New ancient Eastern European *Yersinia pestis* genomes illuminate the dispersal of plague in Europe

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**Supplementary Note 1: Archaeological background of the samples**

**The cemetery of St. Dmitry Rostovsky fortress (Rostov-on-Don, Russia)**

The cemetery of St. Dmitry Rostovsky fortress was excavated in 1998-2004. The cemetery was divided into several segments, with most burials containing multiple individuals. Two of these collective burials had traces of lime present, indicating disinfection of the corpses was likely performed [1-3]. Burial rites were characterized as Christian and have been dated to the second half of the 18th century. A section of the burials was dated more precisely to 1762-1773 AD (Supplementary Table 1). Documentary sources provided a means to connect at least a few of the burials with plague epidemics in Rostov-on-Don in the winter of 1771 (Elena Batieva, personal communication). Teeth from 39 individuals were analyzed from several sectors of the cemetery (Supplementary Table 1). The samples were provided under the Agreement on Scientific collaboration with Southern Scientific Center, Russian Academy of Sciences.

**Azov city (Rostov-on-Don region, Russia)**

Skeletal remains were discovered in 2005-2012 during a rescue excavation in the historical city of Azov located southwest of Rostov-on-Don. Burial artifacts and stratigraphy date the remains to the 15th-18th centuries, after the fall of the Golden Horde (Elena Batieva, personal communication). Four samples were analyzed during this study (Supplementary Table 1). The samples were provided under the Agreement on Scientific collaboration with Southern Scientific Center, Russian Academy of Sciences.
**Gdańsk ossuaries (Gdańsk, Poland)**

The three ossuaries were discovered during archaeological excavations in the Dominican Square in 2009–2011 [4]. Based on the stratigraphy and burial artifacts, the ossuaries were dated to the 15th-18th centuries [5]. In general, the skeletal material in the ossuaries was intermingled and bones were disarticulated. In one of these ossuaries, Ossuarium 3009, significant amounts of lime were found, suggesting a connection to plague epidemics. Teeth from 35 individuals from three ossuaries were analyzed. One pla-positive sample was discovered during the preliminary screening [6]. Additionally, three skull fragments (about one square cm each) from a rat found in the Ossuarium 3009 (15th-16th century) were also sampled for DNA analyses (Supplementary Table 1). For the extraction step, samples from these fragments were combined and extracted together and is referred to the rat sample in this study. All samples from Gdańsk were provided under the Agreement on Scientific collaboration with the Archaeological Museum in Gdańsk.

For the pla-positive human sample, $^{14}$C dating analysis was performed at the Laboratory of Ion Beam Physics, ETH Zurich (lab number ETH-101916). The sample was dated to 1425-1469 AD ($^{14}$C age BP (1950) 443), which corroborates the archaeological data.

**Supplementary Note 2: Methods**

**DNA extraction**

DNA was extracted at the Paleogenetics laboratory at the Institute of Evolutionary Medicine, University of Zurich, Switzerland. Both the laboratory equipment and experimental protocols conform to all requirements for ancient DNA studies [7, 8]. Ancient DNA work was performed with all the precautionary measures needed to prevent contamination: regular UV-treatment and chemical sterilization of all working surfaces and reusable instruments; use of sterile disposable clothes, sterile filtered tips etc.; negative controls were included at each stage of the experiment and carried through.

DNA was extracted using the protocol described by Rogaev and colleagues [9], with minor modifications. Briefly, 0.2–1.0 g of the sample was decontaminated (mechanical removing of the surface using handheld drill (Proxxon), UV irradiation for 15 min), powdered (Cryogenic
SpexMill), decalcified in lysis buffer (0.5 M EDTA, 10% proteinase K), and incubated with rotation for 48 hours at 37°C. The bone powder was then precipitated by centrifugation for 5 min at maximum speed, the supernatant was concentrated using Amicon centrifugal units (30 kD, Millipore) to the final volume 100–150 μl. DNA was then extracted from the filtrate using silica spin columns (Qiagen MinElute PCR Purification Kit) according to the manufacturer’s protocol. The final volume of the extract was 60 μl. DNA quantity was assessed using Qubit fluorometer (Thermo Fisher Scientific). DNA extracts were stored at –20°C.

**Primary screening**

Initial screening for the presence of *Y. pestis* DNA was performed using primers specific to the plasminogen activator (*pla*) gene located on the high-copy pPCP1 plasmid of *Y. pestis* as described elsewhere (52 bp fragments, [10]). In addition, the primers for longer *pla*-fragments (133 bp fragments, [11]) and subsequent Sanger sequencing was done, in order to exclude false-positive results. As a control for the presence of bacterial DNA of the same length in the extracts, a parallel PCR was performed using universal primers for V6 region of bacterial 16S rRNA [12]. *Pla*-positive samples were built into NGS libraries [13, 14]. Due to a small amount of skeletal material for the rat, the screening stage for the rat sample was skipped, and the rat DNA was directly transformed into NGS libraries following the same methods [13, 14].

**NGS Library preparation and shotgun sequencing**

Double-stranded indexed Illumina libraries were constructed according to the protocols [13, 14] specifically developed for ancient DNA. Index combinations containing unique 8 bp barcodes were used for double indexing. Ten PCR cycles were used for the indexing step. Indexed libraries were quantified using Agilent 2200 TapeStation System, and equimolar quantities of every library were pooled together and sequenced on Illumina HighSeq 4000 with 2*75+8+8 cycles. The sequencing was performed at the Functional Genomics Center Zurich, Switzerland.

**Target enrichment**

The five samples showing positive *Y. pestis* signals in shotgun sequencing (Rostov16039, Rostov2033, Rostov2039, Azov38, and Gdansk8) and the rat sample were subjected to target enrichment [15, 16]. The libraries prepared for shotgun sequencing were used for enrichment.
SeqCap EZ Prime Developer Probes (Roche) were used for in-solution capture. Full *Y. pestis* chromosome (NC_003143.1) and three plasmids, pCD1 (NC_003131.1), pMT1 (NC_003134.1), and pPCP1 (NC_003132.1) were used to design the probes. Target enrichment was performed according to the manufacturer’s protocol. Briefly, amplified indexed libraries were mixed in equimolar amounts to a final concentration of 1.5 µg of DNA per capture reaction and hybridized with DNA capture probes using the following regimen: 95°C for 5 min, 47°C for 20 hrs in a thermocycler with heated lid (57°C). After this, the captured DNA samples were washed using HyperCap Beads (Roche). The whole bead-bound DNA samples (about 20 µl) were used for subsequent PCR amplification with the same pair of primers which was used for amplification of indexed DNA libraries prior to enrichment. 14 cycles of amplification were performed. Enriched libraries were quantified using Agilent 2200 TapeStation System and sequenced on Illumina NextSeq500 with 2*75+8+8 cycles (Functional Genomics Center Zurich).

**Data analysis**

**Read processing, mapping, and variant calling**

First, all libraries belonging to the same individual were merged. Then, all samples were processed using EAGER version 1.92.55 [17]. To summarize, the sequencing quality was inspected with FastQC version 0.11.5 [18], the reads were adapter trimmed and read pairs merged with AdapterRemoval version 2.2.1a [19] and subsequently aligned to the *Y. pestis* CO92 chromosome (NC_003143.1) using CircularMapper version 1.0 [17] with a minimum quality score of 37 and a maximum edit distance of n=0.01. Duplicates were removed with MarkDuplicates version 2.15.0 (Picard Tools - By Broad Institute, n.d.), and DamageProfiler version 0.3.12 [20] was used to investigate the damage patterns.

Before variant calling, one base at the 5’ and 3’ end, respectively, was trimmed by one base pair using FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) to remove sites that could have been affected by ancient DNA damage. Subsequently, the reads were re-filtered for length and remapped using the parameters described above. The Genome Analysis Toolkit (GATK) version 3.8.0 [21, 22] was used to generate a mapping assembly and SNP calling. The reference base was called if the position was covered by a read at least three times and the quality score was at least 30. The base was called as a SNP if the quality score was at least 30 and 90% of the mapped reads contained this variant.
In addition, all merged libraries from humans were mapped to the human mitochondrial genome (NC_012920.1) as described above with the exception of variant calling.

To assign the rat to the correct species, this sample was independently mapped against different reference mitochondrial genomes of the genus Rattus, namely Rattus fuscipes (NC_014867.1), Rattus leucopos (NC_014855.1), Rattus norvegicus (NC_001665.2), and Rattus rattus (NC_012374.1) using the parameters described when mapping against Y. pestis, with the exception of the variant calling. In addition, the sample was mapped against Mus musculus (NC_005089.1). Furthermore, these data were mapped to the complete nuclear genome of R. rattus and R. norvegicus, which are the only complete nuclear genomes available for this genus. For this purpose, BWA aln [23], instead of CircularMapper [17], was used for mapping.

