Since the initial identification of the Denisovans a decade ago, only a handful of their physical remains have been discovered. Here we analysed ~3,800 non-diagnostic bone fragments using collagen peptide mass fingerprinting to locate new hominin remains from Denisova Cave (Siberia, Russia). We identified five new hominin bones, four of which contained sufficient DNA for mitochondrial analysis. Three carry mitochondrial DNA of the Denisovan type and one was found to carry mtDNA of the Neanderthal type. The former come from the same archaeological layer near the base of the cave’s sequence and are the oldest securely dated evidence of Denisovans at 200 ka (thousand years ago) (205–192 ka at 68.2% or 217–187 ka at 95% probability). The stratigraphic context in which they were located contains a wealth of archaeological material in the form of lithics and faunal remains, allowing us to determine the material culture associated with these early hominins and explore their behavioural and environmental adaptations. The combination of bone collagen fingerprinting and genetic analyses has so far more-than-doubled the number of hominin bones at Denisova Cave and has expanded our understanding of Denisovans and Neanderthal interactions, as well as their archaeological signatures.

The earliest Denisovans and their cultural adaptation

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Since the initial identification of the Denisovans a decade ago, only a handful of their physical remains have been discovered. Here we analysed ~3,800 non-diagnostic bone fragments using collagen peptide mass fingerprinting to locate new hominin remains from Denisova Cave (Siberia, Russia). We identified five new hominin bones, four of which contained sufficient DNA for mitochondrial analysis. Three carry mitochondrial DNA of the Denisovan type and one was found to carry mtDNA of the Neanderthal type. The former come from the same archaeological layer near the base of the cave’s sequence and are the oldest securely dated evidence of Denisovans at 200 ka (thousand years ago) (205–192 ka at 68.2% or 217–187 ka at 95% probability). The stratigraphic context in which they were located contains a wealth of archaeological material in the form of lithics and faunal remains, allowing us to determine the material culture associated with these early hominins and explore their behavioural and environmental adaptations. The combination of bone collagen fingerprinting and genetic analyses has so far more-than-doubled the number of hominin bones at Denisova Cave and has expanded our understanding of Denisovans and Neanderthal interactions, as well as their archaeological signatures.

The identification and analysis of Pleistocene hominin remains form the basis for unravelling the processes governing human evolution, interaction and adaptation, yet discovery of new human fossils continues to present a substantial hurdle. Recent developments in excavation practices and archaeological science cannot subvert an unavoidable problem—that human remains are rarely identified, especially in prehistoric contexts where formal burials were not observed. This is particularly true for the Denisovans, a sister population to the Neanderthals, whose discovery fundamentally changed our understanding of hominin diversity in Eurasia during the late Pleistocene. The high-coverage nuclear genome of a Denisovan individual (Denisova 3) showed that they diverged from a common ancestor with Neanderthals between 440 and 390 ka (thousand years ago). The identification of Denisovan ancestry in indigenous peoples of Australia and Papua New Guinea and in East and Southeast Asia has led to the inference that modern humans met and admixed with at least two distinct populations of Denisovans. This raises the possibility that Denisovans may have been widespread across continental Asia, island Southeast Asia and near Oceania.

So far, only five small and highly fragmented fossils, all discovered at Denisova Cave (Russian Altai, Siberia, Russia), have been identified as Denisovans on the basis of DNA analyses. These include worn and incomplete molars (Denisova 2, Denisova 4 and Denisova 8), partial phalanges (Denisova 3) and small bone chips (Denisova 11). Only one (Denisova 3) has yielded enough DNA for whole-genome sequencing. Poor DNA preservation and modern contamination has thus far impeded nuclear genome analyses of the other specimens. Outside Denisova Cave, a mandible from Baishiya Cave (Xiahe, China) was tentatively assigned to Denisovans on the basis of proteomic evidence and sediment DNA further confirmed the presence of Denisovans at the site.

