
Supplementary information

The earliest Denisovans and their cultural adaptation

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Supplementary Information

The earliest Denisovans and their cultural adaptation

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Supplementary Information Text

1. Zooarchaeology by Mass Spectrometry (ZooMS)

ZooMS analysis was carried out for 3,791 samples from Denisova Cave's East Chamber.¹ All archaeological layers were investigated resulting in the identification of five Hominidae samples. The majority of bones studied were identified as Bos/Bison (23%), Cervidae/Gazella/Saiga (22%), and Equidae (10%; Fig. S1). 22% of the samples failed ZooMS analysis as a result of poor collagen preservation; these are not shown in Fig. S1. The results of the ZooMS-based fauna identification are in reasonable agreement with fauna previously identified at the site through traditional morphological analysis.¹⁻³ Trends linked to prevailing environmental conditions (interglacial versus glacial periods), ambient temperatures and plant coverage have been reconstructed, although a larger sample size is required to solidify these trends.

2. List of new hominin fossils

Denisova 17, bone fragment (Neanderthal) (Fig. 1).

2011, East Chamber, Sector 6, Layer 12

This specimen was identified among the bulk fragment assemblage from layer 12 of the East Chamber using ZooMS (ZooMS ID: DC4969) and is reported in this paper for the first time. MtDNA analysis, summarised in Section 3 of the Supplementary Text, indicates this individual carries Neanderthal mtDNA.

Denisova 18, bone fragment (Homo sp.) (Fig. 1).

2012-2013, East Chamber, Sector 6, Layer 15

This specimen was identified among the bulk fragment assemblage from layer 15 of the East Chamber using ZooMS (ZooMS ID: DC7277) and is reported in this paper for the first time. MtDNA analysis, summarised in Section 3 of the Supplementary Text, was inconclusive due to present-day human contamination and low aDNA preservation in the sample.

Denisova 19, bone fragment (Denisovan) (Fig. 1).

2012-2013, East Chamber, Sector 6, Layer 15

This specimen was identified among the bulk fragment assemblage from layer 15 of the East Chamber using ZooMS (ZooMS ID: DC8846) and is reported in this paper for the first time. MtDNA analysis, summarised in Section 3 of the Supplementary Text, indicates this individual carries Denisovan mtDNA. The Denisova 19 and 21 mitochondrial sequences are identical and may belong to the same individual or maternal relatives.

Denisova 20, bone fragment (Denisovan) (Fig. 1).

2012-2013, East Chamber, Sector 6, Layer 15

This specimen was identified among the bulk fragment assemblage from layer 15 of the East Chamber using ZooMS (ZooMS ID: DC7795) and is reported in this paper for the first time. MtDNA analysis, summarised in Section 3 of the Supplementary Text, indicates this individual carries Denisovan mtDNA.

Denisova 21, bone fragment (Denisovan) (Fig. 1).

2012-2013, East Chamber, Sector 6, Layer 15

This specimen was identified among the bulk fragment assemblage from layer 15 of the East Chamber using ZooMS (ZooMS ID: DC8591) and is reported in this paper for the first time. MtDNA analysis, summarised in Section 3 of the Supplementary Text, indicates this individual carries Denisovan mtDNA. The mitochondrial sequence of Denisova 21 and Denisova 19 identical and may belong to the same individual or maternal relatives.

3. Mitochondrial DNA analysis

Ancient DNA authenticity

Cytosine residues (C) close to the ends of ancient DNA molecules tend to become deaminated to uracils (U)⁴ and are read by DNA polymerases as thymines (T). Thus we gauged the presence of ancient DNA in a sample based on the frequency of C to T mismatches to the reference at the extremities of the sequences.⁵ After sequencing of the human mtDNA enriched libraries, the data were mapped to the human reference mtDNA sequence rCRS and 26 libraries show evidence for presence of ancient hominin mtDNA as their C to T frequencies at both the 5' and 3' ends are > 10% (between 19.7% and 53.2% at the 5' ends and between 10% and 47.8% at the 3'ends) (Table S3). 3 out of 5 libraries of Denisova 18 have C to T frequency <10% indicating that aDNA is less well preserved in that specimen in comparison to the others.

Determination of mtDNA hominin type

We determined the mtDNA hominin type of the mapped sequences in each library by estimating the frequency of sequences matching the modern human, Neanderthal or Denisovan states at “diagnostic” positions that discriminate each of the different hominin mtDNA lineages.⁶ Considering only sequences carrying a C to T mismatch at one or the other end (deaminated sequences), the libraries of *Denisova 17* have a total of 4,990 deaminated sequences covering the Neanderthal mtDNA diagnostic positions and 94% of them match the Neanderthal state, indicating that the mtDNA of *Denisova 17* is of Neanderthal type. The deaminated sequences of the *Denisova 19*, *20* and *21* libraries match the Denisovan state at 96% (250/260), 92% (425/463) and 87% (381/439), respectively, indicating that these specimens have a mtDNA of Denisovan type. The two libraries of *Denisova 18* with C to T frequencies >10% yield a total of only 248 deaminated sequences with 34 of them covering the diagnostic positions of the Denisovan mtDNA and matching at 70% the Denisovan state, 18 of them covering the diagnostic positions of Neanderthal mtDNA and matching at 0% the Neanderthal state and 8 of them covering the diagnostic positions of modern human mtDNA and matching at 62.5% the modern human state. It is likely that the *Denisova 18* specimen has a mtDNA of the Denisovan type, but due to the small number of mtDNA sequences we cannot rule out cross-contamination from the other Denisovan specimens during the DNA processing.

Re-alignment of the data

For *Denisova 18, 19, 20* and *21*, which were assigned to the Denisovan mtDNA type, we re-aligned all sequences to the mtDNA sequences of *Denisova 3* and *Denisova 8*⁷ in order to recover sequences that might have been lost due to high divergence to the human reference mtDNA. Re-alignment to the *Denisovan 8* mtDNA recovered 4.4% to 9.2 % and 0.4% to 2.6% more mapped sequences than when the sequence of rCRS and the *Denisova 3* mtDNAs were used as reference. Similarly, we re-aligned sequences from the *Denisova 17* libraries to the mtDNA of the Vindija 33.25 individual and recovered between 5.9% and 6.1% more mapped sequences than with the rCRS as reference. C to T frequencies for each library were computed based on the reference genome used (Table S4). After merging the sequences obtained from different libraries from the same specimen, we have 907,043 unique mtDNA sequences longer than 35 bp for *Denisova 17*, and 22,215, 20,741, 40,284, and 41,434 unique mtDNA sequences longer than 35 bp for *Denisova 18, 19, 20* and *21*, respectively. The average rate with which identical sequences were observed was ~2-fold for *Denisova 17* and over 12-fold for the others, indicating that the libraries have been sequenced deeply (Table S4).

Present-day human mitochondrial contamination

We estimated the mitochondrial contamination by determining the proportion of sequences that share the derived modern human states at positions where 311 present-day humans from around the world differ from all Neanderthals and Denisovans. We estimated the average contamination among the libraries prepared from *Denisova 17* to 0.89%, and the average contamination for *Denisova 18, 19, 20* and *21* to 65.9%, 9.4%, 13.4% and 31.9%. Because of the contamination estimates of *Denisova 18, 19, 20* and *21*, we used only deaminated fragments to reconstruct their mtDNA genome sequences.

Mitochondrial genome consensus

Depending on the modern human contamination estimates we used either all sequences or only deaminated sequences to reconstruct mtDNA consensus sequences. Using all sequences we call a mtDNA consensus sequence for *Denisova 17* to an average coverage of 2,695-fold. Complete consensus mtDNAs of *Denisova 20, 21* were called using only deaminated sequences to an average coverage of 31-fold and 28-fold, respectively. For *Denisova 19*, we have an average coverage of 15-fold with three positions that could not be called confidently. The mtDNA consensus of *Denisova 18* could not be called.

Phylogenetic analyses

Phylogenetic analyses (Fig. S3) and pairwise differences (Fig. S4) support the assignment of the mtDNA of *Denisova 17* within the diversity of Neanderthal mtDNAs and the assignment of the mtDNA of *Denisova 19, 20* and *21* within the diversity of Denisovan mtDNAs. The *Denisova 17* mtDNA sequence differs from the mtDNAs of other Neanderthals from Denisova Cave - *Denisova 5, 15* and *11*- by 26, 27 and 28 substitutions, respectively (Fig. S4). Sixteen equally parsimonious trees with an equal length of 253 substitutions can be generated for our dataset of 27 Neanderthal mtDNAs. We called a consensus tree using the 50% majority-rule method (Fig. S5) and counted the number of substitutions accumulated in each mtDNA of the Neanderthal from Denisova cave since they split from their most recent ancestor to

the exclusion of the Hohlenstein-Stadel Neanderthal. Since they diverged from their last common ancestor, *Denisova 5*, *15*, *17* and *11* have accumulated 15, 16, 17 and 21 substitutions, respectively (Fig. S5). The *Denisova 17* mtDNA accumulated 4 substitutions less than the mtDNA of *Denisova 11* and 1 and 2 substitutions more than the mtDNAs of *Denisova 15* and *Denisova 5*, respectively. Using the mitochondrial mutation rate inferred for modern humans of 2.53×10^{-8} (95% HPD: $1.76-3.23 \times 10^{-8}$)⁹ (Table S5) under the assumption that the mitochondrial mutation rate in archaic humans is the same as that in modern human, we estimate that the molecular age of the mtDNA of *Denisova 17* is about 7-14 ky older than *Denisova 11*, 2-3 ky younger than *Denisova 15* and 4-7 ky younger than *Denisova 5* (Table S5).