**Constructing SNP alignment**

The four newly reconstructed and 257 previously published ancient and modern Y. pestis genomes ([2, 15, 24-35], Supplementary Table 2) were used for phylogenetic reconstruction. We only included samples that fulfilled quality criteria of at least 3fold coverage at each called site and at least 60% of the reference genome covered. Also, the strains SCL1006, NAB005, STN011, STN004, NAB005, and BRA003 were excluded due to possible environmental contamination [34]. In addition, we excluded strain TRP002 as it is likely contaminated [27]. All published strains were treated with the EAGER pipeline [16] as described above. For genomes where only the fasta sequence was available, sequencing reads were simulated using Genome2Reads (https://github.com/shendurelab/HybridYeastHiC).

**Metagenomic screening**

To detect the presence of Y. pestis in studied samples and determine the Yersinia species that is most likely present in the rat sample, we performed a comparative mapping with MALT [36] using all complete bacterial, viral, and archaeal genomes in GenBank [37] as a reference (version May 2018). MALT was executed with the following mapping parameters: Only reads with a minimum 85% identity (--minPercentIdentity) were considered as a possible match to the reference. Moreover, the minimum support parameter (--minSupport) was set to 5, i.e. only nodes with minimum support of five reads are kept. BlastN mode and SemiGlobal alignment were applied and
a top percent value (=topPercent) of 1 was set. All other parameters were set to default. MALT results were analyzed and visualized using MEGAN6 [38].

The reference database also includes various Yersinia strains, which were used for the identification of the reads mapping to Yersinia from the rat sample (Y. enterocolitica (NC_008800.1), Y. pseudotuberculosis (NC_010634.1), Y. similis (NZ_CP007230.1), Y. ruckeri (NZ_CP011078.1), Y. frederiksenii (NZ_CP009364.1), Y. rohdei (NZ_CP009787.1), Y. aldovae (NZ_CP009781.1), Y. intermedia (NZ_CP009801.1), and Y. massiliensis (NZ_CP028487.1)).

**Phylogenetic tree reconstruction**

Four newly reconstructed and 257 previously published ancient and modern Y. pestis genomes ([2, 15, 24-35], Supplementary Table 2) were used for phylogenetic reconstruction. For creating consensus sequences, bcftools version 1.7 (http://www.htslib.org/doc/bcftools-1.7.html) was used. Indels were excluded from vcf files before creating consensus sequences. The regions with coverage below three were masked during consensus construction. Next, CDS sequences were extracted from consensus sequences using gffread software from GFF Utilities version 0.11.5 (http://ccb.jhu.edu/software/stringtie/gff.shtml). Concatenation of CDS sequences was used in phylogenetic tree reconstruction using RaXML software version 8.2.4 [39] with parameters “-m GTRCAT -x 123456 -N 100 -p 098765” and outgroup Y. pseudotuberculosis IP32953.’ In the analysis, 100 bootstrap iterations were used.

To access the phylogenetic placement of the partial Yersinia pestis strain reconstructed from the rat sample, a maximum likelihood tree was calculated based on a SNP alignment using positions that were covered at least three times. We added one random strain per branch, the rat strain, Y. pseudotuberculosis, and Y. enterocolitica since they two contained the maximum number of mapped reads after Y. pestis (Supplementary Figure 5). The alignment was created as described above. RAxML version 8.2.12 [39] was used with 100 bootstraps and the GTR - GAMMA model.

**BEAST analysis**

We used the Bayesian framework BEAST v1.10.4 [40] to estimate divergence times and substitution rates. All published modern and ancient strains [2, 15, 24-35] were treated with the EAGER pipeline [17] using the parameters described above. In the analysis, we only included the samples representing branch 1 of the Y. pestis phylogeny that fulfilled quality criteria of at least
3fold coverage at each called site and at least 60% of the reference genome covered. The SNP alignment was built with MUSIAL (https://github.com/Integrative-Transcriptomics/MUSIAL) and a SNP was used when it was called in at least one sample. All positions with more than 3% missing data were excluded. The resulting SNP alignment consisted of 620 SNPs for a total of 82 historical and modern strains [15, 25, 27, 28]. No outgroup sequence was included in compliance with BEAST Bayesian dated phylogeny assumptions.

The GTR nucleotide substitution model was used according to ModelGenerator version 851 [41] analysis applying Bayesian Information criterion. A relaxed uncorrelated log-normal clock with CTMC Rate Reference prior and Bayesian skyline tree model were used. The MCMC chain was run for 300 million steps with sampling every 10,000th step. Convergence and mixing were inspected in Tracer v1.7.1 [42] with all ESS exceeding value 100. The Maximum Clade Credibility tree was built using TreeAnnotator (part of BEAST package) and visualized using FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Temporal signal in the dataset was investigated using Date-Randomisation Test (DRT, [43]; prepared using an unpublished R script by Sebastian Duchene) and Bayesian Evaluation of Temporal Signal (BETS, [44]). In DRT, substitution rate estimates for ten replicates of the BEAST analysis with tip dates randomized among the samples do not overlap with the estimate using the original tip dates (Supplementary Figure 7), indicating sufficient temporal signal for calibrated phylogenetic analysis in the dataset. BETS analysis using Bayes Factor based model selection also detected temporal signal supporting the justification for our tip dating analysis with Bayes Factors of $1.97 \times 10^{96}$ and $2.65 \times 10^{96}$ to support the tip dated versus isochronous phylogeny using path sampling and stepping stone sampling, respectively. Root-to-tip regression was projected using TempEst v1.5.3 [45]; however, it did not support the presence of temporal signal in the data resulting in $R^2 = 0.19$ and a Correlation Coefficient of -0.43.

**Genome coverage**

Per base depth was obtained by using samtools depth software. CG count were counted in 100 bp windows for *Y. pestis* CO92 genome. Circular graphs for depth and GC content were created using CIRCOS software [46]. Full *Y. pestis* chromosome (NC_003143.1) and three plasmids, pCD1 (NC_003131.1), pMT1 (NC_003134.1), and pPCP1 (NC_003132.1) were used for the analysis.
Functional analysis

A dataset of 37 ancient *Y. pestis* genomes (five newly reconstructed and 32 previously published ancient strains [15, 25, 27-30], Supplementary Table 2) was functionally annotated using SnpEff [47] version 4.3t.

Supplementary Note 3: Results phylogenetic timescale reconstruction

Despite the likely issues with phylogenetic dating plague [25], we performed a dated phylogeny estimation similar to previous studies (e.g. Spyrou and colleagues [27]) to explore the possible timescale of *Y. pestis* evolution with the newly generated genetic data. Our Bayesian dated phylogeny for Branch 1 (Supplementary Figure 8) revealed the origin of the branch ~740 years ago (95% CI 670 – 940 years ago), i.e. ca.1270 AD, similar to the age estimated by Spyrou and colleagues [27]. Posterior probability varies highly throughout the tree, with very low support values for the nodes comprising Black Death samples. It was previously indicated that the substitution rates vary drastically among different *Y. pestis* lineages [25]. Therefore, the reconstruction of an uncontroversial genealogical tree seems difficult [25]. Even though our analyses suggest sufficient temporal signal in the dataset for the timescale reconstruction (Supplementary Figure 7 and Supplementary Table 6), we thus urge the readers to interpret the time estimates shown in the tree with caution and as due for further confirmation with future research.