Advances in proteomic research, in particular the increasingly common application of peptide mass fingerprinting (or ZooMS; Zooarchaeology by Mass Spectrometry), has been shown to be an efficient way for determining hominin presence at archaeological sites through the taxonomic identification of bone based on collagen characterization. In vertebrates, it is commonly used to assign genus or family-level identifications and, in some instances, species-specific determinations are possible. The highly time- and cost-efficient nature of ZooMS, its reproducibility and the long-term preservation of collagen compared with other biomolecules, including DNA, make it an invaluable screening tool for the identification of fragmentary, morphologically non-diagnostic bones. ZooMS has been used to successfully identify hominin remains in large assemblages of bones, including Denisova 11, a female individual with a Neanderthal mother and a Denisovan father. Here we present a high-throughput application of peptide mass fingerprinting to unidentified bones from Denisova Cave. Located in the northwest Altai mountains, Denisova Cave preserves the longest archaeological sequence in northern Eurasia dating from the middle Pleistocene to the Holocene. The cave contains a rich stratigraphic record, most notable for its Middle and Late Palaeolithic cultural, faunal and fossil remains. It is the only site where the presence of Denisovans and Neanderthals has been determined on the basis of DNA recovered from both fossils and cave deposits.
sediments in several layers throughout the sequence. In addition, the presence of early modern humans was recently confirmed at the site on the basis of mitochondrial DNA recovered from sediments. The combination of good biomolecular preservation, rich archaeological assemblages and the presence of multiple hominin groups makes Denisova Cave one of the most informative archaeological sites for Pleistocene Eurasia.

Non-diagnostic bone fragments, an important untapped source of potential human fossils, represent 95% of the bones excavated at Denisova Cave. We applied ZooMS to 3,791 bone fragments from the East Chamber, one of the three explored galleries of the cave. The fragments were specifically chosen for their lack of diagnostic features, which precluded macroscopic identification. The analysed bones came from each of the archaeological layers of the East Chamber, specifically layers 9, 11, 12, 13, 14 and 15. We also analysed a small number of bones from layer 17, which contains no archaeological evidence for hominin occupation (layers 10 and 16 are composed of culturally sterile deposits; Supplementary Table 1). The majority of analysed bones were excavated from layers 14 and 15 from which no hominin bones were previously found, although layer 15, the lowermost archaeological layer of the East Chamber, has previously yielded Denisovan sediment DNA. From each bone, a chip of approximately 20 mg was removed and, following established ZooMS protocols, collagen was extracted and analysed using a matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometer to carry out taxonomic identification (Materials and Methods). The vast majority of the analysed bones were assigned to large herbivores (Bos/Bison, Equidae and Cervidae) and carnivores, in reasonable agreement with fauna previously identified at the site through morphological analysis (Supplementary Fig. 1).

**Results**

**ZooMS.** Five bone fragments (Fig. 1a) generated peptide mass fingerprints with characteristic markers corresponding to the Hominidae (Fig. 1b, Supplementary Table 2 and Dataset 1). Four of them come from layer 15 (DC7277, DC7795, DC8591 and DC8846) and one from layer 12 (DC4969). Given that no great apes are known from the region, these bones almost certainly belong to humans. Human fossils identified using ZooMS now account for the majority of the hominin bones discovered at Denisova Cave (9 of the 17 fossils; 52%).

**microCT analysis.** To digitally preserve the morphology of the bone fragments, four of the five new specimens were scanned with a microCT system (Bruker SkyScan 2211 X-ray Nanotomograph). We used image spatial resolutions ranging between 0.020 and 0.023 mm, following the recommendations of Immel et al. to avoid degrading effects of X-ray irradiation on ancient DNA (Materials and Methods). 3D surfaces of the fossil bones were extracted from the microCT scans (Supplementary Fig. 2 and Dataset 2).

