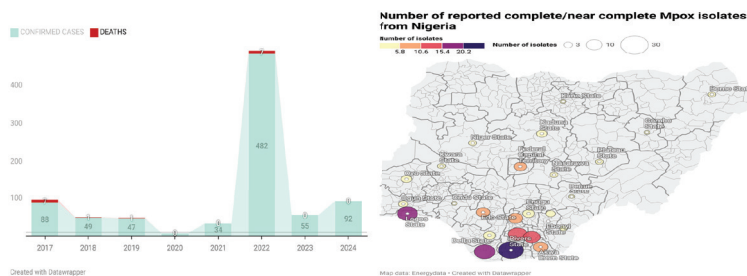




ORIGINAL ARTICLE

Genomic Diversity and Regional Epidemiologic Dynamics of mpox Clade II in Nigeria (2017–2024)

Graphical abstract



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Highlights

- Mpox lineage A.2.3 dominated the 2022 epidemic in Nigeria.
- A total of 96 high-quality mpox genomes obtained between 2017 and 2024 from 25 states were analyzed.
- Mutation was observed more frequently in envelope and DNA polymerase genes.
- South-south and South-east regions recorded a higher prevalence of mpox in comparison to other regions in Nigeria.
- Males aged 21–40 years were the most prominent demographic group with the highest infection rate.

Brief statement

This study presents the genomic and epidemiological dynamics of mpox clade II in Nigeria, identifies lineage A.2.3 as the most prevalent variant during the 2022 epidemic, and discusses the observed regional and demographic disparities. The study emphasizes the need for target-specific intervention and continuous genomic surveillance.

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Genomic Diversity and Regional Epidemiologic Dynamics of mpox Clade II in Nigeria (2017–2024)

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Abstract

Objectives: The reappearance of monkeypox (mpox) in Nigeria since 2017 presented with significant epidemiological shifts which has raised serious public health concern both in the domestic and international front. To implement effective response strategies against the disease, understanding its genomic diversity and transmission dynamics is essential. This study aimed to investigate the genomic diversity and epidemiological patterns of mpox clade II in Nigeria from 2017 to 2024.

Methods: 153 mpox clade II genome sequences obtained from 25 Nigerian states were retrieved from Nextstrain and GenBank. After quality assurance, 96 sequences were selected for further analysis. IQ-TREE and BEAST were used in constructing phylogenetic trees, while Nextclade was used for mutation profiling. Epidemiological data were obtained from NCDC and WHO reports.

Results: Lineage A.2.3 of mpox clade II was observed to be the most prevalent during the outbreak of 2022. This coincides with the highest national incidence of mpox. Several non-synonymous mutations were identified, predominantly in the envelope proteins, and DNA polymerase. Regional disparities were observed, with the south-south and south-east regions reporting the highest case burdens. It was also observed that males aged 21–40 years were the prominently mpox infections age group.

Conclusion: These findings emphasize the significance of continuous genomic surveillance and region-specific interventions for controlling mpox in Nigeria. Understanding local lineage dynamics and demographic risk factors is crucial for targeted outbreak responses.

Key words: mpox, epidemiology, phylogenetic, BEAST, IQ-Tree, FigTree, *Orthopoxvirus*, clade II

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INTRODUCTION

The world is once again confronted with the threat of a significant public health crisis due to the re-emergence of monkeypox (mpox), a viral infection historically

classified as a neglected tropical disease owing to previously low morbidity and mortality rates [1].

After the first report of an mpox infection was reported in the Democratic

Republic of Congo (DRC) in 1970, the disease has spread to different parts of the world including Africa, Europe, North America, South America, Central America, and the United States of America. Mpox has also been reported on several occasions across Asia, Middle East, and Oceania, causing sporadic outbreaks influenced by factors, such as human-animal interactions, ecologic disturbances, and global migration [2–4].

The monkeypox virus (MPXV) is the etiologic agent for mpox infections. It is a large, double-stranded DNA virus belonging to the Poxviridae family and subfamily Chordopoxviridae of genus *Orthopoxvirus*, which includes variola (the causative agent of smallpox), cowpox, and vaccinia viruses. The MPXV genome is linear with approximately 197 kilobases that exhibits complex replication cycles entirely within the host cell cytoplasm. Mpox is a zoonotic pathogen because it is transmitted from animal-to-human and from human-to-human. Infection with mpox presents with clinical symptoms that are similar to smallpox, including headaches, body pain, fatigue, malaise, fevers, myalgias, sore throat, swollen lymph node, and rash that characteristically develop on the face, hands, genitals, and feet. When mpox is improperly managed, it can result in death [3,5].

Phylogenetic classification of MPXV divides the virus into two major groups: clade I MPXV (formerly referred to as the Central African clade); and clade II (formerly known as the West African clade) [6,7]. The clade II group is further sub-grouped into clades IIa and IIb. Clade IIa is comprised of all MPXVs originating from West Africa, while clade IIb is comprised of all sequences isolated during the 2022 global outbreak and sequences isolated from Nigeria [8–10].

Mpox received very little attention after the DRC occurrence in 1970 and therefore did not lead to major public health intervention. Recent global outbreaks, such as the 2022 epidemic, underscore the potential of the virus to cause widespread morbidity and mortality. As a result, public health agencies (e.g., the World Health Organization [WHO] and Centers for Disease Control [CDC]) had reasons for concern and the need for better public health intervention strategies with a focus on communities in endemic regions [11,12].

The 2017 mpox recurrence in Nigeria occurred after many years without mpox infections, marking a critical period in the epidemiologic history of mpox since the first indigenous occurrence report in 1978. The 2017 mpox outbreak resulted in 88 confirmed cases and 8 fatalities, suggesting the critical public health impact of the virus.

Although the number of confirmed mpox cases declined following the 2017 epidemic, by 2022 the number of mpox cases spiked and reached a total of 482 confirmed cases and 7 associated deaths. In 2023 the Nigerian Centres for Disease Control (NCDC) reported a decline in the number of confirmed mpox cases but the number increased slightly by 2024 [13] and by the end of 2024

the NCDC reported a cumulative total of 731 confirmed mpox cases and 16 associated deaths between 2017 and 2024 [14].

It is essential to understand the disease epidemiologic inclination, genomic diversity, and evolutionary dynamics to plan a functional public health intervention to mitigate mpox infection. Mpox clade II is known to be less virulent but more widespread than clade I mpox [15]. Genomic analyses of clade II have identified sub-clades (IIa and IIb) and multiple sub-lineages A, A.1, A.2, A.2.2, A.2.3, A.3, and B.1, each with its own distinct geographic and temporal patterns [16].

The persistent recurrences of mpox in Nigeria have a connection between ecologic, sociocultural, and viral factors, which has vital role in shaping disease dynamics. Because global public health agencies strive to mitigate the effect of mpox, understanding the genomic diversity of the virus and the phylogenetic relationships continues to be of paramount importance. The aim of this study was to add to the growing body of knowledge on mpox by providing epidemiologic and genomic findings that highlight the epidemic patterns of mpox in Nigeria. The objective was to determine the Nigeria mpox profile and identify the leading mpox lineage, therefore providing evidence-based interventions for outbreak prevention and control.

Although several studies have explored the epidemiology of clade II mpox globally, few have provided a detailed, large-scale genomic, and epidemiological assessment specifically focused on Nigeria over an extended period. This study uniquely analyzes 153 mpox genomes collected across 25 Nigerian states from 2017 to 2024, offering the most comprehensive phylogenetic mapping of clade II lineages in Nigeria to date. By identifying the emergence and dominance of lineage A.2.3, regional disparities in disease burden, and shifting demographic patterns, this work provides novel insights into the evolutionary dynamics and epidemiological trends of mpox in an endemic country. These findings contribute critical data needed for developing region-specific public health responses and highlight the importance of sustained genomic surveillance in endemic settings.

METHODS

Data sources

Metadata were obtained from the World Health Organization (WHO), Nigeria Centre for Disease Control (NCDC), National Center for Biotechnology Information (NCBI), and various published literature on mpox, accessed between 2 January and 21 January 2025. All retrieved data were processed and cross-examined for discrepancies using Microsoft Excel 2016 (Microsoft Office Microsoft Corp, Redmond, WA, USA). Graph plotting and geospatial mapping of confirmed mpox cases were performed using the Datawrapper web tool (<https://www.datawrapper.de/>, accessed 16 January 2025).

Retrieval of MPXV sequences

A total of 153 mpox genome sequences from 25 Nigerian states were identified on Nextstrain/Monkeypox (<https://nextstrain.org/Mpox/mpxv>, accessed 2 January 2025) and retrieved from GenBank (<https://www.ncbi.nlm.nih.gov/nuccore/>). Selection criteria included collection date (2017–2024), clade type (clade II), and sequence length ($\geq 19,700$ bp).

All retrieved genome sequences were derived from clinical human samples, as indicated by the associated metadata, with typical sample types including skin lesion swabs, blood, and respiratory specimens.

Quality control and filtering

Duplicate sequences, low-quality sequences, and incomplete genomes were removed using the Biostrings package from Bioconductor (R version 2024.12.0+467). Only 96 high-quality sequences were retained for further analysis.

Multiple sequence alignment (MSA)

Multiple sequence alignment was performed using the MAFFT online tool (<https://mafft.cbrc.jp/alignment/server/index.html>), aligning the retrieved sequences against the reference genome (OP535341.1).

Phylogenetic tree construction

The best-fit substitution model (HKY+F+I) for phylogenetic analysis was determined using IQ-TREE (version 2.3.6). Phylogenetic trees were constructed using BEAST (version 10.5.0) with a strict clock model, Coalescent (Exponential Growth) tree prior, and an MCMC chain length of 1,000,000. Visualization and annotation of trees were performed using FigTree (version 1.4.4) [17].

Mutation analysis of mpox genome sequence (2017–2023)

Mutation analysis of the mpox genome sequences was performed using the Nextclade tool available at <https://clades.nextstrain.org/> (accessed 28 May 2025). The 96 high-quality mpox genome sequences were uploaded in FASTA format and compared against the reference genome (GenBank accession number OP535341.1). Nextclade identified nucleotide substitutions and non-synonymous amino acid mutations relative to the reference. The resulting mutation profiles were downloaded and further analyzed to summarize the most frequent mutations observed across different years (2017–2024). Particular attention was given to the emergence of new mutations during major outbreak periods, especially in 2022. Mutations were mapped to their corresponding viral genes where possible, and their potential functional implications were noted based on the existing literature.

RESULTS

Retrieved metadata and MPXV sequences

Mpox metadata were obtained from public repositories, including the NCBI (<https://www.ncbi.nlm.nih.gov/nuccore/>, accessed on 2 January 2025), nextstrain/monkeypox (<https://nextstrain.org/Mpox/mpxv>, accessed on 2 January 2025), and the NCDC (<https://ncdc.gov.ng/ncdc.gov.ng/>, accessed on 2 January 2025).

Figure 1 illustrates the trend of mpox infections across Nigeria between 2017 and 2024. The year 2022 recorded the highest number of confirmed cases, with a total of 482. This was followed by 92 cases in 2024 and 88 cases in 2017, while no confirmed cases were reported in 2020.

