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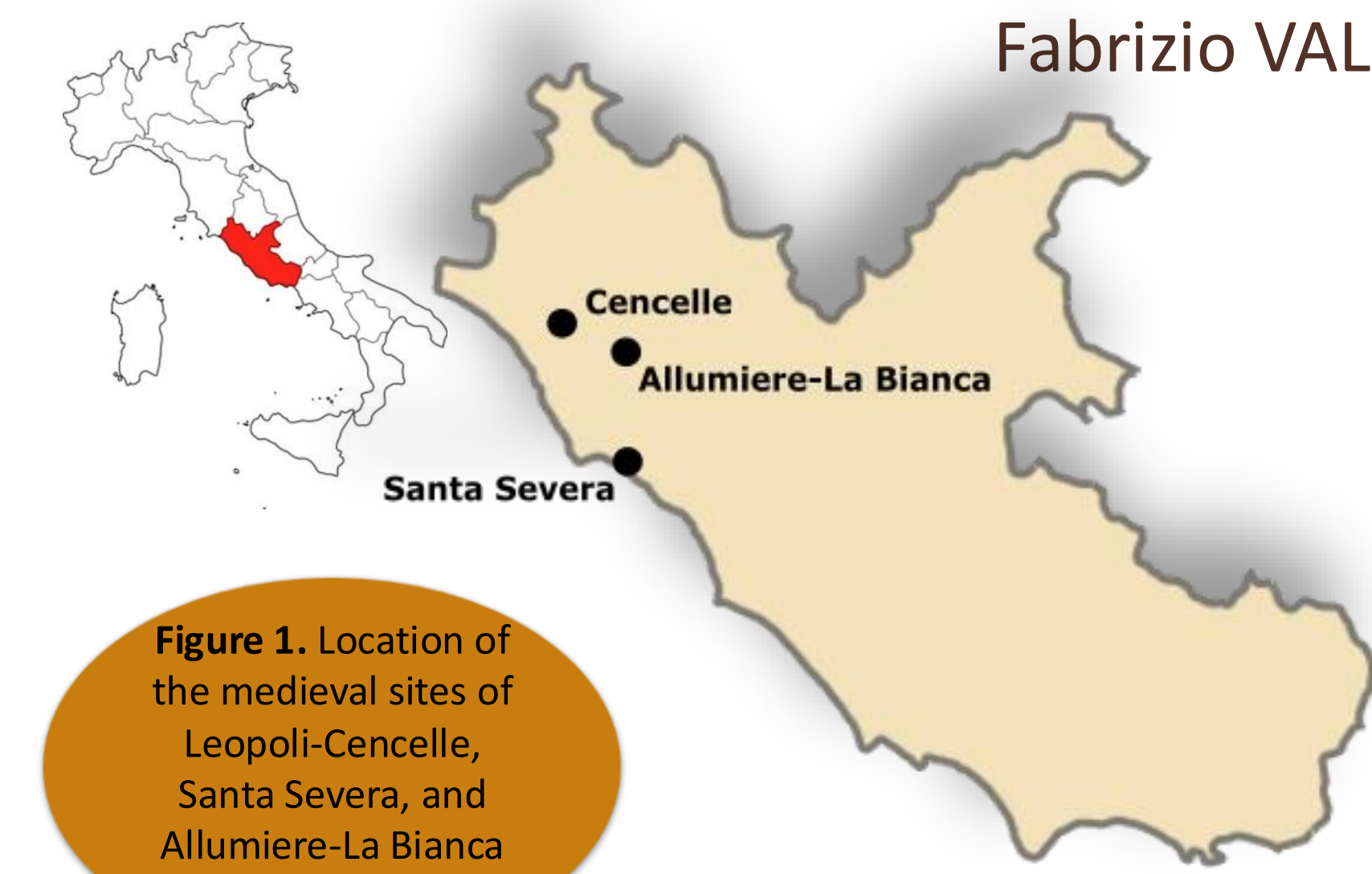


Figure 1. Location of the medieval sites of Leopoli-Cencelle, Santa Severa, and Allumiere-La Bianca in northern Latium.

1. BACKGROUND

Medieval central-northern Latium (**Fig. 1**) represents a key area for investigating population dynamics between the Early and Late Middle Ages. The region was characterized by settlement continuity, territorial reorganization, economic specialization, and different forms of mobility. This study focuses on three medieval communities with distinct archaeological and socio-economic profiles: the coastal settlement of Santa Severa, the mining-related community of Allumiere-La Bianca, and the papal foundation town of Leopoli-Cencelle.

2. ARCHAEOLOGICAL CONTEXTS

Santa Severa

A coastal settlement located along the Tyrrhenian coast [1]. (**Fig. 2**), represented by individuals from Casa del Nostromo and Piazza della Rocca.



Figure 2. Aerial view of Santa Severa Castle and its medieval coastal settlement.

Allumiere-La Bianca

A late medieval mining-related community connected to alum extraction in the Tolfa Mountains. (**Fig. 3**) [2].