**mtDNA analysis.** Since peptide mass fingerprinting cannot be used for a more specific taxonomic assignment than Hominidae,
we used DNA analysis to identify the groups these five bones belonged to on the basis of mtDNA sequences. Extraction, sequenc-
ing and authentication of ancient hominin DNA from each bone followed published procedures (Materials and Methods). Using an
mtDNA enrichment approach, we isolated sufficient ancient hom-
inin DNA and reconstructed the mitochondrial genomes of four of the five specimens; Denisova 17 (DC4969), Denisova 19 (DC8846),
Denisova 20 (DC7795) and Denisova 21 (DC8591) (Supplementary
Tables 3 and 4). These were sequenced to an average coverage of
2,695-fold, 15-fold, 31-fold and 28-fold, respectively. Pairwise dif-
ferences and phylogenetic analyses showed that the mtDNA of
Denisova 17 falls within the diversity of Neanderthal mtDNAs,
while the mtDNAs of Denisova 19, Denisova 20 and Denisova
21 fall within the diversity of Denisovan mtDNAs (Fig. 2 and
Supplementary Tables 5–8). Denisova 18 contains too few ancient
DNA fragments to securely associate its mtDNA with a hominin

group (Materials and Methods and Supplementary Information).

Discussion

The presence of Neanderthals in the Altai was originally identified
in Okladnikov Cave, a site located 50 km to the north of Denisova
Cave, on the basis of mtDNA evidence. Further archaeological
and genetic data suggest that Neanderthals were in Siberia on sev-
eral separate occasions. They appeared at Denisova Cave (layer
12, East Chamber) at least ~150–130 ka. Five Neanderthal fos-
sils have been found in the East Chamber so far, of which three are
from layer 12 (Denisova 9, 11, 17) and two are from the overlying
layer 11.4 (Denisova 5, 15) (Fig. 3a). A single sediment sample
from layer 14 of the East Chamber yielded Neanderthal DNA, but
further work is required to replicate and confirm this signal. We
estimated the molecular age of the mtDNA of the newly identi-
fied Neanderthal (Denisova 17) to ~134 ka (95% height posterior
density (HPD): 94–177 ka) using Bayesian dating as implemented
in BEAST v.1.10.4 and the mtDNA of 12 radiocarbon dated
Neanderthal individuals as calibration points (Supplementary Table
7). Phylogeny inferences show that the mtDNA of Denisova 17 is
more distantly related to the mtDNAs of the two other Neanderthals
from Denisova Cave, Denisova 5 and Denisova 15, who are more
closely related to one another (Fig. 2a) (Supplementary Fig. 4 and
Table 5). In contrast, Denisova 11 mtDNA is more closely related
to the mtDNAs of Neanderthals from western Eurasia and to other
Siberian Neanderthals, such as those from Okladnikov Cave and
Chagyrskaya Cave (Fig. 2a) (Supplementary Fig. 4 and Table 5).

Gene flow between Neanderthals and Denisovans provides additional indirect evidence of earlier interactions between the two groups. Analysis of the genome of a female Denisovan individual (Denisova 2), for example, has revealed that she had Neanderthal ancestry deriving from an intro-
gression ~1,500 years before she lived, as early as 250–200 ka.
Two other Denisovans from higher up the stratigraphic sequence
(Denisova 8 and 3) also show Neanderthal introgression from
two different Neanderthal populations. Although it is not pos-
sible to tell where these interbreeding events occurred, they pro-
vide evidence for potential cohabitation and frequent interactions
between the two hominin groups from >200 ka (Denisova 2)
until their disappearance from the Altai around 50 ka (Denisova
3). Neanderthal presence, while more pronounced during the Last
Interglacial at Denisova Cave (MIS5) (Fig. 3b), is discontinuous in
the Altai region and may reflect occasional eastward migration of
Neanderthal groups across large tracts of Eurasia. Since no gene
flow from Denisovans to late European Neanderthals has been identi-

ced so far, these interactions seem most likely to have occurred
in northeastern Eurasia. The Altai, in particular, appears to be an
overlapping zone for both Denisovan and Neanderthal groups for
over 150,000 years, witnessing and possibly facilitating population
admixture as well as sustaining distinct hominin populations over
this long period.

