# PROGRAM BOOKLET

## Integrating ZooMS and Zooarchaeology (IZAZ) 2nd workshop

19-21st November 2024,
Collège de France,
Paris, FR

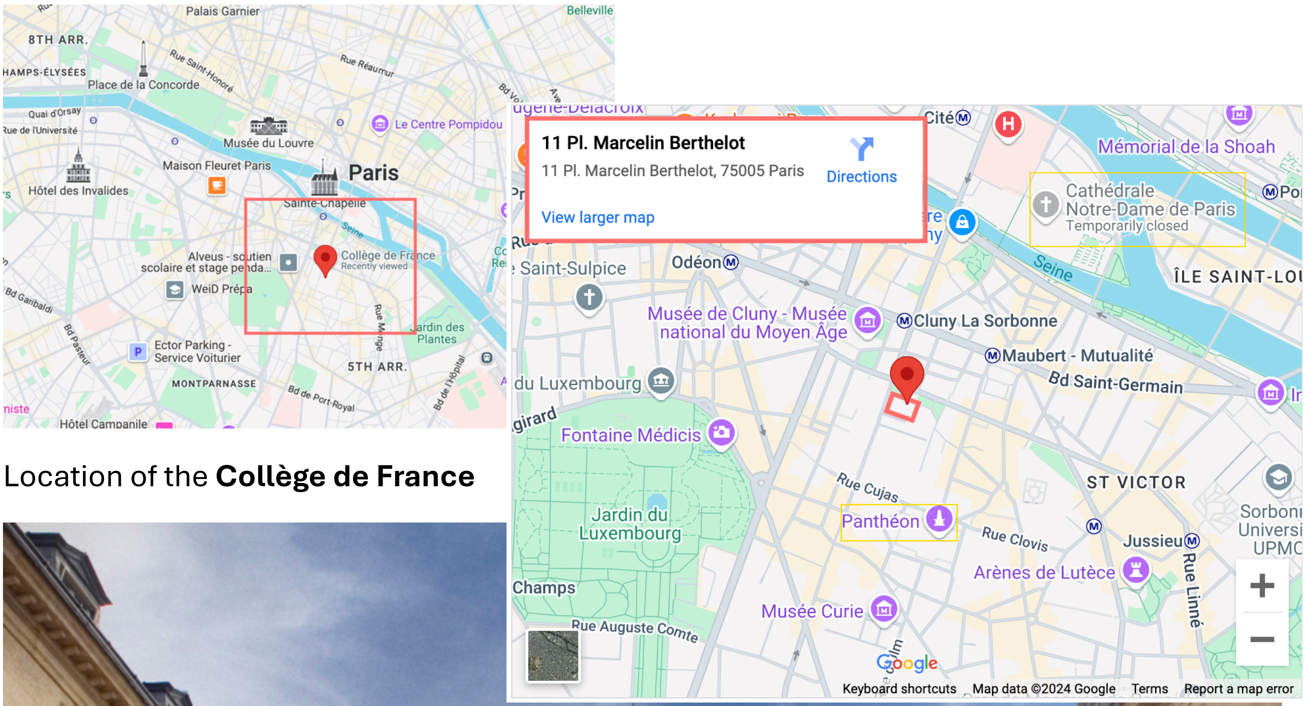
### Organizers:

Aurélie Artizzu
Annelise Binois-Roman
Giulia Gallo
Raija Heikkilä
Pauline Raymond

UNIVERSITÉ PARIS 1
PANTHÉON SORBONNE

COLLÈGE
DE FRANCE
1530

The keynote talks and workshop sessions are held at the
**Collège de France** at **11, place Marcelin-Berthelot (Paris, FR)** in **Salle 4**



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Location of the **Collège de France**

**Salle 4** is in the building to the right of the **main entrance** (shown in image) at **11, place Marcelin-Berthelot** and directions to the room will be indicated by signs near the information desk.

# PROGRAM

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# KEYNOTE SPEAKERS

## November 19

*Dr. Virginie Sinet-Mathiot*

### ZooMS: A Technological Leap in Zooarchaeology

Traditionally reliant on morphological analysis, zooarchaeology is often constrained by fragmentation or the removal of diagnostic features from organic remains. However, over the past fifteen years, the introduction of mass spectrometry techniques has transformed the field, particularly with Peptide Mass Fingerprinting methods emerging as a powerful tool in archaeological sciences and cultural heritage. Zooarchaeology by Mass Spectrometry (ZooMS) uses the persistence and stability of collagen to identify animal species from archaeological materials, offering a complementary tool for zooarchaeologists. As this method becomes widely used by different research groups, multidisciplinary collaborations have been key to the evolution of ZooMS, leading to novel applications across various domains. Methodological developments have refined sampling techniques to preserve the integrity of the analysed objects, simplified sample preparation and data analysis for high-throughput studies of large sample cohorts, and increased the range of identifiable species through the expansion of peptide marker databases. Moreover, the shift towards analyzing non-collagenous proteins, such as those in paint binders or eggshells, has broadened the range of materials that can be examined.

