

UNDERSTANDING THE COMPLEX SOCIAL RELATIONS IN PRE-ROMAN UMBRIAN COMMUNITIES WITH AN INTEGRATED APPROACH OF BIOARCHAEOLOGY

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PIAZZA D'ARMI NECROPOLIS IN SPOLETO objectives of a multidisciplinary study

This project investigates the origins, family relationships, social links and mobility patterns of pre-Roman Umbrian people through the analysis of the Piazza d'Armi necropolis in Spoleto, Central Italy. Discovered in 1982, the site was the focus of several archaeological excavation campaigns conducted until 2011 (Fig. 1). The burials revealed extraordinary artefacts reflecting the power of the Umbrian elite during the Orientalising period (late 8th - early 6th century BCE) [1]. The presence of numerous infants' grave goods rich in weapons and ceremonial ceramic vessels, as well as female burials containing priestly objects, along with many non-local or foreign items, and the four sceptres from a man's grave, makes this site exceptional. These findings suggest that this Umbrian community was notably inclusive, attributing symbolic power to both women and children [2]. To explore the key questions emerging from the archaeological evidence, molecular analyses on skeletal remains have been focused on:

- reconstructing kinship and social relations among individuals buried at Piazza d'Armi;
- evaluating mobility patterns to determine whether foreign artefacts reflect non-local genetic ancestry and/or cultural transmission;
- investigating health and dietary habits, examining variation linked to social status, ancestry and geographical origin.



Figure 1. Geographical location of Piazza d'Armi necropolis in Spoleto (A) and aerial photo of the "CANTIERE DI MARCO" (B).

MATERIALS AND METHODS

Morphological and morphometric methods identified 17 non-adults and 21 adults. **Paleogenomic analysis** was conducted on 28 individuals due to the state of preservation. Ancient DNA (aDNA) was extracted using a silica column-based protocol [3] and converted into double-stranded genomic libraries [4] (Fig. 2). Libraries passing quality check were shotgun sequenced on the Illumina NextSeq550 platform (University of Tor Vergata). Data were processed using a specific aDNA bioinformatics pipeline. Post-mortem damage was assessed through MapDamage 2.0 [5] and mtDNA contamination was estimated using contamMix [6]. Molecular sex determination was performed by mapping reads on sex chromosomes [7] and mitochondrial haplogroup was assessed using HaploGrep2 [8] only for >5X mtDNA coverage. **Principal component analysis (PCA)** was employed to explore Umbrian genetic variation in the context of 969 modern and ancient genomes [9] from 1240K+HO dataset. Pseudo-haploid variant calls were generated using PileupCaller; only samples with >20,000 SNPs were merged with published dataset via PLINK. Conversion to EIGENSTRAT format was required to run smartpca from the EIGENSOFT package [10].

