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# A European perspective of phylogenomics, sublineages, geographical distribution, epidemiology, and mutational landscape of mpox virus: Emergence pattern may help to fight the next public health emergency in Europe

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## ABSTRACT

**Background:** The 2022 outbreak of the mpox virus (previously monkeypox virus, MPXV) in non-epidemic regions has created a global issue. The emergence of MPXV was first reported in Europe, which was described as the MPXV epicenter, however, no reports are available to illustrate its outbreak patterns in Europe.

**Methods:** The study used numerous *in silico* and statistical methods to analyze hMPXV1 in European countries. Here, we used different bioinformatics servers and software to evaluate the spread of hMPXV1 in European countries. For analysis, we use various advanced servers like Nextstrain, Taxonom, MpoxSpectrum, etc. Similarly, for the statistical model, we used PAST software.

**Results:** The phylogenetic tree was depicted to illustrate the origin and evolution of hMPXV1 using a number of genome sequences (n = 675). We found several sublineages in Europe, indicating microevolution. The scatter plot reveals the clustering patterns of the newly developed lineages in Europe. We developed statistical models for the monthly total relative frequency counts of these sublineages. The epidemiology of MPX in Europe was examined in an attempt to capture the epidemiological pattern, total cases, and deaths. Our study noted the highest number of cases was in Spain (7500 cases) and the second-highest in France (4114 cases). The third highest number of cases was in the UK (3730 cases), which was very similar to Germany (3677 cases). Finally, we noted the mutational landscape throughout European genomes. Significant mutations were observed at the nucleotide and protein levels. We identified several unique homoplasmic mutations in Europe.

**Conclusion:** This study reveals several essential aspects of the European outbreak. It might help to eradicate the virus in Europe, assist in strategy formation to fight against the virus, and support working against the next public health emergency in Europe.

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## Introduction

The recent emergence of the mpox virus (previously known as monkeypox virus, MPXV) was first reported in Europe in the UK on

May 4, 2022. A man from the UK visited Nigeria and developed a rash on April 29, 2022, before leaving Nigeria [1]. On May 13, 2022, the first mpox (MPX) case was reported in the UK that was not linked to travel to endemic countries [2]. Before this case, travel-linked cases were often noted from endemic countries. However, in May 2022, a series of confirmed MPX cases were identified in European countries, such as the UK, Italy, and Portugal [2,3]. Approximately one month later, 1654 cases were reported in 25 countries

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throughout Europe on June 15 by the ECDC [4]. The situation has evolved quickly, and the most significant number of cases has been detected in European countries, including the UK, Portugal, Germany, and Spain, while the number of cases has rapidly increased in other European countries. The European Health Authority, ECDC, and the WHO have established that this new outbreak was initiated through a series of cases [5]. Simultaneously, the WHO declared a Public Health Emergency on July 23, 2022 [6]. In Europe, cases originating from different parts of the globe have also increased rapidly.

The recent MPX outbreak in Europe was the most significant outbreak, other than in non-epidemic countries (African regions) and the epicenter of the outbreak; it was noted as Europe's most widespread geographical outbreak [5]. Since the beginning of this outbreak, 11,001 confirmed MPX cases have been reported up to July 28, 2022 in 27 EU/EEA countries [4]. As of November 8, 2022, 20,744 confirmed MPX cases had been reported in 29 European countries [4]. As of January 20, 2023, 114,987 confirmed MPX cases were reported across 111 countries worldwide.

MPX is a viral zoonotic disease that was first reported in monkeys in Denmark in 1958 [7]. Subsequently, MPX outbreaks were noted in captive monkey colonies in the Netherlands and USA between 1960 and 1968 [8]. The first case of MPX in humans was reported in 1970. MPX cases were noted during smallpox eradication, and a surveillance program is underway in Africa. MPX remained an African disease in humans until 2003. Cases were occasionally diagnosed in the forested areas of West Africa and in Central Africa, and some small outbreaks have been noted in the DRC. Subsequently, the first cases outside Africa were reported in the USA, which were linked to the import of rodents from Ghana to Texas. The rodents transmitted MPXV to prairie dogs kept in the same animal facility, which then transmitted the virus to humans, primarily children and young people [9].

In 2003, MPX infections in the USA generated international attention; there were 71 human cases in total [10]. Subsequently, a few travel-related cases were occasionally reported between 2003 and 2022 in North America, Europe, and Asia. However, such cases were also reported in endemic countries. In 2018, five infected persons were identified, one in Israel, three in the UK, and one in Singapore [11,12]. MPX is usually rare and cases sporadic in the USA and UK [12,13]. In a systematic review from 1970 to 2018, Bunge et al. (2022) noted the changing epidemiological pattern of human MPX and researchers have reported the emergence of MPX in Europe and elsewhere [11]. Several studies note that the current emergence of MPX has novel clinical and epidemiological features [14,15]. For example, MPXV Clade 1 caused several outbreaks in the DRC between 1981 and 2017, where high fatality rates were noted (1–12%) [7,16]. However, in 2017, there was a large outbreak in Nigeria, and several PCR-confirmed cases (n = 122) were noted in MPXV Clade 2 [17]. The present global outbreak involves a new MPXV lineage, Clade 2b [18,19]. Therefore, it is clear that the clinical and epidemiological features of MPXV are changing.

In this study, we analyzed the phylogenomics, divergence, and scatter plot of genome clusters, lineages or sublineage relative frequencies over time, epidemiology, geographical distribution, and mutational landscape of the hMPXV1 genome from the European region.

## Methods

### Literature review

This literature review was carried out in order to understand the whole-European region and country-wise MPX occurrence. We searched for information on MPX disease and MPXV from different health authorities, such as the WHO, ECDC, and CDC. Literature

searches were performed using different scientific literature databases, such as Google Scholar, PubMed, and Web of Science.

A single keyword or combination of keywords was used during the literature search, including “monkeypox disease,” “monkeypox virus,” “European region,” “evolution,” “phylogenomics,” “sub-lineages,” “epidemiology,” “geographical distribution,” “entropy diversity,” and “mutation.” A European country-wise keyword search was also performed using Austria, Belgium, France, Germany, Portugal, Slovakia, Slovenia, Spain, and the United Kingdom. In these cases, combinations of keywords were used to search the scientific literature.

We retrieved hMPXV1 genome sequences for all analyses using GenBank databases, NCBI, and NCBI virus.

### Data analysis

#### Phylogenomic analysis of the genome sequence using the whole European region and significant MPX-affected European countries

Phylogenetic Phylogenomic analysis was performed using the whole-genome sequence of MPXV obtained from the European region. Here we have used the two servers and developed two types of phylogenomic trees: The nextstrain server [20] and the Taxonomium server [21]. Similarly, country-wise phylogenomics was also developed for Europe and various European countries, such as Austria, Belgium, France, Germany, Portugal, Slovakia, Slovenia, Switzerland, and the United Kingdom. For country-wise phylogenomics analysis, we have used the nextstrain server [20].

#### Divergence and scatterplot of hMPXV1 genome clusters in different European countries

Here, we have used the nextstrain server [20] to illustrate the divergence and scatterplot of hMPXV1 genome clusters in different European countries.

