Identification of a new Mpox virus lineage during the first occurrence of Mpox in Togo

Abstract

The first case of Mpox was reported by Togo on May 2025 and the outbreak is still in ongoing. Between May and December 2025, 90 cases of Mpox infection were confirmed in the country using Xpert® Mpox kits at the National Reference Laboratory of Tuberculosis at CHU SO (Centre Hospitalier Universitaire Sylvanus Olympio). A subset of positive samples were sequenced to describe the circulating Mpox clades in the country. Here we report high-quality whole-genome sequences generated by the Laboratory of molecular biology at University of Lome (BIOLIM/FSS-UL). Phylogenetic analysis including other previously published sequences obtained from the National public Health Laboratory in Lome, showed that the mpox virus (MPXV) strains clustered with MPXV Clade IIb/sh2017 and form a related but distinct cluster to the newly designated lineage G.1 recently identified in Sierra-Leone and Guinea. Our findings indicate ongoing circulation of clade IIb in West Africa and highlight the critical role of genomic surveillance in supporting timely public health responses.

Introduction

Mpox is an actual public health concern. Since 2022, there is a resurgence of mpox over the world (1) and this intensified in summer 2024, triggering WHO to launch an alert as incidence increased in Africa (2). In WHO 61st situation report on the Mpox epidemic published in December 2025; 2,150 new cases were recorded in November 2025 (3). In general, mpox infection needs to be confirmed by PCR and sequenced to characterize the virus strains. In order to better understand the evolution, identify epidemiological transmission chains and take appropriate measures, virological and genomic surveillance were required by countries as recommended by WHO. The Mpox virus (MPXV) is represented by two clades: clade I and clade II and each clade was divided into two clades (a, b) (4). It was demonstrated that clade IIb was predominant in West Africa since 2014 particularly in Nigeria (5). In Benin, the case was confirmed in 2022 (6). Then from January to July 2025, Sierra Leone became the epicenter of a mpox epidemic due to the novel G.1 sub-lineage within the lineage A.2.2 of clade IIb (7). In Togo, no case was reported until May 2025. The first case was notified in the period of outbreak in Ghana, Nigeria, and when a critical situation arose in Sierra Leone. Till the end of 2025, ninety (90) confirmed cases were recorded in Togo. It was then important to characterize Mpox strains that circulate in the country. Currently, sequencing has been undertaken in two settings in Lome : at the National public Health Lab and at the BIOLIM/FSS-UL lab. This

report describes high-quality sequencing of two samples obtained at BIOLIM/FSS-UL laboratory and the phylogenetic analysis of all of mpox good sequences from Togo.

Methods

Between May and December 2025, as recommended by MOH, mpox suspect cases were enrolled at the infectious disease's unit of CHU SO of Lome and at some health care units in regions where Mpox patients had been followed. For each mpox suspected patient, skin crust and vesicle swab were collected and sent to the National reference Laboratory of Tuberculosis for molecular confirmation with Xpert® Mpox kits (Cepheid, Sunnyvale, CA, USA). Aliquots of skin crust and vesicle swab for positive cases were then transported to the Laboratoire BIOLIM/FSS-UL or the National Public Health Laboratory in Lome, the capital city, for whole-genome sequencing.

BIOLIM/FSS-UL received two mpox positives samples. Nucleic acids were extracted using the NucliSENS EasyMAG platform (BioMérieux, France). The NGS sequencing method was based on probe enrichment using the Twist Biosciences Comprehensive Viral Research Panel on libraries previously prepared with the Twist Library Preparation EF kit v2.0 (Twist Biosciences, USA). The post-capture amplified and purified library was diluted and sequenced on Illumina iSeq100 platform (2 ×150 cycles). Raw data were analyzed online using CZID (https://czid.org) and clade assignment was performed with Nextclade (https://clades.nextstrain.org/). The two sequences were then uploaded on Pathoplexus.

For phylogenetic analysis, we used complete and high coverage sequences available from Pathoplexus : various lineage A.2.2 sequences were selected, and references from lineages A.2.1, A.2.3, A.2.4, A.2.5, A.3 were also retrieved. From GISAID we downloaded four high-coverage sequences from Togo obtained at the National Public Health Laboratory. The sequences were aligned by using Squirrel (https://github.com/aineniamh/squirrel). The phylogenetic tree was drawn with IQ-TREE v.2.0.3, using ModelFinder to set up the best fitting model of nucleotide substitution, then edited under FigTree v1.4.4.

Results and discussion

During the study period, 90 samples out of the 497 from patients with clinical suspicion of mpox infection from different health regions across the country were molecularly confirmed as positive for mpox infection (18,1%). Among positive samples, 13 (14,4%) were sequenced and uploaded on public databases by the two laboratories (2 for Laboratoire BIOLIM/FSS-UL

on Pathoplexus and GISAID and 11 for National Public Health Laboratory on GISAID). All samples sequenced were derived from patients in Lome, the capital city. This rapid sequencing is due to the experience gained during the SARS-CoV-2 pandemic.
The two samples, sequenced at BIOLIM/FSS-UL, with above described methods, yielded high quality complete genome sequences (Table).