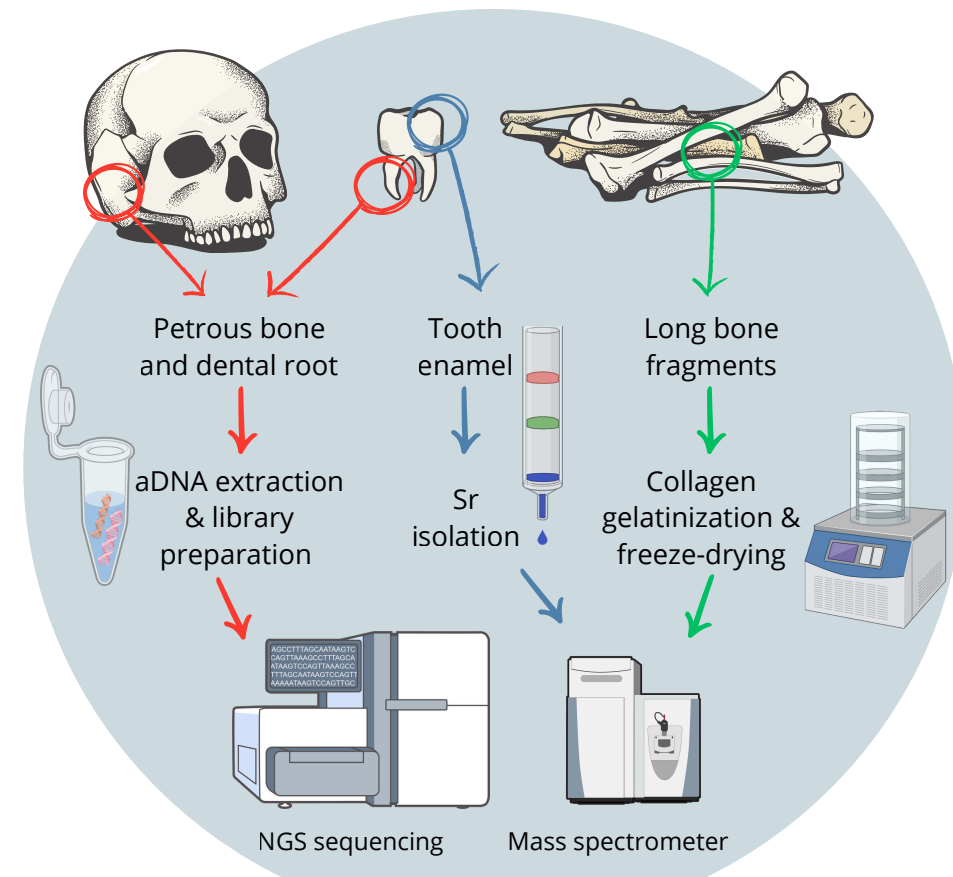


Figure 2. Visual representation of molecular analysis' workflow

Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope analysis for reconstructing dietary habits was performed on 28 long bones samples. Bone collagen was extracted following modified Longin method [11] (Fig. 2). Each extract was analysed in duplicate using a Delta V Advantage isotope ratio mass spectrometer coupled to a Flash 1112 Elemental Analyser via a Conflow III interface (IRMS Laboratory, University of Campania "Luigi Vanvitelli"). Only specimens that met established quality criteria for collagen preservation and contamination [12] were processed. Statistical analyses were applied using the statistical software Past (v. 5.2.1) [13] for the non-parametric Mann-Whitney U-test and the non-parametric one-way Kruskal-Wallis H-test. **Strontium ($^{87}\text{Sr}/^{86}\text{Sr}$) isotope analysis** was conducted to investigate mobility by comparing enamel from 28 human individuals to local baselines derived from 10 soil samples, using preparation methods tailored to each material type [14]. Strontium was isolated via ion exchange chromatography (Fig. 2) and analysed with a FINNIGAN MAT 262RPQ mass spectrometer (Sapienza University of Rome and CNR-IGAG).

RESULTS AND DISCUSSION

Sequencing data reveal low endogenous DNA yield in several samples (Fig. 3), so that we are testing targeted human DNA enrichment (Twist Biosciences) [15]. All samples show post-mortem damage and low mtDNA contamination. Molecular sex determination identified 13 females and 4 males. Despite low DNA quality, molecular results matched morphological data (2 females, 9 males). At the present moment, two mtDNA haplogroups H24 and U2e1b were assigned, both commonly associated with Central European ancestry.

