



# Piazza d'Armi of Spoleto (PG, 720 – 580 BCE): a bioarcheological research to understand the Umbrians origins and their complex social relationships

Arduini Chiara<sup>1</sup>, Pusceddu Allegra<sup>1</sup>, Weidig Joachim<sup>2</sup>, Castorina Francesca<sup>3</sup>, Ottoni Claudio<sup>1</sup>, Mantile Noemi<sup>4</sup>, Giacometti Valentina<sup>4</sup>, Micheli Maria Elisa<sup>5</sup>, Casciarri Silvia<sup>6</sup>, Micozzi Marina<sup>7</sup>, Coen Alessandra<sup>5</sup>, Martinez-Labarga Maria Cristina<sup>1</sup>

Dipartimento di Biologia, Università degli Studi di Roma Tor Vergata<sup>1</sup> - Albert-Ludwigs-Universität Freiburg<sup>2</sup> - Istituto di Geologia Ambientale e Geoingegneria (IGAG), Consiglio Nazionale delle Ricerche (CNR), c/o Università degli Studi di Roma Sapienza<sup>3</sup> - Università degli Studi della Campania "Luigi Vanvitelli"<sup>4</sup> - Università degli Studi di Urbino Carlo Bo<sup>5</sup> - Museo Archeologico Nazionale e Teatro Romano di Spoleto, Direzione regionale musei Umbria MIC<sup>6</sup> - Università degli Studi della Tuscia<sup>7</sup>

## INTRODUCTION

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 (Fig. 2) reflecting the power of the Umbrian elite during the Orientalising period (720-580 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 test these hypotheses and to further investigate Umbrians' society through genetic ancestry, mobility pattern, and diet, a multidisciplinary approach was adopted focusing on the analysis of ancient biomolecules (DNA and stable isotopes).