Excluding the three positions not covered in the mtDNA of the *Denisova 19* individual, its sequence is identical to the mtDNA sequence of *Denisova 21*. Both *Denisova 19* and *21* mtDNAs differ from *Denisova 20* mtDNA by 4 substitutions (Fig. S4). The phylogenetic relationship between the three new Denisovan mtDNAs and the previously published four Denisovan mtDNAs can be described by two equally parsimonious scenarios with a length of 274 substitutions (Fig. S6A and S6B). In both scenarios the 7 Denisovan mtDNAs are distributed in 2 main clades, one composed by the mtDNAs of the *Denisova 19*, *20*, *21*, *2* and *8* individuals and the other composed by the mtDNAs of the *Denisova 3* and *4* individuals. The two maximum parsimony trees differ in their topology and the lengths of their branches. In the tree Fig. S6A, the mtDNAs of *Denisova 19*, *20* and *21* group together in a clade including *Denisova 2* and *8* and the mtDNAs of *Denisova 19*, *20*, *21* and *2* accumulated 21 substitutions since their divergence from a common ancestor with *Denisova 3* and *Denisova 4*. *Denisova 19*, *20*, *21* and *2* are older since they accumulated 12 substitutions less than *Denisova 8* since they diverged from each other. The alternate tree in Fig. S6B suggests that the clade formed by *Denisova 2*, *8*, *19*, *20* and *21* is more structured, with *Denisova 2* and *8* grouping together alongside the group formed by *Denisova 19*, *20* and *21*. In this tree, the mtDNAs of *Denisova 19*, *20* and *21* accumulated 6 and 18 substitutions fewer than the mtDNAs of *Denisova 2* and *Denisova 8*, respectively. Both scenarios show that the branch length leading to the mtDNA of *Denisova 3* and *Denisova 4* are the longest and that the mtDNA of *Denisova 3* and *Denisova 4* accumulated respectively 33 and 31 substitutions more than the mtDNA of *Denisova 19*, *20* and *21* since they diverged from the common ancestor of Denisovan mtDNA. The estimates of the molecular ages of the mtDNAs suggest that *Denisova 19*, *20* and *21* are of similar age or about 11-20 ky older than *Denisova 2*, about 22-62 ky older than *Denisova 8* and about 58-113 ky older than *Denisova 3* and *Denisova 4* (Table S6).

Bayesian analyses

We performed Bayesian analyses on the dataset above plus 60 additional modern human mtDNAs (Table S9) using BEAST v.1.10.4¹⁰ to estimate the molecular ages and divergence times of the mtDNAs of the newly identified individuals. As concerns regarding the reliability of the hypervariable region of the mtDNA of low coverage mtDNAs was raised,^{11,12} we performed the analyses on both the complete mtDNA sequences and only the mtDNA coding sequences (577-16,023 in rCRS coordinates) (Table S7).¹² For

consistency with previous works, we report only the estimates using the coding sequences in the main text.^{11,12}

The best fitting nucleotide substitution model estimated for both datasets is Tamura-Nei 1993¹³ with a fraction of invariable sites and gamma distributed rate variation among sites (TrN+I+G4). A strict clock and Bayesian skyline coalescent model are better supported by Marginal likelihood inferences^{14,15} than a relaxed clock and constant size coalescent models ($\log_{10} \text{BF} > 5.2$).

We estimated the molecular age of the samples by setting the date of present-day individuals to 0 and using uniform priors for the ages of the ancient individuals as in (Table S7).¹¹ Under the assumption that the mitochondrial mutation rate for Neandertals and Denisovans is the same as that of modern humans, we inferred the molecular age of the *Denisova 17* mtDNA to 134 ka (95% HPD: 94 ka – 177 ka) (Table S7) consistent with the association of the individual to the Middle Paleolithic. The lack of direct date estimates for the Denisovan individuals makes the molecular ages inferred for these specimens less reliable (Table S7). However, the relative Bayesian age estimates for the Denisovan individuals are consistent with the branch shortening in the parsimony analyses, suggesting that *Denisova 19*, *20* and *21* are almost contemporaneous with *Denisova 2*, and older than *Denisova 8*, *Denisova 3* and *Denisova 4* (Table S7).

As the new Denisovan specimens were excavated from a secure archaeological context in layer 15 of the East Chamber, we used the optical ages of that layer³ (Table S8) as calibration points for these specimens, together with the ages inferred by Bayesian age modelling for the previously published Denisovan individuals¹⁶ (Table S8) to estimate the time of divergence of the Denisovan mtDNAs to ~229 ka (95% HPD: 206 ka – 252 ka) (Table S8) during the interglacial period of the Marine Isotope Stages 7.

4. Archaeology

The oldest archaeological evidence from Denisova Cave was found in layer 22 of Main Chamber, with an upper age limit of 287 ± 41 ka estimated using a series of optical ages². It is overlain by layers 21 and 20, dated in around 250 ± 44 to 170 ± 19 ka. In the East Chamber, these deposits correspond to layers 15 and 14 dated between 203 ± 14 ka and 187 ± 14 ka during MIS 7².

Based on the chronostratigraphy of cave sediments and the techno-typological appearance of stone tool industries, the material culture from layers 15 and 14 in East Chamber is attributed to the Early Middle Palaeolithic. The stone tool industries of this period are characterized by the presence of discoid cores with unifacial and bifacial variations, as well as parallel unifacial single-platform cores that are mainly produced from massive flakes and larger pebbles or smaller boulders. Flakes are dominated by short pieces with a smooth or natural platform, showing evidence of longitudinal or multidirectional dorsal faceting. Blades are rare and have a smooth striking platform and a longitudinal dorsal scar pattern. Typologically stone artifacts made on standard blanks, such as single, oblique, transverse, convergent, and canted scrapers, including those shaped by stepped Quina-type retouch, as well as spur-like, denticulate and notched forms, are the most common in the lithic inventory. Of particular interest are the numerous ventrally thinned pieces that comprise large, massive flakes with the distal edge and one or two lateral edges intensively reduced by large flake removals on the ventral surface.

Another characteristic type of artifacts includes basally truncated and truncated-faceted flakes, showing indications of ventral or, rarely, dorsal truncation. In addition, the tool assemblage contains naturally backed knives, transverse burins on blade, angle burins on massive flakes, as well as elongated Levallois points with a faceted medially convex striking platform, and flake-blades with occasional retouch.

The Early Middle Paleolithic stone tool assemblages associated with the lower part of Pleistocene deposits from Denisova Cave constitute original lithic industries with no direct equivalents in North and Central Asia. Their closest counterparts may be found in the Early Middle Paleolithic assemblages from the Near East, namely the Acheulo-Yabrudian cultural complex (AYCC) dating back between 350–250 ka. The AYCC has been recovered from caves and open-air sites, such as Tabun, Qesem, Bezez, Hayonim, Azraq, Misliya, Zuttiyeh, Yabrud I, Masloukh, Jerf Ajla, and others. The Early Middle Paleolithic industries present in the Levant for about 100,000 years involved both Levallois and non-Levallois reduction techniques were employed disproportionately, whereas blades and flakes were used as blanks. This variability seems to be associated with changes in adaptation strategies of early hominins living in diverse environments.⁸

There appear to be certain techno-typological features shared between these Levantine assemblages and the early Middle Paleolithic assemblage from Denisova Cave. These include comparable forms of ventrally thinned and basally truncated flakes, Quina scrapers, denticulate and notched tools. The stone tool industry found at Denisova Cave differs principally from the Near Eastern assemblages in the lack of bifacial tools in its composition, however.

The next development phase of the Middle Paleolithic in the East Chamber is associated with the late Middle and early Upper Pleistocene deposits corresponding to MIS 6–4. This period is represented by stone tool industries from layers 13–11.3 and dates to between 156 ± 15 ka and 70 ± 8 ka². The Middle

Paleolithic lithic reduction systems involved radial and parallel flaking; furthermore, edge-faceted and volumetric sub-prismatic cores made on large cobbles and boulders, rarely on large flakes, were also reduced using the same methods. Levallois flaking technique is also present in the assemblage. Scrapers of different modifications were found to be the most typologically diverse tools in the lithic inventory. In additions denticulate, notched, and spur-like pieces are interesting types of tools. Mousterian points, Levallois points and blades, ventrally thinned flakes, and Upper Paleolithic tools, such as end- and canted scrapers, angle burins, borers and truncated flakes, constitute small but rather distinctive groups of the lithic toolkit.

