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NEW INSIGHTS INTO LATE PLEISTOCENE CAVE HYENA CHRONOLOGY AND POPULATION HISTORY—THE CASE OF PERSPEKTYWICZNA CAVE, POLAND

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ABSTRACT. The paper focuses on the Pleistocene deposits in Perspektywiczna Cave, southern Poland, related to cave hyena (*Crocuta crocuta*). We used direct radiocarbon dating of hyena fossils supported by genetic and stable isotope analyses to infer the paleobiology of this population. Radiocarbon dating of 19 hyena remains suggests long inhabitation of the region during early MIS 3, around 50–34 ky cal BP. The youngest among our dates, 34,355–33,725 cal BP (1σ, combined of two dates for the same specimen) points out the latest appearance of a cave hyena north to Carpathians. Beside this long period of occupation, the Perspektywiczna Cave hyenas stayed ecologically stable, but their genetic structure changed. Two mtDNA haplogroups were present, one typical for other Late Pleistocene European populations and the other one known so far only from recent African populations.

KEYWORDS: collagen, *Crocuta*, paleogenetics, paleontology, stable isotopes.

INTRODUCTION

The spotted hyena, *Crocuta crocuta* (Erxleben 1777), is currently native to Africa, but during the Pleistocene the species inhabited large areas of Eurasia (Werdelin and Solounias 1991; Lewis and Werdelin 2022). There it was an important member of the mammoth steppe ecosystem (Kurtén 1968; Kahlke 1999). European populations, often referred to as cave hyenas and sometimes grouped into a separate subspecies or species, *C. c. spelaea* or *C. spelaea*, became extinct by the end of the Late Pleistocene (Stuart and Lister 2014). Their disappearance was among the early signals of the late Quaternary megafaunal mass extinction (Koch and Barnosky 2006; Stuart and Lister 2014).

A large-scale radiocarbon (^{14}C) survey by Stuart and Lister (2014) revealed that cave hyena disappeared from Central and Eastern Europe ca. 40 ka BP and survived until ca. 31 ka BP in the western and southern parts of the continent. However, of the 104 ^{14}C dates available for cave hyena remains from Europe by 2014, only 15 of them were from Central Europe. Recently published ^{14}C dates from Lindenthaler Höhle (Westbury et al. 2020) provided evidence of quite late occurrence of hyena in eastern Germany, as late as ca. 22 ka BP. This suggests that the European cave hyena extinction was more complex, and hyenas could have survived longer in some parts of Central Europe. Therefore, more chronological data from variable regions are

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needed to comprehensively understand the paleobiogeography of the hyena's late populations and the complexity of their extinction.

In this paper, we investigated the population's evolutionary history from a region so far weakly studied, the Polish Jura in southern Poland. We focused here on a Late Pleistocene cave hyena den recently discovered at Perspektywiczna Cave. We used direct ^{14}C dating supported by genetic and stable isotope data to infer the paleobiology of this population. The results brought important evidence of the cave hyena regional survival and its genetic variabilty in Central Europe.

The Site—Perspektywiczna Cave and Its Hyena Den

Perspektywiczna Cave is located in Udorka Valley (50°26'33.5'N, E 19°46'1.5'E), in the middle part of Kraków-Częstochowa Upland, southern Poland. The cave includes two chambers and a gallery between them (Figure 1). Its size is difficult to estimate, as some parts of the cave are totally filled with sediments. Two entrances, facing W and NW, are situated at elevations of 340 m and 345 m above the sea level (Krajcarz et al. 2022).

The excavations began in 2012 and uncovered about 30 m^2 by 2022. The sedimentary fill of the upper chamber is poorly known. The 2013 and 2022 test pittings were the only excavations in this part so far. It revealed Holocene series (layer C1) disturbed by badger burrowing, and gray and yellowish-brown loamy sands with limestone debris (layers C2 and C3) below it (Figure 1). This sediment was dated to early Marine Isotopic Stage (MIS) 3 on the basis of ^{14}C dating of cave bear bones (Krajcarz et al. 2016a). It is rich in coprolite fragments and gnawed and/or digested bone fragments, and few cave hyena bones and teeth—an assemblage typical for hyena den deposits (Brugal et al. 1997; Fosse 1999; Diedrich and Žák 2006; Villa et al. 2010; Orbach and Yeshurun 2021; Palomares et al. 2022).

The lower chamber was intensively excavated in 2012–2019. It contains thick Late Glacial (layers 9, 9a) and Holocene (layers 8–1) series of gravels, silts, loams, and limestone debris (Figure 1), with archaeological material of Late Paleolithic, Mesolithic, and Middle Ages (Krajcarz et al. 2022). The series comprises eolian, alluvial, and colluvial deposits, moderately bioturbated. Within the series there are two lenses of sandy loams (layers 7c and 4a), of similar lithology and faunal composition to the layer C3 from the upper chamber. These strata can be regarded as colluvially re-deposited sediments of the hyena den. Hyena remains and hyena-related (gnawed, digested) bones were found within these strata. Isolated finds were also found among other stratigraphic units of the Holocene series, both in the lower and upper chambers, likely dispersed by post-depositional processes (such as colluvial activity, anthropic digging, and burrowing).

The taphocenosis consisted of remains of adult, senile and juvenile hyena individuals. Remains of prey belonged to a number of large ungulates, including reindeer, woolly rhino, steppe bison, giant deer, and mammoth. Moreover, some cave bear and cave hyena bones were also heavily gnawed.

