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Genomic monitoring and tracking of mpox virus clade Ib in Burundi between july to september 2024

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Abstract

Background Since the first identification of mpox virus (MPXV) clade Ib in Burundi in July 2024, Burundi is one of the most affected countries in this evolving outbreak outside of the DRC.

Methods Here we aimed to understand the route(s) of introduction and spread of MPXV throughout the first months of the outbreak in Burundi. In total, 98 genome sequences from cases diagnosed during the first three months of the outbreak were generated.

Results Here we show using phylogenetic analysis that the virus was introduced from the DRC and Bayesian evolutionary analysis shows that different clusters could be identified in Burundi leading to further spread within the country.

Conclusions In summary, we identify sustained circulation of clade Ib MPXV in Burundi, most likely after several different introductions from the bordering province of South Kivu-DRC. The virus has acquired several additional APOBEC-3 mediated mutations, in line with reported evidence of ongoing human-to-human transmission.

Plain Language Summary

Mpox is a viral infection caused by the mpox virus (MPXV). A specific MPXV strain called Ib, is spreading in the Eastern African region. Here, we aimed to understand how this strain was introduced into Burundi and spread to the rest of the country using genetic sequencing. Using this data, we could track the genetic evolution of the virus and found that there were several different introductions of the virus into Burundi, or that the virus had already been circulating in the country since around May 2024. Additionally, we identified specific mutations in the virus, indicative of human-to-human transmission (APOBEC-3). These findings suggest there is sustained circulation of mpox MPXV Ib in Burundi, largely driven by human-to-human transmission.

Mpox virus (MPXV) is a large double-stranded DNA virus with a genome of approximately 200,000 bp, which causes mpox. The virus was first detected during an outbreak in an animal facility in Denmark in 1958[1] and has since been detected in over 40 different animal species including various monkey species and rodents such as prairie dogs, squirrels, and dormice[2]. Since its first detection in humans in 1970[3], human mpox cases have been consistently reported in regions where the virus remains in enzootic reservoirs, mainly linked to zoonotic transmission and limited household transmission[4,5]. Since 2022, for the first time, a sustained global outbreak occurred predominantly in men having sex with men (MSM), where the virus was mainly transmitted via sexual contact[6,7].

Mpox virus can genetically be subdivided into two different clades. Historically, clade I MPXV has been described to be more pathogenic and with increased mortality rates compared to clade II MPXV[8]. While clade IIa

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is currently known to mainly circulate in West Africa, the global outbreak in 2022 was caused by the clade IIb virus. MPXV clade I is mainly circulating in Central Africa and can, since recently, also be subdivided into clade Ia and clade Ib. The increased detection of the different mpox sub-clades could result from anthropogenic changes, genomic rearrangement, evolution, diminished population-level immunity to Orthopoxviruses and/or increased mobility[9–11]. Additionally, heightened awareness from previous global and regional outbreaks and strengthened diagnostic capabilities could have increased our ability to detect MPXV[9]. Human-to-human transmission via (professional) sexual contact has recently been described for both clade Ia and clade Ib, but the current expansion in Central and East Africa seems to be primarily driven by clade Ib[12–14].

The clade Ib virus lineage emerged in the province of South Kivu in the Democratic Republic of the Congo (DRC) in 2023 and has subsequently expanded to other areas within and outside the DRC, including Burundi, Kenya, Rwanda, and Uganda[15]. In addition, travel-related cases linked to East African countries have been identified in Germany, Kenya, India, Rwanda, Sweden, Thailand, the United Kingdom, the United States, Zambia, and Zimbabwe[15]. During this ongoing clade Ib outbreak, unlike for the ongoing global clade IIb outbreak, both males and females have been affected, and the main mode of transmission seems to be primarily driven through (hetero)sexual contact followed by secondary infections in close (household) contacts[12,16,17].