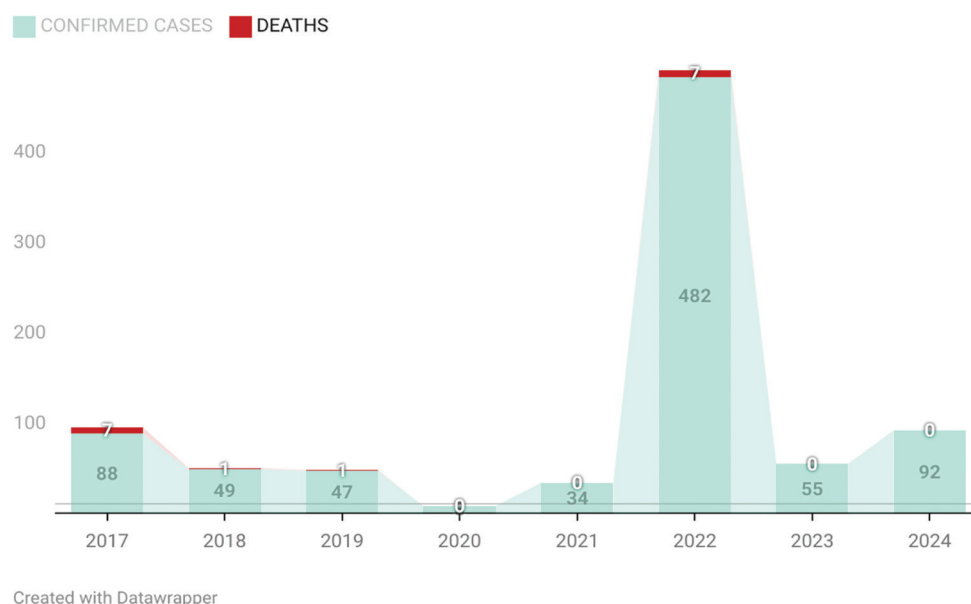


FIGURE 1 | Trends of confirmed mpox clade II case in Nigeria from 2017–2024 (<https://datawrapper.dwcdn.net/DjqtF/1/>, accessed on 21 January 2025).

The years 2018, 2019, 2021, and 2023 recorded 49, 47, 34, and 55 confirmed cases, respectively.

Figures 2 and 3 illustrate the geospatial distribution of confirmed cases of mpox in Nigeria from 2017 to 2024. Fig 2 illustrates the regional distribution of confirmed cases in Nigeria while Fig 3 presents a state-level overview of mpox prevalence in the country. It can be observed that southern and eastern Nigeria recorded the highest burden of the infections, with Rivers and Bayelsa states reporting the highest incidence (29 and 17 cases, respectively). In contrast, the western, north-central, and northern regions experienced comparatively lower number of cases. Notably, Lagos and Abuja reported higher case counts (16 and 6 cases, respectively) relative to other states within their respective regions.

Gender profile and age differences of mpox cases in Nigeria (2017–2024)

Data from the NCDC revealed that males accounted for approximately 70% of the 6001 suspected and confirmed mpox cases in Nigeria between 2017 and 2024 [18]. The major age demographic consistently infected by the MPXV were adults. The specific age group most often affected has always fluctuated with time but reports from the NCDC on the 2017 epidemic showed that individuals within the 21–30 y age group were mostly affected

by the disease outbreak and made up 39% of all reported cases that year [19,20]. Records from the 2018–2021 outbreaks showed that individuals in the 31–40 y age group were predominantly affected, which accounted for 35% and 38% of confirmed cases in 2018 and 2021, respectively [20,21].

By 2022 most affected individuals were 20–41 y of age [19]. However, in 2023 the highest burden of reported cases shifted again to the 21–30 y age group [14]. As of 2024 data for the most affected age group is unavailable.

Genomes and phylogenetic analysis of Nigeria MPXV

The retrieved mpox genome sequenced and deposited on biological databases were obtained from diverse hosts, including *Homo sapiens* (n = 570), *Cricetomys gambianus* (n = 1), *Crocidura littoralis* (n = 1), *Cynomys* (n = 1), *Funisciurus* (n = 1), *Funisciurus anerythrus* (n = 1), *Funisciurus bayonii* (n = 1), *Gliridae* (n = 1), *Malacomys longipes* (n = 1), *Pan troglodytes verus* (n = 12), and *Platyrrhini* (n = 1) [16]. All retrieved genome sequences in the current study were derived from human hosts.

A total of 153 mpox genomes from 25 Nigerian states were retrieved from the NCBI database (accessed 2 January 2025). Selecting these sequences was based on the sequence length ($\geq 19,700$ bp) and confirmation that the

Number of reported complete/near complete mpox isolates from Nigeria

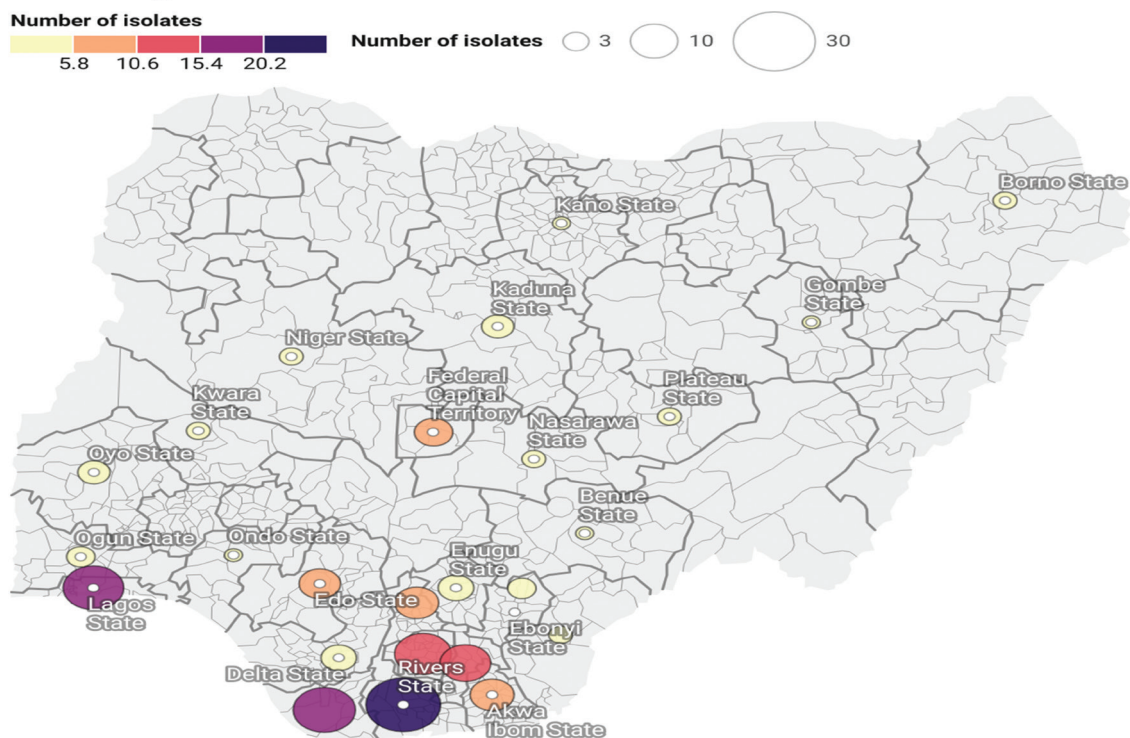


FIGURE 2 | Geospatial distribution of confirmed mpox cases from 2017–2024 (<https://datawrapper.dwcdn.net/FX2MI/1/>, accessed on 21 January 2025).

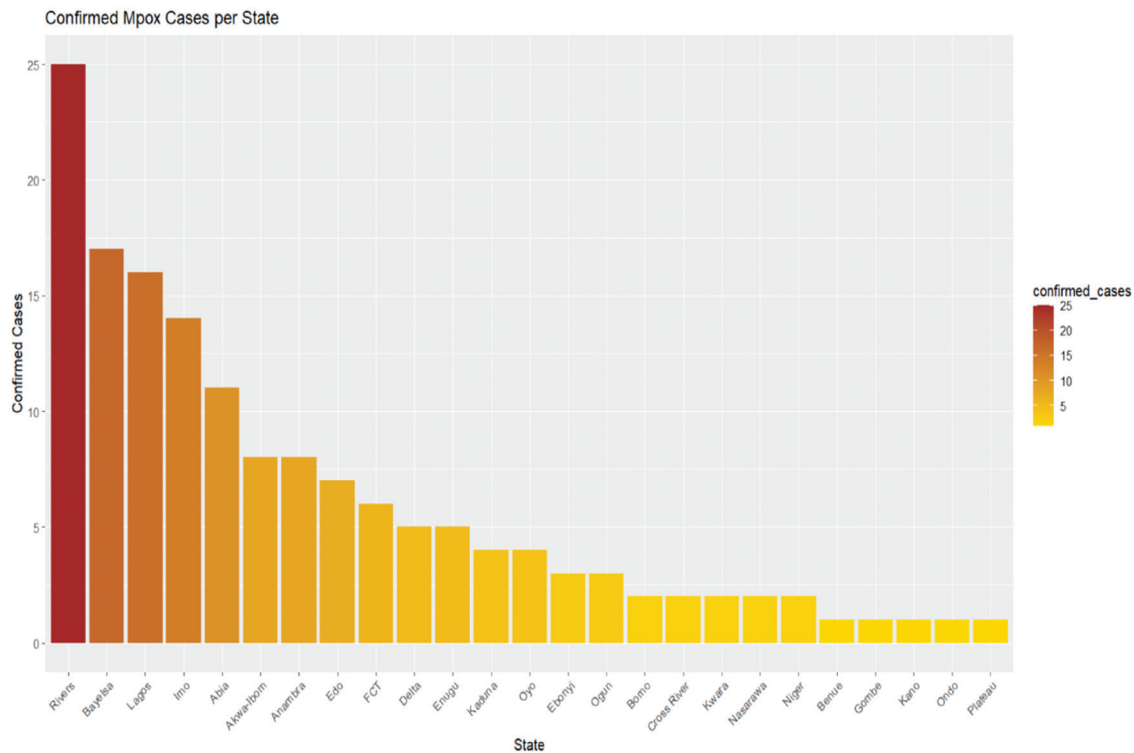


FIGURE 3 | Distribution of the mpox incidence in 25 states in Nigeria.

sequence is an mpox clade IIb genome, which is the predominant clade in Nigeria.

Phylogenetic studies of the mpox clade II genomes revealed the temporal occurrence of mpox clade II lineages in Nigeria from 2017–2024, including A, A.1, A.2, A.2.1, A.2.2, A.2.3, A.3, and B.1. The frequencies of lineage occurrence from Nigeria between 2017 and 2024 were as follows: A ($n = 37$); A.2 ($n = 24$); A.2.2 ($n = 18$); A.2.3 ($n = 53$); A.3 ($n = 11$); and B.1 ($n = 3$). Additionally, four genomes from lacked lineage assignments, which may have been due to incomplete or ambiguous data (Fig 4).

The phylogenetic analysis in Fig 4 showed that while lineage A.2.3 was the most prevalent variant in the 2022 outbreak, other lineages which include lineages A and A.2 had significant circulation; this is represented in the bar chart in Fig 5. The outbreak dynamics were shaped by the co-circulating and temporal interplay of these major and minor lineages, which contributes to the intricate epidemiological landscape of mpox in Nigeria during the study period.

Mutation analysis of mpox genome sequences (2017–2023)

A detailed mutation analysis of 96 mpox clade IIb genome sequences collected across Nigeria between 2017 and 2023 revealed numerous amino acid changes, suggesting ongoing viral adaptation. A total of 361 unique mutations were identified, spanning various viral genes involved in replication, transcription, structural integrity, and immune modulation. This is shown in Table 1.

The most frequently observed mutations were OPG118:K606E with a frequency of 0.049483415%, followed by OPG180:D442N with a frequency of 0.034801523%. These mutations and those with an occurrence frequency of 0.033713975% appeared consistently in sequences from the 2022 outbreak onward and persisted into 2023, indicating their potential role in sustained transmission or selective advantage. Notably, OPG176:Q22 introduced premature stop codons among others, which are likely to result in truncated proteins and possible loss of function.

Table 1 summarizes each identified mutation, including gene locus, amino acid substitution, observed frequency, estimated year of first appearance, and functional interpretation.