In general, as we move up the sequence the Middle Paleolithic industries show expansion in typological diversity of cores and a percentage increase of blades in flakes, whereas the lithic inventory composition shows an increase in the share of Upper Paleolithic tools against the background of a reduction in the Levallois and notch-denticulate components. Despite gaps in the Pleistocene sedimentary sequence, archaeological evidence from the stratified Middle Paleolithic complex of Denisova Cave shows the development of technologies in a wide time range spanning MIS 9–4, from the early Middle Paleolithic through its final stages, demonstrating the continuity of the Middle Paleolithic traditions without abrupt changes in the composition of stone tool industries.

Supplementary Figures

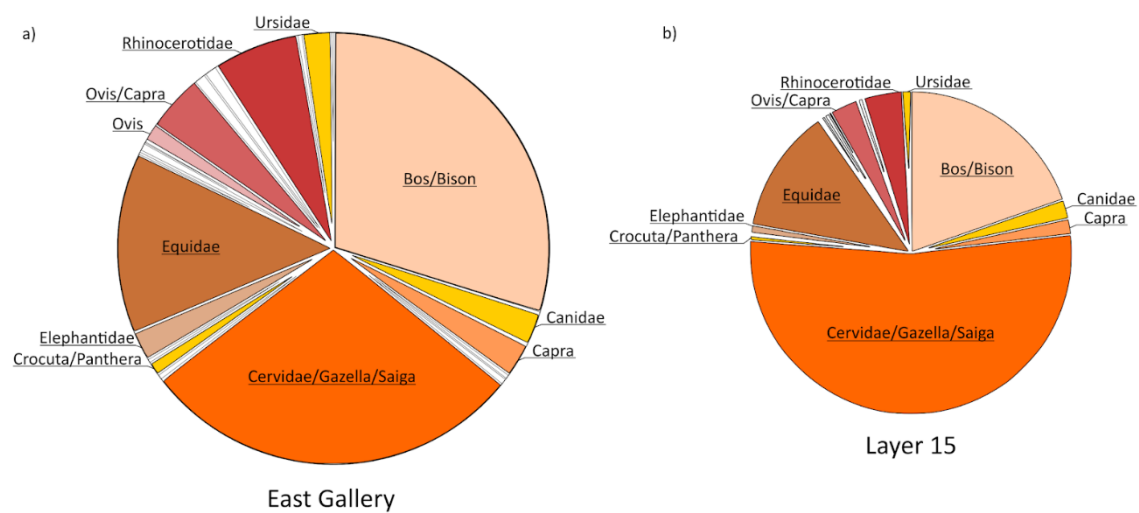


Fig. S1. Pie chart for the largest faunal groups present in the unidentified bone assemblage identified using ZooMS. Graph a) shows the total percentages of fauna identified for all bones analysed for the East Chamber of Denisova Cave, b) shows the total percentage of fauna identified for Layer 15 of the East Chamber only³.

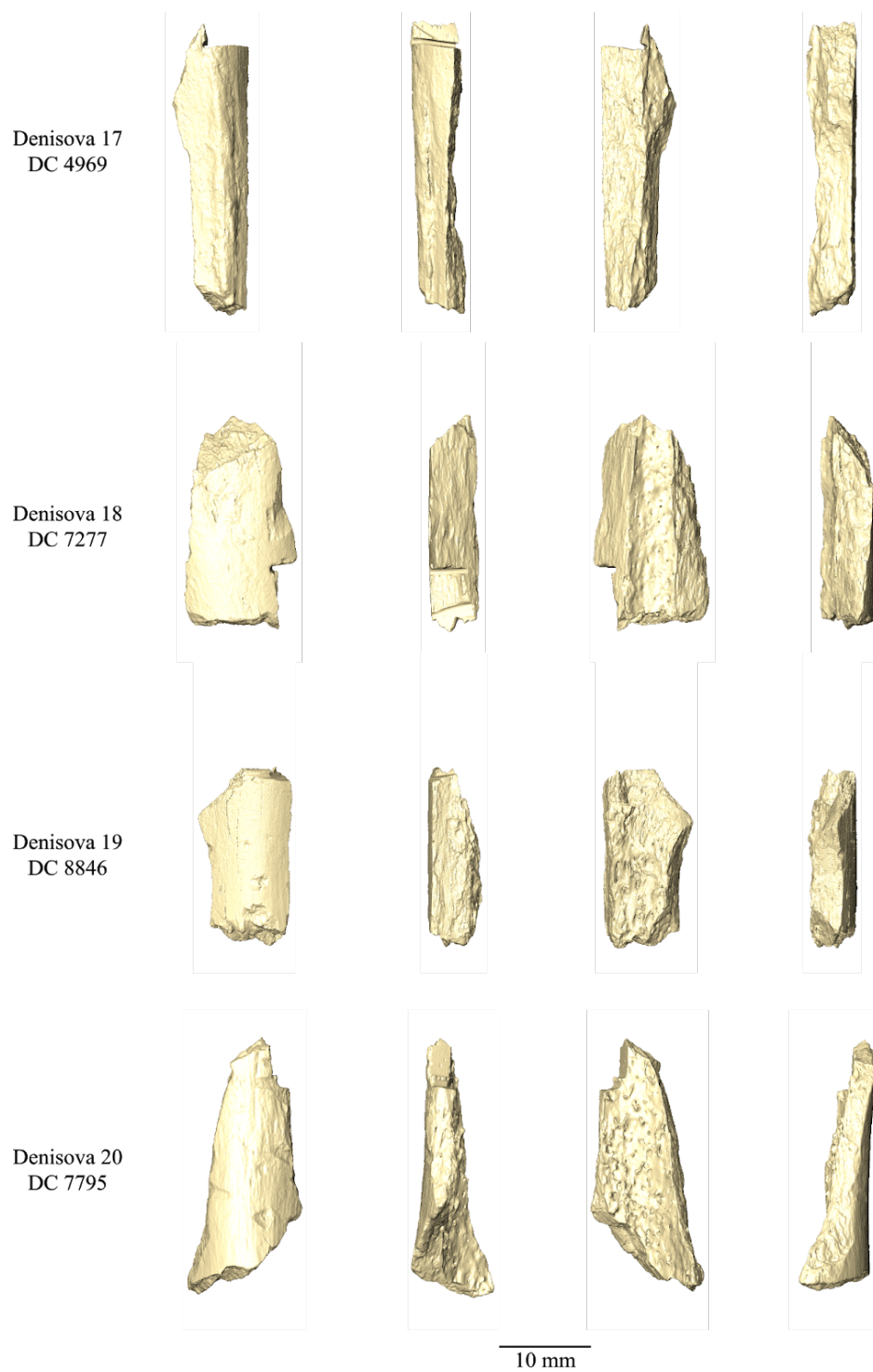


Fig. S2. 3D surface renderings retrieved from the microCT scanning performed for Denisova 17, 18, 19 and 20 (DC4969, DC7277, DC8846, and DC7795).