Especially, Burundi has been severely affected during the current clade Ib outbreak, with 2447 confirmed cases between the first detection on the 25th of July 2024 and the 4th of December 2024. Few mpox cases from East Africa have been sequenced to date despite a substantial number of reported cases. Previously, we have described the MPXV clade Ib introduction into Burundi and the phylogenetic analysis of the first two genome sequences[18]. Here, we describe the sequencing and phylogenetic analysis of an additional 96 genome sequences during the first three months of the outbreak in Burundi to better understand the outbreak dynamics, the source of the outbreak, viral evolution, and genetic diversity of the current ongoing MPXV outbreak. We show that either there were several different introductions of MPXV clade Ib in Burundi or that the virus was cryptically circulating in the country several months before the first detection.

Methods

Study design and samples collection

This is a retrospective study based on a selection of cases from the national surveillance database. All cases were hospitalised and had been diagnosed during the first three months of the epidemic in Burundi. Minimum demographic metadata were collected from the different health districts and included age, occupation, sex, and health district. The swab samples from vesicular lesions were tested in the National Reference Laboratory of the National Public Health Institute of Burundi for MPXV with real-time PCR using the generic US CDC and a clade Ib specific target[19,20]. Positive samples with sufficient remaining sample volume that were collected between 23-07-2024 and 13-10-2024 from different health districts, and with a cycle threshold (Ct) value below 30, were selected for whole-genome amplicon sequencing. Of note, there was a drop in the number of cases diagnosed and sequenced in week 36 because of a nationwide logistical problem through which many alerts were not investigated.

DNA preparation, MPXV amplicon generation, and sequencing

From 96 selected vesicular lesions swabs, genomic DNA was extracted using the QIAamp® DNA Mini Kit (Qiagen). MPXV amplicons were generated as described previously[10]. Sequencing libraries were prepared using the Native Barcoding kit 24 v14 (Oxford Nanopore Technologies) and sequenced on a MinION™ Mk1C (ONT) using R10.4.1 flowcells. Reads were basecalled with the high-accuracy model on Dorado Basecall Server v7.4.13 (ONT). Generation of consensus sequences and phylogenetic analysis was performed as previously described[21]. Briefly, sequencing reads were quality controlled with fastp[22], and primers were trimmed using cutadapt[23] and Ampliclip[24]. Reads were mapped against NC_003310.1 using minimap2, and consensus sequences were generated using Virconsens[25] using a minimal coverage cut-off of 30x. Mutation calling and quality check were performed using Nextclade v3.9.1[26].

Phylogenetic analysis

Generated consensus sequences (horizontal genome coverage between 53% and 95% with an average of 84%) and available clade Ib sequences on GISAID on December 12th were aligned using squirrel v1.0.11 making use of the clade I masking option for low-complexity and repetitive regions as recommended by the WHO collaboratory group[27–29] Phylogenetic analysis was performed with IQ-TREE v2[30] with model K3Pu+F + I and visualised with a custom R script.

BEAST analysis

Generated consensus sequences and available clade Ib sequences on GISAID were aligned using squirrel v1.0.11, making use of the recommended clade I masking option by squirrel. We have employed the generalised stepping-stone sampling (GSS) model selection analysis implemented in BEASTX v10.5.0 to estimate the most appropriate molecular clock model and demographic prior[31]. We tested both the strict molecular clock model and the uncorrelated relaxed molecular clock model with the constant size prior and the exponential growth prior. In addition, we have performed a SkyGrid analysis, which showed the constant size prior was the best demographic prior to use (Supplementary Fig. 2). The GSS analysis indicated the strict molecular clock with constant size prior was the best-fit model for the data set (Supplementary Table 1). The analysis was performed using 200,000,000 iterations, and log files were analysed in Tracer v.1.7.1 to check if ESS values were beyond the threshold (>200). Tree annotator v10.5.0 was used with 10% burn-in and the keep target height option. A similar approach was used for the Bayesian evolutionary analysis on the full dataset, however, for this, the masking option as advised by the WHO collaboratory group was used.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Ethical statement

Ethical approval was given by the National Ethics Committee in Burundi (CNE/10/2024). The consent from participants for genetic analysis and data publication was waived by the National Ethics Committee in Burundi, as the samples were originally collected as part of a national surveillance program.

