

## Research Proposal

### **PALEOGENOMIC INSIGHTS INTO A MEDIEVAL HUMAN SETTLEMENT: THE COMMUNITY OF SANTA SEVERA (ROME, 7<sup>th</sup>–15<sup>th</sup> CENTURY AD)**

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State of art and previous studies: The present study focuses on the Medieval population of Santa Severa (Rome, Italy), a small town 50 km north of Rome. The Castle of Santa Severa was built during the Medieval period on the site of the ancient settlement of *Pyrgi*. Archaeological evidence suggests that *Pyrgi* was active since the Neolithic. During the Etruscan period, *Pyrgi* was noted as one of the most important harbours of ancient *Caere*, currently Cerveteri, which handled a Mediterranean-wide scale trade and was frequented by Greek and Phoenician navigators (Enei, 2013). The Medieval settlement changed the name of the village itself to Santa Severa in honor of the saint's martyrdom (Gnes et al., 2018). The skeletal series is composed of 455 individuals. The research group of Forensic Anthropology and Skeletal Biology laboratory of the University of Rome Tor Vergata has conducted a morphological analysis by estimated sex, age at death, stature, and life conditions. Occupational stress markers, skeletal and dental diseases, and dietary pattern have also been investigated, revealing evidence of periostitis and arthrosis as a manifestation of infectious and degenerative diseases (Gnes et al., 2018, Gismondi et al., 2020).

Until now few studies shed light on Italian Medieval populations through biomolecular analysis, so it would be interesting to characterize the genetic profile of one of the largest Italian Medieval populations. The ability to extract and sequence ancient DNA from skeletal remains (in particular petrous bones and teeth) makes it possible to reconstruct ancient genetic ancestries, investigate the genetic structure of the population, identify the geographic provenance of individuals, their sex and potential kinship relationships with other individuals buried in the same context (Schroeder et al., 2019, Gomes et al., 2020, Vai et al., 2020, Scorrano et al., 2022) not only to address questions about the social organization of ancient groups (Fernandes et al., 2021), but also to analyze the genetic ancestry of the population.