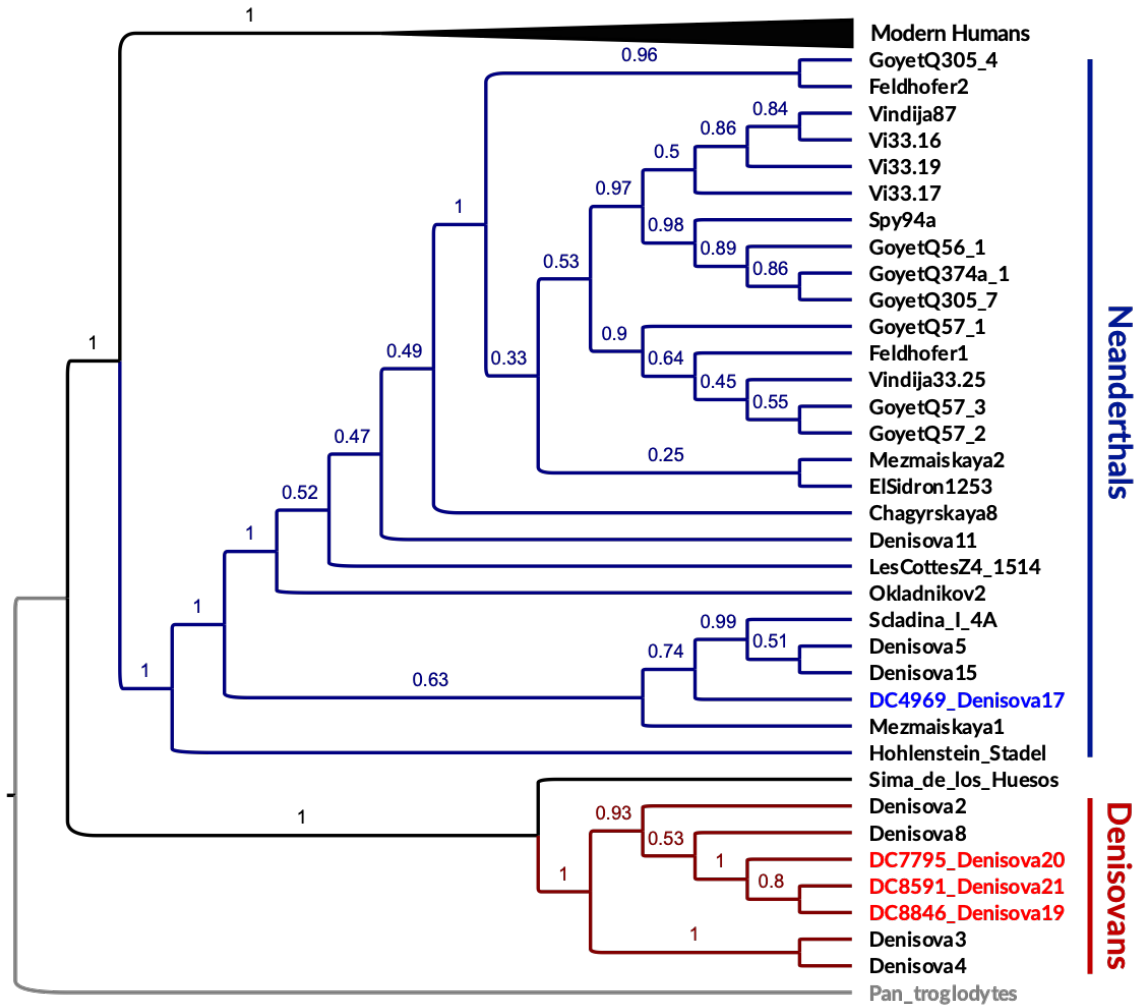


Fig. S3. Neighbor-joining tree relating the mtDNA of Denisova 17, 19, 20 and 21 to other ancient and present-day hominin mtDNAs. The mtDNA of chimpanzee was used as an outgroup. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches.

A)

	Denisova2	Denisova19	Denisova21	Denisova20	Denisova8	Denisova4	Denisova3
Denisova2		20	20	20	29	69	71
Denisova19	20		0	4	29	67	69
Denisova21	20	0		4	30	67	69
Denisova20	20	4	4		30	67	69
Denisova8	29	29	30	30		83	85
Denisova4	69	67	67	67	83		2
Denisova3	71	69	69	69	85	2	

B)

Chromosome 1																												
	Neanderthal 1	Neanderthal 2	Neanderthal 3	Neanderthal 4	Neanderthal 5	Neanderthal 6	Neanderthal 7	Neanderthal 8	Neanderthal 9	Neanderthal 10	Neanderthal 11	Neanderthal 12	Neanderthal 13	Neanderthal 14	Neanderthal 15	Neanderthal 16	Neanderthal 17	Neanderthal 18	Neanderthal 19	Neanderthal 20	Neanderthal 21	Neanderthal 22	Neanderthal 23	Neanderthal 24	Neanderthal 25	Neanderthal 26	Neanderthal 27	Neanderthal 28
Neanderthal 1		90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116
Neanderthal 2	90		35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
Neanderthal 3	91	35		25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Neanderthal 4	92	36	25		19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Neanderthal 5	93	37	26	19		19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
Neanderthal 6	94	38	27	20	19		20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
Neanderthal 7	95	39	28	21	20	20		21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
Neanderthal 8	96	40	29	22	21	21	21		22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
Neanderthal 9	97	41	30	23	22	22	22	22		23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
Neanderthal 10	98	42	31	24	23	23	23	23	23		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
Neanderthal 11	99	43	32	25	24	24	24	24	24	24		25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
Neanderthal 12	100	44	33	26	25	25	25	25	25	25	25		26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
Neanderthal 13	101	45	34	27	26	26	26	26	26	26	26	26		27	28	29	30	31	32	33	34	35	36	37	38	39	40	
Neanderthal 14	102	46	35	28	27	27	27	27	27	27	27	27	27		28	29	30	31	32	33	34	35	36	37	38	39	40	
Neanderthal 15	103	47	36	29	28	28	28	28	28	28	28	28	28	28		29	30	31	32	33	34	35	36	37	38	39	40	
Neanderthal 16	104	48	37	30	29	29	29	29	29	29	29	29	29	29	29		30	31	32	33	34	35	36	37	38	39	40	
Neanderthal 17	105	49	38	31	30	30	30	30	30	30	30	30	30	30	30	30		31	32	33	34	35	36	37	38	39	40	
Neanderthal 18	106	50	39	32	31	31	31	31	31	31	31	31	31	31	31	31	31		32	33	34	35	36	37	38	39	40	
Neanderthal 19	107	51	40	33	32	32	32	32	32	32	32	32	32	32	32	32	32	32		33	34	35	36	37	38	39	40	
Neanderthal 20	108	52	41	34	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33		34	35	36	37	38	39	40
Neanderthal 21	109	53	42	35	34	34	34	34	34	34	34	34	34	34	34	34	34	34	34	34	34		35	36	37	38	39	40
Neanderthal 22	110	54	43	36	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35		36	37	38	39	40
Neanderthal 23	111	55	44	37	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36		37	38	39	40
Neanderthal 24	112	56	45	38	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37		38	39	40
Neanderthal 25	113	57	46	39	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38		39	40
Neanderthal 26	114	58	47	40	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39		40
Neanderthal 27	115	59	48	41	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	

Fig. S4. Number of pairwise nucleotide differences between the mtDNAs of A) 7 Denisovan individuals and B) 27 Neanderthals individuals. Positions containing gaps and missing data between each pair were excluded from the analysis

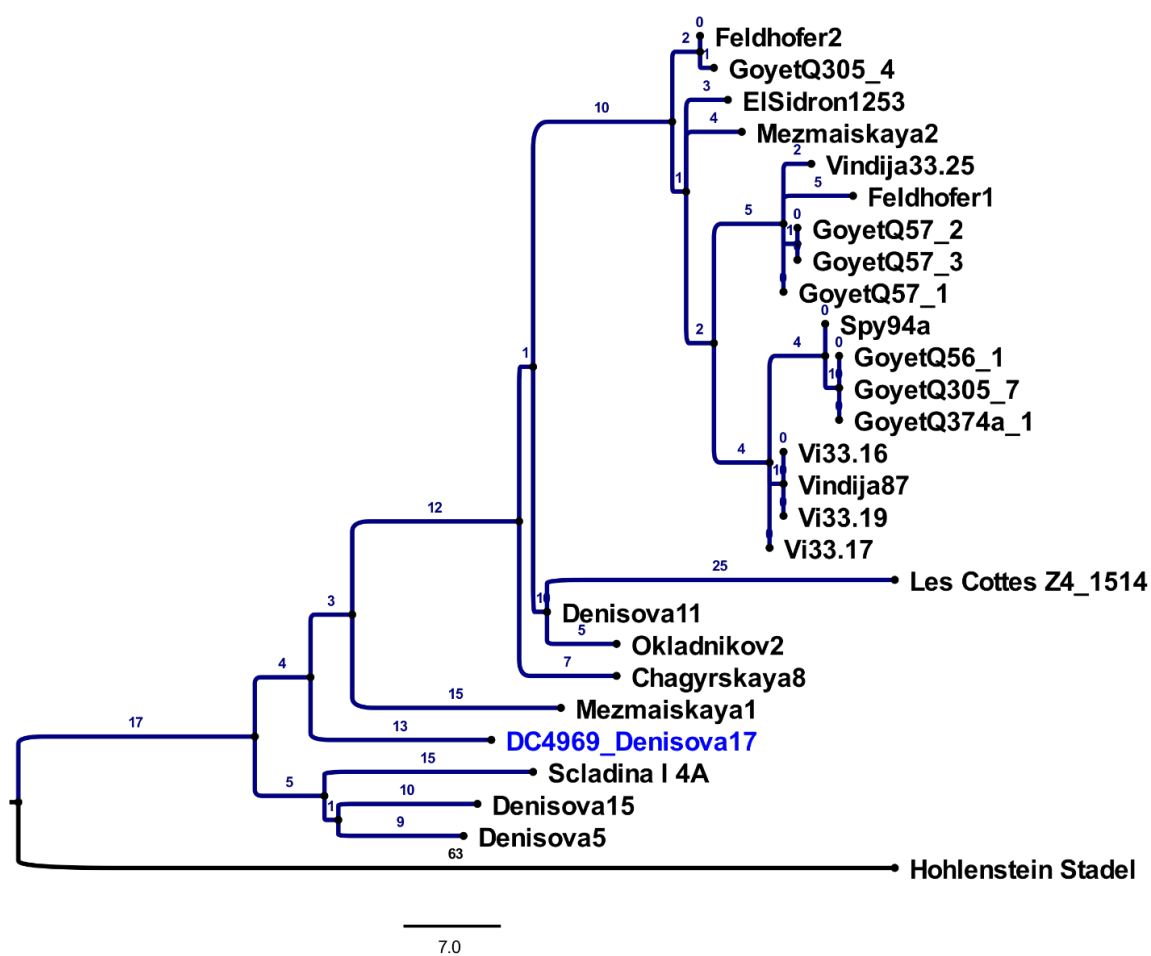
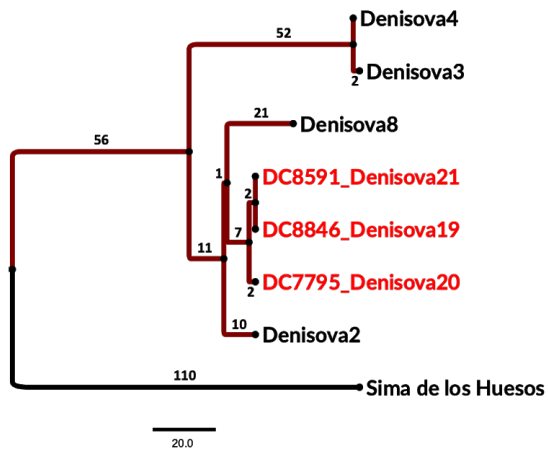


Fig. S5. Consensus parsimony tree obtained by 50% majority rule of 16 equally parsimonious trees relating the mtDNA of Denisova 17 to 26 other Neanderthal mtDNAs. The highly divergent mtDNA of the Hohlenstein Stadel Neanderthal was used as an outgroup. The tree is drawn to scale, with branch lengths computed in number of substitutions.