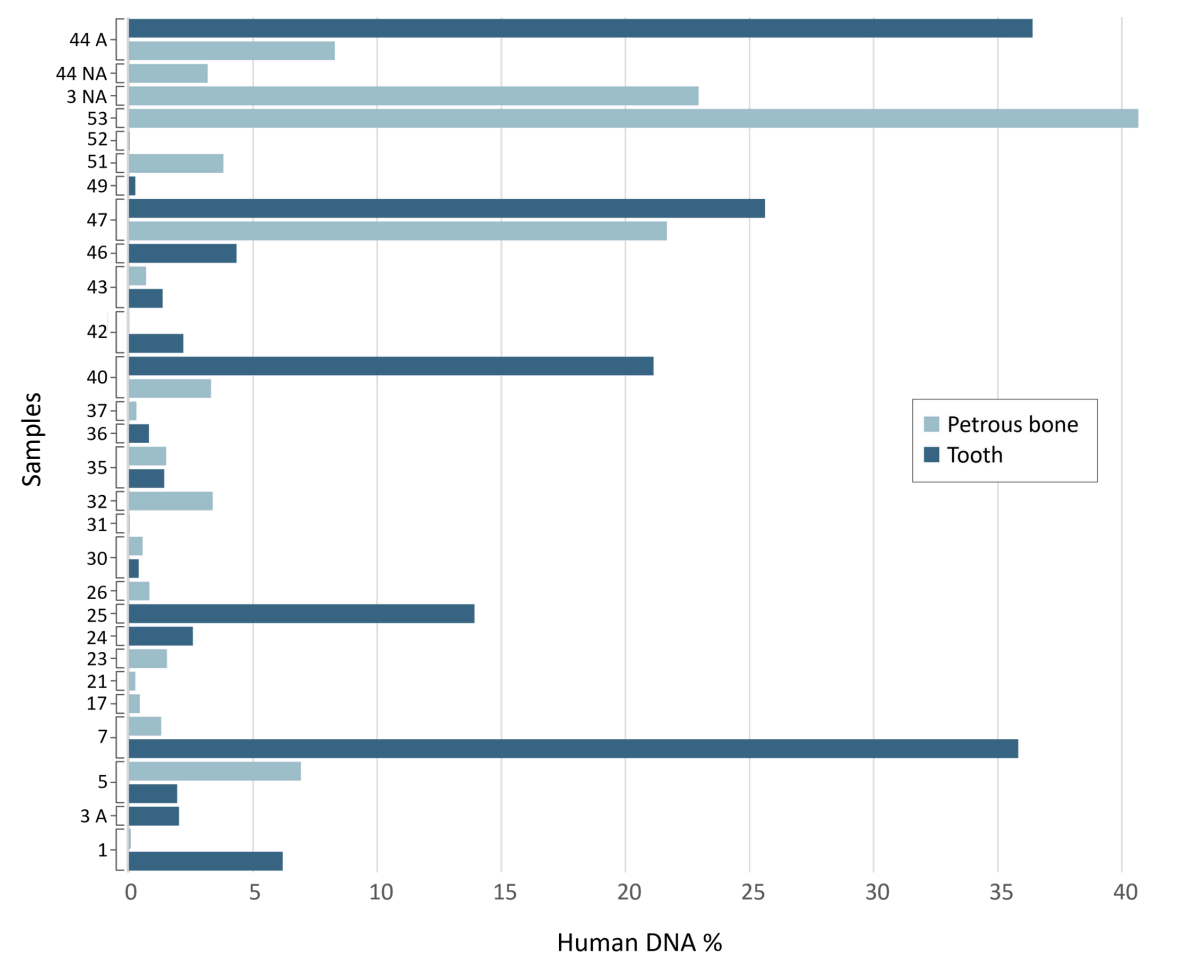


Figure 3. Endogenous DNA yield obtained for each individual. Bars enclosed between two tick marks refer to two different samples - petrous bone and tooth- for the same individual.

PCA shows that, as other Iron age cultural groups, Umbrians (n = 7) cluster predominantly into genetic variation of modern Italian, Northern European, and Balkan populations (Fig. 4). As described by Ravasini [9], Tyrrhenian (Etruscans and Iron Age/Republic Romans) and Adriatic (Iron Age Apulians) groups differ in distribution, with the latter slightly shifted towards Balkan and Northern Europeans. In this context Umbrians appear genetically closer to Etruscans, with a higher Anatolian Neolithic component than Eastern HG/Yamnaya. It confirms the subsequent appearance of Caucasus HG/Iran Neolithic component in Italy.