Results

After the initial detection of MPXV in Burundi on the 25th of July 2024, the virus continued to spread, with a total of 2447 notified cases on the 4th of December 2024. All MPXV cases were classified as clade Ib. During this time period, the epidemic was mainly concentrated in and around the highly densely populated city of Bujumbura, which is geographically located next to the province of South Kivu (DRC). However, infections have also been reported in other health districts in the country, with cases in 45 out of 49 (91.8%) districts. Community transmission, including sexual transmission and household transmission, has been reported throughout the country. As of the 4th of December, one death has been reported from MPXV clade Ib infection in Burundi.

We have in addition to the two previously reported sequences, generated an additional 96 genome sequences obtained from 96 individuals from the first three months of the MPXV clade Ib outbreak in Burundi. In total, 14.1% (98/665) of the detected cases in epi weeks 30−38 were sequenced (Table 1). These sequences mainly originated from patients situated in the most densely populated health district of Bujumbura Nord ($n = 70$), Bujumbura Centre ($n = 15$), Bujumbura Sud ($n = 2$), but also from health districts outside the capital, such as Gitega ($n = 1$), Kabezi ($n = 1$), Kayanza ($n = 5$), Kiremba ($n = 1$), Mpanda ($n = 1$) and Muramvya ($n = 2$)

Table 1 | Number of Burundian MPXV sequences per week per health district compared to the number of cases per health district (week 30−38)

	Week 30	Week 31	Week 32	Week 33	Week 34	Week 35	Week 36	Week 37	Week 38	Total (per health district)
Bujumbura-Nord	4/4 (100%)	4/11 (36%)	7/17 (41%)	15/24 (63%)	9/39 (23%)	12/60 (20%)	0/31 (0%)	11/66 (17%)	8/55 (15%)	70/297 (24%)
Bujumbura-Centre	-	2/5 (40%)	2/5 (40%)	0/7 (0%)	1/3 (33%)	1/6 (17%)	0/4 (0%)	2/16 (13%)	7/25 (28%)	15/68 (22%)
Bujumbura-Sud	-	0/2 (0%)	0/3 (0%)	0/4 (0%)	0/3 (0%)	0/11 (0%)	0/11 (0%)	1/21 (5%)	1/5 (20%)	2/58 (3%)
Gitega	0/1 (0%)	-	0/5 (0%)	0/10 (0%)	0/2 (0%)	0/5 (0%)	-	1/17 (6%)	0/10 (0%)	1/50 (2%)
Kabezi	-	-	0/1 (0%)	-	0/4 (0%)	-	-	-	1/3 (33%)	1/8 (13%)
Kayanza	-	2/4 (50%)	0/2 (0%)	1/3 (33%)	0/7 (0%)	0/5 (0%)	-	-	2/4 (50%)	5/26 (19%)
Kiremba	-	-	0/1 (0%)	0/2 (0%)	0/1 (0%)	-	-	1/1 (100%)	0/1 (0%)	1/5 (20%)
Mpanda	-	-	-	-	-	-	0/2 (0%)	-	1/1 (100%)	1/3 (33%)
Muramvya	-	1/1 (100%)	0/4 (0%)	1/5 (20%)	0/2 (0%)	-	-	0/1 (0%)	-	2/13 (15%)
Unknown	0/4 (0%)	0/13 (0%)	0/5 (0%)	0/15 (0%)	0/17 (0%)	0/20 (0%)	0/9 (0%)	0/57 (0%)	0/28 (0%)	0/138 (0%)
Total	4/9 (44%)	9/31 (29%)	9/43 (21%)	17/70 (24%)	10/78 (13%)	13/97 (13%)	0/57 (0%)	16/179 (9%)	20/132 (15%)	98/696 (14%)

[a]Includes two sequences generated by the Burundian National Public Health Institute during the initial rapid outbreak investigation by Nzoyikorera et al.,202418 (GISAID: EPI_ISL_19469177 − 78).
The percentage indicates the percentage of sequenced cases per health district per week.

(Table 1). Sequences were mainly obtained from adults due to limited sample availability.