Table: Samples and consensus sequence information from CZID platform for BIOLIM/FSS-UL sequences

Sample type	ID	Collection date	Genome coverage	Coverage depth
Skin crust	BL25-M001C	16-05-2025	99.4%	3758.2 X
Skin vesicle	BL25-M014S	07-06-2025	99.4%	3156.6 X

Phylogenetic analysis was performed on a total of six high quality sequences from Togo. The curated alignment was constituted with 112 sequences and was 190010 positions long. All new sequences belong to Clade IIb/sh2017 lineage A.2. They clustered together and form a separate cluster close the cluster constituted predominantly with sequences from Sierra Leone that was designated G.1. The sequences from Togo are the closest relatives to the G.1 clade, and represent a new clade G.2 as already stated in (7).
The limitations of our study included the sampling collection that occurred only in Lome, the capital city. It would be interesting to continue the genomic surveillance by sequencing samples from other health regions and new positive cases. This would enable tracking transmission dynamics, since the circulation of people is well established. The introduction of Mpox virus in Togo must be considered as a threat and be followed up, since neighbouring countries also experienced mpox outbreaks. Also, sequences analysis must be continued in order to study APOBEC mutations.

Conclusion

We undertook the genomic characterization of some Mpox strains involved in the first mpox epidemic in Togo. The Togolese sequences defined a new subclade within lineage A.2.2 of clade IIb, illustrating the persistence and the capacity of evolvement of clade IIb in human populations since its emergence in 2014. These preliminary findings highlight the importance of expanding early warning surveillance system within the country which will enable us to control the outbreaks and track the evolution of the virus.