A)



B)

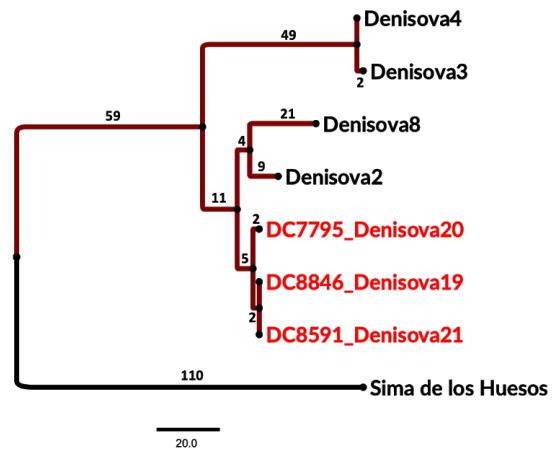


Fig. S6. The two equally parsimonious trees relating the mtDNA of Denisova 19, 20 and 21 to 4 other Denisovan mtDNAs. The mtDNA of the Middle Pleistocene hominin from Sima de los Huesos was used as an outgroup. The trees are drawn to scale, with branch lengths computed in number of substitutions.

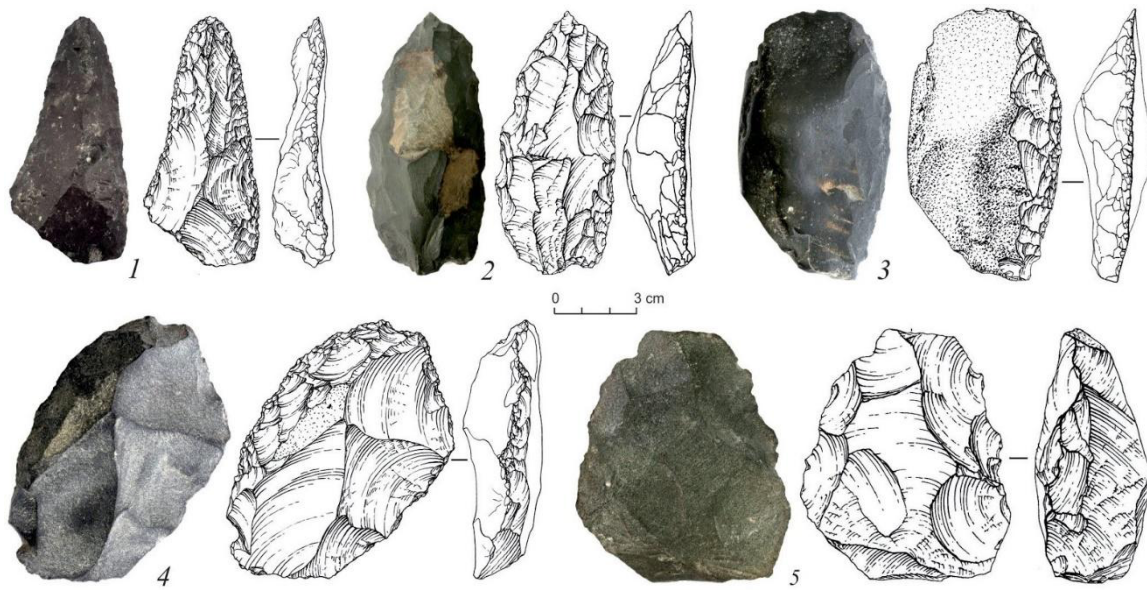


Fig. S7. Illustrations and photographs of artifacts recovered from the Early Middle Paleolithic layers 15-14 attributed to the arrival of Denisovans at Denisova Cave.

Table S1. Number of bones analysed per stratigraphic layer of the East Chamber of Denisova Cave. Layers 10 and 16 are culturally sterile hence no bones were analysed from these.

Stratigraphic Layer	Number of fragmented bones analysed
9.2	89
9.3	196
11.2	566
11.3	20
11.4	353
12	425
13	27
14	1096
15	967
17.1	52
Total	3791

Table S2. Peptide markers¹⁷ used to identify DC4969, DC7277, DC7795, DC8846, and DC8591 as Hominidae.

DC #	ID	COL1a1 508–519	COL1a2 978–990	COL1a2 978–990'	COL1a2 484–498	COL1a2 502–519	COL1a2 292–309	COL1a2 793–816	COL1a2 454–483	COL1a1 586–618	COL1a1 586–618'	COL1a2 757–789	COL1a2 757–789'
4969	Denisova 17	1105.6			1477.7			2115.1		2869.8	2885.3		
7277	Denisova 18	1105.6			1477.4	1580.4		2114.6		2869.9	2885.2		
7795	Denisova 20	1105.6		1235.7	1477.8	1580.8		2115.2		2869.5	2885.5		
8846	Denisova 19	1105.6		1235.6	1477.8	1580.8		2115.2		2869.5	2885.4	2957.6	
8591	Denisova 21	1105.6		1235.6	1477.8	1580.8	1619.8	2115.1		2869.5	2885.4		

Table S3. Summary of the sequence data generated from the libraries that were enriched for hominin mtDNA. The statistics are based on alignment of sequences to the human mtDNA reference sequence (rCRS). Negative controls are included at the bottom of the table.

DC ID	Specimen ID	Sample ID	Extract ID	Library ID	Bleach pre-treated	#library molecules	Unique Mapped fragments >= 35 bp	Duplication rate	5' C to T frequency	3' C to T frequency
DC4969	<i>Denisova 17</i>	SP9673	E16281	B31446	No	1.9E+09	216,937	2.22	32.9	26.4

DC4969	Denisova 17	SP9673	E16282	B31447	No	1.1E+10	341,557	1.78	31.8	25.9
DC4969	Denisova 17	SP9673	E16283	B31448	No	5.6E+09	296,352	1.95	32.8	26.5
DC7277	Denisova 18	SP9674	E16285	B31449	No	3.4E+10	8,493	31.69	9.0	5.8
DC7277	Denisova 18	SP9674	E16286	B31450	No	2.7E+10	4,513	41.57	20.6	12.3
DC7277	Denisova 18	SP9674	E17773	B35203	Yes	1.8E+08	3,352	40.53	0.8	0.7
DC7277	Denisova 18	SP9674	E17774	B35204	Yes	1.6E+08	5,343	64.43	1.5	1.2
DC7277	Denisova 18	SP9674	E17775	B35205	No	2.3E+10	330	1.63	34.3	17.9
DC8846	Denisova 19	SP9677	E16293	B31457	No	8.0E+09	3,294	76.55	40.9	30.6
DC8846	Denisova 19	SP9677	E16294	B31458	No	8.0E+09	6,025	58.19	37.2	29.4
DC8846	Denisova 19	SP9677	E16295	B31459	No	1.3E+10	6,525	31.66	42.3	29.0
DC8846	Denisova 19	SP9677	E17785	B35215	Yes	5.5E+08	371	16.87	31.6	38.8
DC8846	Denisova 19	SP9677	E17786	B35216	Yes	1.6E+08	794	36.07	46.1	41.4
DC8846	Denisova 19	SP9677	E17787	B35217	No	6.4E+09	2,518	1.91	38.0	35.7
DC7795	Denisova 20	SP9675	E16287	B31451	No	1.5E+10	5,323	43.92	43.5	35.4
DC7795	Denisova 20	SP9675	E16288	B31452	No	1.4E+10	8,247	35.57	39.7	31.6
DC7795	Denisova 20	SP9675	E16289	B31453	No	1.6E+10	18,675	15.76	43.6	36.6
DC7795	Denisova 20	SP9675	E17776	B35206	Yes	1.1E+09	853	10.61	52.7	46.2
DC7795	Denisova 20	SP9675	E17777	B35207	No	1.6E+10	2,670	1.79	52.0	47.8
DC7795	Denisova 20	SP9675	E17778	B35208	Yes	3.6E+08	203	21.25	43.8	30.0
DC7795	Denisova 20	SP9675	E17779	B35209	No	8.6E+09	459	1.39	51.2	43.2
DC7795	Denisova 20	SP9675	E17780	B35210	Yes	1.9E+09	255	5.79	20.5	10.0
DC7795	Denisova 20	SP9675	E17781	B35211	No	1.5E+10	1,047	2.18	53.2	47.0
DC8591	Denisova 21	SP9676	E16290	B31454	No	1.6E+10	11,261	30.76	23.5	17.4
DC8591	Denisova 21	SP9676	E16291	B31455	No	1.1E+10	17,626	12.05	45.0	37.6
DC8591	Denisova 21	SP9676	E16292	B31456	No	1.0E+10	8,005	16.43	19.7	12.6
DC8591	Denisova 21	SP9676	E17782	B35212	Yes	3.8E+08	493	35.25	32.0	18.9
DC8591	Denisova 21	SP9676	E17783	B35213	Yes	2.1E+08	262	94.23	50.0	38.2
DC8591	Denisova 21	SP9676	E17784	B35214	No	8.6E+09	1,305	2.68	45.4	32.2
control	control	control	E17788	B35218	Yes	1.3E+08	73	4.42	0	0
control	control	control	-	B31445	No	8.0E+07	209	342.01	0	5.9

Library molecules = qPCR estimates of the number of unique molecules in each library; Unique mapped fragments = number of DNA sequences with a map quality score of 25 or greater retained after duplicate removal;
Duplication rate = Average number of duplicates per unique sequence

Table S4. Summary of the sequence data used to call the mtDNA consensus sequences of *Denisova 17, 18, 19, 20* and *21*.

