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Supplementary Information for

Unraveling ancestry, kinship and violence in a Late Neolithic mass grave

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Other supplementary materials for this manuscript include the following:

Datasets S1 to S7

Supplementary Text

1. Archaeological background

The Globular Amphora culture (GAC) is an archaeological term referring to communities that lived across parts of Central and Eastern Europe during the period 3,300-2,700 BCE. Named after its distinctly shaped ceramic amphora, it is identified archaeologically through the Globular Amphora material culture complex, which also includes certain flint and bone tools, stone battle axes, a range of symbolic or ornamental amber and bone artefacts, as well as specific types of burials and animal depositions.

With earlier roots in present-day Poland, from around 3,100 BCE the GAC spread across larger parts of northern Central Europe and adjacent parts of Eastern Europe, thus becoming a supra-regional phenomenon sharing a more or less similar combination of economic strategies, material culture, social organization, and ritual practices (1). Within the GAC, three regional groups can be identified: the Central group inhabited the Vistula River basin (1), the Eastern group inhabited the region between the Pripyat, Dnieper and Prut Rivers, and the Carpathian Mountains (2), and the Western group inhabited the region between the Oder River basin in the east and the Elbe River basin in the west (3–5).

The economy of the GAC was based mainly on animal husbandry, with crop cultivation playing a minor role. The importance of animal husbandry is shown by several lines of evidence, including bone remains reflecting meat consumption in settlement contexts and, notably, abundant depositions of animals or animal parts in ritual contexts, e.g. joints of meat as accompanying food in human burials, and of more or less whole animals – often cattle – in "animal burials", most often associated with human graves. In addition to cattle, the domestic animals kept included pigs, sheep and goats, and dogs. Horse bones found in GAC contexts cannot be reliably identified as coming from domesticated or wild horses. The few stable isotope analyses (C and N) that have addressed the diet of GAC individuals so far have yielded results which are fully compatible with a diet predominated by meat and dairy from domestic animals (6).

The dominant form of GAC settlement remains found represent single homesteads and camp sites, whereas larger settlements, potentially consisting of several contemporary houses, are rare. Both economic and settlement evidence indicate that GAC groups were relatively mobile (3–5). The basic pattern of mobility appears to have involved a series of relatively short-range shifts made across the annual cycle within large territories, presumably in order to seek out optimal grazing for the animals. Recent isotope analyses ($^{87}Sr/^{86}Sr$ and $\delta^{18}O$) on humans and cattle have come to the same overall conclusion (7, 8). Occasionally, however, mobility also involved farther-reaching movements of small groups, as indicated by minor finds of GAC settlement/activity remains well outside the culture's normal geographical range (9).

GAC communities buried their dead in a variety of different ways, varying partially according to regional group. The most prevalent form is inhumation burial in a stone cist tomb, but other forms, such as tombs with stone kerbs or stone pavement, as well as simple pit graves, also occur regularly. While inhumation burial of more or less intact bodies dominates, interred bodies are sometimes disarticulated, just as partial or complete cremations occur. Where larger funerary areas have been excavated, cemeteries are usually found to comprise burials of fewer than 20 individuals (1, 8).

As in human culture in general, the economic strategy and settlement and mobility patterns of GAC groups appears to have been closely linked to their social organization. The archaeological evidence concerning these patterns, as well as the organization of cemeteries, indicates that the basic organizational unit was the family or extended family group, and that these small groups probably aggregated into larger communities, presumably tied together by kinship, at certain occasions or time(s) of the year (1). These aggregations most likely occurred at or near the cemeteries, which constituted the most stable, cultural element in the GAC landscape. Here, in the presence of ancestors, communal ritual activities probably functioned to reinforce the coherence and identity of a larger group whilst simultaneously regulating the autonomy, activities and social links of the smaller social units through feasts or symbolic consumption of wealth, such as the slaughtering and deposition of cattle and other domestic animals (10, 11). In addition to exchange of individuals through cross-group marriage, one of the concrete organizational aspects probably negotiated at these localities, crucially for a socioeconomic system emphasizing mobility, was the division of land-use between groups, which may have relied partly on the cemeteries' quality as physical markers or fix points in the landscape (4).

In summary, a probable mode of life for GAC groups is as follows: Families or extended families moved their base settlement within a defined, if perhaps rather loosely defined, territory several times in a year, mainly according to needs defined by their most important economic activity, animal husbandry. Potentially, these groups may have split up into even smaller subunits when favorable, e.g. with some individuals staying at the base settlement and some herding animals in the hinterland. At certain times, basic social units aggregated into larger groups at ideologically key points in the landscape, facilitating social, economic and biological exchange and reaffirming socioeconomic rules and agreements. GAC organization thus appears to have combined substantial, to a large extent cyclical mobility with normative and physical elements of stabilization, allowing for a predominantly pastoral land-use while maintaining prosocial interaction between related groups.

The interaction of GAC groups with other, contemporary Late Neolithic communities was multifaceted. In most parts of its distribution area, GAC groups appear to have overlapped in territory and coexisted with other groups, such as TRB (Funnel Beaker) groups, Late Baden groups and, during its final phase, early Corded Ware groups (12). The nature of interaction between GAC groups and these other groups has long been a moot point. To which extent did they compete, cooperate or keep to themselves? In other cases, GAC groups seem to have neighbored other groups along fairly defined territorial borders. A notable case is the situation on the south-east periphery of the GAC where, in the region between the Siret and Moldova rivers in the west and the Dnieper in the east, GAC groups neighbored contemporary Yamnaya groups from around 3,100 BCE. While apparently sticking to themselves territorially, exchange of material objects between these communities (e.g. GAC ceramic vessels in Yamnaya graves) provides evidence for some interaction between them (9, 13). In other cases still, GAC groups have clearly engaged in much more extensive cultural exchange with neighboring groups; a case in point being the Late TRB communities residing in the western and northern parts of the Jutland Peninsula and GAC groups residing southeast of the peninsula (14).

A special case is the so-called Złota group, which emerged around 2,900 BCE in the northern part of the Małopolska Upland and existed until 2,600-2,500 BCE. Originally defined as a separate archaeological "culture" (15), this group is mainly defined by the rather local introduction of a distinct form of burial in the area mentioned. Distinct Złota settlements have not yet been identified. Nonetheless, because of the character of its burial practices and material culture, which both retain many elements of the GAC and yet point forward to the Corded Ware tradition, and because of its geographical location, the Złota group has attracted significant archaeological attention (15, 16).

The Złota group buried their dead in a new, distinct type of funerary structure; so-called niche graves (also called catacomb graves). These structures featured an entrance shaft or pit and, below that, a more or less extensive niche, sometimes connected to the entrance area by a narrow corridor. Local limestone was used to seal off the entrance shaft and to pave the floor of the niche, on which the dead were usually placed along with grave goods. This specific and relatively sophisticated form of burial probably reflects contacts between the northern Małopolska Upland and the steppe and forest-steppe communities further to the east, who also buried their dead in a form of catacomb graves. Individual cases of the use of ochre and of deformation of skulls in Złota burials provide further indications of such a connection (15). At the same time, the Złota niche grave practice also retains central elements of the GAC funerary tradition, such as the frequent practice of multiple burials in one grave, often entailing redeposition and violation of the anatomical order of corpses, and thus differs from the catacomb grave customs found on the steppes which are strongly dominated by single graves. Nonetheless, at Złota group cemeteries single burial graves appear, and even in multiple burial graves the identity of each individual is increasingly emphasized, e.g. by careful deposition of the body and through the personal nature of grave goods (16).