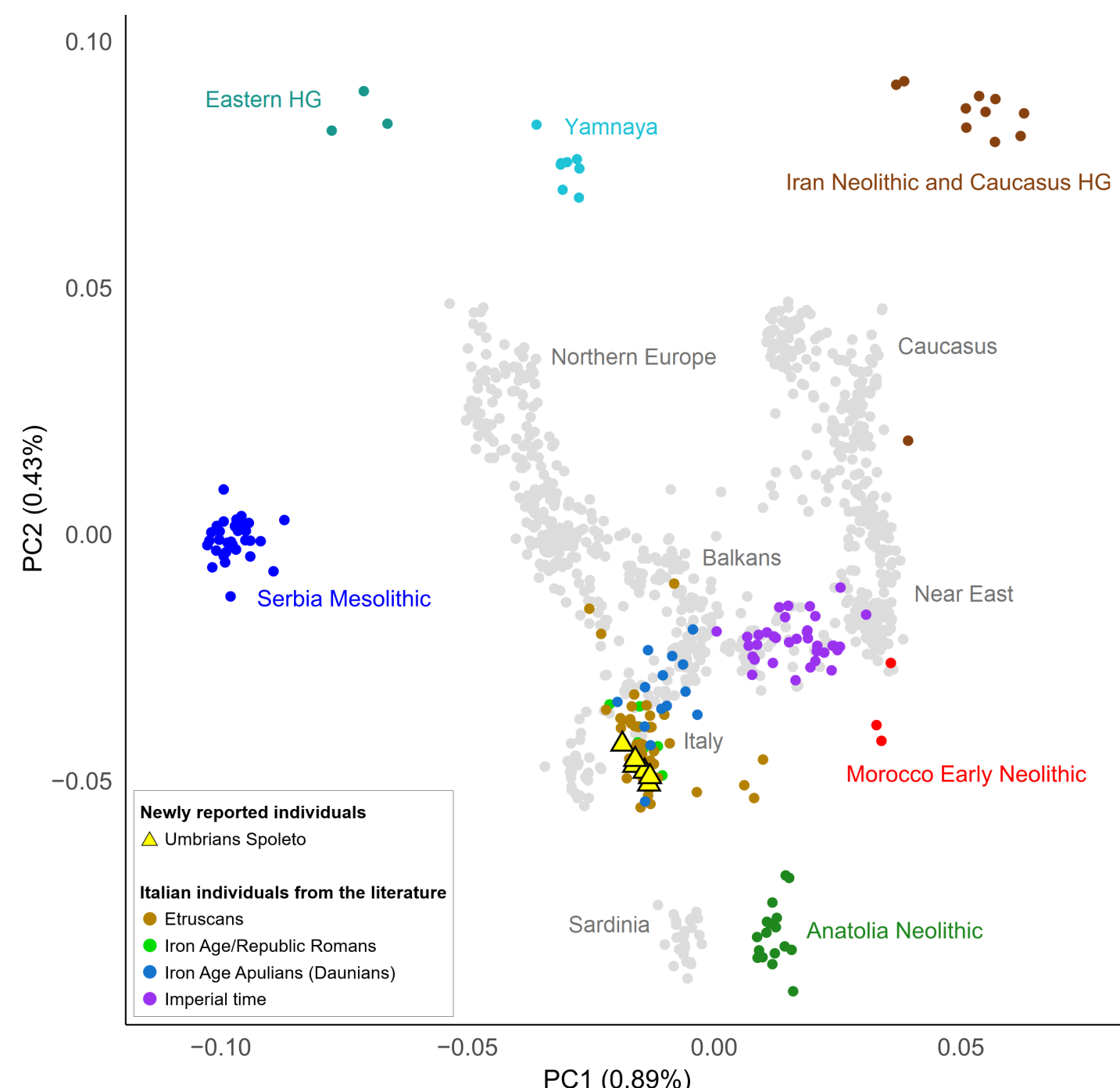


Figure 4. PCA with the newly Spoleto sequences from Piazza d'Armi and 969 modern and ancient sequences from the literature