DISCUSSION

Since the re-emergence of mpox in Nigeria in 2017, the disease has presented complex epidemiologic patterns, manifested by fluctuating incidence rates and regional variations. The 2017 outbreak is an important period in the epidemiologic history of mpox in Nigeria, this outbreak resulted in 88 confirmed cases and 7 deaths, as reported by the NCDC and the WHO [18,22]. This event emphasizes the resurgence of mpox after decades of relative quiescence. This event was followed by repeated spontaneous outbreaks over the next 2 y that were marked by a decline in the number of confirmed cases until 2020 in which there were no reported cases. However, by 2021 the apparent absence of mpox cases was interrupted when

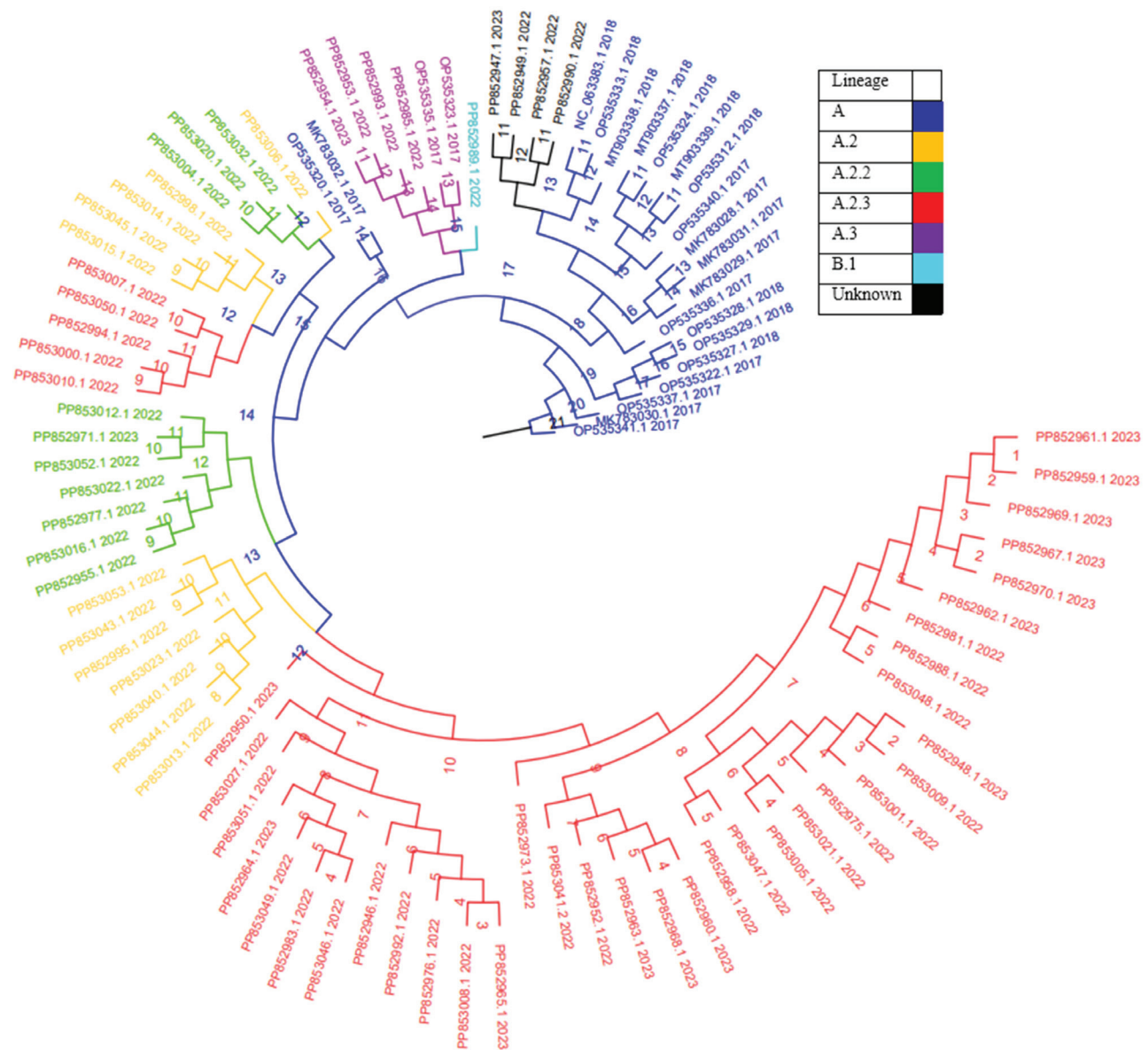


FIGURE 4 | Phylogenetic analysis of 153 MPXV selected genomes within Nigeria from 2017–2024.

the disease resurfaced, resulting in 34 confirmed cases reported from 8 states in the country, although no deaths were recorded (Fig 1). This outbreak highlighted the persistent public health threat posed by mpox in Nigeria, which is characterized by unpredictable outbreak patterns and dynamic transmission dynamics.

In 2022 Nigeria recorded the highest annual incidence of mpox, which led to 482 confirmed cases and 7 deaths. The 2022 outbreak represents a significant surge in disease outbreaks compared to previous years, suggesting a likely shift in the virus transmission dynamics, perhaps driven by changes in viral genetic adaptations, human behavior, or ecologic factors. By 2023 Nigeria recorded a sharp decline in the incidence of mpox with only 55 confirmed cases and no deaths, demonstrating the unpredictable characteristics of mpox outbreaks. However, by the end of 2024

the number of confirmed cases rose again by nearly 100%, resulting in 92 confirmed cases with no reported fatalities. The cumulative total of confirmed mpox cases in Nigeria from 2017–2024 reached 731, with 16 reported deaths [8,14]. These fluctuating trends highlight the complex epidemiologic landscape of mpox in Nigeria and emphasize the necessity for continuous surveillance, genomic monitoring, and adaptive public health interventions.

Observation from the geospatial analysis conducted in this study revealed that significant regional disparities occurred across Nigeria (Fig 2). Our research revealed that the south-south and south-east regions of Nigeria had the highest frequencies of mpox cases, while the southwestern regions recorded lower incidences and the north-central and northern regions recorded the lowest incidences during the study years. For example, Rivers State, which is

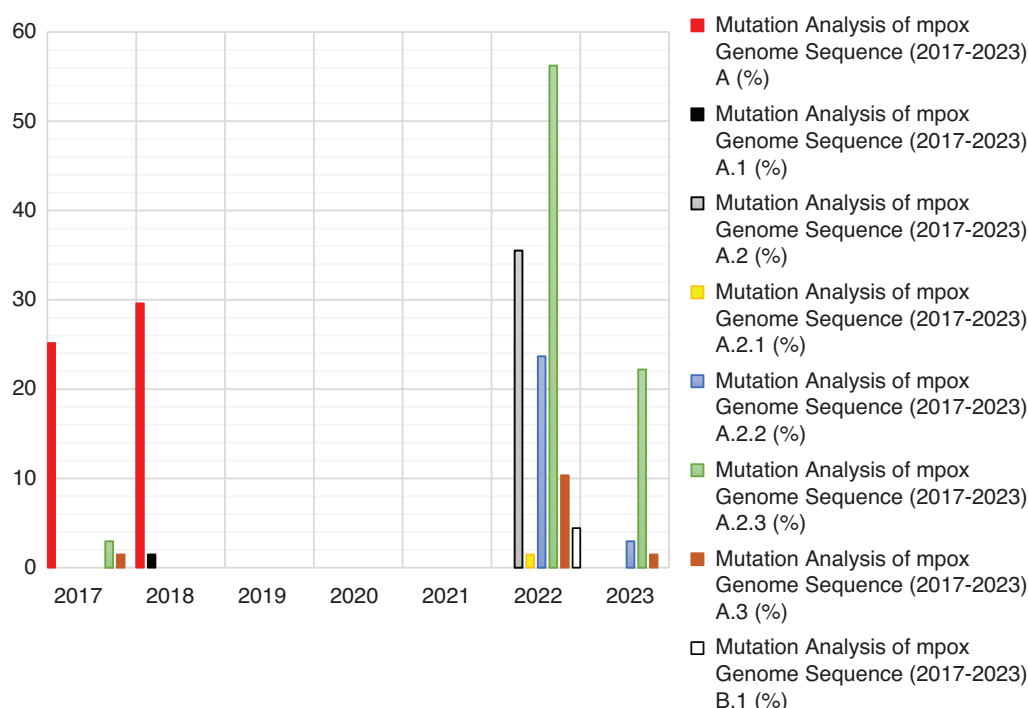


FIGURE 5 | Mutation analysis of mpox genome sequences (2017–2023).

located in the south-south region, recorded the highest number of confirmed cases (25), which was followed by Bayelsa State within the same region (17). In the south-east, Imo State reported the highest incidence of mpox infections (14). Although the south-west region also presented a significant number of cases in Lagos State (16). This number was most likely due to the status of Lagos State as the economic hub in Nigeria, which is typically characterized by a high population density, increased mobility, and complex socio-economic interactions. Internal migration patterns into Lagos State may have catalyzed spread of the disease, which highlights the part of urbanization in shaping disease dynamics. Other south-western states reported fewer to no cases.

Conversely, the north-central and northern regions were the least affected regions, with the exception of the Federal Capital Territory (FCT), which reported a considerable number of cases (6) compared to other north-central and northern states. The higher incidence in the FCT may be linked to factors similar to those observed in Lagos State, including high population mobility and dense socio-economic activities (Fig 3).

These observed regional differences suggest that mpox transmission dynamics in Nigeria could be attributed to a combination of demographic, environmental, socio-economic, and cultural factors, including population density, human-animal interactions, human-human interactions, and healthcare access. In addition, ecologic influences, such as vegetation type and climatic conditions, could have a role in the regional mpox distribution, as observed in other endemic countries, like the DRC [7].

The observed spatial distribution patterns suggests that mpox may be more prevalent in rainforest regions compared to savanna and arid areas, highlighting the need for region-specific public health interventions.

One significant challenge in Nigeria is the problem of underreporting mpox cases. This challenge potentially obscures the true epidemiologic burden of mpox in Nigeria. Contributing factors to this problem include inadequate laboratory infrastructure, limited surveillance systems, and insufficient diagnostic capacities, particularly in rural and remote areas. In addition, socio-cultural factors, such as stigmatization, poverty, and poor health-care-seeking behaviors, further worsen this problem. Other factors include asymptomatic infections and potential sexual transmission, which also complicate accurate case detection and reporting [23,24]. These limitations hinder comprehensive epidemiologic assessments and impede effective public health responses. Addressing these challenges requires strengthening the public health infrastructure in Nigeria, enhancing laboratory capacity and implementing community-based surveillance strategies to improve case detection and reporting accuracy.

Phylogenetic analysis of mpox genomes in Nigeria (Fig 4) revealed the predominance of lineage A.2.3, accounting for 53 genomes, followed by lineage A with 37 genomes and lineage A.2 with 24 genomes. The least represented lineages were A.1 and A.2.1, with only one genome each. The phylogenetic tree showed distinct clustering patterns with lineage A positioned near the base, indicating emergence of lineage A during the early phase of the mpox epidemic in Nigeria between 2017 and 2018.

TABLE 1 | Mutation analysis of mpox genome sequences (2017–2023).

