Plague Denmark Paper

This manuscript ([permalink](https://rawcdn.githack.com/ktmeaton/obsidian-public/d90f1c60/academic/Plague%20Denmark%20Paper.html)) was automatically generated from [ktmeaton/obsidian-public@d90f1c60](https://github.com/ktmeaton/obsidian-public/tree/d90f1c60) on July 13, 2021.

## Authors

* **Katherine Eaton** ORCID icon [0000-0001-6862-7756](https://orcid.org/0000-0001-6862-7756) · GitHub icon [ktmeaton](https://github.com/ktmeaton)  
   McMaster University
* **Ravneet Sidhu**  
   McMaster University
* **Jennifer Klunk** ORCID icon [0000-0002-6521-8516](https://orcid.org/0000-0002-6521-8516)  
   Daicel Arbor Biosciences
* **Julia Gamble** ORCID icon [0000-0001-7486-757X](https://orcid.org/0000-0001-7486-757X)  
   University of Manitoba
* **Jesper Boldsen** ORCID icon [0000-0002-2850-0934](https://orcid.org/0000-0002-2850-0934)  
   University of Southern Denmark; ADBOU
* **Ann Carmichael**  
   Indian University Bloomington
* **Nükhet Varlık** ORCID icon [0000-0001-6870-5945](https://orcid.org/0000-0001-6870-5945)  
   University of South Carolina
* **Sebastian Duchene** ORCID icon [0000-0002-2863-0907](https://orcid.org/0000-0002-2863-0907) · GitHub icon [sebastianduchene](https://github.com/sebastianduchene)  
   University of Melbourne
* **Leo Featherstone** ORCID icon [0000-0002-8878-1758](https://orcid.org/0000-0002-8878-1758)  
   University of Melbourne
* **Vaughan Grimes** ORCID icon [0000-0002-2177-3147](https://orcid.org/0000-0002-2177-3147)  
   Memorial University
* **G. Brian Golding** ORCID icon [0000-0002-7575-0282](https://orcid.org/0000-0002-7575-0282)  
   McMaster University
* **Hendrik N. Poinar** ORCID icon [0000-0002-0314-4160](https://orcid.org/0000-0002-0314-4160)  
   McMaster University

## Abstract

This is a placeholder from the original paper proposal. To be re-written once results are finalized.

### Background

The sequencing of ancient pathogen genomes has resulted in vast advancements to our understanding of the second plague pandemic. However, due to limited sample availability, debate remains about the Plague’s origins, routes of dissemination, genomic diversity, and persistence. Specifically, Scandinavia has a unique history with regards to plague persistence as it is home to the oldest known strain of plague to date and has been ravaged by historic epidemics, only for the plague to have disappeared from this region in the modern era.

### Problem

It is unknown to what extent local plague reservoirs fed the recurring epidemics in Scandinavia as compared to the continual introduction of globally circulated strains. Previously studied historical records primarily derive from large commercial centres such as London, which are contrasted by countries such as Denmark where the archives have retained limited information about the spread of the plague and its impact on society.

### Objectives

To confidently identify and sequence ancient *Yersinia pestis* from Danish archaeological sites across a wide geographic and temporal range. With the aim of performing genomic analyses to estimate the timing, spread, and evolutionary changes occurring within Danish plague strains as compared to neighboring regions.

### Significance

First, there have been relatively few studies that explore the genetics of plague in Scandinavia across time and geography, particularly in Denmark. Second, this paper contributes to a larger body of epidemiological literature that considers the patterns and mechanisms by which diseases emerge, propagate, and go extinct.

## Introduction

1. When does plague appear? The presence of LNBA plague in Sweden raises the possibility that plague may be an endemic disease for the region. However, most European. However, most evidence of plague comes from the pandemic times (Justinian, and Black Death). We want to know if Denmark is the same (novel disease) or different.
2. How is plague in Northern/Western Europe connected to Modern plague. There’s the theory of a back migration out of Europe (Netherlands –> Russia). If this is true, then Denmark may also be involved. At ‘worst’ we expand the relationship of European regions connected to this lineage. At ‘best’ we capture where that transition from the Black Death clonal lineage to the post-Black Death lineage emerges.
3. Longitudinal study: The possibility to capture how plague affected the same people over time. (Highly regional, with 1 Danish strain?), or
4. Document the *pla* depletion/restoration events.

## Results

### Sites and Samples

326 individuals were sampled across 6 regions from 14 archaeological sites (Table [1](#tbl:site_summary)). The site occupation dates span from the 10th to 18th centuries which encompasses the Viking Age (8th - 10th century), the Medieval Period (11th - 16th century) and the Early Modern Period (16th - 19th century) in Denmark.

Table 1: Summary of archaeological sites sampled in this study.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Region | Site Name | Site Code | Site Occupation | Samples | Plague Positive |
| Ribe | Ribe Gräbrødre | ASR 1015 | 1200 - 1560 | 53 | 5 |
|  | Ribe Lindegärden | ASR 2391 | 900 - 1000 | 5 | 0 |
|  |  | ASR 13/13II | 900 - 1000 | 15 | 0 |
|  |  | ASR 13II | 1200 - 1560 | 28 | 1 |
| Viby | Nordby | FHM 3970 | 1050 - 1250 | 36 | 0 |
| Horsens | Monastery Church | HOM 1272 | 1600 - 1800 | 50 | 0 |
|  | Ole Wormsgade | HOM 1649 | 1100 - 1500 | 17 | 2 |
|  | Sejet | HOM 1046 | 1150 - 1574 | 25 | 1 |
|  | Tirup | VKH 1201 | 1150 - 1350 | 12 | 1 |
| Hågerup | Hågerup | ØHM 1247 | 1100 - 1555 | 7 | 1 |
| Refshale | Refshale | Refshale | 1100 - 1350 | 19 | 0 |
| Viborg | Sct. Mikkel | JAH 1-77 | 1000 - 1529 | 4 | 0 |
|  | The Catholic Church | VSM 09264 | 1100 - 1529 | 6 | 0 |
|  | Sct. Mathias | VSM 855F/906F | 1100 - 1529 | 23 | 0 |
|  | Sct. Drotten | VSM 902F | 1100 - 1529 | 8 | 0 |
|  | Faldborg | VSM 29F | 1100 - 1600 | 17 | 2 |
| **Total** |  |  |  | 325 | 13 |



Figure 1: Geographic map of archaeological sites by region. Stars indicate sites where *Yersinia pestis* was detected.

### Plague Detection

13 individuals were identified as plague-positive based on a combination of PCR assays, shotgun sequencing, and targeted enrichment for the *Y. pestis* whole genome (Tables [2](#tbl:plague_positive_high_coverage) and [3](#tbl:plague_positive_low_coverage)). Of the 13 individuals, 9 had chromosomal coverage sufficient for phylogenetic analyses.

Overall, plague was detected in 4% (13/325) of all individuals in this study (Table [1](#tbl:site_summary)). When excluding plague-negative archaeological sites, this estimate rises to 8.2% (13/159) of individuals. *Y. pestis* was observed primarily in the Medieval Period, with no evidence of plague in the Viking Age settlements at Ribe Lindegärden or the Early Modern cemetery at Horsens.

* Absence of plague in Viking/Early Modern could easily be a false negative.
* These periods are sparsely sampled, with fewer sites and individuals sampled.
* Are G25A and G25B two individuals from the same grave?