The specimens with the Denisovan mtDNAs (19, 20 and 21)
come from layer 15 of the East Chamber. The mitochondrial
sequences of Denisova 19 and 21 are identical, indicating that they
may belong to the same individual or be maternal relatives. They
differ from the mtDNA of Denisova 20 by four substitutions. In
phylogenetic trees, the mtDNAs of the newly identified Denisovans
form a clade with the mtDNAs of Denisova 2 (layer 22.1, Main
Chamber) and Denisova 8 (layer 11.4, East Chamber) from which
they differ by 20 and 30 substitutions, respectively (Fig. 2b and
Supplementary Figs. 3 and 6). Parsimony analyses are consistent,
with Denisova 19, 20 and 21 being of similar age or slightly older than Denisova 2, and substantially older than Denisova 8, Denisova 3 and Denisova 4 (layer 11.2, East Chamber, and layer 11.1, South Chamber, respectively).

The mtDNA age estimates for the newly identified fossils (Supplementary Table 6) and their relationship to Denisova 2 agree with the overall stratigraphic context and previous attempts to cross-correlate the three Chambers of Denisova Cave on the basis of absolute dates, archaeological sequence and hominin groups.\(^6\)\(^,9\)\(^,25\). Previously, the earliest Denisovan (Denisova 2) was estimated to date to 122–194 ka using a Bayesian approach incorporating optical, genetic and stratigraphic data\(^9\)\(^,25\) (Fig. 3b), or as early as 280 ka on the basis of optical ages only\(^15\). That specimen was discovered in 1984 in the Main Chamber and its contextual integrity has been questioned, whereas the new fossils reported here were excavated in 2012–13 from a secure context. Layer 15 is the oldest archaeological layer of the East Chamber and is estimated to date to \(~200\) ka (205–192 ka at 68.2% probability, or 217–187 ka at 95.4% probability) on the basis of Bayesian modelling of existing optical ages\(^9\) (Fig. 3a). Using these date estimates as calibration points in a Bayesian statistical framework, we inferred a divergence date for the mtDNAs of the three new and the four previously published Denisovans to \(~229\) (95% HPD 206–252 ka; Supplementary Table 8) during the Interglacial period MIS 7. Both the mtDNA age estimates and the established chronology for layer 15 render Denisova 19, 20 and 21, or their maternal relatives, the oldest Denisovans currently documented (Supplementary Fig. 3).

The presence of individuals carrying Denisovan mtDNA in the lowermost archaeological layer 15 of the East Chamber offers us an opportunity to consider the wider archaeological and subsistence context of this group of hominins. So far, this has not been possible because previous Denisovan fossils were either derived from layers impoverished in archaeological material or from layers where Neanderthal cohabitation could not be excluded\(^11\)\(^,24\). Denisova 19, 20 and 21 date to the Penultimate Interglacial (MIS 7) (Fig. 3b), a warm climatic period with comparable conditions to today that would have rendered the Altai a favourable location for hominin expansion and intensified occupation. During this phase, a mosaic of landscapes can be detected in the vicinity of the cave, including both broad-leaved forests and open steppe landscapes\(^21\). Both traditional zooarchaeological and ZooMS analyses revealed that the inhabitants of the cave targeted a variety of taxa living in these environments, including interglacial forest and forest-steppe species, such as roe deer (Capreolus pygargus), Siberian red deer (Cervus elaphus) and giant deer (Megaceros giganteus), as well as species typical of more open country, such as horse (Equus ovovidi and Equus ferus), bison (Bison priscus), woolly rhinoceros (Coelodonta antiquitatis) and Mongolian gazelle (Gazella gurtassa)\(^9\)\(^,25\) (Supplementary Fig. 1). Frequent anthropogenic impacts on bones, including splitting, burning and butchery cut-marks, confirm that these species were procured regularly. Humans appear not to have been the only occupants of Denisova Cave during this period, however. About a quarter of the macroscopically identified faunal assemblage from layer 15 comprised carnivore remains, predominantly Canis lupus and Cuon alpinus\(^9\)\(^,26\). This high proportion of carnivore taxa suggests that humans may have been actively competing with these predators over resources and perhaps the cave itself.

