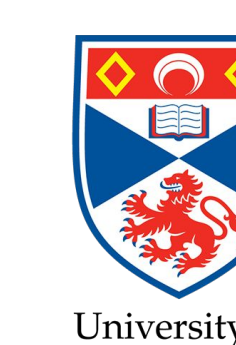


# Combining biomolecular, osteological analysis and historical research for the characterization of a post-medieval monastic community in northern Italy

Sarah Sandron<sup>1</sup>, Cynthia Spiteri<sup>1</sup>, Alessandra Cinti<sup>1</sup>, Emanuele Grotto Maffiotti<sup>1</sup>, Alfredo Santovito<sup>2</sup>, Dominique Scalarone<sup>3</sup>, Meaghan Mackie<sup>1,4,5,6</sup>, Alison Beach<sup>7</sup>, Anita Radini<sup>6</sup>, Beatrice Demarchi<sup>1</sup>, Rosa Boano<sup>1</sup>



<sup>1</sup> ArchaeoBiomics, Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy; <sup>2</sup> Microscopy Laboratory, Genetics and Molecular Zoology group, Department of Life Sciences and Systems Biology, University of Turin, Turin, Italy; <sup>3</sup> Department of Chemistry, University of Turin, Turin, Italy; <sup>4</sup> The Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; <sup>5</sup> The Globe Institute, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; <sup>6</sup> University College Dublin, School of Archaeology, Belfield, Dublin, Ireland; <sup>7</sup> Università di St. Andrews, St. Andrews, United Kingdom



## INTRODUCTION

In the past, religious women were often left out from historical-archaeological narratives, typically focussed on males. Today, interdisciplinary projects are beginning to integrate historical research and cutting-edge human remains analysis to understand female agency in premodern Europe monasticism<sup>1</sup>.