Table 2: Plague positive summary of high coverage genomes. Human (%) and plague (%) derive from shotgun estimates. The mean depth of coverage for the chromosome (CHROM) and the plasmids (pCD1, pMT1, pPCP1) are reported after targeted enrichment.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Arch ID | Project ID | Site | PCR | Human (%) | Plague (%) | CHROM | pCD1 | pMT1 | pPCP1 |
| G16 | D71 | Ribe Gräbrødre | 6/6 | 5.95 | 0.18 | 22.6 | 39.4 | 14.7 | 4.6 |
| G861 x1035 | D75 | Ribe Gräbrødre | 6/6 | 4.42 | 0.23 | 17.4 | 40.2 | 16.5 | 3.4 |
| G25B x98 | R36 | Ribe Gräbrødre | 6/6 | 8.41 | 0.25 | 24.0 | 51.8 | 14.9 | 5.8 |
| G25A | D62 | Ribe Gräbrødre | 6/6 | 1.12 | 0.10 | 3.8 | 10.5 | 2.5 | 0.9 |
| G207 | D72 | Ribe Gräbrødre | 6/6 | 12.94 | 0.04 | 6.0 | 13.5 | 5.8 | 2.2 |
| A146 x3011 | P187 | Sejet | 6/6 | 0.68 | 0.01 | 4.9 | 18.4 | 6.6 | 52.2 |
| G371 | P212 | Tirup | 6/6 | 0.61 | 0.04 | 6.7 | 26.3 | 8.5 | 56.6 |
| Gr GC 15 | D51 | Faldborg | 6/6 | 0.67 | 0.05 | 9.0 | 25.4 | 8.1 | 2.0 |
| A1480 x1480 | P387 | Ole Wormsgade | 6/6 | 0.04 | 0.01 | 6.5 | 21.7 | 5.0 | 75.0 |

Table 3: Plague positive summary of low coverage genomes. Human (%) and plague (%) derive from shotgun estimates. The mean depth of coverage for the chromosome (CHROM) and the plasmids (pCD1, pMT1, pPCP1) are reported after targeted enrichment.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Arch ID | Project ID | Site | PCR | Human (%) | Plague (%) | CHROM | pCD1 | pMT1 | pPCP1 |
| A1155 x1155 | P384 | Ole Wormsgade | 4/6 | 0.11 | 0.01 | 1.1 | 4.8 | 1.4 | 19.6 |
| Gr ID 319 | R21 | Faldborg | 6/6 | 0.85 | 0.01 | 2.6 | 3.8 | 2.3 | 0.4 |
| A19 X21 | D24 | Hågerup | 6/6 | 0.55 | 0.01 | 2.6 | 6.1 | 1.9 | 0.7 |
| X1265 | P246 | Ribe Lindegärden | 6/6 | 0.03 | 0.01 | 0.1 | 0.1 | 0.1 | 3.2 |

### Dating

I’m relying heavily on the discussion in Boldsen (2009) [[1](#ref-eD3kpkYB)], as quoted here:

*“The dating of individual skeletons is a fundamental problem in historical studies like this, and even the period of usage of each cemetery raises some serious problems. However, most cemeteries have at least some documentary sources broadly framing them in time. The most intensely studied skeletal samples, Tirup and Westerhus, are really the only exceptions in being dated solely on archaeological evidence (Kieffer-Olsen et al. 1986, Sivěn 2005).”*

*“In medieval graves the position of the arms in relation to the rest of the skeleton in the grave is the only feature that systematically indicates dating of the burial within the temporal frame provided by the period of usage of the cemetery. Arm position dating is primarily based on work by Redin (1976) and Kieffer-Olsen (1993). The successive stages of arm position from A (the arms besides the body) over B (hand joint over the lower part of the abdomen and usually found in the pelvis) and C (the forearms over the upper part of the abdomen and the elbows flexed in an approximately right angle) to D (the hands placed on the shoulders, forearms often crossed over the chest) have primarily been described by Kieffer-Olsen (1993) but Jantzen et al. (1994) have slightly modified the transition dates between the various stages.”*.

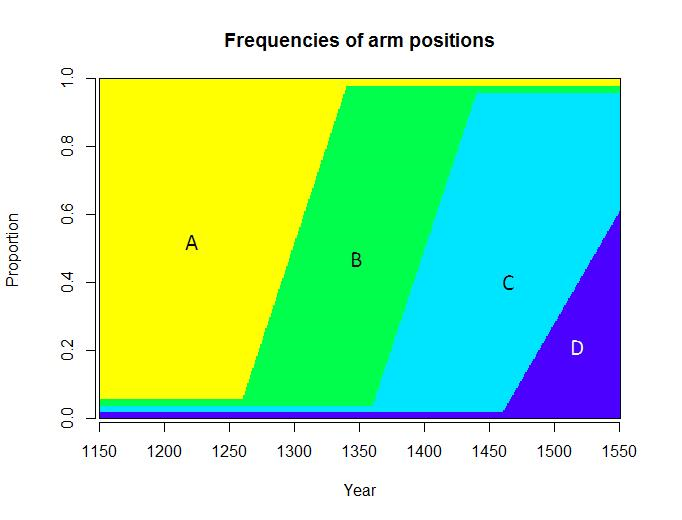


Figure 2: Arm position dating.

**Question for Julia Gamble and Jesper Boldsen.** Can I use this diagram to broadly assign time periods to arm positions? If so, I can add greater resolution to Figure [3](#fig:timeline_sites). - Position A: 1150 - 1325 - Position B: 1250 - 1425 - Position C: 1350 - 1550 - Position D: 1450 - 1550

The skeletal and molecular dates of the 9 high coverage *Y. pestis* genomes are presented in Table [4](#tbl:tip_dating_summary) and Figure [3](#fig:timeline_sites). Almo st all molecular dates had overlap with the archaeological dates as determined by the site occupation period and burial patterns. The exception to this pattern was individual G371 from the site of Tirup.

To investigate the dating disparity for G371, we performed 14C radiocarbon dating. The radiocarbon estimate largely agrees with the archaeological dates, with an estimated mean date of 1260 CE (+/- 75 yrs with 1 sigma). Thus there is robust evidence that the individual lived and died sometime between the late 12th and early 14th century

**Question for Vaughan Grimes.** Is the interpretation and visualization of the radiocarbon date appropriate?

However, the associated *Y. pestis* genome is dated to the 15th century. While the 2 sigma distribution of the radiocarbon date does extends into the 15th century, there remains a substantial conflict between the date of the host and the associated pathogen.

Table 4: Summary of the *Y. pestis* molecular dates. The estimated tip date reflects the 95% highest posterior density.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| ID | Region | Site | Site Occupation | Arm Position | Skeletal Date | Tip Date |
| G16 | Ribe | Ribe Gräbrødre | 1200 - 1560 | C | 1350 - 1550 | 1310 - 1388 |
| G861 x1035 | Ribe | Ribe Gräbrødre | 1200 - 1560 | C | 1350 - 1550 | 1489 - 1567 |
| G25B x98 | Ribe | Ribe Gräbrødre | 1200 - 1560 | C | 1350 - 1550 | 1327 - 1414 |
| G25A | Ribe | Ribe Gräbrødre | 1200 - 1560 | C | 1350 - 1550 | 1295 - 1375 |
| G207 | Ribe | Ribe Gräbrødre | 1200 - 1560 | C | 1350 - 1550 | 1477 - 1551 |
| A146 x3011 | Horsens | Sejet | 1150 - 1574 | B | 1250 - 1425 | 1397 - 1470 |
| A1480 x1480 | Horsens | Ole Wormsgade | 1100 - 1500 | ? | ? | 1384 - 1473 |
| G371 | Horsens | Tirup | 1150 - 1350 | B | 1250 - 1425 | 1419 - 1490 |
| Gr GC 15 | Viborg | Faldborg | 1100 - 1600 | C | 1350 - 1550 | 1539 - 1655 |