Just like its burial practices, the material culture and grave goods of the Złota group combine elements of the GAC, such as amber ornaments and central parts of the ceramic inventory, with elements also found in the Corded Ware tradition, such as copper ornaments, stone shaft-hole axes, bone and shell ornaments, and other stylistic features of the ceramic inventory. In particular, Złota group ceramic styles have been seen as a clear transitional phenomenon between classical GAC styles and the subsequent Corded Ware ceramics, probably playing a key role in the development of the typical cord decoration patterns that came to define the latter (17).

As briefly summarized above, the Złota group displays a distinct funerary tradition and combination of material culture traits, which give the clear impression of a cultural "transitional situation". While the group also appears to have had long-distance contacts directed elsewhere (e.g. to Baden communities to the south), it is the combination of Globular Amphora traits, on the one hand, and traits found among late Yamnaya or Catacomb Grave groups to the east as well as the closely related Corded Ware groups that emerged around 2,800 BCE, on the other hand, that is such a striking feature of the Złota group and which makes it interesting when attempting to understand cultural and demographic dynamics in Central and Eastern Europe during the early 3rd millennium BCE.

2. Site descriptions

The skeletal material sampled for this study was excavated at five different sites in southern Poland, all belonging to the Globular Amphora culture or its so-called Złota group variant. There follow brief site descriptions below.

Koszyce *(site 3, grave 523), Małopolska province.* The mass grave at Koszyce is located on the edge of the Szreniawa river valley in the southern part of the Nida river basin (18). Excavated in 2011, the grave pit, which measured 245 x 135 cm, contained the skeletal remains of 15 individuals including men, women, and children (Fig. S1). The individuals had, apparently, all been killed by blows to the head (Fig. S2) but had subsequently been buried in general accordance with funerary customs of the Globular Amphora culture, i.e. accompanied by ceramic amphorae, flint tools, amber and bone ornaments as well as wild boar tusks and various animal bones. The human bodies had been laid down closely next to and on top of one another, and their arrangement clearly indicated that they were all deposited in the grave at the same time. A nearby pit (feature 506) contained remains of seven pigs, which are likely to have been deposited in connection with the burial of the human individuals (Fig. S1).

Książnice *(site 2, grave 3ZC), Świętokrzyskie province.* This burial, a so-called niche grave of the Złota type (with a vertical entrance shaft and perpendicularly situated niche), was excavated in 2006 and contained the remains of 8 individuals, osteologically identified as three adult females and five children, positioned on limestone pavement in the niche part of the grave (19). Radiocarbon dating of the human remains indicates that the grave dates to 2900-2630 BCE, 95.4% probability (Dataset S1). The grave had an oval entrance shaft with a diameter of 60 cm and depth of 130 cm; the depth of the niche reached to 170 cm (both measured from the modern surface), and it also contained a few animal bones, a few flint artefacts and four ceramic vessels typical of the Złota group. Książnice is located in the western part of the Małopolska Upland, which only has a few Złota group sites but a stronger presence of other, contemporary groups (including variants of the Baden culture) (19).

Mierzanowice *(site 1, grave 3), Świętokrzyskie province.* Excavated in 1936-1938, this burial contained the bones of a single individual, an adult male, who had been placed in an oval pit, measuring approximately 190x150 cm and 100 cm deep, and paved with limestone blocks on the bottom (20). The skeleton was accompanied by an intact ceramic vessel, typical of the Globular Amphora culture. Two flint axes were also found, but they are likely to have been located originally in a later, superposing grave, which had partly disturbed the Globular Amphora feature. Mierzanowice is located in the Sandomierz Upland, an area with substantial presence of both the Globular Amphora culture and Złota group, as well as the Corded Ware culture from 2800 BCE (20).

Sandomierz *(site 78, graves 2 and 8), Świętokrzyskie province.* A rescue excavation carried out in 1984 investigated several burial features of the Globular Amphora culture (21). Grave 2, a rectangular pit measuring approximately 100x250 cm and 90 cm deep, contained the skeleton of one adult individual *in situ*, as well as scattered bones of a second individual, apparently relocated from the southern part of the grave, which had been disturbed by later prehistoric activity. The fully intact, northern part of the grave contained three ceramic vessels, a flint axe, several animal bones and a lump of hematite shaped into a cube. About 3 m west of grave 2 another feature, partly destroyed by construction work, was excavated, containing the remains of at least one cattle and one domestic pig or wild boar, as well as a flint axe. It is likely that this animal deposition was connected to the burial made in grave 2. Grave 8, a rectangular pit measuring approximately 150x250 cm and 145 cm deep, contained the remains of three individuals, which had been placed sequentially within a rectangular stone construction. The skeletal remains of the two individuals (II and III) deposited first had been reordered significantly in connection with the deposition of the final, third individual (I), which was lying in anatomical order, positioned on the back with tightly drawn-up legs. The grave also contained two intact ceramic vessels, a flint axe and a flint chisel, a double-ended point and an awl made of animal bone, an amber disc, and two pig/boar mandibles along with a few other animal bones. Sandomierz is located in the Sandomierz Upland, an area with substantial presence of both the Globular Amphora culture and Złota group, as well as the Corded Ware culture from 2800 BCE (21).

Wilczyce *(site 90, grave 10), Świętokrzyskie province.* A rescue excavation in 2001 uncovered a niche grave of the Złota type, which had a round entrance shaft measuring 90 cm in diameter (22). The grave was some 60-65 cm deep below the modern surface and the bottom of the niche was paved with thin limestone plates, on which remains of three individuals had been placed; two adults, one female and one male, and one child (23). Four ceramic vessels of Złota group type were deposited in the niche along with the bodies. Wilczyce is located in the Sandomierz Upland, an area with substantial presence of both the Globular Amphora culture and Złota group, as well as the Corded Ware culture from 2800 BCE.

3. Osteological evidence of interpersonal violence

The osteological evidence for interpersonal violence at Koszyce has been described in detail by Konopka and colleagues (24). Here we present a brief summary of the evidence. All 15 individuals buried in the mass grave at Koszyce exhibit perimortem injuries (Table S1), i.e. injuries sustained around the time of death, which may well have constituted the cause of death. The most common injuries are cranial fractures (Fig. S2), suggesting that the individuals were killed by blows to the head. The lesions have the form of holes varying in shape and measuring up to 4.5 cm in diameter, with radial cracks extending from the margins of the openings. In some cases, only the fragments of such holes were preserved and seen at the margins of larger bone fractures. In the well-preserved postcranial skeletal remains only one perimortem lesion was found at the level of the elbow of one individual, the upper limb was severed (Table S1; Fig. S2, 4, 4a).