Figure 3. Aerial view of the Allumiere-La Bianca archaeological site

Leopoli-Cencelle

A medieval papal foundation town established in the 9th century CE and occupied until the Late Middle Ages. (**Fig. 4**) [3].



Figure 4. Aerial view of the Sector VI-VII funerary area at Leopoli-Cencelle, including the basilica/cathedral complex and the Early and Late Medieval cemeteries.

3. MATERIALS AND METHODS

A total of **82 individuals** from three medieval burial contexts in central-northern Latium were selected for paleogenomic screening: **32 from Leopoli-Cencelle**, **30 from Santa Severa**, and **20 from Allumiere-La Bianca**.

Samples were selected according to skeletal preservation and the availability of anatomical elements with high ancient DNA preservation potential, primarily **petrous bones** and **teeth** with vertebral bodies sampled in non-adult individuals (**Fig. 5**).



Figure 5. Skeletal elements sampled for ancient DNA analysis: petrous bone, tooth, and vertebral body. Scale bars are in centimetres.

Ancient DNA extraction [4] and library preparation [5] were performed in dedicated clean laboratory facilities. Sequenced libraries were evaluated for endogenous DNA content, *post-mortem* damage patterns, and contamination estimates. (**Fig. 6**).



Figure 6. Schematic workflow of the ancient DNA analysis, including sampling, DNA extraction, library preparation, NGS sequencing, and bioinformatic processing.

Following quality filtering (endogenous DNA >5%, characteristic terminal C→T substitution patterns, and estimated contamination <5%), 36 samples were retained for downstream analyses (**15 from Leopoli-Cencelle**, **15 from Santa Severa**, and **6 from Allumiere-La Bianca**).

5. DISCUSSION

Despite their different settlement histories and economic backgrounds, **the three communities display a broadly shared genetic profile**. The PCA suggests that regional continuity played a major role in shaping the genetic structure of these populations.

The absence of marked genetic differentiation between the sites may indicate that mobility and demographic exchanges occurred mainly within a shared central Italian gene pool. The slight variation observed in **Allumiere-La Bianca** could reflect local demographic dynamics associated with the mining landscape and will require further investigation using **higher-resolution genomic data**.

Genetic characterization

Molecular sex determination identified **16 females** and **20 males** among the 36 individuals analysed. (**Fig. 7**) All individuals from Allumiere-La Bianca were assigned as male, consistent with the interpretation of the site as a mining-related community. [2].

	Female	Male	N
Cencelle (Early and Late Middle Ages)	9	6	15
Santa Severa - Casa del Nostromo (Early Middle Ages)	6	5	11
Santa Severa - Piazza della Rocca (Late Middle Ages)	1	3	4
Allumiere - La Bianca (Late Middle Ages)	0	6	6
	16 females	20 males	

Figure 7. Molecular sex determination of the 36 individuals analysed. Females are shown in pink and males in blue.

Preliminary uniparental marker analyses indicate mitochondrial lineages commonly observed in European and Mediterranean populations, including **H, J, T, U**, and **K**. Y-chromosome lineages also fall within the range commonly observed in European populations.

Principal Component Analysis (PCA)

PCA was performed using the **Human Origins** dataset and the smartpca algorithm [6], with comparison to published ancient individuals from the **Allen Ancient DNA Resource (AADR)** [7] and following previous archaeogenomic studies on ancient Italian populations [8]. **PCA** revealed substantial **genetic overlap** among individuals from the three sites (**Fig. 8**). Santa Severa and Leopoli-Cencelle cluster closely with previously published medieval populations from central Italy, while Allumiere-La Bianca shows a slight displacement within the same regional genetic landscape. Overall, the results indicate **broad genetic homogeneity** across the three communities.