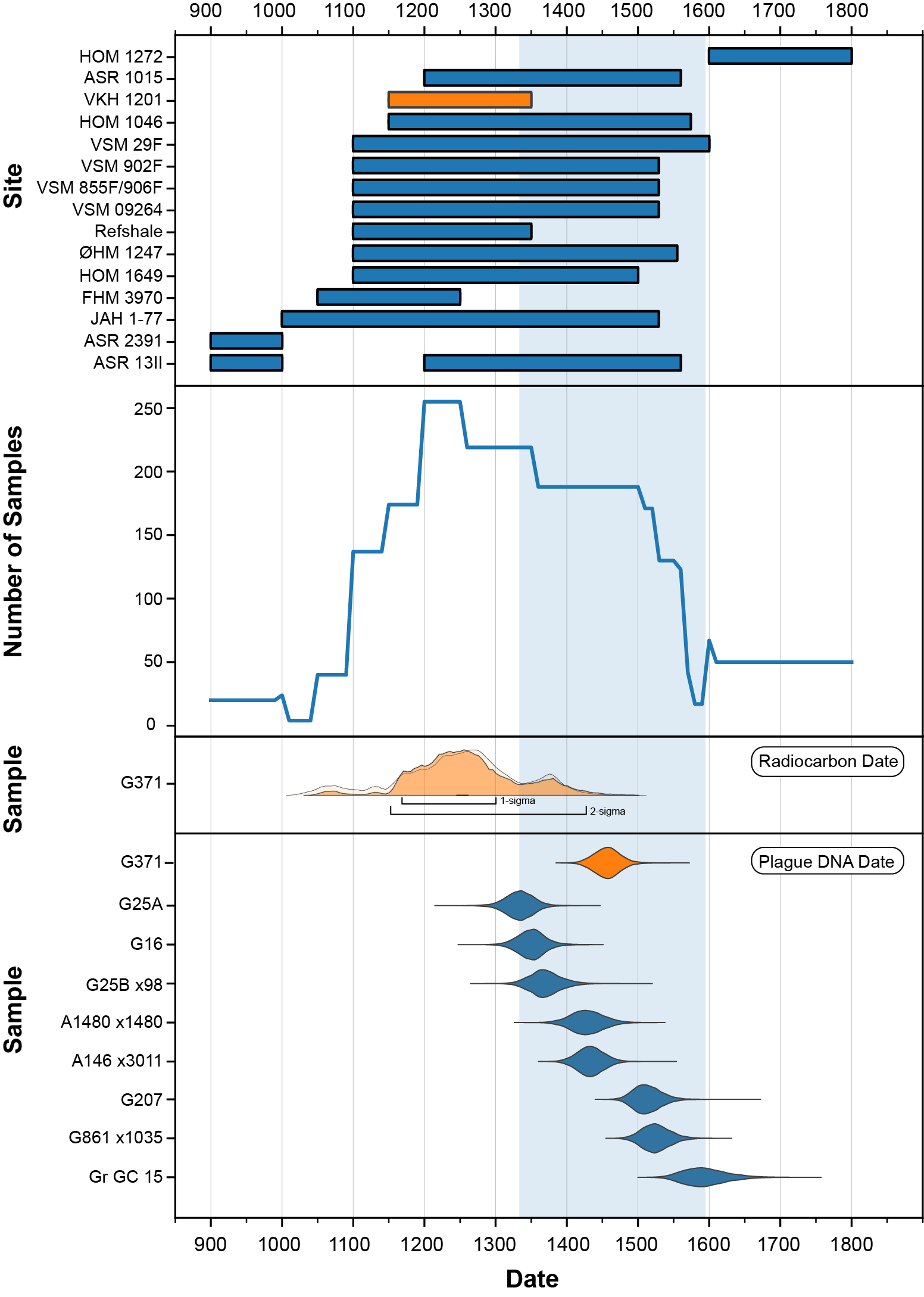


Figure 3: Timeline of archaeological sites and plague-positive individuals. The blue shaded range spans the highest probability period from the oldest to the youngest sample. The color orange indicates the sample and site with disparate dates.

### Phylodynamics

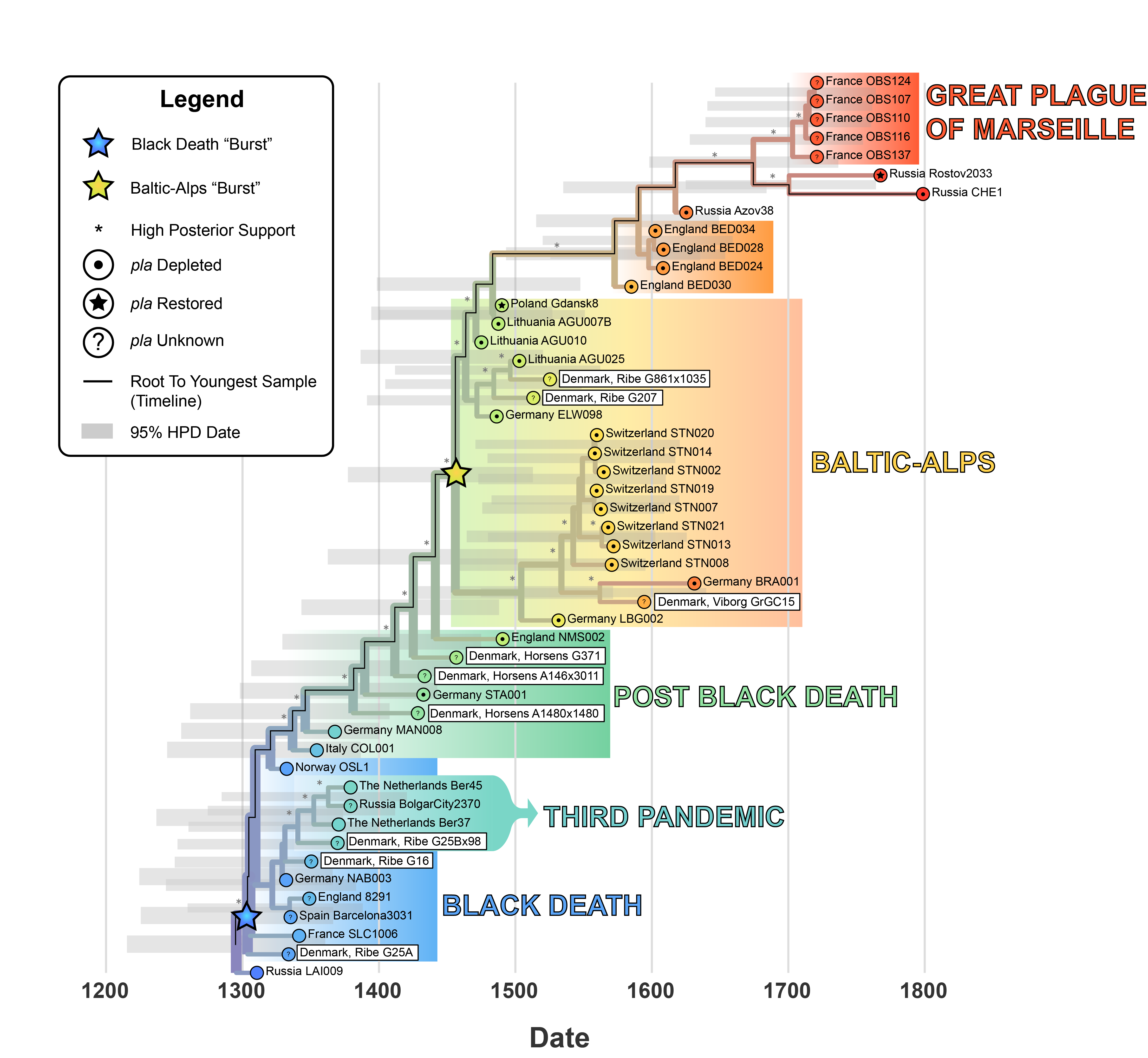


Figure 4: A time-scaled phylogeny of the Second Plague Pandemic.

