PAASTA Symposium 2026

Abstract book

Breaking ZooMS: Improving sample preparation methods for MALDI-based palaeoproteomic analysis of Medieval parchment

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Presenter: Zandra Fagernäs

In-person talk

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Keywords: ZooMS; Parchment; Method development

Zooarchaeology by Mass Spectrometry (ZooMS) has revolutionised the study of our past, as a rapid, low-cost method for identifying the taxonomy of historical, archaeological, and palaeontological materials. For the study of historic manuscripts, ZooMS allows us to identify the species from which the parchment was made, as well as explore manufacturing methods, giving insights into manuscript production and trade. However, many aspects of ZooMS have not been thoroughly optimized for specific material types. Here, we aim to improve upon the current protocols for ZooMS analysis of Medieval parchment by exploring various parts of the sample preparation procedure, from sampling to protein digestion and peptide desalting.

We find that samples can be taken from either the hair- or flesh side of a parchment without affecting the results, with variation between samples not being related to different people conducting sampling, and that the best results are achieved by using a PVC eraser for sampling. Sampling can thereby be carried out by curators at institutions holding manuscripts, without the need for additional specialised training. Although trypsin leads to the highest collagen coverage, alternative enzymes can lead to improved accuracy in species identification. Finally, peptide desalting using ZipTips leads to the best results, but this step can be removed to save time and costs, without significantly impairing our ability to conduct taxonomic identification. To conclude, although the basic, well-established ZooMS method works in many cases, exploring different options can reduce the need for sampling of irreplaceable objects, as well as reduce costs.

Rethinking palaeproteomics

Presenter: Matthew Collins

In-person talk

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Keywords: Proteomics, LC-MS/MS, collagen

Mass spectrometry–based identification of ancient and structurally repetitive collagenous proteins faces two compounding challenges: the absence of matched reference proteomes for extinct or divergent taxa, and the failure of standard target-decoy competition (TDC) when applied to collagen's periodic (Gly-X-Y)□ motif. We present two complementary tools addressing these problems at the database-generation and search stages.

PhASTM (Phylogenetically Anchored, PTM-Explicit Sequence Database Generator) resolves reference bias and PTM combinatorics upstream of searching. Rather than relying on a single modern reference proteome, PhASTM builds phylogenetically anchored, clade-aware peptide databases from a canonical exon architecture, using ancestral sequence reconstruction with paralog-aware clustering to represent divergent or extinct lineages. PTMs are explicitly pre-encoded via a Unicode pseudo-residue alphabet, shifting combinatorics out of the search engine, while an empirically derived missed-cleavage model improves realistic peptide coverage — producing smaller, more representative target databases for improved FDR estimation.

pSAGEd (pSAGE-Decoy) addresses a related failure: naive reversal or shuffling decoys break collagen's Gly-X-Y reading frame, distorting decoy mass and fragmentation distributions and compromising FDR control. pSAGEd implements a native, frame-preserving Gly-X-Y triplet-shuffling algorithm within a high-performance Rust search engine, permuting internal tripeptides and their PTM mass shifts while locking glycine positions in place. Integrated directly into database indexing and fragment scoring, this eliminates prior external-preprocessing overhead, reducing intermediate disk footprint to ~1 MB and completing in-memory indexing in under 2.4 seconds per specimen across a 24-specimen benchmark, with score distributions that better reflect the true null model.

Together, PhASTM and pSAGEd form an end-to-end pipeline — phylogenetically informed database construction through structurally valid decoy generation — purpose-built for palaeoproteomic and matrisome mass spectrometry.