**Fig. 3 | Stratigraphic and chronological relationship of the newly identified fossils from the East Chamber.** a, Stratigraphy of the East Chamber of Denisova Cave. The position of hominin fossils (circles) and sediment DNA (trowel) is shown. The newly identified hominin fossils—Denisova 17, Denisova 18, Denisova 19, Denisova 20 and Denisova 21—are shown next to the relevant stratigraphic layers they were excavated from. To the left, the coloured bars and the numerals represent the age range in thousand years before present (ka \(\pm \)) of each dated layer based on modelled optical ages\(^9\) (Fig. 3a). Using these date estimates as calibration points in a Bayesian statistical framework, we inferred a divergence date for the mtDNAs of all hominins from Denisova Cave, including the newly identified specimens. The ages for Denisova 17, 19, 20 and 21 were derived from the Bayesian statistical treatment of optical ages\(^9\) (Fig. 3b), or as early as 280 ka on the basis of Bayesian modelling of existing optical ages\(^9\) (Supplementary Fig. 1). Frequent anthropogenic impacts on bones, including splitting, burning and butchery cut-marks, confirm that these species were procured regularly. Humans appear not to have been the only occupants of Denisova Cave during this period, however. About a quarter of the macroscopically identified faunal assemblage from layer 15 comprised carnivore remains, predominantly Canis lupus and Cuon alpinus\(^9\)\(^,26\). This high proportion of carnivore taxa suggests that humans may have been actively competing with these predators over resources and perhaps the cave itself.
Archaeologically, layer 15 (and layer 14) of the East Chamber contain the highest frequency of stone artifacts in the entire sequence of the cave, with more than 3,000 pieces per m² (ref. 22). The lithic assemblage comprises discoidal, Levallois, and parallel cores to produce flakes using primary reduction techniques. Scrapers are the dominant tool type, including those shaped by steep Quina-type retouch, as well as spur-like, denticulate and notched forms (Fig. 4). Large ventrally thinned and basally truncated flakes, or truncated-faceted flakes, are typical pieces (Supplementary Information Section 4). A small number of blades with a longitudinal dorsal scar pattern is also present. Analyses of organic residues collected from a retouched flake from layer 15 revealed saturated and unsaturated fatty acids and, alongside the absence of bone and plant micro-residues, its proposed use was for animal skin processing activities, such as scraping, cutting and/or sawing37.

On the basis of their techno-typological characteristics and chrono-stratigraphic position, the lithic assemblage of layers 14 and 15 of the East Chamber is attributed to an early Middle Palaeolithic stone tool industry that has no direct counterparts in North and Central Asia. If we were to look further afield, the closest parallel is the Acheulo–Yabrudian cultural complex (AYCC) from the Near East. The AYCC has been identified at several cave (mostly) and open-air sites such as Tabun, Qesem, Hayonim and Misliya, dating to between 400/350 and 250 ka38. This is a period that marks the transition from the Early to Middle Palaeolithic, and is linked to major transformations in hominid adaptive and cognitive abilities and major technological and subsistence innovations39. These include, among others, the habitual use of fire and the systematic hunting and butchering of medium-size ungulates, such as fallow deer. Techno-typological similarities between the AYCC with Denisova Cave layers 14 and 15 of the East Chamber include comparable forms of ventrally thinned and basally truncated flakes, and the presence of Quina scrapers, denticulate and notched tools (examples in Fig. 4). There are no bifacial tools in the Denisova assemblage; bifaces are a typical element of the Acheulean variant of the AYCC, but are rare or absent in the other two facies of the complex. Yet, since there are no intermediate occurrences of similar traditions between the Levant and the Altai, and no hominin remains that could be directly linked to Denisovans outside the Altai and the Tibetan Plateau, further work is required to resolve issues surrounding Denisovan cultural adaptations and innovations. A focused attempt to characterize the lithic component of the earliest Denisovan layers is currently underway and will allow further understanding of the evolution of the Denisovan toolkit through time.