In total, MPXV genomes from swabs collected from 57 male (59%) and 41 female (43%) cases were sequenced with 85 out of 98 individuals (87%) being adults older than 20 years (Fig. 1). Phylogenetic analysis based on the alignment generated using squirrel[27] with the masking as advised by the WHO collaboratory group (see methods) showed that the branch containing all Burundi sequences was rooted to cases notified in the DRC (Fig. 2). From there, a few different branches with sequences from Burundi were observed. This observed pattern suggests several introductions, one of which mainly seeded the ongoing outbreak. Of note, sample number 362 showed alternative clustering, suggesting a different introduction into the country, which was supported by recorded recent travel history to the DRC. Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3 (APOBEC-3) mutations were also studied as an indicator for sustained human-to-human transmission. The APOBEC-3 ratio among the Burundian clade Ib sequences was 73.8% (45/61) providing further support for the epidemiological observation that transmission was mainly driven by continuous human-to-human spread (Fig. 2). Bayesian evolutionary analysis performed on this dataset showed a similar pattern (Supplementary Fig. 3).

Bayesian evolutionary analysis based on the alignment generated using squirrel[27] (with the masking as advised by squirrel to add more resolution) on the shaded sequences in Fig. 2 showed that there were at least three different clusters identified in Burundi with a most recent common ancestor (MRCA) earlier than July 1st (Fig. 3). Assuming that the virus was introduced into Burundi around July 2024, thereby also taking into account the incubation time of the virus, three different introductions leading to further spread within the country could be identified. Alternatively, based on the Bayesian evolutionary analysis, the virus could have already been present in the country since around May 2024 (95% confidence interval of halfway April to halfway June) but remained unnoticed (Fig. 3) despite the surveillance system for case detection being in place. Only two major clusters were still detected in September 2024 (Fig. 3). The largest cluster consisted of 77 sequences and was first detected in a sample from Bujumbura Nord collected on the 23rd of July. This cluster subsequently rapidly spread further into the country with detections in the provinces of Gitega, Kabezi, Kayanza, Kiremba, Mpanda, and Muramvya. Two other smaller clusters were also first detected in Bujumbura Nord, one comprising 5 sequences, which was first detected in a sample obtained on the 23rd of July without further spread to other provinces while the other was also first detected in a sample collected the 23rd of July and later spread to Bujumbura Centre, Bujumbura Nord, Kiremba and Kebezi (Supplementary Fig. 1).

Discussion

In recent years, the incidence, diversity, and geographical spread of MPXV have been continuously increasing. Additionally, there has been a shift from historically primarily zoonotic spill-over events with limited regional human-to-human transmission events to sustained and global human-to-human transmission. This was reported for the first time during the global outbreak in 2022, with retrospective evidence of ongoing human-to-human transmission of this clade in Nigeria dating back to 2016[32]. Since then, escalating human-to-human transmission has been seen for clade Ia in Kinshasa in 2024 (western DRC)[13], and sustained human-to-human transmission has been documented for clade Ib in South Kivu (eastern DRC) since 2023[12]. This clade Ib virus subsequently spread and resulted in sustained human-to-human transmission chains in Uganda and Burundi in 2024. In this study, we applied whole-genome sequencing of isolates collected from the first three months after the introduction of MPXV into Burundi to help understand the expansion and genomic diversity of the virus.

Based on the molecular clock analysis several different clusters could be identified. Assuming the diagnostic testing successfully detected the first cases and the virus was not present in the country before July 2024, there were at least three different introductions into the country, two of which mainly led to further spread of the virus within the country. This is in contrast with findings elsewhere where the clade Ib cases have been detected but have been contained efficiently with sometimes only a limited number of additional cases[33–35]. Also in Burundi there have been sporadic cases without further spread, suggesting that the establishment of sustained human-to-human transmission is not purely a virus trait but is more dependent on high-risk behaviour patterns. Alternatively, the virus was already present in the country at an earlier time point but remained unnoticed. Although mpox surveillance was already implemented since October 2023 in Burundi it is a stigmatising disease and therefore the first introductions might have been missed.