Guiding paleoproteomic database construction with next-generation MS/MS proteotyping

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Presenter: Jean Armengaud

In-person talk

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Keywords: Next-generation proteotyping, paleoproteomics, database, strategy, DIA

Our cultural and historical heritage encompasses a remarkable diversity of artifacts, many of which consist of complex mixtures of protein residues originating from multiple organisms. Here, we present and illustrate a proteotyping strategy specifically designed for minute sample amounts, for which metagenomic approaches are precluded by limited material availability and/or extensive DNA degradation. Next-generation MS/MS-based proteotyping enables establishing the list of the organisms represented in a sample and provides an estimate of their relative biomass contributions. This information can then be used to construct fine-tuned protein databases for paleoproteomic searches, prioritizing proteins from the most abundant organisms and thereby reducing the substantial false-positive rates that can arise from searches against excessively large or taxonomically inflated databases. We will compare this proteotyping workflow with alternative approaches using a range of historical artifacts as case studies. Notably, our analyses identified peptides specific to clinically relevant pathogens in several specimens, enabling retrospective clinical diagnoses and providing new insights into the potential causes of disease and death in historical individuals. For example, all inferior long bones of Saint Leonard de Noblat, who died in 559 CE, displayed morphological evidence consistent with osteomyelitis. Our proteomic analysis further supported the presence of a bacterium closely related to Actinomadura bangladeshensis, a recognized causative agent of mycetoma, providing molecular evidence for an infectious etiology. The same strategy can be applied to data-independent acquisition datasets, where it enables deeper characterization of the proteomic diversity preserved in historical artifacts and demonstrates the potential of DIA for high-depth paleoproteomic investigations.

Beyond Stockfish: Identifying migratory and freshwater fish within the medieval-early modern ichthyoarchaeological record via high-throughput ZooMS analysis

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Presenter: Liz Quinlan

Post-PhD ECR

Online

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Keywords: ZooMS, fish, medieval

The archaeological study and identification of fish bones from Northern European sites during the high medieval to early modern period (c. 1200-1650) has primarily been focused on two of the most economically important taxonomic groups of marine fish– gadids (cod, haddock, saithe, etc.) and clupeids (herring, shad, sardines, etc.). These species are not only some of the most economically important of this time period, but are also more frequently identified within the archaeological record due various combinations of size, distinct morphology, and sheer frequency. This, combined with the tendency for smaller freshwater and migratory fish species to be less robustly ossified, means that relying solely on morphological identification methods may lead to underrepresentation of these fish in the archaeological record. As part of an ongoing research project intended to shed light on the fisheries for freshwater and migratory species of fish, a large number of unidentifiable fish bone have been sampled and analysed via zooarchaeology by mass spectrometry from three historically and economically important trading centres across Northwestern Europe; Exeter, Lübeck, and Bergen. This paper will present findings from high-throughput applications of ZooMS to a representative sample of fragmentary fish bone, with the intention of capturing a more accurate view of species frequency at various sites throughout the period of interest.

Generation and validation of eggshell protein biomarkers for palaeoenvironmental reconstruction

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Presenter: Jan Dekker

In-person talk

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Keywords: Palaeoproteomics, eggshell, birds

The species-specific identification of bird eggshells in the archaeological record can be extremely valuable for the study of past ecosystems, due to the highly specific environmental requirements for nesting. However, the morphological identification of eggshells is challenging and limited in its specificity. Instead, given the exceptional preservation of proteins in eggshell, taxon-specific differences in the amino acid sequence have been used for determining the bird species. Yet reference protein sequences are currently only available for a small number of bird species, severely limiting the applicability of this method. To address this issue we have analysed 65 eggshell samples from 33 bird species and 9 families from the Natural History Museum in Tring using LC-MS/MS and MALDI-TOF/TOF. Observed PSMs and biomarkers were compared against a custom database of Anatidae protein reference sequences translated from publicly available genomes.
Of the 12 eggshell proteins this study focused on XCA1, XCA2 and ovalbumin were found in all samples, while OC116 showed the most variation between species. Species and genus specific peptides of XCA1/2, OC116, ALB and clusterin were found for 5 of the 16 analysed Anatidae species. However, the MALDI-TOF/TOF analysis revealed distinct signatures of biomarkers for 13 of 16 species with only 3 species showing identical combinations of peptides in the MALDI-TOF-MS spectra.

