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A new assemblage of late Neanderthal remains from Cova Simanya (NE Iberia)

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This study presents an exceptional collection of 54 Late Pleistocene human remains that correspond to at least three Neanderthal individuals from Simanya Gran, the main gallery of Cova Simanya, located in the northeastern Iberian Peninsula. The collection comprised 53 unpublished remains that were unearthed during the 1970s and an additional tooth discovered during 2021 excavations. The specimens represent an adult with a small stature, a periadolescent aged approximately 11.5 years, and an immature individual aged approximately 7.7 years, thus offering a more complete demographic perspective. The collection encompasses diverse anatomical parts including upper and lower dentition, mandible, vertebrae, and limb bones from both the upper and lower extremities. Attempts to extract aDNA were unsuccessful. Renewed archaeological investigations at Cova Simanya have facilitated the reevaluation of the original stratigraphic context of these remains, leading to the discovery of the additional tooth, aligning with the periadolescent individual. This assemblage is currently the most extensive Neanderthal collection from the northeastern Mediterranean Iberia, offering invaluable insights into the morphology and evolutionary trajectory of Late Pleistocene hominins. Hence, Simanya Neanderthals will enhance our understanding of Neanderthal demographics and evolution, paving the way for an in-depth examination of the morphological diversity and evolutionary context of Iberian Neanderthals.

KEYWORDS

Late Pleistocene, Middle Paleolithic archeology, human remains, Neanderthal, Cova Simanya, paleoanthropology, Paleolithic archaeology, Iberian Peninsula