Genomic rearrangement events have been reported for MPXV as a mechanism to compensate for comparatively low SNP rates of DNA viruses[36,37]. A deletion event of approximately 1,140 bp in clade Ib viruses near the 5' terminal region removes the D14L gene, one of five genes

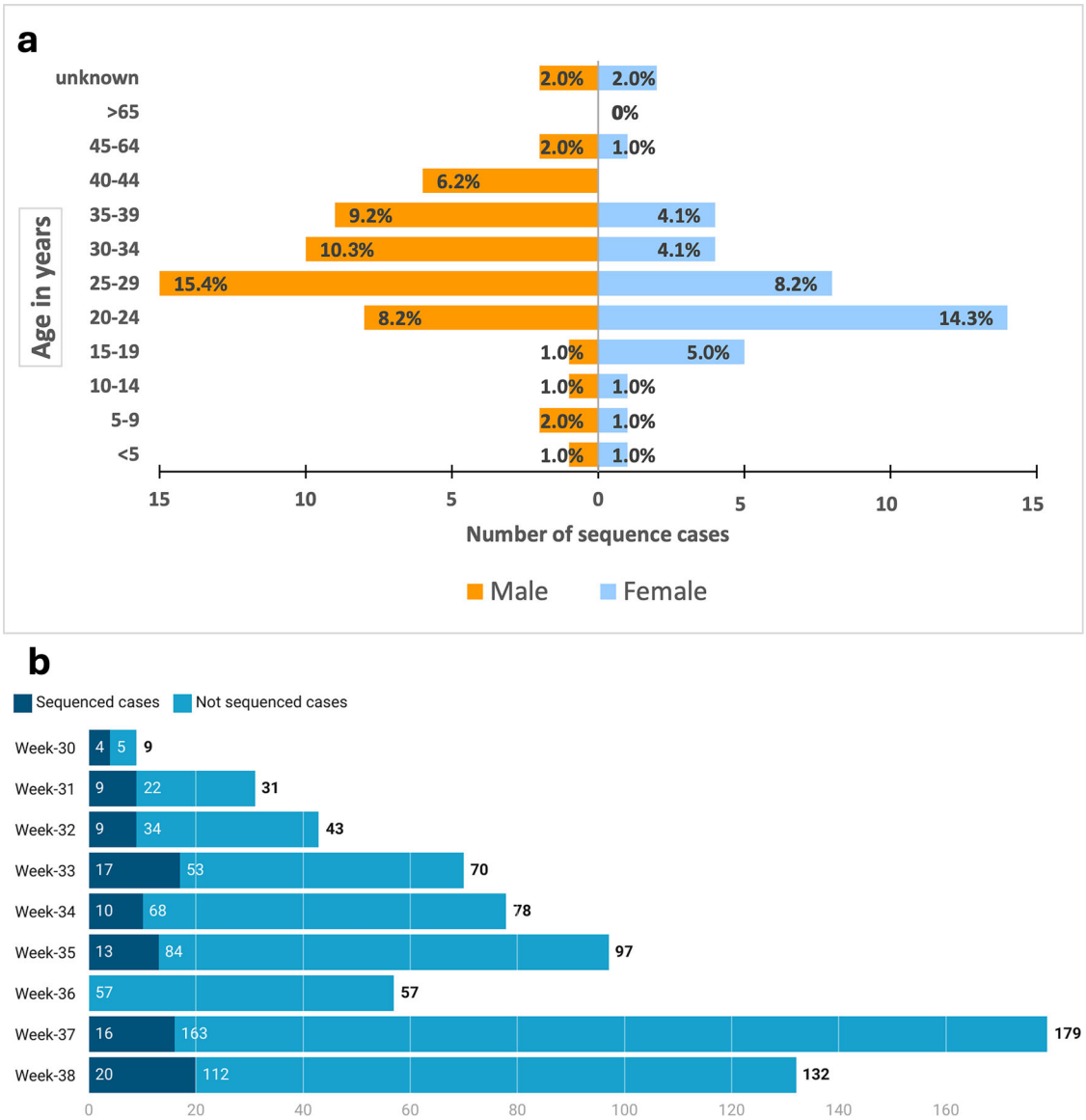

Fig. 1 | Age distribution and weekly number of the sequenced mpox cases. Age distribution of the sequenced cases (**a**) and the number of sequenced samples per epi week (**b**).

believed to be linked to increased virulence in clade Ia viruses[38]. This gene is also missing in the clade II viruses[19,39]. However, recently also a sexual transmission cluster of clade Ia has also been described[40] raising the question if clade Ia MPXV has the potential to be further spread in sexual networks mainly via sexual transmission. There was no indication of genomic rearrangement among the 98 clade Ib sequences analyzed in this study, and to the best of our knowledge, no published genomic rearrangements in MPXV clade Ib have been reported to date. This contrasts with genomic rearrangement events reported during the clade IIb outbreak, which ranged from 0.63% to 3.4%[10,36–38]. However, further and longer sequencing efforts may be required to draw stronger conclusions.