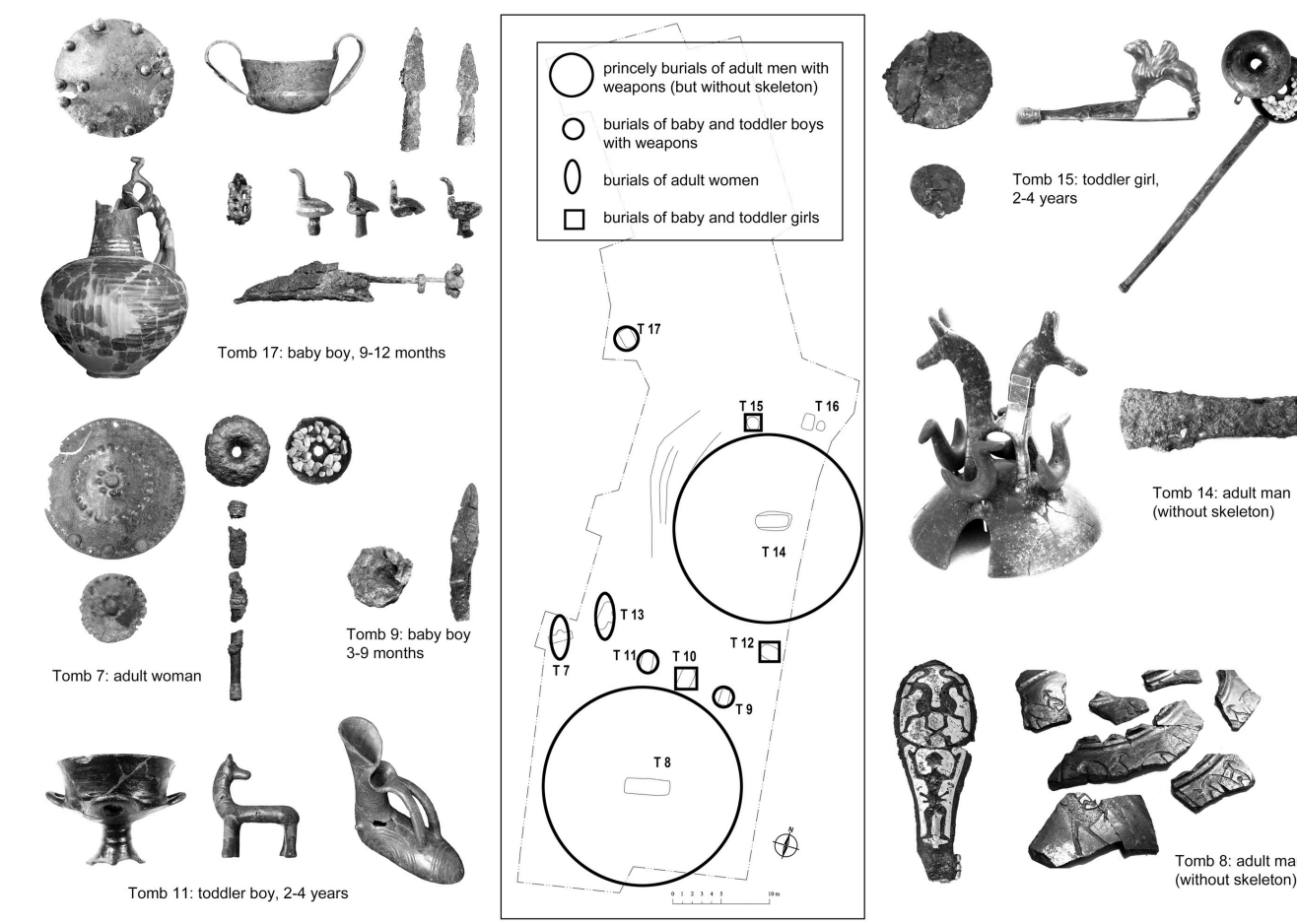


Figure 2. Burials of the aristocratic family in the north-eastern sector (Ater).



Figure 1. The Piazza d'Armi necropolis in Spoleto.

## MATERIALS & METHODS

## RESULTS & DISCUSSION

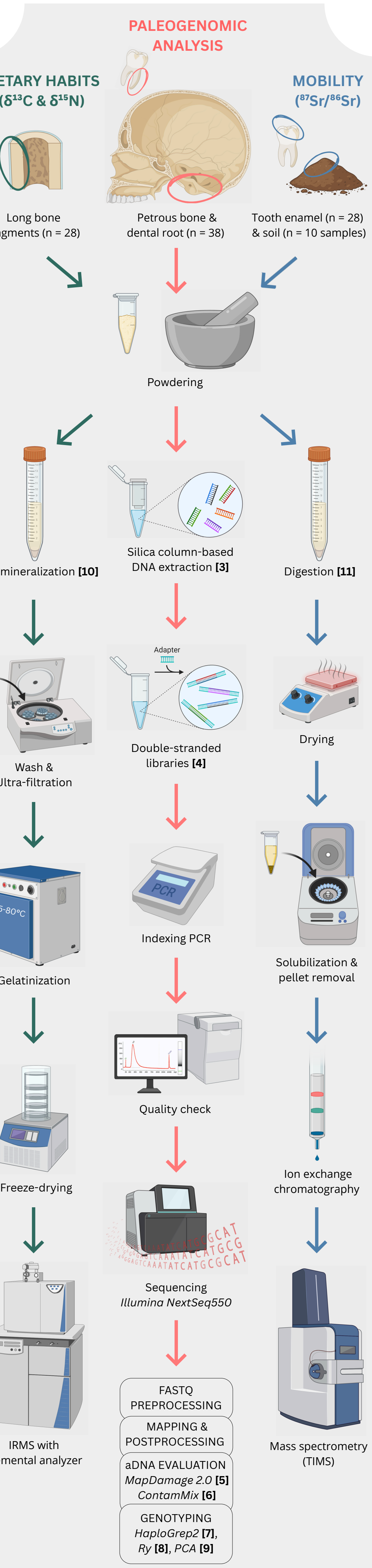


Figure 3. Visual representation of molecular analysis' workflow. Image created in BioRender.com