This talk will review major developments and applications of ZooMS, emphasizing its integration with zooarchaeology for the analysis of faunal assemblages, and will discuss the contributions of palaeoproteomics to understanding human behavior and ecological dynamics through time. Current limitations of the method and its complementary role alongside other proteomic disciplines will also be explored. These advancements collectively represent a substantial leap forward in interpreting the archaeological record and elucidating the complexities of past human-environment interactions.

## Democratizing ZooMS by integrating Shape Analysis

*Dr. Matthew Collins*

Recent advancements in zooarchaeology have significantly enhanced our ability to interpret faunal remains, particularly through the development of ZooMS (Zooarchaeology by Mass Spectrometry) and associated analytical tools. My presentation considers how we can further democratize and expand the accessibility of zooarchaeological methods, with a particular focus on integrating shape analysis with molecular techniques. Building upon the pioneering work of researchers like Thomas Cucchi, who emphasized the importance of shape at the interface of zooarchaeological and biomolecular analysis, there is no better place than Paris to think about how our community can best link molecular records with morphological data. This integration presents the next great challenge in zooarchaeology and has the potential to significantly broaden the field's reach and applicability.

Several developments are in the pipeline or under development already which can be harnessed to this task:

1: Adapt high-speed proteomic sexing methods for teeth to be more ZooMS-compatible.
2: Develop tools that connect morphology with molecular identification, leveraging expertise from existing collections.
3: Create persistent databases capable of handling complex shape file types.
4: Utilize Geometric Morphometrics (GMM) and AI-driven statistical tools to compare morphological and molecular identities.

By bridging the gap between molecular and morphological analyses, we can expand the ZooMS repertoire to include species groups like fish and small mammals, where whole bones are common but destructive analysis is often undesirable. Moreover, this approach could enable zooarchaeological work in regions where collagen preservation is poor or where access to sophisticated instrumentation is limited. This integrative method not only addresses specific archaeological questions but also engages a wider research community, including conservators and researchers from diverse global contexts. By focusing on shape - a fundamental property of almost all archaeological objects - we can create more inclusive and accessible ZooMS.

# ABSTRACTS

## November 20

## First Session: ZooMS Applications Across Diverse Archaeological and Historical Contexts

### The RATTUS project and the role of ZooMS in large multidisciplinary studies

David Orton[1], Sam Presslee[1], Michelle Feider[1], Zachary Sepulveda[1]

*1 : BioArCh, Department of Archaeology, University of York, York*

ZooMS can be an indispensable tool when working with closely related and morphologically similar species such as members of the Rattus genus. In addition to their inherent zooarchaeological value, secure low-cost identifications are invaluable in support of other molecular analyses. In this paper we showcase the role of ZooMS within the interdisciplinary RATTUS project, which aims to explore the history of black rat (*Rattus rattus*) and brown rat (*Rattus norvegicus*) in Europe and adjacent areas over the past c. 2500 years. After introducing the project and its inherent identification challenges - both among Rattus species and between rats and other western Eurasian rodents - we discuss how ZooMS fits into a wider project workflow that variously includes zooarchaeological, isotopic, and genetic analyses, as well as direct radiocarbon dating. We pay particular attention to quantities of bone required, given the small size of the species involved. This is all illustrated with preliminary results from ZooMS-backed metrical and radiocarbon studies, along with an update on efforts to expand the reference database to cover more rat-sized mammals.

## The Contribution of eZooMS to understanding medieval bookbinding production: highlighting the use of wild mammal skins by monks

Élodie Lévêque[1], Sarah Fiddyment[2], Matthew Teasdale[3], Matthew Collins[4,5,6,7]