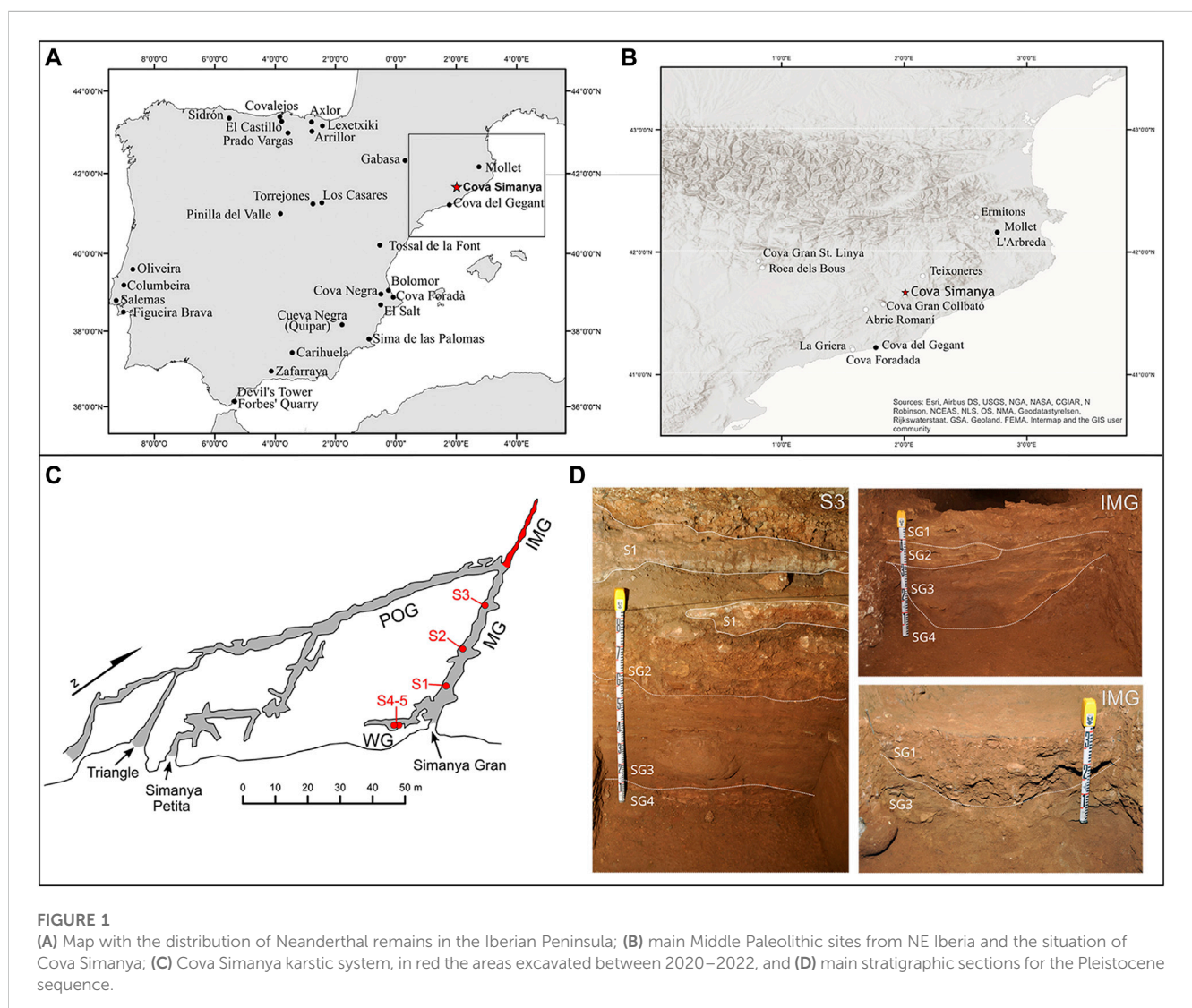
1 Introduction

Neanderthals exhibited distinct genetic histories and cultural adaptations along time and space. In the Iberian Peninsula, the Iberian Mediterranean region holds significant importance in understanding Neanderthal evolution and variability, and its archaeological record provides valuable insights into their biological and cultural development as well as their eventual disappearance during marine isotope stage (MIS) 3.

Middle Paleolithic Mousterian sites can be found along the Mediterranean coast from Gibraltar to the Pyrenees, with Neanderthal remains being documented at several locations (Dean et al., 1986; Arsuaga and Bermúdez de Castro, 1987; Daura et al., 2005; Arsuaga et al., 2007; Walker et al., 2008; Daura et al., 2010; Walker et al., 2010; Rodríguez et al., 2011; Arsuaga et al., 2012; Maroto et al., 2012; Michel et al., 2013;

Aparicio et al., 2014; Garralda et al., 2014; Quam et al., 2015; Bokelmann et al., 2019; Carrión et al., 2019) (Figure 1A).

These data suggest that Mediterranean Iberia was continuously inhabited by Neanderthals from MIS-7 onwards, with northeastern Iberia being an especially dense Mousterian cluster (Martínez-Moreno et al., 2010a; Martínez-Moreno et al., 2010b; Rosell et al., 2010; Mora et al., 2018; Morales et al., 2022) (Figure 1B), including long-term occupation archives such as Abric Romaní (Barcelona) (Bischoff et al., 1988; Vallverdú et al., 2010; Carbonell, 2012) or L'Arbreda (Soler et al., 2014), and the southernmost occurrence of Châtelperronian technologies (Morales et al., 2019; Rodríguez-Hidalgo et al., 2019). However, the paleoanthropological record in this region is restricted to the isolated teeth from Mollet (~215 ka) (Maroto et al., 2012), and the Cova del Gegant assemblage dated back to 55,000–52,000 years ago (Daura et al., 2005; Daura et al., 2010; Quam et al., 2015; Daura et al., 2022) once the long-time



disputed Banyoles mandible does not show any derived Neanderthal features (Keeling et al., 2023).

Herein, we present a novel Neanderthal assemblage from Cova Simanya (Sant Llorenç Savall, Barcelona), first recognized during the analysis of a museum collection and constituting the largest sample documented so far in northeastern Iberia. This discovery has led to a new research project that aimed at reconstructing the genesis, context, and paleobiology of the Neanderthal sample, including renewed excavations at Cova Simanya. These works have provided a robust archeological context for the uncontextualized “Museum Collection” including additional Neanderthal remains.

Simanya’s assemblage may offer insights into the late Neanderthals’ apomorphic traits and their congruence with other known samples, while also raising questions about the genetic diversity and population turnovers within the Iberian Peninsula (Vernot et al., 2021).