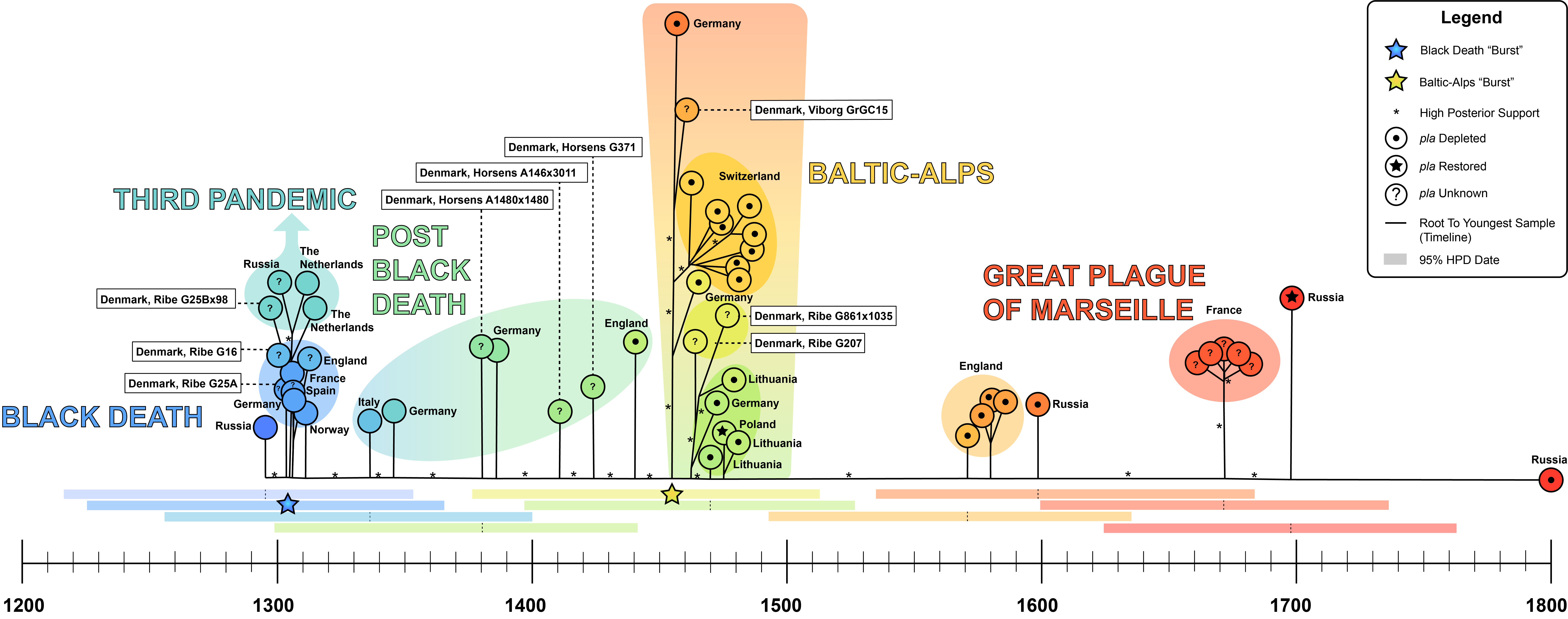


Figure 5: A timeline phylogeny of the Second Plague Pandemic.

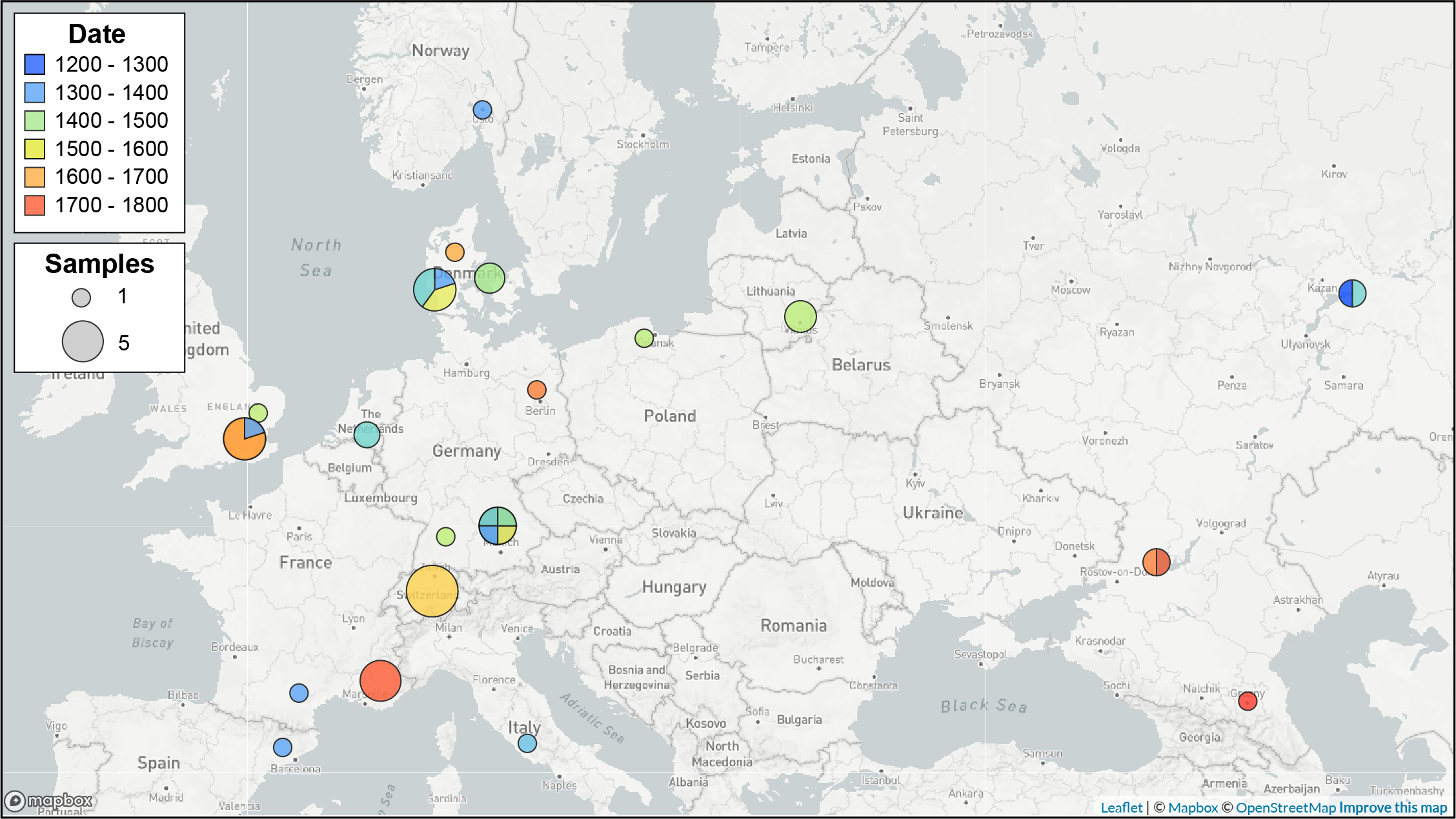


Figure 6: Geographic distribution of Second Pandemic samples used in the *Yersinia pestis* phylogeny.

#### The Black Death

Two individuals from Ribe, G25A and G16, cluster with *Y. pestis* strains dated to the 14th century. This cluster is thought to be associated with the Black Death (1346-1353), as the genomes are nearly identical and have been isolated from all across Europe, including France, Spain, England, Germany, and Norway. These genomes mark the first observation of *Y. pestis* in Denmark and is congruent with historical records that document the arrival of the Black Death in Ribe in 1349 [[2](#ref-1G9pdnarW)]. However, the genome associated with G25A has the lowest sequencing depth, with a mean chromosomal depth of 3.8 molecules per nucleotide.

**Kat’s Note**: - G25A has the lowest sequencing depth, with a mean chromosomal depth of 3.8 molecules per nucleotide. This might mean that diagnostic positions for the post-Black Death clade are missing as “false” negatives. This would make it seem earlier/less-derived if those mutations are ‘missing’. I will investigate!

Similar to the rest of Western Europe, *Y. pestis* appears suddenly in Denmark in the 14th century. No evidence of *Y. pestis* was found in Denmark during the preceding centuries, suggesting plague was a relatively new disease for medieval Danish populations. If true, this would imply an immunologically naive population and may have important implications for the study of health and immunity in both past and present Denmark.

Unfortunately, the high degree of genetic similarity means that the branching patterns, and thus dispersal of *Y. pestis*, cannot be resolved during this period. Our understanding of the Black Death clade is that of a “Burst” radiation, with limited genetic diversity spreading rapidly over a vast geographic area. This pattern is typical of epidemic spread, and has also been observed at the advent of the Third Plague Pandemic.