*1 : Université Paris 1 Panthéon-Sorbonne, Paris, France*
*2 : BioArCh, Department of Archaeology, University of York, York*
*3 : Newcastle University, UK*
*4 : McDonald Institute for Archaeological Research*
*5 : Copenhagen University, Denmark*
*6 : UK & Globe Institute*
*7 : Cambridge University*

eZooMS, a non-invasive variant of Zooarchaeology by Mass Spectrometry (ZooMS) that extracts proteins from the surface of parchment through the gentle application of a PVC eraser, represents a significant methodological advancement in the identification of animal species used in Medieval parchment production. This technique not only provides reliable species identification but, combined with traditional zooarchaeological analysis, offers fresh perspectives into the environmental and economic dynamics of the medieval period. This approach is implemented at Morimond Abbey, a key monastic center of the Cistercian order in North-Eastern France, to investigate the relationship between animal husbandry and parchment production and use between the 12th and 15th centuries. Our study attempts to combine results from the osteological analysis of ca. 8000 animal bones stemming from the archaeological excavation of the abbey's hostelry with an eZooMS analysis of over 300 dated and localized parchment documents selected within the abbey's extensive archival records. Preliminary results indicate a notable shift in parchment production materials, with a transition from predominantly calfskin to sheepskin occurring between 1180 and 1200. This shift may indicate broader changes in Cistercian livestock management practices. The eZooMS findings, supported by zooarchaeological data, are now being further investigated at other significant Medieval abbeys in Northern France to gain deeper insights into our understanding of medieval resource management and economic

## Parchments & Bones: Combined contributions of eZooMS and Zooarchaeology to the study of Medieval Morimond Abbey (Haute-Marne, France)

Aurélie Artizzu[1], Annelise Binois-Roman[1], Benoît Rouzeau[2], Matthew Collins[3,4,5,6]

*1 : Université Paris 1 Panthéon-Sorbonne, Paris, France*
*2 : Université de Picardie-Jules Verne, Amiens, France*
*3 : McDonald Institute for Archaeological Research*
*4 : Copenhagen University, Denmark*
*5 : UK & Globe Institute*
*6 : Cambridge University*

The role of eZooMS is pivotal in identifying the materials used in medieval bookbinding, revealing the surprising prevalence of wild mammal skins in certain periods and geographical areas. Through the analysis of 8th–10th century manuscripts from French religious institutions, eZooMS identified deer and roe deer skins as predominant materials for Carolingian book covers and binding elements, challenging the conventional idea of a reliance on domesticated animals such as calves, sheep, and goats. This finding underscores the significance of the hunting rights granted by Charlemagne to monastries, which supplied skins for both bookbinding and personal items. Additionally, eZooMS analysis uncovered the use of sealskin, present on Romanesque binding covers across Cistercian abbeys in Europe. This discovery, supported by DNA analysis, points to a transcontinental supply chain, possibly originating in Greenland, as seals were not native to France during the 12th and 13th centuries. The material diversity reflects broader ecological and economic networks that transcended political borders, through Norse trading routes that extended down to the Champagne medieval fairs or their connections with Cistercian monasteries. These insights significantly enhance our understanding of medieval bookbinding, highlighting the complexity of material choices and the far-reaching trade and religious networks of the relevant periods.

## Second Session: Pleistocene ZooMS- Exploring Human Subsistence and Species Identification

### Reevaluating Neanderthal Cannibalism: Insights from biomolecular methods (ZooMS) and zooarchaeology

Pauline Raymond[1], Karen Ruebens[1, 2], Geoff Smith[2, 3], Jean-Jacques Hublin[1]

*1 : Paleoanthropology Chair, CIRB, Collège de France, PSL University, CNRS, INSERM, 75005 Paris, France, Chaire de Paléoanthropologie, Collège de France*
*2 : University of Reading, Whiteknights House, RG6 6UR Reading, UK*
*3 : School of Anthropology and Conservation, University of Kent, Giles Ln, CT2 7NZ Canterbury, UK*

Neanderthal cannibalism has implications for our broader understanding of their subsistence and adaptation. The site of Abri Moula (France, MIS-5) has yielded large quantities of bone remains including cut-marked Neanderthal remains. This presentation explores how combining ZooMS with taphonomic and zooarchaeological analyses provides new insights into Neanderthal cannibalism. Morphologically unidentifiable bones (n=1,037) were taxonomically identified with ZooMS. Zooarchaeological and taphonomic analyses were recorded on both morphological and ZooMS identified bone remains. Finally, to better understand the site formation and depositional context of the bone remains, microtomographic virtual histology and glutamine deamidation values were studied. Our ZooMS analysis achieved an identification rate of 87.7% representing a broad taxonomic range consistent with an MIS-5 environnement and including 48 new Neanderthal remains. Taphonomic analysis indicated a high propotion of anthropogenic modifications with minimal carnivore modification. Deamidation values and virtual histology suggested differential depositional contexts for some taxa.Here we discuss the integration of NISP data as well as taphonomic data from morphologically and ZooMS identified remains. To integrate NISP data, we calculated an adjusted NISP (Discamps et al. 2024). We also explore the incorporation of taphonomic data to refine interpretations of human behavior at the site, by experimenting with a modification index (n traces/n specimens (morpho, ZooMS, adjusted)). Reevaluating this collection is a way to explore the phenomenon of cannibalism through innovative questions and methods. Our work provides an in-depth understanding of Neanderthal cannibalism and exemplifies an approach that can be applied to existing collections to enhance our comprehension of past human behavior.