The distribution of Denisovan DNA in present-day humans suggests that Denisovans were widely dispersed, occupying large tracts of Pleistocene Asia, and that there was spatial and temporal structure in their population4,5,33,35. The Denisovan DNA introgressed in present-day humans from Siberia and East Asia, and indigenous Americans share the highest similarity with the high quality genome of Denisova 34,5. However, the mtDNAs of the three older Denisovans we identified here—Denisova 19, 20 and 21—belong to a different mtDNA lineage from that of Denisova 3. Characterization of the nuclear DNA of these individuals is required to determine whether these early Denisovans are more closely related to the Denisovans that admixed with the ancestors of present-day humans living in island Southeast Asia and New Guinea5. Deciphering the relationship of various Denisovan groups is necessary to further understand how their distribution across Central, East and Southeast Asia may reflect the variability in the material culture observed in these regions during the Pleistocene. The challenges encountered by Denisovans while living in extremely diverse and changing environments, from the Altai mountains to the high altitudes of the Tibetan Plateau, and possibly from north China to island Southeast Asia, would have required adaptation in novel ways to survive. The application of state-of-the-art biomolecular approaches, such as palaeoproteomics and DNA analyses, to bone fossils and sediments holds great potential in identifying new hominins dating back to the middle Pleistocene in Central Asia and elsewhere, and provides an opportunity to calibrate past demographic and dispersal events, while also linking them to the development of specific techno-complexes and cultural traditions.
Methods

Zooarchaeology by mass spectrometry (ZooMS). Analysis was carried out at the ZooMS facility of the Department of Archaeology at the Max Planck Institute for the Science of Human History, Jena, Germany. We followed established protocols. In brief, each bone, approximately 10–20 mg was removed using a circular diamond drill bit. Samples were rinsed in ammonium bicarbonate overnight and incubated for 1 h at 65 °C. The supernatant was treated with 0.4 µg trypsin (Thermo Scientific Pierce trypsin protease) and allowed to digest at 37 °C for 18 h. The incubated samples were concentrated and desalted using C18 ziptips (Thermo Scientific Pierce C18 tips) and eluted in a final solution of 50% acetonitrile and 0.1% trifluoroacetic acid. Then, 0.5 µL of the resulting solution was mixed with 0.5 µL α-cyano-4-hydroxycinnamic acid solution (10 mg mL⁻¹) in 50% acetonitrile and 0.1% trifluoroacetic acid and allowed to crystallize. The samples were analysed using a MALDI TOF (Bruker Autoflex Speed LRF) mass spectrometer. The resulting spectra were screened for diagnostic markers using flexAnalysis 3.4 (Bruker Daltonics) and mMass software. The spectra were compared against a reference library of known peptide markers.

MicroCT scanning. Before sampling for ancient DNA analysis, the five bones identified as Hominidae using ZooMS were scanned with Image spatial resolutions ranging between 0.020 and 0.023 mm using the Bruker SkyScan 2211 X-ray Nanotomograph housed at MPI-SHH in Jena, Germany. Following the recommendations of Immel et al. for avoiding the degrading effects of X-ray irradiation, we strictly limited the scan image spatial resolution to 0.020 mm, although smaller voxel sizes could have been achieved. We used a 0.5 mm titanium filter to block the energies of the X-ray spectrum. All scans were acquired at 110 kV source voltage and 170 µA source current. Using the ‘Iosurface’ module of Avizo 9.4 (Visualization Science Group), we extracted 3D surfaces of the fossil bones from the microCT scans.

Mitochondrial DNA analysis. DNA extraction and library preparation. After removing approximately 1 mm surface material using a sterile dentistry drill bit, multiple small samples of ~7–21 mg bone powder were obtained from each specimen. DNA was extracted from each sample (or a subsample thereof, not using more than 15 mg bone powder) with a method that uses silica-coated magnetic particles for the retrieval of short DNA molecules on an automated liquid handling platform. Due to the low quantities of material that were removed, the volume of lysing buffer was reduced to 300 µL, of which 150 µL were used for DNA extraction. For Denisova 18, 19, 20, and 21, additional sampling of 9–17 mg bone powder was performed and the samples were pre-treated with 0.5% sodium hypochlorite (bleach) solution following the protocol developed by Korlević et al. DNA was extracted from the bleach-treated samples following the same silica-based protocol used for non-bleached samples. The entire DNA extracts were converted into single-stranded DNA libraries. Extraction and library negative controls were carried through all steps of the experiments. The libraries were amplified according to a double indexing scheme and purified as described in the aforementioned library preparation protocol.