2 Materials and methods

2.1 Cova Simanya

Cova Simanya is a karstic system (UTM ETRS89 417517–4614066, 885 masl) opened in Montcau mountain, running through the Paleogene conglomerates forming the Sant Llorenç del Munt Massif, on the eastern margin of the Ebro Basin, and has over 300 m of gratulated galleries. The Font del Llor stream cuts the system, creating three consecutive entrances known as Simanya Triangle, Simanya Petita, and Simanya Gran (Figure 1C and Supplementary Figure S1).

Simanya Gran is the larger sector of Cova Simanya, comprising the Western Gallery (WG), Main Gallery (MG), Pas de l’Oca Gallery (POG), and the inner part of the Main Gallery (IMG) (Figure 1C). The cave has been known to scholars at least since the 18th century, and the presence of archeological remains is known since the first excavation in the 1930s.

2.2 The “Museum Collection”

The “Museum Collection” was collected by an amateur, Mr. Miguel Aznar, in 1978–79 and contains a combination of two distinct sources: 1) surface findings and 2) remains from a small excavation. The surface findings include isolated human and animal bones, pottery, and chert fragments. The excavation yielded human and faunal remains, as well as a few samples of charcoal, sediment, and carbonate crusts. The excavation was performed in a breccia deposit of reddish sediments with abundant charcoals located in the IMG sector. No pottery or any other Holocene remains were found during the excavation. The collection containing 143 osteological remains was donated to the Museum of Archaeology of Catalonia in 1986. In 2021, Mr. Aznar delivered two additional bags to us containing small and mostly undiagnostic bone fragments that are not included in the original donation.

Despite being layered in the reddish clay-like sediment, the human bone collection exhibited a notable state of preservation. However, the presence of modern fractures and adhesive-induced alterations from the 1970s necessitated a thorough evaluation of

their conservation status. Following the evaluation, a series of measures were taken to inhibit further deterioration. Cleaning procedures involving water and soft brushes were applied, and outdated adhesives were removed and substituted with Paraloid® B-72, a durable and reversible acrylic resin. This resin was also utilized to mend modern fractures and consolidate the overall structure, ensuring the assemblage’s longevity and integrity for future study and preservation.

2.3 2020–2022 excavations

The 2020–2022 excavation programs targeted Simanya Gran and aimed at identifying the original context of the “Museum Collection” and evaluating the potential of the Pleistocene deposits in the cave. In 2020, five test pits were excavated to reconstruct the longitudinal stratigraphic development along the MG (S1, S2, and S3) and WG (S4–S5). In 2021, a more extensive excavation was carried out in the IMG sector (Figure 1D). The latter provided a similar fossil assemblage to the “Museum Collection,” including a new Neanderthal tooth (SI-60) found in the area reported by Mr. Aznar as the origin of the collection (Supplementary Table S1). In 2022, we continued with the excavation of the IMG and expanded the S3 test pit of the MG to a surface of 10 m². A visual recognition of Mr. Aznar excavations (trenches, sections, or backfills) was not possible due to the homogenization of the sedimentary surface by the unrestricted public access.

2.4 Radiocarbon dating

Eleven bone samples, both anthropized and non-anthropized remains, and five charcoals (*Pinus sylvestris* type) from the “Museum Collection” and our own excavations at the IMG were selected for radiocarbon pretreatment at the University of Bologna’s BRAVHO facilities. Talamo et al. (2021) followed the collagen extraction method, and only a non-anthropized *Capra pyrenaica* humerus (SG21.06.39) from the excavation yielded sufficient collagen for ¹⁴C dating. Charcoal pretreatment used the ABOx method proposed by Tassoni et al. (2022), adapting Bird et al.’s (1999) method to small, poorly preserved samples by adjusting the HCl solution’s molarity and reducing the oxidation step’s reaction time. After the pretreatment, the collagen and charcoal samples were graphitized at the BRAVHO lab (Tassoni et al., 2022). During the charcoal sample SG-CA-200 combustion, the C integral peak obtained was very low for analysis and was excluded. The resulting graphite was pressed into targets and sent to the Mannheim AMS Laboratory at the Curt-Engelhorn-Center for Archeometry for measurement.

All ¹⁴C ages were calibrated with the IntCal20 calibration curve (Reimer et al., 2020) using OxCal v4.4 software (Bronk Ramsey, 2009).

