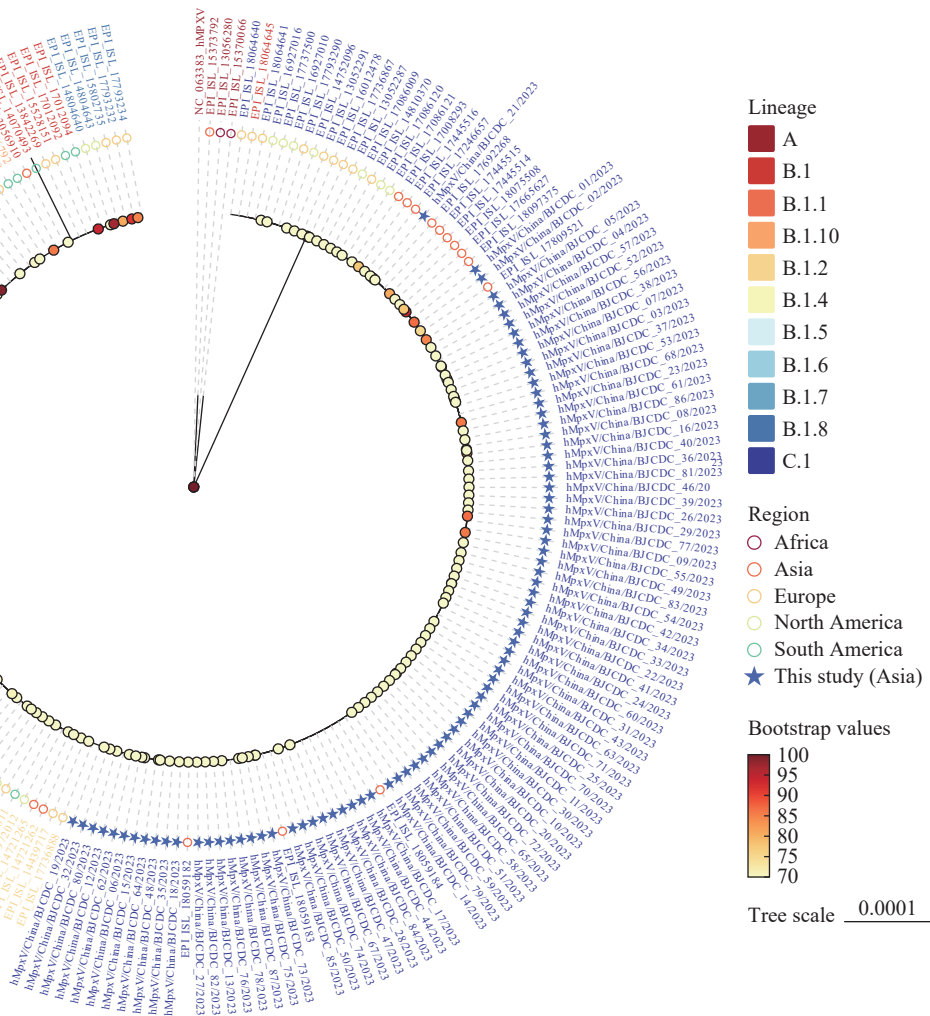


FIGURE 1. Phylogenetic tree derived from the complete Mpxv virus genomes. Note: The phylogenetic analysis of 84 strains in Beijing, conducted from May 31 to July 31, 2023, is presented. Distinct lineages are represented by strain names in varying colors. Bootstrap values are indicated by circles filled with gradient colors. Strains sequenced within the current study are marked with asterisks.

(OPG056:S354L), and C149963T (OPG0176:S52L), which were not present in the previous Beijing case. Overall, our data suggested that the genetic diversity of local MPXVs in Beijing was lower than that of the imported instances.

To date, two separate clades of MPXV have been identified, Clade I (CA-MPXVs/Clade I, previously referred to as the Congo Basin or Central African clade), and Clade II or III (WA-MPXVs/Clade II or III, formerly known as the West African clade) (15–16). The ongoing Mpxv outbreak, beginning in 2022, can be traced back to lineage B.1, Clade II, which shares a genetic connection with a significant outbreak in Nigeria between 2017 and 2018 (17). A noteworthy number of single-nucleotide polymorphisms (SNPs) – over 50 – were identified in the viral genome during this outbreak, a figure

considerably higher than the average estimate based on the *Orthopoxviruses* substitution rate (18). A similar phenomenon is noted in the present study, with 76–87 SNPs discovered in the MPXV genomes of the Beijing cases. It is proposed that an abundance of mutations within viral genomes could be attributed to the interaction between the host’s apolipoprotein B editing complex (APOBEC3), cytosine deaminase, and host adaptation (18–19). APOBEC3’s significant impact results in a strong tendency towards G>A and C>T substitutions in the A:T-rich framework of the MPXV (18). A total of 61 (52.14%) G>A substitutions and 45 (38.46%) C>T substitutions were observed among the 117 SNPs in all MPXV genomes from the Beijing cases. This codon bias aids the virus’s immune evasion through deaminase-dependent or independent processes (19).



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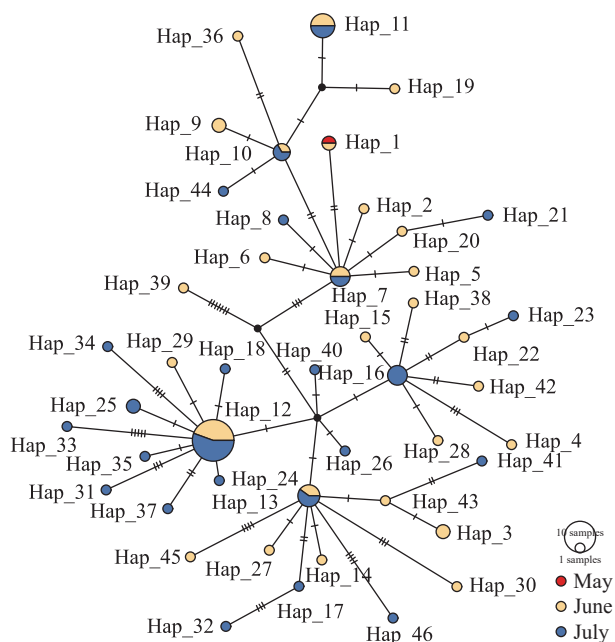


FIGURE 2. Network of Mpox virus haplotypes observed in Beijing from May to July, 2023.

Note: The size of each plot corresponds to the number of cases reported. The collected samples from May, June, and July of 2023, are represented in red, yellow, and blue, respectively.

## CONCLUSIONS

In conclusion, this paper presents the genetic characteristics of the MPXVs currently circulating in Beijing. Our research illustrates that over the past two months since the discovery of the first imported case, local transmission has been significantly restrained. Nevertheless, the extensive genetic diversity displayed in the imported cases underscores the necessity of continuous surveillance for both foreign introductions and local transmission of MPXVs.

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