#### Relative frequencies of hMPXV1 sublineages over time and their total count in the European region and significant MPX-affected European countries

In this analysis, we used open data from GISAID to understand the relative frequencies over time of different hMPXV1 sublineages in the European region and individual European countries [22]. We also used the MpoXpectrum server for different hMPXV1 sublineages in the European region and individual European countries [23].

#### Statistical model of the month-wise total relative frequency count of hMPXV1 sublineages in the European region and significant MPX-affected European countries

The statistical software PAST (version 4.03) was used for statistical analysis and statistical model development of the month-wise total relative frequency count of hMPXV1 sublineages in the European region and significant MPX-affected European countries.

### MPX epidemiology in Europe

We retrieved data from the scientific literature review, the WHO, ECDC, and CDC to understand the epidemiology. The statistical software PAST (version 4.03) was used for the model development of MPX epidemiology in Europe.

### Geographical distribution of hMPXV1 sublineages in Europe

To depict the geographical distribution of hMPXV1 sublineages in Europe, we have also used the nextstrain server [20].

### Entropy diversity of hMPXV1 genomes from European countries

To analyze the entropy diversity of hMPXV1 genomes from European countries, we have also used the open data sources of the nextstrain server [20].

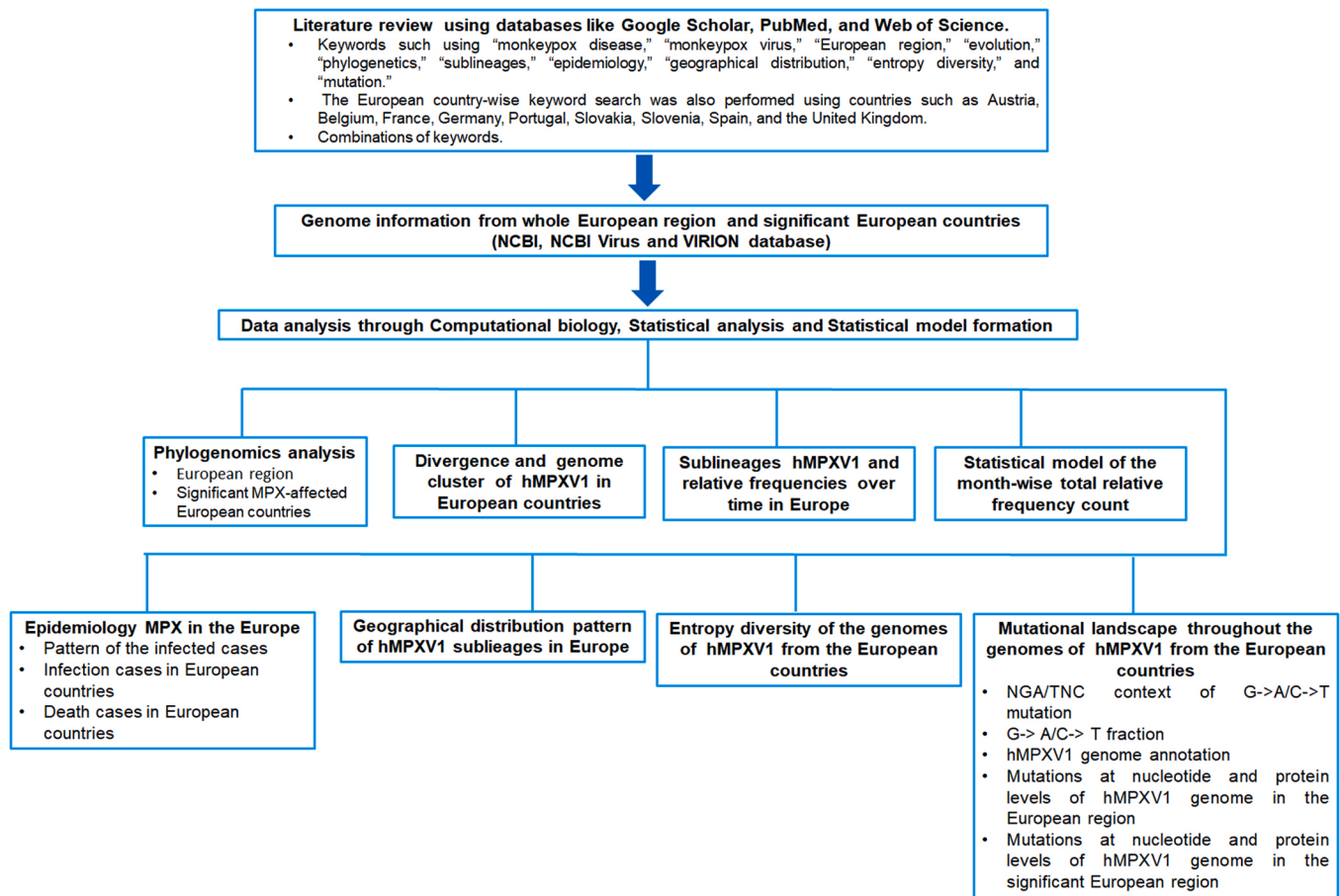


Fig. 1. A flowchart illustrates the step-by-step methods performed during our study.

#### Mutational landscape throughout hMPXV1 genomes from European countries

To evaluate the entropy diversity of hMPXV1 genomes from European countries, we have also used the open data sources of the nextstrain server [20].

All hMPXV1 genome sequences were retrieved from GenBank of NCBI and analyzed using the nextstrain server [20] and other servers. Finally, a flowchart has been depicted to illustrate the different steps of our study (Fig. 1).

## Results

#### Phylogenomic analysis of the genome sequence using the whole European region and significant MPX-affected European countries

We first produced a phylogenetic tree of hMPXV1 in the European region in order to understand the phylogenomics or molecular evolution of hMPXV1 (Fig. 2A) using the hMPXV1 genome sequences ( $n=675$ ) submitted to GenBank from Europe. We have developed another phylogenomics whole world ( $n=5049$ ), focusing on the European region using the Taxonom server platform using the sequences from Europe (Fig. 2B). The tree depicts phylogenomics marked in the European part, which indicates the vast number of genome sequences from Europe.