2.5 aDNA

An attempt to extract aDNA from Simanya Neanderthals was made by sampling a tooth and a phalanx from the “Museum Collection” through shotgun sequencing (Illumina NovaSeq

6000) and mitochondrial DNA (mtDNA) capture with Neanderthal-specific baits (Arbor Biosciences).

3 Results

3.1 Stratigraphic context and archeo-paleontological record

The litho-stratigraphic sequence documented so far for the MG and IMG sectors of Simanya Gran contains a primary Pleistocene sequence capped by a thin Holocene deposit, 5–30 cm thick, which is documented from the entrance up to 53 meter. From that point of the MG to the end of the IMG, the Pleistocene units are uncapped ([Supplementary Figure S2.1](#)) and four main litho-stratigraphic units have been described ([Figure 1D](#)), namely, from the bottom to the top, Unit SG4 (cave clays), Unit SG3 (channel deposits), Unit SG2 (complex unit), and Unit SG1 (diamicton).

Unit SG4 contains at least 60 cm of pure, dark reddish-brown clays with scarce coarse to very coarse gravels, mainly limestone and slate, documented at S1, S2, S3, and the IMG, and is eroded by Unit SG3. Unit SG3 comprised several coarsening upward sequences (silt/clay to coarse sand) that are cut and filled by at least four poorly sorted (silts to coarse sand) channel deposits. The thickness of Unit SG3 varies from 5 to 45 cm, and it has been documented in S1, S2, S3, and the IMG. In the S1 section of the MG sector, Unit SG3 is conformably overlain by Unit SG2-a, an approximately 50 cm-thick matrix-supported conglomerate of slate and limestone clasts that are more abundant toward the top, while in the S3 section, it is overlain by Units SG2.3-to-1, a succession of brown clays, a poorly sorted yellowish sandy deposit, and a very fine gravel conglomerate, and by the S1 flowstone. The Pleistocene sequence ends with Unit SG1, a reddish-brown, matrix-supported polygenic limestone, sandstone, and slate conglomerate with an erosive base that has been documented exclusively at the IMG.

Three archaeological layers have been documented so far within the litho-stratigraphic sequence at the MG-IMG sector. Layer P1 corresponds to the Holocene phase and yields artifacts from the Late Neolithic to the Late Antiquity, including scattered funerary evidence dated back to the 5th century AD, in a condensed sub-superficial sedimentary palimpsest ([Supplementary Figure S2.1](#)). The Pleistocene layer O300-PO400 (Unit SG1) was documented in the IMG and is characterized by a paleontological background composed mostly of *Ursus cf. arctos*, *U. cf. spelaeus*, and *Capra pyrenaica*. The preliminary analysis of the assemblage suggests a combination of taphonomic agents and processes in the formation of the record, including bear deaths during hibernation, accumulation of Iberian ibex by a large carnivore (likely a felid), and transportation and/or reworking by physical processes such as water flow and gravity. This taxonomic profile is consistent with the “Museum Collection,” which mostly consists of *U. cf. arctos* and *Capra pyrenaica*. Evidence of occasional Middle Paleolithic Mousterian occupation has also been found to be interspersed in the paleontological background of O300-PO400, over 70 m inside the cave ([Supplementary Figures S2.2, 2.3, S3](#)). The evidence associated to human occupations contains a small lithic assemblage and some faunal remains (*Sus scrofa* and *Cervus elaphus*) that exhibit cut marks or green breakage patterns.

Within this horizon, a small and flat combustion feature was also documented. The presence of core-trimming flakes, a sidescraper, multifaceted butts, dorso-ventral right angles, and centripetal dorsal scar patterns in the lithic assemblage clearly indicates a Mousterian affinity ([Morales et al., 2022](#)). Additionally, a third upper human molar (SI-60) was found in Layer O300-PO400 (301–80 meter). Finally, Layer P300, documented exclusively at S3, has provided a faunal assemblage composed of highly cemented skeletal remains of *Capra pyrenaica* and isolated remains of *Cervus elaphus*, without any evidence of human activity so far.