The low support values for certain nodes could also be caused by the absence of sufficient data from eastern regions (i.e. Eastern Europe and Asia). Thus, we can expect that the location of some ancient *Y. pestis* samples on the tree may be changed with the addition of new data. These two reasons, as well as the higher quality thresholds for the sequences included in the BEAST analysis which resulted in a much smaller (both lengthwise and in the number of samples included) alignment as compared to the one used for the ML phylogeny, could explain the discrepancies between the ML and BEAST trees (Figure 2 and Supplementary Figure 8).
Supplementary Figure 1. Damage profiles of the studied human samples. Damage profiles for samples Rostov2033, Rostov2039, Rostov1639, Azov38, and Gdansk8. Alignments against Y. pestis are in dashed lines, those against H. sapiens are in solid lines.
Supplementary Figure 2. Genome coverage after enrichment for Y. pestis. The coverage is in red, GC content is in blue. Since the GC content was calculated using a sliding window, it is shown even for uncovered regions. For details, see Supplementary Note 2.
Supplementary Figure 3. Phylogenetic relationships between ancient and modern *Y. pestis* strains. Maximum Likelihood tree was constructed based on four newly reconstructed genomes (Rostov2033, Rostov2039, Azov38, and Gdansk8) and 257 previously published ancient and modern *Y. pestis* genomes ([2, 15, 24-35], Supplementary Table 2). *Y. pseudotuberculosis* genome [48] was used as an outgroup. The newly studied *Y. pestis* genomes (15-18th centuries) are colored in red; the previously published samples dating to the Black Death period (13-14th centuries, [15, 27, 28]) are in brown. Previously published samples dated to the post-Black Death period (15-18th centuries, [24, 27]) are marked in blue.
Supplementary Figure 4. Damage profiles of the rat sample. Alignment to *Y. pestis* is depicted in light-blue, to *R. rattus* is in blue.
Supplementary Figure 5. Mapping the rat sample against different Yersinia species. The number of reads uniquely assigned to the different Yersinia species. MALT [36], with all complete bacterial, viral, and archaeal genomes in GenBank [37] as a reference (version May 2018), was used for comparative mapping. In total, 3,784 reads mapped on genus-level. This also includes measured values that deviated from the species-level because of the same mapping probability.
Supplementary Figure 6. Phylogenetic (ML) location of the rat *Y. pestis* partially reconstructed genome among random ancient and modern *Yersinia* strains. Colors are similar to Supplementary Figure 3: The newly studied *Y. pestis* genomes (15-18th centuries) are in red; previously published samples dating to the Black Death period (13-14th centuries, [15, 27, 28]) in brown; and previously published samples dating to the post-Black Death period (15-18th centuries, [24, 27]) in blue.
Supplementary Figure 7. Results of Date-Randomization Test for the plague BEAST dataset. Estimates for ten replicates with tip dates randomized among the samples (1-10) and for the original data (“data”). The lack of overlap between the original estimate values and the estimates for the replicates indicate that the dataset represents a measurably evolving population, i.e. the temporal signal in the dataset supports the applicability of tip dating analysis.
Supplementary Figure 8. Bayesian dated Maximum Clade Credibility tree for Branch 1 generated using BEAST [40] with tip dating molecular clock calibration. The newly studied Y. pestis genomes (15-18th centuries) are in red and marked by arrows. The previously published samples dating closer to the Black Death period (13-14th centuries) are marked in brown. The previously published samples dated to the post-Black Death period (15-18th centuries) are marked in blue. The modern Y. pestis strains are collapsed to improve the tree visibility. The number of samples inside the collapsed branches are indicated in brackets. For detailed information about the strains included in the analysis, see Supplementary Table 2 and Supplementary Figure 3. Node labels are Bayesian posterior probabilities. Scale shown in years before the present where the present is the date of the youngest sample, i.e. year 2005.
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## Supplementary Table 1. Archaeological information about the studied samples from Eastern Europe

| Sample laboratory ID | Archaeological ID | Archaeological date | Sex | Age | Sample | Abbreviation in the text |
|----------------------|-------------------|---------------------|-----|-----|--------|--------------------------|
| 1128                 | MG-98.sit 1       | 18th century        | M?  | 25-30| Tooth  |                          |
| 1129                 | MG-98.gr.1        | 18th century        | M   | 25-30| Tooth  |                          |
| 1130                 | MG-98.gr.1 middle | 18th century        | F   | 30-35| Tooth  |                          |
| 1131                 | MG-98.gr.1 northern | 18th century    | M?  | 25-30| Tooth  |                          |
| 1132                 | MG-98.gr.4        | 18th century        | M   | 25-30| Tooth  |                          |
| 1133                 | MG-98.gr.5        | 18th century        | M?  | 30-35| Tooth  |                          |
| 1134                 | MG-98.gr.6        | 18th century        | M   | 25-30| Tooth  |                          |
| 1136                 | MG-98.gr.8        | 18th century        | F   | 20-25| Tooth  |                          |
| 1137                 | MG-98.gr.8        | 18th century        | M?  | >45  | Tooth  |                          |
| 1138                 | MG-98.gr.10 upper northern | 18th century | F   | 20-25| Tooth  |                          |
| 1139                 | MG-98.gr.10 lower central | 18th century | M   | 30-35| Tooth  |                          |
| 1140                 | MG-98.gr.10 lower | 18th century        | F   | 18-20| Tooth  |                          |
| 1141                 | MG-98.10 upper southern | 18th century | F   | 20-25| Tooth  |                          |
| 1142                 | MG-98.gr.12       | 18th century        | F?  | 30-35| Tooth  |                          |
| 1145                 | MG-98.           | 18th century        | F   | 25-30| Tooth  |                          |
| 1354                 | MG-2000.gr.1,№7  | 18th century        | M   | 20-25| Tooth  |                          |
| 1625                 | MG -01.gr.5 upper southern (1st tier) | 18th century | M   | 30-35| Tooth  |                          |
| 1627                 | MG -01.gr.5 lower tier (with cross) | 18th century | F   | 35-40| Tooth  |                          |
| 1628                 | MG -01.gr.5 lower tier | 18th century | M   | 25-30| Tooth  |                          |
| 1629                 | MG -01.gr.5 lower tier southward coffin | 18th century | F   | 30-35| Tooth  |                          |
| 1631                 | MG -01.gr.6 northern middle southern | 18th century | F   | 25-30| Tooth  |                          |
| 1632                 | MG -01.gr.6 above the coffin | 18th century | M   | 25-30| Tooth  |                          |
| 1633                 | MG -01.gr.6 above the coffin | 18th century | M   | 35-40| Tooth  |                          |
| 1635                 | MG -01.gr.9 northern | 18th century | F   | 35-40| Tooth  |                          |
| 1636                 | MG -01.gr.13 upper southern | 18th century | M   | 35-40| Tooth  |                          |
| 1639                 | MG -01.gr.16 upper southern | 18th century | M   | 30-35| Tooth  |                          |
| 1640                 | MG -01.gr.16 lower southern | 18th century | F   | 25-30| Tooth  |                          |
### Supplementary Table 1 (continued)

| Identifier | Location/Region | Age | Sex | Age Range | Description | Find Place |
|------------|----------------|-----|-----|-----------|-------------|------------|
| **Azov, Russia** | | | | | | |
| N37 | Azov-2005 Lermontova N37 gr. 25 | 15th-17th century | M | >35 | Petrous bone | |
| N14 | Azov-2008 Chapaeva 14 gr.11 | 16th-18th century | M | 35-45 | Tooth | |
| N27 | Azov-2012 Lermontova 27 sect. 1 gr.7 | 15th-17th century | F | >30 | Petrous bone | |
| N38 | Azov-2012 Kalinina 38 gr.5 | 15th-17th century | M | 25-35 | Tooth | Azov38 |