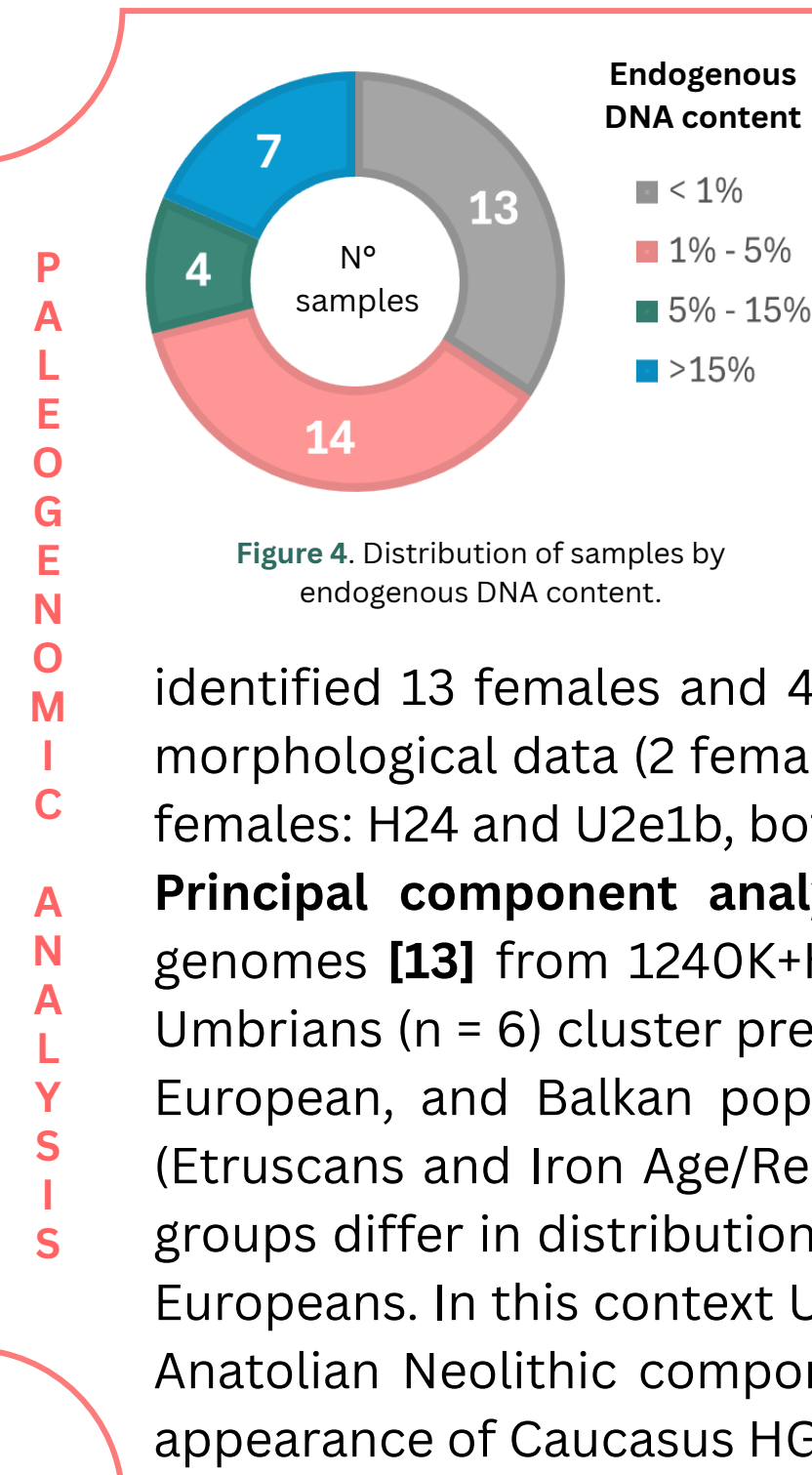


Figure 4. Distribution of samples by endogenous DNA content.

Among the 38 individuals examined (17 non-adults and 21 adults), paleogenomic analyses were conducted on 28 due to the state of preservation. The shotgun sequencing data reveal low endogenous DNA yield in several samples (Fig. 4), which are currently being submitted to targeted enrichment of 1.4 million human polymorphisms (Twist Biosciences) [12]. All samples show typical ancient DNA post-mortem damage (avg. 3'G>A: 32.3%, 5'C>T: 39.2%) and low rates of contamination. Molecular sex determination identified 13 females and 4 males. Despite low DNA quality, molecular results matched morphological data (2 females, 9 males). mtDNA haplogroups were assigned for only two females: H24 and U2e1b, both commonly associated with Central European ancestry.