## The Application of Palaeoproteomics to Late Pleistocene sites in Croatia

Lia Vidas[1], Sara Silvestrini[2] , Federico Lugli[3, 4], Stefano Benazzi[5], Ivor Janković[6]

*1 : Centre for Applied Bioanthropology, Institute for Anthropological Research, Zagreb*
*2 : Department of Cultural Heritage, University of Bologna, Ravenna*
*3 : Department of Chemical and Geological Sciences, University of Modena and Reggio Emilia*
*4 : Institut für Geowissenschaften, Goethe-Universität Frankfurt*
*5 : Department of Cultural Heritage, University of Bologna, Ravenna*
*6 : Centre for Applied Bioanthropology, Institute for Anthropological Research, Zagreb*

The ways in which human groups organised themselves and exploited the resources around them is an ever-fascinating question. In archaeology, this relies heavily on the study of the faunal remains. In the last decades, the field of archaeozoology has been expanding and improving rapidly with the help of other scientific disciplines, one of which is palaeoproteomics. Even though it has been 15 years since its first application to archaeological assemblages, in parts of Europe it still hasn't reached its full potential. In Croatia, the only published data comes from the site of Vindija, where a part of the fauna was selected for a targeted ZooMS analysis. First systematic, non-targeted analysis of faunal assemblages using ZooMS method, is underway for the last two years as a part of the PREHISTRIA (HRZZ IP-2019-04-7821) research project. This study is focused on Late Pleistocene sites of the Istrian peninsula. Two of them are attributed to Late Upper Palaeolithic period (Ljubi ćeva pe ćina, Pupi ćinape ć), one to the Middle Upper Palaeolithic (Abri Kontija 002) and the last one to Middle Palaeolithic (Romualdova pe ćina). As these sites also differ in their type and geographical location, it poses interesting questions, both in terms of methodology and interpretation of human behaviour. Here we present current results on taxa composition, calculation of the deamidation of glutamine, as well as the efforts of integrating those with the archaeozoological data, all in hope of shedding new light into human behaviour in the Northern Adruatic during Late Pleistocene.

# Integrating ZooMS and zooarchaeology to study human subsistence strategies at Istykskaya Cave, Tajikistan

Sveta Shnaider[1], S. Baranova[2], A. Chernonosov[3], V. Koval[3], Nuritdin N. Sayfulloev[3], William Rendu[1]

*1: International Research Laboratory ZooStan - ArchaeoZoological Center for the study of Central Asia - CNRS – Al-Farabi Kazakh National University, Almaty, Kazakhstan*
*2 :Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia*
*3 : Institute of History, Archaeology, and Ethnography, Dushanbe, Tajikistan*

The Istykskaya Cave, located in the southwestern part of the Pamir Plateau at an altitude of 4060 m, contains the earliest evidence for human occupation in the Pamir highlands (15-12 kBP). The site has great potential to reveal important insights into the adaptive strategies developed by the first inhabitants of high-altitude environments. However, the faunal material is highly fragmented due to human activities and post-depositional alterations. Less than 6% of the remains are morphologically identifiable, yet human impact is observable on 80% of the non-identified (NID) fragments. To gain better insights into human subsistence strategies at the site, we conducted a targeted ZooMS (Zooarchaeology by Mass Spectrometry) study on bone fragments with anthropogenic modifications. Our results show that most of the hunted prey belonged to the genus Ovis. By combining traditional zooarchaeological methods with ZooMS analysis, we were able to interpret the site as a seasonal hunting camp. Additionally, two specimens were identified as canids, suggesting the potential presence of domesticated dogs, which is an interesting hypothesis given the limited carnivore marks on the faunal remains. Further studies will be done to test this hypothesis. ZooMS also allowed to identify an earlier presence of wild yak at the site, challenging previous assumptions that yaks appeared in the region during the Bronze Age as a result of human migration from eastern territories. This finding necessitates further research into the genetic history of yaks in Eastern Pamir.