Most of the cranial lesions (with the exception of individuals 2, 3 and 7) appear to have been caused by a blow with the same type of weapon – possibly a flint axe/chisel or stone axe. It is noteworthy that individual 3, an adult female, who does not seem to be closely related (genetically) to anyone in the group, exhibits lesions that differ from those of the other individuals (Fig. S2, 2). The opening in the frontal squama appears to have resulted either from two blows of similar force inflicted closely together with a tool with a round cross-section and a diameter of 1.5-2 cm, or from one blow with a tool having two such points, with bone fragments crumbling in between the two sites of impact. In the former case, these closely located lesions reflecting similar force would suggest that these blows were deliberately struck with greater precision than applied in the cases of the other skulls.

The character of the lesions and the absence of any signs of healing or tissue regeneration indicate that the injuries were sustained around the time of death and it seems reasonable to assume that they also constitute the cause of death. Alternatively, they may have been inflicted soon after death, in which case the individuals must have been killed in a manner leaving no osteological trace (e.g. by strangling). The distribution of blow marks suggests that many of the individuals were struck more than once and the fact that many of the lesions are found at the back of the head suggests that they were struck down from behind. Overall, the nature of the injuries and the near absence of so-called parry fractures (i.e. injuries sustained to the upper limbs) indicates that the individuals were executed rather than killed in hand-to-hand combat (24).

4. Radiocarbon dating

Bone samples were cleaned and dissolved in hydrochloric acid at 4°C for 2-3 days. Humic substances were removed using 0.2M NaOH at 4°C, with frequent changes of the NaOH until the solution stayed clear and subsequently rinsed with 0.01M HCl. Samples were gelatinized in 0.01M HCl at 58°C overnight, with an additional 3-day gelatinization afterwards. The extracted collagen was ultrafiltered in pre-cleaned Amicon Ultra Centrifugal Filters Ultracel 30K. All samples produced enough collagen for ^{14}C age analysis (>0.1% collagen yield) and %C (35-45 %wt), %N (13-16 %wt) and C:N ratios (2.9-3.5) were within the acceptable range (Dataset S1). Collagen was converted to CO_2 by combustion in sealed evacuated quartz tubes with 200 mg CuO and silver wires. The CO_2 was reduced to graphite by the H_2 reduction method using an iron catalyst and $MgClO_4$ to remove water (25). The graphites were then dated using an HVE 1MV Tandetron accelerator AMS (26). Radiocarbon dates are reported as uncalibrated radiocarbon ages BP (Dataset S1). Dates in the article have been calibrated with OxCal v. 4.3 (27) using the calibration curve IntCal13 (28) and are reported as calibrated ages BC (Dataset S1). Stable isotopes $\delta^{13}C$ and $\delta^{15}N$ were measured using a continuous-flow IsoPrime IRMS coupled to an Elementar PyroCube elemental analyser. The results indicate that the Koszyce individuals largely relied on a terrestrial diet (Fig. S3). Therefore, no reservoir correction was applied.

Both the archaeological and aDNA evidence strongly suggest that humans found in the Koszyce grave were killed and buried within a very short period of time. Hence, in terms of radiocarbon their death can be considered to represent one single event and therefore their ^{14}C ages should represent similar values. Considering the AAR ^{14}C samples, all but three individuals are in statistical agreement producing a weighted average ^{14}C age of 4209 ±10 ^{14}C years BP (Fig. S4B). Individuals 8 and 14 yielded ^{14}C ages that are "too old" when compared to the average ^{14}C age, yielding ^{14}C age differences of 121 and 170 ^{14}C years respectively. These ^{14}C age differences may indicate radiocarbon reservoir effects influencing the accuracy, however, the Ua-45620 ^{14}C age of individual 14 agrees with the average ^{14}C age suggesting that AAR-28710 is an outlier. Whether the older age of individual 8 (AAR-26317) is suggestive of an undetected reservoir age offset cannot be determined with the present dataset.

Individual 6, genomically identified as the daughter of individual 12 and sister of individual 4, appears younger than the calculated average ^{14}C value and thus cannot be explained with ^{14}C reservoir effects unless it is assumed that all other individuals are influenced by a ^{14}C reservoir age offset (Fig. S4B). However, the stable isotope evidence indicates a terrestrial diet, and moreover, the ^{14}C age of the sheep (AAR-27613) is in agreement with the calculated average ^{14}C age. Sheep obtain their ^{14}C content from terrestrial plants and hence are not influenced by possible ^{14}C dietary reservoir offsets. The Ua-45618 ^{14}C age of individual 6 is furthermore in agreement with AAR-26316 suggesting that the younger ^{14}C age is probably best explained by an undetected sample contamination. Thus, individual 6 is considered an outlier in any further analysis. Considering all available ^{14}C age information of the Koszyce grave produce a weighted average ^{14}C age of 4198 ±8 ^{14}C years BP (Fig. S4C). This yields a calibrated 68.2% confidence age range of 2880-2868 BCE (24.9%) and 2804-2776 BCE (43.3%) BCE (Fig. S5).

5. Strontium isotope analyses

Analytical procedure. Tooth enamel surfaces were cleaned off and a few milligram of clean enamel was dissolved in a 1:1 solution of 0.5 ml 6N HCl and 0.5 ml 30% H_2O_2. Subsequently, the solutions were dried on a hotplate at 80°C and the residue taken up in a few drops of 3M HNO_3 and loaded onto disposable extraction columns into which we fitted a frit which retained a 0.2 ml stem volume of intensively pre-cleaned mesh 50-100 SrSpec™ resin (Eichrome Inc.) (29). The Sr isotope ratios were measured using Thermal ionization mass spectrometry. Samples were dissolved in 2.5 µl of a Ta_2O_5-H_3PO_4-HF activator solution and directly loaded onto previously outgassed 99.98% single rhenium filaments. Samples were measured at 1250-1300°C in dynamic multi-collection mode on a VG Sector 54 IT mass spectrometer equipped with eight faraday detectors. Five nanogram loads of the NBS 987 Sr standard that we ran during the time of the project yielded $^{87}Sr/^{86}Sr$ = 0.710241 +/- 0.000011 (n=5, 2). Total procedure blanks were less than 150 pg, an amount that is insignificant compared to the amount of sample strontium.

The environmental samples were analyzed at the Isotope Laboratory of the Adam Mickiewicz University at Poznań, Poland, following established protocols (30). 15 ml of water was evaporated on a hot plate in a PFA vial. Subsequently, 340 ml of 2N HNO_3 was added to the sample and left overnight to equilibrate. The powdered sediment samples (~50 mg) were dissolved on a hot plate (~100°C, three days) in closed PFA vials using a mixture of concentrated hydrofluoric and nitric acid (4:1). Strontium was separated from matrix elements on PFA columns filled with SrSpec™ resin using miniaturized chromatographic technique developed by Pin et al. (31). Strontium was loaded with a $TaCl_5$ activator on a single Re filament and analyzed in dynamic multi-collection mode on a Finnigan MAT 261 mass spectrometer. During the course of this study, the NBS987 Sr standard yielded $^{87}Sr/^{86}Sr$

= 0.710228 ± 9 (2σ, n = 26). The measured ratios were normalized to a nominal value of 0.710240 for the standard NBS987. Total procedure blanks were less than 80 pg.