Biological age-estimation of archaeological human remains using protein degradation patterns

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Presenter: Celine Chong

Online talk

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Keywords: AGEs, age-estimation, LC-MS/MS

Biological age-estimation of archaeological human remains is an important area in archaeology that requires further development of more accurate methods. However, despite consensus about the limitations of osteological age estimation, there is not much research on alternative methods of age estimation for archaeological human remains, such as using biomolecular techniques. In clinical literature, protein aging clocks based on protein degradation patterns are used to measure the biological age of patients. This study used Liquid Chromatography – Tandem Mass Spectrometry (LC-MS/MS) to identify correlations between protein degradation patterns and age in archaeological human remains. This study also aims to answer a methodological question of whether the type of enzyme digestion – trypsin, elastase, or trypsin and elastase – used for sample preparation in LC-MS/MS can affect the detection of protein degradation patterns. The results suggest that trypsin is the most effective enzyme digestion method for LC-MS/MS, and we found putative evidence of post-translational modifications that, if confirmed in a larger dataset, could be potential age predictors based on their positive correlation with age.

Reevaluating Human and Carnivore Presence in El Mirón Cave (Cantabria, Spain) through the Integration of Paleoproteomic and Taphonomic Approaches

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Presenter: Carla Gómez-Montes

Online talk

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Keywords: Upper Paleolithic, Cantabrian Region, Paleoproteomics, Taphonomy

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Reconstructing human–carnivore ecological dynamics during the Upper Paleolithic is essential for understanding settlement systems, subsistence strategies, and human–animal interactions in the Cantabrian Region of northern Spain. El Mirón Cave (eastern Cantabria) provides an exceptional record, with a well-preserved stratigraphic sequence spanning the Mousterian to the Azilian (>46,000–12,000 cal BP), including particularly rich archaeofaunal assemblages.

This study focuses on the Solutrean occupation (24,500–21,500 cal BP), coinciding with the Last Glacial Maximum (LGM), and integrates previous archaeozoological, taphonomic, and sedimentary DNA (sedaDNA) datasets with newly generated ZooMS (Zooarchaeology by Mass Spectrometry) results. A total of 363 highly fragmented mammalian bone remains from Gravettian, Solutrean, and Magdalenian contexts were analysed. These specimens lacked diagnostic morphological characteristics and therefore could not be taxonomically identified through conventional archaeozoological approaches.

Previous research has shown that sedaDNA preserves a taxonomic signal that partially differs from the morphological record. By integrating ZooMS and sedaDNA, we aim to identify taxa detected exclusively through molecular approaches and assess their contribution to the reconstruction of site formation and human–carnivore interactions. ZooMS has already revealed previously undetected

taxonomic information, including Vulpes vulpes from Solutrean Level 125 of El Corral (22,980–22,240 cal BP).

Future radiocarbon and ancient DNA analyses will further investigate the chronology and ecological significance of these carnivore remains. Overall, this study highlights the potential of integrating paleoproteomics, sedaDNA, and taphonomic data to improve taxonomic resolution and reconstruct human–animal interactions and site-use dynamics in Upper Paleolithic contexts.

Evaluating Minimally Destructive Sampling Methods for Proteomic Analysis of Historic Marine Shagreen

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Presenter: Rachel Winter

In-person talk

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Keywords: elasmobranchs, marine shagreen, ZooMS, minimally destructive methods, historic ecology