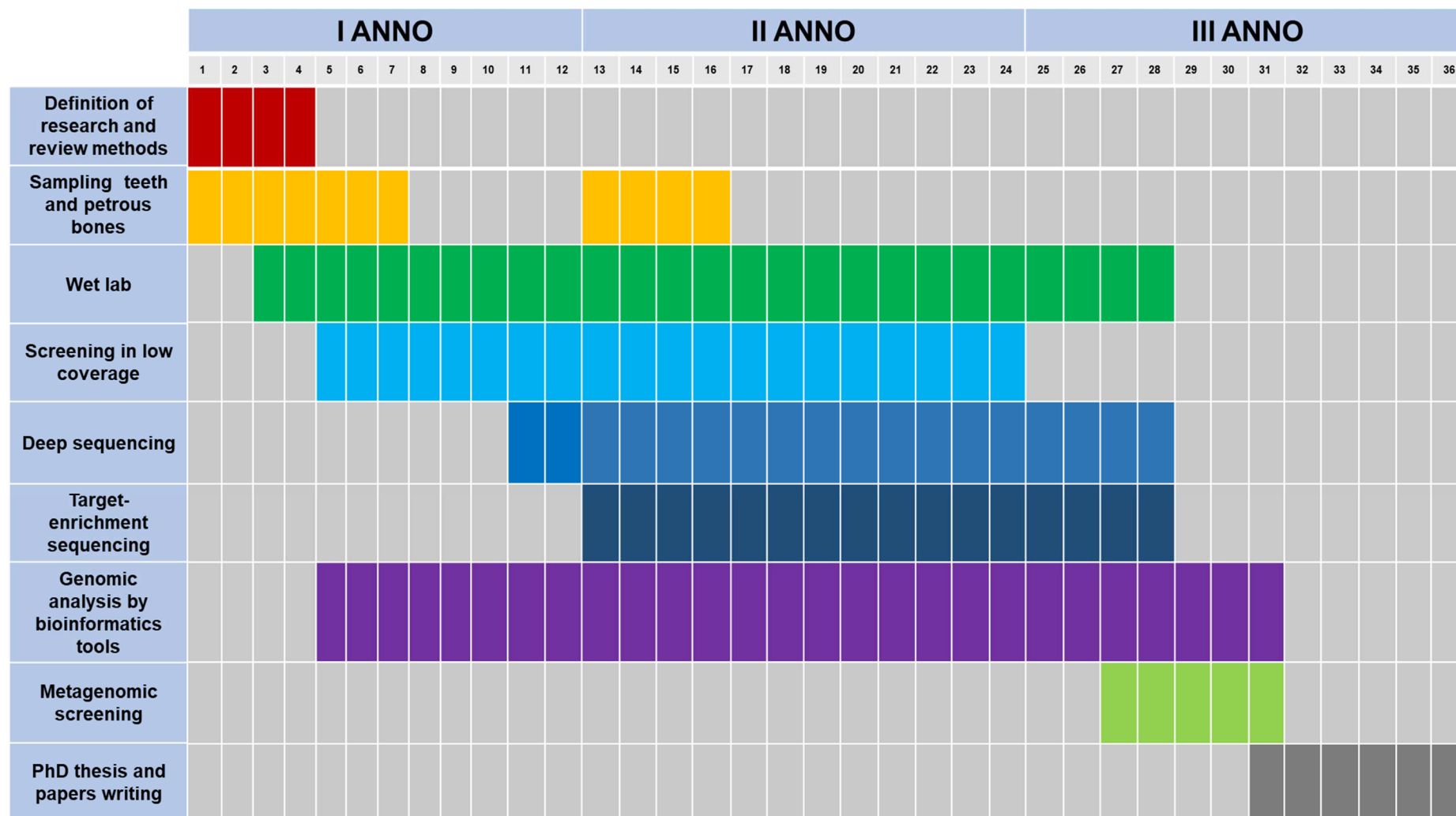
Previous studies reconstructed nutritional and medicinal habits through isotope analysis from bone proteins and investigations on dental calculus. An interesting aspect that has emerged from these analyses is the use of artemisinin presenting antimalarial properties (Desrosiers et al., 2016, Gismondi et al., 2020). The malaria hypothesis in Central Italy could be supported by records of swampy areas surrounding the Santa Severa Castle during the Medieval period (Fatucci and Cerilli, 2016), a condition which favoured the survival of the *Plasmodium falciparum*. A paleopathological and metagenomic screening will also be carried out to investigate the presence of pathogens and health status (Mbouaka et al., 2021, Scorrano et al., 2022). Some of the Medieval samples of Santa Severa are included in the PRIN2017 Project (20177PJ9XF), during which were conducted genomic analysis and strontium isotope analysis to obtain information on the mobility. Feasibility of the project is supported by the yields of endogenous DNA observed so far up to 59%.

Research Question: Genomic analysis of skeletal remains is essential for reconstructing genetic structure, kinship, and geographic origin. The archaeological examination highlighted that a few sarcophagi were reused for multiple burials (Enei, 2013). It would be interesting to understand if the spatial distribution of the burials in the cemetery areas is associated with any kinship relationship among the individuals. Also, according to bioarchaeological and archaeobotanical evidence, it would be interesting to evaluate the health status of one of the largest Italian Medieval populations to provide interesting information about the presence of disease and the diffusion of pathologies.

Objective and Aims: This research aims at investigating through paleogenomic analysis human remains from the Medieval population of Santa Severa (RM) recovered during the archaeological excavation at two cemetery areas known as “Casa del Nostromo” and “Piazza della Rocca”. The skeletal collection that will be analyzed in this project is housed at Museo Civico di Santa Marinella “Museo del Mare e della Navigazione Antica”, Castello di Santa Severa (Roma–Italia; Responsible: Dr. Flavio Enei).

Research Methods: Ancient DNA (aDNA) will be extracted from teeth and petrous bone samples (Saag et al., 2017, Saupe et al., 2021). The DNA extracted will be converted into Illumina double-stranded libraries, as described in Meyer and Kircher (2010), and shotgun sequenced on NovaSeq 6000Dx. DNA extraction and library preparation will be conducted in dedicated clean laboratory at the Center of Molecular Anthropology for Ancient DNA Studies, Villa Mondragone (Monte Porzio Catone, Roma). The reads will be aligned to the reference genome using BWA (Li and Durbin, 2009, Li et al., 2009). The sequences will be subjected to strict quality control for contamination (Korneliussen et al., 2013) and to authentication of the reads aligned to assess the *post-mortem* damage through mapDamage 2.0 (Ginolhac et al., 2011, Jonsson et al., 2013). After an initial screening in low coverage, samples that will have an endogenous DNA greater than 15 % will be deep sequenced, while for intermediate samples could be done an enrichment using myBaits Expert Human Affinities Complete Kit. This target set includes 1.240.000 SNP sites (Mathieson et al., 2015) useful to determine genetic ancestry and kinship estimation (Gretzinger et al., 2022). Target-enrichment approach could be useful to focus sequencing efforts on library content of particular interest (Orlando et al., 2021), such as pathological markers and SNPs useful for predicting external phenotypes (Gomes et al., 2020, Villalba-Mouco et al., 2021, Bardan et al., 2023). A metagenomic screening on shotgun sequence will be done to understand infectious human disease (Wood and Salzberg, 2014, Truong et al., 2015, Orlando et al., 2021).