Local baseline. The Koszyce site is located on the edge of the Małopolska Upland, where the plateau is cut by the Vistula River and its tributary the Szreniawa. Their valleys are filled with Holocene alluvial sediments, up to 60 m thick, mostly clays with intercalations of sands, muds and peats. The upland area around Koszyce is covered by a Pleistocene sequence composed of loess, generally only a few meters in thickness. Underlying is a monotonous series of marine Miocene claystones (Krakowiec Beds), which constitute the topmost part of the sedimentary infill of the Carpathian Foredeep. The Miocene clays of the area are characterized by $^{87}Sr/^{86}Sr$ values of around 0.714-0.715, whereas the loess sediments are more radiogenic, with Sr isotope signatures between 0.721 and 0.724 (Fig. S6). In contrast to the sediments, the water of the Szreniawa River measured in this study, yields a signature of 0.7086. Although a small lowering of the $^{87}Sr/^{86}Sr$ ratio due to impact of fertilizers is possible (32), the non-radiogenic composition of the Szreniawa can be explained by the composition of the geological substrate in the river catchment area. The Szreniawa River, which constituted most probably the main water source for the prehistoric community of Koszyce, drains in its upper course (20-25 km before Koszyce) Campanian and Maastrichtian sequences, composed exclusively of marine carbonates. Hence, it can be assumed that these Cretaceous sediments show compositions that reflect the marine Sr isotope values for this period, i.e. Sr signatures between 0.7075 and 0.7078 (33). Because Miocene and Pleistocene rocks likely provide a radiogenic strontium component into the lower course of the river, the composition of the Szreniawa river water at Koszyce could therefore be shifted to a slightly higher Sr signature of 0.7086 (Dataset 2).

In summary, the complexity of the geology in the area combined with the few baseline samples at hand prevents us to presently constrain the baseline. However, it appears likely that the local bioavailable fraction is a mixture of radiogenic strontium liberated from the bulk rocks (the less significant component) and non-radiogenic strontium from surface waters that potentially are dominated by strontium from carbonates (the significant component). Three fauna samples (pigs) yielded $^{87}Sr/^{86}Sr$ values between 0.7095 to 0.7113 (Dataset S2). However, these should be considered with caution when characterizing the local baseline as they may have been imported (34). The surface water value of 0.7086 can be considered a lower bound for the area around Koszyce (Dataset 2).

Additional data derives from recent Sr isotope study of human remains from a Late Neolithic group that lived in an area located about 10 km north of Koszyce, and which has virtually the same geological substrate. This group yielded a relatively narrow range of $^{87}Sr/^{86}Sr$ values, ranging between 0.7095 and 0.7105 (35). If we assume that these values are representative of the local bioavailable $^{87}Sr/^{86}Sr$ range, including that for Koszyce, then it seems plausible that some of the Koszyce family members (those with a similar Sr isotope signature) were born in the area around Koszyce, while the rest (n=8) who show a slightly more radiogenic Sr isotope composition ($^{87}Sr/^{86}Sr$ = 0.7113-0.7120), might have been born elsewhere in the region (Fig. S6). However, given the geological complexity of the region and the limitations of the $^{87}Sr/^{86}Sr$ isotope method it is currently difficult to determine where exactly they might have originated.

6. Ancient DNA analysis

Lab work. DNA was extracted from petrous bones or tooth roots using established protocols specifically designed for ancient DNA (36). Between 100-200 mg of sample material was crushed into a coarse powder and incubated for 10 min at 45°C in 1 ml of an EDTA-based lysis buffer containing 0.25 mg/mL Proteinase K. After 10 min, the buffer was replaced with fresh lysis buffer and the samples digested overnight at 45°C. The DNA was then purified using 50 µl of silica pellets and 40 ml of binding buffer containing 5 M Gu-HCl, 100 mM NaOAc, 20 mM NaCl and 30% isopropanol, as described in (37). The silica-bound DNA was washed twice in 80% ethanol eluted in 60 µl EB. Twenty µl of each DNA extract was then built into double-stranded DNA libraries using the NEBNext DNA Sample Prep Master Mix Set 2 kit (E6070) and Illumina specific adapters (38). The optimal number of PCR cycles was determined by qPCR and the DNA libraries were then amplified and indexed using a dual-indexing protocol (39). The indexed and amplified libraries were then purified and quantified on an Agilent Bioanalyzer 2100 before being pooled in equimolar amounts.

Sequencing and data processing. The pooled libraries were sequenced (80 bp, single read) on Illumina HiSeq 2500 platforms at the Danish National High-throughput DNA Sequencing Centre. Base-calling was done using CASAVA 1.8.2. Adapter sequences and leading/trailing stretches of Ns were trimmed using AdapterRemoval-2.1.3 (40). Reads of at least 30 bp were mapped to the human reference genome GRCh37 using bwa-0.7.10 (41), with the seed disabled and alignments were processed using samtools-1.3.1 (42) removing reads with a mapping quality lower than 30. Duplicates were removed using picard-1.127 MarkDuplicates and libraries merged to sample level and realigned using GATK-3.3.0 (43). Realigned bams had the md-tag updated and extended BAQs calculated using samtools calmd. Read depth and coverage were determined using BEDtools 2.27.0 genomecov (44). The sequencing results are reported in Dataset S3.

Genotype likelihoods were determined using the UnifiedGenotyper from GATK-3.7.0 (45). We only called biallelic sites present in the 1000G dataset and only the observed alleles (--genotyping_mode GENOTYPE_GIVEN_ALLELES). Hereafter we filtered the VCF files to set genotype likelihoods to 0 for all three genotypes (e.g. hom ref, het and hom alt) for sites with potential deamination (C>T and G>A). Subsequently, the per-individual vcfs were merged using bcftools-1.3.1. The combined VCF were then split into 200,000 markers each and imputed separately using beagle-4.0 (r1399)(46) using the 1000 Genomes (47) phase 3 map included with beagle (*.phase3.v5a.snps.vcf.gz and plink.chr*.GRCh37.map) with input through the genotype likelihood option.