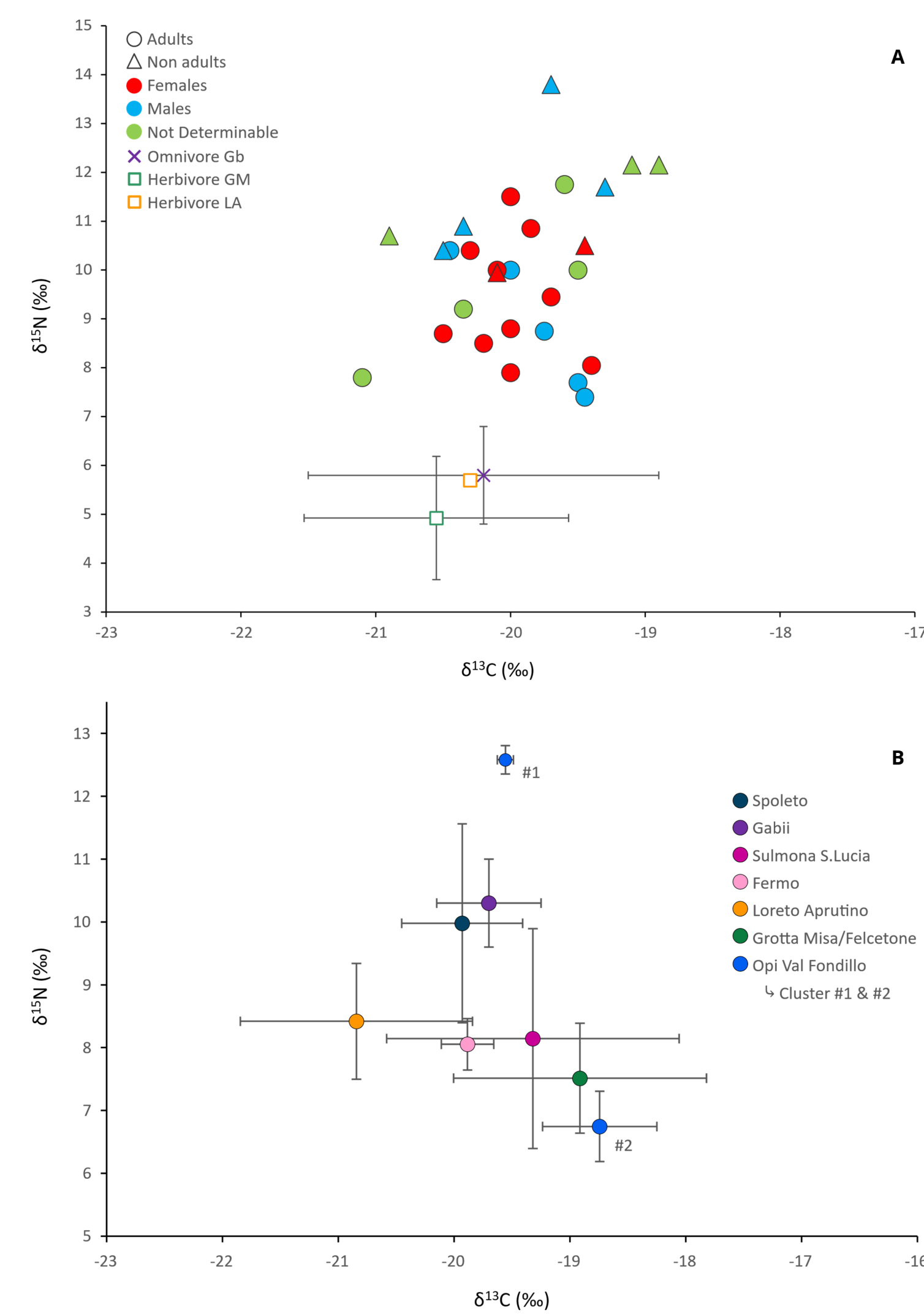


Figure 5. Plot of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of individuals from this study compared to faunal isotopic data from the sites of Loreto Aprutino (LA), Grotta Misa (GM) and Gabii (G) (A). Mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of Spoleto compared to coeval sites in Central Italy (B).