A total of 29 single-stranded DNA libraries were made for the five samples, including nine for which extracts were pre-treated with bleach (Supplementary Table 3). Using quantitative PCR, we estimated the number of DNA molecules including nine for which extracts were pre-treated with bleach (Supplementary Table 9) in a Bayesian statistical framework. We inferred the age of the most recent common ancestor of Denisovans 2,6,8, the middle Pleistocene hominin from Sima de los Huesos 59, six present-day humans 60–62, six ancient modern humans 63–65, in the Neanderthal 13,36,49–58, four Neanderthals from Middle Pleistocene hominins Homo heidelbergensis 66,67, four Neanderthals from Denisova 8, six ancient modern humans 68–71, six present-day humans 60–62, and a chimpanzee 72 using marginal likelihood estimations (path sampling method) to determine the best-fitting clock model and tree model. For each model combination, we used an MCMC chain of 500,000,000 iterations with a burn-in representing 10% of the chain. We used a mutation rate of 2.53 × 10⁻⁸ (95% confidence interval [CI] [1.76 × 10⁻⁸, 3.33 × 10⁻⁸]) substitutions per site per year for the whole mtDNA genome and 1.57 × 10⁻⁸ (95% CI [1.17 × 10⁻⁸, 1.98 × 10⁻⁸]) substitutions per site per year for the coding region. Following the scale of Kass and Raftery, a strict clock and Bayesian skyline tree model was supported over the other model combinations (logBF > 5.2). The molecular age of Denisova 17 mtDNA was determined using the uniform priors for the ages of the Neanderthals as defined in Peyrégne et al. (Supplementary Information) as calibration points. We inferred the age of the most recent common ancestor of Denisovan mtDNAs using the 95% CIs of archaeological date estimates for the Denisovan remains from Douka et al. (Supplementary Information). We then performed six Max k chain Monte Carlo runs of 75,000,000 iterations for the complete mitochondrial genome and the coding region. We sampled trees every 2,500 iterations after a burn-in of 10% of the number of iterations, and merged the runs using the BEAST post-analysis programme, LogComber.

Data availability

The mtDNA consensus sequences generated for the current study are available in NCBI GenBank under accession numbers MT576650–MT576653.

Dataset 1

Raw MALDI-TOF files from ZooMS analysis of the hominin bones DC4969 and DC8486 (Denisova 19), DC7795 (Denisova 20) and DC8591 (Denisova 21) converted to open source format. Files have been uploaded to: https://doi.org/10.17617/3.44.
62. Fu, Q. et al. The genetic history of Ice Age Europe. Nature 534, 200–205 (2016).
63. Devièse, T. et al. Compound-specific radiocarbon dating and mitochondrial DNA analysis of the Pleistocene hominin from Salkhit Mongolia. Nat. Commun. 10, 274 (2019).
64. Sikora, M. et al. Ancient genomes show social and reproductive behavior of early Upper Paleolithic foragers. Science 358, 659–662 (2017).
65. Green, R. E. et al. A draft sequence of the Neandertal genome. Science 328, 710–722 (2010).
66. Horai, S. et al. Man's place in Hominioidea revealed by mitochondrial DNA genealogy. J. Mol. Evol. 37, 89 (1993).
67. Sievers, F. et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol. Syst. Biol. 7, 539 (2011).
68. Stecher, G., Tamura, K. & Kumar, S. Molecular evolutionary genetics analysis (MEGA) for macOS. Mol. Biol. Evol. 37, 1237–1239 (2020).
69. Swoford, D. L. PAUP: phylogenetic analysis using parsimony, version 4.0 b10. (Sinuara, 2002).
70. Suchard, M. A. et al. Bayesian phylogenetic and phylogenetic data integration using BEAST 1.10. Virus Evol. 4, vey016 (2018).
71. Darrow, D. et al. ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. Mol. Biol. Evol. 37, 291–294 (2020).
72. Baele, G. et al. Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. Mol. Biol. Evol. 29, 2157–2167 (2012).
73. Baele, G., Li, W. L. S., Drummond, A. J., Suchard, M. A. & Lemey, P. Accurate model selection of relaxed molecular clocks in Bayesian phylogenetics. Mol. Biol. Evol. 30, 239–243 (2013).
74. Fu, Q. et al. A revised timescale for human evolution based on ancient mitochondrial genomes. Curr. Biol. 23, 535–539 (2013).
75. Kass, R. E. & Raftery, A. E. Bayes factors. J. Am. Stat. Assoc. 90, 773–795 (1995).
76. Listerick, L. E. & Raymo, M. E. A Pliocene–Pleistocene stack of 57 globally distributed benthic δ18O records. Paleoclimatography 20, PA1003 (2005).