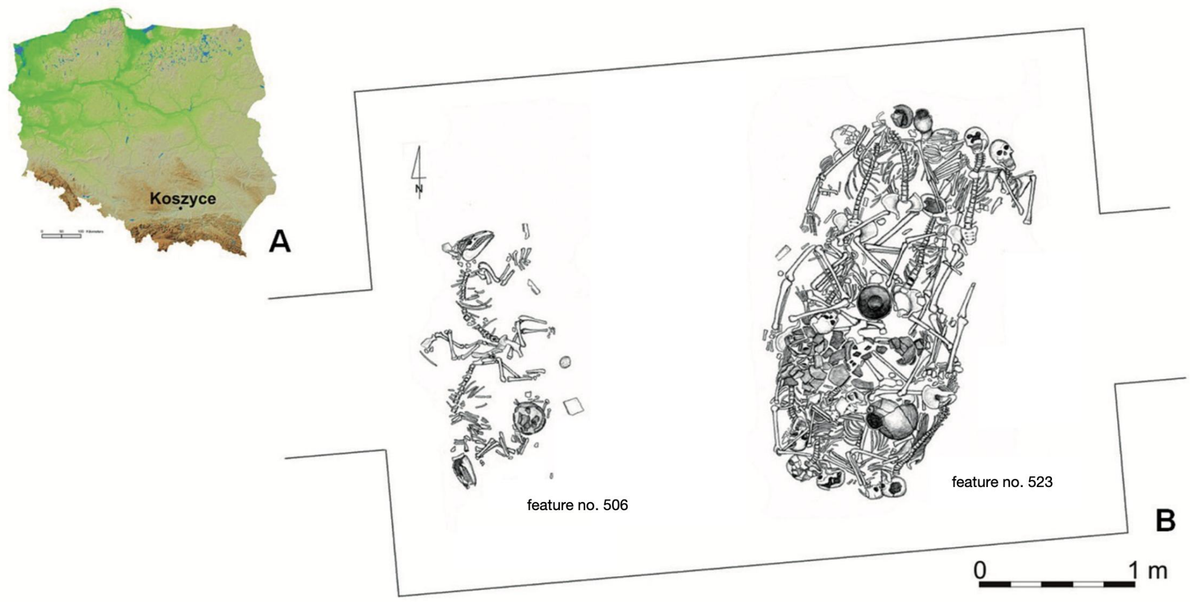

Fig. S1. Location **(A)** and site plan **(B)** of the multiple burial at Koszyce (reproduced with permission from ref. (24).

Fig. S2. Examples of bone lesions found in individual skeletons of the Koszyce mass grave. 1. The skull of individual 1; 1a. Reconstruction using fragment of bone found inside the cranial vault; 2. The skull of individual 3; 3. The skull of individual 5; 3a-3b. Internal views of the skull, 3c. Oval cracking of the external table of the right parietal bone; 4. The right radius and ulna of individual 5; 4a. Upper parts of these bones bearing cut marks; 5. The skull of individual 11; 6. The skull of individual 12; 6a. A partially charred clavicle; 7. The skull of individual 14. All photographs by A. Szczepanek.

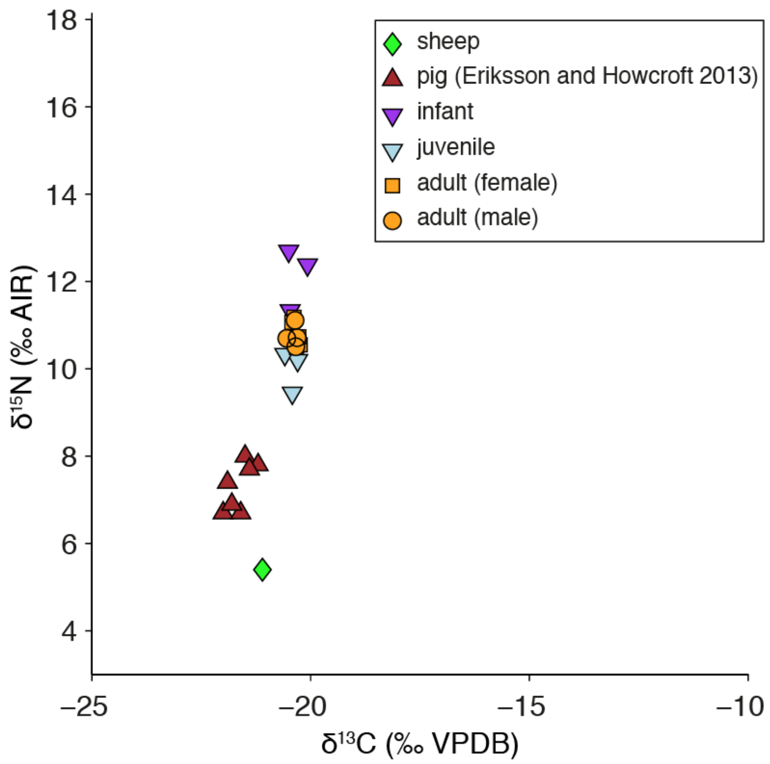

Fig. S3. Mean stable isotope ($\delta^{13}C$ and $\delta^{15}N$) values of faunal and human remains from Koszyce (additional data from ref. (6)).