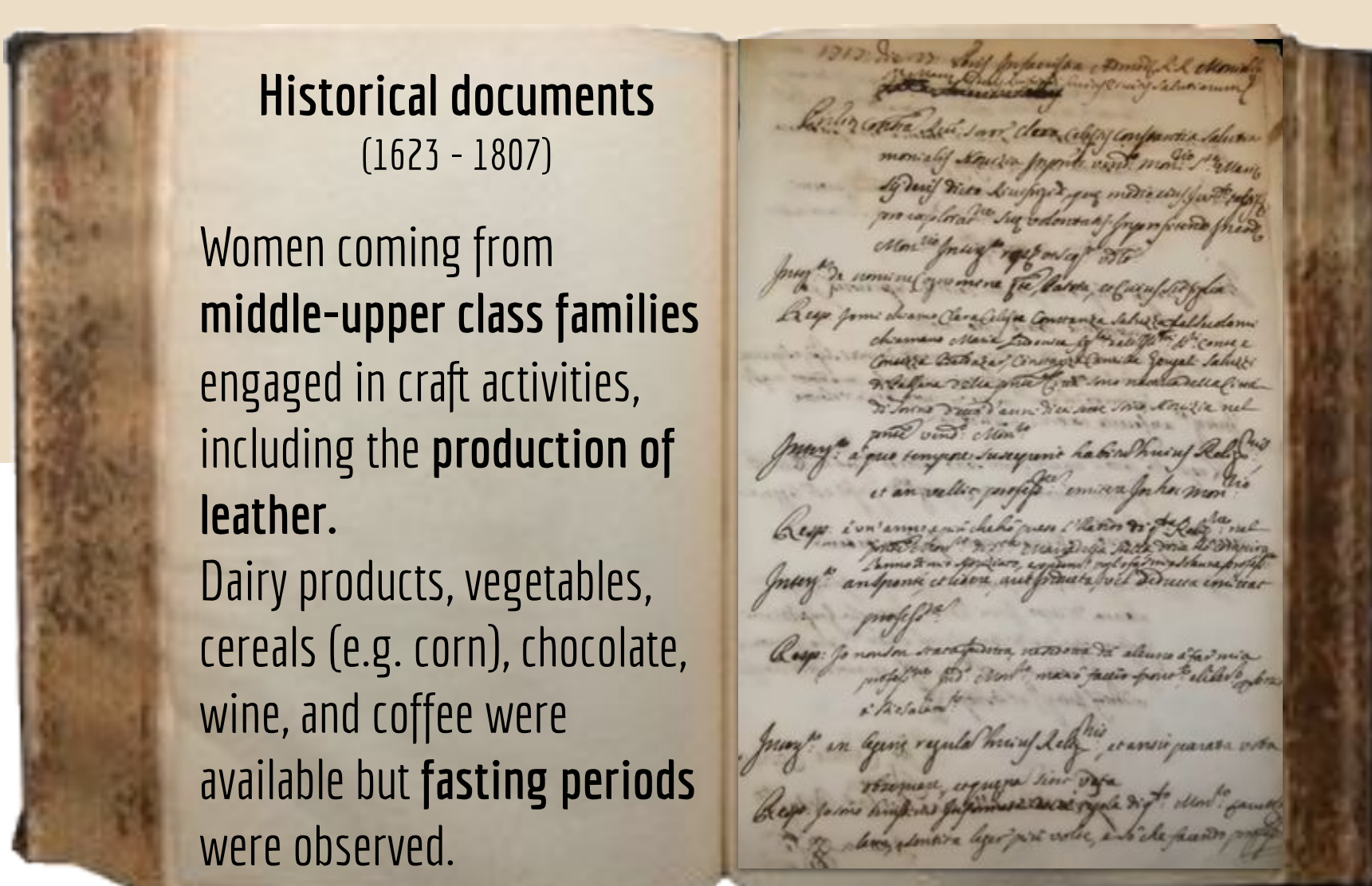


Fig. 1 - Historical data from S. Maria della Stella's archival documents

The female Cistercian cloistered community of Santa Maria della Stella (Saluzzo, Italy) left abundant written documentation (fig.1). We are combining this with bioarchaeological data collected from 48 burials from the 18th century, to produce individual **osteobiographies** and to reconstruct "hidden" aspects of the nuns' lifeways. According to the Rule of St Benedict, *ora et labora* (pray and work) should govern monastic life.