#### Ancestors of the Third Pandemic

Interestingly, the *Y. pestis* genome associated with individual G25Bx98 (who was found in the same grave as G25A?) is genetically distinct from the earlier strains from Ribe. This isolate falls within a clade of high epidemiological significance, which is the ancestral group giving rise to the Third Pandemic of plague and the rest of Branch 1.

The phylogenetic position of samples within this clade has been hypothesized to reflect a “backward” migration of plague from Northern Europe into Asia. The *Y. pestis* genome retrieved from G25Bx98 tentatively supports this hypothesis, as it falls basal to the more derived strains from The Netherlands and Russia. However, directionality cannot be robustly inferred from four samples alone, particularly given the strong Western European sampling bias of Second Pandemic samples. It will be an important avenue of future research to further develop the relationship between Northern European plague and the only Second Pandemic lineage that is known to persist until the present.

#### Post Black Death

In contrast to the genetic homogeneity observed across Europe during the Black Death period, isolates of plague in the post-Black Death period are easily distinguished. Three genomes collected from three different sites near Horsens derive from independent emergences, despite having temporal overlap. This has also been observed in Germany during this period, as samples collected in relatively close proximity are genetically distinct. A product of this increased genetic diversity means that branching patterns are well resolved in the post-Black Death period. Unfortunately, the geographic origins and dispersal of plague are still challenging to reconstruct, as the number of genomes (N=7) is sparsely sampled relative to the minimum number of countries (N=4) that are implicated.

The pattern of independent emergence is the defining dynamic of plague during this period as all *Y. pestis* collected after the Black Death, but prior to the Early Modern Period, are unique lineages. The transition captured here, from little genetic diversity spread across a continent to significant diversity accumulating within a country, may indicate “boom-bust” dynamics [[3](#ref-11wSWLa1D)].

The epidemiological interpretation of this transition requires more thought.

Another defining characteristic of the post-Black Death period is depletion of a key virulence factor, the *plasminogen activator* (*pla*). Figure [7](#fig:pla_ratio) compares the sequencing depth of the *pla* gene to its corresponding plasmid pPCP1 across samples from the Second Pandemic. Two linear trends are observed separating samples with ‘normal’ gene-to-plasmid ratios from samples that have a ‘depleted’ gene-to-plasmid ratio. This event was previously observed [Susat 2020 Yersinia Pestis Strains|[[4](#ref-OLBXS56Z)]]], and found in samples from as early as the 15th century.

The three Danish genomes from Horsens have temporal overlap with the 15th century and may potentially capture the transition from to a *pla*-depleted state. Unfortunately, the sequencing depth of the pPCP1 plasmid in these samples is insufficient for statistical analysis. The results of a targeted enrichment for the pPCP1 plasmid will be the subject of a forthcoming publication.

Ravneet has completed this experiment and has exciting results, stay tuned!

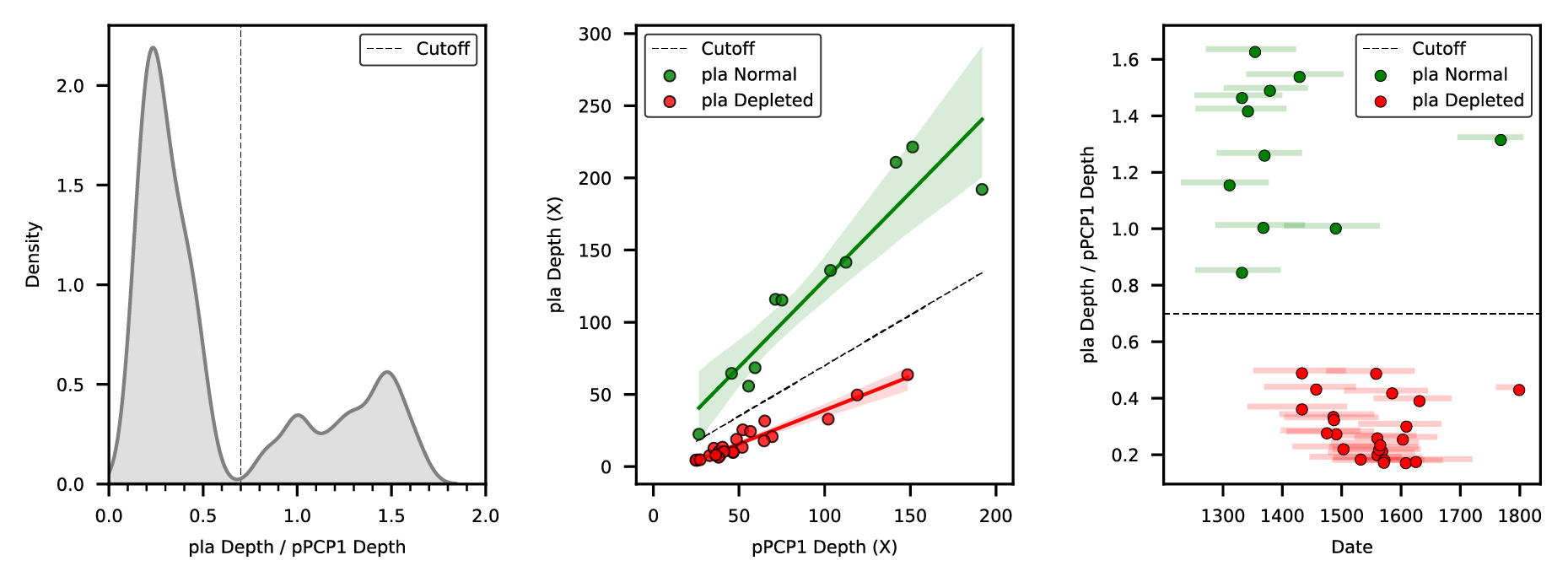


Figure 7: Depletion of the *pla* virulence factor. Left: The observed multimodal distribution of *pla*/pPCP1 ratios across the Second Pandemic. Middle: Linear regressions of *pla* depth on pPCP1 depth. Right: *pla* depletion events over time.

#### Early Modern Plague

Following the Medieval Period, the evolutionary dynamic of *Y. pestis* changes yet again and a second “Burst” event occurs. Similar to the Black Death “Burst”, this event begins with a series of divergences in quick succession, producing highly similar genomes in contemporaneous individuals from Germany, Lithuania, and Poland. Following these divergences, two major lineages emerge.

The first lineage, termed the Baltic-Alps, has been previously observed in the Baltic countries (Lithuania, Poland, Germany) and the Alps (Switzerland). Three Danish genomes fall within the Baltic-Alps clade, including two samples from coastal Ribe and one sample from inland Viborg. The addition of these samples strengthens the geographic ties of this lineage to the Baltic Region. The epidemiological significance of this localization has been speculated to indicate the formation of a novel plague reservoir within Europe.

The second lineage that emerges in this period gives rise to later epidemics of plague in Russia, England, and France, including the Great Plague of Marseille (1720-1721). No Danish plague in this study is associated with this lineage. As sampling efforts intensify, it will be interesting to see if these two lineages remain geographically distinct, and to investigate what might be obstructing the flow of plague between them.

## Conclusion

To Be Done post co-author discussion of results.

## Methods

### DNA Extraction

Ancient DNA laboratory work was performed in dedicated clean-room facilities at the McMaster Ancient DNA Centre (Hamilton, ON). A single root of each individual’s molar was sectioned into two subsamples (50-100 mg) using a circular Dremel. Demineralisation and digestion were performed as previously described [[5](#ref-CToe6ZKY)] and DNA extraction was conducted using a specialized protocol designed for ancient DNA [[6](#ref-9kFCN7oR)]. Reagent blanks were introduced as negative controls to monitor DNA contamination in subsequent steps.

### *Yersinia pestis* PCR screening

An initial plague-screening PCR was performed in duplicate on 1:10 extract dilutions using a *pla* assay [[7](#ref-ACt53Sow)]. In brief, the PCR primers used in this study target the 3’UTR of the *pla* gene which has reduced sequence similarity in non-*Yersinia* species. The forward primer used is thus far known to be identical to *Yersinia pestis* only. A second round of pla PCR was performed for all extracts that amplified in the initial PCR, using the 1:10 dilution in duplicate and the original concentration in duplicate. In total, 6 PCR replicates were performed for each plague-positive sample.

### Shotgun Sequencing

The extracted DNA of plague-positive individuals was converted into Illumina sequencing libraries using a modified protocol [[8](#ref-uH8TFQKI)] and quantified using an Illumina library qPCR assay. Sample libraries were then pooled at equimolar concentrations while negative controls were sequenced at maximum volume input to maximize detection of contaminant organisms. Paired-end sequencing was performed on an Illumina HiSeq 1500 platform (Farncombe Family Digestive Health Research Institute, Hamilton, ON).

### Targeted Sequencing

In-solution enrichment for the pan-genome of *Yersinia pestis* was performed with a previously designed bait-set [[7](#ref-ACt53Sow)] and using the myBaits v4 protocol. The following modifications were incorporated to improve recovery of degraded and divergent DNA sequences: 5uL library input, 100ng bait concentration, hybridisation at 60°C, 16–24h hybridisation capture, and two rounds of enrichment. The enriched libraries were quantified using an Illumina library quantification qPCR assay and pooled at maximum input volume (13uL) due to low concentration. Following pooling, libraries were size-selected on anagarose gel to retain 150–500bp fragments which corresponds to molecule lengths of approximately 15–365bp without the adapter sequences. Paired-end sequencing was performed on an Illumina HiSeq 1500 platform at the Farncombe Metagenomics Facility (Hamilton, ON).

### Comparative Genomes

Ancient unassembled genomes from the Second Plague Pandemic were identified using NCBImeta [10](#ref-6YubepsW) and downloaded from the SRA database in FASTQ format using the SRA Toolkit [[11](#ref-puYDXtJ9)] (Table [7](#tbl:genome_second_pandemic)).

*Y. pestis* strain CO92 was used as the reference genome for sequence alignment and annotation (Table [8](#tbl:genome_reference)). All assembled genomes belonging to phylogenetic branch 0.ANT3 were downloaded and used as an outgroup to root the maximum likelihood phylogeny (Table [9](#tbl:genome_outgroup)).

### Genomic Alignment

Pre-processing and alignment to the reference genome was performed using the nf-core/eager pipeline, a reproducible workflow for ancient genome reconstruction [[12](#ref-17yD9OrGW)]. A multiple sequence alignment was constructed using the Snippy Core module of the Snippy pipeline [[13](#ref-1DR126iIZ)]. The output alignment was filtered to only include chromosomal variants and to exclude sites that had no more than 30% missing data (ie. no more than 30% of samples having an ambiguous nucleotide) . The value of 30% was selected as the most permissive threshold where the number of shared, parsimony-informative sites (240) was still larger than the number of singleton sites (234) which are observed in a single genome (Figure [8](#fig:aln_missing_data)). Furthermore, a 30% threshold was selected as ambiguous nucleotides made up no more than 10% of the alignment ([9](#fig:aln_ambig_sites)).

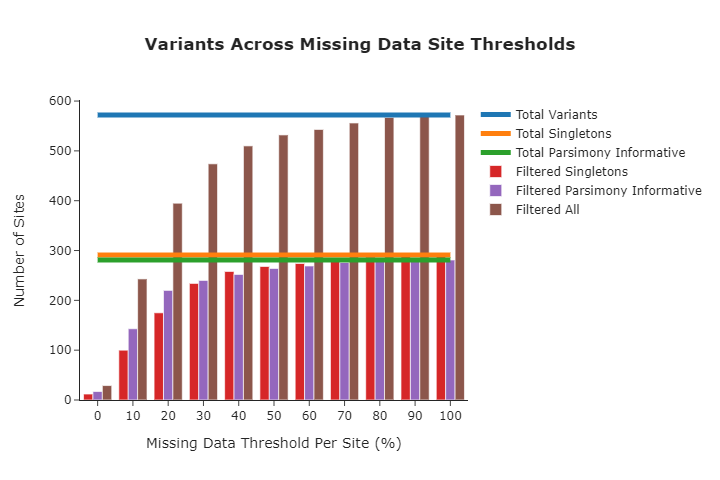


Figure 8: The number of variant positions used in the multiple alignment according to different missing data thresholds.

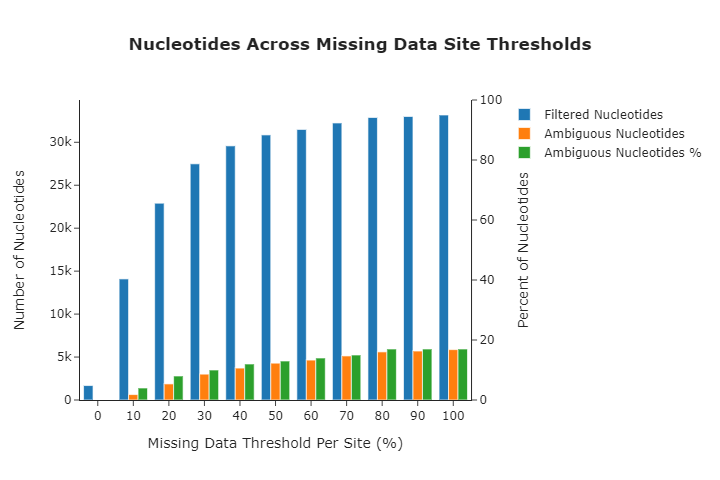


Figure 9: The number of ambiguous nucleotides used in the multiple alignment according to different missing data thresholds.

### Phylogeny

Model selection was performed using Modelfinder which identified the K3Pu+F+I model as the optimal choice based on the Bayesian Information Criterion (BIC) [[14](#ref-QZIPWLUx)]. A maximum-likelihood phylogeny was then estimated across 10 independent runs of IQTREE [[15](#ref-mkkgRhHT)]. Branch support was evaluated using 1000 iterations of the ultrafast bootstrap approximation, with a threshold of 95% required for strong support [[16](#ref-12SvE6y3A)].

### Phylodynamics

To evaluate the degree of temporal signal present, a Bayesian Evaluation of Temporal Signal (BETS) was first performed. Briefly, each candidate model was tested using the correct collection dates of all samples and then compared to the same model with all collection dates assumed to be contemporaneous. Bayes factors (BF) were calculated by comparing the marginal likelihoods of each model, as estimated with a generalized stepping stone (GSS) computation across 100 chains each sampled over 1,000,000 generations.

The BETS analysis revealed decisive support for temporal signal (dates vs. no dates) using both the strict clock (SC) and the uncorrelated lognormal relaxed clock (UCLN) (Table [5](#tbl:bets_summary)). A comparison of the strict vs. relaxed clocks using collection date produced decisive support for the relaxed clock. Therefore, a time-scaled phylogeny with tip-dating was estimated using a relaxed clock and diffuse normal priors centered around the mean collection date.