**Principal component analysis (PCA)** includes a total of 1029 ancient and modern genomes [13] from 1240K+HO dataset. It shows that, as other Iron age cultural groups, Umbrians (n = 6) cluster predominantly into genetic variation of modern Italian, Northern European, and Balkan populations (Fig. 5). As described by Ravasini [13], Tyrrhenian (Etruscans and Iron Age/Republic Romans) and Adriatic (Picenes and 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.

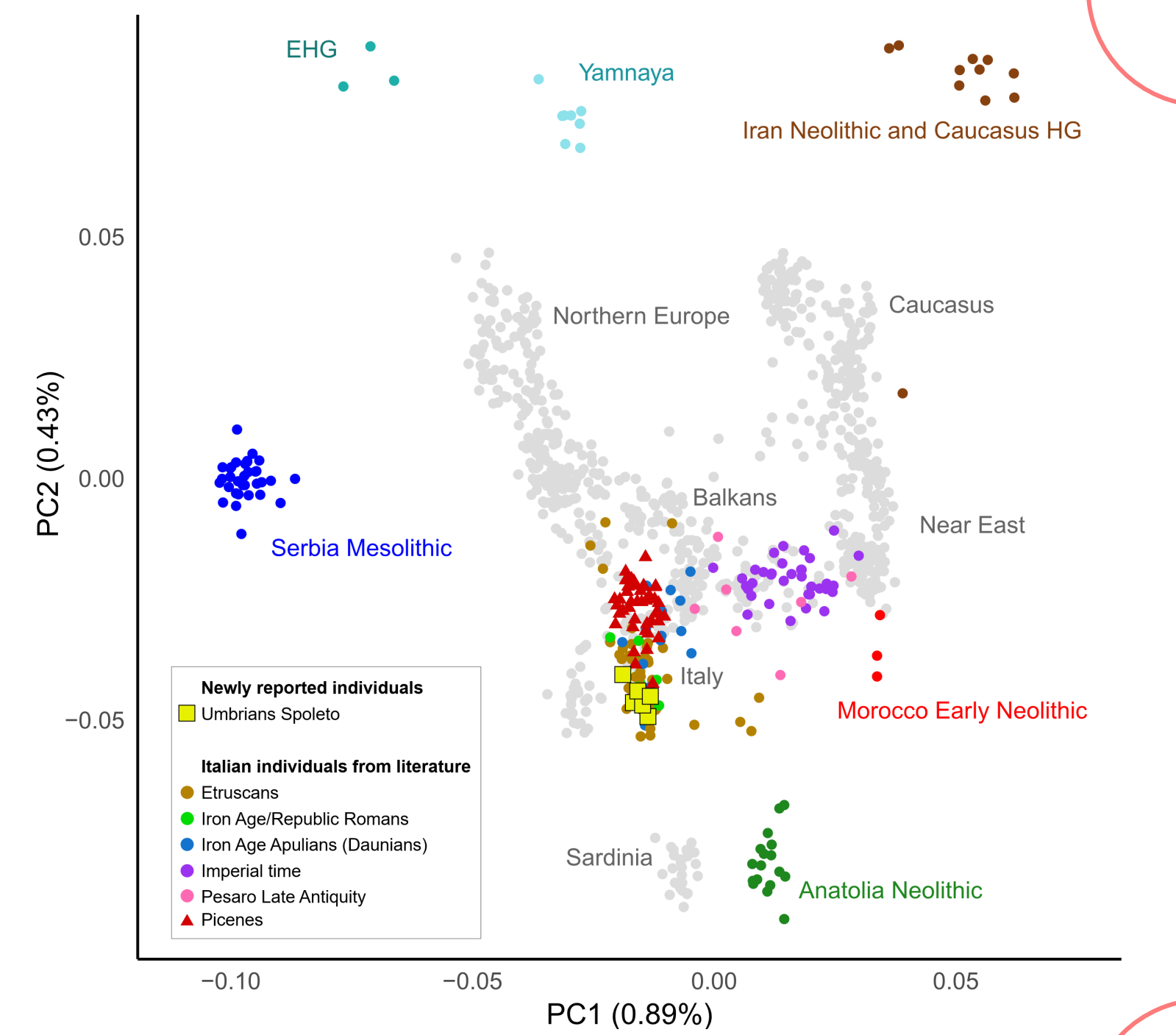


Figure 5. PCA with the newly Spoleto samples from Piazza d'Armi and 1023 modern and ancient sequences from the literature.