METHODS

Material

Our research materials were hyena bones and teeth coming from several stratigraphic contexts in Perspektywiczna Cave: (1) as *in situ* deposit in the upper chamber (layer C3); (2) in a

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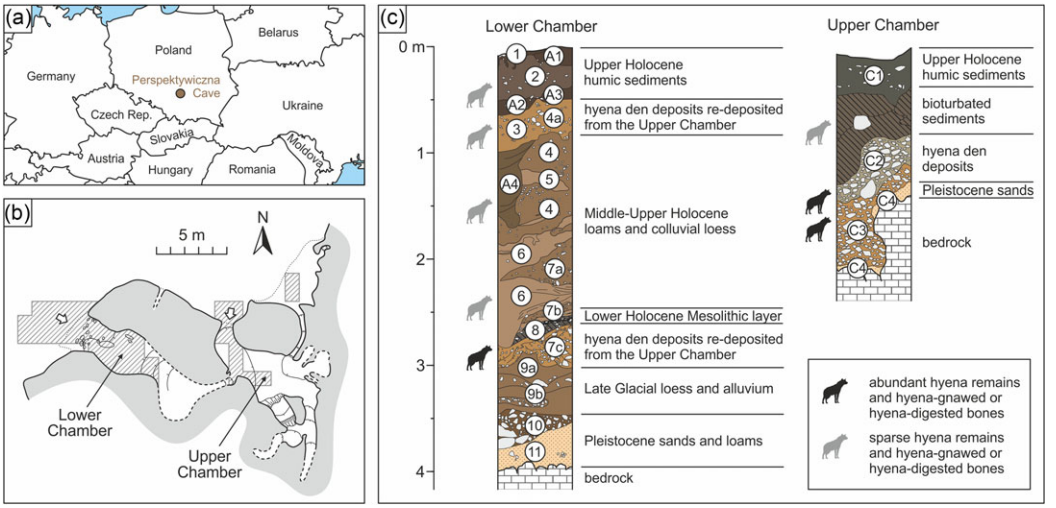

Figure 1 The site, Perspektywiczna Cave. Localization map and plan of the cave are shown in panels (a) and (b); hatched fields in (b) are for excavated area. Panel (c) shows simplified stratigraphic logs of the sedimentary fills of cave chambers (after Krajcarz et al. 2022) and the distribution of hyena-related fossils within the stratigraphy.

secondary position within compact packets of colluvially re-deposited deposits (layers 7c and 4a); and (3) secondarily dispersed among the cave fill. Despite their stratigraphic positions, we considered all these fossils to represent a single original taphocenosis. Attribution to a single assemblage was suggested by: (i) the presence of one original context (layer C3); (ii) distribution of bones in the Holocene series, so in sediments likely much younger than a hyena extinction in Europe, and thus indicating the post-depositional dispersal; and (iii) similar appearance of bones from variable stratigraphic contexts (similar color, bone density, external surface preservation state). Nevertheless, all studied bones were assumed to be directly ^{14}C dated to check their chronological attribution.

The taxonomy was preliminary identified on the basis of bone/tooth morphology and then checked by mtDNA analysis (see the Paleogenetic Analysis section). We also selected 100 unidentifiable digested bone fragments to be checked by ZooMS proteomics method (at BioArCh, University of York) and those exhibiting carnivoran affiliation were then tested by mtDNA analysis. Only those confirmed to be hyenas were taken for further analyses. The details of our research material are presented in the Appendix.

^{14}C Dating

We performed ^{14}C dating directly on 19 hyena remains (18 bones and 1 tooth); two more bone fragments were too small to be sampled, so only mtDNA and stable isotope analyses were done on them. The analyzed fraction was collagen. First, the macroscopic sediment remains were drilled off from the external surfaces with a diamond-coated bit. Then, fossils were bathed in an ultrasonic cleaner, first in acetone to break down clay mineral aggregates, then several times in demineralized water, until water was visually clean, to remove any remained sediment and dirt. The collagen extraction followed Longin (1971) with further modifications according to Piotrowska and Goslar (2002). Briefly: mechanical crushing to grain size <0.3 mm; 2M HCl treatment (room temp., 20 min); centrifugation and residuum collection; 0.1M NaOH

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treatment (room temp., 1 hr); centrifugation and residuum collection; HCl pH=3 maceration (temp. 80°C, 10 hr); centrifugation and supernatant collection. The extracts were ultrafiltered on pre-cleaned Vivaspin 15 MWCO 30 kD filters (Bronk Ramsey et al. 2004). Quality of the collagen was assessed basing on C:N atomic ratio (interval of acceptance: 2.9–3.6) (DeNiro 1985; Ambrose 1990) and collagen extraction yield (acceptance threshold: 0.5%).

For most samples, CO_2 was produced by combustion in closed (sealed under vacuum) quartz tubes with CuO and Ag wool (temp. 900°C, 10 hr). The gas was dried in a vacuum line and reduced with hydrogen (H_2), using 2 mg of Fe powder as a catalyst. For samples processed more recently (in this paper: all samples with Poz- numbers above 100000) combustion and reduction of the obtained CO_2 was performed with automated graphitization system AGE (Wacker et al. 2010) coupled with elemental analyser vario ISOTOPE select (Elementar). The obtained carbon and iron mixture was then pressed into aluminum holder (Goslar and Czernik 2000; Czernik and Goslar 2001).

Content of ^{14}C, ^{13}C, and ^{12}C in a carbon sample was measured in the spectrometer "Compact Carbon AMS" (National Electrostatics Corporation, USA) (Goslar et al. 2004). "Oxalic Acid II" and ^{14}C-free coal were used as standards. Conventional ^{14}C age was calculated using correction for isotopic fractionation (Stuiver and Polach 1977), basing on $^{13}C/^{12}C$ and $^{14}C/^{12}C$ ratios. The 1σ uncertainty of conventional ^{14}C age were given.

A specimen that showed the youngest ^{14}C age was cross-checked by dating the collagen in an independent facility, the Laboratory of Ion Beam Physics, Zurich (Switzerland). The remaining portion of collagen of less than 0.1 mg, which was prepared for stable isotopes analysis at Tübingen, was analyzed using the gas ion source (Ruff et al. 2010) of MICADAS (Synal et al. 2007). ^{14}C age of such small sample required a correction for constant contamination (Haghipour et al. 2019).

We calibrated the obtained ^{14}C ages with the IntCal20 ^{14}C calibration curve (Reimer et al. 2020), using OxCal ver. 4.4.4 software (Bronk Ramsey 2009). For summarizing the dating results, we used sum distributions and kernel density model (KDE) in OxCal following Bronk Ramsey (2017). In KDE we used default parameters, as recommended (Bronk Ramsey 2017; Carleton and Groucutt 2021).