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Marine shagreen is a manipulated rawhide made of the skin of sharks and rays. This material peaked in popularity in northwestern Europe between the 17th and early 19th centuries. It was used to adorn items ranging from eyeglass cases and scientific instruments to sword handles. Some of these skins were being imported from around the world, while some may have been procured locally. Recent research comparing historic documents and analysis of the objects themselves finds a mismatch between the species and habitats that were used to create these objects. Historic records suggest more coastal and shallow water species, whereas analysis of the dermal denticles, tooth-like scales on sharks and some rays, on historic objects indicates predominantly deep-sea sharks being utilised. Due to limitations with comparative data available and diversity of denticle morphologies, the taxonomic specificity of non-destructive methods, which is often to the level of one or multiple families, leaves remaining questions of which species were being used. However, sampling museum objects prompts questions of maintaining the integrity of the object. Prior applications of minimally invasive sampling methods (e.g. eZooMS) to skin-based materials have been successful. This paper thus trials five minimally invasive sampling methods for MALDI-ToF analysis on pieces of shark and ray skin to ascertain which approach maximises proteins retrieved with minimal alteration or damage to the skins. Additionally, this work will discuss the challenges of working on a taxonomic group (elasmobranchs) with minimal proteomic or genetic reference data available.

Osteology constrains, proteomics refines

Presenter: Joan Femenias-Gual

Online talk

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Keywords: Palaeoproteomics; Osteology; Taxonomic identification; MALDI-TOF MS; Alaudidae

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Taxonomic identification of fossil birds remains challenging when morphologically similar taxa cannot be distinguished from preserved skeletal elements. In such cases, palaeoproteomics can provide additional discriminatory information. To explore whether combining both sources of evidence could improve taxonomic resolution in Passeriformes, we used Mediterranean larks as a pilot study.
To develop a molecular reference framework, collagen sequence variation was screened for potentially informative amino-acid differences among larks, and predicted peptide-mass differences were tested by MALDI-TOF MS on modern osteological specimens. Several candidate differences were detected, including signals observed in multiple individuals of the same species, while others remain tentative or require further replication, with LC-MS/MS planned to confirm peptide identity.
This approach was applied to Late Pleistocene-Holocene Alaudidae from Es Pouàs (Eivissa, Balearic Islands), where many remains are morphologically identifiable only to family level. Fossil spectra preserved distinct peptide profiles compatible with different lark taxa, allowing preliminary taxonomic refinement beyond the identifications provided by osteology alone.
These results provide a proof of concept for integrating osteological constraints with proteomic evidence to refine taxonomic identification. The modern reference dataset is now being expanded to include additional Mediterranean lark species and individuals per species to assess marker reproducibility, inter- and intraspecific variation, and achievable taxonomic resolution.

Minimally invasive ZooMS and community partnership in Aotearoa New Zealand

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Monica Tromp
Online talk

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Keywords: ZooMS, minimally invasive sampling, whale bone, museum collections, Indigenous partnership, repatriation

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Taonga tūturu (treasured cultural objects) made from bone are held in museum collections across Aotearoa New Zealand, yet many were collected without documentation of where they came from or who made them. Ika Moana Ika Whenua applies Zooarchaeology by Mass Spectrometry (ZooMS) to identify the genus/species used to make these taonga, with the dual aims of supporting mātauranga Māori (Indigenous Māori knowledge) revitalisation and building evidence for future repatriation. We treat minimally invasive sampling as a values position rather than a technical constraint: taonga are culturally significant in their own right, not specimens awaiting identification, and sampling should leave no visible trace. Tikanga (protocols) developed with our partners shaped every stage, from selecting taonga and handling collagen extracts to interpreting and sharing results.

Of the samples from unprovenanced taonga at Te Papa, New Zealand's national museum, most were identified as sperm whale, as expected; however, several other species were also identified, including humpback, blue and southern right whales, and non-cetacean taxa including cow, sheep, moa and human. Many identifications contradict museum records, and the recognition of kōiwi (human bone) has implications for care and repatriation.

We reflect on what it means to embed proteomic research within community relationships, including shared data governance, and argue that partnership and mātauranga Māori should be treated as co-equal to the science. Results were interpreted alongside mātauranga Māori experts before wider sharing, so that the science informs, rather than overrides, cultural understandings of these taonga.