All samples analysed for dietary reconstruction meet quality criteria. **$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ human values** were compared to faunal isotopic data of coeval site of Gabii [16] and Loreto Aprutino [17] but also of Grotta Misa/Felcetone [18] located closest geographically to Spoleto. The $\delta^{13}\text{C}$ values for individuals from Spoleto range from -21.1‰ to -18.9‰ while $\delta^{15}\text{N}$ range from 7.4‰ to 13.8‰ (Fig. 5A). No significant differences for $\delta^{13}\text{C}$ (Mann-Whitney U-test = 48.000; $p = 0.694$) and $\delta^{15}\text{N}$ values (Mann-Whitney U-test = 44.500; $p = 0.522$) are observed between sexes. The comparison of age groups shows significant differences for $\delta^{15}\text{N}$ (Kruskal-Wallis H-test = 12.110; $p = 0.001$) but not for $\delta^{13}\text{C}$ (Kruskal-Wallis H-test = 3.605; $p = 0.458$). However, given the proportion of nonadults likely still breastfeeding, the statistically significant difference for $\delta^{15}\text{N}$ values evaluated against other age groups (Mann-Whitney U-test = 11.000; $p = 0.000$), can be probably attributed to nursing practices. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ mean values of Umbrians from Spoleto compared to coeval sites of Central Italy (Fig. 5B), including also Fermo [19] and Sulmona S. Lucia - Opi Val Fondillo [20], suggest a diet based primarily on C_3 plants and terrestrial protein similar as that reported in the archaeological site of Gabii. No evidence suggests significant C_4 plant or marine resource consumption.

The area around Spoleto, located along the Apennine Mountain range, is mainly dominated by carbonate formations that generate a $^{87}\text{Sr}/^{86}\text{Sr}$ signal relatively homogeneous in Umbria ranging from 0.7080 to 0.7089, as defined by soil samples. The $^{87}\text{Sr}/^{86}\text{Sr}$ data show 4 of 38 individuals outside the bioavailable strontium isotope baseline (Fig. 6). In cases like tomb 5, this was archaeologically suggested by grave goods with references to the Etruscan and Sabine cultures. Unfortunately, low endogenous DNA content precluded genetic comparisons with PCA results. Further analysis will be necessary to understand migration patterns and determine whether cultural factors influenced mobility.