DC ID	Sample ID	Library ID	ref. sequence	Unique Mapped fragments >= 35 bp	Duplication rate	mtDNA coverage (x)	Human Contamination Estimate	5' C to T frequency	3' C to T frequency	#deaminated fragments	mtDNA coverage deaminated (x)
DC4969	<i>Denisova 17</i>	B31446	<i>Vindija3325</i>	231,199	2.2	774	2.5%	32.9	26.1	46583	165
DC4969	<i>Denisova 17</i>	B31447	<i>Vindija3325</i>	361,920	1.8	1238	0.1%	31.6	25.4	69016	256
DC4969	<i>Denisova 17</i>	B31448	<i>Vindija3325</i>	313,924	2.0	1060	0.1%	32.8	26.2	61211	221
DC7277	<i>Denisova 18</i>	B31449	<i>Denisova8</i>	8,777	31.8	33	68.6%	11.9	8	676	2.5
DC7277	<i>Denisova 18</i>	B31450	<i>Denisova8</i>	4,634	39.6	19	37.6%	23	12.6	488	1.8
DC7277	<i>Denisova 18</i>	B35203	<i>Denisova8</i>	3,270	40.1	14	87.8%	2.4	3.1	118	0.5
DC7277	<i>Denisova 18</i>	B35204	<i>Denisova8</i>	5,211	63.1	20	89.4%	3.4	2.9	201	0.8
DC7277	<i>Denisova 18</i>	B35205	<i>Denisova8</i>	323	1.6	1	45.9%	35.5	15.7	51	0.2
DC8846	<i>Denisova 19</i>	B31457	<i>Denisova8</i>	3,576	74.4	12	0.0%	43.1	30.5	855	3.1
DC8846	<i>Denisova 19</i>	B31458	<i>Denisova8</i>	6,327	57.8	23	0.0%	38.4	28.5	1392	5.1
DC8846	<i>Denisova 19</i>	B31459	<i>Denisova8</i>	7,073	31.2	25	6.5%	42.4	29.8	1622	5.8
DC8846	<i>Denisova 19</i>	B35215	<i>Denisova8</i>	383	16.1	1	34.1%	36.6	41.9	96	0.3
DC8846	<i>Denisova 19</i>	B35216	<i>Denisova8</i>	821	35.0	3	10.9%	46.1	38.4	251	0.9
DC8846	<i>Denisova 19</i>	B35217	<i>Denisova8</i>	2,561	1.9	9	4.8%	38.6	34.8	636	2.4
DC7795	<i>Denisova 20</i>	B31451	<i>Denisova8</i>	5,814	43.3	19	8.6%	44.2	34.7	1508	4.9
DC7795	<i>Denisova 20</i>	B31452	<i>Denisova8</i>	8,696	35.3	29	5.1%	41.4	33.3	2197	7.3
DC7795	<i>Denisova 20</i>	B31453	<i>Denisova8</i>	20,233	15.6	67	2.9%	46	38.2	6252	21.2
DC7795	<i>Denisova 20</i>	B35206	<i>Denisova8</i>	860	10.1	3	1.1%	51	49.1	291	0.9
DC7795	<i>Denisova 20</i>	B35207	<i>Denisova8</i>	2,719	1.8	8	4.0%	53.3	47.5	1006	3.1
DC7795	<i>Denisova 20</i>	B35208	<i>Denisova8</i>	204	20.7	1	21.7%	45.2	35.5	61	0.2
DC7795	<i>Denisova 20</i>	B35209	<i>Denisova8</i>	462	1.4	1	0.0%	55.7	41.7	144	0.4
DC7795	<i>Denisova 20</i>	B35210	<i>Denisova8</i>	243	5.7	1	62.4%	26.1	15.3	39	0.1
DC7795	<i>Denisova 20</i>	B35211	<i>Denisova8</i>	1,053	2.1	3	14.8%	53.9	49.4	360	1.1
DC8591	<i>Denisova 21</i>	B31454	<i>Denisova8</i>	11,874	30.9	46	30.2%	26.3	19.6	1649	6.1

DC8591	<i>Denisova 21</i>	B31455	<i>Denisova8</i>	19,149	12.1	65	0.1%	45.6	38.1	5351	19.1
DC8591	<i>Denisova 21</i>	B31456	<i>Denisova8</i>	8,339	16.3	33	48.9%	24.1	14.7	1089	4.0
DC8591	<i>Denisova 21</i>	B35212	<i>Denisova8</i>	495	33.7	2	39.0%	32.8	15.4	73	0.2
DC8591	<i>Denisova 21</i>	B35213	<i>Denisova8</i>	266	93.5	1	45.3%	48.3	45.5	73	0.2
DC8591	<i>Denisova 21</i>	B35214	<i>Denisova8</i>	1,311	2.6	4	27.8%	44.2	32.7	332	1.1

Unique mapped fragments = number of DNA sequences with a map quality score of 25 or greater retained after duplicate removal; Duplication rate = Average number of duplicates per unique sequence

Table S5. Inferred number of substitutions on the branches leading to the mtDNA sequences of Neanderthals individuals from Denisova Cave since their last common to the exclusion of the mtDNA of the *Hohlenstein Stadel* Neanderthal and their approximate relative molecular ages.

mtDNA type	Specimen	Substitutions since common ancestor	Comparison of inferred molecular age (Mutation rate: 2.53×10^{-8} (95% HPD: 1.76×10^{-8} - 3.23×10^{-8}))
Neanderthal mtDNA	<i>Denisova 17</i>	17	3.7-6.8 ky younger than <i>Denisova 5</i> 1.9-3.4 ky younger than <i>Denisova 15</i> 7.4-13.7 ky older than <i>Denisova 11</i>
	<i>Denisova 5</i>	15	1.9-3.4 ky older than <i>Denisova 15</i> 11.2-20.5 ky older than <i>Denisova 11</i>
	<i>Denisova 15</i>	16	9.3-17.1 ky older than <i>Denisova 11</i>
	<i>Denisova 11</i>	21	-

Table S6. Inferred number of substitutions on the branches leading to the mtDNA sequences of Denisovan individuals since their last common ancestor to the exclusion of the mtDNA of the Middle Pleistocene Hominin from Sima de los Huesos and their approximate relative molecular ages.