Mutation Analysis of mpox Genome Sequence (2017–2023)					
Gene/ORF	Mutation	No. of Mutations	Frequency (%)	First Appearance	Common After 2022?
OPG021	A66T	3	0.001631321	MK783029.1	2017 No
OPG015	D334N	3	0.001631321	MK783029.1	2017 No
OPG015_dup	D334N	3	0.001631321	MK783029.1	2017 No
OPG025	D491N	1	0.000543774	MK783029.1	2017 No
OPG019	R62K	3	0.001631321	MK783029.1	2017 No
OPG175	E112K	1	0.000543774	MK783030.1	2017 No
OPG150	S43L	11	0.005981512	MK783030.1	2017 No
OPG209	E104K	1	0.000543774	MK783032.1	2017 No
OPG042	R366C	1	0.000543774	MK783032.1	2017 No
OPG180	D442N	64	0.034801523	OP535320.1	2017 Yes
OPG031	D44N	2	0.001087548	OP535320.1	2017 No
OPG165	L52F	2	0.001087548	OP535320.1	2017 No
OPG208	M258I	2	0.001087548	OP535320.1	2017 No
OPG005	R40K	2	0.001087548	OP535320.1	2017 No
OPG170	E58K	3	0.001631321	OP535322.1	2017 No
OPG133	G619E	3	0.001631321	OP535322.1	2017 No
OPG039	D256N	7	0.003806417	OP535323.1	2017 No
OPG117	D265N	6	0.003262643	OP535323.1	2017 No
OPG146	D50N	6	0.003262643	OP535323.1	2017 No
OPG120	E260K	6	0.003262643	OP535323.1	2017 No
OPG071	R877Q	6	0.003262643	OP535323.1	2017 No
OPG074	S53L	6	0.003262643	OP535323.1	2017 No
OPG085	D427N	1	0.000543774	OP535336.1	2017 No
OPG145	D453N	1	0.000543774	OP535336.1	2017 No
OPG129	E227K	1	0.000543774	OP535336.1	2017 No
OPG050	R26Q	1	0.000543774	OP535336.1	2017 No
OPG136	R830Q	1	0.000543774	OP535336.1	2017 No
				Alanine to threonine at position 66	Hypothetical or uncharacterized protein; functional role unclear
				Aspartic acid to asparagine at position 334	Replication-associated protein; could influence viral genome replication
				Aspartic acid to asparagine at position 334	Hypothetical or uncharacterized protein; functional role unclear
				Aspartic acid to asparagine at position 491	Hypothetical or uncharacterized protein; functional role unclear
				Arginine to lysine at position 62	Hypothetical or uncharacterized protein; functional role unclear
				Glutamic acid to lysine at position 112	Hypothetical or uncharacterized protein; functional role unclear
				Serine to leucine at position 43	DNA helicase; mutation could impair replication or repair
				Glutamic acid to lysine at position 104	Structural virion assembly protein; truncation could impact particle formation
				Arginine to cysteine at position 366	Hypothetical or uncharacterized protein; functional role unclear
				Aspartic acid to asparagine at position 442	Structural scaffold; mutation may affect particle assembly
				Aspartic acid to asparagine at position 44	Immune evasion protein; mutation may alter interaction with host defenses
				Leucine to phenylalanine at position 52	DNA-binding protein; mutation may affect regulatory binding efficiency
				Methionine to isoleucine at position 258	Involved in DNA metabolism or repair; mutation impact unclear
				Arginine to lysine at position 40	Entry-fusion complex component; may affect viral entry efficiency
				Glutamic acid to lysine at position 58	Hypothetical or uncharacterized protein; functional role unclear
				Glycine to glutamic acid at position 619	Hypothetical or uncharacterized protein; functional role unclear
				Aspartic acid to asparagine at position 256	Poorly characterized; potential regulatory or accessory protein
				Aspartic acid to asparagine at position 265	Hypothetical or uncharacterized protein; functional role unclear
				Aspartic acid to asparagine at position 50	Unknown regulatory function; mutation impact unclear
				Glutamic acid to lysine at position 260	Hypothetical or uncharacterized protein; functional role unclear
				Arginine to glutamine at position 877	Hypothetical or uncharacterized protein; functional role unclear
				Serine to leucine at position 53	Hypothetical or uncharacterized protein; functional role unclear
				Aspartic acid to asparagine at position 427	Hypothetical or uncharacterized protein; functional role unclear
				Aspartic acid to asparagine at position 453	DNA-binding protein; mutation may alter early viral transcription
				Glutamic acid to lysine at position 227	Hypothetical or uncharacterized protein; functional role unclear
				Arginine to glutamine at position 26	Immunomodulatory protein; possible impact on evasion of host immunity
				Arginine to glutamine at position 830	Hypothetical or uncharacterized protein; functional role unclear

TABLE 1 | (continued)

Mutation Analysis of mpox Genome Sequence (2017–2023)						Notes
Gene/ORF	Mutation	No. of Mutations	Frequency (%)	First Appearance	Common After 2022?	
OPG031	D13N	62	0.033713975	OP535337.1	2017 Yes	Immune evasion protein; mutation may alter interaction with host defenses
OPG061	D150N	8	0.00435019	OP535337.1	2017 No	Hypothetical or uncharacterized protein; functional role unclear
OPG047	D187N	62	0.033713975	OP535337.1	2017 Yes	Envelope or virion protein; may alter virion structure or assembly
OPG190	D252N	3	0.001631321	OP535337.1	2017 No	Host range determinant; mutation could influence interspecies transmission
OPG145	D29N	62	0.033713975	OP535337.1	2017 Yes	DNA-binding protein; mutation may alter early viral transcription
OPG023	D623N	8	0.00435019	OP535337.1	2017 No	Hypothetical or uncharacterized protein; functional role unclear
OPG023	E159K	8	0.00435019	OP535337.1	2017 No	Hypothetical or uncharacterized protein; functional role unclear
OPG022	E22K	8	0.00435019	OP535337.1	2017 No	Hypothetical or uncharacterized protein; functional role unclear
OPG113	E553K	62	0.033713975	OP535337.1	2017 Yes	Late transcription factor or helicase; mutation may affect viral gene regulation
OPG053	E87K	10	0.005437738	OP535337.1	2017 No	Membrane or glycoprotein component; may affect virus-host attachment
OPG053	G38E	62	0.033713975	OP535337.1	2017 Yes	Membrane or glycoprotein component; may affect virus-host attachment
OPG188	H120Y	62	0.033713975	OP535337.1	2017 Yes	Accessory or structural protein; mutation may affect stability
OPG005	L54F	8	0.00435019	OP535337.1	2017 No	Entry-fusion complex component; may affect viral entry efficiency
OPG061	M222I	1	0.000543774	OP535337.1	2017 No	Hypothetical or uncharacterized protein; functional role unclear
OPG190	P221S	62	0.033713975	OP535337.1	2017 Yes	Host range determinant; mutation could influence interspecies transmission
OPG176	Q22*	62	0.033713975	OP535337.1	2017 Yes	Truncating mutation (stop codon); likely loss of function
OPG019	Q89*	8	0.00435019	OP535337.1	2017 No	Hypothetical or uncharacterized protein; functional role unclear
OPG205	R689C	62	0.033713975	OP535337.1	2017 Yes	Envelope glycoprotein; mutation likely affects immune evasion or viral entry
OPG172	S140F	8	0.00435019	OP535337.1	2017 No	Surface antigen; mutation may impact immune recognition
OPG082	S140L	8	0.00435019	OP535337.1	2017 No	Envelope-associated protein; mutation may influence membrane fusion
OPG174	S44L	62	0.033713975	OP535337.1	2017 Yes	Membrane-associated structural protein; mutation may affect virion stability
OPG113	V90I	8	0.00435019	OP535337.1	2017 No	Late transcription factor or helicase; mutation may affect viral gene regulation
OPG208	E207K	5	0.002718869	OP535340.1	2017 No	Involved in DNA metabolism or repair; mutation impact unclear
OPG080	E366K	6	0.003262643	OP535340.1	2017 No	Late transcription factor; role in viral assembly or structural gene expression
OPG205	E452K	1	0.000543774	OP535340.1	2017 No	Envelope glycoprotein; mutation likely affects immune evasion or viral entry
OPG118	K606E	91	0.049483415	OP535341.1	2017 Yes	Involved in viral DNA metabolism; mutation could disrupt replication processes
OPG056	S609F	7	0.003806417	OP535341.1	2017 No	Glycoprotein precursor; mutation might influence virion morphogenesis
OPG149	S17L	1	0.000543774	MT903338.1	2018 No	Hypothetical or uncharacterized protein; functional role unclear

TABLE 1 | (continued)

Mutation Analysis of mpox Genome Sequence (2017–2023)								
Gene/ORF	Mutation	No. of Mutations	Frequency (%)	First Appearance	Common After 2022?	Protein Change	Notes	
OPG156	R276W	1	0.000543774	MT903339.1	2018	No	Arginine to tryptophan at position 276	Hypothetical or uncharacterized protein; functional role unclear
OPG139	P41H	1	0.000543774	NC_063383.1	2018	No	Proline to histidine at position 41	Envelope-associated protein; may impact virus-cell fusion
OPG105	V113I	1	0.000543774	NC_063383.1	2018	No	Valine to isoleucine at position 113	Hypothetical or uncharacterized protein; functional role unclear
OPG037	S148L	11	0.005981512	OP535312.1	2018	No	Serine to leucine at position 148	Transcription modulator; may influence viral gene expression timing
OPG133	D168Y	1	0.000543774	OP535324.1	2018	No	Aspartic acid to tyrosine at position 168	Hypothetical or uncharacterized protein; functional role unclear
OPG176	D24N	4	0.002175095	OP535324.1	2018	No	Aspartic acid to asparagine at position 24	Truncating mutation (stop codon); likely loss of function
OPG024	G6D	1	0.000543774	OP535324.1	2018	No	Glycine to aspartic acid at position 6	Function poorly characterized; likely a hypothetical or regulatory protein
OPG150	N100D	4	0.002175095	OP535324.1	2018	No	Asparagine to aspartic acid at position 100	DNA helicase; mutation could impair replication or repair
OPG084	Q620R	4	0.002175095	OP535324.1	2018	No	Glutamine to arginine at position 620	DNA replication or repair protein; potential effect on replication fidelity
OPG104	T12A	4	0.002175095	OP535324.1	2018	No	Threonine to alanine at position 12	Hypothetical or uncharacterized protein; functional role unclear
OPG187	V251I	1	0.000543774	OP535324.1	2018	No	Valine to isoleucine at position 251	Hypothetical or uncharacterized protein; functional role unclear
OPG052	R11*	1	0.000543774	OP535333.1	2018	No	Arginine to stop codon at position 11	Hypothetical or uncharacterized protein; functional role unclear
OPG143	A3E	2	0.001087548	PP852946.1	2022	No	Alanine to glutamic acid lysine at position 3	Hypothetical or uncharacterized protein; functional role unclear
OPG106	A94T	2	0.001087548	PP852946.1	2022	No	Alanine to threonine at position 94	Hypothetical or uncharacterized protein; functional role unclear
OPG156	D100N	2	0.001087548	PP852946.1	2022	No	Aspartic acid to asparagine at position 100	Hypothetical or uncharacterized protein; functional role unclear
OPG068	D341N	2	0.001087548	PP852946.1	2022	No	Aspartic acid to asparagine at position 341	Hypothetical or uncharacterized protein; functional role unclear
OPG057	E353K	3	0.001631321	PP852946.1	2022	No	Glutamic acid to lysine at position 353	Hypothetical or uncharacterized protein; functional role unclear
OPG176	E40K	2	0.001087548	PP852946.1	2022	No	Glutamic acid to lysine at position 40	Truncating mutation (stop codon); likely loss of function
OPG122	E99K	2	0.001087548	PP852946.1	2022	No	Glutamic acid to lysine at position 99	Hypothetical or uncharacterized protein; functional role unclear
OPG205	A35V	40	0.021750952	PP852949.1	2022	No	Alanine to valine at position 35	Envelope glycoprotein; mutation likely affects immune evasion or viral entry
OPG170	D186N	40	0.021750952	PP852949.1	2022	No	Aspartic acid to asparagine at position 186	Hypothetical or uncharacterized protein; functional role unclear
OPG198	D94N	40	0.021750952	PP852949.1	2022	No	Aspartic acid to asparagine at position 94	Hypothetical or uncharacterized protein; functional role unclear
OPG047	E176K	40	0.021750952	PP852949.1	2022	No	Glutamic acid to lysine at position 176	Envelope or virion protein; may alter virion structure or assembly
OPG117	E244K	3	0.001631321	PP852949.1	2022	No	Glutamic acid to lysine at position 244	Hypothetical or uncharacterized protein; functional role unclear
OPG195	E46K	40	0.021750952	PP852949.1	2022	No	Glutamic acid to lysine at position 46	Hypothetical or uncharacterized protein; functional role unclear
OPG123	M463I	11	0.005981512	PP852949.1	2022	No	Methionine to isoleucine at position 463	Hypothetical or uncharacterized protein; functional role unclear
OPG209	Q57*	39	0.021207178	PP852949.1	2022	No	Glutamine to stop codon at position 57	Structural virion assembly protein; truncation could impact particle formation
OPG075	R72K	40	0.021750952	PP852949.1	2022	No	Arginine to lysine at position 72	Hypothetical or uncharacterized protein; functional role unclear

TABLE 1 | (continued)