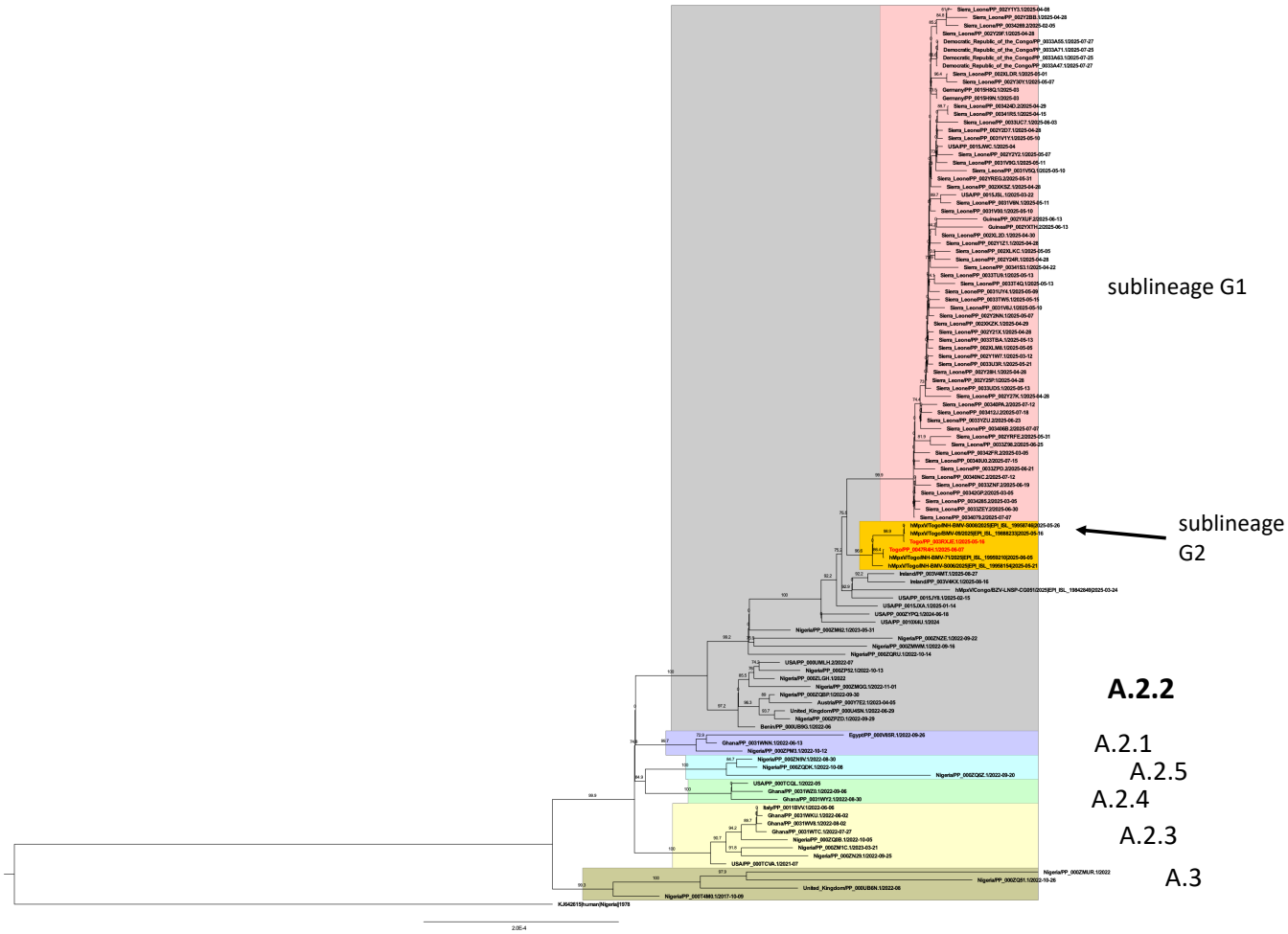

Figure: Phylogenetic tree of mpox in Togo

Maximum likelihood tree based on 112 complete genome sequences. The tree was drawn using IQ-TREE for 1000 ultrafast bootstrap replicates under HKY+F+I as the best fit substitution model according to BIC. The topology was visualized by FigTree, version 1.4.4. The genome sequences obtained in this study are written in red, within the entire sequences of Togo whose sub-lineage is highlighted in orange. Each lineage A was highlighted with a specific color. The tree was rooted with an ancestral clade II reference sampled in 1978.

Data available

New sequences were submitted to Pathoplexus under accession numbers PP_003RXJE.1 and PP_0047R4H.1.

Authors Contributions

Sequencing: Abla Ahouefa KONOU[1], Essowasim ESSO[1], Kokou TEGUENI[1], Emma SAGNA[1]; **Data analysis**: Abla Ahouefa KONOU[1], Nicole VIDAL[2]; **Validation**: Nicole VIDAL[2], Mounerou SALOU[1]; **Supervision**: Nicole VIDAL[2], Ayouba AHIDJO[2]; Martine PEETERS[2], Eric DELAPORTE[2], Mounerou SALOU[1], Anoumou DAGNRA[1]

Authors affiliations

1-Laboratoire de Biologie moléculaire et d'Immunologie-Université de Lomé (BIOLIM/FSS-UL)/Togo

2- Unité TransVIHMI-IRD-Université de Montpellier- INSERM/Montpellier, France

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References

1-Ndodo N, Ashcroft J, Lewandowski K, Yinka-Ogunleye A, Chukwu C, Ahmad A, et *al*. Distinct monkeypox virus lineages co-circulating in humans before 2022. Nat Med. 2023 Sep;29(9):2317–24.

2- WHO Director-General declares mpox outbreak a public health emergency of international concern [Internet]. [cited 2025 Jul 2]. Available from: https://www.who.int/news/item/14-08-2024-who-director-general-declares-mpox-outbreak-a-public-health-emergency-of-international-concern

3- Mpox : multi-country external situation report N°61. [cited 2025 December 27] Available from : https://cdn.who.int/media/docs/default-source/_sage-2026/multi-country-outbreak-of-mpox--external-situation-report--61.pdf

4- Wawina-Bokalanga T, Kinganda-Lusamaki E, Ngandu C, Akil-Bandali P, Kundey-Mafu J , Ngombe N, et *al*. Mpox Clade IIb Virus Introduction into Kinshasa, Democratic Republic of the Congo, July 2025. Viruses. 2026, *18*, 87. https://doi.org/10.3390/v18010087

5-Parker, E, Omah IF, Djuicy DD, Magee A, Tomkins-Tinch CH, Otieno JR et *al.* Genomics reveals zoonotic and sustained human mpox spread in West Africa. Nature. 2025 Jul;643(8074):1343-1351. doi: 10.1038/s41586-025-09128-2.

6- Yadouleton A, Faye M, Tchibozo C, Oke M, Faye O, Lawale T, et *al*. Introduction of monkeypox virus in Benin, 2022. Mil Med Res. 2022 Nov 9;9(1):63.

7- Campbell AKO, Sandi JD, Omah IF, Faye M, Parker E, Brock-Fisher T. et *al*. Genomic epidemiology uncovers the origin of the mpox epidemic in Sierra Leone. medRxiv 2025.10.15.25337823

Supplementary Appendix

All genome sequences and associated metadata supporting the findings of this study can be accessed through the persistent digital object identifier

https://doi.org/10.55876/gis8.260112vh

In addition to the minted DOI, GISAID also communicates the aggregation of GISAID accession numbers (EPI_ISL_IDs) through the corresponding EPI_SET_260112vh identifier to facilitate both, the acknowledgment of all data contributors and the direct retrieval of the underlying data from GISAID used in this study.

Mpox Virus data summary

GISAID identifier	Digital object identifier	Number of individual viruses	Data collection range	Number of countries/territoires
EPI_SET_260112vh	https://doi.org/10.55876/gis8.260112vh	4	2025-05-16 to 2025-06-05	1