### Gdańsk, Poland

| O5 | 255/05/08 Oss. 3009 w. 3010 k. 272/2014 No.1 | 15th-16th centuries | ? | 20-30 | Tooth | |
| O6 | 255/05/08 Oss. 3009 w. 3010 k. 287/2014 No.2 | 15th-16th centuries | ? | 35-45 | Tooth | |
| O7 | 255/05/08 Oss. 3009 w. 3010 k. 440/2014 No.3 | 15th-16th centuries | M | 35-50 | Tooth | |
| O21 | 255/05/08 Oss. 3009 w. 3010 k. 317/2014 No.5 | 15th-16th centuries | ? | 25-30 | Tooth | |
| O19 | 255/05/08 Oss. 3009 w. 3010 k. 317/2014 No.6 | 15th-16th centuries | F | 15-18 | Tooth | |
| O22 | 255/05/08 Oss. 3009 w. 3010 k. 274/2014 No.7 | 15th-16th centuries | M | 30-40 | Tooth | |
| O24 | 255/05/08 Oss. 3009 w. 3010 k. 344/2014 No.8 | 15th-16th centuries | M | 20-30 | Tooth | |
| O23 | 255/05/08 Oss. 3009 w. 3010 k. 318/2014 No.9 | 15th-16th centuries | ? | 30-40 | Tooth | |
| O9 | 255/05/08 Oss. 3009 w. 3010 k. 271/2014 No.10 | 15th-16th centuries | ? | 30-35 | Tooth | |
| **O8** | **255/05/08 Oss. 3009 w. 3010 k. 243/2014 No.11** | **1425-1469*** | M | **25-30** | Tooth | **Gdansk8** |
| O20 | 255/05/08 Oss. 3009 w. 3010 k. 243/2014 No.12 | 15th-16th centuries | M | 30-35 | Tooth | |
| O18 | 255/05/08 Oss. 3009 w. 3010 k. 349/2014 No.13 | 15th-16th centuries | F | 30-40 | Tooth | |
| Rat  | Oss. 3009 w. 2010 | 15th-16th centuries | NA | NA | Skull | Rat  |
|------|------------------|---------------------|----|----|-------|------|
| O4   | 255/05/08 Oss. 2006 w. 2007 k. 91/2014 No.1 | 18th century | M  | 30-40 | Tooth |
| O11  | 255/05/08 Oss. 2006 w. 2007 k. 91/2014 No.2 | 18th century | M  | 30-40 | Tooth |
| O29  | 255/05/08 Oss. 2006 w. 2007 k. 91/2014 No.3 | 18th century | M  | 30-40 | Tooth |
| O25  | 255/05/08 Oss. 2006 w. 2007 k. 92/2014 No.4 | 18th century | M  | 30-35 | Tooth |
| O3   | 255/05/08 Oss. 2006 w. 2007 k. 92/2014 No.5 | 18th century | M  | 30-40 | Tooth |
| O2   | 255/05/08 Oss. 2006 w. 2007 k. 92/2014 No.6 | 18th century | F  | 18-20 | Tooth |
| O10  | 255/05/08 Oss. 2006 w. 2007 k. 92/2014 No.7 | 18th century | M  | 40-50 | Tooth |
| O28  | 255/05/08 Oss. 2006 w. 2007 k. 92/2014 No.8 | 18th century | F  | 20-30 | Tooth |
| O26  | 255/05/08 Oss. 2006 w. 2007 k. 92/2014 No.9 | 18th century | M  | 30-40 | Tooth |
| O1   | 255/05/08 Oss. 2006 w. 2007 k. 92/2014 No.10 | 18th century | F  | 16-18 | Tooth |
| O31  | 255/05/08 Oss. 2046 w. 2047 k. 246 No.1 | 18th century | ?  | 30-40 | Tooth |
| O30  | 255/05/08 Oss. 2046 w. 2047 k. 246 No.2 | 18th century | ?  | 50+  | Tooth |
| O33  | 255/05/08 Oss. 2046 w. 2047 k. 304/2014 No.3 | 18th century | M? | 45-55 | Tooth |
| O12  | 255/05/08 Oss. 2046 w. 2047 k. 304/2014 No.4 | 18th century | F  | 45-55 | Tooth |
| O27  | 255/05/08 Oss. 2046 w. 2047 k. 246 No.5 | 18th century | M  | 20-30 | Tooth |
| O34  | 255/05/08 Oss. 2046 w. 2047 k. 246 No.6 | 18th century | M? | 45-55 | Tooth |
| O32  | 255/05/08 Oss. 2046 w. 2047 k. 246 No.7 | 18th century | M  | 35-45 | Tooth |
| O13  | 255/05/08 Oss. 2046 w. 2047 k. 246 No.8 | 18th century | M  | 30-35 | Tooth |
| O15  | 255/05/08 Oss. 2046 w. 2047 k. 246 No.9 | 18th century | F  | 16-18 | Tooth |
| O16  | 255/05/08 Oss. 2046 w. 2047 k. 246 No.11 | 18th century | F  | 35-45 | Tooth |
| O17  | 255/05/08 Oss. 2046 w. 2047 k. 246 No.12 | 18th century | M  | 30-40 | Tooth |
| O14  | 255/05/08 Oss. 2046 w. 2047 k. 246 No.14 | 18th century | M? | 30-40 | Tooth |
| O35  | 255/05/08 Oss. 2046 w. 2047 k. 182 No.15 | 18th century | M  | 30-35 | Tooth |

Note. Plague-positive samples are in bold

*The dates are based on $^{14}$C dating. All other dates are based on archaeological information.
### Supplementary Table 2. Published data used in phylogenetic analysis

| Sample          | Used for       | Publication (or NCBI accession)          | Dating    | Isolate       | Geographic location                  |
|-----------------|----------------|------------------------------------------|-----------|---------------|--------------------------------------|
| Azov38          | BEAST / ML tree| This study                               | 1400-1700 | Ancient plague| Azov, Russian Federation             |
| Gdansk8         | BEAST / ML tree| This study                               | 1400-1600 | Ancient plague| Gdańsk, Poland                       |
| Rostov2033      | BEAST / ML tree| This study                               | 1762-1773 | Ancient plague| Rostov-on-Don, Russian Federation    |
| Rostov2039      | BEAST / ML tree| This study                               | 1762-1773 | Ancient plague| Rostov-on-Don, Russian Federation    |
| 1.ANT1_Antiqua  | BEAST / ML tree| NC_008150                                | 1965      | 1.ANT1        | Congo                                |
| 1.ANT1_UG05-0454| BEAST / ML tree| NZ_AAYR01000000                          | 2004      | 1.ANT1        | Uganda                               |
| 1.IN1a_CMCC11001 | BEAST / ML tree| Cui et al., 2013                         | 1954      | 1.IN1a        | Qinghai, China                       |
| 1.IN1b_780441   | BEAST / ML tree| Cui et al., 2013                         | 1978      | 1.IN1b        | Qinghai, China                       |
| 1.IN1c_K21985002| BEAST / ML tree| Cui et al., 2013                         | 1985      | 1.IN1c        | Xinjiang, China                      |
| 1.IN2a_CMCC640047| BEAST / ML tree| Cui et al., 2013                         | 1964      | 1.IN2a        | Qinghai, China                       |
| 1.IN2b_30017    | BEAST / ML tree| Cui et al., 2013                         | 1976      | 1.IN2b        | Tibet, China                         |
| 1.IN2c_CMCC31004| BEAST / ML tree| Cui et al., 2013                         | 1990      | 1.IN2c        | Tibet, China                         |
| 1.IN2d_C1975003 | BEAST / ML tree| Cui et al., 2013                         | 1975      | 1.IN2d        | Qinghai, China                       |
| 1.IN2e_C1989001 | BEAST / ML tree| Cui et al., 2013                         | 1989      | 1.IN2e        | Qinghai, China                       |
| 1.IN2f_710317   | BEAST / ML tree| Cui et al., 2013                         | 1971      | 1.IN2f        | Qinghai, China                       |
| 1.IN2g_CMCC05013| BEAST / ML tree| Cui et al., 2013                         | 1988      | 1.IN2g        | Qinghai, China                       |
| 1.IN2h_5        | BEAST / ML tree| Cui et al., 2013                         | 2004      | 1.IN2h        | Qinghai, China                       |
| 1.IN2i_CMCC11002| BEAST / ML tree| Cui et al., 2013                         | 1964      | 1.IN2i        | Qinghai, China                       |
| 1.IN2j_CMCC27002| BEAST / ML tree| Cui et al., 2013                         | 1991      | 1.IN2j        | Qinghai, China                       |
| 1.IN2k_970754   | BEAST / ML tree| Cui et al., 2013                         | 1997      | 1.IN2k        | Qinghai, China                       |
| 1.IN2l_D1991004 | BEAST / ML tree| Cui et al., 2013                         | 1991      | 1.IN2l        | Qinghai, China                       |
| 1.IN2m_D1964002b| BEAST / ML tree| Cui et al., 2013                         | 1964      | 1.IN2m        | Qinghai, China                       |
| 1.IN2n_CMCC02041| BEAST / ML tree| Cui et al., 2013                         | 1965      | 1.IN2n        | Qinghai, China                       |
| 1.IN2o_CMCC03001| BEAST / ML tree| Cui et al., 2013                         | 1954      | 1.IN2o        | Qinghai, China                       |
| 1.IN2p_D1982001 | BEAST / ML tree| Cui et al., 2013                         | 1982      | 1.IN2p        | Gansu, China                         |
| 1.IN2q_D1964001 | BEAST / ML tree| Cui et al., 2013                         | 1964      | 1.IN2q        | Qinghai, China                       |
| 1.IN3a_F1954001 | BEAST / ML tree| Cui et al., 2013                         | 1954      | 1.IN3a        | Yunnan, China                        |
| 1.IN3b_E1979001 | BEAST / ML tree| Cui et al., 2013                         | 1979      | 1.IN3b        | Yunnan, China                        |
| 1.IN3c_CMCC84038b| BEAST / ML tree| Cui et al., 2013                         | 1982      | 1.IN3c        | Yunnan, China                        |
**Supplementary Table 2 (continued)**