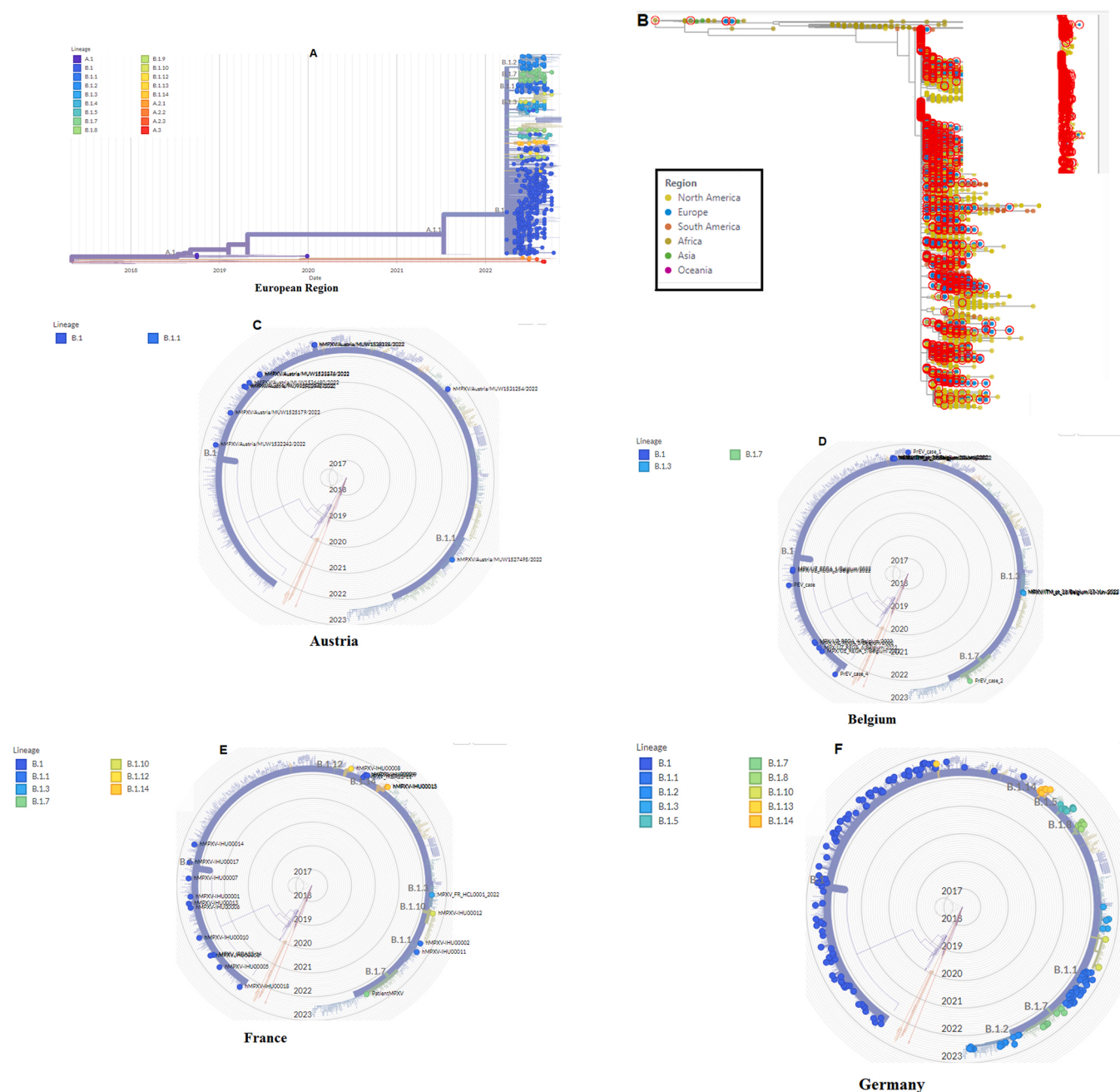
We also produced phylogenetic trees of significant European countries using the submitted hMPXV1 genome sequences. This includes the country-wise phylogenetic trees of Austria ( $n=11$ , Fig. 2C), Belgium ( $n=18$ , Fig. 2D), France ( $n=22$ , Fig. 2E), Germany ( $n=190$ , Fig. 2F), Portugal ( $n=183$ , Fig. 2G), Slovakia ( $n=11$ , Fig. 2H), Slovenia ( $n=36$ , Fig. 2I), Spain ( $n=9$ , Fig. 2J), and the United

Kingdom ( $n=172$ , Fig. 2K). The maximum number of genome sequences was obtained in Germany ( $n=190$ ). Although the number of cases was high in Spain, the number of genome sequences submitted to GenBank was low ( $n=9$ ).

The phylogenetic trees reveal that several sublineages, located at the terminal nodes, have emerged in the European region owing to micro-evolution over time. We noted numerous sublineages, namely B.1, B.1.1, B.1.2, B.1.3, B.1.4, B.1.5, B.1.7, B.1.8, B.1.9, B.1.10, B.1.12, B.1.13, and B.1.14 (Fig. 2A). B.1 and B.1.1 were generated in Austria (Fig. 2B), while B.1, B.1.3, and B.1.7 originated in Belgium (Fig. 2C). B.1, B.1.1, B.1.3, B.1.7, B.1.10, B.1.12, and B.1.14 originated in France (Fig. 2D), and B.1, B.1.1, B.1.2, B.1.3, B.1.5, B.1.7, B.1.8, B.1.10, B.1.13, and B.1.14 developed in Germany (Fig. 2E). Likewise, the sublineages produced in Portugal were B.1, B.1.1, B.1.3, B.1.5, B.1.7, B.1.9, and B.1.14 (Fig. 2F), while B.1 and B.1.3 originated in Slovakia (Fig. 2G), and B.1, B.1.2, B.1.3, and B.1.14 originated in Slovenia (Fig. 2H). B.1, B.1.3, B.1.8, and B.1.10 originated in Spain (Fig. 2I) and B.1, B.1.1, B.1.2, B.1.3, B.1.4, B.1.5, B.1.7, B.1.8, B.1.10, and B.1.12 were produced in the UK (Fig. 2J).

#### Divergence and scatterplot of hMPXV1 genome clusters in different European countries

We evaluated the divergence of hMPXV1 evolution in the European region, and a genome cluster scatterplot was produced using 675 samples. The divergence model was developed with a regression line, which indicates the pattern of divergence in the European region (Fig. S1A). The model indicates that 675 hMPXV1 genomes developed a distinct cluster on the higher side of the regression line. We also assessed the divergence of hMPXV1 evolution in significant European countries, such as Austria (Fig. S1B), Belgium



**Fig. 2.** Phylogenetic tree illustrates the hMPXV1 evolution in the European region and significant MPX-affected European countries during the 2022 outbreak. (A) The phylogenetic tree depicts the hMPXV1 in the European region. The tree was developed using the hMPXV1 genome sequences ( $n = 675$ ) submitted in GenBank from Europe. (B) Another result of the phylogenomics analysis of whole world, focusing on the European region ( $n = 5049$ ) using the Taxonium server platform (C) Depicted phylogenetic tree of Austria (D) Depicted phylogenetic tree of Belgium (E) Depicted radial phylogenetic tree of France (F) Depicted phylogenetic tree of Germany (G) Depicted phylogenetic tree of Portugal (H) Depicted phylogenetic tree of Slovakia (I) Depicted phylogenetic tree of Slovenia (J) Depicted phylogenetic tree of Spain (K) Depicted phylogenetic tree of United Kingdom. Here, we developed different radial phylogenetic trees to illustrate the hMPXV1 evolution for each European country.

(Fig. S1C), France (Fig. S1D), Germany (Fig. S1E), Portugal (Fig. S1F), Slovakia (Fig. S1G), Slovenia (Fig. S1H), Spain (Fig. S1I), and the United Kingdom (Fig. S1J). There was approximately  $5.70 \times 10^{-5}$  or  $5.70 \text{ e-}5$  substitutions per site per year.

