

Reporting Summary

Nature Research 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 Research policies, see [Authors & Referees](#) 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 (n) 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
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- 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. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- 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 d , Pearson's r), 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 Avizo 6.3; Geomagic Studio 2015.1.3; E4 software (<http://www.oldstoneage.com/software/e4.shtml>) and Microsoft Access 2016;

Data analysis BATS 4.0; OxCal V4.3; Wolfram Mathematica 10; bam-rmdup 0.6.3; SAMtools 1.3.1; MAFFT v7.271; MEGA7, jModelTest2, mMass 5.5.0, R 3.4.2 and R 3.6.2, R package ggplot 2 3.2.1, R, package hexbin 1.28.0, RStudio 1.2.5033.

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [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 list of figures that have associated raw data
- A description of any restrictions on data availability

Sequence reads from all libraries and corresponding negative controls are deposited at ENA under the study accession number PRJEB35466.

The FASTA files of the mitochondrial genomes are deposited in GenBank with the accession numbers MN706602-MN706607.

Details are as follows:

BachoKiro_AA7_738	MN706602
BachoKiro_BB7_240	MN706603
BachoKiro_BK_1653	MN706604
BachoKiro_CC7_335	MN706605
BachoKiro_CC7_2289	MN706606

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)

Ecological, evolutionary & environmental sciences study design

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

Study description	Analysis of the palaeontological and archaeological material discovered in the course of a new excavation at the site of Bacho Kiro Cave (Bulgaria).
Research sample	The archaeological material (artifacts and faunal remains) was extracted from two areas (Main Sector and Niche 1) in the Bacho Kiro cave. Sediment samples were collected from each layer to perform micromorphological analyses. Collagen was extracted from archaeological bones from all stratigraphic levels of Bacho Kiro Cave to perform ZooMS and radiocarbon analyses.
Sampling strategy	For ZooMS, a random selection of morphologically unidentifiable bone specimens with a maximum length of over 2 centimeters was conducted. For radiocarbon dating and ancient DNA analyses, all human remains identified by ZooMS were sampled. For the morphological study of the human tooth F6-620, all comparative samples are detailed in the methods section.
Data collection	M.H., T.T., N.S., VA, S.S., R.S. Z.R. and S.P.M. collected field data; S.B., M.S. and J.J.H. collected morphological data on the hominin remains; V.S.M., L.P., F.W. and A.W. collected bone samples to perform ZooMS; M.H extracted mtDNA from hominin bone samples; H.F. and S.T. collected bone samples to perform radiocarbon dating of hominin remains; G.S., R.S., V.P., N.M. collected morphological data on the faunal assemblage using the faunal reference collection stored at the Bulgarian National Museum of Natural History was used to accurately identify species and skeletal element; T.T. and S.P.M. collected metrical data on the lithic assemblage; V.A. collected micromorphological and sedimentological data in the site.
Timing and spatial scale	Bones were excavated from the Niche 1 and Main Sector areas of Bacho Kiro Cave during the 2015/2016/2017 field seasons. Bone pretreatment, and ancient DNA analyses were carried out over the course of 2016-2018.
Data exclusions	For the EDJ analysis, fossil teeth that were highly worn were excluded. For Radiocarbon dating two AMS dating methods across three AMS labs were used to check reproducibility. 11 collagen extracts from different layers were dated with graphite targets on a MICADAS AMS at two labs (ETH-Zurich and MAMS). Results were in statistical agreement for 8 of the extracts. Dates from the two labs were outside 2 sigma for 3 of the oldest extracts (all >40,000 BP). These samples were excluded from further analysis in the companion paper by Fewlass et al. Collagen from two human bones was dated with graphite targets at ETH-Zurich and in replicate with the gas ion source of the Aix-MICADAS AMS at CEREGE. All measurements were in statistical agreement.
Reproducibility	The mitochondrial genome sequences of Bacho Kiro Cave hominin specimens are deposited in GenBank.
Randomization	ZooMS samples were randomly analyzed. For the EDJ study we cannot determine any reason to apply randomization.
Blinding	For the EDJ study, blinding would be inappropriate given the small sample sizes and the relatively simple inferences made from the results of the principal component analysis.
Did the study involve field work?	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

Field work, collection and transport

Field conditions	Yearly excavation inside the Bacho Kiro Cave of about one month between 2015-2018
Location	Bacho Kiro Cave, near Dryanovo (Bulgaria)
Access and import/export	The archaeological material was studied in Bulgaria. Temporary exports of some items were organized between Bulgaria and Germany. Permit delivered by National Museum of Natural History (Sofia) Nr. 4CH30/04.01.19.
Disturbance	The samples were obtained through archaeological excavation of two sections in the cave. At the end of the project (summer 2020) measures will be taken to protect the stratigraphic profiles.

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
- Animals and other organisms
- Human research participants
- Clinical data

Methods

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

Palaeontology

Specimen provenance	Excavation of the Bacho Kiro Cave authorized by the Bulgarian Ministry of the culture, delivered by NAIM-BAS: Nr124/11.05.2015; Nr225/28.04.2016; Nr47/02.05.2017; Nr99/17.04.2018/ Nr120/2019.
Specimen deposition	The palaeontological material will be deposited at the National Museum of Natural History in Sofia and the lithic material at the History Museum of Dryanovo (Bulgaria)
Dating methods	Small aliquots (80-110 mg) of the six ZooMS identified hominin bone fragments were sampled to preserve as much material as possible for further analyses. Collagen was extracted using a technique based on a modified Longin collagen extraction protocol followed by an ultrafiltration step. The gelatinized collagen samples were then passed through an Ezee Filter (Elkay labs, UK) to remove large particles (>80 µm) and separated by molecular weight with pre-cleaned Sartorius VivaSpin Turbo 15 ultrafilters (30 kD MWCO). The samples were freeze dried and the large molecular fraction (>30 kD) was graphitised using the Automated Graphitisation Equipment III and measured using the latest model of the MICADAS AMS in the Laboratory of Ion Beam Physics at ETH-Zurich (lab code: ETH). The dates were calibrated using the IntCal13 dataset in OxCal v4.3.

Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.