Gantt Chart of the project:



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CITTÀ DI SANTA MARINELLA  
POLO MUSEALE CIVICO  
MUSEO DEL MARE E DELLA NAVIGAZIONE ANTICA  
MUSEO DEL CASTELLO DI SANTA SEVERA

**Alla Scuola di Dottorato  
di Biologia Evoluzionistica e Ecologia**  
Università degli Studi di Roma "Tor Vergata"  
Roma

Sua sede

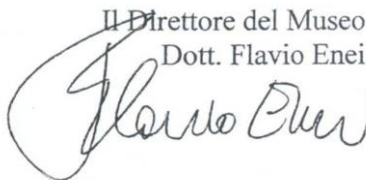
Santa Severa 16.6.2023

Prot. 1006/23

Oggetto: parere favorevole e supporto archeologico alla ricerca "Paleogenomic insights into a medieval settlement – the community of Santa Severa (rome, 7th–15th centuries)"

Il sottoscritto Dott. Flavio Enei, Direttore Polo Museale Civico del Castello di Santa Severa, "Museo del Mare e della Navigazione Antica" e "Museo del Castello di Santa Severa" responsabile della collezione dei resti scheletrici rinvenuti presso il Castello di Santa Severa e conservata presso il Museo del Castello di Santa Severa (Roma-Italia) con la presente esprimo un parere favorevole allo svolgimento della ricerca e do il mio supporto archeologico al progetto di dottorato di ricerca dal titolo "PALEOGENOMIC INSIGHTS INTO A MEDIEVAL SETTLEMENT – THE COMMUNITY OF SANTA SEVERA (ROME, 7th–15th CENTURIES)" che la studentessa Orefice Giulia vuole presentare presso l'Università degli Studi di Roma "Tor Vergata". Tale progetto, sotto la direzione della prof.ssa M. Cristina Martinez-Labarga, è destinato ad approfondire gli studi di paleogenomica dei resti scheletrici della popolazione medievale di Santa Severa (RM) e ad indagare la struttura genetica, i rapporti di parentela e l'origine biogeografica del campione in esame.

Cordiali Saluti

Il Direttore del Museo  
Dott. Flavio Enei  


Roma, 22 giugno 2023

### A CHI DI COMPETENZA

La Dott.ssa Giulia Orefice, attualmente frequenta il Centro di Antropologia molecolare per lo studio del DNA antico del Dipartimento di Biologia dell'Università di Roma "Tor Vergata". La dottoressa Orefice è risultata vincitrice di una borsa di studio dal titolo "*ALUM Archeoantropologia molecolare e gamification per riscoprire la storia della produzione dell'allume*" progetto finanziato dal MUR 113/91 (PANN20\_00370) di cui ero responsabile. In questo periodo ha svolto l'estrazione di DNA da campioni archeologici, ha preparato le librerie genomiche e analizzato con tool bioinformatici i dati di sequenziamento al fine di fornire informazioni sulla popolazione medievale di Allumiere.

La dottoressa vorrebbe proseguire e svolgere nell'ambito del dottorato una ricerca dal titolo: "PALEOGENOMIC INSIGHTS INTO A MEDIEVAL HUMAN SETTLEMENT: THE COMMUNITY OF SANTA SEVERA (ROME, 7<sup>th</sup>–15<sup>th</sup> CENTURY AD)" sotto la mia supervisione.

L'impressione che mi sono fatta della Dott.ssa Orefice, nei mesi di tirocinio sotto la direzione della prof.ssa Rickards e in questi mesi di borsa di studio, mi spingono a esprimere nei suoi confronti un giudizio favorevole, sottolineando in particolar modo le sue doti di persona estremamente valida e autonoma, matura per assumere impegni di ricerca, e pertanto idonea per essere inserita in un dottorato di ricerca e/o laboratorio della comunità scientifica.

In fede,



Cristina Martínez-Labarga  
Prof. Associato di Antropologia