# Sequencing of MPXV positive samples in Berlin 2022 to 2025

P0359

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## AIM

From May 2022, there was a major outbreak of MPXV of the West African Clade II variant. The epidemic quickly subsided in Europe after vaccination programmes in the summer of 2022, but sporadic positive MPXV detections remain to this day, even in vaccinated individuals. This raises the question of genetic evolution of the virus and phylogenetic development.

## METHODS

For this study, 43 samples from 2022 that tested positive for MPXV and 33 samples from 2023/24 were sequenced. The utilized xGen Monkeypox Virus Amplicon Panel (IDT®) is based on a primer design that generates short fragments and amplifies the MPXV genome without inverted terminal repeats (ITRs). Three different library preparations were tested. Sequencing was performed with the MiSeq (Illumina®) and with the MinION (Oxford Nanopore®). Bioinformatic processing was carried out using a self-generated pipeline, followed by phylogenetic analysis using Nextclade (Nextstrain).

## RESULTS

The comparison between the sequences based on the three library preparations showed large differences in e.g. coverage or frame shifts (see table 1). The sequencing worked better with the MiSeq, as the xGen Amplicon Panel provides many short sequence segments that are less suitable for Nanopore Rapid Barcoding. However, the Nanopore Native Barcoding showed similarly good results as the sequencing with the MiSeq. Subtype identification showed that genetic development diverged over the course of the pandemic. While 97.2% of subtypes belonged to the B lineage in 2022, far more subtypes were identified in 2023/25 (see Fig. 2). In the phylogenetic analysis all patient samples were found to belong to MPXV clade IIb. A known positive sample clade Ia and a cell culture supernatant clade I were sequenced as controls. Some clusters can be recognized (see Fig. 3) within the patient samples, which are probably due to the repeated introduction of different viruses into the Berlin MSM community.