mtDNA type	Specimen	Substitutions since common ancestor		Comparison of inferred molecular age (Mutation rate: 2.53x10 ⁻⁸ (95% HPD: 1.76x10 ⁻⁸ - 3.23x10 ⁻⁸))	
		Scenario 2	Scenario 1	Scenario 2	Scenario 1
Denisovan mtDNA	<i>Denisova 19</i>	21	18	similar age to DC7795 <i>Denisova 20</i> similar age to DC8591 <i>Denisova 21</i> similar age to <i>Denisova 2</i> 22.4-41.1 ky older than <i>Denisova 8</i> 57.9-106.3 ky older than <i>Denisova 4</i> 61.6-113.2 ky older than <i>Denisova 3</i>	similar age to DC7795 <i>Denisova 20</i> similar age to DC8591 <i>Denisova 21</i> 11.2-20.6 ky older than <i>Denisova 2</i> 33.6-61.7 ky older than <i>Denisova 8</i> 57.9-106.3 ky older than <i>Denisova 4</i> 61.6-113.2 ky older than <i>Denisova 3</i>
	<i>Denisova 20</i>	21	18	similar age to DC8591 <i>Denisova 21</i> similar age to <i>Denisova 2</i> 22.4-41.1 ky older than <i>Denisova 8</i> 57.9-106.3 ky older than <i>Denisova 4</i> 61.6-113.2 ky older than <i>Denisova 3</i>	similar age to DC8591 <i>Denisova 21</i> 11.2-20.6 ky older than <i>Denisova 2</i> 33.6-61.7 ky older than <i>Denisova 8</i> 57.9-106.3 ky older than <i>Denisova 4</i> 61.6-113.2 ky older than <i>Denisova 3</i>
	<i>Denisova 21</i>	21	18	similar age to <i>Denisova 2</i> 22.4-41.1 ky older than <i>Denisova 8</i> 57.9-106.3 ky older than <i>Denisova 4</i> 61.6-113.2 ky older than <i>Denisova 3</i>	11.2-20.6 ky older than <i>Denisova 2</i> 33.6-61.7 ky older than <i>Denisova 8</i> 57.9-106.3 ky older than <i>Denisova 4</i> 61.6-113.2 ky older than <i>Denisova 3</i>
	<i>Denisova 2</i>	21	24	22.4-41.1 ky older than <i>Denisova 8</i> 57.9-106.3 ky older than <i>Denisova 4</i> 61.6-113.2 ky older than <i>Denisova 3</i>	22.4-41.1 ky older than <i>Denisova 8</i> 46.7-85.7 ky older than <i>Denisova 4</i> 50.4-92.6 ky older than <i>Denisova 3</i>
	<i>Denisova 8</i>	33	36	35.5-65.2 ky older than <i>Denisova 4</i> 39.2-72.0 ky older than <i>Denisova 3</i>	24.3-44.6 ky older than <i>Denisova 4</i> 28.0-51.4 ky older than <i>Denisova 3</i>
	<i>Denisova 4</i>	52	49	3.7-6.8 ky older than <i>Denisova 3</i>	3.7-6.8 ky older than <i>Denisova 3</i>
	<i>Denisova 3</i>	54	51	-	-

Table S7. Bayesian estimates of the molecular age (with 95% HPD) of the newly identified individuals from Denisova Cave and other undated archaic individuals using both the complete mitochondrial sequences and the mitochondrial coding region sequences only.

Sample	mtDNA type	Priors (CI)	Bayesian Age Estimates - complete mtDNA (95% HPD)	Bayesian Age Estimates - coding region (95% HPD)
TMRCAs Human/Chimp	-	-	5,751,500 (4,007,300 - 7,489,100)	6,193,500 (4,912,200 - 7,558,100)
TMRCAs Denisovan-Sima/Neandertal-ModernHuman	-	-	670,525 (543,813 - 848,881)	909,301 (750,575 - 1,080,678)
TMRCAs Denisovan/Sima	-	-	571,723 (457,635 - 705,789)	716,044 (576,661 - 864,135)
TMRCAs Neandertal/ModernHuman	-	-	397,110 (282,930 - 502,280)	526,889 (445,135 - 608,394)
TMRCAs Denisovan	-	-	273,620 (207,670 - 339,100)	292,190 (235,040 - 341,510)
TMRCAs Neandertal	-	-	259,860 (185,560 - 331,310)	297,110 (235,070 - 360,120)
TMRCAs Modern Human	-	-	141,940 (86,257-186,750)	156,690 (121,180 - 193,120)
<i>Chagyrskaya8</i>	Neandertal	115,000 (30,000- 200,000)	85,138 (58,086 - 113,400)	85,766 (57,261 - 115,870)
<i>Denisova5</i>	Neandertal	115,000 (30,000- 200,000)	134,110 (91,603 - 179,380)	143,390 (100,280 - 187,660)
<i>Denisova11</i>	Neandertal	115,000 (30,000- 200,000)	101,820 (77,209 - 127,730)	103,430 (75,892 - 131,000)
<i>Denisova15</i>	Neandertal	115,000 (30,000- 200,000)	134,340 (91,294 - 178,730)	139,360 (95,510 - 183,340)
<i>Denisova17</i>	Neandertal	115,000 (30,000- 200,000)	132,540 (92,223 - 175,520)	134,870 (94,600 - 177,770)
<i>GoyetQ305_7</i>	Neandertal	115,000 (30,000- 200,000)	40,588 (33,826 - 45,651)	40,308 (32,177 - 46,603)
<i>GoyetQ374a_1</i>	Neandertal	115,000 (30,000- 200,000)	40,602 (33,888 - 45,788)	40,328 (32,034 - 46,470)
<i>Hohlenstein_Stadel</i>	Neandertal	115,000 (30,000- 200,000)	119,460 (66,453 - 177,450)	141,460 (89,164 - 199,910)
<i>Mezmaiskaya1</i>	Neandertal	115,000 (30,000- 200,000)	115,420 (78,148 - 155,280)	108,490 (68,582 - 149,800)
<i>Okladnikov2</i>	Neandertal	115,000 (30,000- 200,000)	89,573 (65,668 - 116,550)	96,404 (67,993 - 126,060)
<i>Sladina_I_4A</i>	Neandertal	115,000 (30,000- 200,000)	122,230 (80,993 - 167,170)	131,780 (87,928 - 176,670)
<i>Vindija33.17</i>	Neandertal	115,000 (30,000- 200,000)	49,380 (41,964 - 57,619)	49,949 (40,383 - 58,989)
<i>Vindija33.19</i>	Neandertal	115,000 (30,000- 200,000)	44,890 (37,466 - 51,782)	44,461 (35,029 - 52,457)
<i>Vindija33.25</i>	Neandertal	115,000 (30,000- 200,000)	43,877 (33,208 - 53,478)	44,145 (34,017 - 54,223)
<i>Denisova2</i>	Denisovan	165,000 (30,000- 300,000)	238,080 (182,370 - 299,790)	250,400 (199,620 - 300,000)
<i>Denisova3</i>	Denisovan	65,000 (30,000- 100,000)	81,271 (56,029 - 99,999)	80,422 (55,773 - 99,998)
<i>Denisova4</i>	Denisovan	165,000 (30,000- 300,000)	86,538 (57,188 - 110,650)	84,728 (55,449 - 109,870)
<i>Denisova8</i>	Denisovan	165,000 (30,000- 300,000)	223,310 (160,820 - 288,720)	225,920 (163,680 - 286,480)
<i>Denisova20</i>	Denisovan	165,000 (30,000- 300,000)	227,060 (165,250 - 285,180)	255,790 (208,690 - 299,840)
<i>Denisova21</i>	Denisovan	165,000 (30,000- 300,000)	243,900 (164,250 - 284,280)	247,660 (196,940 - 293,640)
<i>Denisova19</i>	Denisovan	165,000 (30,000- 300,000)	224,350 (162,900 - 282,940)	247,630 (195,950 - 292,940)
<i>Sima de los Huesos</i>	Sima de los Huesos	520,000 (260,000- 780,000)	407,860 (272,200 - 545,910)	406,450 (273,220 - 546,850)

Table S8. Bayesian estimates of the divergence date (with 95% HPD) between each hominin group using as calibration priors for the previously published individuals from Denisova Cave their inferred ages by Bayesian age modelling and for *Denisova 17* and *Denisova 19, 20* and *21* the optical dates of the layers 15 and 12 of the East Chamber, respectively.