| Accession | Reference | Date | Location |
|-----------|-----------|------|----------|
| 1.IN3d_YN1683 | BEAST / ML tree Cui et al., 2013 | 1977 | Yunnan, China |
| 1.IN3e_YN472 | BEAST / ML tree Cui et al., 2013 | 1957 | Yunnan, China |
| 1.IN3f_YN1065 | BEAST / ML tree Cui et al., 2013 | 1954 | Yunnan, China |
| 1.IN3g_E1977001 | BEAST / ML tree Cui et al., 2013 | 1977 | Yunnan, China |
| 1.IN3h_CMCC84033 | BEAST / ML tree Cui et al., 2013 | 1979 | Yunnan, China |
| 1.IN3i_CMCC84046 | BEAST / ML tree Cui et al., 2013 | 1984 | Yunnan, China |
| 1.ORI1a_CMCC114001 | BEAST / ML tree Cui et al., 2013 | 1952 | Fujian, China |
| 1.ORI1b_India195 | BEAST / ML tree NZ_ACNR00000000 | 1898 | India |
| 1.ORI1c_F1946001 | BEAST / ML tree Cui et al., 2013 | 1946 | Fujian, China |
| 1.ORI1d_CA88 | BEAST / ML tree NZ_ABCD00000000 | 1988 | California, USA |
| 1.ORI1e_CO92 | BEAST / ML tree NC_003143 | 1992 | Colorado, USA |
| 1.ORI2a_YN2179 | BEAST / ML tree Cui et al., 2013 | 1995 | Myanmar |
| 1.ORI2b_CMCC110001 | BEAST / ML tree Cui et al., 2013 | 1991 | Yunnan, China |
| 1.ORI2c_YN2551 | BEAST / ML tree Cui et al., 2013 | 2002 | Yunnan, China |
| 1.ORI2d_YN2588 | BEAST / ML tree Cui et al., 2013 | 2000 | Guangxi, China |
| 1.ORI2e_F1991016 | BEAST / ML tree NZ_ABAT00000000 | 1991 | Yunnan, China |
| 1.ORI2f_CMCC870001 | BEAST / ML tree Cui et al., 2013 | 1982 | Yunnan, China |
| 1.ORI2g_F1984001 | BEAST / ML tree Cui et al., 2013 | 1984 | Yunnan, China |
| 1.ORI2h_YN663 | BEAST / ML tree Cui et al., 2013 | 1982 | Yunnan, China |
| 1.ORI2i_CMCC100001 | BEAST / ML tree Cui et al., 2013 | 1984 | Yunnan, China |
| 1.ORI3a_EV76 | BEAST / ML tree Cui et al., 2013 | 1922 | Madagascar |
| 1.ORI3b_MG05-1020 | BEAST / ML tree NZ_AAYS00000000 | 2005 | Madagascar |
| 1.ORI3c_IP275 | BEAST / ML tree AAOS02000088 | 1995 | Madagascar |
| Barcelona_3031 | BEAST / ML tree Spyrou et al., 2016 | 1300-1420 | Ancient plague Barcelona, Spain |
| BED024 | BEAST / ML tree Spyrou et al., 2019 | 1560-1635 | Ancient plague London, Great Britain |
| BED028 | BEAST / ML tree Spyrou et al., 2019 | 1560-1635 | Ancient plague London, Great Britain |
| BED030 | BEAST / ML tree Spyrou et al., 2019 | 1560-1635 | Ancient plague London, Great Britain |
| BED034 | BEAST / ML tree Spyrou et al., 2019 | 1560-1635 | Ancient plague London, Great Britain |
| Ber37 | BEAST / ML tree Namouchi et al., 2018 | 1358-1360 | Ancient plague Bergen op Zoom, Netherlands |
| Ber45 | BEAST / ML tree Namouchi et al., 2018 | 1358-1360 | Ancient plague Bergen op Zoom, Netherlands |
| Sample ID | Methodology | Authors | Date Range | Description | Location |
|-----------|-------------|---------|------------|-------------|----------|
| Bolgar_2370 | BEAST / ML tree | Spyrou et al., 2016 | 1362-1400 | Ancient plague | Bolgar, Russian Federation |
| BRA001 | BEAST / ML tree | Spyrou et al., 2019 | 1618-1648 | Ancient plague | Brandenburg, Germany |
| Ellwangen | BEAST / ML tree | Spyrou et al., 2016 | 1485-1627 | Ancient plague | Ellwangen, Germany |
| LAI009 | BEAST / ML tree | Spyrou et al., 2019 | 1300-1400 | Ancient plague | Laishevo, Russian Federation |
| LBG002 | BEAST / ML tree | Spyrou et al., 2019 | 1455-1634 | Ancient plague | Landsberg, Germany |
| London_11972_8124_8291 | BEAST / ML tree | Bos et al., 2011 | 1348-1350 | Ancient plague | London, Great Britain |
| MAN008 | BEAST / ML tree | Spyrou et al., 2019 | 1283-1390 | Ancient plague | Manching, Germany |
| NAB003 | BEAST / ML tree | Spyrou et al., 2019 | 1292-1392 | Ancient plague | Nabburg, Germany |
| NMS002.A | BEAST / ML tree | Spyrou et al., 2019 | 1475-1536 | Ancient plague | Cambridge, Great Britain |
| OBS107 | BEAST / ML tree | Bos et al., 2016 | 1722 | Ancient plague | Marseille, France |
| OBS110 | BEAST / ML tree | Bos et al., 2016 | 1722 | Ancient plague | Marseille, France |
| OBS116 | BEAST / ML tree | Bos et al., 2016 | 1722 | Ancient plague | Marseille, France |
| OBS124 | BEAST / ML tree | Bos et al., 2016 | 1722 | Ancient plague | Marseille, France |
| OBS137 | BEAST / ML tree | Bos et al., 2016 | 1722 | Ancient plague | Marseille, France |
| STA001 | BEAST / ML tree | Spyrou et al., 2019 | 1433-1523 | Ancient plague | Strarnberg, Germany |
| STN002 | BEAST / ML tree | Spyrou et al., 2019 | 1485-1635 | Ancient plague | Stans, Switzerland |
| STN007 | BEAST / ML tree | Spyrou et al., 2019 | 1485-1635 | Ancient plague | Stans, Switzerland |
| STN008 | BEAST / ML tree | Spyrou et al., 2019 | 1485-1635 | Ancient plague | Stans, Switzerland |
| STN013 | BEAST / ML tree | Spyrou et al., 2019 | 1485-1635 | Ancient plague | Stans, Switzerland |
| STN014 | BEAST / ML tree | Spyrou et al., 2019 | 1485-1635 | Ancient plague | Stans, Switzerland |
| STN019 | BEAST / ML tree | Spyrou et al., 2019 | 1485-1635 | Ancient plague | Stans, Switzerland |
| STN020 | BEAST / ML tree | Spyrou et al., 2019 | 1485-1635 | Ancient plague | Stans, Switzerland |
| STN021 | BEAST / ML tree | Spyrou et al., 2019 | 1485-1635 | Ancient plague | Stans, Switzerland |
| 0.ANT1a_42013 | ML tree | Cui et al., 2013 | 0.ANT1a | Xinjiang, China |
| 0.ANT1b_CMCC49003 | ML tree | Cui et al., 2013 | 0.ANT1b | Xinjiang, China |
| 0.ANT1c_945 | ML tree | Cui et al., 2013 | 0.ANT1c | Xinjiang, China |
| 0.ANT1d_164 | ML tree | Cui et al., 2013 | 0.ANT1d | Xinjiang, China |
| 0.ANT1e_CMCC8211 | ML tree | Cui et al., 2013 | 0.ANT1e | Xinjiang, China |
| 0.ANT1f_42095 | ML tree | Cui et al., 2013 | 0.ANT1f | Xinjiang, China |
| 0.ANT1g_CMCC42007 | ML tree | Cui et al., 2013 | 0.ANT1g | Xinjiang, China |
**Supplementary Table 2 (continued)**

| Sample ID | Type | Location Details |
|-----------|------|------------------|
| 0.ANT1h_CMCC43032 | ML tree | Cui et al., 2013, Xinjiang, China |
| 0.ANT2a_2330 | ML tree | Cui et al., 2013, Xinjiang, China |
| 0.ANT2a_B42003004 | ML tree | Cui et al., 2013, Xinjiang, China |
| 0.ANT3_231 | ML tree | Eroshenko et al. 2017, Aksai high-mountain focus |
| 0.ANT3_790 | ML tree | Zhgenti et al., 2015, Kyrgyzstan |
| 0.ANT3_A-1486 | ML tree | Eroshenko et al. 2017, Aksai high-mountain focus |
| 0.ANT3a_CMCC38001 | ML tree | Cui et al., 2013, Xinjiang, China |
| 0.ANT3b_A1956001 | ML tree | Cui et al., 2013, Xinjiang, China |
| 0.ANT3c_42082 | ML tree | Cui et al., 2013, Xinjiang, China |
| 0.ANT3d_CMCC21106 | ML tree | Cui et al., 2013, Xinjiang, China |
| 0.ANT3e_42091 | ML tree | Cui et al., 2013, Xinjiang, China |
| 0.ANT5_262 | ML tree | Eroshenko et al. 2017, Upper-Naryn high-mountain focus |
| 0.ANT5_5M | ML tree | Eroshenko et al. 2018, Upper-Naryn high-mountain focus |
| 0.ANT5_A-1691 | ML tree | Eroshenko et al. 2017, Sarydzhaz high-mountain focus |
| 0.ANT5_A-1836 | ML tree | Eroshenko et al. 2017, Sarydzhaz high-mountain focus |
| 0.PE2_1412 | ML tree | Zhgenti et al., 2015, Georgia |
| 0.PE2_1413 | ML tree | Zhgenti et al., 2015, Georgia |
| 0.PE2_14735 | ML tree | Zhgenti et al., 2015, Armenia |
| 0.PE2_1522 | ML tree | Zhgenti et al., 2015, Armenia |
| 0.PE2_1670 | ML tree | Zhgenti et al., 2015, Georgia |
| 0.PE2_3067 | ML tree | Zhgenti et al., 2015, Georgia |
| 0.PE2_3544 | ML tree | Kutyrev et al., 2018, Leninakan mountain, Armenia |
| 0.PE2_3551 | ML tree | Kutyrev et al., 2018, Prisevansky mountain, Armenia |
| 0.PE2_3770 | ML tree | Zhgenti et al., 2015, Georgia |
| 0.PE2_835_BPC | ML tree | Kutyrev et al., 2018, Leninakan mountain, Armenia |
| 0.PE2_8787 | ML tree | Zhgenti et al., 2015, Georgia |
| 0.PE2_741 | ML tree | Kutyrev et al., 2018, Russian Federation |
| 0.PE2_KM874 | ML tree | Kutyrev et al., 2018, Armenia |
| 0.PE2_M-986 | ML tree | Kutyrev et al., 2018, Armenia |
| 0.PE2_SCPM-O-B-6176_C-535 | ML tree | PRJNA269675, Dagestan, Russian Federation |
## Supplementary Table 2 (continued)