#### Relative frequencies of hMPXV1 sublineages over time and their total count in the European region and significant MPX-affected European countries

We analyzed the lineages and sublineages of hMPXV1 and their relative frequencies over the entire European region and in significant European countries. We found a significant frequency of B.1,

B.1.7, and B.1.10 in the European region (Fig. 3A), with maximum frequencies of 0.7692, 0.0769, and 0.1538, respectively. However, the relative frequencies of the B.1 lineage were the greatest throughout the study period. We also found the highest relative frequencies of B.1, B.1.1, B.1.2, and B.1.5 in Austria, with frequencies of 0.7778, 0.5, 0.111, and 0.111, respectively (Fig. 3B). Similarly, the highest frequencies of B.1 and B.1.3 were observed in Belgium. In both cases, a maximum relative frequency of 1 was observed (Fig. 3C). The highest relative frequencies over time of B.1 and B.1.7 were in France: 0.6 and 0.2, respectively (Fig. 3D). Similarly, the highest relative frequencies of B.1, B.1.7, and B.1.10 were in Germany: 0.7143, 0.1429, and 0.1429, respectively (Fig. 3E). In Portugal, we noted the highest relative

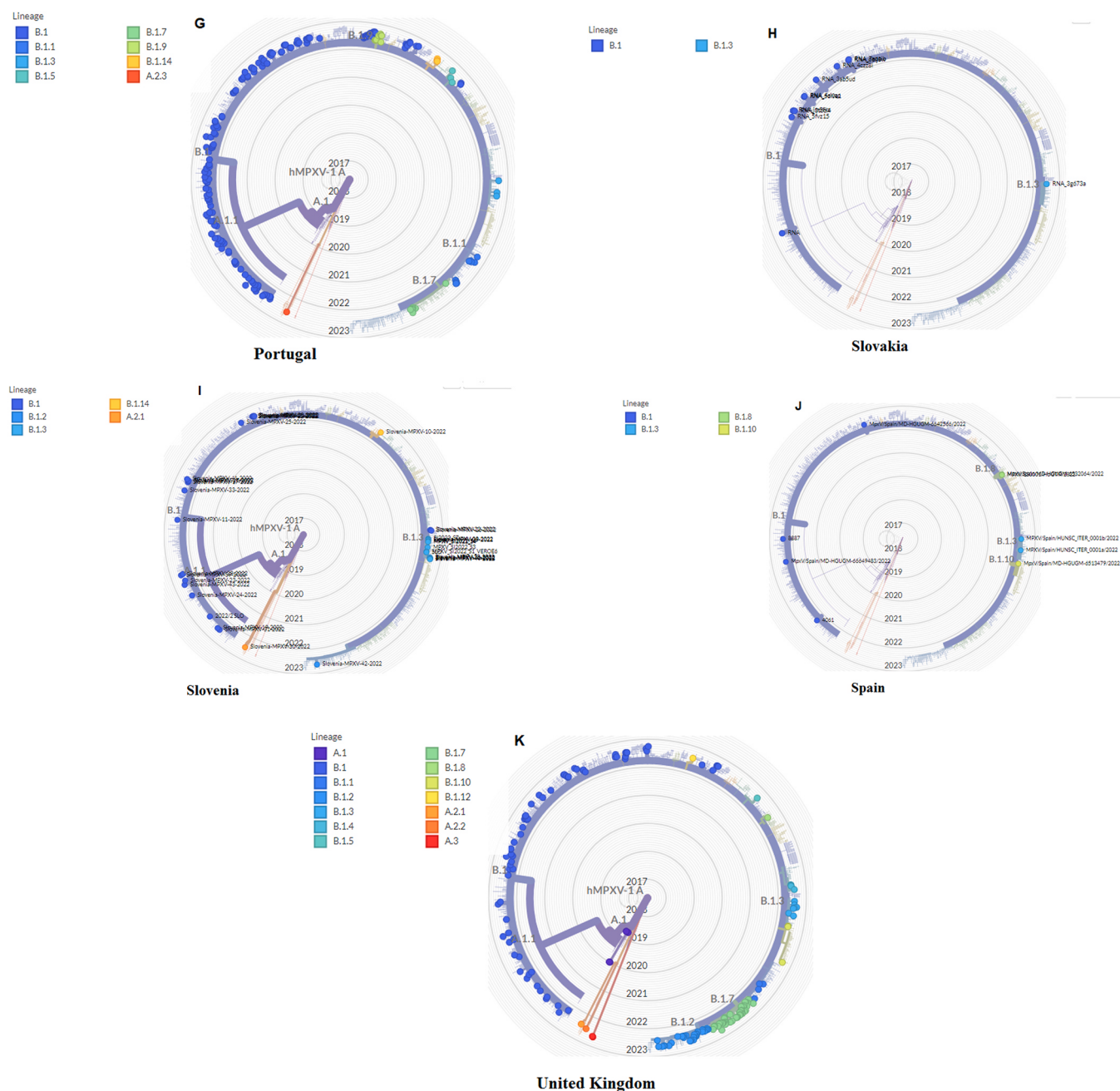


Fig. 2. (continued)

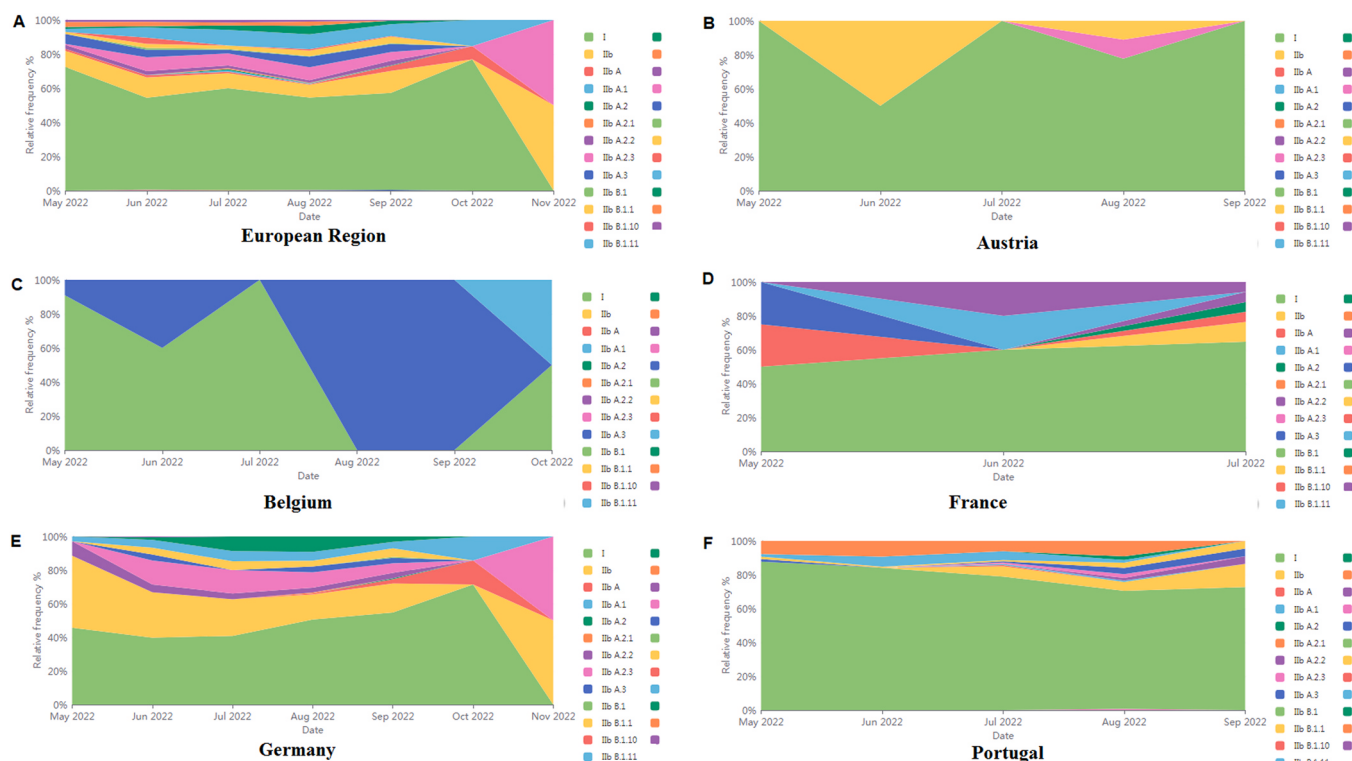
frequencies of sublineages B.1, B.1.1, B.1.2, B.1.3, B.1.7, B.1.8, B.1.9, and B.1.10 (Fig. 3F): 0.8769, 0.053, 0.0227, 0.0379, 0.0152, 0.0227, 0.0909, and 0.0075, respectively. In Slovakia, we noted the highest relative frequencies of lineage or sublineages B.1 and B.1.3 (Fig. 3G) at 0.8333 and 0.1667, respectively.