## METHODS

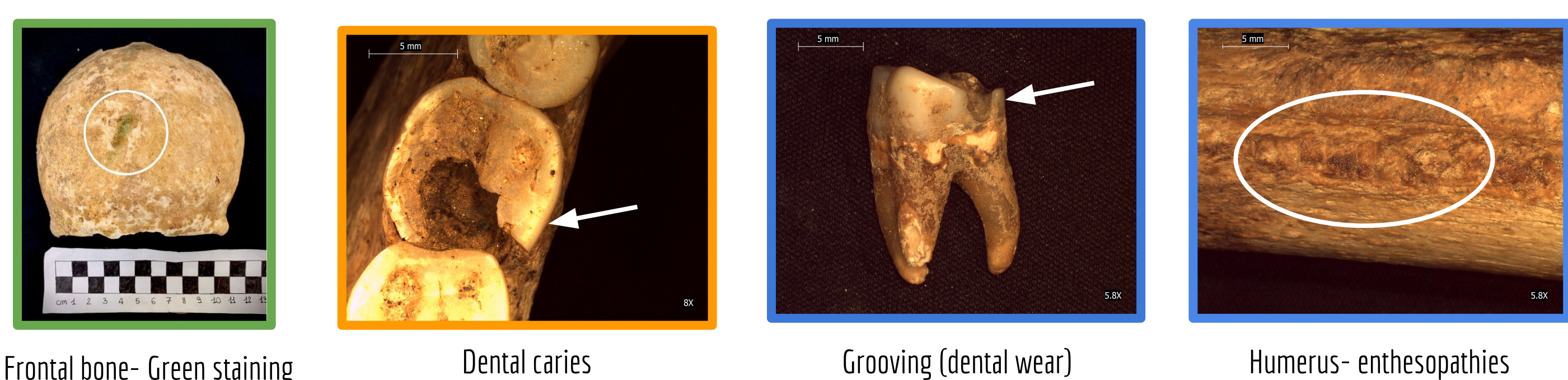
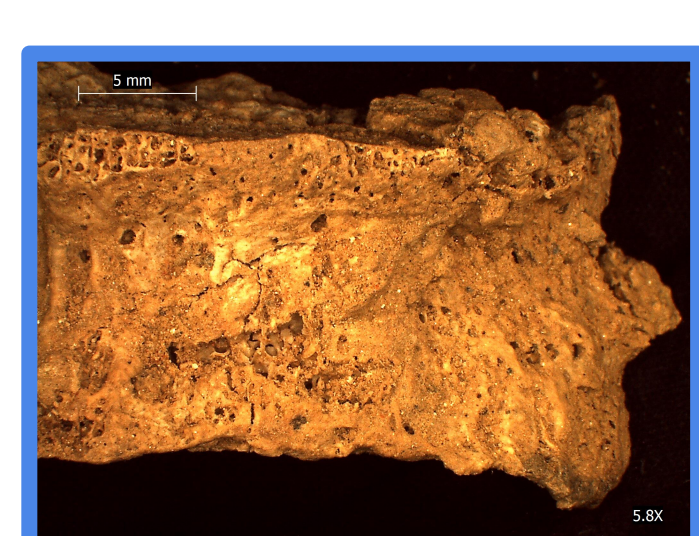
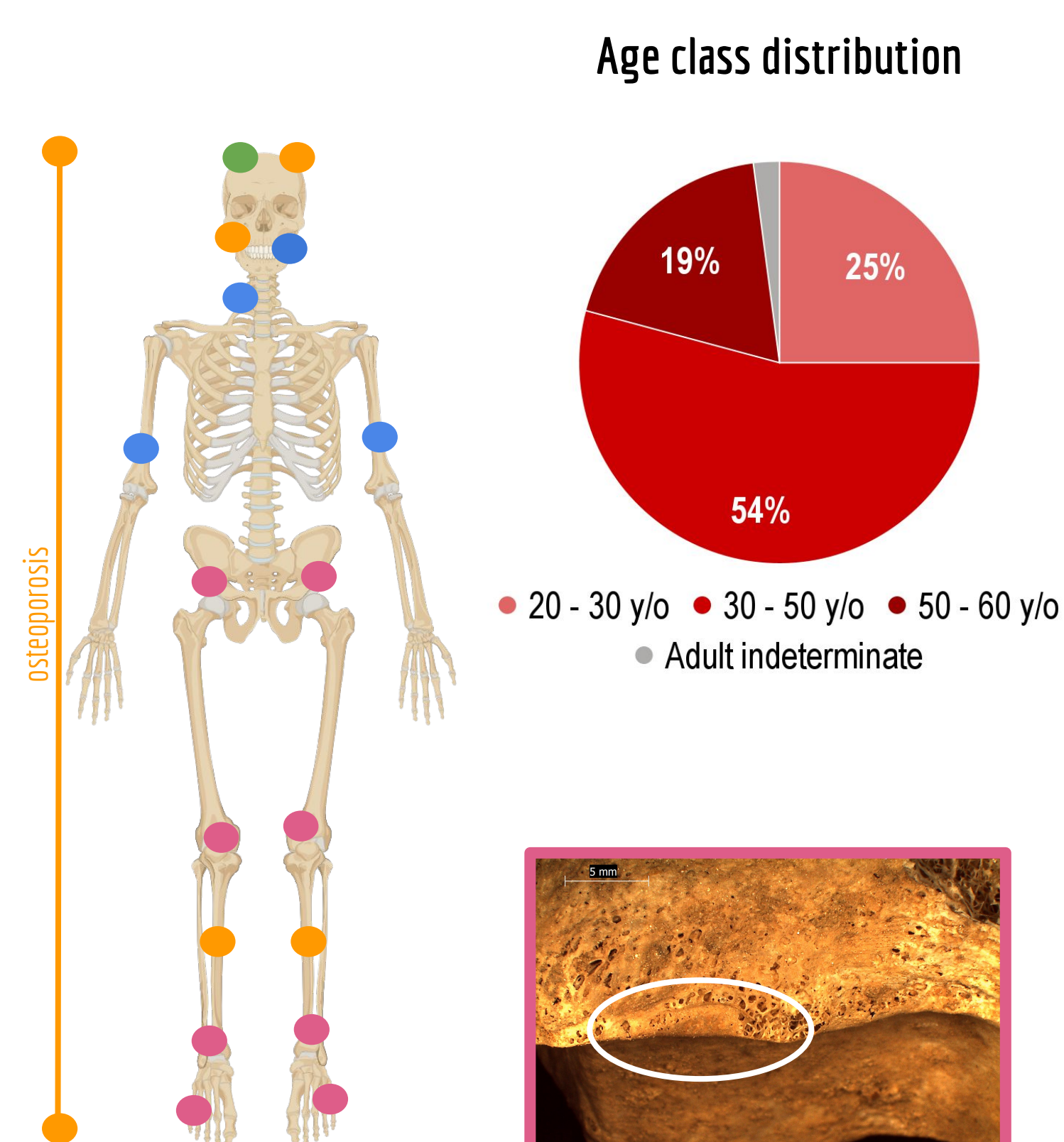
Osteological analyses were conducted on the 48 individuals in order to determine biological sex, age class, presence of pathologies or any activity-related markers. Dental calculus was analysed in order to characterise micro-débris (12 samples - micromorphology<sup>2</sup> by polarized transmitted light microscopy) and to recover ancient proteins (7 samples, SP3 protocol, LC-MS/MS<sup>3</sup>) & ancient lipids (10 samples by TD/Py-GC-MS).