3.2 Dating

Calibrated dates at 68.3% and 95.4% probability are provided in [Table 1](#). The bone sample is the only one that results in a final calibrated range of 42,440–42,110 cal BP (68.3%). All the other four charcoal samples are older than the radiocarbon method (>49,000).

3.3 Paleoanthropology

A collection of 53 human remains was recovered by Mr. Aznar in 1978–79, to which an upper third molar (SI-60) found *in situ* during the excavations ([Supplementary Table S2](#)) must be added. The assemblage includes upper and lower dentition, a fragment of the mandible, vertebrae, the left and right upper limb (humeri and hand bones), and foot bones. Some of the specimens are preserved in complete and good condition, while others are fragmentary with old dry (diagenetic) and modern fractures ([Figure 2](#)) (probably broken during the excavation). No evidence of pathological processes has been detected in the sample, while the preserved dentition does not display strong evidence of the use of the mouth in heavy-duty tasks.

At least three individuals can be identified in the sample. The most complete one is defined by a complete left humerus SI-1 ([Figure 2A](#)). Several other postcranial remains (right proximal humerus, hand 1 and hand 2, vertebra; [Figures 2B–D](#)) can be securely associated based on the size and taphonomic features. It corresponds to an adult, with a stature estimation for the humerus of 154 ± 4.89 cm based on the [Sjøvold \(1990\)](#) equation (max. length: 293 mm), which is consistent with a Neanderthal female individual.

SI-7 is an upper left P3 at the Rc growth stage [according to the work of [Moorrees et al. \(1963\)](#)], with the root length completed with parallel ends ([Figure 2E](#)). This provides an estimated age of approximately 11.5 years by modern human standards [according to the work of [AlQahtani et al. \(2010\)](#)]. SI-60 is a complete upper left M³ crown ([Figure 2E](#)) with a defined pulp roof [CRC, according to the work of [Moorrees et al. \(1963\)](#)], which would provide an age of 12.5–13.5 years [according to the work of [AlQahtani et al. \(2010\)](#)]. However, M3 development is thought to be accelerated in Neanderthals as compared to modern humans ([Guatelli-Steinberg, 2009](#)), which makes it probable that the M3 (SI-60) and P3 (SI-7) may come from a single individual of approximately 11.5 years old.

Two other immature remains are also preserved in the sample: SI-29 is a hand first phalanx of ray V, whose size is like Juvenile 1 from El Sidrón ([Rosas et al., 2017](#)) ([Figure 2F](#)). This provides an approximate age of 7.61–7.78 years for this individual. SI-37 is a

TABLE 1 Samples selected for ¹⁴C dating pretreated at the University of Bologna and AMS results.

Bologna Code	Sample ID	Unit	Sector	Material	Species	Collagen (mg)	Collagen (%)	AMS Code	d13C	F14C	C:N	14C age	1s Error	Cal BP age (68.3%)	Cal BP age (95.4%)
BRA-6108	SG21.06.39	O300-PO400	301-70	Bone	Caprini	4.8	1%	MAMS 56486	-18.21	0.0088	2.89	38009	410	42440–42110	42620–41930
BRA-6109	SG21.11.08	O300-PO400	301-71	Bone	Suidae	0.7	0%	—							
BRA-6110	SG21.06.78	O300-PO400	301-82	Bone	Caprini	0.6	0%	—							
BRA-6111	SG21.10.58	O300-PO400	301-82	Bone	Caprini	0.2	0%	—							
BRA-6112	SG21.12.10	O300-PO400	301-82	Tooth	Caprini	—	—	—							
BRA-6113	SG21.10.30	O300-PO400	301-83	Tooth	Caprini	—	—	—							
BRA-6114	121_1978	Museum Collection	IMG	Tooth	Ursidae	0.8	0%	—							
BRA-6115	SG-CA-125	Museum Collection	IMG	Tooth	Ursidae	0.5	0%	—							
BRA-6116	SG-CA-10	Museum Collection	IMG	Tooth	Ursidae	0.5	0%	—							
BRA-6117	SG-CA-62	Museum Collection	IMG	Bone	Indeterminate	0	0%	—							
BRA-6118	SG-CA-137	Museum Collection	IMG	Bone	Caprini	1.2	0%	—							
						Carbon (mg)	Carbon (%)								
BRA-6106	SG21.11.53	O300-PO400	301-72	Charcoal	<i>Pinus sylvestris</i> type	33.3	35%	MAMS 56487	-22.61	0.0009	137.59	>49000	—	—	—
BRA-6107	SG21.03.05	O300-PO400	301-86	Charcoal	<i>Pinus sylvestris</i> type	50.8	49%	MAMS 56488	-23.43	0.0012	129.79	>49000	—	—	—
BRA-6264	SG-CA-200	Museum Collection	IMG	Charcoal	<i>Pinus sylvestris</i> type	4.6	7%	—							
BRA-6265	SG-CA-210	Museum Collection	IMG	Charcoal	<i>Pinus sylvestris</i> type	11.7	21%	MAMS 62623	-24.17	0.0020	87.05	>49000	—	—	—
BRA-6266	SG-CA-207	Museum Collection	IMG	Charcoal	<i>Pinus sylvestris</i> type	6.2	16%	MAMS 62624	-23.79	0.0013	172.17	>49000	—	—	—