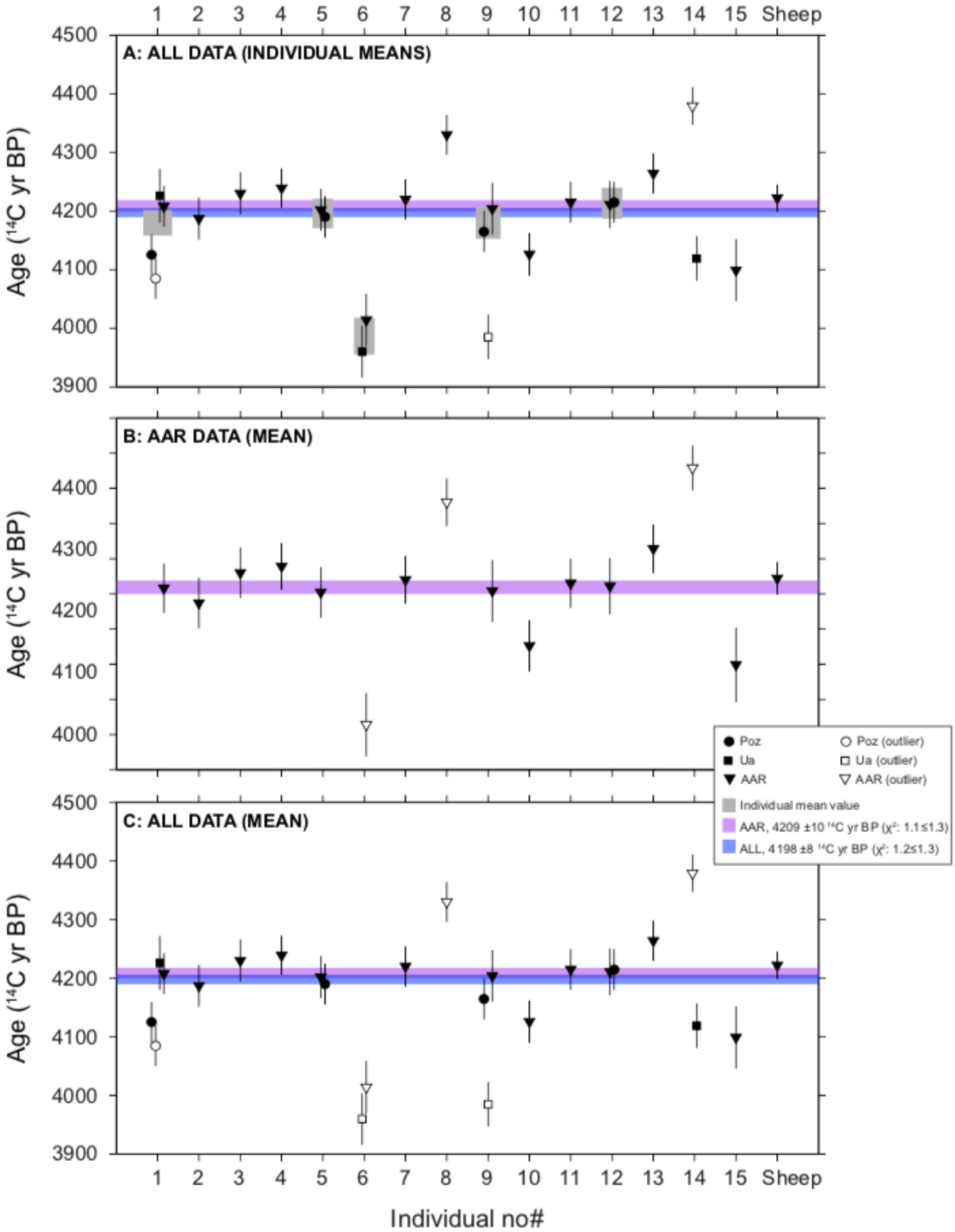

Fig. S4. A) ^{14}C age of all individuals from the Koszyce grave. Individual mean ^{14}C age is indicated with grey bars (±1σ); **B)** ^{14}C age of all individuals from this study. The weighted ^{14}C age average is indicated by the purple bar (±1σ); **C)** All ^{14}C age of all individuals from this and previous studies. The weighted ^{14}C age average is indicated by the blue bar (±1σ). Radiocarbon dates are listed in Dataset S1.

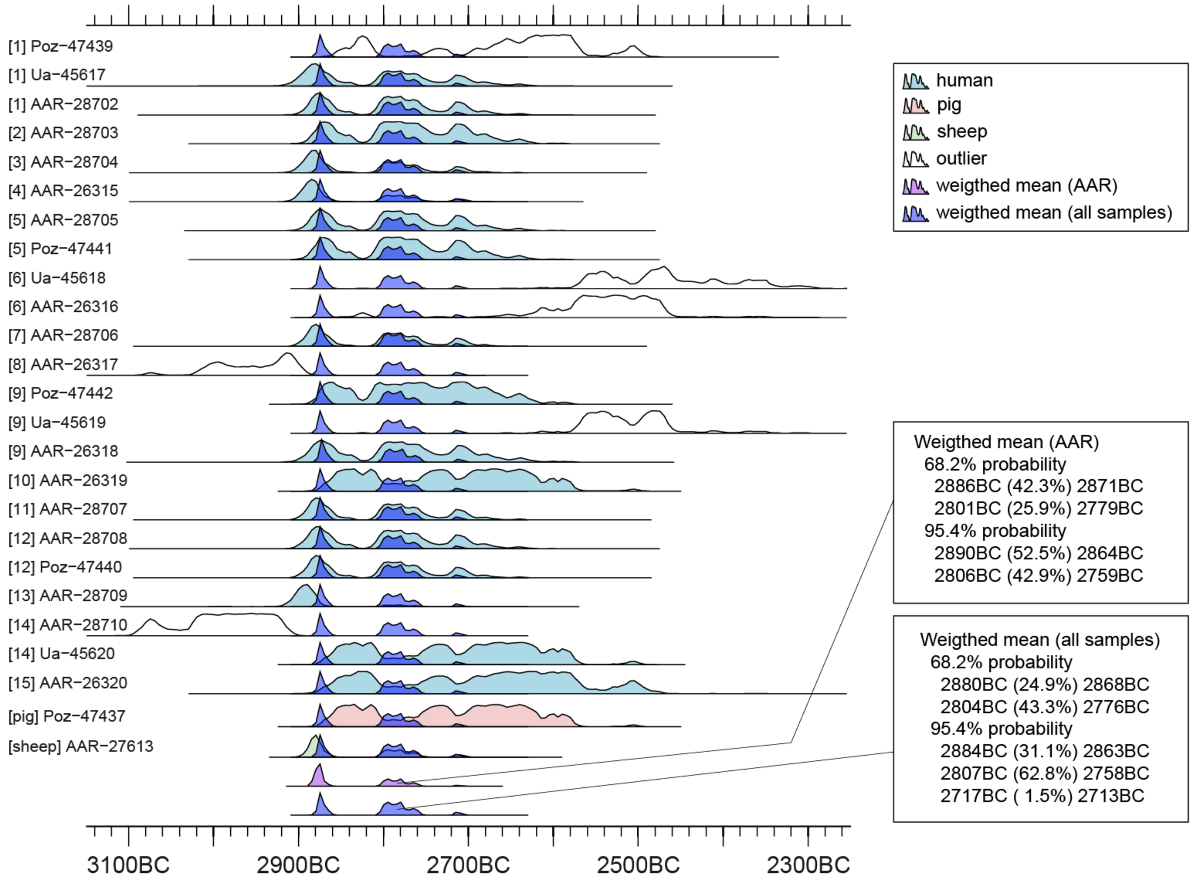

Fig. S5. Calibrated probability distributions of all radiocarbon dates from Koszyce. Individual dates are listed in Dataset S1.

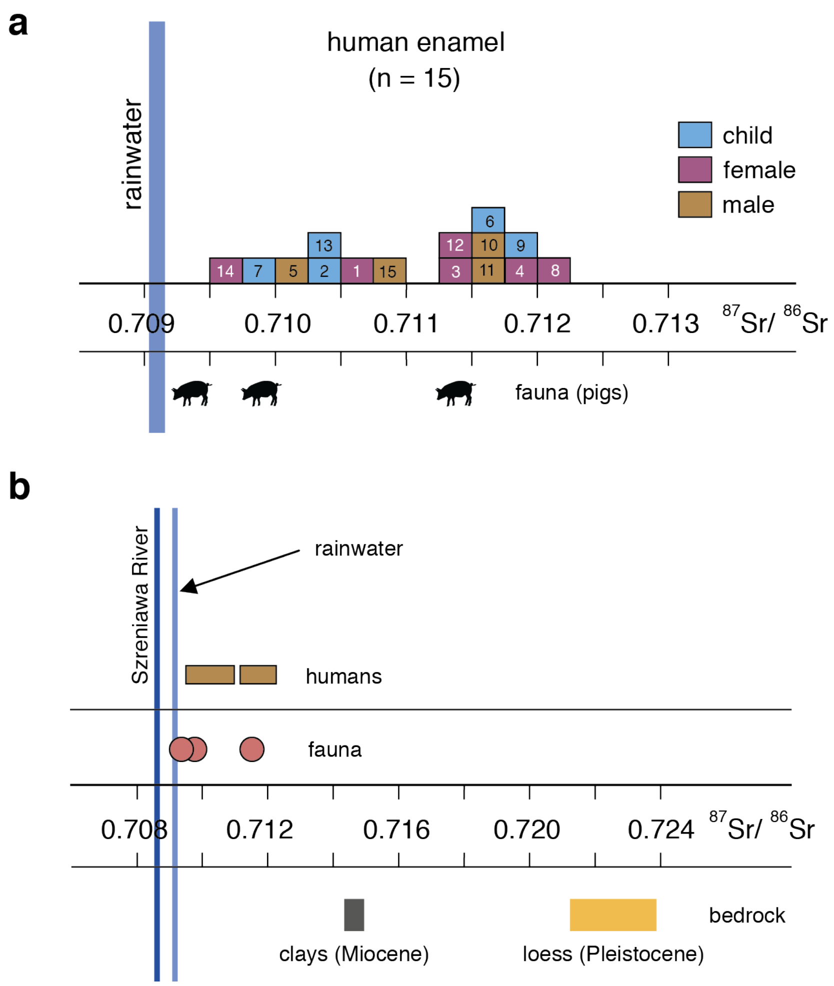

Fig. S6. A) Variation in $^{87}Sr/^{86}Sr$ isotope values in human and animal enamel at Koszyce. The individual no.# are indicated. **B)** Ranges of $^{87}Sr/^{86}Sr$ ratios in human and animal enamel at Koszyce, along with values for the Szreniawa River, geological bedrock and rainwater in the studied area. Data are shown in Dataset S2.