Paleogenetic Analysis

Mitochondrial DNA (mtDNA) analyses were performed in dedicated ancient DNA facilities at the Centre of New Technology, University of Warsaw, following strict rules recommended to work with aDNA preventing any kind of contamination. The small fragment of bones has been cut off, washed with ultrapure water and 0.05% bleach, UV-irradiated (250 nm) for 10 minutes from each side and powdered in a cryogenic mill (SPEX CentriPrep, Stanmore, UK). Around 100 mg of the bone powder was used for DNA extraction, which was carried out using silica magnetic beads according to Rohland et al. (2018). DNA extracts were directly converted to double-indexed single-strand libraries according to Gansauge et al. (2020) with prior half-UDG treatment. Target enrichment of mtDNA was performed following Horn (2012). To obtain bait we amplified, sonicated, and enzymatically modified mitogenome of modern brown hyena. High-throughput sequencing was performed on Illumina NextSeq and NovaSeq platforms (paired mode, 2x75bp and 2x50bp, respectively).

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Raw Illumina reads were demultiplexed using bcl2fastq v2.20 (Illumina Inc.). Adapter sequences were trimmed and paired-end collapsed with Adapter Removal v2.2.3 (Schubert et al. 2016). Filtered reads were mapped to mitochondrial genome of European cave hyena (GenBank accession number JF894379). Duplicates, short reads (<30) and reads with low mapping quality (Q<30) were removed using SAMtools v1.9 (Li et al. 2009). We applied *bcftools* package to call consensus sequences and positions covered with less than 3 reads were masked with N. All BAM files were manually checked using Tablet v1.17 (Milne et al. 2010).

Authenticity of the obtained sequences was evaluated with MapDamage2.0 (Jónsson et al. 2013). Phylogenetic analyses were performed using BEAST v.1.10.4 (Suchard et al. 2018) and dataset of extant and ancient hyenas analyzed by Hu et al. (2021) (see Supplementary Information for details about the analysis).

Stable Isotopes Analysis

We tested the studied specimens for carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$) and sulfur ($\delta^{34}S$) stable isotope signal in bone collagen. We did not analyze the tooth specimen due to isotopic inconsistency between bone collagen and dentine collagen ecophysiological record (Bocherens 2015).

First, the fossils were cleaned as for ^{14}C dating. Then ca. 0.5 g samples cut off using a rotary diamond-coated saw and crushed to grain size <0.7 mm. The extraction followed Bocherens et al. (1997). Briefly: 1M HCl treatment (room temp., 20 min); filtration and residuum collection; 0.125M NaOH treatment (room temp., 20 hr); filtration and residuum collection; pH=2 HCl gelatinization (temp. 100°C, 17 hr); filtration and supernatant collection; lyophilization. Internal standards of modern elk and seal bones underwent collagen extraction and measurements together with fossil samples, to control the process.

Quality of extracted collagen was checked by measuring the C and N content and calculating the C:N atomic ratio. The reproducibility error for the amounts of C and N was lower than 1%, and lower than 2% for S. The acceptable ranges were >5% for the N content and 2.9–3.6 for C:N atomic ratio (DeNiro 1985; Ambrose 1990).

Elemental analysis and isotopic measurements were performed at facilities of the Geography working group of the University of Tübingen (Germany) using a CHNOS Vario Isotope Cube elemental analyzer (Elementar) in conjunction with an IsoPrime visION isotope ratio mass spectrometer (Elementar). The international laboratory standards USGS-40 and USGS-41a and two in-house reference materials (modern collagen of camel and elk) were used to calibrate the results. An analytical error below 0.1‰, 0.2‰, and 0.4‰ respectively (1σ) was determined for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in all the repeated analyses.

For cross-laboratory checking, one randomly selected collagen sample was measured in the Institute of Geological Sciences of the Polish Academy of Sciences (Warsaw, Poland). Isotopic measurements were performed in Flash EA 1112HT elemental analyzer (Thermo Scientific) connected to a Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific). International standards USGS-40, USGS-41 and IAEA-600 were used for 3-point calibration. Mean standard errors produced by the device over a long time is <0.33‰ for $\delta^{13}C$ and <0.43‰ for $\delta^{15}N$.

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Isotopic values were expressed as δ (delta; an isotopic ratio over the ratio of an appropriate reference) in parts per mille (‰), according to the formula: $\delta^{i}E = (R(^{i}E/^{j}E)_{sample} - R(^{i}E/^{j}E)_{reference}/R(^{i}E/^{j}E)_{reference}) \cdot 1000$, where ^{i}E is ^{13}C, ^{15}N or ^{34}S, and $R(^{i}E/^{j}E)$ is a $^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$ ratio (Coplen 2011). The references were V-PDB for carbon, atmospheric nitrogen (AIR N_2) for nitrogen, and V-CDT for sulfur.

We reconstructed isotopic niches (an equivalent of ecological trophic niches) in the C-N, C-S and N-S isospaces, using SIBER (Jackson et al. 2011) in R (Version 4.1.3. in RStudio 2022.12.0 Build 353). For this purpose, we constructed convex hulls (TA: Total niche), 40% (core niche), and 95% standard ellipse areas (SEA) (Layman et al. 2007; Jackson et al. 2011). In the case of the core niches, we constructed two variants: corrected for small sample sizes (SEAc) and calculated using Bayesian statistics (SEAb).

RESULTS

^{14}C Dating Results

We obtained ^{14}C dates for 19 cave hyena remains from Perspektywiczna Cave (Table 1 and Supplementary Figure s1). Dates span from Poz-149862: 29,300 ± 350 BP, or cal BP 34,242–33,443 (1σ), to Poz-148305: 46,000 ± 3000 BP, or cal BP 52,118–46,059 (1σ). One bone gave infinite date, Poz-148266: >44,000 BP. The specimen that yielded the latest age (CRO001) was initially dated to Poz-91892: 25,900 ± 300 BP (Krajcarz et al. 2020). However, that collagen extraction omitted the NaOH step, therefore we exclude this date as potentially compromised by humic contaminations. The same specimen was dated again twice in two independent laboratories, providing quite earlier but still relatively late ages (Table 1).

Sum of the probability distribution of all the Perspektywiczna Cave hyena calibrated dates exhibits tri-modal shape and KDE model shows more robust bi-modal distribution (Figure 2, panel a), with the last modes represented by single dates. The observed modes correspond roughly with the mtDNA variability, described below (see also Figure 2, panels b and c).

