**First paleogenetic evidence from Middle Bronze Age human remains in Grotta della Monaca, Calabria (Southern Italy).**

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**File S1**

*Archaeological context and anthropological analyses*

Located 600m asl close to the Esaro River (Cosenza, north-western Calabria), Grotta della Monaca extends for 360 m in Triassic dolomitic limestone, developing into galleries, chambers, and narrow passages. It shows the presence of iron ore (limonite, lepidocrocite, goethite, hematite, yukonite) and copper ores (malachite, azurite, brochantite, libethenite, sampleite) [1–5]. The cave is conventionally divided into three main sectors: *Pregrotta* (the entrance gallery), *Sala dei Pipistrelli* (a very large hypogeum sector), *Cunicoli terminali* (two narrow tunnels in the innermost part of the cave) [6] (Figure 1).

The first survey of burial areas was conducted in 2000-2003 and allowed the discovery of twelve human skeletons. Out of these, only five have been diagnosed for sex due to the poor preservation conditions (4 males and 1 female) [7]. Further archaeological investigations, conducted until 2010, resulted in the finding of a mass grave with numerous human skeletal remains in a niche of the area of Cunicoli terminali (called *m5v*). The archaeological deposit was excavated using the “arbitrary level excavation method”, applied for mass grave burials in commingled contexts [8]. This archaeological sector was conventionally divided into three areas: *China* (the uppermost part of the deposit), *Sella* (an area of accumulation of commingled remains) and *Buca di L5* (the deepest part of the deposit).The remains were in a poor state of preservation due to several taphonomic factors. Among these factors, human frequentation has above all caused fragmentation of bones and their critical state of preservation [9]. In this area, the pottery assemblage is poor and commingled, but it was possible to recognize a typology of vessels used for funerary rituals, even if not directly attributable to the burials [10].

## *Osteological study*

The osteological remains buried in *m5v* of Grotta della Monaca were examined by traditional anthropological methodologies at the Laboratory of Archaeo-Anthropology and Forensic Anthropology of the Ferrara University (Italy). As suggested by protocols employed to reconstruct skeletons in collective and multiple burials [11–14], bones were grouped by typology (humerus, femur, tibia, etc.) and by side (right/left). The MNI (Minimum Number of Individuals) was assessed based on the anatomical features, considering bone recurrence, age-at-death, sex [15,16]. Skeletons were assembled by the articulation of bones, sex and age, specific features (diseases, bone robustness, muscle insertions, etc.). In compliance with the estimated age, we used the following categories [15] implemented with additional infant categories in accordance with Krenzer [17]: foetus (before birth), infant 1 (0 to 6 years old) (I1), infant 2 (7 to 12 years old) (I2), juvenile (12 to 20 years old) (J), young adult (20 to 35 years old) (YA), middle adult (35 to 50 years old) (MA), old adult (>50 years old) (OA. Age at death in subadults was estimated by dental eruption [18], epiphyseal fusion and dimensions of long bones [18–20], stage of bone ossification [21–24]. Cranial sutures [25,26], changes in the morphology of pubic symphyseal surface [27,28] and auricular surface [26,29], tooth wear [29,30] were used for the age-at-death assessment in adults. In adults, sex diagnosis was performed by the skull and pelvis morphology [15,25,31,32]. Whenever possible, the osteometric characters of the skull [33,34], mandible [35], scapula [36], humerus [24,31,37], radius [38], ulna [39], femur [37], tibia [31] and talus [40] have also been used for sex diagnosis. Although the method is considered less precise, sex was also assigned in subadults according to morphology of the mandible and ilium [41].

We examined macroscopically each specimen from a paleopathological point of view, detecting any osteological changes, and non-metric features. In particular, the presence of cribra cranii [42–44], cribra orbitalia (according to Knipp method reported by Brothwell, [30]), humeral and femoral cribra [45–47] have been investigated. Concerning the analysis of teeth, dental calculus was examined according to the scale of Brothwell [30] and successive modifications [48]. The degree of occlusal wear was scored according to Smith [49]. We recorded hypoplastic defects according to the standard published by Fédération Dentaire Internationale (FDI 1982). We also paid particular attention to the investigation of inherited features possibly present on teeth and skeletal remains.

The mass grave contained a minimum number of 24 individuals: 4 males, 6 females, and 14 undetermined. Regarding age at death, 75% of individuals were subadults (I1:7; I2:8; J:3; YA:4; OA:1; unknown:1).  Table S1 shows a summary of the main results concerning the osteological analysis of seven individuals (N. 5, 6, 7, 11, 12, 14, 24) involved in the molecular study. Porotic lesions were found to be the most common pathological condition: cribra were present in all these individuals although with different intensity and location of the affected areas.

The analysis of inherited skeletal traits led to the identification of some interesting markers among the human remains from the area *m5v*. In particular, three adult individuals show the congenital axis dysmorphism of the odontoid process – ossification is generally within five years. The attribution of these vertebrae to specific individuals was impossible as these vertebrae belonged to the same archaeological level (at the base of the Sella). From the comparison of the vertebrae with other specimens of known age at death, it resulted that they related to three young individuals with the first degree of expression of the pathology: the odontoid process is partially ossified, thus resulting in bifid dens (according to Aufderheide and Rodriguez-Martin[50]).

Carabelli’s tubercle, an additional dental cusp, was found on the first maxillary molar of individual 14. It is a well-formed cusp, corresponding to the 4th degree of expression following the classification of Borgognini Tarli and Pacciani [12]. We had seen several cases of this congenital trait on first molars in this necropolis, even if not associable to any of the other six individuals involved in the genetic analysis.

Human remains from Grotta della Monaca, as well as those of six other southern Italian prehistoric sites from Neolithic to Bronze Age, have also been recently analyzed through stable isotope analysis of bone collagen to reconstruct the diet of these agricultural-pastoral communities [51]. Inherited traits found in individuals from *m5v* have allowed us to advance the hypothesis of multiple family burials.  Other Italian Bronze Age sites corroborate this pattern. Specifically, multiple burial graves in the cave with related individuals were found at Grotta Manaccora in Apulia and at Grotta di Re Tiberio in Emilia-Romagna. In the latter, the remains of young women and newborn infants (0-3 months), probably interrelated, were found [52–57]. Moreover, several Middle Bronze Age burials -not in cave- involving family groups have been found at Toppo Daguzzo, Murgia Timone and Lavello in Basilicata, Ipogeo dei Bronzi (Madonna di Loreto, Trinitapoli) and Ipogeo degli Avori (Terra di Corte, San Ferdinando), Dolmen (Bisceglie), Santa Sabina (Carovigno) in Apulia [56,58].

Isotopic analysis of bone collagen [51] suggested that these communities had a varied diet consisting of terrestrial resources with a prevalent intake of vegetal proteins (cereals and legumes) and a modest intake of animal proteins for all the periods examined. During the Bronze Age, subsistence strategies were mainly based on cattle breeding rather than hunting. Some cereals (such as millet), already cultivated in north-central Italy, had not yet reached the south of the peninsula. As a result, a protein consumption of C4 plants-related was demonstrated for central northern Italy and C3 plants-related for southern Italy.

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