## New insights into the occupation history and subsistence strategies of Denisovans at Baishiya Karst Cave

Huan Xia[1], Jian Wang[1], Zandra Fagernäs[2], Fahu Chen[3], Dongju Zhang[1], Frido Welker[2]

*1 : College of Earth and Environmental Sciences, Lanzhou University*
*2 : Globe Institute, University of Copenhagen*
*3 : Institute of Tibetan Plateau Research, Chinese Academy of Sciences*

Genetic and fragmented palaeoanthropological data suggest that Denisovans were once widely distributed across eastern Eurasia. Despite limited archaeological evidence, this indicates that Denisovans were capable of adapting to a highly diverse range of environments. Here, we integrate proteomic and zooarchaeological analysis of 2,567 bone fragments collected from Baishiya Karst Cave (BKC) on the Tibetan Plateau where the Xiahe Denisovan mandible and Denisovan sedimentary mtDNA were found. Using Zooarchaeology by Mass Spectrometry, a hominin fossil (Xiahe 2) was successfully screened from layer 3 (40.1±8 thousand years ago, ka). Shotgun proteomic analysis assigns this specimen to the Denisovan lineage, extending their fossil presence at BKC well into the Late Pleistocene. Throughout the stratigraphic sequence (layers 11-3, > 224 ka to 32 ka) at BKC, medium-sized caprines (*Pseudois nayaur*) are the most abundant taxa, followed by the wild yak (*Bos cf. mutus*) and equids (*Equus sp.*). Large carnivores, megaherbivores, small mammals and birds also appear in some layers. The high intensity of anthropogenic modifications on the bone surfaces suggests that Denisovans were the primary agent of faunal accumulation and utilization of most of the abovementioned animals. Abundant hominin activities throughout the stratigraphy, including skinning, dismembering, filleting, bone marrow extraction and expedient bone tool manufacture, suggest Denisovans fully exploited the available animal resources. Therefore, our results provide insight into Denisovan behaviour and their adaptations to the diverse and fluctuating environments of the late Middle and Late Pleistocene in eastern Eurasia.

## Third Session: Innovative Approaches in Palaeoproteomics

### A comparative study of commercially available, minimally invasive, sampling methods on Early Neolithic humeri analysed via palaeoproteomics

Jakob Hansen[1, 2], Joannes Dekker[2, 3], Gaudry Troché[2] , Zandra Fagernäs[2] , Jesper Olsen[4], Maria Saña Seguí[5], Frido Welker[2]

*1 : Universitat Autònoma de Barcelona [Barcelona] (UAB) UAB Campus 08193 Bellaterra Barcelona - Espagne*
*2 : Globe Institute, University of Copenhagen*
*3 : Departments of Archaeology, University of York*
*4 : Novo Nordisk Foundation Center for Protein Research, University of Copenhagen*
*5 : Departament de Prehistòria,Universitat Autònoma de Barcelona Barcelona - Espagne*

Due to methodological advances in archaeological sciences, an increasing number of archaeological specimens undergo destructive sampling. However, preserving the cultural heritage remains a primary concern, causing a dilemma between accessing the sample material and obtaining sufficient information for a meaningful analytical outcome. Ideally, sampling a specimen would preserve the object for further macro, micro, and molecular analyses. For palaeoproteomics, several minimally invasive sampling approaches have been proposed, each with different benefits and limitations. While studies have compared some of these protocols, they have often focused on specimens from homogeneous preservation environments using Zooarchaeology by Mass Spectrometry (ZooMS). This study expands on previous work by comparing specimens from two highly different preservation environments using both ZooMS and liquid chromatography-tandem mass spectrometry (LC-MS/MS). We evaluate five sampling approaches and seven extraction protocols on 10 Bos sp. humeri from the Early Neolithic site of La Draga, Spain, utilizing MALDI-ToF MS and LC-MS/MS to generate proteomic data, while assessing the protocol's invasiveness by using microscopy and 3D imaging. Five humeri come from Sector A, characterized by dry, terrestrial preservation conditions, and the other five from Sector B, characterized by phreatic/aquatic conditions. Our findings reveal significant differences in protein recovery and taxonomic specificity between the sampling techniques and burial conditions. Additionally, various surface modifications were observed depending on the sampling technique used. Therefore, it is essential to assess protein preservation for each sedimentological context within an archaeological site before performing extensive sampling, as protein preservation can be highly inter- and intra-site- specific.