Paleogenetic Results

We obtained almost complete sequences of mitochondrial genomes for 19 specimens with mean coverage >85%. The damage pattern and read lengths corroborated the authenticity of the obtained data (Appendix). In the obtained phylogeny, five main lineages correspond well with five haplogroups defined earlier by Westbury et al. (2020) and Hu et al. (2021), i.e., A1, A2, B, C, and D. Samples from Perspektywiczna Cave cluster within a haplogroup A (*sensu* Westbury et al. 2020). Most of the samples are located in lineage A1, consisting of Late Pleistocene European *Crocuta*. Three specimens are located in lineage A2, which up to date consisted of modern African hyenas (see Supplementary Figure s2) (Table 1).

Based on both the ^{14}C age and the mtDNA phylogenetics, we identified three groups within the Perspektywiczna Cave hyenas:

1. "Main A1", the largest group (N=13, including three juveniles), gathering all specimens of A1 sub-haplogroup, all with the ages of >37 ka cal BP (for 1σ);
2. "Early A2", a single specimen (CRO016) of A2 sub-haplogroup and early ^{14}C age of cal BP 48,269–44,055 (1σ) or >44,000 BP; and

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Table 1 Results of ^{14}C dating, genetic attribution to mtDNA haplogroups and collagen stable isotope composition of hyenas from Perspektywiczna Cave (n.d. = not determined). For detailed information see the Appendix.

Sample ID	^{14}C lab no.	^{14}C date	mtDNA haplogroup	$\delta^{13}C$	$\delta^{15}N$	$\delta^{34}S$
CRO001	Poz-149863; ETH-130958	29300 ± 350 BP; 30100 ± 800 BP[a]	A2	–19.5[c]	9.1[c]	2.5
CRO010	Poz-131676	31800 ± 600 BP	A2	–19.3[c]	10.3[c]	2.0
CRO009	Poz-129667	33700 ± 600 BP	A1	–19.8	8.2	0.5
CRO002	Poz-91940	34700 ± 600 BP[b]	A1	–19.2	8.4	1.6
CRO003	Poz-91895	35500 ± 700 BP	A1	–19.5	8.3	–0.2
CRO004	Poz-91939	35500 ± 700 BP	n.d.	n.d.	n.d.	n.d.
CRO005	Poz-91941	36500 ± 800 BP[b]	A1	–19.9	9.5	–3.2
CRO019	Poz-148301	37300 ± 800 BP	A1	–19.4	8.3	1.4
CRO021	Poz-148303	38100 ± 900 BP	A1	–19.7	8.9	0.9
CRO006	Poz-91893	39000 ± 1000 BP	A1	–19.2[c]	9.4[c]	1.0
CRO013	Poz-148265	39800 ± 1200 BP	A1	–20.0	10.8	–2.1
CRO007	Poz-61113	39900 ± 1100 BP	A1	–19.6	10.6	0.4
CRO008	Poz-91942	41500 ± 1500 BP	n.d.	–20.6[d]	9.8[d]	–0.6[d]
CRO012	Poz-148257	41700 ± 1400 BP	A1	–19.8	11.8	0.9
CRO011	Poz-148256	41900 ± 1400 BP	A1	–19.7	10.5	0.8
CRO022	Poz-148306	43100 ± 1600 BP	A1	–19.6	9.2	–1.0
CRO017	Poz-148304	43100 ± 1900 BP	A1	–20.9[d]	10.7[d]	–0.4[d]
CRO018	Poz-148305	46000 ± 3000 BP	A1	–19.5	9.0	–0.3
CRO016	Poz-148266; Poz-149863	>44000 BP; 43000 ± 2000 BP	A2	–19.5	9.5	1.3
CRO015	—	n.d.	A1	–19.5	10.1	0.6
CRO020	—	n.d.	A1	–19.7	8.7	1.2

[a]Gas Ion Source AMS.
[b]Published in Czernielewski et al. (2020).
[c]Published in Krajcarz et al. (2016a).
[d]Results not reliable due to weak collagen quality.

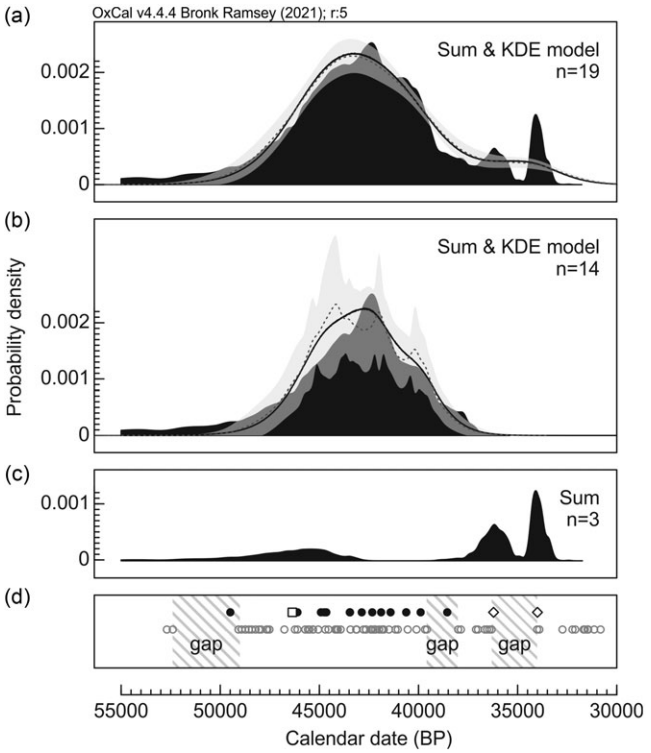

Figure 2 Summarizing of ^{14}C dates for the Perspektywiczna Cave hyenas. Panel (a) shows the OxCal sum distribution (black field), the OxCal KDE estimated distribution (kernel density model, the black line), and the OxCal mean ±1σ for snapshots of the KDE distribution generated during the MCMC process (the dotted line and transparent gray band) for all dated Perspektywiczna Cave hyenas. Panel (b) is the same for A1 sub-haplogroup hyenas only, and panel (c) for A2 sub-haplogroup hyenas only (without KDE model due to low sample size). Panel (d) shows the distribution of calibrated date medians (black dots—A1 sub-haplogroup, open diamonds—A2 sub-haplogroup, open gray circles—dates from Europe after Stuart and Lister (2014). Gaps in the European dataset are cross-hatched.

3. "Late A2", two specimens (CRO001 and CRO010) of A2 sub-haplogroup and late ^{14}C age (the latest within the taphocenosis) of <37 ka cal BP (for 1σ).

