

## Supplementary material

### SUPPLEMENTARY TEXT A: Method and results of the sex determination of the two individuals

Poor preservation of the skeletal remains did not permit DNA extraction; therefore, sex determination was obtained by liquid chromatography-mass spectrometry analysis of the tooth enamel peptides (Stewart et al., 2016; Ziganshin et al., in press). The maturation of tooth enamel occurs with the participation of a number of proteins secreted by specialized cells: ameloblasts. Amelogenins are major structural proteins that represent 90% of the tooth enamel organic matrix protein matter. Amelogenin genes are located in both the X-chromosome (AMELX gene) and the Y-chromosome (AMELY). The protein structures encoded by

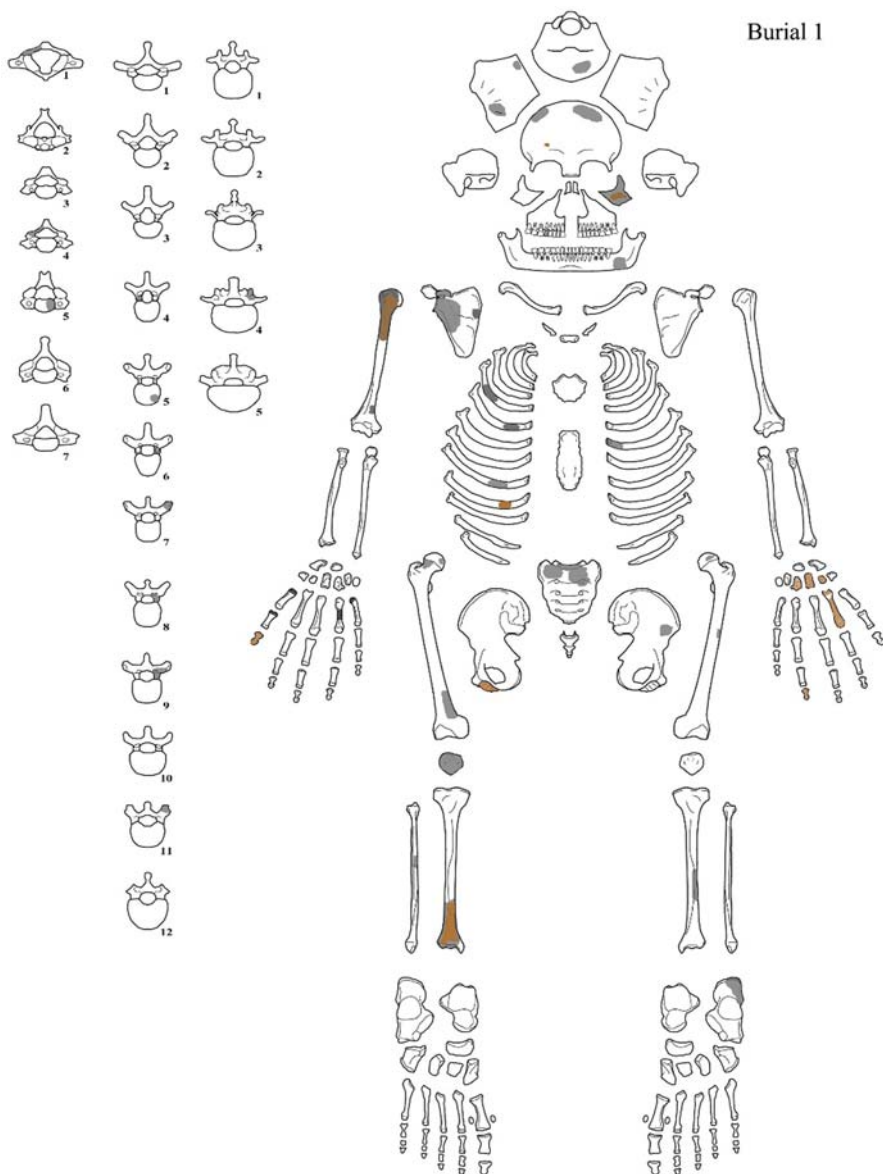
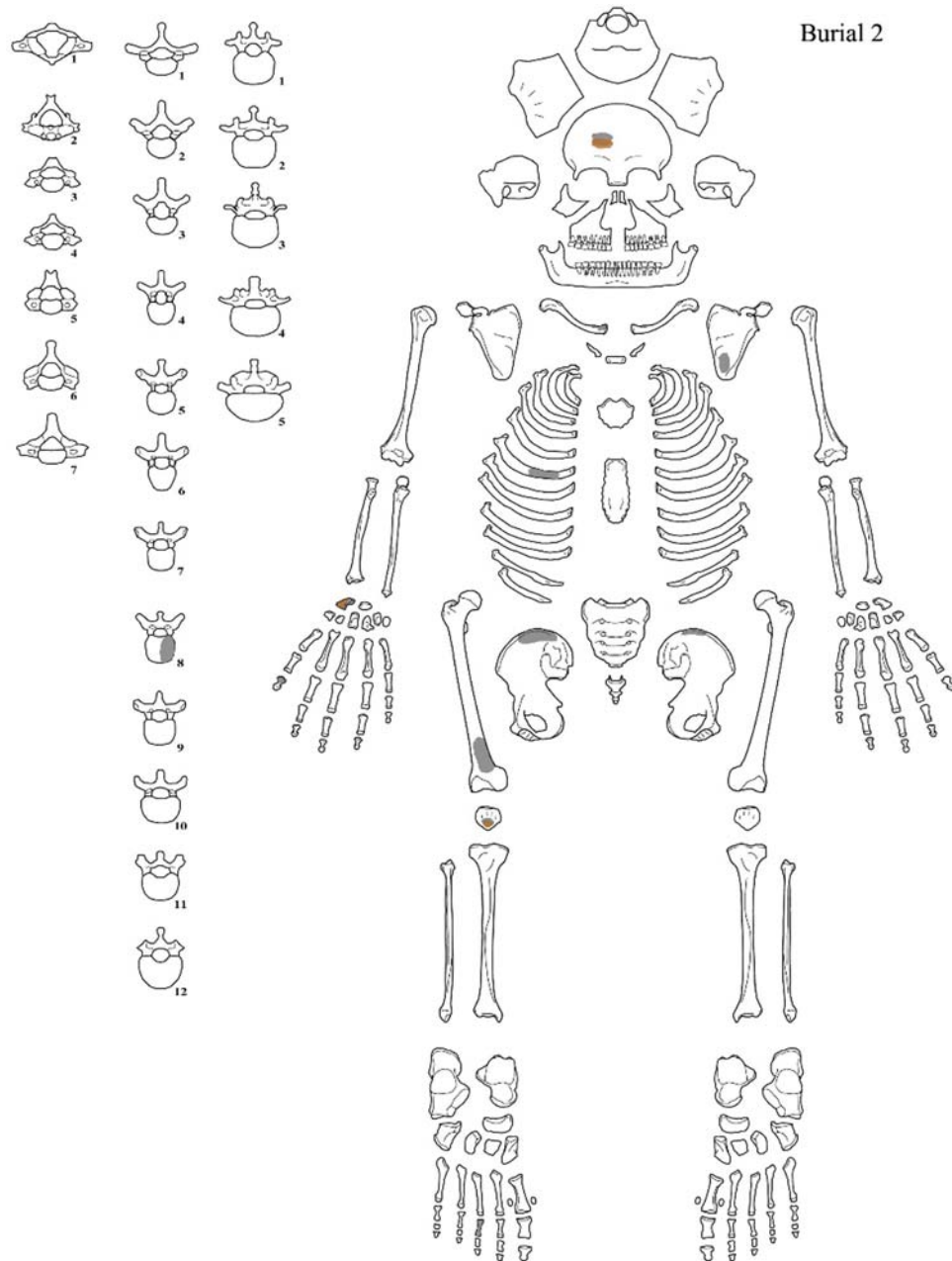