| Accession                  | Type      | Location                  | Country         |
|----------------------------|-----------|---------------------------|-----------------|
| 0.PE2 SCPM-O-B-6992_C-700  | ML tree   | PRJNA269675               | Dagestan, Russia|
| 0.PE2 SCPM-O-B-6994_C-739  | ML tree   | PRJNA269675               | Dagestan, Russia|
| 0.PE2 SCPM-O-B-7005_C-824  | ML tree   | PRJNA269675               | Dagestan, Russia|
| 0.PE2 SCPM-O-B-7037_C-370  | ML tree   | PRJNA269675               | Dagestan, Russia|
| 0.PE2 SCPM-O-B-7040_C-678  | ML tree   | PRJNA269675               | Dagestan, Russia|
| 0.PE2 SCPM-O-B-7042_C-712  | ML tree   | PRJNA269675               | Dagestan, Russia|
| 0.PE2 SCPM-O-B-7111_C-746  | ML tree   | PRJNA269675               | Dagestan, Russia|
| 0.PE2a Pestoides_F         | ML tree   | NC_009381.1               | Former Soviet Union |
| 0.PE2b_G8786               | ML tree   | Cui et al., 2013          | Georgia         |
| 0.PE3a_Angola              | ML tree   | NC_010159                 | Africa          |
| 0.PE4a_B1313               | ML tree   | Kutyrev et al., 2018     | Russian Federation |
| 0.PE4a_I-2751-55           | ML tree   | Kutyrev et al., 2018     | Russian Federation |
| 0.PE4a_I-2998              | ML tree   | Kutyrev et al., 2018     | Russian Federation |
| 0.PE4a_12                  | ML tree   | Cui et al., 2013          | Qinghai, China  |
| 0.PE4Ab_9                  | ML tree   | Cui et al., 2013          | Qinghai, China  |
| 0.PE4Ba_PestoidesA         | ML tree   | ACNT01000009.1            | Former Soviet Union |
| 0.PE4Ca_CMCCN010025        | ML tree   | Cui et al., 2013          | Sichuan, China  |
| 0.PE4Cb_M0000002           | ML tree   | Cui et al., 2013          | Qinghai, China  |
| 0.PE4Cc_CMCC18019          | ML tree   | Cui et al., 2013          | Qinghai, China  |
| 0.PE4Cd_CMCC93014          | ML tree   | Cui et al., 2013          | Inner Mongolia, China |
| 0.PE4Ce_CMCC91090          | ML tree   | Cui et al., 2013          | Inner Mongolia, China |
| 0.PE4CF_Microtus91001      | ML tree   | NC_005810                 | Inner Mongolia, China |
| 0.PE4h_A-1249              | ML tree   | Eroshenko et al., 2017   | Sogdyskaya Region, Tajikistan |
| 0.PE4m_I-3086              | ML tree   | Kutyrev et al., 2018     | Bayan-Khongor aimak, Mongolia |
| 0.PE4t_A-1815              | ML tree   | Eroshenko et al., 2017   | Talas high-mountain focus |
| 0.PE5 SCPM-O-B-6212_I-2238 | ML tree   | PRJNA269675               | --              |
| 0.PE5 SCPM-O-B-6301_I-2231 | ML tree   | PRJNA269675               | --              |
| 0.PE5 SCPM-O-DNA-15_I-2236 | ML tree   | PRJNA269675               | --              |
| 0.PE5 SCPM-O-DNA-16_I-2422 | ML tree   | Kislichkina et al., 2015 | --              |
| 0.PE7a_CMCC05009           | ML tree   | Cui et al., 2013          | Qinghai, China  |
| Accession | Type     | Location                          |
|-----------|----------|-----------------------------------|
| 0.PE7b_620024 | ML tree  | Qinghai, China                    |
| 2.ANT1a_34008  | ML tree  | Tibet, China                      |
| 2.ANT1b_34202  | ML tree  | Tibet, China                      |
| 2.ANT1c_Nepal516 | ML tree  | Nepal                             |
| 2.ANT2a_2      | ML tree  | Qinghai, China                    |
| 2.ANT2b_351001 | ML tree  | Tibet, China                      |
| 2.ANT2c_CMCC347001 | ML tree | Tibet, China                      |
| 2.ANT2d_G1996006 | ML tree | Tibet, China                      |
| 2.ANT2e_G1996010 | ML tree | Tibet, China                      |
| 2.ANT2f_CMCC348002 | ML tree | Tibet, China                      |
| 2.ANT3_KM682_11996 | ML tree | Trans-Baikal steppe, Russian Federation |
| 2.ANT3a_CMCC92010 | ML tree  | Inner Mongolia, China             |
| 2.ANT3b_CMCC95001 | ML tree  | Inner Mongolia, China             |
| 2.ANT3c_CMCC96001 | ML tree  | Inner Mongolia, China             |
| 2.ANT3d_CMCC96007 | ML tree  | Inner Mongolia, China             |
| 2.ANT3e_CMCC67001 | ML tree  | Inner Mongolia, China             |
| 2.ANT3f_CMCC104003 | ML tree  | Inner Mongolia, China             |
| 2.ANT3g_CMCC51020 | ML tree  | Jilin, China                      |
| 2.ANT3h_CMCC106002 | ML tree  | Inner Mongolia, China             |
| 2.ANT3i_CMCC64001 | ML tree  | Inner Mongolia, China             |
| 2.ANT3j_H1959004 | ML tree  | Jilin, China                      |
| 2.ANT3k_5761  | ML tree  | St.Petersbg, Russian Federation   |
| 2.ANT3l_735   | ML tree  | St.Petersbg, Russian Federation   |
| 2.MED0_C-627_KM919 | ML tree  | Russian Federation                |
| 2.MED1_1045  | ML tree  | Azerbaijan                        |
| 2.MED1_1116-D | ML tree  | Russian Federation                |
| 2.MED1_1240  | ML tree  | Azerbaijan                        |
| 2.MED1_139   | ML tree  | Taucum desert, Kazakhstan         |
| 2.MED1_173   | ML tree  | Mangyshlaksy desert, Kazakhstan   |
| 2.MED1_1906  | ML tree  | Precaspian sandy, Russian Federation |
### Supplementary Table 2 (continued)