Stable Isotope Results

The $\delta^{13}C$ and $\delta^{15}N$ data for three specimens were published before (Krajcarz et al. 2016a) (Table 1). The isotopic measurements for other specimens and all $\delta^{34}S$ results are new data. The collagen quality check allowed regarding 18 collagen extracts as well-preserved and thus, stable isotope results as reliable (details are provided in the Appendix). Two other collagen extracts had high C:N atomic ratio, beyond the acceptance range (3.63 and 3.73). Both of these samples had also quite low collagen yield (<7%). A randomly selected specimen CRO015 was cross-checked in the laboratory in Warsaw. The found inter-lab difference was 0.52‰ for $\delta^{13}C$ and 0.03‰ for $\delta^{15}N$.

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Adult/subadult hyenas gave rather narrow range of $\delta^{13}C$ values, varying from -19.9 to -19.2 ‰ V-PDB, while their $\delta^{15}N$ values ranged from 8.2 to 10.5 ‰AIR (Table 1). Three juveniles had similar to adults $\delta^{13}C$ values, from –20.0 to –19.6 ‰V-PDB, but clearly higher $\delta^{15}N$ values, from 10.6 to 11.8 ‰AIR. The $\delta^{34}S$ values are quite variable, ranging from –3.2 to 2.5 ‰ V-CDT, with the highest values recorded in the A2 sub-haplogroup specimens.

Since the studied hyenas divided into genetic-chronological groups (see the section Paleogenetic Results), they possibly represent different paleoecologies. Therefore, the calculation of isotopic niche and niche overlaps should be conducted individually for groups rather than for the entire collection. However, considering statistical representation, we could do it only for the "Main A1" group, as the two others are represented by only one and two specimens. We further excluded juveniles from ecological analyses, as their isotopic signal may likely record more physiology-related than ecology-related habits, such as milk consumption (Jenkins et al. 2001; Dalerum et al. 2007; Bocherens 2015).

Specimens of the "Early A2" and "Late A2" groups are within the 95% SEA of the "Main A1" group (Figure 3). One individual of "Early A2" and one of "Late A2" are even within the 40% SEA, i.e., the core niche of the "Main A1" group. Juveniles of the "Main A1" group show significantly higher $\delta^{15}N$ values and are therefore outside the 95% SEAc of the "Main A1" group's adults.

DISCUSSION

Chronology of the Perspektywiczna Cave hyenas

The range of the ^{14}C age of Perspektywiczna Cave hyenas covered almost the entire MIS 3 period (*sensu* Lisiecki and Raymo 2005) (Figure 2), except for its last several millennia. This may point toward a long occupation of the cave by hyenas. Analogies may be found e.g. in another Late Pleistocene hyena den in Caverne Marie-Jeanne (Belgium) that was interpreted to be regularly reused as a birth den over a long period of time, around 47.6–43 ka cal BP (Jimenez et al. 2022).

Our latest dates are significantly younger than previously published hyena dates from Poland (Stuart and Lister 2014), which were: OxA-11062: 46,100 ± 900 BP (49,770–47,466 cal BP, 1σ, may be out of the calibration range); OxA-11158: 42200 ± 800 BP (45,582–44,366 cal BP, 1σ), and OxA-11161: 41,700 ± 1100 BP (45448–43417 cal BP, 1σ), all from Komarowa Cave. The youngest one among our dates, i.e., the combined Poz-149862 and ETH-130958 obtained for one specimen CRO001: 34,355–33,725 cal BP (1σ), is later by 10 millennia and points out the latest directly dated remain of a cave hyena north to Carpathians. The taxonomic attribution of this specimen is undoubted, both from morphological point of view (it is a well preserved proximal part of ulna with typical hyenid morphology; see Supplementary Figure s3) and genetically (mtDNA proved an A2 sub-haplogroup of *Crocuta crocuta*; see Supplementary Figure s2). Also, the second specimen of the "Late A2" group, the CRO010, provided much younger date than known from the previous studies.

In the distribution of our ^{14}C ages (sum of date probabilities in Figure 1, panels a and d) we can detect two gaps. The first is situated around 39–37 ka cal BP, and the second around 35 ka cal BP. Only single dates appear after these intervals, so we shall regard these gaps with caution. Noteworthy is, however, a coincidence with gaps found in the dataset of the pan-European ^{14}C record of hyenas presented by Stuart and Lister (2014). In that dataset, the most recent gaps

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between calibrated date medians are within 39.6–38.0 ka cal BP and 36.3–34.0 ka cal BP (Figure 1, panel d). Similarity between the gaps in the general European and in local Perspektywiczna Cave datasets may point toward a decline of hyena populations during these periods in Europe. This hypothesis finds an additional support in the mtDNA results, discussed below (see section Genetic structure of the Perspektywiczna Cave hyenas). This also suggests that the temporal distribution of hyenas in southern Poland followed the general continental pattern.

Remarks on the Chronology of Cave Hyenas in Poland

Two more hyena dens are known from Poland, both situated in the same region of Kraków-Częstochowa Upland. One is Komarowa Cave, layers C, D, and E (Wojtal 2007; Nadachowski et al. 2009; Nadachowski et al. 2015), situated 46 km away; another one is Biśnik Cave, layers 7–5 (Stefaniak and Marciszak 2009; Marciszak et al. 2011), situated 7 km away. Both are dated to MIS 3: Komarowa Cave den to early MIS 3 on the basis on ^{14}C dating (Wojtal 2007; Nadachowski et al. 2009), and Biśnik Cave den broadly to MIS 4/3 and MIS 3 based on the chronology of stratigraphic units (Krajcarz et al. 2014). Hyena remains of unprecise chronology are also know from other sites (Nadachowski et al. 2015). All these data suggest quite intense inhabitation of the region by hyenas during MIS 3.