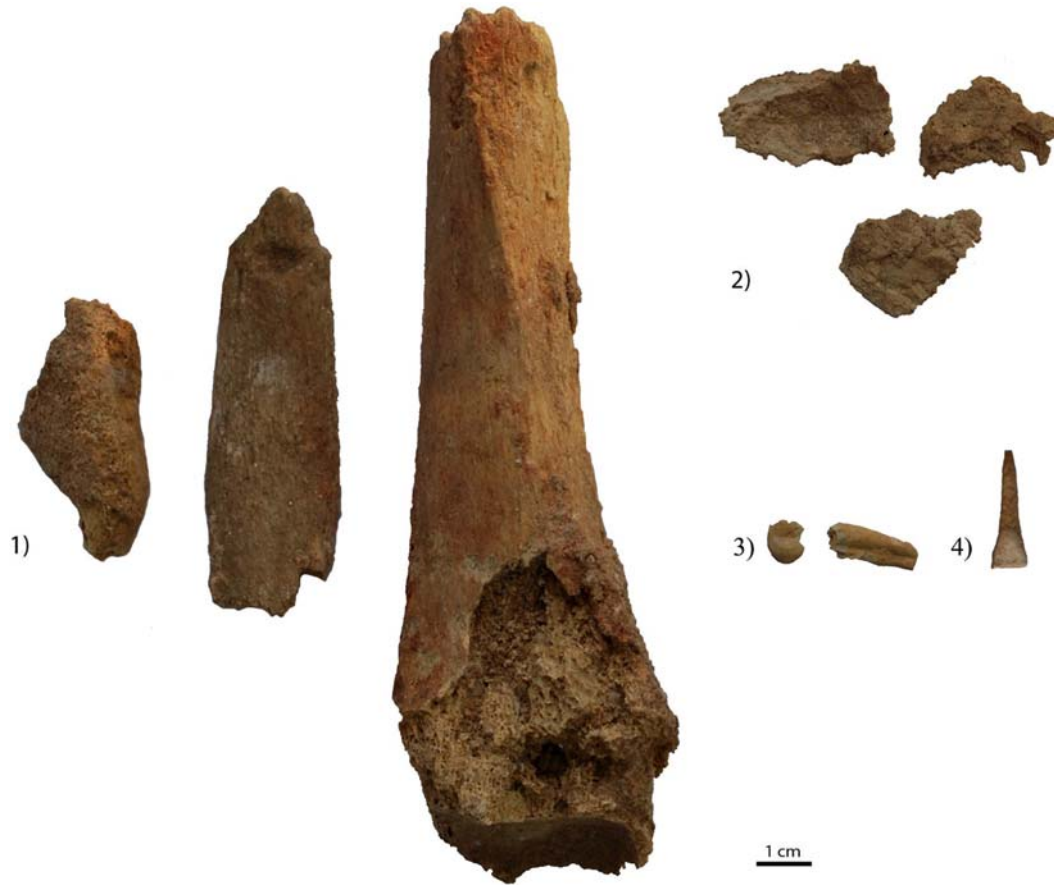
Figure S1: Burial 1, skeleton preservation scheme in gray, ochre residue is color coded in brown.

these genes are dimorphic, with different amino acid sequences that differentiate these proteins from each other. Both genes express their own products – female tooth enamel includes the product of the AMELX gene expression, and males the products of the AMELX and AMELY gene expression (Figure S4).

The list of tooth enamel proteins was identified for both individuals (Tables S1 and S2) during the analysis, but fragments unique to the Y isoform of amelogenin were revealed only for the individual from burial 1 (Tables S1 and S3). This result clearly indicates that the tooth from burial 1 belongs to a man, and the second one belongs to a woman (Table S2).