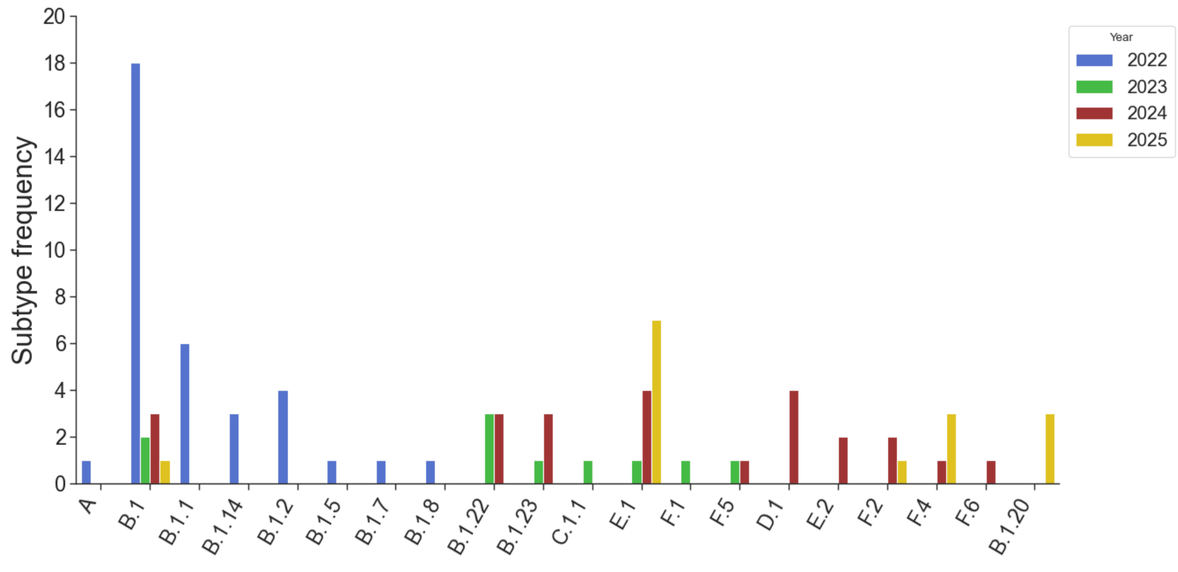


**Fig. 2: Subtype distribution in 2022 and follow up to 2025**

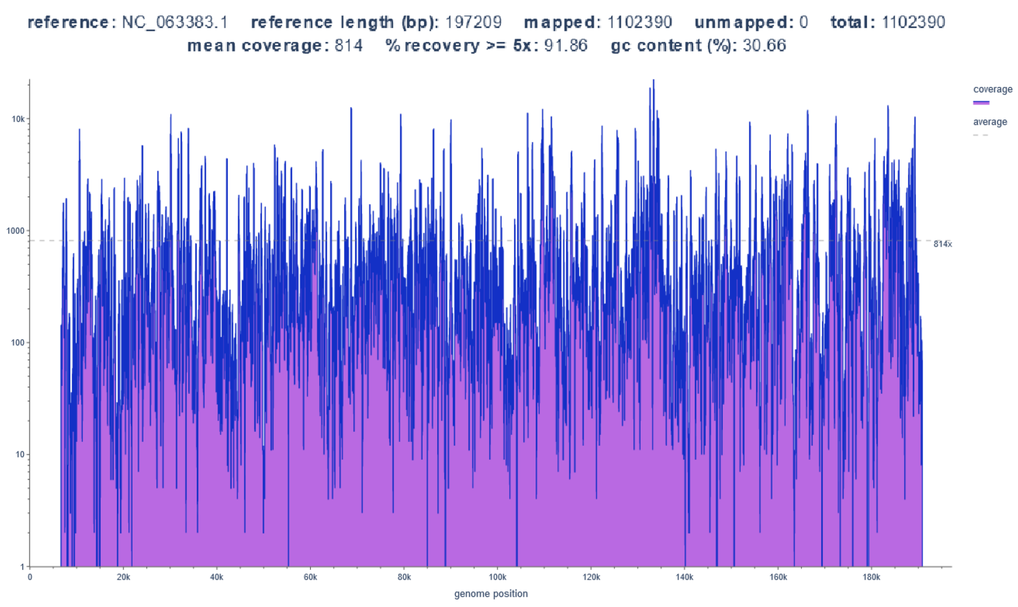


**Fig. 1: Coverage plot MPXV genome**

**Tab. 1: comparison between MiSeq and Nonopore sequencing**

| method | coverage [%] | missing nt | nt-Subst. | nt-InDel | AA Subst. | AA InDel | Frame Shift |
|---|---|---|---|---|---|---|---|
| MinION (N=80) Nanopore (RBK) | 56,96 | 84870 | 39,64 | 76,58 | 15,83 | 16,56 | 18,94 |
| standardeviation Nanopore (RBK) | 34,62 | 68274 | 24,82 | 91,46 | 10,13 | 13,26 | 14,03 |
| MinION Nanopore (N=24) (NBK) | 93,33 | 12923 | 64,57 | 53 | 27,3 | 8,09 | 23,83 |
| standardeviation Nanopore (NBK) | 0,66 | 1247 | 5,61 | 26,15 | 2,94 | 4,45 | 12,89 |
| MiSeq (N=80) Illumina | 86,43 | 19488 | 64,47 | 16,47 | 29,25 | 0,31 | 0,89 |
| standardeviation Illumina | 11,71 | 6025 | 10,08 | 11,09 | 3.93 | 1,49 | 1,29 |