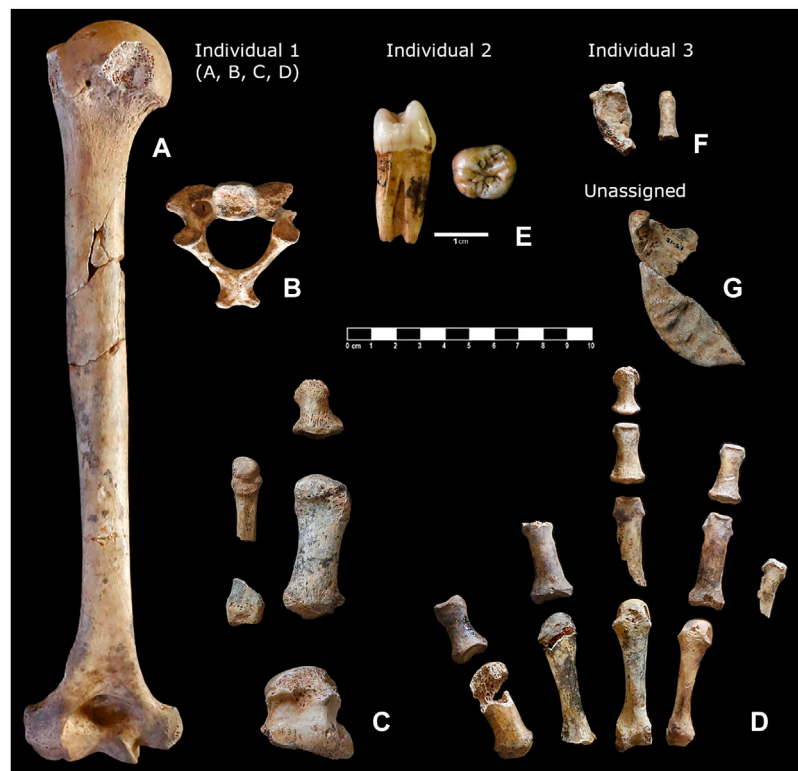


FIGURE 2

Foremost Neanderthal remains found at the Simanya Gran gallery from Cova Simanya (Barcelona, Spain). Skeletal remains are distributed according to their attribution to specific individuals. (A) Left humerus; (B) shaft, caudal view; (C) bones of the foot; (D) bones of the right hand; (E) upper P³ and M³; (F) fragment of the atlas and first phalanx of the hand; and (G) fragment of the ascending branch.

fragment of an atlas from the right side, preserving upper and lower articular facets. The surface of the neurocentral junction can be appreciated, meaning that the anterior arch of the atlas was still unfused even though the size of the specimen is relatively large. This is fully compatible with the growth pattern established for Neanderthals by Rosas et al. (2017) and congruent with the age of 7.7 years established by the SI-37 phalanx.