A large proportion of the sequenced cases were adults above 20 years of age, representing 86,9% of the sequences. This does not necessarily reflect the current epidemiological situation in Burundi, where predominantly children have been infected with clade Ib MPXV with 39,5% of the cases aged below 15[18]. The reason for the bias is that only samples with enough sample volume were sequenced. However, we still think this sequence data is representative of the situation in the country in the first few months after the introduction of the virus because these adults can be used as a proxy for what is going on in the entire population since adults are most probably driving the transmission while children most probably acquire the infection via community transmission though direct contact with infected individuals.

There was one reported death among the 2447 confirmed clade Ib MPXV cases in Burundi (0,04%). This contrasts with the initial case fatality rates reported in the DRC with 1% for clade Ib in South Kivu province (670 cases) and 4.6% in the nationwide previously predominantly clade Ia outbreak[16,21]. However, the case fatality rate is difficult to attribute in different settings, possibly due to age differences, overrepresentation of detections in hospitalized cases, surveillance coverage and/or co-morbidities. In the first three months of the outbreak in Burundi, mainly younger children were infected, which might account for this difference, although all cases investigated here were hospitalised.

In Burundi, after the initial introductions of MPXV the virus continued to spread within the country, mostly in an apparent monophyletic cluster, although Bayesian evolutionary analysis revealed that there were at least three different introductions with subsequent spread within the country. However, these results have to be interpreted with care, and more sequence data should be generated and shared from the current outbreak in neighbouring countries and the DRC. Likewise, some infections might have been