# Increasing sustainability in palaeoproteomics by optimizing digestion times for large-scale archaeological bone analyses

Frido Welker[1], Louise Le Meillour[1], Virginie Sinet-Mathiot[2], Ragnheiður Diljá Ásmundsdóttir[1], Jakob Hansen[1, 3], Dorothea Mylopotamitaki[4], Gaudry Troché[1], Huan Xia[5], Jorsua Herrera Bethencourt[1], Karen Ruebens[6, 7], Geoff M. Smith[6, 8,] Zandra Fagernäs[1]

*1 : Globe Institute, University of Copenhagen*
*2 : University of Bordeaux University of Bordeaux, CNRS UMR 5287, 33076 Bordeaux, France*
*3 : Departament de Prehistòria, Universitat Autònoma de Barcelona*
*4 : Chaire de Paléoanthropologie, CIRB, Collège de France, Université PSL, CNRS Centre de Recherche Inserm, PSL*
*5 : Lanzhou University*
*6 : Department of Archaeology, University of Reading*
*7 : Chaire de Paléoanthropologie, CIRB, Collège de France, Université PSL, CNRS Centre de Recherche Inserm*
*8 : School of Anthropology and Conservation, University of Kent*

The ongoing climate crisis has become an important part of government, economic, and scientific policies worldwide. The scientific community can take an active part in addressing this challenge by building sustainable approaches to conducting science. One way of transitioning to a more sustainable science is by reducing electricity and plastic consumption in the laboratory context. ZooMS (Zooarchaeology by Mass Spectrometry) and SPIN (Species by Proteome INvestigation) are increasingly performed on hundreds or thousands of bone specimens in individual studies. The climate impact of their increasing usage in bioarchaeology are, therefore, increasing proportionally as well. We explored the effects of reducing digestion time from 18 hours (overnight digestion) to 3 hours on herbivore skeletal element from Baiyisha Karst Cave (China) and La Draga (Spain), covering a chronological range from the Middle Pleistocene to the Early Holocene. Each sample was powdered, homogenized, and split between digestion conditions (3, 6 and 18 hour digestions). To each extract, both MALDI-ToF MS (ZooMS) and LC-MS/MS (SPIN) were applied. Our results indicate no statistical changes in terms of attained proteomic species identifications for either proteomic method. The 6-fold reduction in digestion time would equate to a 6-fold reduction in electricity consumption per sample digest. In addition, we demonstrate that working in 96-well plates further reduces electricity consumption, per sample, by 60% in comparison to individual microtubes. Thus, our analyses show that the environmental impact of our research can be reduced without affecting data quality, thereby taking an important step towards more sustainable scientific practices.

## Experimental protein extraction for the ZooMS analysis of bones with poor biomolecular preservation

Nikolitsa Nikolakopoulou[1], Britt M. Starkovich[2, 3], Keiko Kitagawa[2, 3], Salma Ibrahim[1], George Konidaris[4, 5], Dimitrios S. Kostopoulos[6], Katerina Harvati[7, 8, 9], Samantha Brown[10]

*1 : Institute for Archaeological Sciences, Department of Geosciences, University of Tübingen , Tübingen*
*2 : Institute for Archaeological Sciences, Department of Geosciences, University of Tübingen , Tübingen*
*3 : Senckenberg Center for Human Evolution and Palaeoenvironment, University of Tübingen, Tübingen*
*4 : Senckenberg Center for Human Evolution and Palaeoenvironment, University of Tübingen, Tübingen*
*5 : Paleoanthropology, Institute for Archaeological Sciences, Department of Geosciences, University of Tübingen, Tübingen*
*6 : School of Geology, Aristotle University of Thessaloniki, Thessaloniki*
*7 : Paleoanthropology, Institute for Archaeological Sciences, Department of Geosciences, University of Tübingen, Tübingen*
*8 : DFG Centre for Advanced Studies 'Words, Bones, Genes, Tools', University of Tübingen, Tübingen*
*9 : Senckenberg Center for Human Evolution and Palaeoenvironment, University of Tübingen, Tübingen*
*10 : Institute for Archaeological Sciences, Department of Geosciences, University of Tübingen , Tübingen*

Biomolecular preservation is particularly challenging in warm and tropical climates, and therefore ZooMS has struggled to detect surviving collagen in archaeological assemblages from these regions. Comparisons of existing ZooMS extraction methods have frequently highlighted acid-based protocols as the most effective for low-collagen bones (see for example Culley et al., 2021; Wang et al., 2021). However, demineralization is known to adversely impact protein survival. This study modified an existing extraction protocol aimed at protein precipitation without demineralization (Cleland and Vashishth 2015; 2017) to make it suitable for ZooMS analysis. To test this method, bones were selected from three archaeological sites in warm climates: Sakai Cave in Thailand, Wadi Madamagh and Ghwair I in Jordan, and the palaeontological site Tsiotra Vryssi (TSR) in Greece. These sites span a wide temporal range, from the ancient TSR site in Greece, dated at 1.78-1.50 million years old, to the relatively recent site in Thailand, approximately 50-100 years old. Samples were studied using three established ZooMS protocols (van Doorn et al., 2011; van der Sluis et al., 2014; Welker et al., 2015) and this novel approach (AmPhos adapted from Cleland and Vashishth 2015; 2017) to determine each protocol's effectiveness. By applying different protocols to areas with low biomolecular preservation, this study introduces the AmPhos method as an alternative to acid-based protocols in ZooMS research and demonstrates the utility of exploring a demineralization-free approach for bones with low protein concentrations. Our research aims to maximize collagen retrieval to expand the range of samples suitable for ZooMS research.