The size of lower dentition I₁ (SI-3) and M₃ is relatively small, while P₃ (SI-5) falls at the upper limit and the upper I¹ (SI-45) in the middle of the Neanderthal distribution, while the mesiodistal crown diameter of the I² (SI-4 and SI-31) falls well above the Neanderthal mean [according to the work of Pinilla and Trinkaus (2017)] (Supplementary Table S3, Supplementary Figure S4). Historical and taphonomic conditions of the assemblage make it feasible to assign some of these teeth to individual 1. However, because of the noted size differences, the presence of other individuals cannot be ruled out. For the time being, no individual has been assigned to adult dentition. The upper and lower permanent teeth exhibit moderate occlusal wear [stage 5 of the work of Skinner (1997)], indicating a mature adult. Considering that Neanderthal anterior permanent teeth wear faster or commence wear earlier (Skinner, 1997), an age of 30–40 years may be considered.

In order to assess the location of the Simanya Gran specimen SI-1 in the morphospace (Figure 3), we used 3D geometric

morphometric techniques based on landmark configurations, according to Rosas et al.'s (2015) procedures and comparative samples (see Supplementary Material).

Taxonomically, the human remains from Simanya Gran present a large series of Neanderthal apomorphic features distributed across different anatomical systems. Upper central incisors are large with a robust root, a strong dental tubercle, and characteristic flat wear (Supplementary Figure S4). The upper lateral incisors present a conspicuous and characteristic shovel shape, where the marginal ridges are extremely thickened and invade the lingual fossa, circumscribing a narrow longitudinal groove. The mandible shows a large, moderately truncated gonial area, with a large medial pterygoid tubercle, and a lateral position of the condyle in relation to the mandibular notch (Figure 2G). Humerus SI-1 (Figures 2A, 3; Supplementary Material) displays a massive and anteriorly projecting lesser tubercle. A deep olecranon fossa, which is elliptical in shape, wider than high, and a marked medial epicondyle are moderately prominent. The long lateral supracondylar crest and coracobrachial insertion are located more distally than the distal limit of the deltoid tuberosity, while the deltoid tuberosity displays two parallel and proximal ridges. The humeral torsion is moderate. The first metacarpal is short and displays a flange of bone along the distodorsoradial margin, marking the insertion of the opponens pollicis muscle. The first pollex phalanx is relatively short. The first phalanx of the second ray

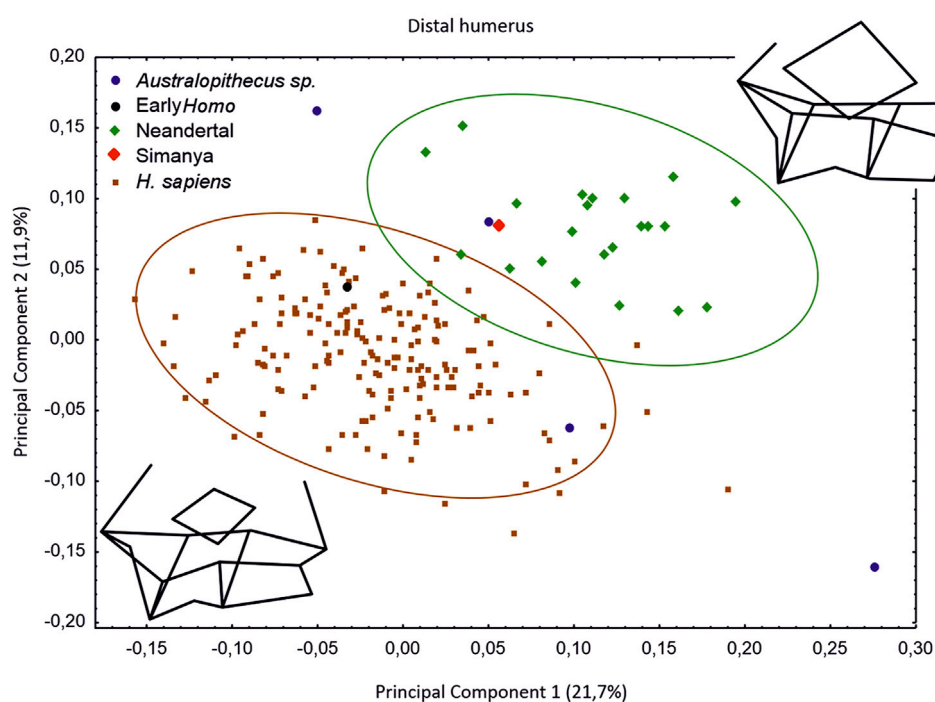


FIGURE 3

Scatter plot of the PCA (PC1–PC2) shape space of the distal humerus performed on 18 landmarks and a large comparison sample from the work of Rosas et al. (2015). Landmark 8 of the Simanya humerus has been estimated by the thin plate spline method. Ellipses for the prediction interval of a single new observation (0.95% probability) are shown only for the Neanderthal and modern human samples. Note the position of the Simanya's humerus clearly within the Neanderthal distribution.