Table 5: Bayesian Evaluation of Temporal Signal (BETS) summary.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Model | Abbrev. | Dates | Likelihood | Bayes Factor (Dates) | Bayes Factor (Model) |
| Strict Clock | SC | Yes | -5948088 | 749 | – |
|  |  | No | -5948837 | – | – |
| Relaxed Clock | UCLN | Yes | **-5947948** | 715 | 140 |
|  |  | No | -5948663 | – | – |

## References

1. **Leprosy in Medieval Denmark — Osteological and epidemiological analyses**   
Jesper L. Boldsen  
*Anthropologischer Anzeiger* (2009) <http://www.jstor.org/stable/29543069>

2. **The Black Death**   
Kristina Lenz, Nils Hybel  
*Scandinavian Journal of History* (2016) <https://journals.scholarsportal.info/details/03468755/v41i0001/54_tbd.xml>   
DOI: [10.1080/03468755.2015.1110533](https://doi.org/10.1080/03468755.2015.1110533)

3. **Boom-bust dynamics in biological invasions: towards an improved application of the concept**   
David L. Strayer, Carla M. D’Antonio, Franz Essl, Mike S. Fowler, Juergen Geist, Sabine Hilt, Ivan Jarić, Klaus Jöhnk, Clive G. Jones, Xavier Lambin, … Jonathan M. Jeschke  
*Ecology Letters* (2017-10)   
DOI: [10.1111/ele.12822](https://doi.org/10.1111/ele.12822) · PMID: [28834087](https://www.ncbi.nlm.nih.gov/pubmed/28834087)

4. ***Yersinia pestis* strains from Latvia show depletion of the pla virulence gene at the end of the second plague pandemic**   
Julian Susat, Joanna H. Bonczarowska, Elīna Pētersone-Gordina, Alexander Immel, Almut Nebel, Guntis Gerhards, Ben Krause-Kyora  
*Scientific Reports* (2020-09-03) <https://www.nature.com/articles/s41598-020-71530-9>   
DOI: [10.1038/s41598-020-71530-9](https://doi.org/10.1038/s41598-020-71530-9)

5. **New insights from old bones: DNA preservation and degradation in permafrost preserved mammoth remains**   
Carsten Schwarz, Regis Debruyne, Melanie Kuch, Elizabeth McNally, Henry Schwarcz, Andrew D. Aubrey, Jeffrey Bada, Hendrik Poinar  
*Nucleic Acids Research* (2009-06-01) <https://doi.org/10.1093/nar/gkp159>   
DOI: [10.1093/nar/gkp159](https://doi.org/10.1093/nar/gkp159)

6. **Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments**   
J. Dabney, M. Knapp, I. Glocke, M.-T. Gansauge, A. Weihmann, B. Nickel, C. Valdiosera, N. Garcia, S. Paabo, J.-L. Arsuaga, M. Meyer  
*Proceedings of the National Academy of Sciences* (2013-09-24) <http://www.pnas.org/cgi/doi/10.1073/pnas.1314445110>   
DOI: [10.1073/pnas.1314445110](https://doi.org/10.1073/pnas.1314445110)

7. ***Yersinia pestis* and the Plague of Justinian 541–543 AD: a genomic analysis**   
David M Wagner, Jennifer Klunk, Michaela Harbeck, Alison Devault, Nicholas Waglechner, Jason W Sahl, Jacob Enk, Dawn N Birdsell, Melanie Kuch, Candice Lumibao, … Hendrik Poinar  
*The Lancet Infectious Diseases* (2014-04) <https://linkinghub.elsevier.com/retrieve/pii/S1473309913703232>   
DOI: [10.1016/s1473-3099(13)70323-2](https://doi.org/10.1016/S1473-3099(13)70323-2)

8. **Illumina Sequencing Library Preparation for Highly Multiplexed Target Capture and Sequencing**   
M. Meyer, M. Kircher  
*Cold Spring Harbor Protocols* (2010-06-01) <http://www.cshprotocols.org/cgi/doi/10.1101/pdb.prot5448>   
DOI: [10.1101/pdb.prot5448](https://doi.org/10.1101/pdb.prot5448)

9. **Double indexing overcomes inaccuracies in multiplex sequencing on the Illumina platform**   
Martin Kircher, Susanna Sawyer, Matthias Meyer  
*Nucleic Acids Research* (2012-01-01) <https://academic.oup.com/nar/article/40/1/e3/1287690>   
DOI: [10.1093/nar/gkr771](https://doi.org/10.1093/nar/gkr771)

10. **NCBImeta: efficient and comprehensive metadata retrieval from NCBI databases**   
Katherine Eaton  
*Journal of Open Source Software* (2020) <https://doi.org/10.21105/joss.01990>   
DOI: [10.21105/joss.01990](https://doi.org/10.21105/joss.01990)

11. **ncbi/sra-tools**   
NCBI - National Center for Biotechnology Information/NLM/NIH  
(2021-05-18) <https://github.com/ncbi/sra-tools>

12. **Reproducible, portable, and efficient ancient genome reconstruction with nf-core/eager**   
James A. Fellows Yates, Thiseas C. Lamnidis, Maxime Borry, Aida Andrades Valtueña, Zandra Fagernäs, Stephen Clayton, Maxime U. Garcia, Judith Neukamm, Alexander Peltzer  
*PeerJ* (2021-03-16) <https://peerj.com/articles/10947>   
DOI: [10.7717/peerj.10947](https://doi.org/10.7717/peerj.10947)

13. **Snippy: Rapid haploid variant calling and core genome alignment.**   
Torsten Seemann  
(2020-03-08) <https://github.com/tseemann/snippy>

14. **ModelFinder: fast model selection for accurate phylogenetic estimates**   
Subha Kalyaanamoorthy, Bui Quang Minh, Thomas K. F. Wong, Arndt von Haeseler, Lars S. Jermiin  
*Nature Methods* (2017-06) <http://www.nature.com/articles/nmeth.4285>   
DOI: [10.1038/nmeth.4285](https://doi.org/10.1038/nmeth.4285)

15. **IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era**   
Bui Quang Minh, Heiko A. Schmidt, Olga Chernomor, Dominik Schrempf, Michael D. Woodhams, Arndt von Haeseler, Robert Lanfear  
*Molecular Biology and Evolution* (2020-05-01) <https://academic.oup.com/mbe/article/37/5/1530/5721363>   
DOI: [10.1093/molbev/msaa015](https://doi.org/10.1093/molbev/msaa015)

16. **UFBoot2: Improving the Ultrafast Bootstrap Approximation**   
Diep Thi Hoang, Olga Chernomor, Arndt von Haeseler, Bui Quang Minh, Le Sy Vinh  
*Molecular Biology and Evolution* (2018-02-01) <https://academic.oup.com/mbe/article/35/2/518/4565479>   
DOI: [10.1093/molbev/msx281](https://doi.org/10.1093/molbev/msx281)

## Appendix

### Plague Detection

Table 6: Plague false positive summary.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Arch ID | Project ID | Site Code | PCR | Human (%) | Plague (%) | Chrom | pCD1 | pMT1 | pPCP1 |
| G70 x212 | R44 | ASR 1015 | 3/6 | 1.48 | 0.00 | 0.1 | 0.1 | 0.1 | 0.0 |
| G860 | R39 | ASR 1015 | 5/6 | 0.09 | ? | ? | ? | ? | ? |
| G364 | R43 | ASR 1015 | 4/6 | ? | ? | ? | ? | ? | ? |
| K1167 x1167 | P235 | ASR 13 II | 3/6 | ? | ? | ? | ? | ? | ? |
| A21 x23 | D25 | ØHM 1247 | 4/6 | 0.01 | 0.00 | 0.05 | 0.1 | 0.0 | 0.0 |
| G260 K539 x876 | R27 | VSM 09264 | 3/6 | ? | ? | ? | ? | ? | ? |