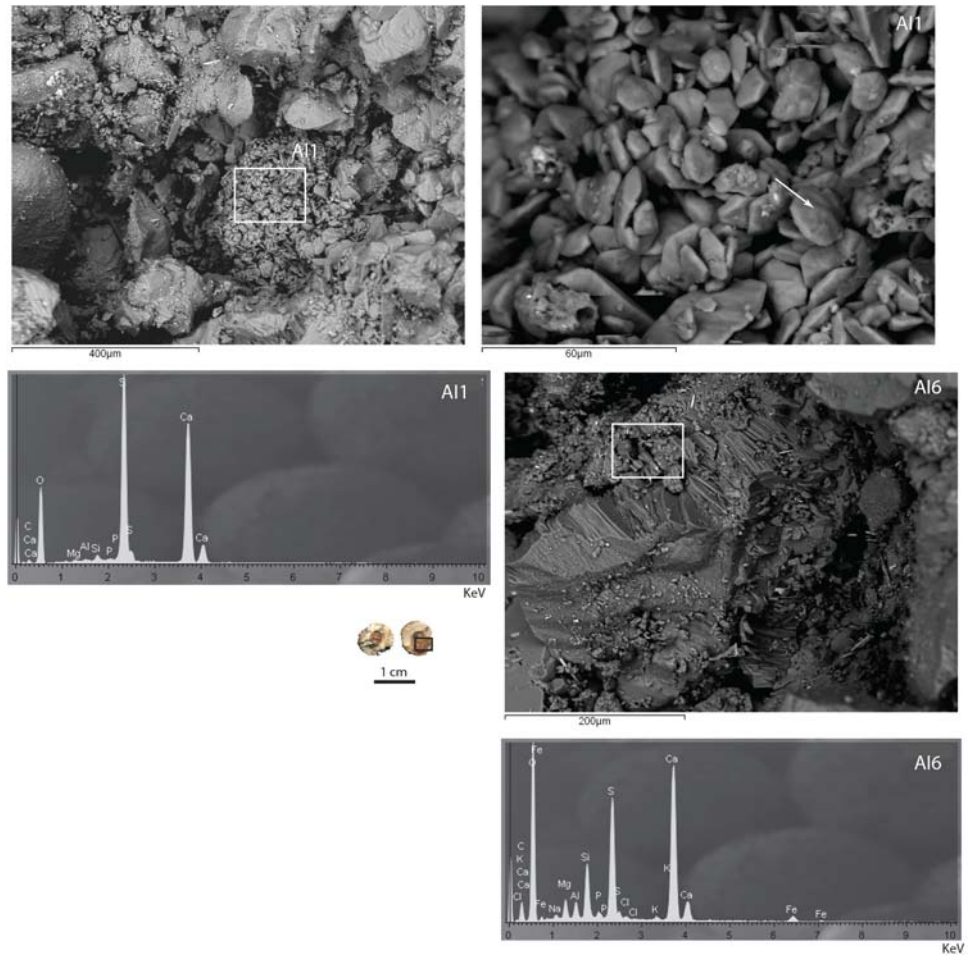
**Figure S2:** Burial 2, skeleton preservation scheme in gray, ochre residue is color coded in brown.