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Author contributions
K.D. designed the study; S.B., D.M., B.J.-S. and A.S. performed the laboratory work; S.B., D.M., A.S., M.M., J.K., S.P. and K.D. analysed the data; M.B.K., M.V.S. and A.P.D. provided samples and site-specific expertise; S.B., D.M., T.H. and K.D. wrote the paper with the assistance and input of all co-authors.

Competing interests
The authors declare no competing interests.

Additional information
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- 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
- 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: No custom software or code was required in this project

Data analysis: No commercial or custom code was utilised to analyse our data

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

Raw MALDI-TOF files from ZooMS analysis of the hominin bones DC4969 (Denisova 17), DC7277 (Denisova 18), DC8846 (Denisova 19), DC7795 (Denisova 20), and DC8591 (Denisova 21) converted to open source format. Files have been uploaded to: https://dx.doi.org/10.17617/3.44. MicroCT Scan files of the hominin bones DC4969 (Denisova 17), DC7277 (Denisova 18), DC8846 (Denisova 19), and DC7795 (Denisova 20). Files have been uploaded to: https://dx.doi.org/10.17617/3.45. All other data has been supplied as part of 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

Ecological, evolutionary & environmental sciences study design

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

Study description
Analysis of 3800 non-diagnostic bone fragments using collagen peptide mass fingerprinting to locate new hominin remains from Denisova Cave (Siberia, Russia). Five new hominin bones were identified, four of which contained sufficient DNA for mitochondrial analysis. This indicated that three bones carry mtDNA of the Denisovan type and one carries mtDNA of the Neanderthal type.

Research sample
All samples included in the study are Pleistocene-age bones, some of which were identified as being hominin remains.

Sampling strategy
Sampling was done randomly from amongst large assemblages of non-diagnostic bone fragments.

Data collection
Data was obtained by Samantha Brown and Diyendo Massilani.

Timing and spatial scale
November 2017-October 2020

Data exclusions
No data was excluded

Reproducibility
Samples were analysed in triplicates and experiments were conducted several months apart to test data reproducibility.

Randomization
N/A

Blinding
N/A

Did the study involve field work?  ☒ Yes  ☐ No

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 |
| ☐ Human research participants |
| ☐ Clinical data |
| ☒ Dual use research of concern |

Methods

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

Palaeontology and Archaeology

Specimen provenance
Bones were excavated from Denisova Cave, Siberia, Russia and were studied in collaboration with the Institute of Archeology and Ethnography of the Siberian Branch of the Russian Academy of Sciences.

Specimen deposition
All data has been uploaded to Mendeley Data.

Dating methods
No new dates are provided. Previously published OSL dates are incorporated in a Bayesian model using OxCal v.4 to estimate the age of the newly discovered fossils.

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

Ethics oversight
No ethical approval or guidance was required to study these archaeological specimens.

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