## OSTEOLOGY

### ORA ET LABORA: Markers

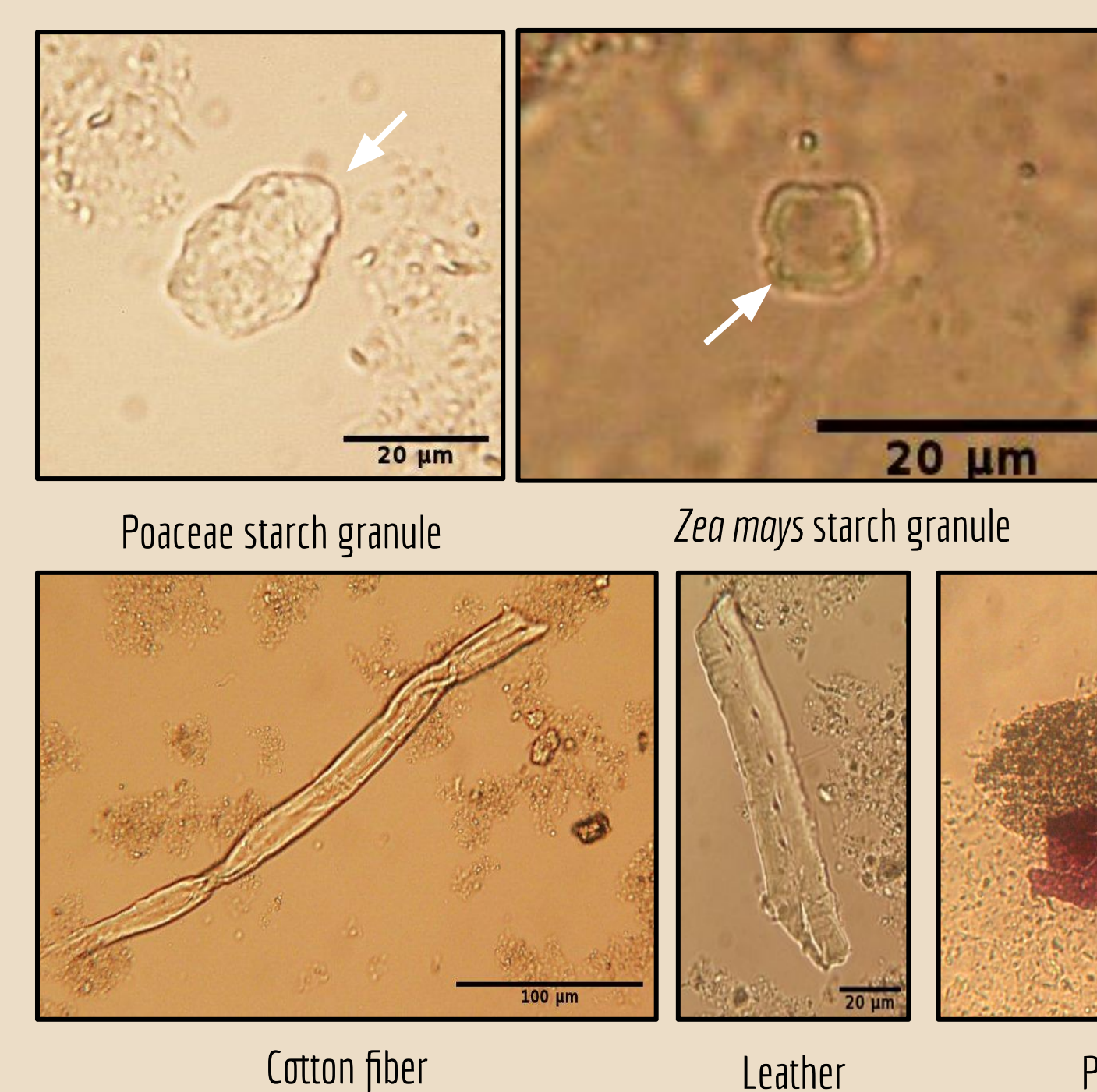
- **Religious activities**
  - Knee arthropathy
  - Kneeling markers<sup>4-6</sup> (tibia, metatarsal)
  - Enthesopathies (pelvis)
- **Other labour**
  - Articular degenerative disease cervical (vertebrae)
  - Enthesopathies (upper limbs)
  - Dental wear
- **Health**
  - Diffuse bone mass reduction: hypothetical osteoporosis in all age classes (skeletal metabolic disease)<sup>7</sup>
  - Diploe hyperplasia (skull)
  - Periosteal reaction (lower limbs)
  - Dental caries, intra-vitam loss of teeth, alveolar retraction
- **Taphonomic alteration** - contact with hair-veil pins