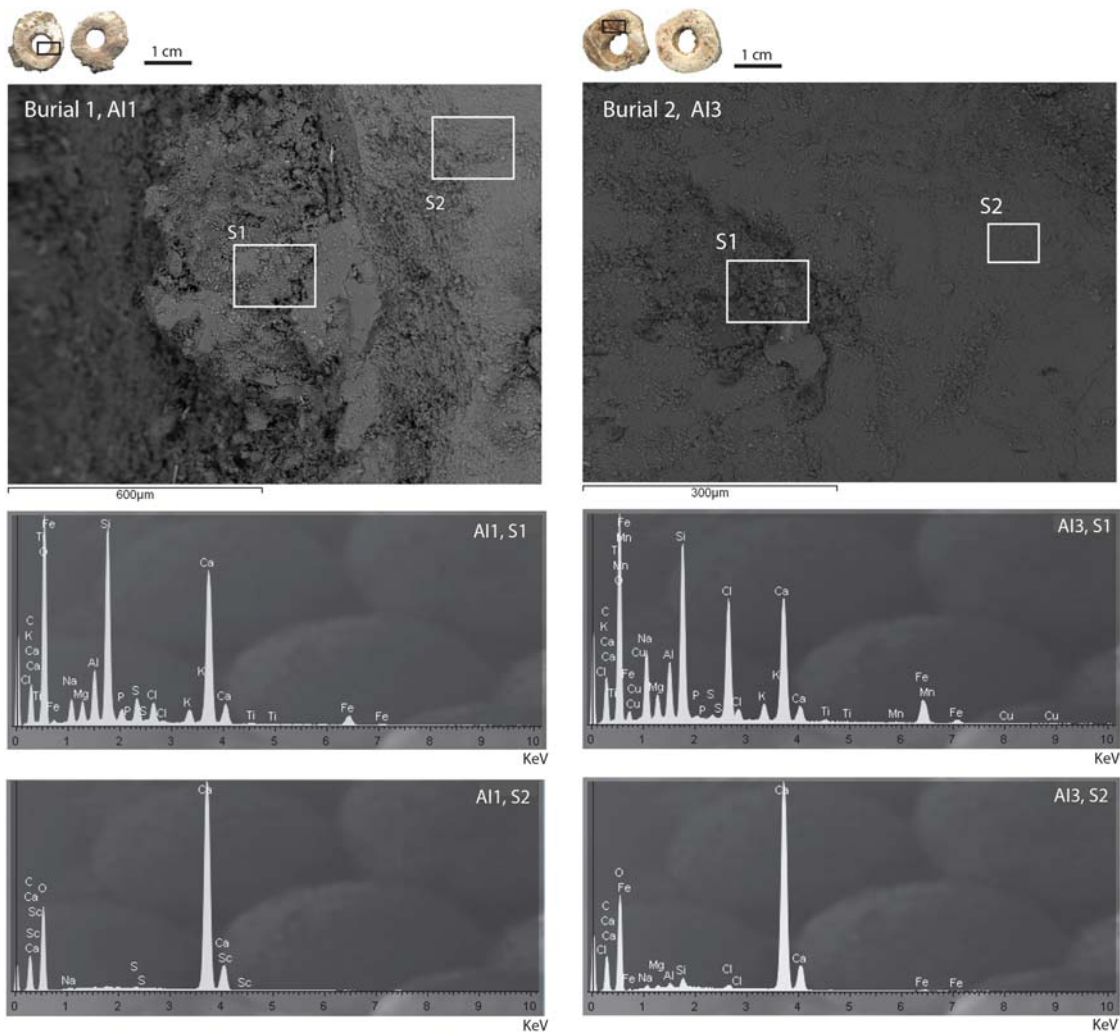
**Figure S3:** Example of human bone preservation. Burial 1: 1) distal left tibia diaphysis fragments, 2) skull fragments, 3) upper right premolar; Burial 2: 4) lower right incisor.



**Figure S4:** Amino acid sequence of amelogenin Y isoform (AMELY, UNIPROT KB accession number Q99218). Peptide fragments identified by LC-MS/MS in the tooth sample from burial 1 are indicated by colored segments under the sequence. AMELY peptides are indicated by red colored segments. The letters inside the segments indicate the modification of the amino acid residue: **d** – deamidation (N/Q); **p** – phosphorylation (S); **o** – oxidation (M).



**Figure S5:** SEM-EDS analysis performed on one small bead. AI: Area of Interest. AI1: gypsum; AI6: gypsum.



**Figure S6:** SEM-EDS analysis performed on one mid-sized bead from burials 1 and 2. AI: Area of Interest; S: Spectrum. AI1-S1: salt, sand, ochre, gypsum, calcite; AI1-S2: aragonite; AI3-S1: mixed minerals; AI3-S2: aragonite from the shell.

**Table S1:** List of the proteins identified in the premolar enamel from burial 1. Chromato-mass spectrometric analysis of the acid etching products collected from the tooth shows the presence of fragments unique to the amelogenin Y isoform (see table 3), clearly attributable to a man. 10lgP - PEAKS protein score

Accession	-10lgP	#Peptides	#Unique	Description
Q99217-3	164.46	74	34	Isoform 3 of Amelogenin X isoform
Q99218	150.25	48	8	Amelogenin Y isoform
Q9NRM1	147.52	61	61	Enamelin
Q9NP70	135.45	50	50	Ameloblastin
O60882	53.26	3	3	Matrix metalloproteinase-20
P35527	51.8	3	3	Keratin type I cytoskeletal 9
Q9BYX7	40.06	2	2	Putative beta-actin-like protein 3
P02765	34.84	2	2	Alpha-2-HS-glycoprotein
P02458	30.83	1	1	Collagen alpha-1(II) chain
P02768	29.94	1	1	Serum albumin
Q6UX39	27.47	1	1	Amelotin
Q9C0K0	27.24	1	1	B-cell lymphoma/leukemia 11B
P02452	25.36	1	1	Collagen alpha-1(I) chain

