

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our [Editorial Policies](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☐	☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	Illumina HiSeq X10 platform
Data analysis	FastQC v0.11.8 (reads quality assessment) AdapterRemoval v2.3.1 (reads merge and shorter adapter removal) BWA v0.7.12 (reads alignment) EAGER1 v1.92.37 (reads procession pipeline) samtools v1.14 (reads view and extraction, coverage calculation) bedtools v2.25.0 (bam to fastq) HOPS v0.34 (taxonomic assignment) Trimmomatic v0.38 (adapter and quality trimming) Picard MarkDuplicates v2.21.2 (PCR duplicates removal) GATK HaplotypeCaller v3.8 (variant calling) BLASTN v2.13.0+ (variant identification) Tandem Repeats Finder v4.07b (repetitive regions identification) IQ-TREE v1.6.5 (ML tree construction) FigTree v1.4.4 (ML tree visualization) GrapeTree v1.5.0 (schematic generation) OxCal v4.4.4 (14C date calibration) TempEst v1.5.3 (temporal signal assessment)

BEAST v2.6.7 (time-calibrated phylogenetic inference)
 Tracer v1.7.2 (MCMC convergence confirmation)
 TreeAnnotator v2.6.7 (MCC tree generation)
 Prokka v1.14.6 (gene annotation and coding sequence prediction)
 Panaroo v1.2.8 (pangenome inference)
 SPAdes v3.13.0 (de novo assemble)
 Python packages in Python3 v3.10.3:
 pandas v2.3.0 (data handling)
 matplotlib v3.10.3 (data visualization)
 NumPy v2.3.1 (probability density mapping)
 R packages in R v4.2.3:
 maps v3.4.1 (map plotting)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The raw *Y. pestis* FASTQ data generated in this study have been deposited in the Genome Sequence Archive (GSA) at the National Genomics Data Center, China National Center for Bioinformation / Beijing Institute of Genomics, Chinese Academy of Sciences under accession code CRA027461, and are publicly accessible via BioProject PRJCA042579. Accession numbers for all newly sequenced and publicly available *Y. pestis* genomes are provided in Supplementary Data 9. Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	Not applicable
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable
Population characteristics	Not applicable
Recruitment	Not applicable
Ethics oversight	Not applicable

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to predetermine sample size. Sample size was determined by the availability of archaeological material and the quality of DNA preservation.
Data exclusions	All ancient <i>Y. pestis</i> genomes were included in the analysis.
Replication	The sequencing data and analysis software are publicly available, as described in the Methods section, enabling independent reproduction of the analyses. All analyses were performed using established and reproducible bioinformatic workflows.
Randomization	This is not applicable to our study. There were no experimental groups. Samples were selected based on their archaeological context, suitability for ancient DNA analysis, and subsequent detection of <i>Y. pestis</i> DNA.

Blinding

This is not applicable to our study. There were no experimental groups, and blinding was therefore not relevant.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☐	☒ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Palaeontology and Archaeology

Specimen provenance	The specimens reported in this study come from 3 archaeological sites: Uybet-Charkov, Itkol-II, and Zhengjiagou site. The archaeological context are described in the Supplementary note. The ancient samples newly reported in this research were collected with the approval of the sample custodians, who are archaeologists or museums from each of the countries for which we analyzed the data. Each newly reported ancient sample in this study has received analysis permission from its custodians, who are collaborators and confirm that conducting ancient DNA analysis on these samples is appropriate.
Specimen deposition	The specimens reside under the stewardship of the archaeologists and affiliated cultural institutions from which they were sourced. They are available for re-examination upon formal request directed to the respective archaeologists.
Dating methods	Dating work for individuals CY470 and C276 was carried out at the AMS-14C Graphitization Lab, Guangzhou Carbonage Technology Co., Ltd. The C14 dates were calibrated using OxCal v.4.4.4 using the IntCal20 atmospheric curve. This is followed by the median probability calibrated age before the present (cal BP). The radiocarbon (14C) dating for individual B275 has been previously published; therefore, we utilized these established dating results.
☒ Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.	
Ethics oversight	This study relies on archaeological remains previously excavated and incorporates neither new excavation endeavors nor research involving living human or animal subjects. Every newly reported ancient sample in this study has permission for analysis from custodians of the samples who are co-authors and who affirm that ancient DNA analysis of these samples is appropriate.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable