Scientific Reports
https://doi.org/10.1038/s41598-025-34905-4

Article in Press

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Received: 14 July 2025
Accepted: 31 December 2025
Published online: 17 February 2026
Cite this article as: Barta P., Dörnhöferová M., Baldovič M. *et al.* Multidisciplinary identification of human skeletal remains from the karst abyss in Demänovská Valley (19th–20th century calCE, Slovakia). *Sci Rep* (2026). https://doi.org/10.1038/s41598-025-34905-4

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Abstract
The subject of this multidisciplinary forensic archaeological-anthropological research is a near-complete skeleton of a woman aged 40–49 years with possible perimortal cranial trauma, found within a known archaeological site from the 7th–3rd century calBCE. The skeleton (without any artefacts) was exhumed by speleologists from a depth of 14 metres, 2.8 meters below the sedimentary deposit, in a deep and narrow karst abyss known as Studňa na Jame, which is located in the district of Liptovský Mikuláš in the Low Tatra Mountains (Liptov Region, north Slovakia). Enthesopathies rank her among strong, physically-working individuals living in a mountainous terrain. Analysis of $\delta^{13}C$ and $\delta^{15}N$ testify to a terrestrial diet with high animal protein. A Bayesian chronological model with two ^{14}C dates and osteological prior information suggested her death occurred in the 19th–20th centuries calCE. Oral history research in the nearby village concluded that reportedly, after 1870, an adult woman of a known name had gone missing. Genealogical and archival research produced her date of birth and later corrected the earliest possible date of missing to 1891. Analysis of nuclear and mitochondrial aDNA extracted from her molar were used for identification of the skeletal remains. Her only found living matrilinear female offsprings could not be sampled for DNA due to ethical reasons. Therefore, profound archival genealogical research was conducted and two living distant matrilinear relatives were identified. They were chosen as probands and were DNA matched as relatives of the studied woman, whom we refer to as LM. Hence, we could add her date of birth as new

prior information in the Bayesian chronological model and, eventually, estimate her date of death to 1891–1927 calCE. In Slovakia, this is the first forensic archaeological-anthropological case of successful identification of a missing person from skeletal remains using a strong, multidisciplinary, case-specific research toolkit rooted both in sciences and humanities.

Keywords: skeletal remains; missing person; anthropological and palaeopathological analyses; DNA analysis; ^{14}C dating; C and N stable isotopes, archival and genealogical research; forensic archaeology and anthropology; Slovakia

INTRODUCTION

Caves are a specific part of our natural environment, with over 7,000 currently known in Slovakia. From an anthropological point of view, about one-third of the total number are potentially interesting [1].

The Liptov Basin is surrounded by limestone formations of the outer and inner zones of the Western Carpathians and is characterized by the occurrence of drier, predominantly horizontal caves. Since prehistoric times, they have been used as temporary human settlements and, in exceptional cases, as places of cultic activity [2]. Caves in the Liptov region were used from the Eneolithic Period until Modern Era [3], with the intensity of settlement being proportional to their accessibility [2]. In prehistoric times, caves were mainly used as temporary settlements, while later, in the Neolithic Period, they also became sanctuaries and places of various rituals [1]. In the Middle Ages, caves were used as temporary shelters in times of turbulence, or as short-term refuges for shepherds or groups of bandits [3, 4].

The caves in Demänovská Valley contained human skeletal remains discovered by speleologists during explorations in the 1990s. In the abyssal caves, skulls and parts of skeletons of several individuals were found. According to anthropological analyses, skeletal remains are of younger date and possibly belonged to victims of accidents or ambushes [3]. A similar find came from the abyss of Studňa na Jame (in English "Well at the Pit hill"), which was explored by volunteer speleologists in 2016–2017 (Figure 1). Studňa na Jame is a karst abyss at an altitude of 1,445 meters [5]. It lies within an extreme upland site Na Jame fortified by a rampart [6] with finds from the Iron Age, 7th–3rd cent. cal BCE [3, 6, 7]. In the Liptov basin, the extreme upland sites were parts of the Iron Age settlement system along the mountain ridges consisting of unfortified settlement on the valley floor, hillfort, and extreme upland site on the highest elevation [7, 8].

The name most likely comes from oral tradition passed down by residents. The circumference of the abyss is outlined with a low embankment. Kettner [9] claimed that it was a classic karst abyss. In contrast, Droppa [10] believed that it was dug out in search of water. He supported his claim by pointing out that prehistoric settlement had been found in the immediate vicinity. On the basis of these conflicting opinions, in 1999, cavers decided to explore Studňa na Jame for the very first time. They agreed that it was not an artificial structure, but rather a classical abyss that had been partially altered by the builders of the fortified settlement Na Jame [11]. A year later, the abyss was mapped, and its natural origin was confirmed [12].

The location of the abyss on the prehistoric fortified extreme upland site, suggests that further interesting finds may be uncovered in the future [3]. This was confirmed in 2015 and 2016 by archaeological survey, during which burnt sandstone slabs in the rampart were observed and several iron objects, fragments of pottery from the Late Hallstatt and Early La Tène Period, and granite pebbles (sling stones) were found [6, 13].

Figure 1. Map of Slovakia with location of karst abyss Studňa na Jame (Demänovská Valley, Slovakia; status according to Third military mapping of 1875–1884). 1 – karst abyss Studňa na Jame; 2 – village Pavčina Lehota; 3 – group of settlements around and of present-day town Liptovský Mikuláš. Sources: Third military mapping – WMS services arc.sazp.sk (https://arc.sazp.sk/arcgis/rest/services/uses/ruses/MapServer); base layers – ArcGIS Pro from Esri (https://www.esri.com/en-us/home). Author of the map: Jakub Tamaškovič.

BACKGROUND

During the first expedition, a human skeleton was discovered at a depth of 14 meters, 2.8 meters below the sedimentary deposit (Figure 2). The remains were in an anatomical prone position, with the right upper limb extended forward. There were no accompanying finds in the immediate vicinity of the skeletal remains that would allow their dating. Approximately 10 cm below the skeleton were limestone stones 10–50 cm in diameter without sharp edges, most likely from the surface of the abyss. They may have been thrown there by shepherds cultivating the surrounding pastures. Thus, the stratigraphy of the findspot suggested that the skeleton may not have been associated with the Lusatian-culture hillfort, but perhaps with much younger layers [5].

Figure 2. Find context – cross section with stratigraphy of karst abyss Studňa na Jame.

Given the stratigraphic indications that the skeletal remains may date back to the Modern Period, the speleologists began to investigate in the neighbouring villages whether people could remember the disappearance of any local citizens. They discovered that in the nearby village of Pavčina Lehota, there is a belief among the locals that sometime after 1870, an elderly woman went missing and most likely perished on the Studňa na Jame abyss.

This information was subsequently confirmed to us in 2019 by the Village Mayor. At that time, in fact, there was even a granddaughter of the missing woman still living in the village, who later died in 2022 at the age of 96.

We decided to launch a research project aimed at identification of the skeletal remains and clarification whether the deceased represented the disappeared woman from the village of Pavčina Lehota. We designed the project in the theoretical framework of forensic archaeology and anthropology by employing graphic and written documentation of controlled exhumation of the remains, human osteology and palaeopathology, ^{14}C and stable isotopes (C, and N) analyses, oral history in the micro-region, archival genealogical research, as well as ancient and modern nuclear and mitochondrial DNA analyses of the skeletal remains and living probands.

MATERIALS AND METHODS

Anthropological analysis

Skeletal remains were analysed using standard morphometric and morphoscopic methods [14, 15]. Sex was determined based on the degree of development of morphological traits in the skull and the postcranial skeleton [16, 17]. The age at death of the individual was estimated based on suture obliteration and age changes in the *symphysis* and *facies auricularis* of the pelvic bones [16, 18, 19]. Stature was calculated according to the method of Sjøvold [20] using the equations for the Caucasoid variety. Developmental anomalies and pathological changes in the skeleton were assessed macroscopically. Enthesopathies in the postcranial bones were evaluated according to Villotte [21, 22].

Radiocarbon dating, Bayesian modeling and stable isotopes (C, N) analysis

To estimate the time of death, the root of lower right second molar and a rib were sampled by Dremel circular saw with a clean diamond cutting disc. The collagen from both samples was extracted by modified Longin method [23, 24]. The collagen quality was analysed by %C, %N, and C:N ratio [24, 25, 26]. The potential freshwater reservoir effect was investigated by means of $\delta_{IRMS}{}^{13}C$ and $\delta_{IRMS}{}^{15}N$. Two conventional radiocarbon ages (CRA) were measured by accelerator mass spectrometry (AMS) on the rib and tooth-root collagen. The collagen extraction, graphitization, stable isotopic analysis, and CRA measurements were performed at the Beta Analytic laboratory (Miami, USA). Two CRAs were used in a Bayesian model built in OxCal program v4.4.4 [27, 28] using IntCal20 radiocarbon calibration curve [29]. In the V_Sequence model [27], prior information included the skeletal age of the deceased and the estimated periods of biological activity of the sampled tissues. CRAs and calibrated radiocarbon dates are reported according to Millard [30].

Genealogical and archival research

Genealogical research was conducted through oral history fieldwork and archival investigation at the State Archives in Žilina, headquartered in Bytča, Slovak Republic. The aim was to compile the family tree of the disappeared woman and subsequently to identify persons suitable for DNA analysis.

DNA analysis

DNA analysis included analysis of ancient DNA (aDNA) and contemporary DNA.

Ancient DNA was isolated from the upper left first molar (tooth 26). Nuclear aDNA was used to verify sex determined by anthropological analysis. Mitochondrial aDNA was used to identify skeletal remains.

The contemporary DNA was obtained from buccal swabs and was used to confirm the identity of the skeleton. Two persons – siblings (male and female) – were selected based on the compiled family tree. Using a sterile applicator, scrapings were taken from the buccal mucosa surface. Both probands consented to the examination by signing an informed consent form. All experiments using human dental tissues and buccal scrapings were performed in accordance with regular guidelines and regulations valid in the Slovak Republic. DNA sampling and analysis were approved by the Ethics Committee of the Faculty of Natural Sciences, Comenius University in Bratislava (application number ECH19018).

Sample decontamination and aDNA extraction

Molar tooth sample was decontaminated by its submersion in a bleach solution (sodium hypochlorite) for one minute. Afterwards, the tooth was washed, dried, and irradiated by UV-light (254 nm) for 30 min on each side. The crown was removed by a rotatory saw (Dremel 542). Subsequently the remaining roots were irradiated by UV-light (254 nm) for 30 min and mechanically milled to fine powder separated into two roughly the same volumes for independent extraction. Specimen was prepared using sterile tools to avoid the risk of contamination with contemporary DNA or cross contamination between the samples. In this study, we used the aDNA extraction method from bones and teeth described by Rohland and Hofreiter [31].

Sex determination, Real-time PCR analysis of amelogenin

Sex determination, along with aDNA quantification, were realized by Real-Time PCR of a 70 bp long segment of the amelogenin gene, which has slightly different homologues on the X and Y chromosomes. Sequences of designed primers, labelled X and Y probes (FAM, HEX) along with optimized PCR conditions, are available upon request. The amplification was performed in an Mx3005P (Stratagene); data were analysed in MxPro software. DNA quantification calculation was based on standard curves and detected Ct values.

HVS analysis and PCR amplification

Five pairs of primers were designed for the overlapping amplification of the hypervariable segment I (HVR I) and hypervariable segment II + III of mtDNA control region between positions 15,985–16,398, 69–361, and 437–590 (primers available upon request); PCR conditions of each amplicon were optimized previously [32]. The yield of amplification was evaluated by electrophoretic separation of 10 µL of PCR product on 2% agarose gel (Sea Kem LE agarose, Lonza). In order to detect possible contamination, both negative extraction control and negative PCR control were conducted for each PCR amplicon. PCR products were purified using ExoSAP-IT™ (Thermo Fisher Scientific) and sequenced using

BigDye™ Terminator v1.1 chemistry following the manufacturer's protocol (Life Technologies). Sequencing products were analysed on SeqStudio instrument. Obtained electrophoretograms were analysed in ChromasPro software (Technelysium) using rCRS as reference sequence (Supplemetary Figure 1) [33]. Haplogroup determination was conducted using HaploGrep tool [34] and Phylotree 17 mtDNA haplogroups nomenclature [35].

RESULTS AND DISCUSSION

Preservation and biological profile

The skeletal remains were incomplete, the 5th cervical vertebra, one left rib, both patellae and most of the small bones of the hands and legs were missing (Figure 3). The condition of the bones was relatively good, with only moderate damage (missing right zygomatic arch, few fragmented ribs, separated head of left humerus and incomplete fibulae). The preservation of dentition was very poor, with only four molars present (17, 16, 26 and 47), while four teeth were lost antemortem (14, 25, 37 and 46) and the remaining teeth were lost postmortem.

Figure 3. Skeletal remains of a 40–49-year-old female excavated from the karst abyss of Studňa na Jame

The skull was medium-robust while the mandible was gracile, both with medium-robust muscle attachments. Morphological sexual-diagnostic traits in the skull were evaluated as follows: the surface of *squama occipitalis* was slightly arched; *protuberantia occipitalis externa* was slightly marked; *processus mastoidei* were medium-sized; *tubera parietalia* slightly were marked; *frons* (forehead) was arched; *tubera frontalia* were medium-sized; *glabella* was type III; *arcus superciliares* were medium-sized; *margo supraorbitales* were sharp; orbits were squared; *arcus zygomatici* were thin; *ossa zygomatici* were high with irregular surface; *processus marginales* were medium-sized, and *crista supramastoidea* was marked. In the mandible, the *corpus mandibuale* in the place of the 2nd molar was medium-thick, the *angulus mandibulae* had medium elevation, the *trigonum mentale* was medium-marked, and the *processus articulares mandibulae* was small.

Based on metric characteristics (Supplementary Table 1), the skull was short, medium-wide and medium-high; the face was low; orbits were narrow and very low; the nose was narrow and medium-high.

Postcranial bones were gracile and medium-robust with medium-robust muscle attachments. The sexual-diagnostic traits in the pelvic bones were as follows: the pelvis was low and wide, while the entrance to the small pelvis was medium-wide and round; the *foramen obturatum* was triangular with a sharp edge; the *incisura ischiadica major dexter* was indifferent; the *incisura ischiadica major sinister* was slightly U-formed; the *angulus pubis* was approximately 90˚; the *arc composé* was double-arched; the *corpus ossis ischii dexter* was medium-thick; the *corpus ossis ischii sinister* was very narrow with weakly-formed *tuber ischiadicum*; the *crista iliaca was* pronounced S-shape; the *fossa iliaca* was high and narrow; the *tuberculum musculi piriformis* was absent; the *sulcus preauricularis* was deep and well-defined; the *spina ischiadica* was angular and the *acetabulum* large and anterior-lateral oriented. Metric characteristics of the postcranial bones can be found in Supplementary Table 2.

The skeletal remains belonged to a female (DS score=-0.28, [16]) who died at the age of 40–49 years. The stature of the woman was calculated to be 157.3±6.8 cm.

Palaeopathological analysis

Several (pathological) changes were present in the skeletal remains, which we classified according to the period of their occurrence as antemortem (clearly occurred before death and were healed), perimortem (occurred very shortly before or at the time of death), and postmortem (occurred after death as taphonomic changes, Supplementary Figure 2).

A depressed lesion was present on the right parietal bone near the coronal suture (Figure 4A, B, C). The lesion diameters were 22×19 mm, and the bone fragments were slightly depressed into the cranial cavity.

The shape of the lesion, the impacted fragments into the cranial cavity, and the character and coloration of the fracture surfaces do not exclude a possible perimortem injury. Depressed skull fractures occur after a hit with a blunt object, e.g. a hammer or a stone, either intentionally or accidentally. They have the character of circumscribed lesions with a circular fracture line around the depressed site and impaction of bone fragments into the intracranial space. However, distinguishing perimortem fractures on the skull from postmortem fractures, which occurred long before exhumation, is quite problematic [36]. Radial fissures, which are often a concomitant feature in impressive injuries to the flat bones of the skull, are not present in this case, and for this reason our conclusions are not clear. The head injury may have occurred shortly before death, but it must be considered that the skeleton was lying in the abyss, into which stones and soil were falling or sliding from the surface of the abyss. A lesion in the parietal bone could have occurred postmortem because of a stone striking the skull vault.

In the skull, another lesion located on the right side of the squamous part of the frontal bone just above the frontal eminence was present. The perforation was 15×10 mm in size and a bone fragment was missing. Since its edges were sharp, irregular, and of a lighter colour in the place of the lesion than the surrounding bone (Figure 4A, B, D), it is evident that the lesion occurred certainly postmortem. We suppose that the formation of this perforation may have occurred during the exhumation of the skeletal remains or even after the exhumation from the abyss. The environment in the abyss was humid, which thus made the bones soft, and the lesion in the skull may have occurred as a result of careless handling.

Figure 4. A, B – Lesions on the cranial vault – in the right parietal bone (white arrow) and in the frontal bone (yellow arrow). C – The perforation in the right parietal bone. Time of its origin cannot be estimated with certainty, perimortem interval is possible but not provable. D – The lesion in the frontal bone occurred postmortem.

A healed compression fracture of the vertebral body was recorded on the first lumbar (L1) vertebra (Figure 5A). The vertebral body is slightly wedge-shaped from the side view, and the top surface is slightly concave. According to Genant´s scoring method [37, 38], this is a grade 1 wedge fracture. The vertebral body is mildly-deformed and decreased by 20–25% in height. Vertebral compression fractures represent the most common osteoporotic fractures. The incidence increases markedly with age and is slightly more common in women due to lower bone mineral density [39]. Typically occurring due to biomechanical failure under axial or compressive loading, these fractures predominantly involve the anterior column of the vertebral body, usually resulting in a characteristic wedge-shaped deformity [36]. In younger people, vertebral compression fractures typically result from high-energy traumatic events, e.g., fall from significant heights. Another etiological factor is osseous involvement in neoplastic pathologies, such as multiple myeloma [39]. Vertebral injuries in the thoracolumbar region are also indicative of chronic loading of the spine during strenuous work activities [40].

Another healed fracture was present on the 5th right-sided rib at the site of the costal angle, where the callus is well-recognized (Figure 5B). Rib fractures are caused by direct or indirect violence. While the ribs are broken in the anterior axillary line in direct violence, the line is in a perpendicular direction to the application of pressure in indirect violence. Fractures near the costal angle also occur with anteroposterior compression of the thorax. The most common cause of rib fractures are direct impacts and blows to the chest, often because of fights and falls, while occurring more rarely as a result of repeated stress on the ribs as so-called stress fractures, or with severe coughing. Fractures most commonly occur in the middle segment of the thorax, i.e. from the fourth to the tenth rib [41].

Clinical studies have shown patients with L1 vertebra burst fractures frequently associated with rib fractures, notably in the lower rib cage due to this mechanism of injury and force. The thoracolumbar junction, including L1 vertebra, acts as a biomechanical transition zone between the relatively rigid thoracic spine (with ribs attached) and the more flexible lumbar spine. Due to this transition, axial compressive forces (such as those from a fall on the feet or buttocks) are concentrated here, leading to burst fractures. During the axial compression that causes the L1 vertebra burst fracture, multiple fracture lines may have propagated throughout the vertebral body, which is transmitted through adjacent structures. Since the ribs are attached to the thoracic spine just above the L1 vertebra, the energy from the axial load can extend to the lower ribs leading to rib fractures, usually in the lower thoracic ribs (around the 9th to 12th ribs) which are anatomically close to the thoracolumbar junction [42]. When taking into consideration the fact that the woman from Studňa na Jame had a fracture in the upper chest segment, we hypothesize that fractures of the 5th rib and the L1 vertebra did not occur simultaneously in a single accident.

A congenital defect and degenerative-productive changes were present in the postcranial skeleton as well.

A posterior midline cleft was present on the first cervical vertebra (Figure 5C). The width of the cleft is approximately 3 mm. According to a system proposed by Currarino et al. [43], congenital defect of the posterior atlas arch was classified as type A, which is a common type (approximating 80% of cases) and is encountered in 4% of the general population.

Osteoarthrosis on the tuberculum costae was present on three right-sided and seven left-sided ribs. Most of vertebrae were affected by the spondylosis deformans (Figure 5D). Parts of the cartilage attaching the ribs to the sternum were ossified on four ribs.

Figure 5. Pathological and congenital changes in the postcranial bones. A – Healed compression fracture of the L1 vertebral body. B – Healed fracture (callus) on the fifth right rib (white arrow). C – Posterior midline cleft in the first cervical vertebra. D – Spondylosis deformans in the lumbar vertebrae (white arrow).

Distinct enthesopathy was present on the postcranial skeletal bones, with more pronounced enthesopathy on the right-sided bones than on the left-sided bones (Table 1). Enthesopathies are considered markers of physical stress, thus indicating the degree of physical stress in an individual. However, it should be noted that the development of enthesopathies is also dependent on the age and sex of the individual. In younger individuals, enthesopathies are predominant on one side of the skeleton and are associated specifically with physical activity, whereas in older individuals, they generally occur bilaterally on most limbs and are related to age-related changes [44]. In the female from the Demänovská Valley, enthesopathy was more pronounced on the right side, indicating a preference for the right limbs. The presence of enthesopathies on the bones of the lower limbs and spondylosis in the lower part of the spine may indicate walking in difficult terrain and carrying heavy loads on the back. However, degenerative-productive changes should be interpreted regarding the age of the individual, as they are also a typical manifestation of physiological aging of the organism.

Table 1. Enthesopathies in the postcranial bones of the skeleton from the karst abyss Studňa na Jame (evaluated according to Villotte [21, 22]).

Stable isotopes analysis and radiocarbon dating

According to measured bone collagen quality indicators (Table 2), the collagen is well preserved [25, 26]. The atomic C/N ratio of the extracted collagen together with the %C shows that the samples are degraded but not contaminated [26]. Considering only the C/N ratio [45], the samples can be considered well preserved. Both δ^{13}C values of the collagen indicate C3 biome. Enriched δ^{15}N in the samples indicates a higher proportion of animal proteins in the diet, which we relate to the traditional subsistence in northern Slovakia typical for sheep breeding and consumption of sheep meat and milk products.

Table 2. Collagen quality indicators, stable isotopes, and conventional radiocarbon ages.

Due to the absence of δ^{13}C and δ^{15}N data on aquatic biota from the region and time of our interest, it is not possible to identify any freshwater diet component in the measured values, as exampled by recent studies from elsewhere [46, 47]. Even if sometimes δ^{13}C of

European freshwater fish are significantly less negative [48], without environmental data from the region, we must rely on information about traditional regional subsistence. Considering the traditional subsistence, we assume that the measured values represent C3 cereals, vegetables, cow and sheep milk, as well as meat.

As the source of carbon is thought to be terrestrial without any measurable freshwater reservoir component, the CRAs can be used for chronological purposes without further corrections. Due to atmospheric ^{14}C concentration in the Northern Hemisphere in 1650–1950 CE (IntCal2020) [29], vague-prior calibrated ^{14}C dates have wide distributions. Tooth 47 is dated to three intervals from 1675 to 1942 calCE (95.4% probability), and the rib from 1679 to 1941 calCE (95.4 probability). In the Bayesian chronological model for the time of death (V_Sequence; Figure 6) we used as prior information anthropologically estimated age at death 40–49 (44.5±4.5) years and eruption of analysed tooth 47 (12±1 yrs). The resulting maximum probability density for the time of death of the deceased woman is in 1838–1978 calCE (59.6% at 68.3% probability level).

Figure 6. Bayesian chronological model with radiocarbon dates taking into account the carbon turnover estimates in sampled hard tissues and the skeletal age at death of the analysed individual. End boundary indicates the time of death.

Genealogical and archival research

In the village of Pavčina Lehota, it is said that after 1870 a woman disappeared from the village (pers. comm. Deputy Mayor of the municipality 2019).

Through archival research, we were able to establish that the missing woman was born in 1848. At the age of 16, she married for the first time and, after 16 years of marriage, gave birth to an only son. She was widowed in 1889. In 1891, at the age of 42, she married a second time to a 25-year-old man. This marriage was childless.

From the above facts, it follows that the woman from Pavčina Lehota could have disappeared in 1891 at the earliest (after the marriage), i.e. at least 20 years later than the legend says.

It should be noted that at the time of our research (2019) the only living granddaughter of the missing woman was still living in the village of Pavčina Lehota. Her relatives did not want to traumatize her due to her old age and therefore she did not cooperate with us in the identification of the disappeared grandmother. To obtain samples of genetic material suitable for identification, we were forced to create a family tree of the disappeared woman using vital records. Based on the family tree, two persons suitable for DNA analysis were subsequently identified.

Creation of the family tree was a difficult task mainly due to two major obstacles. The first problem was with the namesakes, meaning people with the same first and last names. Since Pavčina Lehota is situated in the Demänovská Valley surrounded by mountains, the population that lived there in the past showed signs of genetic isolation. For a long time, there was a relatively closed community of people who intermarried, and as a result, there were more people living there who not only had the same last name, but also the same first name.

The second problem was related to genetic analysis. Since the identification of the missing woman was to be made using mitochondrial DNA, we needed living individuals who were related only through the female lineage. Unfortunately, many of the women from the previous two–three generations had died in infancy, i.e. without descendants, or had male offsprings. We found a common female ancestor six generations back. This was a woman who lived in the second half of the 18th century and gave birth to eight children (three boys and five girls). The missing woman from Pavčina Lehota was matrilineally related to the daughter born to this woman as the third child. Comparative genetic material was obtained from siblings (male and female) matrilineally-related to the youngest daughter (Figure 7).

Figure 7. Family tree compiled on the basis of archival research. Arrows designate DNA-analysed individuals. Deceased woman from the karst abyss Studňa na Jame is marked with a yellow arrow; the living matrilinear related individuals with blue arrows.

aDNA sex determination

Sex estimation from the skeletal remains was also verified by DNA analysis. In the aDNA sample obtained from the upper left first molar, the amplification curve in both duplicates was detected only for the target sequence AMELX (FAM) using RT-PCR analysis. The measured average Ct value was 29.4, which, based on the constructed calibration curve, represents a copy number of the target X chromosome sequence of 128.6. Control reactions without added DNA template (NTC), as well as with added "blank" DNA isolation sample, were negative, i.e. without amplification. Thus, the results indicate that the skeletal remains indeed belonged to a female.

mtDNA identification

Detected haplotype of mitochondrial DNA belonging to H1c haplogroup (Table 3) is relatively common (frequency of 2.9%) in Central European populations. However, estimated frequency of detected combined HVS1 and HVS2+3 haplotype in the Slovak population based on our database, which consists of more than 2,000 records, is below 0.5%. According to the EMPOP database, which consists of approximately 48,000 records, complete mtDNAs frequency of detected combined haplotype of HVS1 and HVS2+3 data is circa 0.43% (69 out of 15,782) in the European metapopulation.

Assuming haplotype frequency of 0.5%, it is 200 times more likely that observed haplotypes of surveyed individuals are consistent due to virtual matrilinear kinship as a result of random match; thus, kinship probability is 99.5%.

Table 3. Detected haplotype of mitochondrial DNA in the skeleton from the karst abyss Studňa na Jame and matrilineally-related man and woman.

Discussion

Initially, the analysed find was without contextual information, which demanded case-tailored research methodology [49]. The skeletonized body without artefacts was in a remote karst abyss under 2.8 m of sediment, within an upland hillfort from the 11th–5th century calBCE.

However, the oral history in the micro-region turned out to be sufficient in contextualising the find. Accordingly, we formulated our research question whether the skeletal remains belonged to the missing woman reported about in the nearby village.

The woman, aged 40–49 years with cranial and possibly perimortem trauma, had enthesopathies which testified to severe physical labour in a mountainous terrain, thus implying her non-privileged status in the local population. Radiocarbon excluded the prehistoric dating of the find. A Bayesian chronological model from two CRAs with an uncertainty of 30 BP-years brought a wide range (1700s to 1900s calCE, cf. Table 2 and Figure 6), which is typical for the post-1650 CE period with oscillating atmospheric ^{14}C concentration.

The chronometric date of the remains defines the find as a forensic archaeological-anthropological case. Even if forensic archaeological-anthropological research in the Slovak Republic has still not been formally established, the present work represents this very type of investigation [50]. The used case-specific research toolkit gathered diverse approaches from sciences and humanities, all aiming at the identification of the deceased.

The results of anthropological and palaeopathological analyses proved to be with the oral tradition in the nearby village reporting about a woman known by name and surname, who went missing reportedly after 1870. The subsequent archival research brought the date of birth of the missing woman (1848) and corrected the earliest possibility for the date of disappearance (1891). Next, skeletal remains were genetically characterized by mitochondrial and nuclear aDNA analyses. Living matrilinear relatives of the missing woman were searched for to genetically identify the skeletal remains. The screening of the living population in the village pinpointed her only elderly granddaughter who, on ethical grounds, could not be approached as a DNA proband.

Accordingly, profound genealogical research in archives was launched and resulted in the identification of two living distant relatives of the missing woman in the female lineage from their common female ancestor born in the mid-18th century. The living probands were successfully sampled, and the DNA analysis brought a match between them and the studied skeleton. Ultimately, the skeletal remains were identified as the missing woman known from the local oral tradition and we refer to her [cf. 50] further as LM.

With this knowledge, we have attempted to refine the estimate of LM's date of death, which apparently vanished in the village. The date of birth was put as prior information into the existing Bayesian chronological model and reduced the date of death estimate to 1880–1927 calCE (68.3% probability). The archival information about LM's second marriage in 1891 enables the further shortening of this interval to 1891–1927 calCE. The highest probability density of the posterior density function is around 1900 calCE (Figure 8), which corroborates to the later part of the anthropologically-ascertained skeletal age of the LM (40–49 years), who was born in 1848.

Figure 8. Posterior density function for the event of death after formation of the tooth root and rib tissue represented as end boundary in V_Sequence (OxCal: Bronk Ramsey 2024 [27]). The death of LM is estimated to 1880–1927 calCE (68.3% probability level).

As for the cause of LM's death, she succumbed to injuries sustained by a 14-meter-deep fall into a narrow, well-like karst abyss. The only skeletal injury possibly of perimortal origin is a fracture in the right parietal bone. If this was a result of inter-personal violence, accidental fall or – according to the village oral tradition – a suicidal motive, it cannot be conclusively proven by the physical and archival evidence gathered by the present research. Not even the organization of the bones in the sediment documented in the course of the exhumation has brought any conclusive argument about the perimortal activity of the wounded woman. It might have been premortal in-vivo extension movement of the right upper limb or a result of the archaeologization of the body.

Since we consider the human remains important for their cultural, symbolic, and spiritual value, as well as feel ethical responsibility [50], the final stage of our endeavour, beyond the scientific research, is appropriate disposal of LM's body. We envisage that after both LM's relatives and local religious authorities will have been contacted, a burial site in the micro-region will be sought to bury LM's body in appropriate interment.

Conclusions

Speleological investigation of the karstic abyss Studňa na Jame near the village of Pavčina Lehota (district Liptovský Mikuláš, Slovakia) resulted in an inadvertent find of human bones. In the course of excavation in 2016 under extremely difficult conditions, a nearly complete skeleton of an adult woman was exhumed and documented. Subsequently, a synergic research project was launched in the theoretical framework of forensic archaeology and anthropology employing documentation of controlled exhumation of the find, human osteology and palaeopathology, ^{14}C and stable isotopes (C, N) analyses, use of oral tradition research in the micro-region, archival genealogical research, and ancient and modern nuclear and mitochondrial DNA analyses of the skeletal remains and living probands. The collected evidence resulted in successful identification of the deceased as a missing woman still known in the village by name and surname, who reportedly disappeared in the late 19th century. We have corrected the earliest possible year of her disappearance, estimated date of her death, and discussed the cause of death with possible motives leading to this tragic occurrence. In Slovakia, without any formally-constituted forensic archaeological-anthropological research, we have successfully identified a person from their skeletal remains for the first time who went missing more than a century ago.

Data availability

The mitochondrial DNA sequences (HVR1–HVR3) generated in this study have been deposited in GenBank under accession numbers PX571993–PX571995 and are publicly available at:
https://www.ncbi.nlm.nih.gov/nuccore/PX571993
https://www.ncbi.nlm.nih.gov/nuccore/PX571994
https://www.ncbi.nlm.nih.gov/nuccore/PX571995

Supplementary Information

Supplementary Figure 1 presents a demonstrative image of PCR amplicons from hypervariable region 1 (HVR1) of mitochondrial DNA, specifically targeting positions 16159–16264 and 16282–16400. The gel image includes amplification products from multiple ancient DNA (aDNA) samples, including the sample from Demänovská Valley (labelled DJ; in the manuscript referred to as LM), positive controls, and a negative extraction control. The image serves to illustrate the success and specificity of the amplification process across the targeted mtDNA regions.

Supplementary Figure 2 presents schematic illustration of antemortem, perimortem and postmortem changes in the skeletal remains.

Supplementary Figure 1. Gel electrophoresis image of PCR amplicons targeting HVR1 regions of mtDNA (positions 16159–16264 and 16282–16400). Samples include multiple ancient DNA extracts, the Demänovská Valley sample (DJ), positive controls, and a negative control. The figure demonstrates successful amplification and absence of contamination.

Supplementary Figure 2. Antemortem, perimortem and postmortem changes in the skeleton. Green arrows – antemortem traumas in L1 vertebra and 5th right rib; Blue arrow – perimortem trauma in the right parietal bone; Yellow arrows – postmortem lesions in the frontal bone, right zygomatic arch, ribs, left humerus and both fibulae.

Supplementary Tables

Supplementary Table 1. Metric characteristics in the skull.

Supplementary Table 2. Metric characteristics in postcranial bones.

Declarations

Ethical declaration

The authors confirm that all experiments using human dental tissues and buccal scrapings were performed in accordance with regular guidelines and regulations valid in the Slovak Republic. DNA sampling and analysis were approved by the Ethics Committee of the Faculty of Natural Sciences, Comenius University in Bratislava (application number ECH19018).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding declaration

This research was supported by:

- the EU NextGenerationEU through the Recovery and Resilience Plan for Slovakia under the project No. 09I03-03-V04-00672 – **Peter Barta**,
- Slovak Research and Development Agency (APVV-23-0299) – **Marian Baldovič, Silvia Bodoriková and Michaela Dörnhöferová,**
- The Cultural and Educational Grant Agency of the Ministry of Education, Research, Development and Youth of the Slovak Republic (KEGA 028UK-4/2023) - **Michaela Dörnhöferová and Silvia Bodoriková,**
- Scientific Grant Agency of the Ministry of Education, Research, Development and Youth of the Slovak Republic and the Slovak Academy of Sciences (VEGA 1/0333/26) – **Silvia Bodoriková and Michaela Dörnhöferová.**

Gabriela Bľandová, Zuzana Šimková, Michaela Kerešová, Pavel Herich, and Peter Laučík are not members of the listed research grants covering the presented research and were not supported by any special funding focused on the presented research.

Acknowledgments

We would like to thank Jakub Tamaškovič for creating a map with the location of the karst abyss Studňa na Jame, and Michael Sabo for proofreading the manuscript. Radiocarbon and stable isotopic analyses were covered by a donor from the Slovak Republic.

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Gabriela Bľandová (GB): Methodology, DNA analysis.

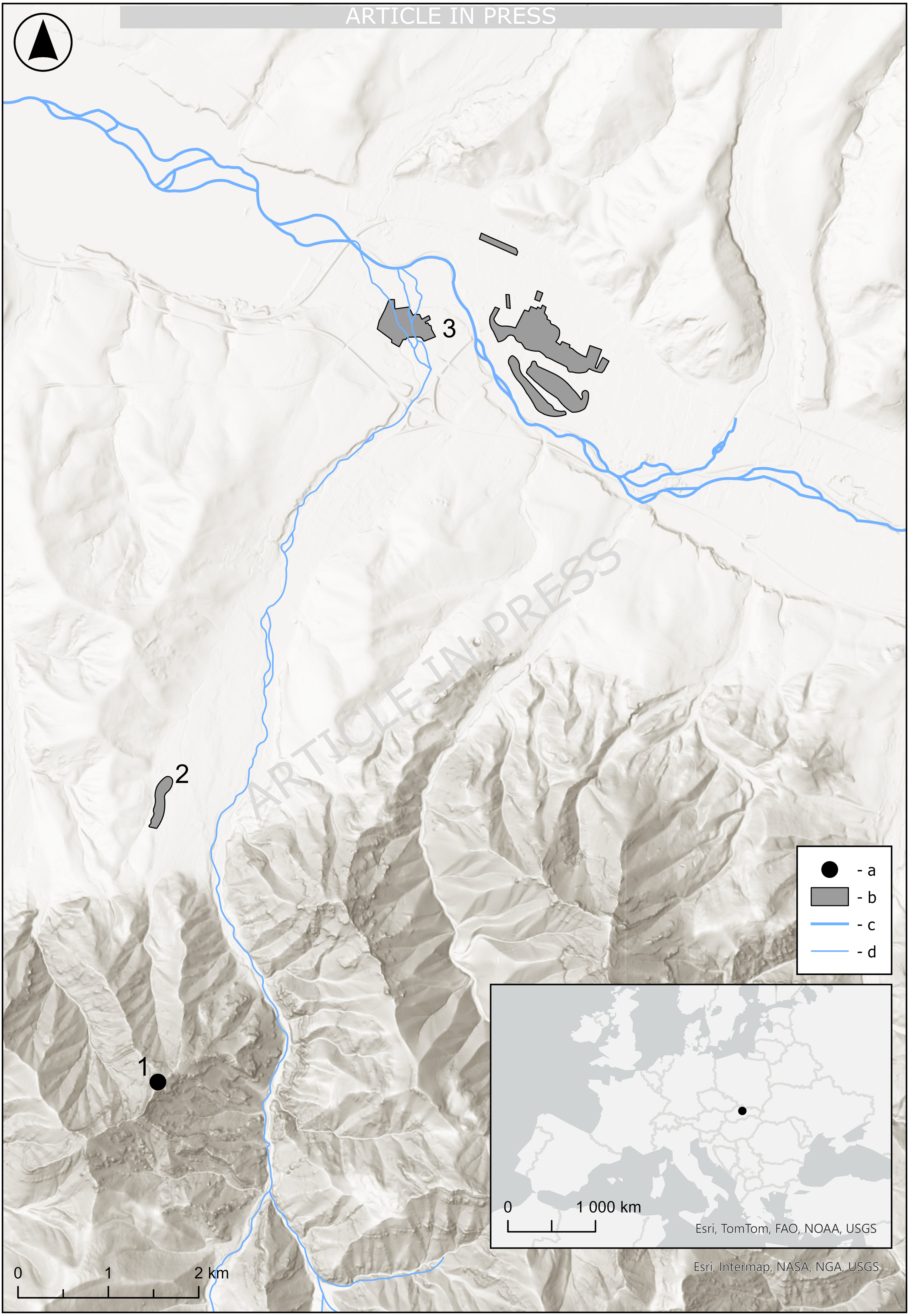
Zuzana Šimková (ZŠ): Methodology, Archaeological analysis, Validation, Formal analysis.

Michaela Kerešová (MK): Methodology, Genealogical analysis, Validation

Pavel Herich (PH): Methodology, Speleological analysis and excavation, Validation, Formal analysis.

Peter Laučík (PL): Methodology, Speleological analysis and excavation, Validation, Formal analysis.

Silvia Bodoriková (SB): Writing – review and editing, Writing – original draft, Visualization, Methodology, Anthropological and palaeopathological analysis, Formal analysis, Conceptualization.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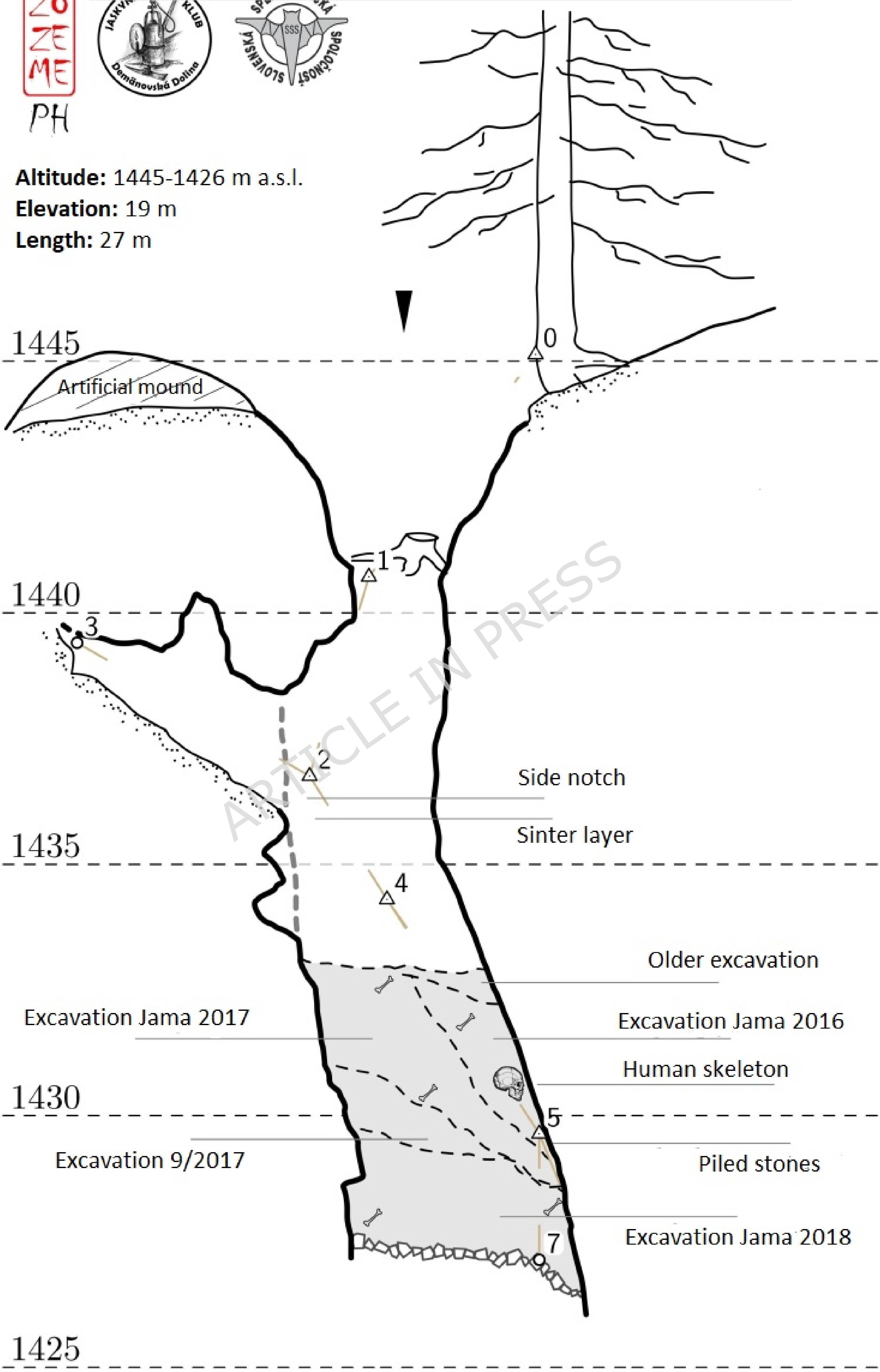
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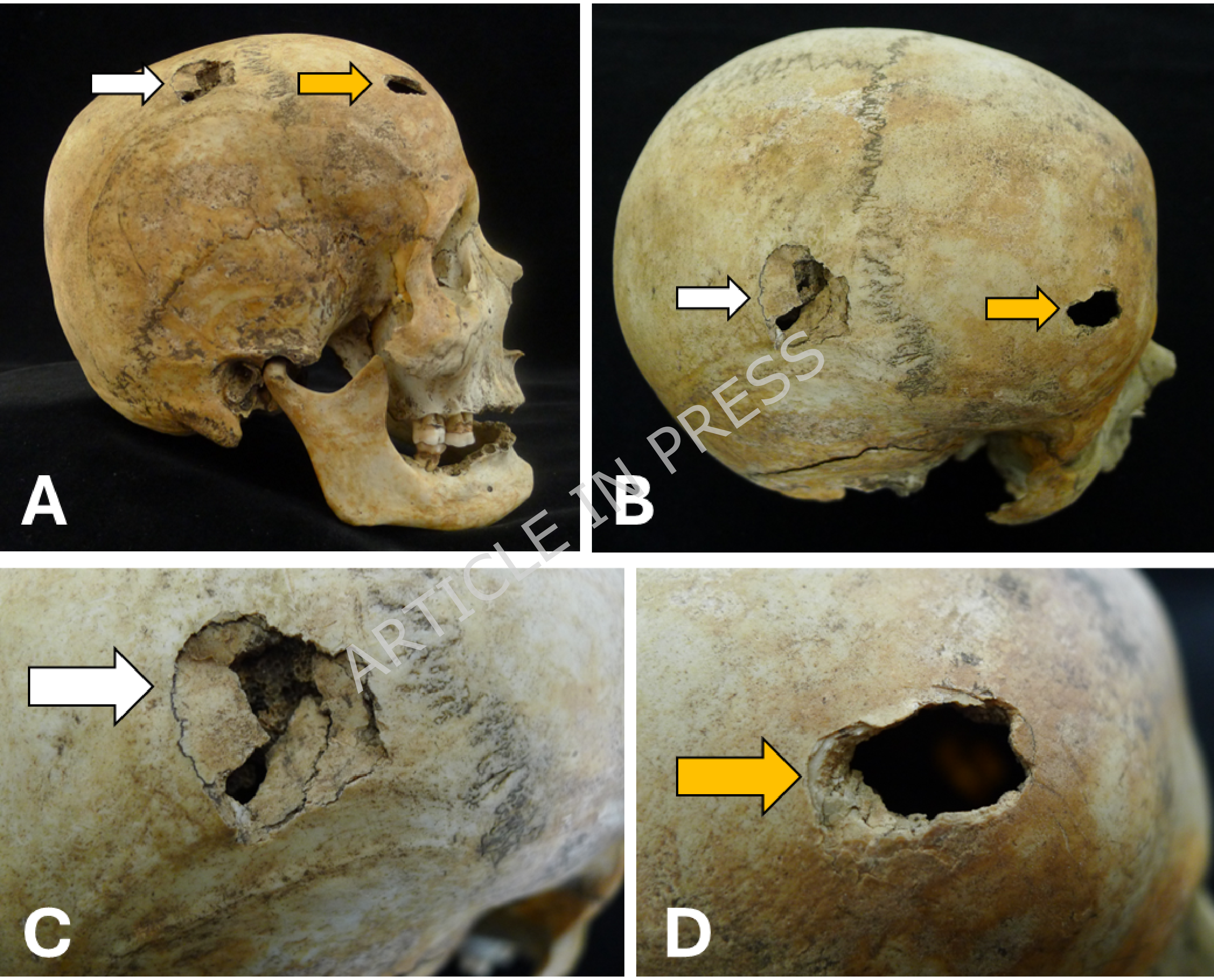
Excavation 9/2017
Piled stones
Excavation Jama 2018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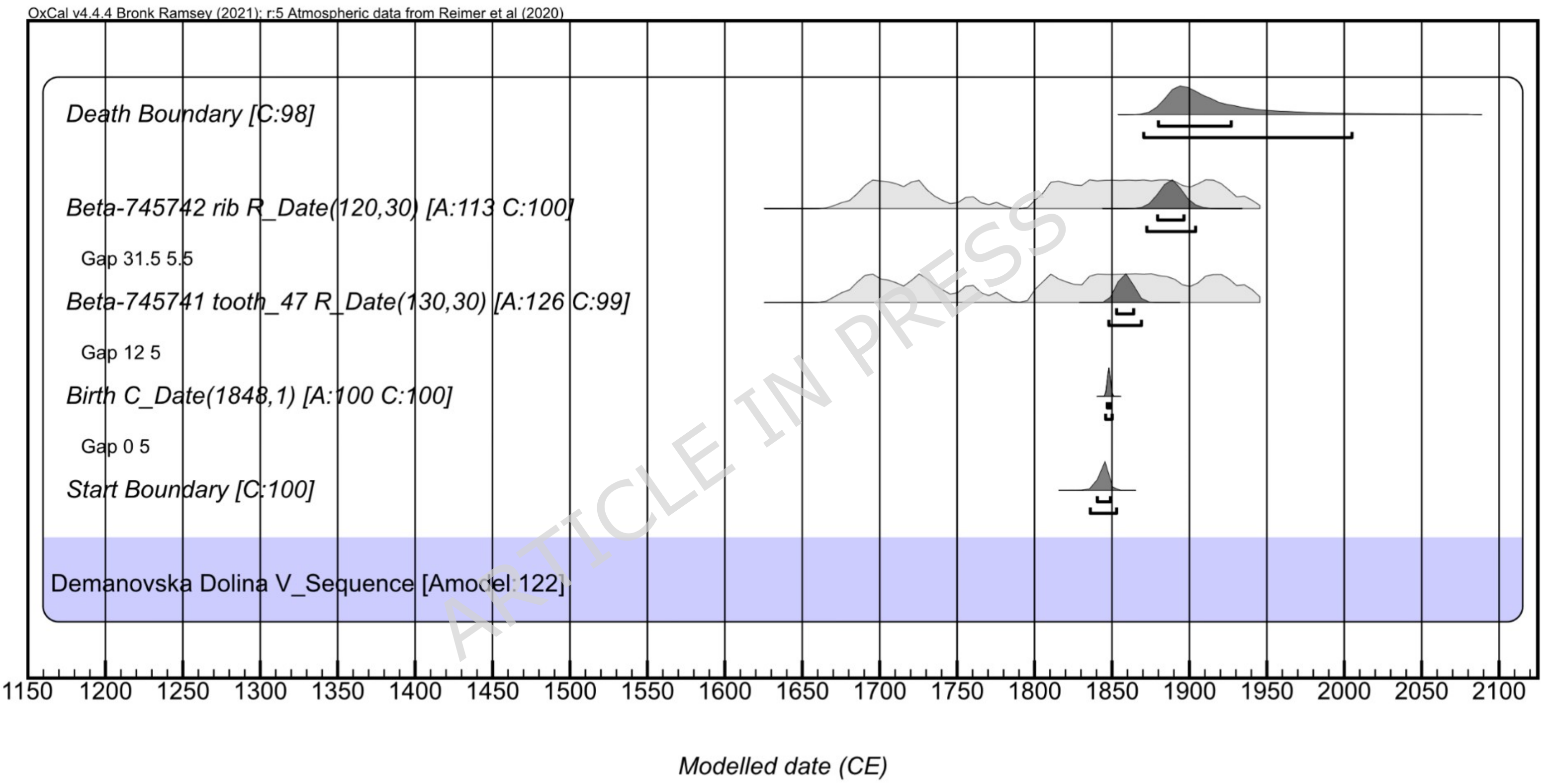

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Gap 31.5 5.5
Beta-745741 tooth_47 R_Date(130,30) [A:126 C:99]
Gap 12 5
Birth C_Date(1848,1) [A:100 C:100]
Gap 0 5
Start Boundary [C:100]
Demanovska Dolina V_Sequence [Amodel:122]
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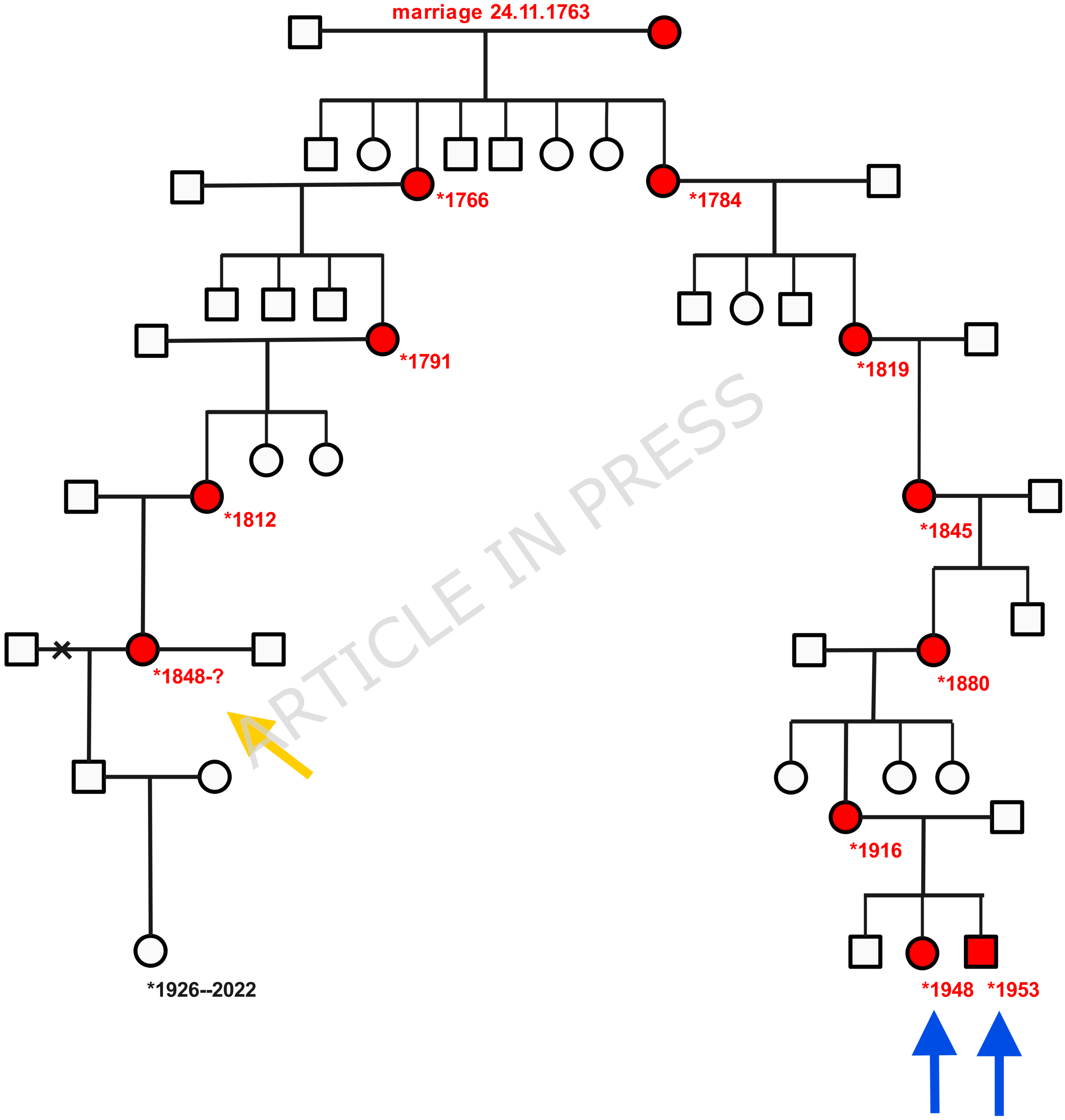
Modelled date (CE)