**Table S2:** List of the proteins identified in the incisor enamel from burial 2. Chromato-mass spectrometric analysis of the acid etching products collected from the tooth shows the absence of peptide unique to the amelogenin Y isoform, clearly attributable to a woman. 10lgP - PEAKS protein score

Accession	-10lgP	#Peptides	#Unique	Description
Q99217-3	122.25	45	45	Isoform 3 of Amelogenin X isoform
Q9NRM1	86.6	20	20	Enamelin
P02452	82.82	12	12	Collagen alpha-1(I) chain
P08123	64.42	9	9	Collagen alpha-2(I) chain
Q9NP70	63.64	9	9	Ameloblastin
Q96B54	40.51	3	3	Zinc finger protein 428
P02461	35.06	2	2	Collagen alpha-1(III) chain
P99999	26.88	1	1	Cytochrome c
P02647	26.47	1	1	Apolipoprotein A-I
P02458	24.73	1	1	Collagen alpha-1(II) chain
Q9UM01	22.66	1	1	Y+L amino acid transporter 1
Q02388	20.87	1	1	Collagen alpha-1(VII) chain
Q9H7N4	20.77	1	1	Splicing factor arginine/serine-rich 19
Q9C0K0	20.61	1	1	B-cell lymphoma/leukemia 11B
Q9N2K0	20.42	1	1	HERV-H_2q24.3 provirus ancestral Env polyprotein



**Table S3:** List of peptide fragments unique to the amelogenin Y isoform identified in the acid etching products collected from the premolar from burial 1. 10lgP - PEAKS peptide score; ppm - part per million, difference of the determined peptide mass from the theoretical value; m/z - mass to charge ratio; z - charge state

Protein Accession	Peptide	10lgP	Mass	ppm	m/z	z	RT	Peak Area	Fragment	PTM
Q99218	YQ(+0.98)SMIRPPY	27.14	1154.543	0.6	578.2791	2	57.69	$4.85 \times 10^5$	56-64	Deamidation (NQ)
Q99218	YQ(+0.98)SM(+15.99)IRPPY	27.1	1170.538	-0.2	586.2761	2	50.78	$1.35 \times 10^6$	56-64	Deamidation (NQ); Oxidation (M)
Q99218	SM(+15.99)IRPPYS	28.05	965.4641	0.7	483.7396	2	41.66	$6.05 \times 10^6$	58-65	Oxidation (M)
Q99218	SM(+15.99)IRPPY	26.73	878.432	0	440.2233	2	41.68	$9.17 \times 10^7$	58-64	Oxidation (M)
Q99218	SMIRPPY	21.19	862.4371	-0.7	432.2255	2	51.77	$1.21 \times 10^7$	58-64	
Q99218	HQ(+0.98)IIPVVSQ(+0.98)Q(+0.98)H	24.24	1287.646	0	644.8302	2	49.05	$1.10 \times 10^5$	78-88	Deamidation (NQ)
Q99218	IIPVVSQ(+0.98)Q(+0.98)H	24.48	1021.544	0.5	511.7797	2	48.15	$5.17 \times 10^5$	80-88	Deamidation (NQ)
Q99218	LPPILPDLH	22.2	1013.591	-0.3	507.8026	2	67.07	$3.36 \times 10^5$	182-190	
Q99218	PATDKTKQ(+0.98)EEVD	24.67	1360.636	0.6	681.3256	2	21.41	$3.50 \times 10^5$	195-206	Deamidation (NQ)
Q99218	ATDKTKQ(+0.98)EEVD	21.92	1263.583	-0.1	632.7987	2	20.11	$4.18 \times 10^5$	196-206	Deamidation (NQ)

## References

- Stewart, N. A., Molina, G. F., Issa, J. P. M., Yates, N. A., Sosovicka, M., Vieira, A. R., ... Gerlach, R. F. (2016) The identification of peptides by nanoLC-MS/MS from human surface tooth enamel following a simple acid etch extraction. *RSC Advances*, 6, 61673–61679. doi: 10.1039/c6ra05120k.
- Ziganshin R. H., Berezina N. Y., Alexandrov P. L., Ryabinin V. V., & Buzhilova A. P. (in press). *Optimization of the method of human sex identification by peptidomic analysis of teeth enamel of different biological generation, archaeological age and taphonomic preservation of teeth*. Biochemistry (Moscow).