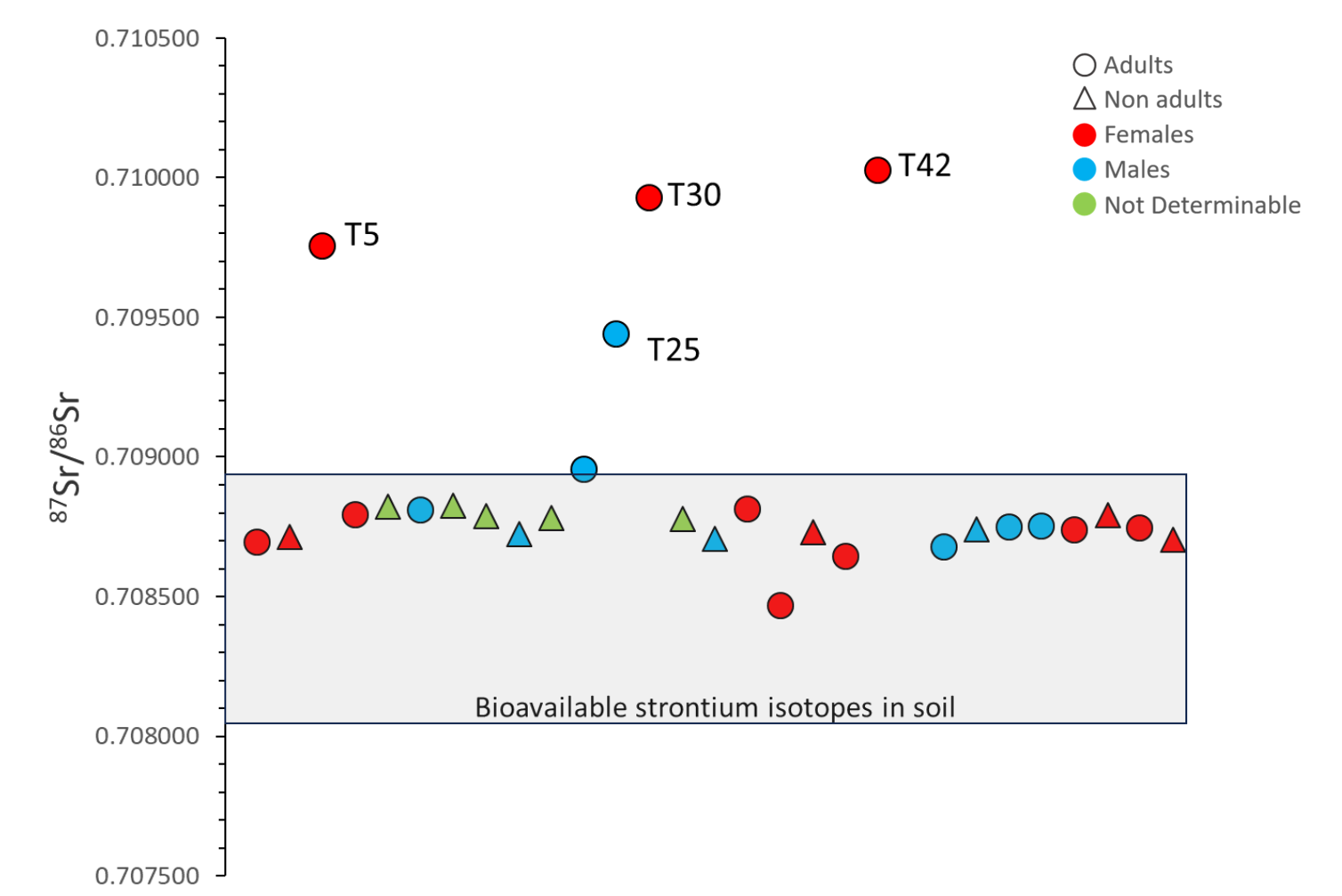


Figure 6. Plot of $^{87}\text{Sr}/^{86}\text{Sr}$ of individuals from this study compared to bioavailable strontium isotopes normal distribution in soil.

PRELIMINARY CONCLUSIONS

This study provides a first overview of pre-Roman Umbrian elite from Spoleto, contributing to understand its social complexity. The inclusion of individuals who grew up elsewhere among Spoleto's upper classes points to a community open to external influences. This cultural diversity may have played a role in shaping not only its social dynamics but also its language. Ongoing analyses of migration pathways and population genetics will help to clarify whether these individuals align with the established genetic pool or constitute clear outliers. Targeted enrichment of human genome will be crucial to the progress of research and to the resolution of some questions surrounding this society. The reason behind the unusually high representation of non-adult individuals associated with wealthy social classes remains unclear. The integration of archaeological, morphological and molecular data will offer insights into one of the Iron Age populations of Central Italy less known. The research will also explore their interactions and connections with better-known populations such as the Etruscans and the Picenes, contributing to the broader picture of cultural dynamics in pre-Roman Central Italy.