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*1948
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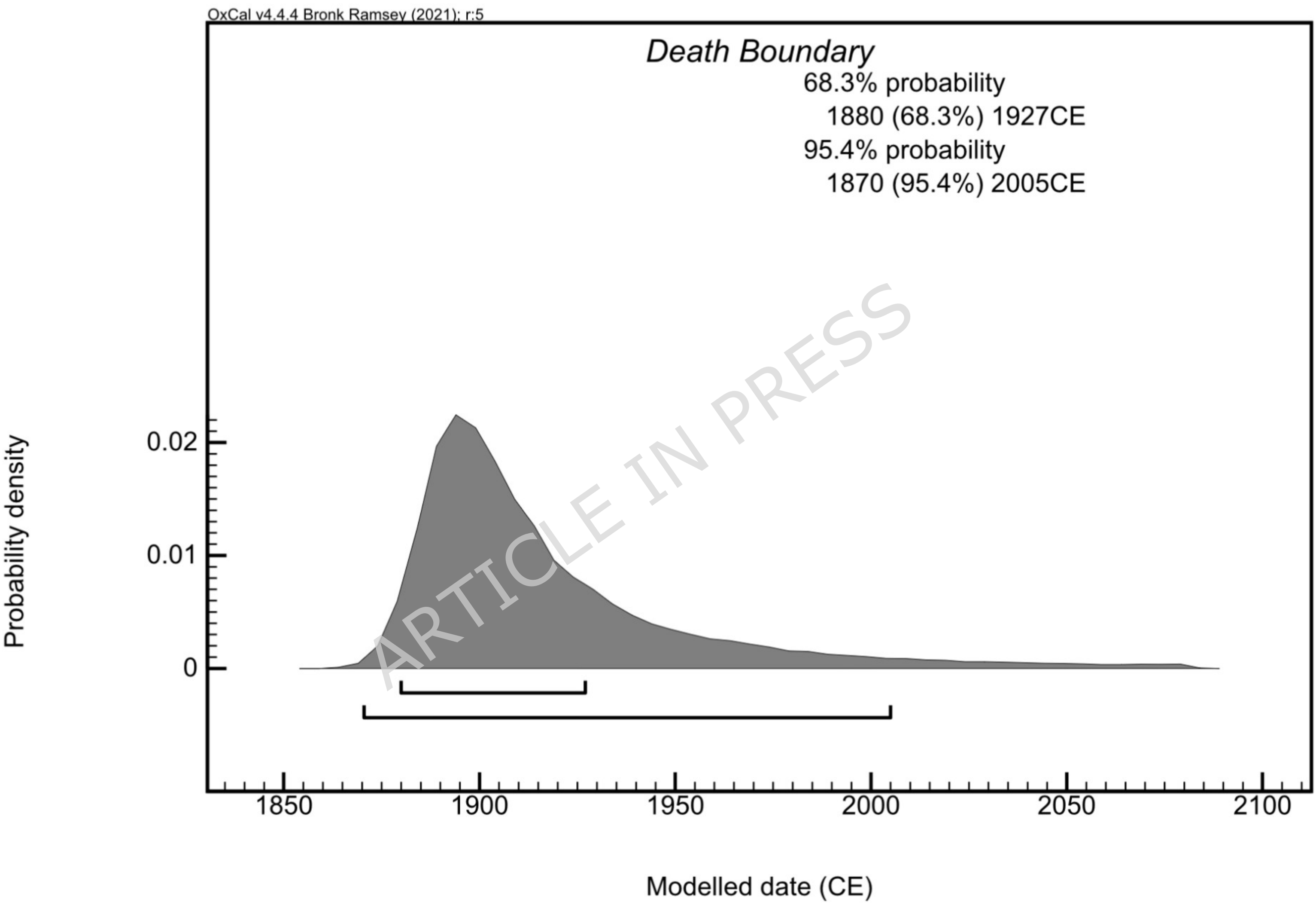
OxCal v4.4.4 Bronk Ramsey (2021); r:5
Death Boundary
68.3% probability
1880 (68.3%) 1927CE
95.4% probability
1870 (95.4%) 2005CE
Probability density
0.02
0.01
0
1850
1900
1950
2000
2050
2100
Modelled date (CE)

List of figures

Figure 1. Map of Slovakia with location of karst abyss Studňa na Jame (Demänovská Valley, Slovakia; status according to Third military mapping of 1875–1884). 1 – karst abyss Studňa na Jame; 2 – village Pavčina Lehota; 3 – group of settlements around and of present-day town Liptovský Mikuláš. Sources: Third military mapping – WMS services arc.sazp.sk (https://arc.sazp.sk/arcgis/rest/services/uses/ruses/MapServer); base layers – ArcGIS Pro from Esri (https://www.esri.com/en-us/home). Author of the map: Jakub Tamaškovič.

Figure 2. Find context – cross section with stratigraphy of karst abyss Studňa na Jame.

Figure 3. Skeletal remains of a 40–49-year-old female excavated from karst abyss Studňa na Jame.

Figure 4. A, B – Lesions on the cranial vault – in the right parietal bone (white arrow) and in the frontal bone (yellow arrow). C – The perforation in the right parietal bone. Time of its origin cannot be estimated with certainty, perimortem interval is possible but not provable. D – The lesion in the frontal bone occurred postmortem.

Figure 5. Pathological and congenital changes in the postcranial bones. A – Healed compression fracture of the L1 vertebral body. B – Healed fracture (callus) on the fifth right rib (white arrow). C – Posterior midline cleft in the first cervical vertebra. D – Spondylosis deformans in the lumbar vertebrae (white arrow).

Figure 6. Bayesian chronological model with radiocarbon dates taking into account the carbon turnover estimates in sampled hard tissues and the skeletal age at death of the analysed individual. End boundary indicates the time of death.

Figure 7. Family tree compiled on the base of archival research. Arrows designate DNA-analysed individuals. Deceased woman from karst abyss Studňa na Jame is marked with yellow arrow, the living matrilinear related individuals with blue arrows.

Figure 8. Posterior density function for the event of death after formation of the tooth root and rib tissue represented as end boundary in V_Sequence (OxCal: Bronk Ramsey 2024). The death of LM is estimated to 1880–1927 calCE (68.3% probability level).

Supplementary Figures

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Valley sample (DJ), positive controls, and a negative control. The figure demonstrates successful amplification and absence of contamination.

Supplementary Figure 2. Antemortem, perimortem and postmortem changes in the skeleton. Green arrows – antemortem traumas in L1 vertebra and 5th right rib; Blue arrow – perimortem trauma in the right parietal bone; Yellow arrows – postmortem lesions in the frontal bone, right zygomatic arch, ribs, left humerus and both fibulae.

Table 1. Enthesopathies in the postcranial bones of the skeleton from the karst abyss Studňa na Jame (evaluated according to Villotte [21, 22]).

Location	**Right**	**Left**
Extremitas acromialis claviculae	B	A
Tuberositas deltoidea humeri	B	B
Epicondylus lateralis humeri	B	B
Olecranon ulnae	B	A
Tuberositas radii	C	A
Symphysis ossis pubis anterior	C	B
Crista phalica	C	B
Tuber ischiadica	C	B
Trochanter major femori	C	B
Tuberositas glutea	C	B
Linea sapéra	C	B
Tuberositas tibiae	C	B
Tuber calcanei	N	B

A – enthesopathies not present; B – enthesopathies slightly created; C – enthesopathies significantly created; N – not evaluable.

Table 2. Collagen quality indicators, stable isotopes and conventional radiocarbon ages.

Sample code and description	**Labcode**	**Coll. yield (%)**	**C:N**	**C (%)**	**N (%)**	**δ_{IRMS} 13C (‰)**	**δ_{IRMS}15N (‰)**	**Conv. ^{14}C age (BP) and fraction modern carbon**
DEDO_47_1 root of tooth 47	Beta-745471	NA	3.0	40.91	15.96	-19.72±0.30	12.13±0.40	130±30 0.9839±0.0037
DEDO_RIB_2 rib	Beta-745472	NA	2.9	41.12	16.48	-20.15±0.30	11.55±0.40	120±30 0.9852±0.0037

Table 3. Detected haplotype of mitochondrial DNA in skeleton from the karst abyss Studňa na Jame and matrilineally-related man and woman.

Sample name	HVS1 region positions sequenced	HVS1 haplotype – differences comparing to rCRS	HVS2+3 region positions sequenced	HVS2+3 haplotype – differences comparing to rCRS	Haplogroup
LM	15995–16398	CRS	69–361; 437–590	263G.315+C.477C	H1c
PG	15830–16527	16519C	60–620	263G.315+C.477C	H1c
AG	15830–16527	16519C	60–620	263G.315+C.477C	H1c

LM – skeleton from the karst abyss Studňa na Jame; PG – male selected based on family tree; AG – female selected based on family tree; PG and AG are siblings.