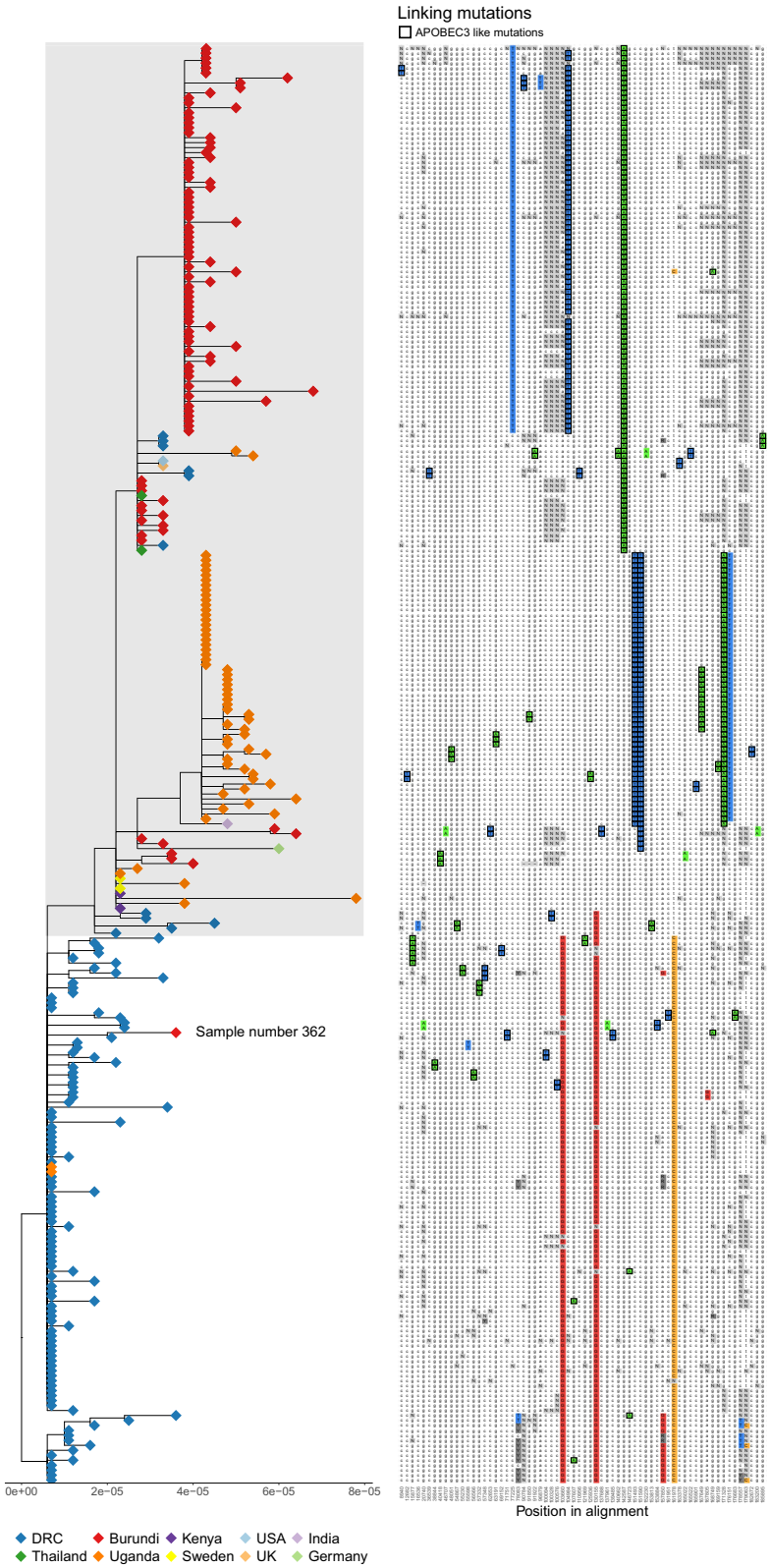

Fig. 2 | Phylogenetic analysis of newly generated sequences from Burundi and all MPXV clade Ib sequences available on GISAID. Tip shapes are coloured by country ($n = 289$ in total). Linking mutations are shown in capital font, and unique mutations are plotted next to the phylogenetic tree. Different nucleotides are coloured differently and APOBEC-3 signature mutations are highlighted with a black box. The light grey box indicates the cluster in which almost all samples from Burundi are located and represents the sequences which were used for the BEAST analysis.

missed, and we did not sequence all positive cases from Burundi, through which we also might have missed some different introductions into the country. Comparing our sequences to those publicly available, we noted the cases in Uganda are not linked to the cases detected in Burundi. However, more data are needed to draw any clear conclusions on this and to allow for further elucidation of potential between country transmission.

In conclusion, here we show the sustained circulation of the clade Ib MPXV in Burundi, most likely after initial introduction from the bordering province of South Kivu (DRC). The virus acquired several additional APOBEC-3 mediated mutations, in line with further ongoing human-to-human transmission[28,41]. More genomic sequencing is needed from neighbouring countries and more recent time points, but it seems that the