| Accession   | Type    | Authors                  | Location                |
|-------------|---------|--------------------------|-------------------------|
| 2.MED1_244  | ML tree | Kutyrev et al., 2018     | North-Aral desert, Kazakhstan |
| 2.MED1_261  | ML tree | Kutyrev et al., 2018     | Kobystan plain-piedmont, Azerbaijan |
| 2.MED1_2944 | ML tree | Zhgenti et al., 2015     | Russian Federation      |
| 2.MED1_44   | ML tree | Kutyrev et al., 2018     | Azerbaijan              |
| 2.MED1_A-1809 | ML tree | Eroshenko et al., 2017  | Talas high-mountain, Kyrgyzstan |
| 2.MED1_A-1825 | ML tree | Kutyrev et al., 2018     | Kazakhstan, Turkmenistan |
| 2.MED1_A-1920 | ML tree | Kutyrev et al., 2018     | Pribalkhashky desert, Kazakhstan |
| 2.MED1_C-791 | ML tree | Kutyrev et al., 2018     | Russian Federation      |
| 2.MED1_KM816 | ML tree | Kutyrev et al., 2018     | Karakum desert, Turkmenistan |
| 2.MED1_KM918 | ML tree | Kutyrev et al., 2018     | Russian Federation      |
| 2.MED1_M-1448 | ML tree | Kutyrev et al., 2018     | Trans-Ural steppe, Kazakhstan |
| 2.MED1_M-1453 | ML tree | Kutyrev et al., 2018     | Ural-Embensky desert, Kazakhstan |
| 2.MED1_M-1484 | ML tree | Kutyrev et al., 2018     | Kazakhstan              |
| 2.MED1_M-1524 | ML tree | Kutyrev et al., 2018     | Mujunkumsky desert, Kazakhstan |
| 2.MED1_M-1763 | ML tree | Kutyrev et al., 2018     | Aral-Karakum desert, Kazakhstan |
| 2.MED1_M-1773 | ML tree | Kutyrev et al., 2018     | Kazakhstan              |
| 2.MED1_M-1864 | ML tree | Kutyrev et al., 2018     | Precaspian sandy, Russian Federation |
| 2.MED1_M-519  | ML tree | Kutyrev et al., 2018     | Kopetdagsky desert, Turkmenistan |
| 2.MED1_M-549  | ML tree | Kutyrev et al., 2018     | Uzbekistan, Turkmenistan |
| 2.MED1_M-595  | ML tree | Kutyrev et al., 2018     | Precaspian sandy, Russian Federation |
| 2.MED1_M-978  | ML tree | Kutyrev et al., 2018     | Mangyshlaksky desert, Kazakhstan |
| 2.MED1a_KIM  | ML tree | NC_004088                | Iran/Kurdistan          |
| 2.MED1b_2506 | ML tree | Cui et al., 2013         | Xinjiang, China        |
| 2.MED1c_2654 | ML tree | Cui et al., 2013         | Xinjiang, China        |
| 2.MED1d_2504 | ML tree | Cui et al., 2013         | Xinjiang, China        |
| 2.MED2a_I160001 | ML tree | Cui et al., 2013         | Xinjiang, China        |
| 2.MED2b_91   | ML tree | Cui et al., 2013         | Xinjiang, China        |
| 2.MED2c_K11973002 | ML tree | Cui et al., 2013 | Xinjiang, China        |
| 2.MED2d_A1973001 | ML tree | Cui et al., 2013 | Xinjiang, China        |
| 2.MED2e_7338 | ML tree | Cui et al., 2013         | Xinjiang, China        |
| 2.MED3a | ML tree | Cui et al., 2013 | Gansu, China |
| 2.MED3b | ML tree | Cui et al., 2013 | Ningxia, China |
| 2.MED3c | ML tree | Cui et al., 2013 | Ningxia, China |
| 2.MED3d | ML tree | Cui et al., 2013 | Ningxia, China |
| 2.MED3e | ML tree | Cui et al., 2013 | Jilin, China |
| 2.MED3f | ML tree | Cui et al., 2013 | Inner Mongolia, China |
| 2.MED3g | ML tree | Cui et al., 2013 | Inner Mongolia, China |
| 2.MED3h | ML tree | Cui et al., 2013 | Inner Mongolia, China |
| 2.MED3i | ML tree | Cui et al., 2013 | Inner Mongolia, China |
| 2.MED3j | ML tree | Cui et al., 2013 | Shaanxi, China |
| 2.MED3k | ML tree | Cui et al., 2013 | Qinghai, China |
| 2.MED3l | ML tree | Cui et al., 2013 | Hebei, China |
| 2.MED3m | ML tree | Cui et al., 2013 | Shaanxi, China |
| 2.MED3n | ML tree | Cui et al., 2013 | Shaanxi, China |
| 2.MED3o | ML tree | Cui et al., 2013 | Inner Mongolia, China |
| 2.MED3p | ML tree | Cui et al., 2013 | Inner Mongolia, China |
| 3.ANT1a | ML tree | Cui et al., 2013 | Qinghai, China |
| 3.ANT1b | ML tree | Cui et al., 2013 | Gansu, China |
| 3.ANT1c | ML tree | Cui et al., 2013 | Gansu, China |
| 3.ANT1d | ML tree | Cui et al., 2013 | Gansu, China |
| 3.ANT2a | ML tree | Cui et al., 2013 | Dornogovi, Mongolia |
| 3.ANT2b | ML tree | Cui et al., 2013 | Dornogovi, Mongolia |
| 3.ANT2c | ML tree | Cui et al., 2013 | Govi-Altai, Mongolia |
| 3.ANT2d | ML tree | Cui et al., 2013 | Bayan-Ölgii, Mongolia |
| 3.ANT2e | ML tree | Cui et al., 2013 | Govi-Altai, Mongolia |
| 4.ANT | ML tree | Kutyrev et al., 2018 | Russian Federation |
| 4.ANT | ML tree | Kutyrev et al., 2018 | Russian Federation |
| 4.ANT | ML tree | Kutyrev et al., 2018 | Russian Federation |
| 4.ANT | ML tree | Kutyrev et al., 2018 | Tuva mountain, Russian Federation |
| 4.ANT | ML tree | Kutyrev et al., 2018 | Tuva mountain, Russian Federation |
| Sample ID   | Method     | Reference          | Date Range | Event       | Location                           |
|-------------|------------|--------------------|------------|-------------|------------------------------------|
| 4.ANT1a_MGJZ12 | ML tree    | Cui et al., 2013   | 426-571    | Ancient plague | Bayan-Ölgii, Mongolia               |
| Altenerding | ML tree    | Feldman et al., 2016 | 550–700    | Ancient plague | Altenerding, Germany               |
| DIT003.B    | ML tree    | Keller et al., 2019 | 500–650    | Ancient plague | Edix Hill, Great Britain           |
| EDI001.A    | ML tree    | Keller et al., 2019 | 500–650    | Ancient plague | Edix Hill, Great Britain           |
| EDI003.A    | ML tree    | Keller et al., 2019 | 500–650    | Ancient plague | Edix Hill, Great Britain           |
| EDI004.A    | ML tree    | Keller et al., 2019 | 500–650    | Ancient plague | Edix Hill, Great Britain           |
| LSD001.A    | ML tree    | Keller et al., 2019 | 530–1200   | Ancient plague | Le Pressoir, France                |
| LSD019.A    | ML tree    | Keller et al., 2019 | 530–1200   | Ancient plague | Le Pressoir, France                |
| LSD020.A    | ML tree    | Keller et al., 2019 | 530–1200   | Ancient plague | Le Pressoir, France                |
| LSD021.A    | ML tree    | Keller et al., 2019 | 530–1200   | Ancient plague | Le Pressoir, France                |
| LSD023.A    | ML tree    | Keller et al., 2019 | 530–1200   | Ancient plague | Le Pressoir, France                |
| LVC         | ML tree    | Keller et al., 2019 | 400–600    | Ancient plague | Lunel-Viel, France                 |
| OSL1        | ML tree    | Namouchi et al., 2018 | 1349-1350   | Ancient plague | Oslo, Norway                       |
| PET004.A    | ML tree    | Keller et al., 2019 | 530–730    | Ancient plague | Petting, Germany                   |
| RISE505     | ML tree    | Rasmussen et al., 2015 | BC 1746-1626 | Ancient plague | Russian Federation                 |
| RISE509     | ML tree    | Rasmussen et al., 2015 | BC 2887-2677 | Ancient plague | Bateni Afanasievo, Russian Federation |
| RT5         | ML tree    | Spyrou et al., 2018 | ~3800 BP   | Ancient plague | Samara region, Russian Federation  |
| TRP002.A    | ML tree    | Spyrou et al., 2019 | 1347-1350  | Ancient plague | Toulouse, France                   |
| UNT003.A    | ML tree    | Keller et al., 2019 | 525–680    | Ancient plague | Unterthürheim, Germany             |
| UNT004.A    | ML tree    | Keller et al., 2019 | 525–680    | Ancient plague | Unterthürheim, Germany             |
| VAL001.B    | ML tree    | Keller et al., 2019 | 500–700    | Ancient plague | Valencia, Spain                    |
| Y_pseudotuberculosis_IP32953 | ML tree | NC_006155 | Y. pseudotuberculosis IP32953 |  |  |
## Supplementary Table 3. Data on genome coverage in human plague-positive samples after target enrichment

| Sample Name | Nmb of reads after C&M prior mapping | Mapped Reads after RMDup | Endogenous DNA (%) | Mean Coverage | Coverage \(\geq 1X\) (%) | Coverage \(\geq 3X\) (%) | Coverage \(\geq 5X\) (%) | Average fragment length | GC content (%) |
|-------------|-------------------------------------|--------------------------|--------------------|--------------|---------------------|---------------------|---------------------|---------------------|----------------|
| Rostov1639  | 14069919 | 4221 | 1.628 | 0.0637 | 0.99 | 0.38 | 0.28 | 70.32 | 49.45 |
| Rostov2033  | 32386617 | 868003 | 55.631 | 12.6812 | 94.48 | 93.53 | 92.13 | 67.99 | 46.15 |
| Rostov2039  | 13910206 | 226632 | 3.783 | 4.3242 | 88.4 | 60.21 | 33.16 | 88.8 | 48.85 |
| Azov38      | 16023607 | 454551 | 37.354 | 5.1775 | 91.74 | 76.49 | 52.65 | 53.01 | 46.58 |
| Gdansk8     | 343180945 | 7908081 | 77.868 | 184.0869 | 95.99 | 95.87 | 95.81 | 108.33 | 47.21 |
| Rat         | 17626308 | 913 | 0.435 | 0.0144 | 1.24 | 0.03 | 0.01 | 73.35 | 50.64 |