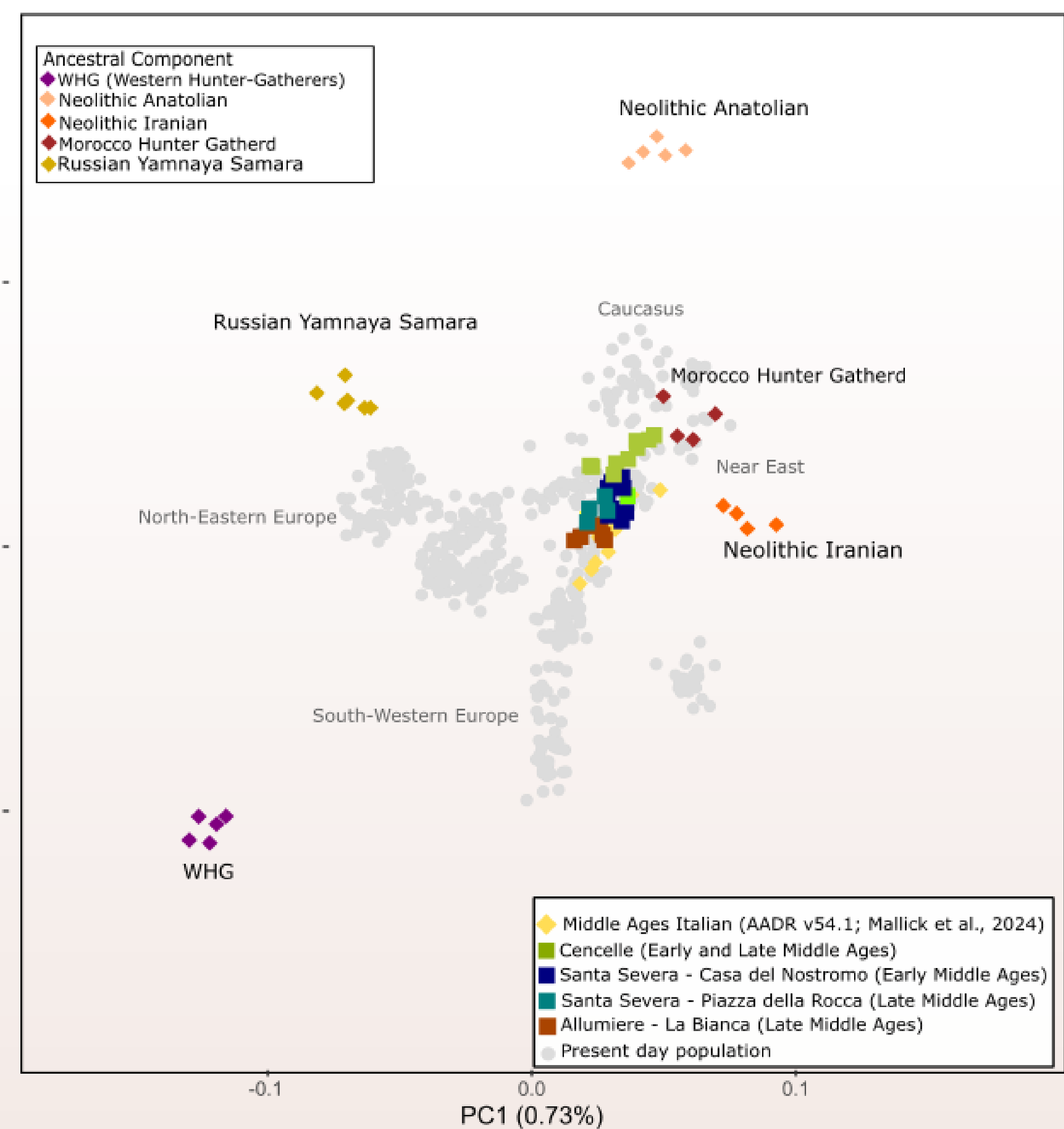


Figure 8. Principal Component Analysis (PCA) of individuals from the three medieval sites projected onto modern populations from the Human Origins dataset. Grey dots represent present-day populations, colored diamonds indicate ancestral reference populations (Western Hunter-Gatherers, Anatolian Neolithic, Iranian Neolithic, Morocco Hunter-Gatherers, and Russian Yamnaya Samara), yellow diamonds represent published medieval Italian individuals from the Allen Ancient DNA Resource (AADR v54.1) [7], and colored squares represent the individuals analysed in this study. PC1 and PC2 are shown with the percentage of explained variance.

6. CONCLUSIONS

This preliminary paleogenomic study supports a pattern of broad **regional genetic homogeneity** among the three medieval communities, despite their different settlement and socio-economic contexts.

Paleogenomic data provide a valuable tool for investigating **biological structure, mobility, and population dynamics** in medieval central Italy.

7. FUTURE PERSPECTIVES

Future analyses will include **additional samples, higher-resolution genomic data**, and further population-genetic approaches to better investigate **mobility, biological continuity, and fine-scale population structure**.

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7. REFERENCES

- Gnes M. et al. Bioarchaeological approach to the study of the medieval population of Santa Severa (Rome, 7th–15th centuries). *Journal of Archaeological Science: Reports*, 18:11–25, 2018.
- Baldoni M. et al. Who were the miners of Allumiere? A multidisciplinary approach to reconstruct the osteobiography of an Italian worker community. *PLoS ONE*, 13:e0205362, 2018.
- Baldoni M. et al. Leopoli-Cencelle (9th–15th centuries CE), a centre of Papal foundation: bioarchaeological analysis of the skeletal remains of its inhabitants. *Annals of Human Biology*, 47(6):522–540, 2020.
- Dabney J. et al. Complete mitochondrial genome sequence of a Middle Pleistocene cave bear reconstructed from ultrashort DNA fragments. *Proceedings of the National Academy of Sciences USA*, 110(39):15758–15763, 2013.
- Meyer M., Kircher M. Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harbor Protocols*, 2010(6):pdb.prot5448, 2010.
- Patterson N. et al. Population Structure and Eigenanalysis. *PLoS Genetics*, 2:e190, 2006.
- Mallik S. et al. The Allen Ancient DNA Resource (AADR): a curated compendium of ancient human genomes. *Scientific Data*, 11, 2024.
- Antonio M. et al. Ancient Rome: A genetic crossroads of Europe and the Mediterranean.