## DENTAL CALCULUS ANALYSIS

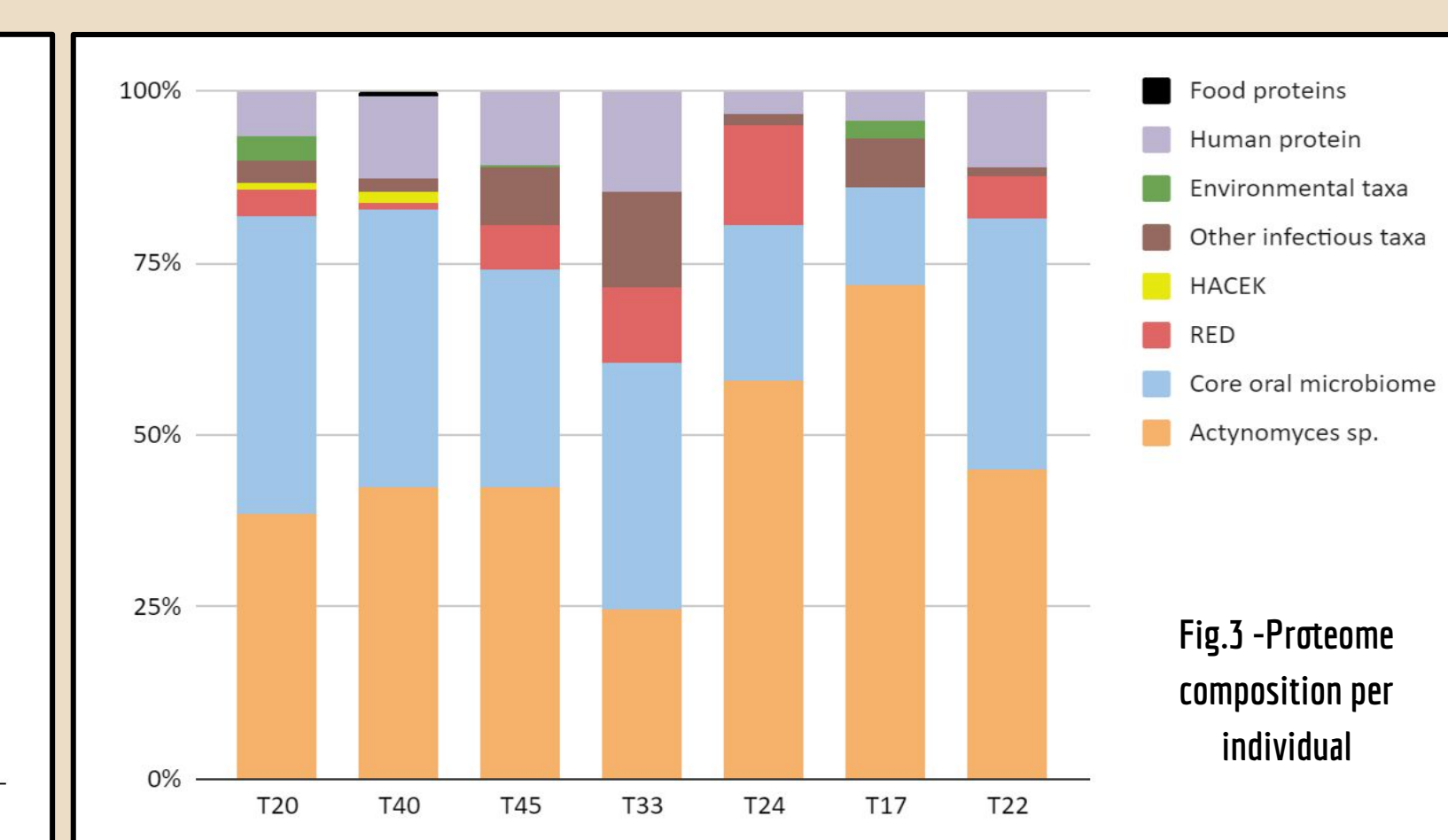
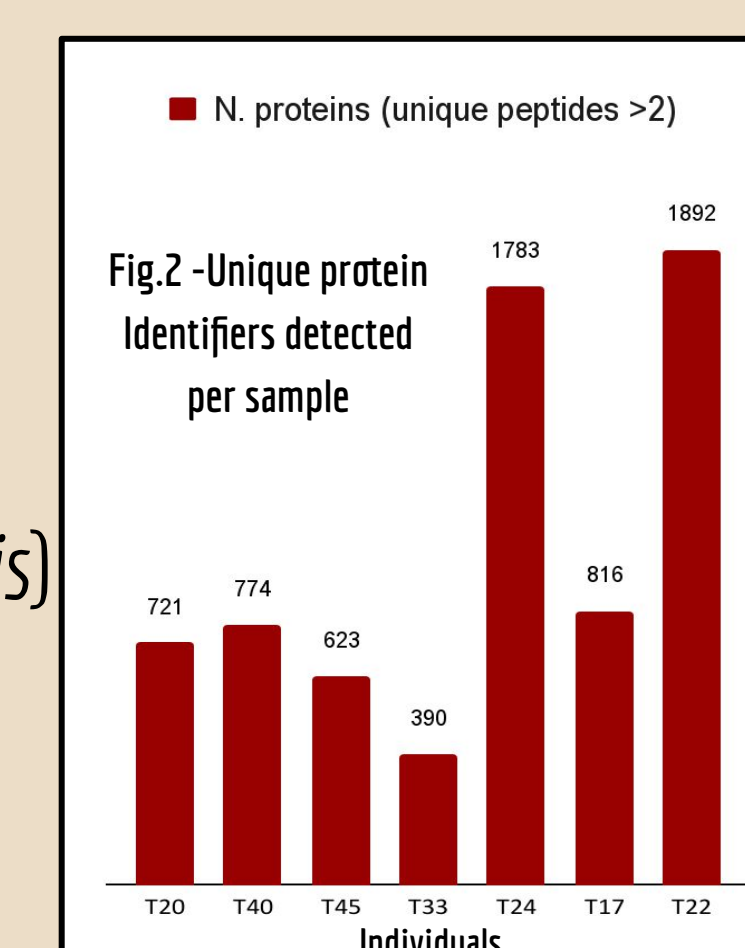
60 micro-débris morphotypes (animal, mineral, plant), were documented. These were compared to a reference collection and some could be identified as:

- Starch granules - **Poaceae** and **Fabaceae**
- Starch granules and phytoliths of corn, *Zea mays* (imported from the New World, typical in the area today)
- Textile fibers (e.g. cotton), leather (?)
- Natural purple colorant (?)



TD/PY-GC-MS biomarker analysis **did not detect evidence for chocolate**, namely theobromine, theophylline and caffeine, in the dental calculus, possibly due to low consumption of chocolate.