Mutation Analysis of mpox Genome Sequence (2017–2023)						Notes
Gene/ORF	Mutation	No. of Mutations	Frequency (%)	First Appearance	Common After 2022?	
OPG030	L13F	1	0.000543774	PP852953.1	2022 No	Leucine to phenylalanine at position 13
OPG190	P126S	1	0.000543774	PP852953.1	2022 No	Proline to serine at position 126
OPG187	R292Q	1	0.000543774	PP852953.1	2022 No	Arginine to glutamine at position 292
OPG210	D32N	1	0.000543774	PP852955.1	2022 No	Aspartic acid to asparagine at position 32
OPG015	E242K	1	0.000543774	PP852955.1	2022 No	Glutamic acid to lysine at position 242
OPG015_dup	E242K	1	0.000543774	PP852955.1	2022 No	Glutamic acid to lysine at position 242
OPG068	E353K	1	0.000543774	PP852955.1	2022 No	Glutamic acid to lysine at position 353
OPG003	H502Y	1	0.000543774	PP852955.1	2022 No	Histidine to tyrosine at position 502
OPG003_dup	H502Y	1	0.000543774	PP852955.1	2022 No	Histidine to tyrosine at position 502
OPG085	P376H	1	0.000543774	PP852955.1	2022 No	Proline to histidine at position 376
OPG101	G162S	2	0.001087548	PP852958.1	2022 No	Glycine to serine at position 162
OPG031	D196N	1	0.000543774	PP852973.1	2022 No	Aspartic acid to asparagine at position 196
OPG065	R28C	2	0.001087548	PP852973.1	2022 No	Arginine to cysteine at position 28
OPG198	Q133*	2	0.001087548	PP852976.1	2022 No	Glutamine to stop codon at position 133
OPG210	D159N	5	0.002718869	PP852977.1	2022 No	Aspartic acid to asparagine at position 159
OPG025	D227N	1	0.000543774	PP852977.1	2022 No	Aspartic acid to asparagine at position 227
OPG023	D341N	5	0.002718869	PP852977.1	2022 No	Aspartic acid to asparagine at position 341
OPG115	E61K	1	0.000543774	PP852977.1	2022 No	Glutamic acid to lysine at position 61
OPG178	P39S	1	0.000543774	PP852977.1	2022 No	Proline to serine at position 39
OPG117	R479K	1	0.000543774	PP852977.1	2022 No	Arginine to lysine at position 479
OPG210	S1194F	1	0.000543774	PP852977.1	2022 No	Serine to phenylalanine at position 119
OPG036	S51F	5	0.002718869	PP852977.1	2022 No	Serine to phenylalanine at position 51
OPG049	S95L	5	0.002718869	PP852977.1	2022 No	Serine to leucine at position 95
OPG037	V359I	5	0.002718869	PP852977.1	2022 No	Valine to isoleucine at position 359
OPG136	G509D	1	0.000543774	PP852981.1	2022 No	Glycine to aspartic acid at position 509
OPG074	S45L	1	0.000543774	PP852981.1	2022 No	Serine to leucine at position 45
OPG209	R137*	1	0.000543774	PP852983.1	2022 No	Arginine to stop codon at position 137

DNA-directed RNA polymerase; possible effect on viral transcription

Host range determinant; mutation could influence interspecies transmission

Hypothetical or uncharacterized protein; functional role unclear

Large multi-domain protein; mutations likely affect multiple viral processes

Replication-associated protein; could influence viral genome replication

Hypothetical or uncharacterized protein; functional role unclear

Hypothetical or uncharacterized protein; functional role unclear

Involved in host range determination or protein interaction; duplication may enhance viral adaptation

Hypothetical or uncharacterized protein; functional role unclear

Hypothetical or uncharacterized protein; functional role unclear

Hypothetical or uncharacterized protein; functional role unclear

Immune evasion protein; mutation may alter interaction with host defenses

Hypothetical or uncharacterized protein; functional role unclear

Hypothetical or uncharacterized protein; functional role unclear

Large multi-domain protein; mutations likely affect multiple viral processes

Hypothetical or uncharacterized protein; functional role unclear

Hypothetical or uncharacterized protein; functional role unclear

Hypothetical or uncharacterized protein; functional role unclear

Hypothetical or uncharacterized protein; functional role unclear

Hypothetical or uncharacterized protein; functional role unclear

Large multi-domain protein; mutations likely affect multiple viral processes

Hypothetical or uncharacterized protein; functional role unclear

Hypothetical or uncharacterized protein; functional role unclear

Transcription modulator; may influence viral gene expression timing

Hypothetical or uncharacterized protein; functional role unclear

Hypothetical or uncharacterized protein; functional role unclear

Structural virion assembly protein; truncation could impact particle formation

TABLE 1 | (continued)

Mutation Analysis of mpox Genome Sequence (2017–2023)						Notes
Gene/ORF	Mutation	No. of Mutations	Frequency (%)	First Appearance	Common After 2022?	
OPG139	A17T	1	0.000543774	PP852985.1	2022 No	Envelope-associated protein; may impact virus-cell fusion
OPG025	A423D	1	0.000543774	PP852985.1	2022 No	Hypothetical or uncharacterized protein; functional role unclear
OPG092	D196N	1	0.000543774	PP852985.1	2022 No	Hypothetical or uncharacterized protein; functional role unclear
OPG210	D209N	1	0.000543774	PP852985.1	2022 No	Large multi-domain protein; mutations likely affect multiple viral processes
OPG072	D56N	1	0.000543774	PP852985.1	2022 No	Hypothetical or uncharacterized protein; functional role unclear
OPG136	D98N	1	0.000543774	PP852985.1	2022 No	Hypothetical or uncharacterized protein; functional role unclear
OPG056	E125K	1	0.000543774	PP852985.1	2022 No	Glycoprotein precursor; mutation might influence virion morphogenesis
OPG098	E162K	1	0.000543774	PP852985.1	2022 No	Hypothetical or uncharacterized protein; functional role unclear
OPG079	E176V	1	0.000543774	PP852985.1	2022 No	Hypothetical or uncharacterized protein; functional role unclear
OPG145	E435K	1	0.000543774	PP852985.1	2022 No	DNA-binding protein; mutation may alter early viral transcription
OPG145	E62K	1	0.000543774	PP852985.1	2022 No	DNA-binding protein; mutation may alter early viral transcription
OPG176	H221Y	1	0.000543774	PP852985.1	2022 No	Truncating mutation (stop codon); likely loss of function
OPG109	H740Y	1	0.000543774	PP852985.1	2022 No	Large structural or scaffolding protein; mutation might affect virion formation
OPG079	I192S	1	0.000543774	PP852985.1	2022 No	Hypothetical or uncharacterized protein; functional role unclear
OPG071	L108F	1	0.000543774	PP852985.1	2022 No	Hypothetical or uncharacterized protein; functional role unclear
OPG079	L177I	1	0.000543774	PP852985.1	2022 No	Hypothetical or uncharacterized protein; functional role unclear
OPG193	L263F	1	0.000543774	PP852985.1	2022 No	Virion-associated protein; could alter assembly dynamics
OPG094	M142I	1	0.000543774	PP852985.1	2022 No	Hypothetical or uncharacterized protein; functional role unclear
OPG210	M174I	1	0.000543774	PP852985.1	2022 No	Large multi-domain protein; mutations likely affect multiple viral processes
OPG210	P722S	1	0.000543774	PP852985.1	2022 No	Large multi-domain protein; mutations likely affect multiple viral processes
OPG053	P78S	1	0.000543774	PP852985.1	2022 No	Membrane or glycoprotein component; may affect virus-host attachment
OPG145	R243Q	1	0.000543774	PP852985.1	2022 No	DNA-binding protein; mutation may alter early viral transcription
OPG047	R48C	1	0.000543774	PP852985.1	2022 No	Envelope or virion protein; may alter virion structure or assembly
OPG001_dup	S105L	1	0.000543774	PP852985.1	2022 No	Hypothetical or uncharacterized protein; functional role unclear
OPG001	S105L	1	0.000543774	PP852985.1	2022 No	Hypothetical or uncharacterized protein; functional role unclear
OPG130	S121F	1	0.000543774	PP852985.1	2022 No	Host range factor; mutation might alter host specificity or infectivity
OPG189	S255L	1	0.000543774	PP852985.1	2022 No	Envelope protein; mutation might influence host cell tropism
OPG150	S307L	1	0.000543774	PP852985.1	2022 No	DNA helicase; mutation could impair replication or repair

TABLE 1 | (continued)

Mutation Analysis of mpox Genome Sequence (2017–2023)						Notes
Gene/ORF	Mutation	No. of Mutations	Frequency (%)	First Appearance	Common After 2022?	
OPG093	S30L	1	0.000543774	PP852985.1	2022 No	Serine to leucine at position 30
OPG002	S54F	1	0.000543774	PP852985.1	2022 No	Serine to phenylalanine at position 54
OPG002_dup	S54F	1	0.000543774	PP852985.1	2022 No	Serine to phenylalanine at position 54
OPG105	S734L	1	0.000543774	PP852985.1	2022 No	Serine to leucine at position 734
OPG079	T193S	1	0.000543774	PP852985.1	2022 No	Threonine to serine at position 193
OPG025	D406N	1	0.000543774	PP852988.1	2022 No	Aspartic acid to asparagine at position 406
OPG042	R263K	1	0.000543774	PP852993.1	2022 No	Arginine to lysine at position 263
OPG198	S179L	1	0.000543774	PP852993.1	2022 No	Serine to leucine at position 179
OPG188	S213L	1	0.000543774	PP852993.1	2022 No	Serine to leucine at position 213
OPG023	D372N	1	0.000543774	PP852994.1	2022 No	Aspartic acid to asparagine at position 372
OPG119	D132N	1	0.000543774	PP852995.1	2022 No	Aspartic acid to asparagine at position 132
OPG082	D105N	2	0.001087548	PP852998.1	2022 No	Aspartic acid to asparagine at position 105
OPG084	D392N	2	0.001087548	PP852998.1	2022 No	Aspartic acid to asparagine at position 392
OPG205	S144L	1	0.000543774	PP852998.1	2022 No	Serine to leucine at position 144
OPG176	S6L	1	0.000543774	PP852998.1	2022 No	Serine to leucine at position 6
OPG083	R341K	1	0.000543774	PP853000.1	2022 No	Arginine to lysine at position 341
OPG021	S196L	1	0.000543774	PP853000.1	2022 No	Serine to leucine at position 196
OPG046	D139N	1	0.000543774	PP853001.1	2022 No	Aspartic acid to asparagine at position 139
OPG134	E124D	1	0.000543774	PP853004.1	2022 No	Glutamic acid to aspartic acid at position 124
OPG144	E175K	1	0.000543774	PP853004.1	2022 No	Glutamic acid to lysine at position 175
OPG016	E96K	1	0.000543774	PP853004.1	2022 No	Glutamic acid to lysine at position 96
OPG134	H125S	1	0.000543774	PP853004.1	2022 No	Histidine to serine at position 125
OPG134	K127N	2	0.001087548	PP853004.1	2022 No	Lysine to asparagine at position 127
OPG134	S126N	1	0.000543774	PP853004.1	2022 No	Serine to asparagine at position 126
OPG031	D244N	2	0.001087548	PP853005.1	2022 No	Aspartic acid to asparagine at position 244
OPG074	E544G	1	0.000543774	PP853006.1	2022 No	Glutamic acid to glycine at position 544
OPG074	G540R	1	0.000543774	PP853006.1	2022 No	Glycine to arginine at position 540
OPG074	H541D	1	0.000543774	PP853006.1	2022 No	Histidine to aspartic acid at position 541

Mutation Analysis of mpox Genome Sequence (2017–2023)