The highest relative frequencies of sublineages in Slovenia were B.1, B.1.2, and B.1.3 (Fig. 3H); the maximum relative frequencies were 0.6667, 0.111, and 1, respectively. In Spain, we noted the highest relative frequencies of lineage or sublineages B.1, B.1.3, B.1.8, and B.1.10 (Fig. 3I): 0.1667, 0.3333, 0.3333, and 0.1667, respectively. In the United Kingdom, we found B.1, B.1.1, B.1.2, B.1.3, B.1.4, B.1.5, B.1.6, B.1.7, B.1.8, and B.1.10 (Fig. 3J); their relative frequencies were 0.4103, 0.0256, 0.1368, 0.1282, 0.0171, 0.0171, 0.21137, 0.0085, and 0.0085, respectively.

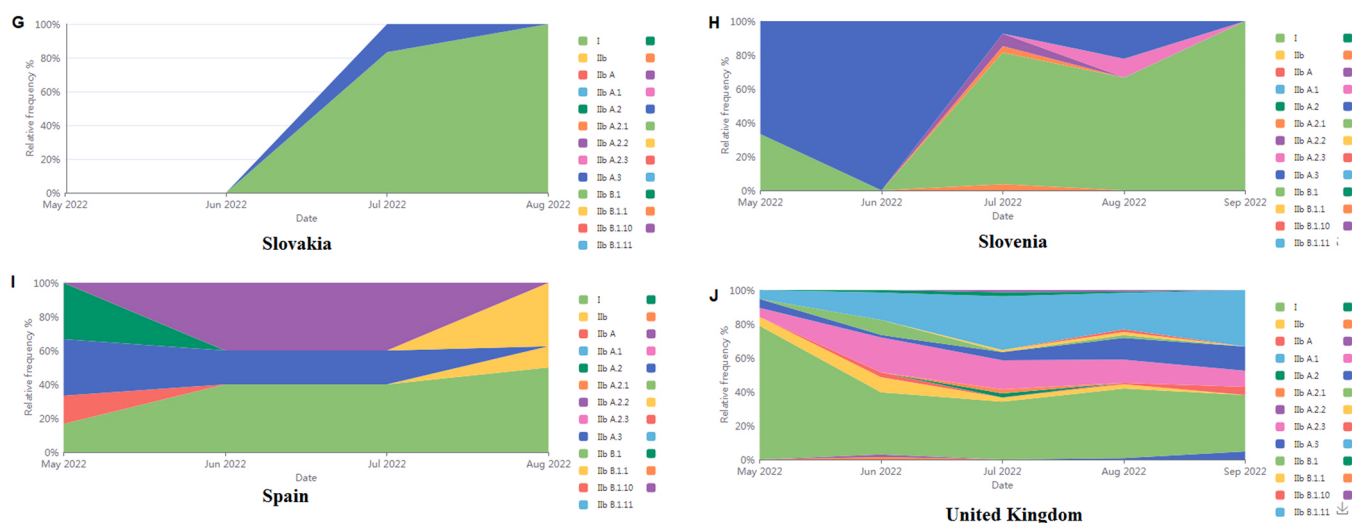
We have also developed the different hMPXV1 sublineages plot in the European region (Fig. S2A). The model indicates that dominance in the B.1 sublineage, such as B.1.5, B.1.7, B.1.9, B.1.10, etc. We also assessed the hMPXV1 sublineages plot in some significant European countries, such as Austria (Fig. S2B), Belgium (Fig. S2C), France (Fig. S2D), Germany (Fig. S2E), Portugal (Fig. S2F), Slovakia (Fig. S2G), Slovenia (Fig. S2H), Spain (Fig. S2I), and the United Kingdom (Fig. S2J).

In all cases, we found that the genome frequencies decreased in October, and no genome frequencies were found after November 2022 in Germany.

Next, we noted the highest total count of different sublineages every month from May 2022 to November 2022 across the entire European region (Fig. S3A), Austria (Fig. S3B), Belgium (Fig. S3C),



**Fig. 3.** The relative frequencies of hMPXV1 sublineages overtime during the 2022 outbreak in Europe (A) The relative frequencies of the sublineages illustrated from the hMPXV1 genome from the European region during the 2022 outbreak. (B) The relative frequencies of the sublineages of hMPXV1 illustrated from the genome from Austria (C) The relative frequencies of the sublineages of hMPXV1 illustrated from the genome from Belgium (D) The relative frequencies of the sublineages of hMPXV1 illustrated from the genome from France (E) The relative frequencies of the sublineages of hMPXV1 illustrated from the genome from Germany (F) The relative frequencies of the sublineages of hMPXV1 depicted from the genome from Portugal (G) The relative frequencies of hMPXV1 of the sublineages described from the genome from Slovakia (H) The relative frequencies of hMPXV1 of the sublineages illustrated from the genome from Slovenia (I) The relative frequencies of hMPXV1 of the sublineages illustrated from the genome from Spain (J) The relative frequencies of hMPXV1 of the sublineages illustrated from the genome from United Kingdom.