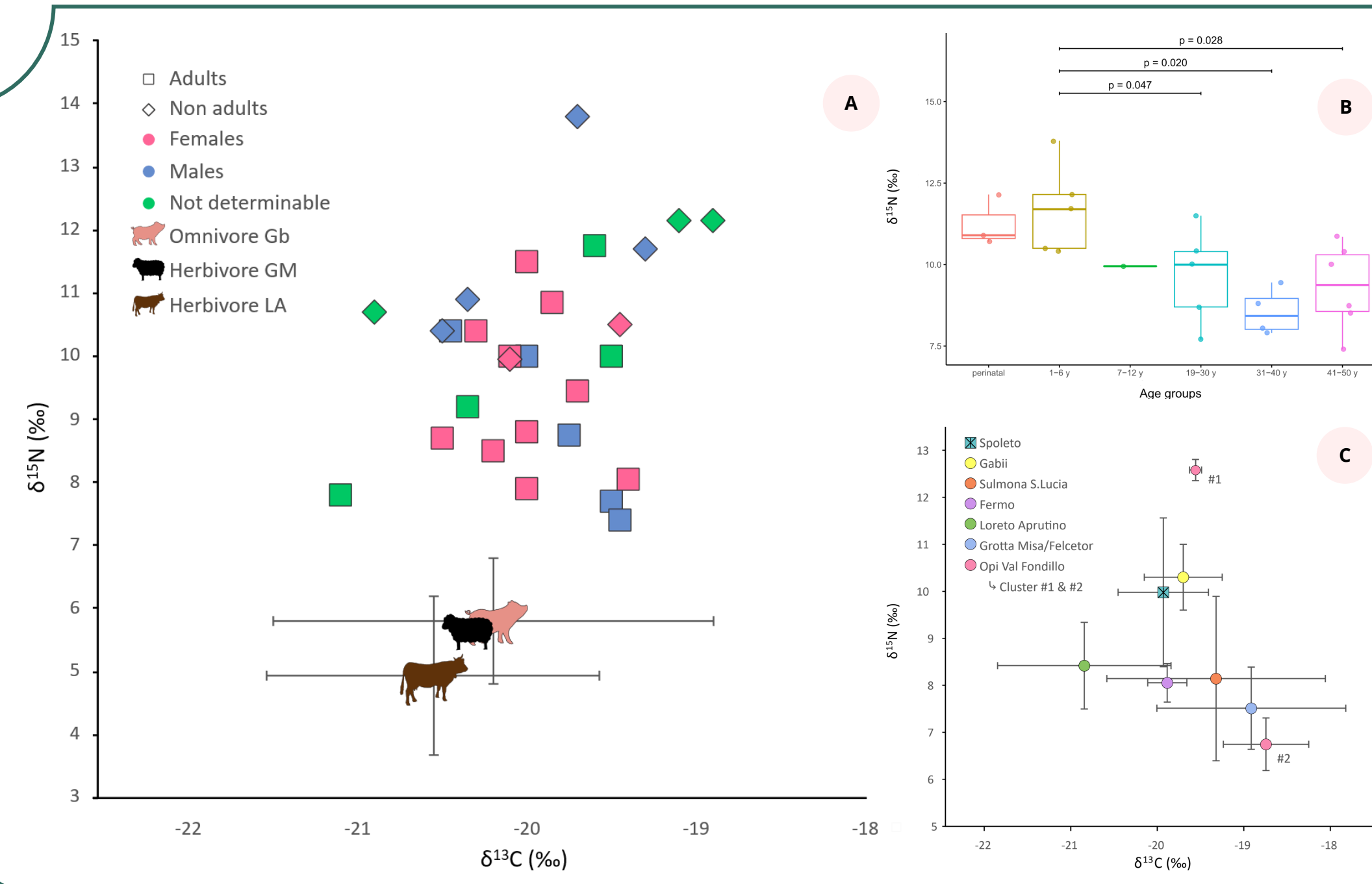


Figure 6. Plot of  $\delta^{13}C$  and  $\delta^{15}N$  values of individuals from this study compared to faunal isotopic data from the sites of Loreto Aprutino (LA), Grotta Misa (GM) and Gabii (Gb) (A). Boxplots for  $\delta^{15}N$  values of Umbrians by age groups (B). Mean  $\delta^{13}C$  and  $\delta^{15}N$  values of Spoleto compared to coeval sites in Central Italy (C).