### Comparative Genomes

Table 7: Second Pandemic genomes metadata.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Strain | Date | Branch | Country | Accession |
| STN021 | 1485 - 1635 | 1.PRE1 | Switzerland | SAMEA5818830 |
| STN020 | 1485 - 1635 | 1.PRE1 | Switzerland | SAMEA5818829 |
| STN019 | 1485 - 1635 | 1.PRE1 | Switzerland | SAMEA5818828 |
| STN014 | 1485 - 1635 | 1.PRE1 | Switzerland | SAMEA5818826 |
| STN013 | 1485 - 1635 | 1.PRE1 | Switzerland | SAMEA5818825 |
| STN008 | 1485 - 1635 | 1.PRE1 | Switzerland | SAMEA5818822 |
| STN007 | 1485 - 1635 | 1.PRE1 | Switzerland | SAMEA5818821 |
| STN002 | 1485 - 1635 | 1.PRE1 | Switzerland | SAMEA5818818 |
| STA001 | 1420 - 1630 | 1.PRE1 | Germany | SAMEA5818817 |
| NMS002 | 1475 - 1536 | 1.PRE1 | England | SAMEA5818815 |
| NAB003 | 1292 - 1392 | 1.PRE1 | Germany | SAMEA5818811 |
| MAN008 | 1283 - 1390 | 1.PRE1 | Germany | SAMEA5818809 |
| LBG002 | 1455 - 1632 | 1.PRE1 | Germany | SAMEA5818808 |
| LAI009 | 1300 - 1400 | 1.PRE0 | Russia | SAMEA5818806 |
| ELW098 | 1485 - 1627 | 1.PRE1 | Germany | SAMEA5818805 |
| BRA001 | 1618 - 1648 | 1.PRE1 | Germany | SAMEA5818803 |
| BED034 | 1560 - 1635 | 1.PRE1 | England | SAMEA5818801 |
| BED030 | 1560 - 1635 | 1.PRE1 | England | SAMEA5818800 |
| BED028 | 1560 - 1635 | 1.PRE1 | England | SAMEA5818799 |
| BED024 | 1560 - 1635 | 1.PRE1 | England | SAMEA5818798 |
| SLC1006 | 1279 - 1389 | 1.PRE1 | France | SAMEA5054093 |
| OSL1 | 1270 - 1390 | 1.PRE1 | Norway | SAMEA5054092 |
| Ber45 | 1300 - 1400 | 1.PRE2 | The Netherlands | SAMEA5054090 |
| Ber37 | 1300 - 1400 | 1.PRE2 | The Netherlands | SAMEA5054089 |
| BolgarCity2370 | 1362 - 1400 | 1.PRE3 | Russia | SAMEA3937654 |
| Barcelona3031 | 1300 - 1420 | 1.PRE1 | Spain | SAMEA3937653 |
| OBS137 | 1720 - 1720 | 1.PRE1 | France | SAMEA3713715 |
| OBS124 | 1720 - 1720 | 1.PRE1 | France | SAMEA3713714 |
| OBS116 | 1720 - 1720 | 1.PRE1 | France | SAMEA3713713 |
| OBS110 | 1720 - 1720 | 1.PRE1 | France | SAMEA3713712 |
| OBS107 | 1720 - 1720 | 1.PRE1 | France | SAMEA3713711 |
| 8291 | 1348-1350 | 1.PRE1 | England | SAMN00715800 |
| COL001 | 1300 - 1400 | 1.PRE1 | Italy | SAMEA7293136 |
| CHE1 | 1500 - 1800 | 1.PRE1 | Russia | SAMEA7293135 |
| Rostov2033 | 1762 - 1773 | 1.PRE1 | Russia | SAMEA7313236\_38 |
| Azov38 | 1400 - 1700 | 1.PRE1 | Russia | SAMEA7313243\_45 |
| Gdansk8 | 1400 - 1700 | 1.PRE1 | Poland | SAMEA7313246\_49 |
| AGU010 | 1435 - 1477 | 1.PRE1 | Lithuania | SAMEA6651390 |
| AGU025 | 1441 - 1612 | 1.PRE1 | Lithuania | SAMEA6637004 |
| AGU007B | 1463 - 1632 | 1.PRE1 | Lithuania | SAMEA6637002 |

Table 8: Reference genome metadata.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Strain | Date | Branch | Country | Accession |
| CO92 | 1992 | 1.ORI1 | United States of America | SAMEA1705942 |

Table 9: Outgroup genomes metadata.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Strain | Date | Branch | Country | Accession |
| 231 | 1947 | 0.ANT3 | Kyrgyzstan | SAMN02777961 |
| A-1486 | 1966 | 0.ANT3 | Kyrgyzstan | SAMN05149973 |
| 790 | 1961 - 1976 | 0.ANT3 | Kyrgyzstan | SAMN02769799 |
| CMCC38001 | 1979 | 0.ANT3 | China | SAMN02403043 |
| CMCC21106 | 2001 | 0.ANT3 | China | SAMN02403038 |
| A1956001 | 1956 | 0.ANT3 | China | SAMN02403019 |
| 42091 | 1999 | 0.ANT3 | China | SAMN02403004 |
| 42082 | 1995 | 0.ANT3 | China | SAMN02403003 |

### Phylogeny

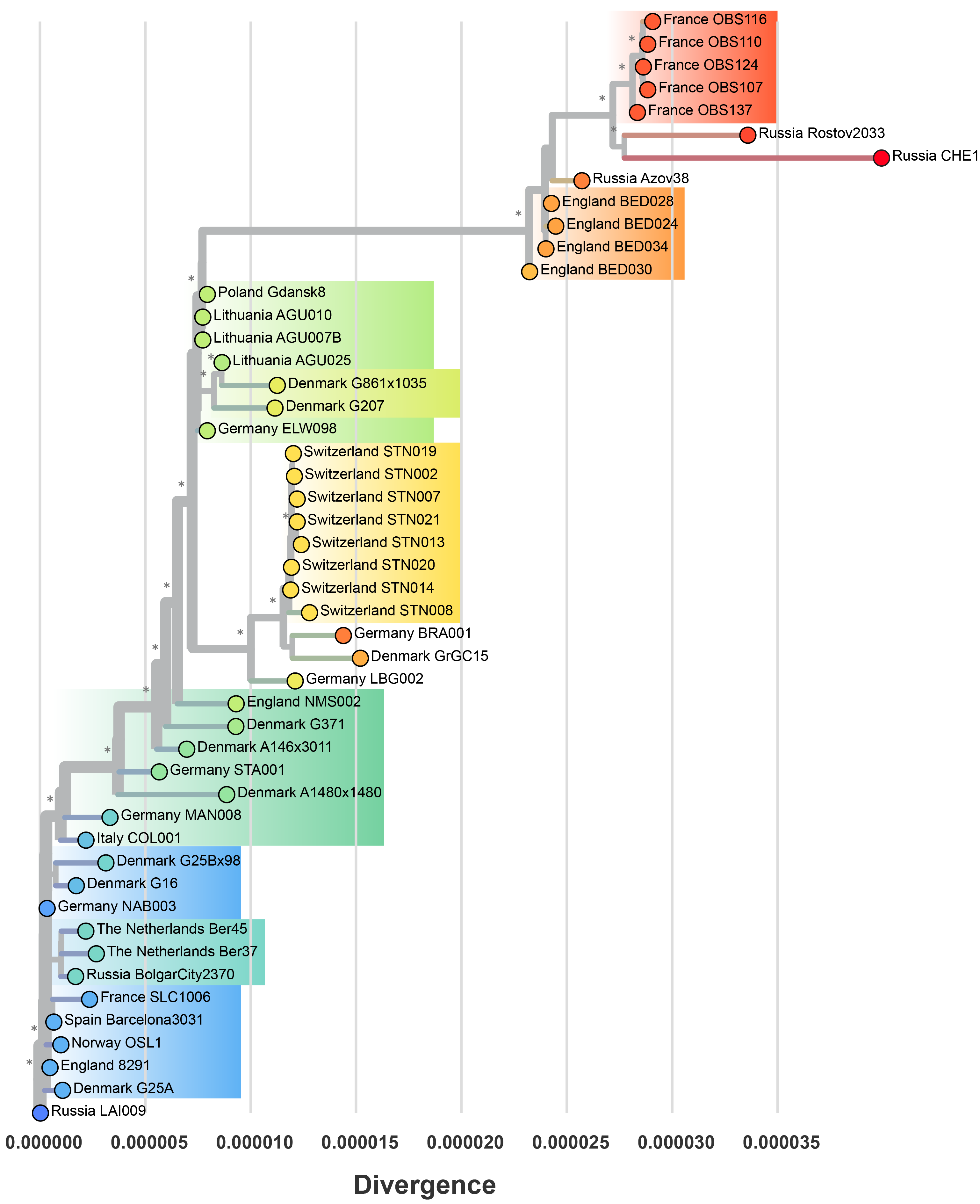


Figure 10: A divergence-scaled phylogeny of the Second Plague Pandemic. Asterisks indicate branches with strong statistical support.