**CO92 chromosome (NC 003143.1)**

| Sample Name | Nmb of reads after C&M prior mapping | Mapped Reads after RMDup | Endogenous DNA (%) | Mean Coverage | Coverage \(\geq 1X\) (%) | Coverage \(\geq 3X\) (%) | Coverage \(\geq 5X\) (%) | Average fragment length | GC content (%) |
|-------------|-------------------------------------|--------------------------|--------------------|--------------|---------------------|---------------------|---------------------|---------------------|----------------|
| Rostov1639  | 14069919 | 27 | 0 | 0.0291 | 1.87 | 0.23 | 0.01 | 75.81 | 51.88 |
| Rostov2033  | 32386617 | 32971 | 14.252 | 34.4148 | 97.94 | 97.12 | 96.45 | 73.38 | 44.47 |
| Rostov2039  | 13910206 | 9157 | 0.475 | 12.8305 | 95.02 | 93.01 | 93.01 | 56.99 | 44.89 |
| Azov38      | 16023607 | 27211 | 10.481 | 224.7357 | 98.68 | 98.68 | 98.68 | 56.99 | 44.89 |
| Gdansk8     | 343180945 | 132181 | 10.481 | 224.7357 | 98.68 | 98.68 | 98.68 | 56.99 | 44.89 |
| Rat         | 17626308 | 121 | 0.001 | 0.1427 | 4.12 | 2.04 | 1.57 | 82.96 | 50.56 |

**pCD1 plasmid (NC 003131.1)**

| Sample Name | Nmb of reads after C&M prior mapping | Mapped Reads after RMDup | Endogenous DNA (%) | Mean Coverage | Coverage \(\geq 1X\) (%) | Coverage \(\geq 3X\) (%) | Coverage \(\geq 5X\) (%) | Average fragment length | GC content (%) |
|-------------|-------------------------------------|--------------------------|--------------------|--------------|---------------------|---------------------|---------------------|---------------------|----------------|
| Rostov1639  | 14069919 | 24 | 0 | 0.0193 | 1.58 | 0.11 | 0 | 77.25 | 49.62 |
| Rostov2033  | 32386617 | 12727 | 18.693 | 9.7797 | 67.35 | 52.77 | 50.63 | 73.93 | 47.4 |
| Rostov2039  | 13910206 | 9665 | 0.684 | 9.4204 | 92.21 | 74.67 | 49.03 | 93.78 | 50.12 |
| Azov38      | 16023607 | 19643 | 13.346 | 11.0281 | 95.16 | 80.98 | 54.01 | 48.84 | 44.89 |
| Gdansk8     | 343180945 | 174766 | 15.285 | 204.0773 | 96.35 | 96.35 | 96.35 | 112.35 | 49.74 |
| Rat         | 17626308 | 70 | 0.001 | 0.0633 | 3.51 | 0.69 | 0.2 | 86.96 | 49.15 |

**pMT1 plasmid (NC 003134.1)**

| Sample Name | Nmb of reads after C&M prior mapping | Mapped Reads after RMDup | Endogenous DNA (%) | Mean Coverage | Coverage \(\geq 1X\) (%) | Coverage \(\geq 3X\) (%) | Coverage \(\geq 5X\) (%) | Average fragment length | GC content (%) |
|-------------|-------------------------------------|--------------------------|--------------------|--------------|---------------------|---------------------|---------------------|---------------------|----------------|
| Rostov1639  | 14069919 | 27 | 0 | 0.2066 | 13.11 | 1.65 | 0.04 | 73.59 | 53.12 |
| Rostov2033  | 32386617 | 16207 | 13.611 | 144.6295 | 100 | 99.99 | 85.77 | 45 |
| Rostov2039  | 13910206 | 4890 | 0.427 | 54.3572 | 98.03 | 85.46 | 73.09 | 106.85 | 50.34 |
| Azov38      | 16023607 | 8249 | 8.058 | 54.4563 | 99.42 | 95.65 | 90.83 | 63.46 | 47.21 |
| Gdansk8     | 343180945 | 18679 | 9.018 | 245.4897 | 100 | 100 | 100 | 126.32 | 45.12 |
| Rat         | 17626308 | 118 | 0.001 | 1.0261 | 27.5 | 14.91 | 11.52 | 83.58 | 50.68 |

**pPCP1 plasmid (NC 003132.1)**

| Sample Name | Nmb of reads after C&M prior mapping | Mapped Reads after RMDup | Endogenous DNA (%) | Mean Coverage | Coverage \(\geq 1X\) (%) | Coverage \(\geq 3X\) (%) | Coverage \(\geq 5X\) (%) | Average fragment length | GC content (%) |
|-------------|-------------------------------------|--------------------------|--------------------|--------------|---------------------|---------------------|---------------------|---------------------|----------------|
| Rostov1639  | 14069919 | 27 | 0 | 0.2066 | 13.11 | 1.65 | 0.04 | 73.59 | 53.12 |
| Rostov2033  | 32386617 | 16207 | 13.611 | 144.6295 | 100 | 99.99 | 85.77 | 45 |
| Rostov2039  | 13910206 | 4890 | 0.427 | 54.3572 | 98.03 | 85.46 | 73.09 | 106.85 | 50.34 |
| Azov38      | 16023607 | 8249 | 8.058 | 54.4563 | 99.42 | 95.65 | 90.83 | 63.46 | 47.21 |
| Gdansk8     | 343180945 | 18679 | 9.018 | 245.4897 | 100 | 100 | 100 | 126.32 | 45.12 |
| Rat         | 17626308 | 118 | 0.001 | 1.0261 | 27.5 | 14.91 | 11.52 | 83.58 | 50.68 |
**Supplementary Table 5.** Mapping the rat sample against different Rattus and Mus species

| Ref genome                                      | Nmb of reads after C&M prior mapping | Mapped Reads after RMDup | Endogenous DNA (%) | Coverage >= 1X (%) | Coverage >= 3X (%) | Coverage >= 5X (%) | Average fragment length | GC content (%) |
|------------------------------------------------|--------------------------------------|--------------------------|--------------------|-------------------|-------------------|-------------------|-------------------------|----------------|
| *Mus musculus* (NC_005089)                     | 17032499                             | 168                      | 0.001              | 16.34             | 8.06              | 4.22              | 52.68                   | 40.29          |
| *Rattus fuscipes* (NC_014867)                   | 17032499                             | 490                      | 0.004              | 42.44             | 24.88             | 15.35             | 55.95                   | 37.84          |
| *Rattus leucopus* (NC_014855)                   | 17032499                             | 473                      | 0.004              | 40.61             | 22.51             | 13.08             | 55.48                   | 37.85          |
| *Rattus norvegicus* (NC_001665.2)               | 17032499                             | 569                      | 0.004              | 44.66             | 28.51             | 19.48             | 55.69                   | 37.55          |
| *Rattus rattus* (NC_012374)                     | 17032499                             | 2419                     | 0.016              | 99.78             | 98.96             | 94.35             | 62.07                   | 38.13          |
| *Rattus norvegicus* complete genome (GCF_000001895.5) | 17032499                         | 480782                   | 2.823              | 1.02              | 0                 | 0                 | 62.07                   | 41.68          |
| *Rattus rattus* complete genome (GCF_011064425.1) | 17032499                          | 681206                   | 3.999              | 1.78              | 0                 | 0                 | 63.86                   | 41.64          |
**Supplementary Table 6.** Temporal signal analysis using BETS (Duchene et al. 2019 preprint*). Temporal signal in the dataset is assessed by performing Bayesian phylogenetic reconstruction using tipdating and with samples constrained to be contemporaneous (isochronous). Strong Bayes Factor support for the tipdated reconstruction indicates sufficient temporal signal for the timescale estimation to be performed reliably.

|                    | log marginal likelihood (using path sampling) from pathLikelihood.delta | Bayes Factor - tipdated vs isochronous | log marginal likelihood (using stepping stone sampling) from pathLikelihood.delta | Bayes Factor - tipdated vs isochronous |
|--------------------|-----------------------------------------------------------------------------|----------------------------------------|--------------------------------------------------------------------------------|----------------------------------------|
| Tipdated           | -4399.65                                                                   | 1.97E+96                               | -439.40                                                                        | 2.65E+96                               |
| isochronous        | -4621.38                                                                   |                                        | -4621.42                                                                        |                                        |

*Duchene S, Lemey P, Stadler T, Ho SYW, Duchene D, Dhanasekaran V, Baele G. 2019. Bayesian Evaluation of Temporal Signal in Measurably Evolving Populations. bioRxiv 810697; doi: https://doi.org/10.1101/810697*