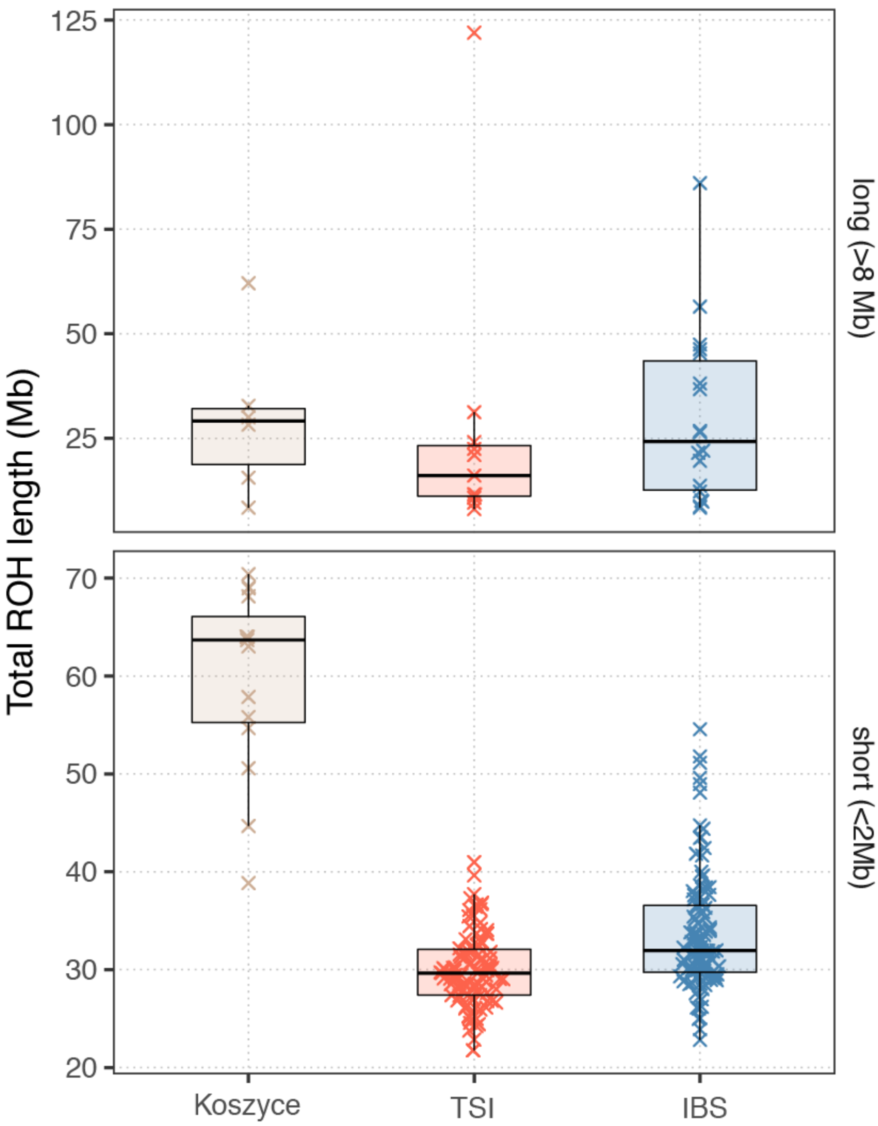

Fig. S7. Distribution of the total length of short (<2Mb) and long (>8Mb) runs of homozygosity (in Mb) in the Koszyce genomes, as well as genomes from two modern populations (IBS = Iberian, and TSI = Toscani) in the 1000 Genomes Project (47).

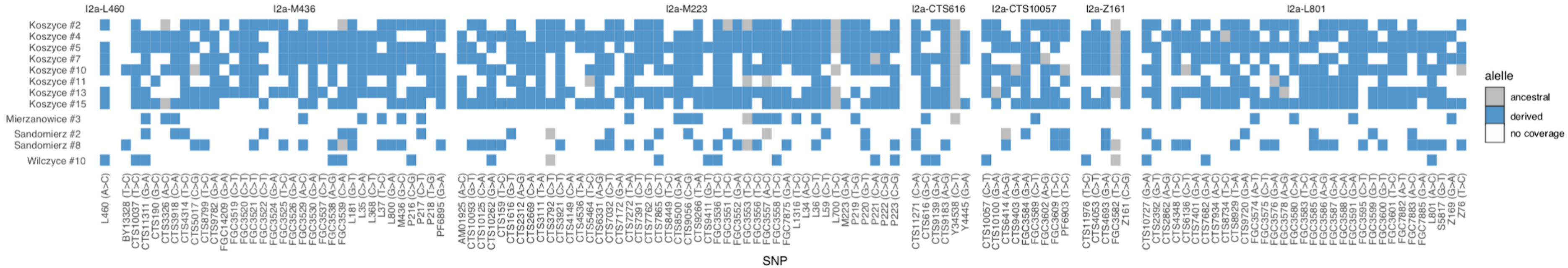

Fig. S8. Alleles observed in the Koszyce male genomes for Y-Chromosome SNPs defining sub-haplogroups of I2a. The missing data for the individuals from Mierzanowice, Sandomierz and Wilczyce can be explained by low coverage. Data are reported in Dataset S7.