presents a robust and prominent radial tubercle, and the distal phalanges show an extremely wide distal end (Figure 2D). The big toe metatarsal and distal phalanx are gracile, but the distal head of the metatarsal is proportionally bulky (Figure 2C).

Ultimately, no positive results for aDNA were obtained, neither for shotgun sequencing nor for the mtDNA capture.

4 Discussion and conclusion

Excavations at Simanya Gran revealed Pleistocene fossil-bearing deposits, primarily preserved in the layer O300-PO400 from the IMG and likely dismantled from the cave entrance. Its sub-superficial position aligns with Mr. Aznar's report, and similarities in the taxonomic structure and taphonomic features connect the "Museum Collection" to the documented contexts. The discovery of a Neanderthal upper M3 that is compatible with "Museum Collection" individual 2 supports this interpretation, which is further corroborated by Mr. Aznar identifying the excavation area.

The ^{14}C chronology provided by charcoals from our excavations are compatible with those from the charcoals included in the sediment samples associated to the human remains from the "Museum Collection," potentially placing Simanya Neanderthals beyond 49,000 years BP. A minimum age of *circa* 42,000 cal BP has been obtained for the paleontological assemblage that is also present in the layer O300-PO400. The discrepancy between both ages is

consistent with the open character of the layer that could have been deposited during a relatively long period including different time-segregated events.

The Simanya sample is attributed to Neanderthals due to distinct apomorphic traits (see Figure 3). It includes at least three individuals—an adult, a periadolescent (~11.5 years), and an immature (~7.5 years)—with preserved dentition, mandible, vertebrae, upper limbs, and foot bones. Some specimens are complete and well preserved, while others are fragmentary with diagenetic and modern fractures. Taphonomic conditions allow assigning some teeth to individual 1, but based on size differences, a fourth individual is possible.

Simanya Neanderthals' apomorphic traits align with other late Neanderthals, supporting, together with the chronological data from the excavation, its location in a final evolutionary phase of the evolution of western Neanderthals. However, different genetic backgrounds within Iberian Neanderthal populations cannot be ruled out. The substitution of Neanderthal populations in Western Europe (Dalén et al., 2012) and a population turnover with mtDNA diversity loss (~100 ka ago) have been identified (Vernot et al., 2021). The presence of primitive features in other samples, such as the 49,000 old from El Sidrón (Rosas et al., 2020), allows for hypothesizing the potential persistence of relict groups. Comparative studies with other Iberian samples may clarify late Iberian Neanderthals' phylogeography.

The analysis of the Cova Simanya assemblage has allowed the identification of a new Neanderthal sample that will provide

essential data on the morphological traits and evolutionary context of late Neanderthals in the Iberian Mediterranean region. Further comparative studies, incorporating morphometric and genetic analyses, with other Iberian samples will elucidate their phylogeography and population dynamics to shed light on a complex evolutionary history comprising different genetic backgrounds and relict genetic groups of late Iberian Neanderthals.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#); further inquiries can be directed to the corresponding authors.

Author contributions

Conceptualization: JM and AR. Investigation: JM, AC, MS, AR-H, RH, EM-R, DL, JR, DM-P, AG-T, EA, AG-B, EL, TM-B, ST, CL-F, and AR. Writing—original draft: JM and AR. Writing—review and editing: JM, AC, MS, AR-H, RH, EM-R, DL, JR, DM-P, AG-T, EA, AG-B, EL, TM-B, ST, CL-F, JF, and AR. Project administration: JM, JF, and AR. Funding acquisition: JM, JF, AR, MS, ST, and CL-F. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/feart.2023.1230707/full#supplementary-material>

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