Mutation Analysis of mpox Genome Sequence (2017–2023)								
Gene/ORF	Mutation	No. of Mutations	Frequency (%)	First Appearance	Common After 2022?	Protein Change	Notes	
OPG074	I543D	1	0.000543774	PP853006.1	2022	No	Isoleucine to aspartic acid at position 543	Hypothetical or uncharacterized protein; functional role unclear
OPG074	L537M	1	0.000543774	PP853006.1	2022	No	Leucine to methionine at position 537	Hypothetical or uncharacterized protein; functional role unclear
OPG074	M536S	1	0.000543774	PP853006.1	2022	No	Methionine to serine at position 36	Hypothetical or uncharacterized protein; functional role unclear
OPG074	T546M	1	0.000543774	PP853006.1	2022	No	Threonine to methionine at position 546	Hypothetical or uncharacterized protein; functional role unclear
OPG074	Y539H	1	0.000543774	PP853006.1	2022	No	Tyrosine to histidine at position 539	Hypothetical or uncharacterized protein; functional role unclear
OPG074	Y542 *	1	0.000543774	PP853006.1	2022	No	TYrosine to stop codon at position 542	Hypothetical or uncharacterized protein; functional role unclear
OPG096	D10N	1	0.000543774	PP853007.1	2022	No	Aspartic acid to asparagine at position 10	Hypothetical or uncharacterized protein; functional role unclear
OPG166	D17N	3	0.001631321	PP853007.1	2022	No	Aspartic acid to asparagine at position 17	Hypothetical or uncharacterized protein; functional role unclear
OPG027	D51N	1	0.000543774	PP853007.1	2022	No	Aspartic acid to asparagine at position 51	Hypothetical or uncharacterized protein; functional role unclear
OPG025	D55N	1	0.000543774	PP853007.1	2022	No	Aspartic acid to asparagine at position 55	Hypothetical or uncharacterized protein; functional role unclear
OPG109	D67N	1	0.000543774	PP853007.1	2022	No	Aspartic acid to asparagine at position 67	Large structural or scaffolding protein; mutation might affect virion formation
OPG151	E511K	1	0.000543774	PP853007.1	2022	No	Glutamic acid to lysine at position 511	Putative helicase or unwinding factor; possible role in transcriptional control
OPG025	M540I	3	0.001631321	PP853007.1	2022	No	Methionine to isoleucine at position 540	Hypothetical or uncharacterized protein; functional role unclear
OPG019	Q81 *	1	0.000543774	PP853007.1	2022	No	Glutamine to stop codon at position 81	Hypothetical or uncharacterized protein; functional role unclear
OPG023	R268 *	1	0.000543774	PP853007.1	2022	No	Arginine to stop codon at position 268	Hypothetical or uncharacterized protein; functional role unclear
OPG094	S74L	1	0.000543774	PP853007.1	2022	No	Serine to leucine at position 74	Hypothetical or uncharacterized protein; functional role unclear
OPG074	V637A	1	0.000543774	PP853007.1	2022	No	Valine to alanine at position 637	Hypothetical or uncharacterized protein; functional role unclear
OPG149	D133N	4	0.002175095	PP853008.1	2022	No	Aspartic acid to asparagine at position 133	Hypothetical or uncharacterized protein; functional role unclear
OPG210	D1877N	14	0.007612833	PP853008.1	2022	No	Aspartic acid to asparagine at position 1877	Large multi-domain protein; mutations likely affect multiple viral processes
OPG068	P511S	7	0.003806417	PP853008.1	2022	No	Proline to aerine at position 511	Hypothetical or uncharacterized protein; functional role unclear
OPG100	R95M	25	0.013594345	PP853008.1	2022	No	Arginine to methionine at position 95	Hypothetical or uncharacterized protein; functional role unclear
OPG156	S269F	1	0.000543774	PP853008.1	2022	No	Serine to phenylalanine at position 69	Hypothetical or uncharacterized protein; functional role unclear
OPG190	S37L	4	0.002175095	PP853008.1	2022	No	Serine to leucine at position 37	Host range determinant; mutation could influence interspecies transmission
OPG118	S521F	4	0.002175095	PP853008.1	2022	No	Serine to phenylalanine at position 521	Involved in viral DNA metabolism; mutation could disrupt replication processes
OPG087	S63L	4	0.002175095	PP853008.1	2022	No	Serine to leucine at position 63	Hypothetical or uncharacterized protein; functional role unclear
OPG089	E118K	1	0.000543774	PP853009.1	2022	No	Glutamic acid to lysine at position 118	Hypothetical or uncharacterized protein; functional role unclear
OPG172	S29L	1	0.000543774	PP853009.1	2022	No	Serine to leucine at position 29	Surface antigen; mutation may impact immune recognition
OPG105	K972I	1	0.000543774	PP853010.1	2022	No	Lysine to isoleucine at position 972	Hypothetical or uncharacterized protein; functional role unclear

TABLE 1 | (continued)

Mutation Analysis of mpox Genome Sequence (2017–2023)						Notes
Gene/ORF	Mutation	No. of Mutations	Frequency (%)	First Appearance	Common After 2022?	
OPG105	K987T	1	0.000543774	PP853010.1	2022 No	Lysine to threonine at position 987 Hypothetical or uncharacterized protein; functional role unclear
OPG105	L988Y	1	0.000543774	PP853010.1	2022 No	Leucine to tyrosine at position 988 Hypothetical or uncharacterized protein; functional role unclear
OPG105	S971V	1	0.000543774	PP853010.1	2022 No	Serine to valine at position 971 Hypothetical or uncharacterized protein; functional role unclear
OPG180	D153N	1	0.000543774	PP853012.1	2022 No	Aspartic acid to asparagine at position 153 Structural scaffold; mutation may affect particle assembly
OPG128	E31K	1	0.000543774	PP853012.1	2022 No	Glutamic acid to lysine at position 31 Hypothetical or uncharacterized protein; functional role unclear
OPG015	D16N	1	0.000543774	PP853013.1	2022 No	Aspartic acid to asparagine at position 16 Replication-associated protein; could influence viral genome replication
OPG015_dup	D16N	1	0.000543774	PP853013.1	2022 No	Aspartic acid to asparagine at position 16 Hypothetical or uncharacterized protein; functional role unclear
OPG023	D243N	1	0.000543774	PP853013.1	2022 No	Aspartic acid to asparagine at position 243 Hypothetical or uncharacterized protein; functional role unclear
OPG079	N222I	1	0.000543774	PP853013.1	2022 No	Asparagine to isoleucine at position 222 Hypothetical or uncharacterized protein; functional role unclear
OPG003_dup	Q297K	1	0.000543774	PP853013.1	2022 No	Glutamine to lysine at position 297 Hypothetical or uncharacterized protein; functional role unclear
OPG148	R227C	1	0.000543774	PP853013.1	2022 No	Arginine to cysteine at position 227 Late gene regulator; mutations may alter expression timing
OPG165	S234L	1	0.000543774	PP853013.1	2022 No	Serine to leucine at position 234 DNA-binding protein; mutation may affect regulatory binding efficiency
OPG189	Q447*	1	0.000543774	PP853014.1	2022 No	Glutamine to stop codon at position 447 Envelope protein; mutation might influence host cell tropism
OPG068	E286K	1	0.000543774	PP853015.1	2022 No	Glutamic acid to lysine at position 286 Hypothetical or uncharacterized protein; functional role unclear
OPG125	E314P	1	0.000543774	PP853015.1	2022 No	Glutamic acid to proline at position 314 Hypothetical or uncharacterized protein; functional role unclear
OPG125	G301R	1	0.000543774	PP853015.1	2022 No	Glycine to arginine at position 301 Hypothetical or uncharacterized protein; functional role unclear
OPG174	M167I	1	0.000543774	PP853015.1	2022 No	Methionine to isoleucine at position 167 Membrane-associated structural protein; mutation may affect virion stability
OPG125	P302A	1	0.000543774	PP853015.1	2022 No	Proline to alanine at position 302 Hypothetical or uncharacterized protein; functional role unclear
OPG125	P303K	1	0.000543774	PP853015.1	2022 No	Proline to lysine at position 303 Hypothetical or uncharacterized protein; functional role unclear
OPG040	Q122*	1	0.000543774	PP853015.1	2022 No	Glutamine to stop codon at position 122 Hypothetical or uncharacterized protein; functional role unclear
OPG181	E17K	1	0.000543774	PP853016.1	2022 No	Glutamic acid to lysine at position 17 Hypothetical or uncharacterized protein; functional role unclear
OPG098	M41*	1	0.000543774	PP853016.1	2022 No	Methionine to stop codon at position 41 Hypothetical or uncharacterized protein; functional role unclear
OPG162	R39K	1	0.000543774	PP853016.1	2022 No	Arginine to lysine at position 39 Hypothetical or uncharacterized protein; functional role unclear
OPG098	S40*	1	0.000543774	PP853016.1	2022 No	Serine to stop codon at position 40 Hypothetical or uncharacterized protein; functional role unclear
OPG066	E133K	1	0.000543774	PP853020.1	2022 No	Glutamic acid to lysine at position 133 Hypothetical or uncharacterized protein; functional role unclear
OPG119	G59R	1	0.000543774	PP853020.1	2022 No	Glycine to arginine at position 59 Virion core or packaging protein; could alter virus particle stability
OPG119	I61L	1	0.000543774	PP853020.1	2022 No	Isoleucine to leucine at position 61 Virion core or packaging protein; could alter virus particle stability
OPG119	N63Y	1	0.000543774	PP853020.1	2022 No	Asparagine to tyrosine at position 63 Virion core or packaging protein; could alter virus particle stability

TABLE 1 | (continued)

Mutation Analysis of mpox Genome Sequence (2017–2023)								
Gene/ORF	Mutation	No. of Mutations	Frequency (%)	First Appearance	Common After 2022?	Protein Change	Notes	
OPG119	V62F	1	0.000543774	PP853020.1	2022	No	Valine to phenylalanine at position 62	Virion core or packaging protein; could alter virus particle stability
OPG198	D95N	4	0.002175095	PP853021.1	2022	No	Aspartic acid to asparagine at position 95	Hypothetical or uncharacterized protein; functional role unclear
OPG163	D98N	2	0.001087548	PP853021.1	2022	No	Aspartic acid to asparagine at position 98	Hypothetical or uncharacterized protein; functional role unclear
OPG148	E157K	4	0.002175095	PP853021.1	2022	No	Glutamic acid to lysine at position 157	Late gene regulator; mutations may alter timing of expression
OPG040	M245I	5	0.002718869	PP853021.1	2022	No	Methionine to isoleucine at position 245	Hypothetical or uncharacterized protein; functional role unclear
OPG181	R229K	3	0.001631321	PP853021.1	2022	No	Arginine to lysine at position 229	Hypothetical or uncharacterized protein; functional role unclear
OPG063	S80G	2	0.001087548	PP853021.1	2022	No	Serine to glycine at position 80	Hypothetical or uncharacterized protein; functional role unclear
OPG036	A55T	1	0.000543774	PP853022.1	2022	No	Alanine to threonine at position 55	Hypothetical or uncharacterized protein; functional role unclear
OPG123	P507S	1	0.000543774	PP853022.1	2022	No	Proline to serine at position 507	Hypothetical or uncharacterized protein; functional role unclear
OPG052	R55Q	1	0.000543774	PP853023.1	2022	No	Arginine to glutamine at position 55	Hypothetical or uncharacterized protein; functional role unclear
OPG208	S19F	2	0.001087548	PP853023.1	2022	No	Serine to phenylalanine at position 19	Involved in DNA metabolism or repair; mutation impact unclear
OPG022	D116N	1	0.000543774	PP853027.1	2022	No	Aspartic acid to asparagine at position 116	Hypothetical or uncharacterized protein; functional role unclear
OPG167	F139L	2	0.001087548	PP853032.1	2022	No	Phenylalanine to leucine at position 139	Hypothetical or uncharacterized protein; functional role unclear
OPG170	G31D	2	0.001087548	PP853032.1	2022	No	Glycine to aspartic acid at position 31	Hypothetical or uncharacterized protein; functional role unclear
OPG199	H237R	2	0.001087548	PP853032.1	2022	No	Histidine to arginine at position 221	Hypothetical or uncharacterized protein; functional role unclear
OPG084	H51Y	2	0.001087548	PP853032.1	2022	No	Histidine to tyrosine at position 51	DNA replication or repair protein; potential effect on replication fidelity
OPG193	I108R	2	0.001087548	PP853032.1	2022	No	Isoleucine to arginine at position 108	Virion-associated protein; could alter assembly dynamics
OPG084	K214R	1	0.000543774	PP853032.1	2022	No	Lysine to arginine at position 214	DNA replication or repair protein; potential effect on replication fidelity
OPG199	K230E	2	0.001087548	PP853032.1	2022	No	Lysine to glutaminc acid at position 230	Hypothetical or uncharacterized protein; functional role unclear
OPG210	K424R	2	0.001087548	PP853032.1	2022	No	Lysine to arginine at position 230	Large multi-domain protein; mutations likely affect multiple viral processes
OPG161	K67E	2	0.001087548	PP853032.1	2022	No	Lysine to glutamic acid at position 67	Hypothetical or uncharacterized protein; functional role unclear
OPG151	K993R	2	0.001087548	PP853032.1	2022	No	Lysine to arginine at position 993	Putative helicase or unwinding factor; possible role in transcriptional control
OPG071	L411W	2	0.001087548	PP853032.1	2022	No	Leucine to tryptophan at position 411	Hypothetical or uncharacterized protein; functional role unclear
OPG089	L419M	1	0.000543774	PP853032.1	2022	No	Leucine to methionine at position 419	Hypothetical or uncharacterized protein; functional role unclear
OPG153	M14T	2	0.001087548	PP853032.1	2022	No	Methionine to threonine at position 14	Hypothetical or uncharacterized protein; functional role unclear
OPG118	N413S	2	0.001087548	PP853032.1	2022	No	Asparagine to serine at position 413	Involved in viral DNA metabolism; mutation could disrupt replication processes
OPG133	T562S	2	0.001087548	PP853032.1	2022	No	Threonine to serine at position 562	Hypothetical or uncharacterized protein; functional role unclear
OPG049	T60N	2	0.001087548	PP853032.1	2022	No	Threonine to asparagine at position 60	Hypothetical or uncharacterized protein; functional role unclear