Remarks on the Perspektywiczna Cave Chronostratigraphy

Obtained dates allow for approximation of the chronostratigraphy of Perspektywiczna Cave sediments. Most of our dates were obtained for bones from two contexts: sandy loam with limestone debris in the upper chamber (layers C1/C2 and C1/C3) and layer 7c in the lower chamber. Date sets are consistent and point toward the mid-Upper Pleistocene (MIS 3) age of the sediments. We can regard the upper chamber sediments as a remnant of hyena den deposits likely preserved in their original context, while layer 7c as a re-deposited colluvium of these deposits. This is based on a topographic position (the upper chamber strata elevated by around 4 m above the layer 7c), sedimentological observations (lithological similarities of both units; large thickness and no sedimentary structures in the upper chamber strata; clear disconformity at the bottom of layer 7c), and stratigraphy (layer 7c situated above the Late Glacial layer 9, evidenced by the ^{14}C dated muskox bone, see Stefaniak et al. 2021). The ^{14}C ages allow the following reconstruction of sedimentological processes: (1) accumulation of hyena den deposits during early MIS 3, as evidenced by the new data presented here and by Krajcarz et al. (2016a); (2) post-MIS 3 erosion and removal of hyena den deposits and other sediments from the lower chamber; (3) accumulation of the lower part (below the layer 7c) of the lower chamber sequence, likely during the Late Glacial; (4) Late Glacial or post-Late Glacial colluvial re-deposition of a part of hyena den deposits from the upper to the lower chamber, resulting in formation of layer 7c; (5) accumulation of the Holocene series with a post-depositional dispersal of fossils deriving from the hyena den context.

Genetic Structure of the Perspektywiczna Cave Hyenas

Previous genetic studies of extant and Pleistocene *Crocuta* based on a short fragment of cytochrome b (Rohland et al. 2005) and almost complete mitochondrial genomes (Westbury et al. 2020; Hu et al. 2021) recognized four main mtDNA haplogroups: A, B, C, and D. Haplogroup C was found only within extant hyenas, while B and D were found only in Pleistocene hyenas. Haplogroup A was found to be the most widespread and occurred in both European cave hyenas and modern African hyenas (Westbury et al. 2020).

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All new samples from Perspektywiczna Cave cluster within haplogroup A. We recognized two sub-haplogroups: A1 and A2 (Supplementary Figure s2). Most of the Perspektywiczna Cave hyenas belong to the A1 sub-haplogroup. This sub-haplogroup was known to date from the Upper Pleistocene of western Europe (France, Germany) and eastern Europe (Ukraine) (Westbury et al. 2020). The presence of the A1 sub-haplogroup in Perspektywiczna Cave, situated in the central part of the continent, suggests that this sub-haplogroup was likely widespread throughout Europe.

Three out of the 19 studied specimens represent the A2 sub-haplogroup. Interestingly, these three specimens were either of the latest age in our dataset or among the earliest ones. The sample CRO016 revealed ^{14}C age close to or beyond the method limit (Poz-149863: 43,000 ± 2000 BP and Poz-148266: > 44,000 BP). Therefore, the exact chronology of this specimen cannot be established. Two others revealed unexpectedly late age (CRO001, combined dates Poz-149862 and ETH-130958: cal BP 34,355–33,725 for 1σ; and CRO010, Poz-131676: cal BP 36,804–35,494 for 1σ), which is the latest among Perspektywiczna Cave hyenas and among the latest in Europe. It is noteworthy that this sub-haplogroup appears after one of the gaps in the temporal distribution of the hyenas (see the section: Chronology of the Perspektywiczna Cave hyenas). A possible hypothesis includes the change of population genetic structure after the pan-European decline. Answering the question of possible population replacement requires further studies of well-dated specimens from nearby regions.

Noticeably, the A2 sub-haplogroup was previously found only in extant African hyenas (Westbury et al. 2020). The Perspektywiczna Cave collection is the first known case of cave hyenas with this sub-haplogroup, as well as the first case of this sub-haplogroup out of Africa. Paleogenomic studies of modern and Pleistocene hyena genomes suggested bidirectional gene flow between African and Eurasian populations after the split of European and Chinese lineages and subsequent unidirectional gene flow from European to northern African spotted hyenas before the divergence of A1 and A2 sub-haplogroups (that happened around 570 to 388 ka BP; Westbury et al. 2020). The latter divergence was an effect of cessation of gene flow from the European to north African populations. There are at least two alternative explanations for the presence of the A2 sub-haplogroup in the Late Pleistocene European hyenas. First, the divergence of the A1 and A2 lineages was not related to the stalling of gene flow between European and African populations, but the gene flow continues or took place after the divergence; as the consequence, the observed pattern is an effect of incomplete lineage sorting. The other explanation is that there was another, later episode of gene flow that introduced the African A2 sub-haplogroup into European populations. Westbury et al. (2020) suggested that admixture of African and European hyenas might have had some adaptive advantages. It is possible that the specimens that carried the A2 sub-haplogroup had a greater adaptive potential and appeared after the pan-European decline. However, further paleogenomic studies are necessary to confirm this hypothesis.

Ecological Stability of the Perspektywiczna Cave Hyenas

In terms of collagen C and N stable isotopes, the Perspektywiczna Cave hyenas are similar to other Late Pleistocene hyenas from Europe (Bocherens et al. 1995; Bocherens et al. 1997; Bocherens et al. 2005; Bocherens et al. 2011; Bocherens 2015; Krajcarz et al. 2016a; Flower et al. 2021). Moreover, the collagen $\delta^{15}N$ and $\delta^{13}C$ values in Perspektywiczna Cave hyenas revealed no difference between the sub-haplogroups (Figure 3, panel a) and no change within the entire dated period (Figure 4, panels a and b). This points toward ecological stability of