All samples analysed for dietary reconstruction meet quality criteria for collagen preservation and contamination [14].  $\delta^{13}C$  and  $\delta^{15}N$  human values were compared to faunal isotopic data of coeval site of Gabii [15] and Loreto Aprutino [16] but also of Grotta Misa/Felcetone [17] located closest geographically to Spoleto. The  $\delta^{13}C$  values for individuals from Spoleto range from -21.1‰ to -18.9‰ while  $\delta^{15}N$  range from 7.4‰ to 13.8‰ (Fig. 6A). No significant differences for  $\delta^{13}C$  (Mann-Whitney U-test = 48.000; p = 0.694) and  $\delta^{15}N$  values (Mann-Whitney U-test = 44.500; p = 0.522) are observed between sexes. The comparison of age groups shows significant differences only for  $\delta^{15}N$  (Fig. 6B). However, given the proportion of nonadults likely still breastfeeding (~78%), it can be probably attributed to nursing practices.  $\delta^{13}C$  and  $\delta^{15}N$  mean values of Umbrians from Spoleto compared to coeval sites of Central Italy (Fig. 6C), including also Fermo [18] and Sulmona S. Lucia - Opi Val Fondillo [19], 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.

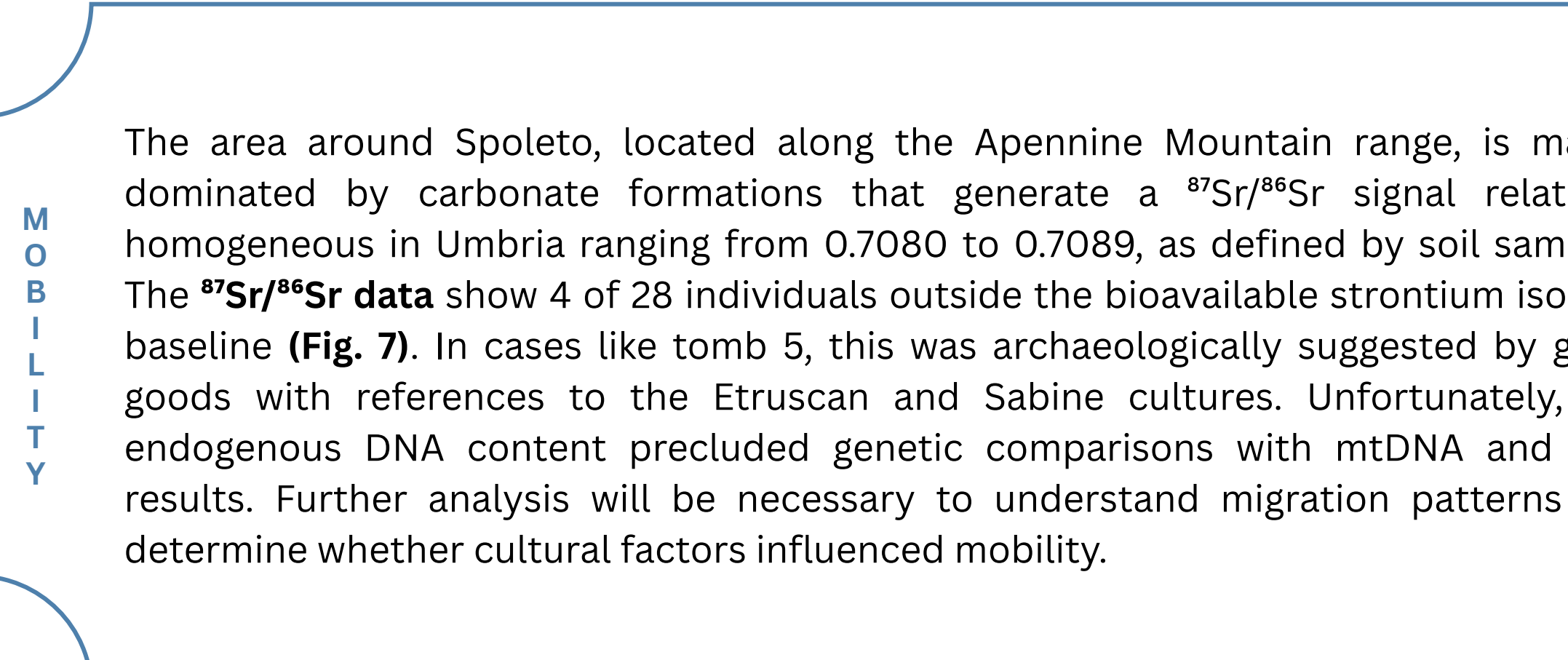


Figure 7. Plot of  $^{87}Sr/^{86}Sr$  of individuals from this study compared to bioavailable strontium isotopes normal distribution in soil.

The area around Spoleto, located along the Apennine Mountain range, is mainly dominated by carbonate formations that generate a  $^{87}Sr/^{86}Sr$  signal relatively homogeneous in Umbria ranging from 0.7080 to 0.7089, as defined by soil samples. The  $^{87}Sr/^{86}Sr$  data show 4 of 28 individuals outside the bioavailable strontium isotope baseline (Fig. 7). 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 mtDNA and PCA results. Further analysis will be necessary to understand migration patterns and determine whether cultural factors influenced mobility.