Ancient proteins (fig.2) were mainly assigned to **core oral microbiome taxa**, e.g. *Actinomyces sp.* (fig.3). **β-lactoglobulin** (Bovinae/Ovis) was found in only one sample (T40). Most individuals yielded evidence of **Red Complex**



**bacteria** (periodontal disease), and two (T20 and T40) of taxa from the **HACEK group**, potentially related to infective endocarditis<sup>8</sup>. Many human proteins detected are linked to the **host defense** (e.g. myeloperoxidase), consistent with the poor health conditions of the monastic women. Interestingly, **zinc-α-2-glycoprotein** (T40) is a lipid and glucose-metabolism protein which is overexpressed in patients with cachexia<sup>9</sup> - we are investigating if this could be related to fasting.

## DISCUSSION

Morphological signs of **malnutrition** (fasting?) combined with the lack of dietary proteins in dental calculus may reflect a **poor diet**, even if the women had access to "exotic" and nutritious foods (e.g. chocolate, corn), as reported in historical texts. The micromorphological analysis of dental calculus also confirms the consumption of **cereals** and **legumes**.

Osteological markers reflect **spiritual** (kneeling) as well as **manual labour** (biomechanical disorders). Dental wear and micro-débris possibly related to leather and textiles suggest **craft activities**, as testified by the written documents. No significant differences between age groups were found, indicating that all were involved in religious-occupational activities, typical of Cistercian "equal labour" rules.

Dental calculus proteins related to the host immune response as well as the frequency of pathogenic bacteria support general **dysbiosis**, with diffuse **periodontal infections**, reflected by osteological data.



## CONCLUSIONS

A comprehensive osteological assessment, combined with micromorphology and biomolecular techniques applied to dental calculus, has given us glimpses of the **social-economic role** of the monastery and of the **impact of cloistered life on women's health**.

- Overall good congruence between archival documents and bioarchaeological data **supports the application of osteology and biomolecular archaeology** to case studies where textual information is not available.
- Bioarchaeology revealed the presence of diseases not mentioned in the texts.
- Ongoing work: lipid analysis for detecting plant-based foodstuffs in dental calculus and aDNA analysis to reveal kinship in the monastery and to investigate the presence of genetic diseases detected osteologically.

## REFERENCES

1 - Radini, A. et al., 2019. Medieval women's early involvement in manuscript production suggested by lapis lazuli identification in dental calculus. *Science Advances* 5.1: eaau7126 ; 2 - Cristiani, E. et al., 2016. Dental calculus reveals Mesolithic foragers in the Balkans consumed domesticated plant foods. *Proceedings of the National Academy of Sciences*, 113(37), pp. 10298–10303 ; 3 - Wilkin, S. et al., 2021. SP3 (Single-Pot, Solid-Phase, Sample-Preparation) Protein Extraction for Dental Calculus. *protocols.io* ; 4 - Sarkic, N., 2017. A study of the lifestyle and health parameters of nuns from convents on the Iberian Peninsula in modern times. PhD dissertation ; 5 - Ubelaker, D.H., 1979. Skeletal evidence for kneeling in prehistoric Ecuador. *Am. J. Phys. Anthropol.*: 51, 679–685 ; 6 - Iscan, M.Y., Kennedy, K.A.R., 1989. Reconstruction of life from the skeleton. Alan R. Liss, New York ; 7 - Curate, F., 2014. Osteoporosis and paleopathology: a review. *Journal of Anthropological Sciences*: 1–28. ; Khaledi, M. et al., 2022. Infective endocarditis by HACEK: a review. *J Cardiothorac Surg* 17, 185. ; Hassan MI, et al., 2008. Zinc alpha 2-glycoprotein: a multidisciplinary protein. *Mol Cancer Res. Jun*;6(6):892-906

## ACKNOWLEDGEMENTS

Project "From Text to Teeth: The Nuns of Santa Maria della Stella" funded by the Royal Society of Edinburgh as collaborative project between University of St. Andrews and University of Turin. The research is also funded by EXARC community. Superintendence of archaeology, fine art and landscape for the provinces of Alessandria, Asti and Cuneo; Fondazione Cassa di Risparmio di Saluzzo and Dr. Michele Scanavino.