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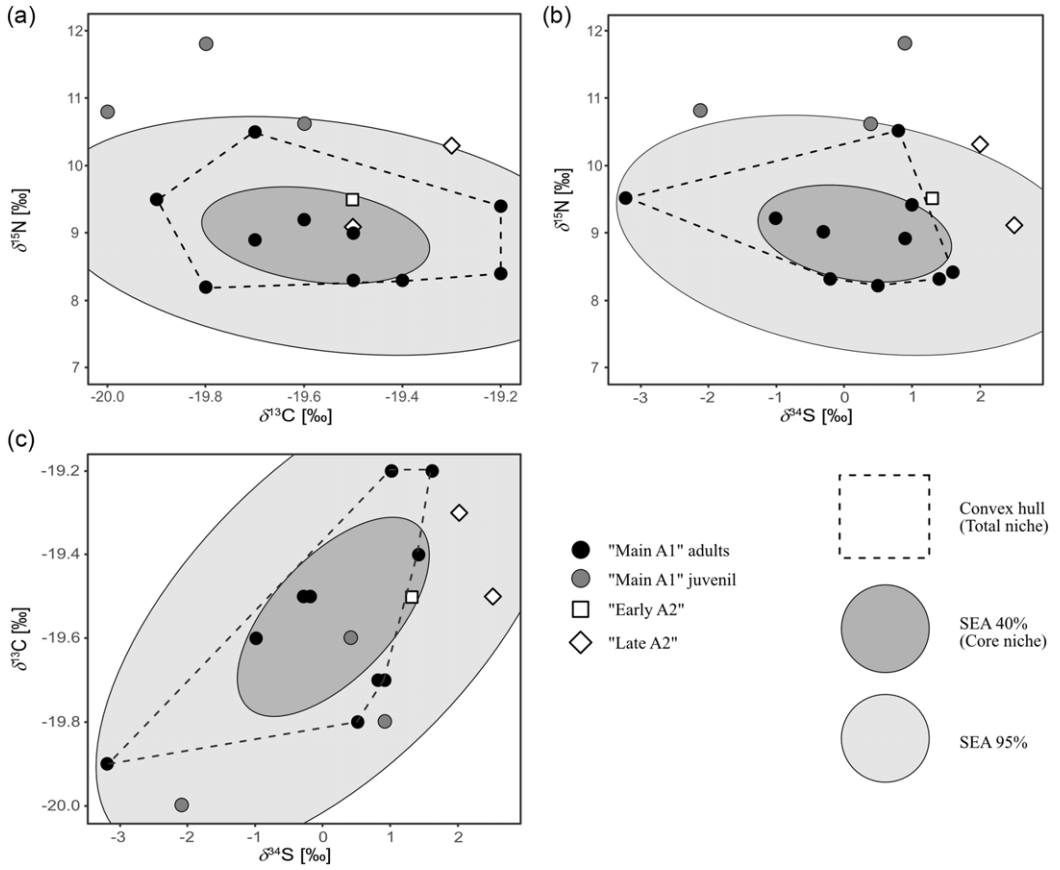

Figure 3 Isotopic values (δ^{15}N, δ^{13}C, and δ^{34}S) of bone collagen of the Perspektywiczna Cave hyenas plotted in (a) C-N, (b) S-N, and (c) S-C isospaces. SEA and convex hull are only shown for adult specimens of the "Main A1" group.

hyena populations through millennia. The only difference is exhibited by juveniles, who are increased in ^{15}N and depleted in ^{13}C in comparison to the most of adults. This complies with expected trend in juvenile carnivorans, who are known to record the non-adult-like isotopic values due to nursing effect (Jenkins et al. 2001).

A slight diachronic change is visible in δ^{34}S results (Figure 4, panel c). In particular, the two latest individuals, both belonging to the "Late A2" group, show higher δ^{34}S values than all other specimens. The δ^{34}S values in ecosystems are known to be related to geologic bedrock and vary between stratigraphic units and lithologies (Richards et al. 2001; Nehlich 2015; Paytan et al. 2020); they also depend on soil activity (Drucker et al. 2011). Sulfur isotopes lack fractionation in trophic chain (Richards et al. 2003; Arneson and MacAvoy 2005; Tanz and Schmidt 2010; Webb et al. 2017; Krajcarz et al. 2019), therefore in terrestrial environments the body δ^{34}S signal directly reflects a local geology or soil geochemistry (Peterson and Fry 1987; Nehlich 2015). A possible hypothesis explaining the change in δ^{34}S in the Perspektywiczna Cave hyenas may be, therefore, a different mobility patterns between earlier and later hyenas, i.e., exploitation of territories with different geologic backgrounds and/or soils. Also, the herbivores hunted by earlier and later hyenas could have followed different mobility patterns

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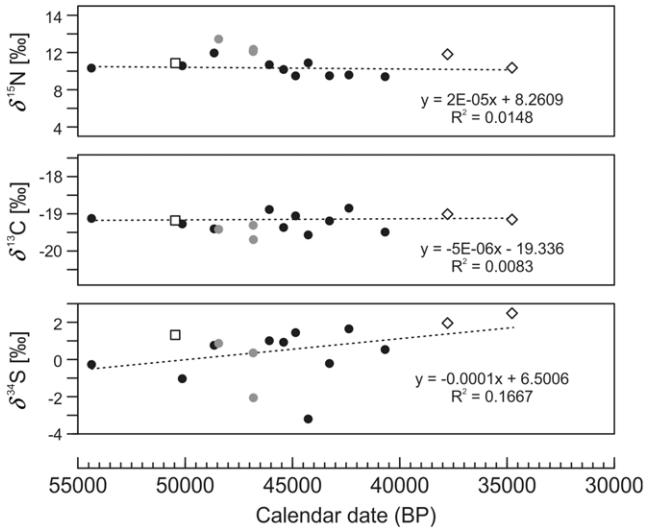

Figure 4 Isotopic values (δ^{15}N, δ^{13}C and δ^{34}S) of bone collagen of the Perspektywiczna Cave hyenas across the chronology (calibrated date medians taken for the time scale; for CRO001 the median of a combined date is taken). Black dots – "Main A1" group, adults; gray dots – "Main A1" group, juveniles; diamonds and square – "Late A2" and "Early A2" groups respectively, adults. Trend lines are calculated for all adults.

and migrated from regions with different δ^{34}S signals. This seems likely, as Perspektywiczna Cave is situated close to the boundary between the Upper Jurassic limestones and Lower Cretaceous sands and sandstones (Bukowy 1968; Bednarek et al. 1978). However, a lack of any abrupt shift in δ^{34}S, but rather a gradual long-lasting change (Figure 4, panel c), suggests another explanation: a slow change of local environmental conditions. This could have happened through the climate change, e.g., the increasing aridity during the MIS 3 period (Fuhrmann et al. 2020) that likely could change the soil geochemistry toward more oxidizing conditions and thus higher soil δ^{34}S values. Alternatively, this could have also happened through the change of geological background, which was not unlikely considering the loess accumulation that is known to be the most intense in the region during the late MIS 3 and MIS 2 (Krajcarz et al. 2016b). All these hypotheses are not mutually exclusive.