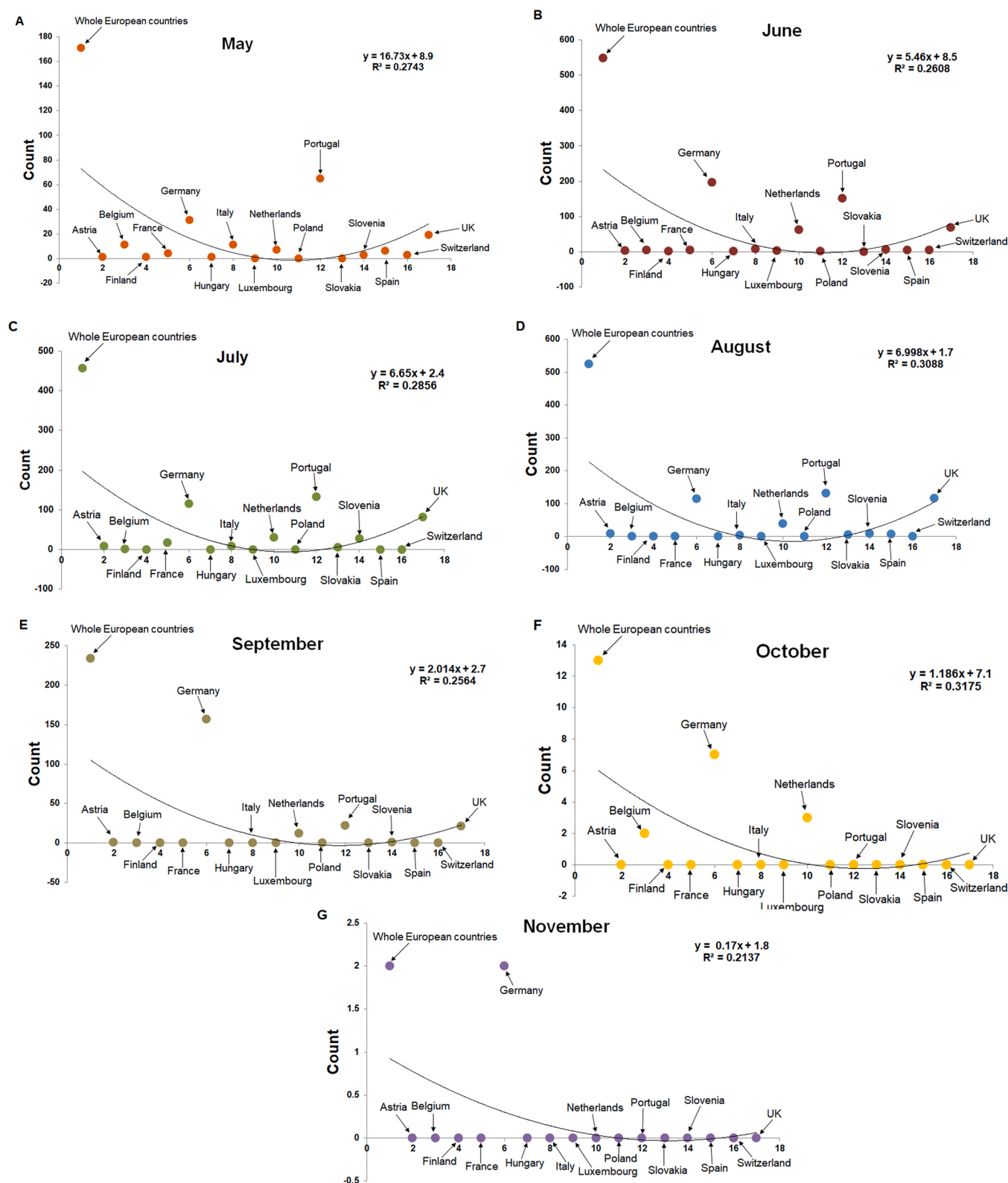


**Fig. 3.** (continued)

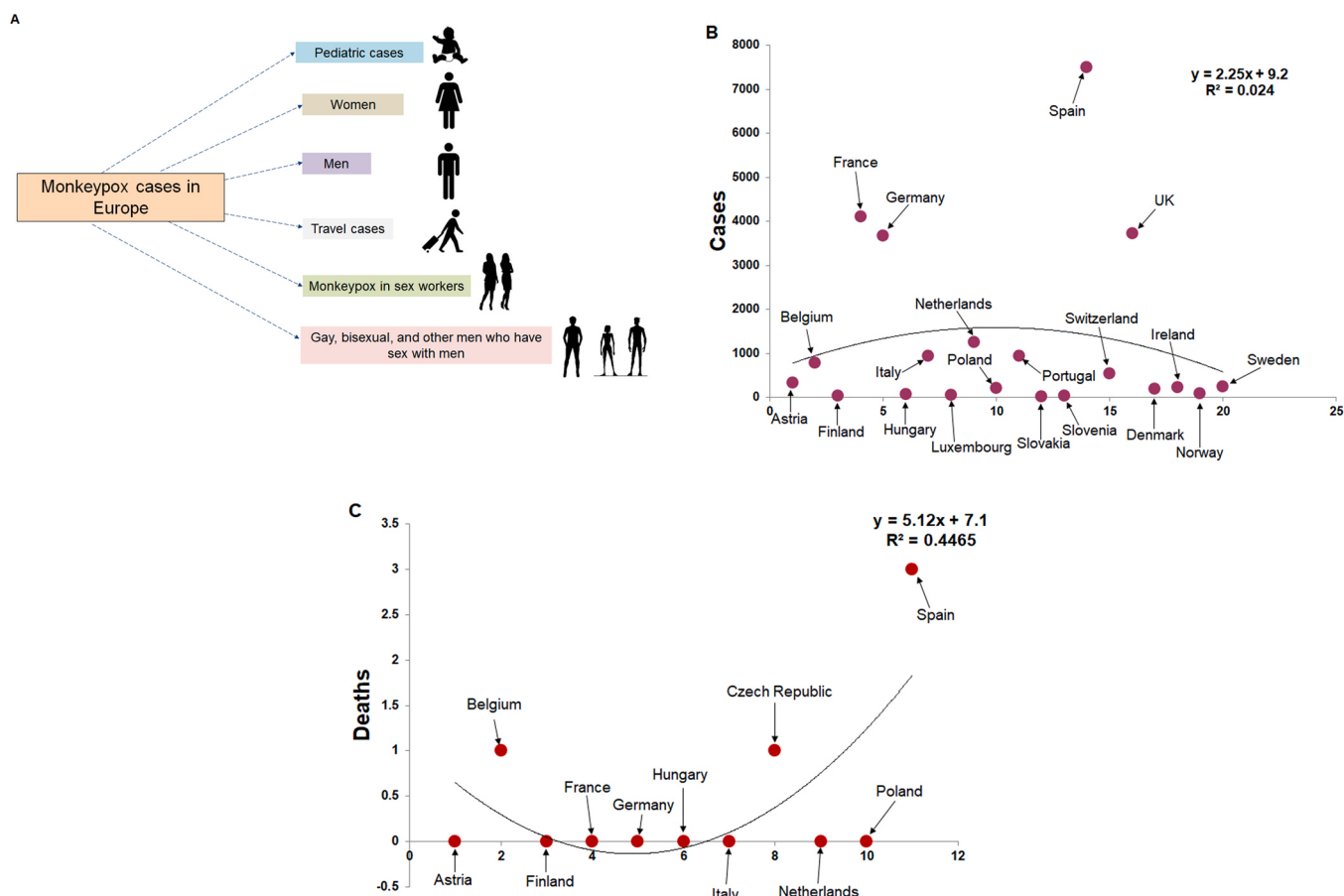
France (Fig. S3D), Germany (Fig. S3E), Portugal (Fig. S3F), Slovakia (Fig. S3G), Slovenia (Fig. S3H), Spain (Fig. S3I), and Portugal (Fig. S3J). Across the whole European region, we found the highest count in June 2022 of approximately 549. In France, the count spanned three months (May to July 2022), which was low compared to other countries. In November, there were no counts in significant European countries other than Germany, where we found two counts. The maximum count per month was found in June 2022 in Germany, at 179.