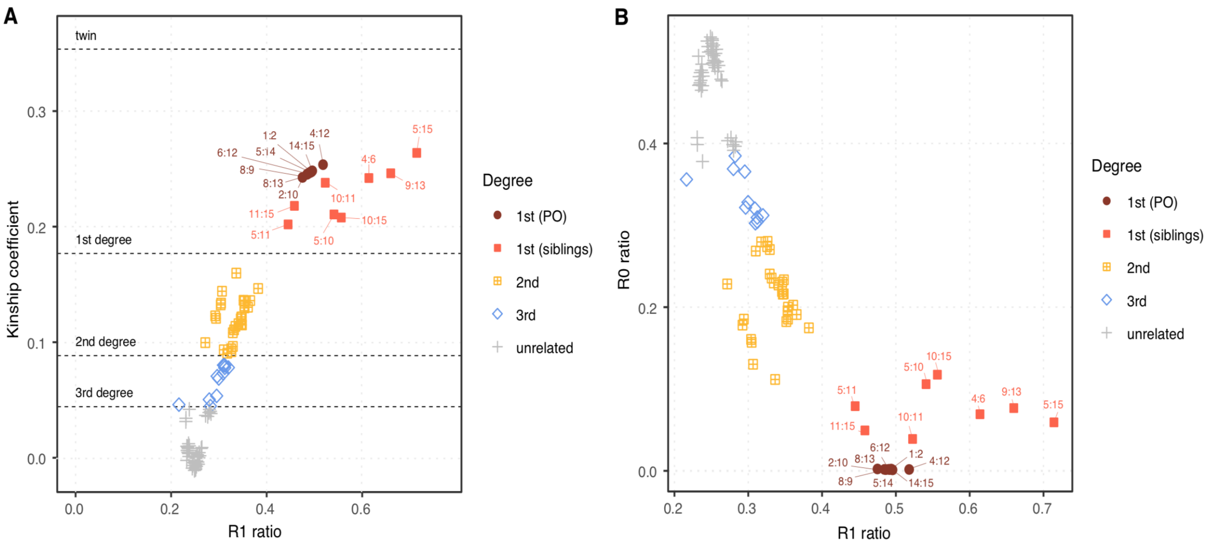

Fig. S9. Relatedness among the Koszyce individuals. A) Kinship coefficients and R1 ratios inferred from IBS scores for pairs of Koszyce individuals. **B)** R0 and R1 ratios inferred from IBS scores for pairs of Koszyce individuals. For clarity pairs of individuals have only been marked with numbers for 1st degree relationships. Data are listed in Dataset S6.

Table S1. Osteological and palaeopathological information for the Koszyce individuals. Age at death and sex were estimated based on standard osteological methods (48-51).

	age	sex	lesions
1	25-30	f	1. round opening, 4 cm in diameter, in the anterior part of the right parietal bone (Fig. S2, 1, 1a); 2. oval opening measuring 3x3.5 cm in the superior left part of the occipital squama; 3. lesion in the margin of a larger bone fracture in the posterior part of the right parietal bone, with a 3.5 cm long arch
2	1.5-2	m	several skull fractures, 4-5 cm in width
3	30-35	f	1. rounded opening above the left superciliary arch, measuring 3.5x2 cm (Fig. S2, 2); 2. rounded opening in the anterior part of the right parietal bone measuring 5x3 cm; 3. rounded lesion in the central part of the right parietal bone measuring 2.5x2 cm
4	16-17	m	1. rectangular opening in the center of the right parietal bone measuring 2.5x0.5 cm; 2. irregular opening in the anterior part of the right parietal bone measuring 2x1 cm; 3. irregular opening at the junction of the right parietal bone and the occipital squama measuring approximately 1x1 cm
5	20-25	m	1. blow marks arranged symmetrically on the lateral surfaces of the head (Fig. S2, 3): drop-shaped fracture measuring 3.5x2 cm in the central part of the left parietal bone (Fig. S2, 3, 3b), oval fracture in the center of the right parietal bone, measuring 3x2 cm, linear scratch of the bone surface in the central part of this oval crack (Fig. S2, 3, 3c); 2. small irregular lesion in the frontal squama near the bregma point (Fig. S2, 3, 3a); 3. irregular opening in the central part of the occipital squama measuring approximately 2x1 cm; 4. defect of the proximal epiphysis of the right humeral bone with visible parallel flaking of small bone fragments; fractures of the proximal epiphyses of the right radial and ulnar bones at the same level (Fig. S2, 4a)
6	13-14	f	1. small semi-circular opening in the central part of the right parietal bone, in the margin of a larger bone defect, measuring 17-18 mm in width; 2. two separate arch-shaped lesions in the lateral part of the right parietal bone in the margin of a larger bone defect, measuring 3 and 4 cm in length, with margins expanding towards the interior of the cranium; 3. irregular small defect in the anterio-lateral part of the left parietal bone
7	2-2.5	m	numerous fractures to the fragile skull but no lesions can be identified unambiguously
8	30-35	f	round fracture on the left side of the occipital squama, ca. 3 cm in diameter
9	15-16	f	regular rectangular opening measuring 3.5x1 cm in the left lambdoid suture
10	18-20	m	1. two round openings 3.5 cm and 4.5 cm in diameter in the right and left part of the occipital squama; 2. opening in the posterio-lateral part of the right parietal bone, in the margin of a larger bone defect, measuring approximately 3 cm in diameter
11	40-50	m	1. drop-shaped lesion in the central part of the right parietal bone, measuring 2.5x3.5 cm (Fig. S2, 5); 2. irregular opening in the left part of the frontal squama
12	30-40	m	1. irregular lesion in the right frontal squama, in the margin of a larger bone defect; 2. round lesion approximately 4 cm in diameter in the left occipital squama; 3. charred fragments of the cranial vault at the margins of the defect spanning the major part of it (Fig. S2, 6); 4. partially charred left clavicle (Fig. S2, 6a)
13	5-6	m	1. large fragment of a round opening in the left parietal bone, close to the coronal suture measuring ca. 4 cm in diameter; 2. irregular lesion in the posterior part of the left parietal bone, measuring 1x1.5 cm
14	50-60	f	1. irregular opening in the posterior-lateral part of the left parietal bone measuring 2x1 cm; 2. irregular lesion in the central part of the occipital squama measuring 5x4 cm; 3. irregular lesion in the central part of the frontal squama measuring 1x0.5 cm; 4. partially charred fragment of the right parietal bone (Fig. S2, 7)
15	40-50	m	1. oval lesion in the center of the left parietal bone, in the margin of a larger bone defect, measuring 1.5 cm in width; 2. irregular lesion in the center of the right parietal bone measuring 3x2.5 cm; 3. irregular opening in the margin of a larger bone fracture in the area of the left lambdoid suture; 4. fracture of the left mandibular ramus, the margin of the fracture is not perpendicular but has an oblique course, expanding internally

Table S2. qpADM results. The ancestry of most Globular Amphora/Złota group individuals can be modelled as a two-way mixture of Mesolithic western hunter-gatherers (WHG), and early Anatolian Neolithic farmers (Barcın). The five individuals from Książnice (Złota group) show evidence for additional gene flow, most likely from an eastern source.

Test population	Reference population	Admixture proportion	SE	N SNPs	Chi square	p-value
2-way model						
Koszyce (GAC)	WHG	0.208	0.022	822,986	3.88	5.68E-01
	Barcın	0.792	0.022			
Mierzanowice (GAC)	WHG	0.113	0.055	405,463	3.90	5.63E-01
	Barcın	0.887	0.055			
Sandomierz (GAC)	WHG	0.159	0.040	681,122	7.19	2.07E-01
	Barcın	0.841	0.040			
Książnice (Złota)	WHG	0.238	0.035	772,946	16.67	5.16E-03
	Barcın	0.762	0.035			
Wilczyce (Złota)	WHG	0.107	0.052	407,508	5.47	3.62E-01
	Barcın	0.893	0.052			
3-way model						
	WHG	0.235	0.035			
Książnice (Złota)	Barcın	0.670	0.049	740,316	8.91	6.33E-02
	Yamnaya	0.096	0.037			
	WHG	0.187	0.040			
Książnice (Złota)	Barcın	0.739	0.034	704,788	6.38	1.73E-01
	EHG	0.074	0.025			

Additional data tables (separate files)

Dataset S1 Radiocarbon dates

Dataset S2 Strontium isotope data

Dataset S3 Ancient genome data

Dataset S4 Mitochondrial DNA and Y chromosome SNPs

Dataset S5 Phenotype data

Dataset S6 Reference panel

Dataset S7 Kinship data

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