To consider the trophic ecology of the Perspektywiczna Cave hyenas in a broader European context, we adopted a niche overlapping approach (Baumann et al. 2020). We calculated the core niches (40% SEA) for C and N isotopic ranges of other Late Pleistocene hyena populations taken from literature, following the same method as for the Perspektywiczna Cave hyenas. We included sites with isotopic results for at least 3 hyena specimens: Goyet and Scladina, Belgium; La Berbie and Camiac, France; Kents Cavern and Sandford, UK (Bocherens et al. 1995, 1997, 2005, 2011; Bocherens 2015; Flower et al. 2021). In addidtion, we plotted the single specimens from Hohlenstein-Stadel, Germany, and Peştera cu Oase, Romania (Bocherens et al. 2011; Trinkaus and Richards 2013), without niche reconstruction. The Kents Cavern hyenas show the widest range of δ^{13}C and δ^{15}N values (Figure 5), which can be interpreted as a broad niche. The other populations, including Perspektywiczna Cave, are similar to each other in terms of isotopic variability (Figure 5). Moreover, their SEAs overlap, which point toward ecological

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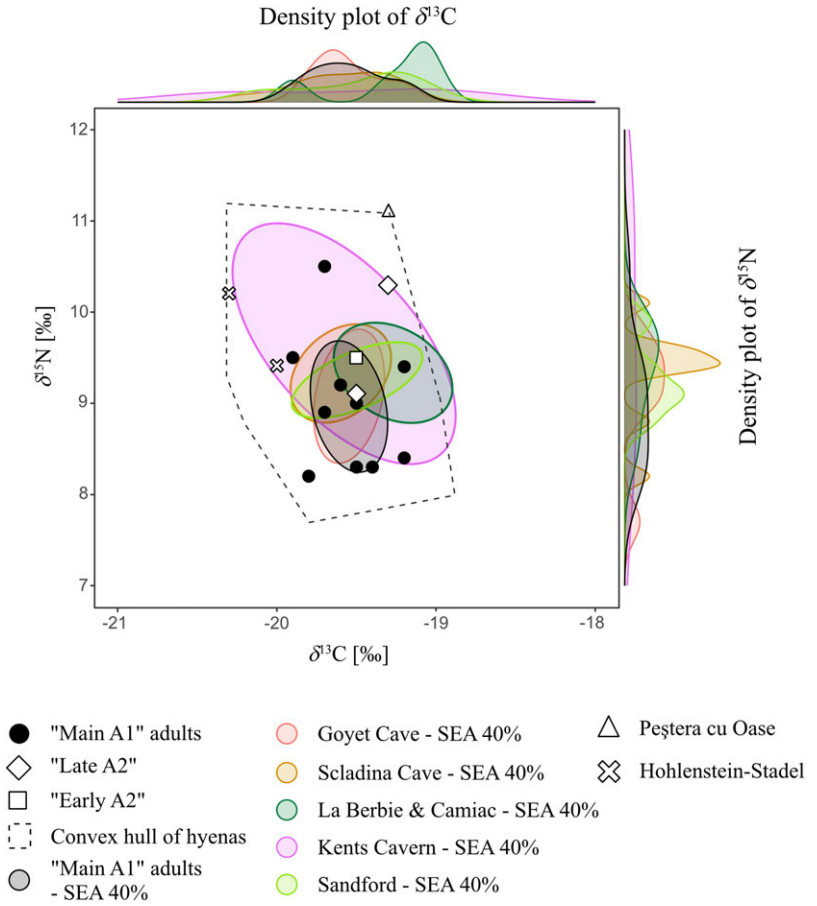

Figure 5 Isotopic values (δ^{15}N and δ^{13}C) of the Perspektywiczna Cave hyenas and other Late Pleistocene European cave hyenas (input values and references are provided in the Appendix). A convex hull covers all hyenas, while core niches (SEA 40%) and density plots are shown for sites with more than 3 specimens.

similarities and place all the Perspektywiczna Cave hyenas well within the European ecological variability.

CONCLUSIONS

Our results for Perspektywiczna Cave provide a geographical complementary data for the Late Pleistocene European cave hyenas, both in terms of the chronology, genetic structure and paleoecology. The case of Perspektywiczna Cave proves that hyenas were still present north of Carpathians during the late MIS 3, as late as ~34 ky cal BP. This information shifts forward the last confirmed appearance of hyena in this region by nearly 10 millennia and may have an important impact on the archaeological interpretations of paleoecology of the Upper Paleolithic people. Our data shows also that the genetic structure of European hyenas was more complex than previously documented. The Perspektywiczna Cave results reveal close affinity of local hyenas with the mtDNA lineage considered so far to be restricted to Africa, as well as with the one known in other Late Pleistocene European populations. Preliminary conclusions

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about the temporal changes in the genetic structure can also be postulated. Noticeably is an ecological stability of the Perspektywiczna Cave hyenas through the millennia and their similarity to the most of other Late Pleistocene European populations.

Even if our results are limited to one region and one site, they are of wide importance. To understand comprehensively the temporal changes in cave hyena's distribution, genetic structure and ecology across the Eurasia, more data from other sites and other regions are needed. Nevertheless, the complex pattern of the hyena extinction in Europe has been revealed. Southern Poland is another region outside of the southern Europe, along eastern Germany, Belgium, and southern Great Britain (Stuart and Lister 2014; Westbury et al. 2020), where hyenas survived at least until the late MIS 3.

SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit https://doi.org/10.1017/RDC.2023.89

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AUTHOR CONTRIBUTIONS

MTK and MK—conceptualization; MTK, MK, and MSP—excavation and archaeological context; MK—collection curation, bone identification, sampling; TG—radiocarbon dating; MB and DP—genetic analysis; MTK, MK, and HB—isotopic analysis; MTK, CB, MB, and DP – data processing, illustrations; MTK, MK, and MSP—financing; all authors—manuscript preparation and reviewing.

DATA AVAILABILITY STATEMENT

The consensus mtDNA sequences generated in this study were deposited in the GenBank under accession numbers from OQ947728 to OQ947746. The sequencing reads mapped to cave hyena mtDNA are available from the European Nucleotide Archive under accession PRJEB61948. The mtDNA alignment used for phylogenetic reconstruction is available from RepOD: https://doi.org/10.18150/VMJZNK. All isotopic and radiocarbon determination data are provided within the paper and its supplementary materials.

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