TABLE 1 | (continued)

Mutation Analysis of mpox Genome Sequence (2017–2023)							Notes
Gene/ORF	Mutation	No. of Mutations	Frequency (%)	First Appearance	Common After 2022?	Protein Change	
OPG056	V323A	2	0.001087548	PP853032.1	2022	No	Valine to alanine at position 323
OPG161	V88A	2	0.001087548	PP853032.1	2022	No	Valine to alanine at position 88
OPG084	D217N	4	0.002175095	PP853040.1	2022	No	Aspartic acid to asparagine at position 217
OPG148	E109K	1	0.000543774	PP853040.1	2022	No	Glutamic acid to lysine at position 109
OPG210	G76V	1	0.000543774	PP853041.2	2022	No	Glycine to valine at position 76
OPG210	G89T	1	0.000543774	PP853041.2	2022	No	Glycine to threonine at position 89
OPG210	I87G	1	0.000543774	PP853041.2	2022	No	Isoleucine to glycine at position 87
OPG210	K83G	1	0.000543774	PP853041.2	2022	No	Lysine to glycine at position 83
OPG210	L92S	1	0.000543774	PP853041.2	2022	No	Leucine to serine at position 92
OPG210	N88V	1	0.000543774	PP853041.2	2022	No	Asparagine to valine at position 88
OPG210	T90C	1	0.000543774	PP853041.2	2022	No	Threonine to cysteine at position 90
OPG210	V77G	1	0.000543774	PP853041.2	2022	No	Valine to glycine at position 77
OPG210	Y91S	1	0.000543774	PP853041.2	2022	No	Tyrosine to serine at position 91
OPG125	D189N	4	0.002175095	PP853043.1	2022	No	Aspartic acid to asparagine at position 189
OPG143	E106K	4	0.002175095	PP853043.1	2022	No	Glutamic acid to lysine at position 106
OPG133	L604I	4	0.002175095	PP853043.1	2022	No	Leucine to isoleucine at position 604
OPG205	M237I	4	0.002175095	PP853043.1	2022	No	Methionine to isoleucine at position 237
OPG191	P103S	4	0.002175095	PP853043.1	2022	No	Proline to serine at position 103
OPG151	R1144Q	4	0.002175095	PP853043.1	2022	No	Arginine to glutamine at position 1144
OPG210	R441K	4	0.002175095	PP853043.1	2022	No	Arginine to lysine at position 441
OPG113	S108L	4	0.002175095	PP853043.1	2022	No	Serine to leucine at position 108
OPG133	S122F	4	0.002175095	PP853043.1	2022	No	Serine to Phenylalanine at position 122
OPG205	S683L	4	0.002175095	PP853043.1	2022	No	Serine to leucine at position 683
OPG143	T230M	4	0.002175095	PP853043.1	2022	No	Threonine to methionine at position 230
OPG147	E79K	1	0.000543774	PP853044.1	2022	No	Glutamic acid to lysine at position 79
OPG200	D67N	1	0.000543774	PP853045.1	2022	No	Aspartic acid to asparagine at position 67
OPG051	E36K	1	0.000543774	PP853045.1	2022	No	Glutamic acid to lysine at position 36
OPG074	G161E	1	0.000543774	PP853045.1	2022	No	Glycine to glutamic acid at position 161

Glycoprotein precursor; mutation might influence virion morphogenesis

Hypothetical or uncharacterized protein; functional role unclear

DNA replication or repair protein; potential effect on replication fidelity

Late gene regulator; mutations may alter expression timing

Large multi-domain protein; mutations likely affect multiple viral processes

Large multi-domain protein; mutations likely affect multiple viral processes

Large multi-domain protein; mutations likely affect multiple viral processes

Large multi-domain protein; mutations likely affect multiple viral processes

Large multi-domain protein; mutations likely affect multiple viral processes

Large multi-domain protein; mutations likely affect multiple viral processes

Large multi-domain protein; mutations likely affect multiple viral processes

Large multi-domain protein; mutations likely affect multiple viral processes

Large multi-domain protein; mutations likely affect multiple viral processes

Hypothetical or uncharacterized protein; functional role unclear

Hypothetical or uncharacterized protein; functional role unclear

Hypothetical or uncharacterized protein; functional role unclear

Envelope glycoprotein; mutation likely affects immune evasion or viral entry

Envelope-associated protein; mutation may affect cell entry

Putative helicase or unwinding factor; possible role in transcriptional control

Large multi-domain protein; mutations likely affect multiple viral processes

Late transcription factor or helicase; mutation may affect viral gene regulation

Hypothetical or uncharacterized protein; functional role unclear

Envelope glycoprotein; mutation likely affects immune evasion or viral entry

Hypothetical or uncharacterized protein; functional role unclear

Structural protein; may affect virion integrity or packaging

Hypothetical or uncharacterized protein; functional role unclear

Hypothetical or uncharacterized protein; functional role unclear

Hypothetical or uncharacterized protein; functional role unclear

TABLE 1 | (continued)

Mutation Analysis of mpox Genome Sequence (2017–2023)							Notes
Gene/ORF	Mutation	No. of Mutations	Frequency (%)	First Appearance	Common After 2022?	Protein Change	
OPG210	L151F	1	0.000543774	PP853045.1	2022	No	Leucine to phenylalanine at position 151
OPG043	P99S	1	0.000543774	PP853045.1	2022	No	Proline to serine at position 99
OPG047	R219K	1	0.000543774	PP853045.1	2022	No	Arginine to lysine at position 219
OPG142	R68C	1	0.000543774	PP853045.1	2022	No	Arginine to cysteine at position 68
OPG200	R69C	1	0.000543774	PP853045.1	2022	No	Arginine to cysteine at position 69
OPG016	S142L	1	0.000543774	PP853045.1	2022	No	Serine to leucine at position 142
OPG054	S432L	1	0.000543774	PP853045.1	2022	No	Serine to leucine at position 432
OPG170	S57F	1	0.000543774	PP853045.1	2022	No	Serine to phenylalanine at position 55
OPG084	D563N	1	0.000543774	PP853046.1	2022	No	Aspartic acid to asparagine at position 563
OPG188	E317K	2	0.001087548	PP853046.1	2022	No	Glutamic acid to lysine at position 317
OPG025	S187L	2	0.001087548	PP853046.1	2022	No	Serine to leucine at position 187
OPG136	A160V	1	0.000543774	PP853047.1	2022	No	Alanine to valine at position 160
OPG037	L55F	1	0.000543774	PP853047.1	2022	No	Leucine to phenylalanine at position 55
OPG188	P314T	1	0.000543774	PP853047.1	2022	No	Proline to threonine at position 314
OPG200	R99K	1	0.000543774	PP853047.1	2022	No	Arginine to lysine at position 99
OPG120	S206F	1	0.000543774	PP853047.1	2022	No	Serine to leucine at position 206
OPG128	T34N	1	0.000543774	PP853047.1	2022	No	Threonine to asparagine at position 34
OPG163	D22N	1	0.000543774	PP853051.1	2022	No	Aspartic acid to asparagine at position 22
OPG068	D393N	1	0.000543774	PP853051.1	2022	No	Aspartic acid to asparagine at position 393
OPG064	E291K	1	0.000543774	PP853051.1	2022	No	Glutamic acid to lysine at position 291
OPG056	E379K	1	0.000543774	PP853051.1	2022	No	Glutamic acid to lysine at position 379
OPG098	E56K	1	0.000543774	PP853051.1	2022	No	Glutamic acid to lysine at position 56
OPG185	S76F	1	0.000543774	PP853051.1	2022	No	Serine to phenylalanine at position 76
OPG204	A64T	2	0.001087548	PP853052.1	2022	No	Alanine to threonine at position 64
OPG025	E528K	2	0.001087548	PP853052.1	2022	No	Glutamic acid to lysine at position 528
OPG015_dup	I291V	1	0.000543774	PP853052.1	2022	No	Isoleucine to valine at position 192
OPG084	P341H	1	0.000543774	PP853052.1	2022	No	Proline to histidine at position 314
OPG106	S48F	1	0.000543774	PP853052.1	2022	No	Serine to phenylalanine at position 48

TABLE 1 | (continued)

Mutation Analysis of mpox Genome Sequence (2017–2023)							Notes
Gene/ORF	Mutation	No. of Mutations	Frequency (%)	First Appearance	Common After 2022?	Protein Change	
OPG109	D70N	2	0.001087548	PP853053.1	2022	No	Aspartic acid to asparagine at position 70
OPG025	D160N	10	0.005437738	PP852950.1	2023	No	Aspartic acid to asparagine at position 160
OPG153	D369N	1	0.000543774	PP852950.1	2023	No	Aspartic acid to asparagine at position 369
OPG036	E162K	3	0.001631321	PP852950.1	2023	No	Glutamic acid to lysine at position 162
OPG124	E24K	1	0.000543774	PP852950.1	2023	No	Glutamic acid to lysine at position 24
OPG174	G11R	10	0.005437738	PP852950.1	2023	No	Glycine to arginine at position 11
OPG188	G349S	5	0.002718869	PP852950.1	2023	No	Glycine to serine at position 349
OPG015	H14Y	1	0.000543774	PP852950.1	2023	No	Histidine to tyrosine at position 14
OPG015_dup	H14Y	1	0.000543774	PP852950.1	2023	No	Histidine to tyrosine at position 14
OPG122	H198Y	10	0.005437738	PP852950.1	2023	No	Histidine to tyrosine at position 198
OPG132	M357I	10	0.005437738	PP852950.1	2023	No	Methionine to isoleucine at position 357
OPG108	P138S	1	0.000543774	PP852950.1	2023	No	Proline to serine at position 38
OPG105	R1160C	1	0.000543774	PP852950.1	2023	No	Arginine to cysteine at position 1160
OPG056	R38C	5	0.002718869	PP852950.1	2023	No	Arginine to cysteine at position 38
OPG023	S318F	10	0.005437738	PP852950.1	2023	No	Serine to phenylalanine at position 318
OPG143	V143M	1	0.000543774	PP852950.1	2023	No	Valine to methionine at position 143
OPG205	V206I	1	0.000543774	PP852950.1	2023	No	Valine to isoleucine at position 206
OPG113	V528I	10	0.005437738	PP852950.1	2023	No	Valine to isoleucine at position 528
OPG086	Y73H	1	0.000543774	PP852954.1	2023	No	Tyrosine to histidine at position 73
OPG015	D317N	1	0.000543774	PP852959.1	2023	No	Aspartic acid to asparagine at position 317
OPG015_dup	D317N	1	0.000543774	PP852959.1	2023	No	Aspartic acid to asparagine at position 317
OPG083	D94N	1	0.000543774	PP852959.1	2023	No	Aspartic acid to asparagine at position 94
OPG074	R180C	1	0.000543774	PP852959.1	2023	No	Arginine to cysteine at position 180
OPG003	D264N	7	0.003806417	PP852960.1	2023	No	Aspartic acid to asparagine at position 264
OPG003_dup	D264N	7	0.003806417	PP852960.1	2023	No	Aspartic acid to asparagine at position 264
OPG074	E23K	5	0.002718869	PP852960.1	2023	No	Glutamic acid to lysine at position 23
OPG118	G4E	1	0.000543774	PP852960.1	2023	No	Glycine to glutamic acid at position 4