#### Statistical model of the month-wise total relative frequency count of hMPXV1 sublineages in the European region and significant MPX-affected European countries

We developed a second-order polynomial equation-based statistical model of the monthly total relative frequency count of hMPXV1 sublineages across the entire European region and significant European countries. All models were developed for May (Fig. 4A), June (Fig. 4B), July (Fig. 4C), August (Fig. 4D), September



**Fig. 4.** Statistical model of the month-wise total relative frequency count of the sublineages of hMPXV1 during the 2022 outbreak in Europe (A) Statistical model of the total relative frequency count of the sublineages of hMPXV1 in all European countries of hMPXV1 during May 2022. Here,  $R^2$  is noted as 0.2743 (B) Statistical model of the total relative frequency count of the sublineages of hMPXV1 in all European countries of hMPXV1 during June 2022. Here,  $R^2$  is noted as 0.2608 (C) Statistical model of the total relative frequency count of the sublineages of hMPXV1 in all European countries of hMPXV1 during July 2022. Here,  $R^2$  is noted as 0.2856 (D) Statistical model of the total relative frequency count of the sublineages of hMPXV1 in all European countries of hMPXV1 during August 2022. Here,  $R^2$  is noted as 0.3088 (E) Statistical model of the total relative frequency count of the sublineages of hMPXV1 in all European countries of hMPXV1 during September 2022. Here,  $R^2$  is noted as 0.2564 (F) Statistical model of the total relative frequency count of the sublineages of hMPXV1 in all European countries of hMPXV1 during October 2022. Here,  $R^2$  is noted as 0.3175 (G) Statistical model of the total relative frequency count of the sublineages of hMPXV1 in all European countries of hMPXV1 during November 2022. Here,  $R^2$  noted as 0.2137.



**Fig. 5.** Epidemiology of MPX cases in Europe (A) The pattern of the epidemiology of MPX indicates the infectious disease has spread in different groups of people, such as pediatric groups, women, men, traveler group, Sex workers, and the group related gay or bisexual or the MSM (B) The total MPX infected cases during the 2022 outbreak in the different countries of Europe (C) The total MPX death cases during the 2022 outbreak in the different countries of Europe.

(Fig. 4E), October (Fig. 4F), and November (Fig. 4G) 2022; the  $R^2$  values were 0.2743, 0.2608, 0.2856, 0.3088, 0.2564, 0.3157, and 0.2137, respectively.

#### MPX epidemiology in Europe

We assessed the epidemiology of recent outbreaks of hMPXV1 infection in different European countries, specifically the spread pattern of cases. We found that the infection spread to different communities or groups.

Cases were documented in pediatric groups, women, men, travelers, sex workers, and gay/bisexual/men who have sex with men (MSM) (Fig. 5A). We noted different infection cases from different European countries till January 4, 2023, and developed a second-order polynomial equation-based ( $y = 2.25x + 9.2$ ) statistical model (Fig. 5B); the  $R^2$  value of the equation was 0.024. The highest number of cases was in Spain (7500 cases) and second-highest in France (4114 cases). The third highest number of cases was in the UK (3730 cases), which was very similar to Germany (3677 cases); both points are located in the same region of the graph.

We also noted deaths in Europe as of January 4, 2023. We developed a second-order polynomial equation-based ( $y = 5.12x + 7.1$ ) statistical model (Fig. 5C); the  $R^2$  value was 0.4465. Here, we noted the highest number of deaths in Spain (three cases) and the second highest in Belgium and the Czech Republic (one case each). However, this study revealed the changing virulence patterns of MPX, showing that recent sublineages are less fatal than Clade 1, as several other studies also reported [19].

Our extensive literature search revealed significant publications on European MPX cases. Some publications noted MPX cases in the United Kingdom, Spain, Portugal, Italy, Germany, and the Netherlands, where researchers have occasionally reported MPX patients (Table 1).

#### Geographical distribution of hMPXV1 sublineages in Europe

We found a geographical distribution pattern of hMPXV1 sublineages in the European region (Fig. S4A). We also noted the geographical distribution pattern of the lineages or sublineages of the virus in Austria (Fig. S4B), Belgium (Fig. S4C), France (Fig. S4D), Germany (Fig. S4E), Portugal (Fig. S4F), Slovakia (Fig. S4G), Slovenia (Fig. S4H), Spain (Fig. S4I), and the United Kingdom (Fig. S4J).

From our geographical analysis of the data, we found three significant clusters in Germany (Fig. S4E), Portugal (Fig. S4F), and the United Kingdom (Fig. S4J).

#### Entropy diversity of hMPXV1 genomes from European countries

We assessed the entropy diversity of the entire European region and several different European countries. Entropy diversity was noted in viral genome sequences ( $n = 675$ ) of the entire European region (Fig. S5A), Austria ( $n = 11$ , Fig. S5B), Belgium ( $n = 18$ , Fig. S5C), France ( $n = 22$ , Fig. S5D), Germany ( $n = 190$ , Fig. S5E), Portugal ( $n = 183$ , Fig. S5F), Slovakia ( $n = 11$ , Fig. S5G), Slovenia ( $n = 36$ , Fig. S5H), Spain ( $n = 9$ , Fig. S5I), and the United Kingdom ( $n = 172$ , Fig. S5J). The hMPXV1 genome is approximately 198 kb long. We observed several positional entropy changes throughout the genome at

**Table 1**  
Monkeypox infection cases were reported from European countries from time to time during the 2022 outbreak.