# Integrating ZooMS and stable isotope zooarchaeology: A case study using lyophilized collagen extracts from Late Pleistocene France

Raija Heikkilä[1], Sarah Barakat[2], Pauline Raymond[1], Karen Ruebens[1, 3], Jean-Jacques Hublin[1, 4], Kate Britton[2]

*1 : Chaire de Paléoanthropologie, Collège de France, Oncologie Cellulaire et Moléculaire, PSL University, INSERM UMR 1050, CNRS UMR 7241, Paris, France*
*2 : Department of Archaeology, University of Aberdeen*
*3 : Department of Archaeology, University of Reading*
*4 : Max Planck Institute for Evolutionary Anthropology*

Zooarchaeology can provide a wealth of information on Late Pleistocene ecosystems, environments, and human-animal interactions. This is especially the case when combined with stable isotope analysis (SIA), which is used to understand past faunal spatial and dietary ecology. Morphological analysis can be further complemented with Zooarchaeology by Mass Spectrometry (ZooMS), which is capable of identifying highly fragmentary and worked animal remains lacking diagnostic features. The use of ultrafiltration during collagen extraction and recent mass spectrometry developments allow the extraction of greater quantities of high-quality collagen, and its analysis in smaller amounts. Thus, researchers can utilise collagen extracts for multiple analyses, including SIA, ZooMS, and 14C dating. Using ZooMS alongside SIA can provide a more accurate species identification of small bone fragments which can be difficult to assign using traditional techniques, guaranteeing an optimised interpretation of palaeoecological data and human-animal-environment interactions. Here we present the results of ZooMS analysis applied to lyophilized collagen originally extracted for SIA from several Late Pleistocene sites from France. We show that ZooMS can be successfully applied to low and high molecular weight collagen, and to near-empty Eppendorf and Falcon tubes, where extracted collagen was stored. These results demonstrate that ZooMS can be targeted at collagen with varying molecular weight, and to determine species identifications even when there is a low collagen yield or after extracts are exhausted.

## Fourth Session: Navigating ZooMS Challenges-
## Software Innovation and Methodological Refinement

### PAMPA: a software suite for peptide markers and taxonomic assignment in ZooMS

Fabrice Bray[1], Yohan Hernandez-Courbevoie, Areski Flissi, Helene Touzet[2]

*1 : Miniaturisation pour la Synthèse, l'Analyse et la Protéomique - USR 3290 (MSAP), Université de Lille, Centre National de la Recherche Scientifique - CNRS : USR3290*
*2 : Centre de Recherche en Informatique, Signal et Automatique de Lille - UMR 9189, Centrale Lille, Université de Lille, Centre National de la Recherche Scientifique*

When working in ZooMS, the availability of reliable and effective automatic methods for data analysis is becoming critical to guarantee high-quality taxonomic assignments, and to ensure speed and reproducibility of the analytical process. We have developed a versatile software suite, called PAMPA (Protein Analysis by Mass Spectrometry for Ancient Species), that is able to perform a large range of tasks associated with ZooMS mass spectra, such as MALDI-TOF or MALDI-FTICR spectra. This involves conducting taxonomic assignments based on known peptide markers, identifying marker sequences in new species by homology through inference from closely related species, or performing de novo prediction from the amino-acids sequences alone. PAMPA also allows in-depth exploration of diverse assignment possibilities within the taxonomic space. It is open source (https://github.com/touzet/pampa) and can be used through a user-friendly web interface featuring intuitive visualization tools for mass spectra, peptide markers and taxonomies (https://bioinfo.univ-lille.fr/pampa/). PAMPA has been successfully tested on a large range of archeological and paleontological samples from terrestrial and marine mammals, provided by the Lille ZooMS platform or found in the literature. This shows that this software can help democratize the analysis of ancient protein data, making this type of analysis accessible to researchers with no bioinformatics background.