TABLE 1 | (continued)

Mutation Analysis of mpox Genome Sequence (2017–2023)							Notes
Gene/ORF	Mutation	No. of Mutations	Frequency (%)	First Appearance	Common After 2022?	Protein Change	
OPG104	D50N	1	0.000543774	PP852961.1	2023 No	Aspartic acid to asparagine at position 50	Hypothetical or uncharacterized protein; functional role unclear
OPG093	D88N	2	0.001087548	PP852961.1	2023 No	Aspartic acid to asparagine at position 88	Hypothetical or uncharacterized protein; functional role unclear
OPG193	D114N	6	0.003262643	PP852962.1	2023 No	Aspartic acid to asparagine at position 114	Virion-associated protein; could alter assembly dynamics
OPG159	D21N	1	0.000543774	PP852962.1	2023 No	Aspartic acid to asparagine at position 21	Hypothetical or uncharacterized protein; functional role unclear
OPG063	D6N	1	0.000543774	PP852962.1	2023 No	Aspartic acid to asparagine at position 6	Hypothetical or uncharacterized protein; functional role unclear
OPG204	E314K	1	0.000543774	PP852962.1	2023 No	Glutamic acid to lysine at position 314	Pro-apoptotic regulator; mutation may influence host cell death response
OPG145	E7K	1	0.000543774	PP852962.1	2023 No	Glutamic acid to lysine at position 7	DNA-binding protein; mutation may alter early viral transcription
OPG129	S619L	1	0.000543774	PP852962.1	2023 No	Serine to leucine at position 619	Hypothetical or uncharacterized protein; functional role unclear
OPG138	S77L	1	0.000543774	PP852962.1	2023 No	Serine to leucine at position 77	Hypothetical or uncharacterized protein; functional role unclear
OPG022	D78N	1	0.000543774	PP852963.1	2023 No	Aspartic acid to asparagine at position 78	Hypothetical or uncharacterized protein; functional role unclear
OPG015	E166K	1	0.000543774	PP852963.1	2023 No	Glutamic acid to lysine at position 166	Replication-associated protein; could influence viral genome replication
OPG015_dup	E166K	1	0.000543774	PP852963.1	2023 No	Glutamic acid to lysine at position 166	Hypothetical or uncharacterized protein; functional role unclear
OPG167	S79L	1	0.000543774	PP852963.1	2023 No	Serine to leucine at position 79	Hypothetical or uncharacterized protein; functional role unclear
OPG002_dup	S70L	5	0.002718869	PP852964.1	2023 No	Serine to leucine at position 70	Hypothetical or uncharacterized protein; functional role unclear
OPG002	S70L	5	0.002718869	PP852964.1	2023 No	Serine to leucine at position 70	Hypothetical or uncharacterized protein; functional role unclear
OPG178	T18I	5	0.002718869	PP852964.1	2023 No	Threonine to isoleucine at position 18	Hypothetical or uncharacterized protein; functional role unclear
OPG071	S420L	1	0.000543774	PP852965.1	2023 No	Serine to leucine at position 420	Hypothetical or uncharacterized protein; functional role unclear
OPG101	D176N	1	0.000543774	PP852967.1	2023 No	Aspartic acid to asparagine at position 176	Hypothetical or uncharacterized protein; functional role unclear
OPG112	D65N	1	0.000543774	PP852967.1	2023 No	Aspartic acid to asparagine at position 65	Hypothetical or uncharacterized protein; functional role unclear
OPG102	D7N	1	0.000543774	PP852968.1	2023 No	Aspartic acid to asparagine at position 7	Hypothetical or uncharacterized protein; functional role unclear
OPG072	D82N	1	0.000543774	PP852969.1	2023 No	Aspartic acid to asparagine at position 82	Hypothetical or uncharacterized protein; functional role unclear
OPG064	R652K	1	0.000543774	PP852969.1	2023 No	Arginine to lysine at position 652	Hypothetical or uncharacterized protein; functional role unclear
OPG113	S172F	1	0.000543774	PP852969.1	2023 No	Serine to phenylalanine at position 172	Late transcription factor or helicase; mutation may affect viral gene regulation
OPG085	S424L	1	0.000543774	PP852969.1	2023 No	Serine to leucine at position 424	Hypothetical or uncharacterized protein; functional role unclear
OPG021	D4N	1	0.000543774	PP852970.1	2023 No	Aspartic acid to asparagine at position 4	Hypothetical or uncharacterized protein; functional role unclear
OPG195	E26K	1	0.000543774	PP852970.1	2023 No	Glutamic acid to lysine at position 26	Hypothetical or uncharacterized protein; functional role unclear
OPG071	R776Q	1	0.000543774	PP852970.1	2023 No	Arginine to glutamine at position 776	Hypothetical or uncharacterized protein; functional role unclear
Total		1836					

This temporal clustering suggests that lineage A represents the ancestral variant of the epidemic. In contrast, lineage A.2.3 was predominantly observed during the 2022 outbreak, coinciding with the largest recorded epidemic of mpox in Nigeria. The predominance of lineage A.2.3 during this period suggests potential genetic adaptations that may have enhanced transmissibility or fitness, contributing to the observed epidemiologic surge.

Furthermore, other lineages within clade IIb, including A.2, A.2.2, and A.3, were identified during the peak of the 2022 outbreak, reflecting the evolutionary dynamics and adaptation of the virus to changing epidemiologic landscapes. Notably, lineage A.3, which was sparsely recorded in 2017, showed a marked increase in 2022, highlighting the temporal and genetic diversity of mpox in Nigeria (Fig 5). The lineage clustering during specific periods suggests that different lineages may have distinct epidemiologic characteristics, influenced by selective pressures, host-pathogen interactions, and environmental factors. These findings provide valuable insights into the evolutionary dynamics of mpox in Nigeria, emphasizing the importance of genomic surveillance to monitor emerging lineages and potential variants of concern.

Demographic analysis revealed that males accounted for 70% of mpox cases with the majority within the 21–40 y age range [14,22]. This trend may be linked to occupational exposure and behavioral factors, such as hunting, farming, and increased mobility, associated with this demographic group. Additionally, socio-cultural practices and gender-specific roles may contribute to the observed gender disparity. The geographic clustering of cases in urban centers like Lagos State and the FCT suggests that high population density, urbanization, and increased socio-economic interactions have significant roles in mpox transmission dynamics. These findings highlight the need for targeted public health interventions, including risk communication, vaccination campaigns, and community engagement strategies, tailored to high-risk populations.

Recent clinical trial results, notably the PALM007 and STOMP trials, have raised concerns regarding the reduced efficacy of tecovirimat, the primary antiviral agent used for mpox treatment (DOI: 10.1016/j.ejim.2025.01.031) [25]. These findings suggest that reliance on tecovirimat alone may not be sufficient for managing future mpox outbreaks, especially with the rising prevalence of clade I mpox, which disproportionately affects children < 15 y of age. While this study focused on clade II mpox dynamics in Nigeria, the insights gained from genomic surveillance are equally critical for clade I monitoring. Continuous genomic surveillance can help detect emerging antiviral resistance mutations, track the spread of more virulent lineages, and inform the design of updated therapeutic strategies. Strengthening molecular surveillance efforts for clades I and II mpox is essential to ensure early detection of clinically significant viral adaptations and to guide

effective outbreak responses and clinical management protocols.

The mutation analysis (Fig 5) revealed several recurrent non-synonymous mutations in the mpox clade IIb genomes from Nigeria (Table 1). The emergence and rise in frequency of mutations, such as K606E, D442N, and E553K, during the 2017 outbreak period that persisted after 2022 suggest possible adaptive changes that may have contributed to increased transmission or viral fitness.

Although the functional impacts of these specific mutations require further experimental validation, similar mutations in other orthopoxviruses have been associated with host immune evasion and enhanced replication efficiency [26].

Many of the mutations affected genes linked to critical viral functions, such as mutations in OPG113 and OPG145 which are associated with transcription regulation, and mutations here may influence the timing or efficiency of viral gene expression. OPG191 and OPG205 encode envelope-associated proteins; mutations in these regions (E452K, P103S, respectively) impact viral entry, membrane fusion, or immune evasion. The OPG118:K606E mutation affects replication fidelity or host adaptation.

Approximately 70% of all observed mutations first appeared in or after 2022, suggesting that the virus has undergone notable genomic changes since the start of the 2022 mpox resurgence in Nigeria. Several of these mutations became prevalent, supporting the hypothesis that they may confer adaptive advantages in transmission, host interaction, or immune evasion (Table 1).

LIMITATIONS

The study was limited by the reliance on publicly available genomic sequences, which may result in underrepresentation of certain regions with limited sequencing capacity, leading to potential biases in lineage distribution analysis. Additionally, the absence of functional analyses to correlate genetic variations with phenotypic outcomes, such as transmissibility or virulence, limited the ability of the study to draw definitive conclusions about lineage behavior.

CONCLUSION

This analysis provided crucial insights into the complex epidemiology, genomic diversity, and spatial distribution of mpox in Nigeria, emphasizing the dynamic interplay between viral genetics, human behavior, and environmental factors. The identification of lineage A.2.3 as the most prevalent variant during the 2022 outbreak underscores a potential role in the observed epidemiologic surge. These findings underscore the importance of continuous genomic surveillance, region-specific public health strategies, and integrated socio-epidemiologic approaches to effectively manage and control mpox outbreaks in Nigeria. Addressing underreported challenges, enhancing laboratory capacity, and strengthening surveillance

systems are critical to achieving effective mpox control and prevention in Nigeria.

DATA AVAILABILITY STATEMENT

All genome sequences analyzed in this study are available on the public databases GenBank and Nextstrain. Epidemiological data were obtained from the Nigerian Centre for Disease Control (<https://ncdc.gov.ng>) and the World Health Organization (<https://www.who.int>). The accession numbers for the sequences used are listed in S1 Table. Additional data used in this study can be obtained from the corresponding author upon reasonable request.

ETHICS STATEMENT

No direct interactions with human or animal subjects were involved. Therefore, ethical approval and informed consent were not required.

AUTHORS CONTRIBUTIONS

Louis O. Ezediuno

Conceived and designed the study, collected data, performed analyses, and drafted the manuscript, and provided overall supervision, technical support, and final approval of the manuscript.

Michael A. Ockiya

Critically reviewed the manuscript and contributed to methods and discussion refinement.

Oluwaseun O. Taofeek

Contributed to the phylogenetic analysis and figure preparation.

Tosin Y. Senbadejo and Sangodare O. Adeola

Assisted with data curation, mutation profiling, and interpretation of genomic results.

Evans M. Elcanah

Assisted in literature review and contributed to the epidemiological interpretation.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

SUPPLEMENTARY MATERIAL

Supplementary Material can be downloaded from https://zoones-journal.org/wp-content/uploads/2025/07/zoones20250010_Suppl.zip.

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