| Sl. No.                              | Country name           | Monkeypox cases                                                                                        | Remarks                                                                                                                                                                                                                                                                                                                                                                         | Ref.                                 |
|--------------------------------------|------------------------|--------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|
| [29]<br>[30]<br>[31]                 | UK                     | 3439 confirmed cases was reported<br>Three cluster (86 cases) recorded<br>Total 181 patients confirmed | Most cases were reported in London during 6 May 2022–16 September 2022.<br>From 17 April to 25 May, 2022 laboratory confirmed MPXV infection without documented history of travel to any endemic countries.<br>The prospective and observational cohort study performed from May 11 to June 29, 2022 at three hospitals in Spain for conformation of monkeypox virus infection. | [29]<br>[30]<br>[31]                 |
|                                      | Spain                  | 37 patients were infected by MPXV<br>185 cases were confirmed<br>96 confirmed cases were recorded      | Cases are noted from 27 May to 24 June, 2022 in Madrid by examine the anal and oropharyngeal swabs.<br>Cases were reported by prospective cross-sectional study in multiple medical facilities in Spain due to high risk sexual behavior.<br>The socio-demographic and clinical characteristics of confirmed cases (between 5 and 27 May 2022) were studied.                    |                                      |
| [32]<br>[33]<br>[34]<br>[35]<br>[36] | Portugal               | A total of 33 numbers of cases reported<br>521 cases were notified                                     | The laboratory-confirmed-cases' are reported by anal and urethral swabs between 15 May to 7 July, 2022.<br>The autochthonous transmission was reported within median at Berlin during May to June 2022.                                                                                                                                                                         | [32]<br>[33]<br>[34]<br>[35]<br>[36] |
|                                      | Italy                  | The pediatric monkeypox patient (10 years old) was reported                                            | The case was noted at Amsterdam, the Netherlands on June 2022, possible cause was the foreign visit.                                                                                                                                                                                                                                                                            |                                      |
|                                      | Germany<br>Netherlands |                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                 |                                      |

different nucleotide positions, including 22917–22919 (entropy, 0.173) and 186165–186167 (entropy, 0.257). Nevertheless, high entropy diversities were noted throughout the hMPXV1 genome from European countries in both codon and nucleic acid contexts.

*Mutational landscape throughout hMPXV1 genomes from European countries*

The mutational landscape of the hMPXV1 genome was evaluated. First, we depicted the NGA/TNC context of the G- > A/C- > T mutation in different European regions. We found that the frequencies were positioned between 0.4 and 1 (Fig. S6A). We also studied the G- > A/C- > T fraction, and found several frequencies of 1 (Fig. S6B). The mutational landscape of the hMPXV1 genome was analyzed for the entire European region (Fig. S7A), Austria (Fig. S7B), Belgium (Fig. S7C), France (Fig. S7D), Germany (Fig. S7E), Portugal (Fig. S7F), Slovakia (Fig. S7G), Slovenia (Fig. S7H), Spain (Fig. S7I), and the United Kingdom (Fig. S7J). We recorded significant mutations at the nucleotide and protein levels in hMPXV1 genome sequences in the European region (Table S1) and in several European countries (Table S2). Here, we noted several unique homoplastic mutations in the genome.

**Discussion**

Europe was described as the epicenter of the 2022 global MPXV outbreak. In our study we analyzed the phylogenomics, divergence, and scatter plot of the relative frequencies of genome clusters, lineages, and sublineages over time, as well as the epidemiology, geographical distribution, and mutational landscape of the hMPXV1 genome from Europe. We also analyzed several significant MPX-affected European countries and the pattern of the recent MPX outbreak. Previously, we analyzed the phylogenetics, sublineages, epidemiology, geographical distribution, and mutational landscape of the recent hMPXV1 global outbreak and revealed the microevolution of hMPXV1 [19]. However, here, we found microevolution of hMPXV1 in Europe and several sublineages generated within Europe. Recently, we also urged the researchers to understand more about the origin and spread of the sublineages and the microevolution of monkeypox [24].

During phylogenomics analysis, we found that the highest number of genome sequences was submitted from Germany (n = 190). Due to an increased number of infection cases, the submission of genome sequences in GenBank of NCBI was more from Germany. At the same time, from Spain, we noted that the number of genome sequences submitted to GenBank was low (n = 9). However, we found that infection cases were high in Spain from further data analysis and the literature survey. Therefore, more genome sequences are needed to submit from Spain. Although, we found from our analyzed data that Germany and UK have deposited more than 150 sequences. Simultaneously, other countries have submitted less number of genome sequences even though their infection cases were high. More genome sequences were needed to submit in GenBank from these countries. Therefore, authorities of those countries need to take care of faster genome submission in the GenBank or other genome databases during future epidemics or pandemics, so it should help the scientist to analyze the genome sequences and forecast the trend of the epidemic or pandemic as well as to provide their recommendations to stop the pandemic.

Several studies have attempted to analyze the MPXV situation in Europe over time; MPXV has spread to different communities, for example, pediatric MPXV cases have been reported in the Netherlands, including a male child < 10 years old for whom the viral source was not identified [25]. Vivancos et al. reported community viral transmission in the UK and reported 86 cases between May 7 and 25, 2022. They analyzed the cases and found that one was

associated with travel to an endemic country and 79 cases were in men, 66 of whom were gay, bisexual, or MSM [2]. However, a report that evaluates phylogenomics from the genome sequences of the virus from Europe is yet to be found.

Our epidemiological evaluation indicated that monkeypox has spread in some European countries immensely. From our analyzed European countries, we found more than 3500 infection cases in some countries like Spain, France, the UK, and Germany. The highest number of cases noted was in Spain (7500 cases). Similarly, we noted a similar pattern of infection cases in the UK and Germany; in these two countries, the number of cases are 3730 and 3677, respectively. Our study revealed that the monkeypox virus has spread to different groups of populations in Europe, from children to travelers. During the literature search, we found several reports about the virus spread virus has spread in sex workers. However, monkeypox has spread in Europe very quickly, and the WHO declared an epidemic due to the faster spread of the virus in different European countries and populations. Kluge and Ammon suggested tackling the spread of Monkeypox in Europe and beyond all together [26]. We should not again neglect the neglected diseases and must learn a lesson about the consequences of ignoring a neglected infectious disease like monkeypox [27,28]. However, regulatory authorities of European countries must take a lesson from the spread of the virus. However, they must be prepared for the next epidemic of other infectious diseases to understand the pattern of infection and transmission dynamics utilizing our data and other data.

During this monkeypox epidemic, Europe was the central hub of the infection. No in-depth study analyzed the whole scenario of monkeypox infection in Europe. In this study, we tried to explore the entire monkeypox scenario infection in Europe. Unfortunately, until now, no study has illustrated the virus sublineages, epidemiology, and mutational landscape from a European perspective. So our study has immense importance from a European perspective because we analyze all these significant aspects of the MPX outbreak in Europe and found an outbreak pattern.

## Conclusion

Many aspects of MPX outbreaks in Europe remain unclear. Our data reveal several essential aspects of this outbreak. Several knowledge gaps have been addressed and the outbreak pattern, phylogenomics, divergence, genome clustering, lineage and sub-lineage relative frequencies over time, and epidemiology were assessed. However, several questions remain unanswered, such as whether different sublineages manifest different outcomes or milder clinical appearances; whether there are any associations between the respiratory or dermal route that contributed to the spread of MPX infection in the European outbreak; or if there are any mutations in the hMPXV1 genome associated with nAb or vaccine escape. Future research must address these questions urgently.

This study may help to eradicate the virus in Europe and assist in strategy formation to fight against the virus. Understanding the outbreak pattern in Europe and the lessons learned may assist in reacting to the next public health emergency in Europe.

## CRedit authorship contribution statement

CC supervised and coordinated the whole project, analysed the data and drafted the main manuscript. MB, GS, AA, MA, and SS-L performed the validation. KD reviewing the manuscript. All authors provided substantial contributions and approved the final version of the manuscript.

## Data availability

All data included within the manuscript and supplementary materials.

## Declaration of Competing Interest

We have no conflict of interest to declare.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2023.04.017.

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