Sample	mtDNA type	Priors (CI)	Bayesian Age Estimates - complete mtDNA (95% HPD)	Bayesian Age Estimates - coding region (95% HPD)
TMRCa Human/Chimp	-	-	6,011,100 (4,735,700 - 7,359,700)	5,936,600 (4,824,100 - 7,054,200)
TMRCa Denisovan-Sima/Neandertal-ModernHuman	-	-	767,529 (642,676 - 914,761,700)	850,275 (720,313 - 996,772)
TMRCa Denisovan/Sima	-	-	614,280 (507,590 - 729,070)	648,120 (535,830 - 762,610)
TMRCa Neandertal/ModernHuman	-	-	411,270 (339,020 - 486,530)	433,767 (355,616 - 508,283)
TMRCa Denisovan	-	-	237,040 (211,150 - 265,660)	229,010 (206,700 - 252,690)
Divergence <i>Denisova 2</i>	-	-	74,075 (61,146 - 85,675)	71,911 (59,676 - 85,145)
Divergence <i>Denisova 3</i>	-	-	205,456 (203,238 - 239,508)	208,652 (180,078 - 237,333)
Divergence <i>Denisova 4</i>	-	-	198,779 (193,228 - 219,043)	201,836 (189,451 - 215,259)
Divergence <i>Denisova 8</i>	-	-	207,861 (182,129 - 235,948)	212,995 (197,620 - 228,080)
Divergence <i>Denisova 19-21</i>	-	-	264,040 (220,520 - 311,510)	282,840 (234,890 - 332,330)
Divergence <i>Denisova 20</i>	-	-	152,790 (123,630 - 185,120)	150,420 (120,960 - 180,550)
TMRCa Neandertal	-	-	264,040 (220,520 - 311,510)	282,840 (234,890 - 332,330)
TMRCa ModernHuman	-	-	152,790 (123,630 - 185,120)	150,420 (120,960 - 180,550)
Chagyrskaya8	Neandertal	115,000 (30,000- 200,000)	84,387 (61,320 - 107,160)	82,326 (58,767 - 106,240)
Denisova5	Neandertal	110,000 (90,900- 130,000)	118,95 (102,850 - 130,000)	118,890 (101,970 - 130,000)
Denisova11	Neandertal	100,000 (79,300- 118,100)	101,880 (87,864 - 118,030)	99,207 (82,787 - 116,620)
Denisova15	Neandertal	110,000 (91,400- 130,300)	119,270 (103,330 - 130,300)	117,190 (99,476 - 130,300)
Denisova17	Neandertal	140,000 (130,000- 150,300)	137,090 (130,000 - 147,220)	136,890 (130,000 - 147,020)
GoyetQ305_7	Neandertal	115,000 (30,000- 200,000)	40,506 (33,617 - 45,778)	40,400 (32,503 - 46,413)
GoyetQ374a_1	Neandertal	115,000 (30,000- 200,000)	40,493 (33,609 - 45,850)	40,399 (32,619 - 46,445)
Hohlenstein_Stadel	Neandertal	115,000 (30,000- 200,000)	116,940 (65,736 - 167,620)	135,510 (81,106 - 193,770)
Mezmaiskaya1	Neandertal	115,000 (30,000- 200,000)	112,720 (86,631 - 138,640)	101,990 (70,982 - 131,310)
Okladnikov2	Neandertal	115,000 (30,000- 200,000)	89,161 (69,935 - 109,060)	92,356 (70,204 - 114,840)
Scladina_I_4A	Neandertal	115,000 (30,000- 200,000)	111,930 (84,389 - 138,230)	114,420 (84,770 - 142,670)
Vindija33.17	Neandertal	115,000 (30,000- 200,000)	49,669 (42,204 - 57,899)	49,580 (40,896 - 57,998)
Vindija33.19	Neandertal	115,000 (30,000- 200,000)	44,928 (37,238 - 51,933)	44,379 (35,505 - 51,870)
Vindija33.25	Neandertal	115,000 (30,000- 200,000)	43,811 (32,981 - 53,635)	43,933 (34,189 - 53,712)
Denisova2	Denisovan	160,000 (122,700- 194,400)	184,420 (150,270 - 194,400)	185,720 (148,070 - 194,400)
Denisova3	Denisovan	62,500 (51,600- 76,200)	66,420 (54,965 - 76,199)	64,913 (54,192 - 76,198)
Denisova4	Denisovan	70,000 (55,200- 84,100)	71,331 (59,035 - 84,043)	68,384 (55,200 - 80,196)
Denisova8	Denisovan	120,000 (105,600- 136,400)	129,38 (116,090 - 136,400)	127,350 (111,960 - 136,400)
Denisova20	Denisovan	200,000 (187,000- 216,900)	198,080 (187,000 - 211,270)	203,180 (190,280 - 216,890)
Denisova21	Denisovan	200,000 (187,000- 216,900)	196,450 (187,000 - 209,260)	198,420 (187,000 - 211,520)
Denisova19	Denisovan	200,000 (187,000- 216,900)	196,420 (187,000 - 209,070)	198,390 (187,000 - 211,440)
Sima de los Huesos	Sima de los Huesos	490,000 (260,000- 780,000)	382,070 (272,480 - 492,230)	346,900 (227,650 - 468,400)

Table S9. Mitochondrial DNA genomes used for the genetic analyses. The age in CalBP corresponds to the radiocarbon date of the specimen as reported in the accession number corresponding reference.

Sample	Accession Number	mtDNA type	Origin (country)	Age in CalBP (95% CI)
<i>Pan troglodytes</i>	NC_001643	Chimpanzee	-	-
53 present day	AF346963 to AF347015	Modern Human	Worldwide	0
<i>Kostenki14</i>	FN600416	ancient Modern Human	Russia	37,473 (36,262- 38,684)
<i>Oase</i>	Fu et al. 2014 (18)	ancient Modern Human	Romania	39,536 (41,761 - 37,311)
<i>Salkhit</i>	MT561166	ancient Modern Human	Mongolia	34,425 (33,900 - 34,950)
<i>Sunghir3</i>	Sikora et al. 2017 (19)	ancient Modern Human	Russia	34,093 (33,031 - 35,154)
<i>Tianyuan</i>	KC417443	ancient Modern Human	China	39,008 (37,761- 40,254)
<i>Ust_Ishim</i>	Fu et al. 2015 (9)	ancient Modern Human	Russia	45,045 (43,212- 46,878)
<i>BS11</i>	KC521454	ancient Modern Human	China	8,182 (8,048 - 8,316)
<i>Loschbour</i>	KC521455	ancient Modern Human	Luxembourg	8,054 (7,948 – 8,160)
<i>IceMan</i>	Ermini & al. 2008 (20)	ancient Modern Human	Italy	5,191 (5,067 – 5,315)
<i>Eskimo_Saqqaq</i>	EU725621	ancient Modern Human	Greenland	4,603 (4,423 – 4,783)
<i>Oberkassel998</i>	KC521457	ancient Modern Human	Germany	14,130 (13,758 – 14,501)
<i>DolniVestonice13</i>	KC521459	ancient Modern Human	Czech Republic	30,868 (30,666 – 31,070)
<i>DolniVestonice14</i>	KC521458	ancient Modern Human	Czech Republic	30,868 (30,666 – 31,070)
<i>BLA7</i>	KF523402	ancient Modern Human	Germany	5,571 (5,488 – 5,653)
<i>BLA20</i>	KF523407	ancient Modern Human	Germany	10,577 (10,441 – 10,713)
<i>Brillenhohle</i>	KU534947	ancient Modern Human	Germany	14,640 (14,245 – 15,034)
<i>GoyetQ55-2</i>	KU534948	ancient Modern Human	Belgium	27,521 (27,311 – 27,731)
<i>GoyetQ116-1-3</i>	KU534952	ancient Modern Human	Belgium	34,801 (34,436 – 35,166)
<i>HohleFels79</i>	KU534962	ancient Modern Human	Germany	14,669 (14,267 – 15,071)
<i>HohlensteinStadel</i>	KU534979	ancient Modern Human	Germany	8,713 (8,448 – 8,713)
<i>Chagyrskaya8</i>	MK388903	Neandertal	Russia	NA
<i>Denisova5</i>	KC879692	Neandertal	Russia	NA
<i>Denisova11</i>	KU131206	Neandertal	Russia	NA
<i>Denisova15</i>	MK033602	Neandertal	Russia	NA
<i>Denisova17</i>	MT576650	Neandertal	Russia	NA
<i>ElSidron1253</i>	FM865409	Neandertal	Spain	39,450 (32,600 – 46, 300)
<i>Feldhofer1</i>	FM865407	Neandertal	Germany	43,707 (42,670-44,744)
<i>Feldhofer2</i>	FM865408	Neandertal	Germany	43,268 (42,193-44,342)
<i>GoyetQ305_4</i>	KX198087	Neandertal	Belgium	44,236 (43,386-45,085)
<i>GoyetQ305_7</i>	KX198086	Neandertal	Belgium	NA
<i>GoyetQ374a_1</i>	KX198085	Neandertal	Belgium	NA
<i>GoyetQ56_1</i>	KX198082	Neandertal	Belgium	42,515 (42,063-42,967)
<i>GoyetQ57_1</i>	KX198082	Neandertal	Belgium	44,696 (43,834-45,558)
<i>GoyetQ57_2</i>	KX198088	Neandertal	Belgium	41,185 (40,595-41,775)
<i>GoyetQ57_3</i>	KX198083	Neandertal	Belgium	42,407 (41,946-42,867)
<i>Hohlenstein_Stadel</i>	KY751400	Neandertal	Germany	NA
<i>LesCottesZ4_1514</i>	MG025536	Neandertal	France	43,230 (42,720-43,740)
<i>Mezmaiskaya1</i>	FM865411	Neandertal	Russia	NA
<i>Mezmaiskaya2</i>	MG025537	Neandertal	Russia	43,834 (42,038-45,630)
<i>Okladnikov2</i>	KF982693	Neandertal	Russia	NA
<i>Scladina_I_4A</i>	MK123269	Neandertal	Belgium	NA
<i>Spy94a</i>	MG025538	Neandertal	Belgium	40,463 (39,840-41,085)

<i>Vindija33.16</i>	NC_011137	Neandertal	Croatia	43,707 (39,234-48,179)
<i>Vindija33.17</i>	KJ533544	Neandertal	Croatia	NA
<i>Vindija33.19</i>	KJ533545	Neandertal	Croatia	NA
<i>Vindija33.25</i>	FM865410	Neandertal	Croatia	NA
<i>Vindija87</i>	MG025539	Neandertal	Croatia	NA
<i>Denisova2</i>	KX663333	Denisovan	Russia	NA
<i>Denisova3</i>	NC_013993	Denisovan	Russia	NA
<i>Denisova4</i>	FR695060	Denisovan	Russia	NA
<i>Denisova8</i>	KT780370	Denisovan	Russia	NA
<i>Denisova19</i>	MT576651	Denisovan	Russia	NA
<i>Denisova20</i>	MT576652	Denisovan	Russia	NA
<i>Denisova21</i>	MT576653	Denisovan	Russia	NA
<i>Sima de los Huesos</i>	NC_023100	Sima de los Huesos	Spain	NA

Dataset S1.

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>

Dataset S2.

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>

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