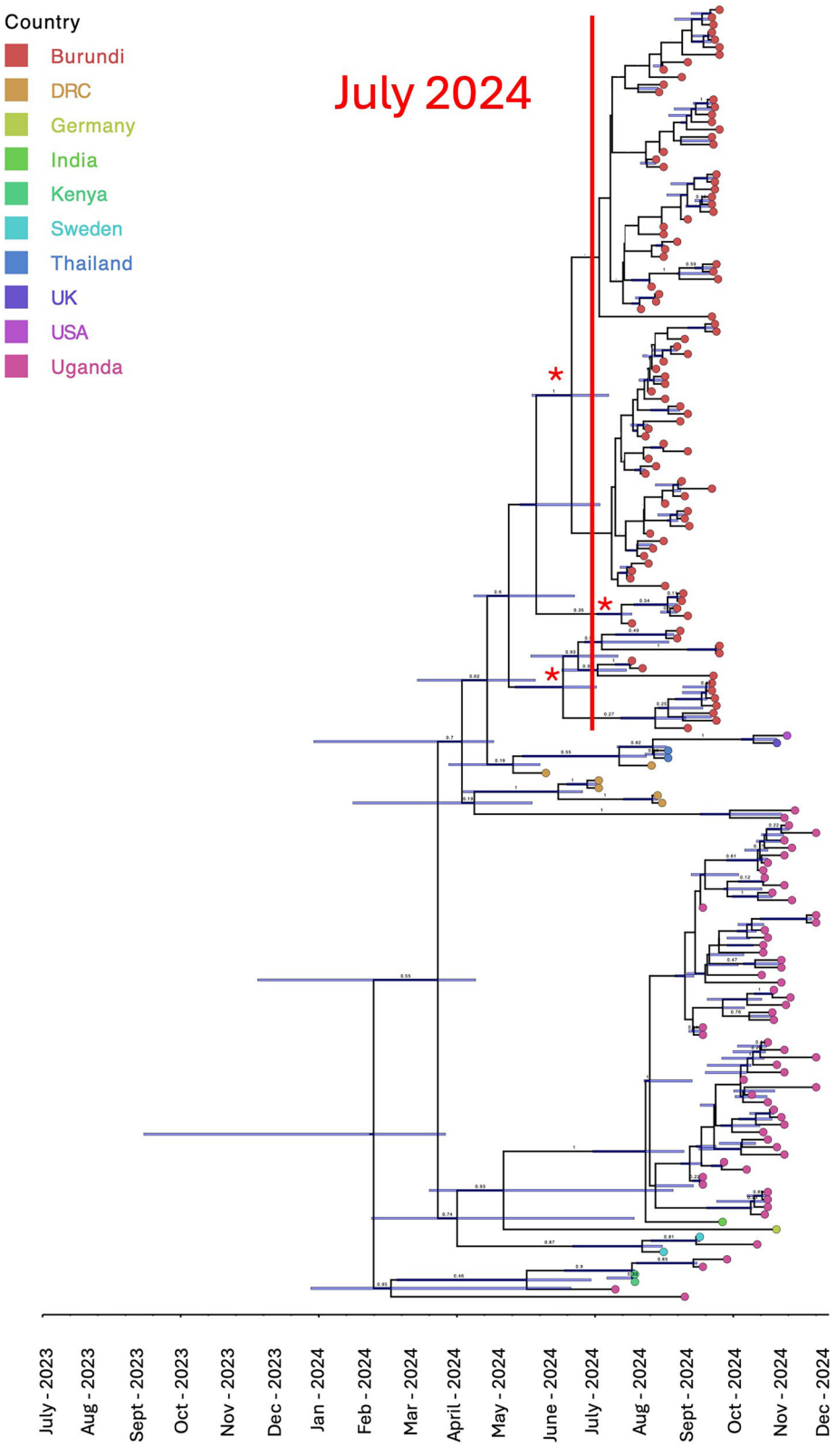

Fig. 3 | BEAST analysis on the sequence cluster containing the sequences generated during this study. The tips are coloured based on the country. Node bars represent the 95% confidence intervals, and the asterisk represents the three potential different introductions. The branch label represents the posterior values, displaying only values > 0.1. The red line presents the date of the 1st of July 2024, the presumed date of entry of the virus into Burundi.

outbreak in Burundi is caused by in-country transmission after several different introductions of the virus into Burundi.

Data availability

The consensus sequences generated in this study have been deposited on GISAID under the accessions: EPI_ISL_19599234 - EPI_ISL_19599325. The numerical data underlying the figures are available in Supplementary Data 1.

Code availability

The BEAST XML and log files are available via GitHub at https://github.com/BOM86/Genomic-monitoring-mpox-in-Burundi

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Author contributions
N.N., J.N., B.B.O.M., M.K., M.N.U., I.D., D.N., F.M.A. conceptualised and designed the study. N.N., L.S., D.F.N., C.N., T.I., A.N.I., B.O.M., H.C., M.B., A.N., M.K., S.O., I.D., D.N., R.M. contributed to data acquisition, analysis, and interpretation. N.N., C.N., T.I., D.N., A.N.I., A.N. were involved in sample collection and investigation. All authors approved the final version.

Competing interests
The authors declare no competing interests.

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