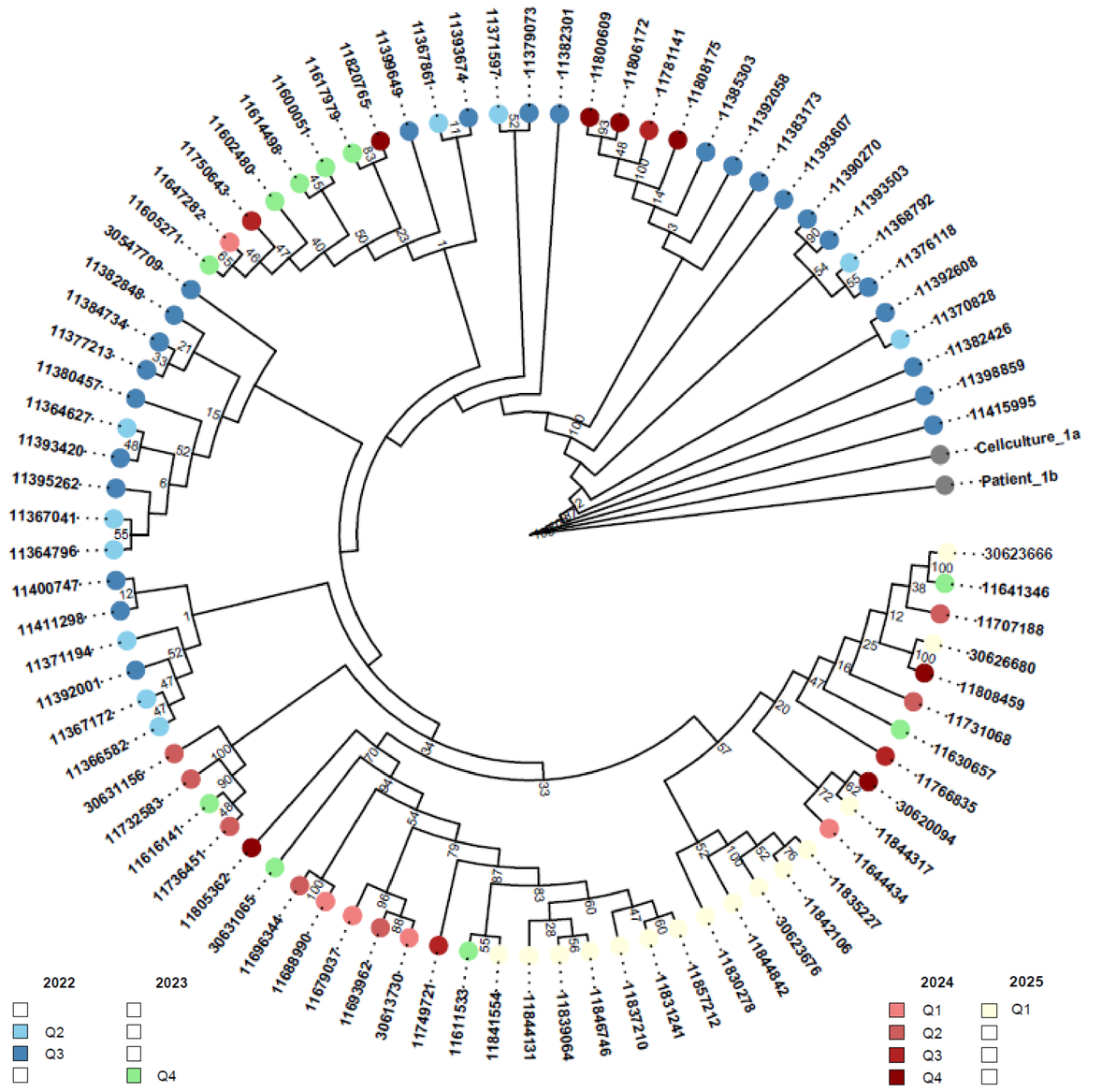


**Fig. 3: Phylogenetic analysis of all sequenced MPXV samples**

## CONCLUSIONS

The sequencing of the amplicons of the xGen MPXV panel performed better on MiSeq® with PCR-based barcoding and MinION with native barcoding, while rapid barcoding did not provide consistent results.
The sequences of the Berlin MSM cohort can all be assigned to MPXV clade IIb, the subtypes diverged over the course of the pandemic. A development in the MPXV with a view to a vaccination escape has not yet been detected. The investigation will be continued.