## 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. 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 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 with better-known populations such as the Etruscans and the Picenes, contributing to a broader picture of cultural dynamics in pre-Roman Central Italy.

## REFERENCES

## ACKNOWLEDGEMENTS

[1] Weidig, J. (2015). Studi sulla necropoli orientalizzante di Spoleto, Piazza d'Armi. Una visione preliminare. In Sui due versanti dell'Appennino. Necropoli e distretti culturali tra VII e VI sec.a.C., Gilotta F. and Tagliamonte G., eds. (Roma: Giorgio Bretschneider Editore); [2] Weidig, J. (2021). Lutto, rito funerario e status sociale. Considerazioni sulle sepolture infantili in Umbria e nelle aree limitrofe dalla prima età del Ferro all'epoca arcaica. In BIRTH. Archeologia dell'infanzia nell'Italia preromana. Govi E. eds. (Bologna: Bononia University Press); [3] Dabney, J., et al. (2013). Proc. Natl. Acad. Sci. 110:15758-63; [4] Meyer M, and Kircher M. (2010). Cold Spring Harb Protoc., 2010: 5448; [5] Jónsson, H., et al. (2013). Bioinformatics, 29: 1682-1684; [6] Fu, Q., et al. (2013). Curr. Biol., 23: 553-559; [7] van Oven, M. (2015). Forens. Sci. Int. Gen., 5: 392-394; [8] Skoglund, P., et al. (2013). J. Archaeol. Sci., 40: 4477-4482; [9] Patterson, N., et al. (2006). PLoS Genet., 2: e190; [10] Brown, T. A., et al. (1988). Radiocarbon, 30: 171-177; [11] Marsteller, S.J., et al. (2017). J. Archaeol. Sci., 13: 535-546; [12] Rohland, N., et al. (2022). Genome Res., 32: 2068-2078; [13] Ravasini, F., et al. (2024). Genome Biol., 25:292; [14] Van Klinken, G. J. (1999). J. Archaeol. Sci., 26: 687-695; [15] Acosta, A. N., et al. (2019). J. Archaeol. Sci., 27: 101962; [16] Triozzi, B. (2021). Ph.D. Thesis (Sheffield, UK: University of Sheffield); [17] Varalli, A., et al. (2016). Int. J. Osteoarchaeol., 26: 431-446; [18] Esposito, C., et al. (2023). Sci. Rep., 13: 3632; [19] Icaro, I., et al. (2023) Biology (Basel), 12: 1382.

This research is funded by MUR "PRIN 2022 PNRR- Prot. P2022LATB9, "Powerful women and outstanding children in Pre-Roman Umbria: Understanding inclusive societies with foreign peoples through an integrative approach of molecular anthropology, archaeology and virtual design". The authors gratefully acknowledge the financial support of Regione Lazio through IS15@MACH ITALIA Research Infrastructure.