## Mastering ZooMS challenges

Dorothea Mylopotamitaki[1, 2] , Sara Silvestrini[3], Gaudry B. Troché[4], Angelica Ferracci[5] , Florians. Harking[6], Jesper V. Olsen[6], Maurizio Gatta[5] , Mario F. Rolfo[5], Matteo Romandini[3], Jean-Jacques Hublin[1, 2], Frido Welker[4], Stefano Benazzi[1, 3]

*1 : Max Planck Institute for Evolutionary Anthropology Leipzig - Allemagne*
*2 : Chaire de Paléoanthropologie, CIRB (UMR 7241 – U1050), CIRB, Collège de France, Université PSL, CNRS, INSERM, 75005 Paris Centre National de la Recherche Scientifique – CNRS*
*3 : Department of Cultural Heritage, University of Bologna, Via Degli Ariani 1, Ravenna, 48121*
*4 : Globe institute, University of Copenhagen, Copenhagen - Danemark*
*5 : Department of History, Culture, and Society, University of Rome Tor Vergata. Rome*
*6 : Center for Protein Research, University of Copenhagen, Copenhagen*

ZooMS (Zooarchaeology by Mass Spectrometry) has provided crucial evidence in multidisciplinary studies (Hublin et al. 2020) However, like any advanced technique, it shows challenges and limitations (Mylopotamitaki et al. 2023). Mastering these challenges is essential for maximizing the potential of palaeoproteomics in palaeoanthropological research. This study explores innovative strategies and technologies overcoming ZooMS limitations, utilizing morphologically unidentified bone fragments recovered from the new excavation (2019-2023) at Grotta Guattari (Italy) (Rolfo et al. 2023). Grotta Guattari is a well-known Neanderthal site previously believed to symbolize Neanderthal cannibalism (Petronio et al. 2021). Preliminary taphonomic and stratigraphic results indicate the accumulation of large mammal bones was related to cave hyenas. After the initial unsuccessful applications of ZooMS (Buckley et al. 2009), the utilization of LC-MS/MS (Rüther et al. 2022) provides updated taxonomic identifications of 489 highly-degraded skeletal remains. We followed a recent protocol that suggests simultaneous ammonium bicarbonate and acid extraction as the most suitable approach for highly-degraded skeletal remains (Mylopotamitaki et al. 2023). We retrieved taxonomic identifications for 29% of 490 skeletal remains. Even though we could not identify all the skeletal remains, we recovered various mammalian taxa in agreement with previous studies (Petronio et al. 2021; Rolfo et al. 2023), including cervids and bovids, and four hominin bone fragments. Notably, this study showcased the effect of environmental conditions on the proteome of archaeological skeletal remains providing optimal proteomic extraction conditions that can yield high-resolution mass spectra for taxonomic identification of morphologically unidentified bone fragments.

## Embracing uncertainty to accurately pinpoint species from bone samples through LC-MSMS

Ian Engels[*1], Alexandra Burnett[*1,3], Prudence Robert[3], Camille Pironneau[3], Robbin Bouwmeester[2], Isabelle De Groote[3], Peter Van der Plaetsen, Marcel Otte[4], Lawrence Straus[5], Valentin Fischer[6], Gregory Abrams[3], Dieter Deforce[1], Fabrice Bray[7], Simon Daled[**1], Maarten Dhaenens[**1].

*1: ProGenTomics, Laboratory of Pharmaceutical Biotechnology, Ghent University, Ghent, Belgium*
*2 : Compomics, VIB-UGent Center for Medical Biotechnology, VIB, Ghent, Belgium*
*3 : ArcheOs Research Laboratory for Biological Anthropology, Department of Archaeology, Ghent University, Ghent, Belgium*
*4 : Archéologie préhistorique, Département des sciences historiques, Université de Liège, Belgium*
*5 : Dept. of Anthropology, MSC01 1040, University of New Mexico, Albuquerque, NM 87131-0001 USA*
*6 : Evolution & Diversity Dynamics Lab, UR Geology, Université de Liège, Belgium*
*7 : Univ. Lille, CNRS, UAR 3290 - MSAP - Miniaturisation pour la Synthèse, l'Analyse et la Protéomique, F-59000 Lille, France*

An LC-MSMS system extends on the peptide mass fingerprinting (PMF) ZooMS MALDI-TOF approach by measuring not only the intensity and m/z of the precursor masses, but also providing a fragmentation pattern for each peptide. For identifying these MSMS spectra, the target-decoy approach is at the heart of most algorithms, for score thresholding and/or to improve the feature weights used for scoring. However, this presents palaeoproteomics with a conundrum: ancient bone samples generate sparse datasets built from only a few proteins, disabling the use of target-decoy strategies. In other words, where the simplicity of the protein composition enables fast and effective ZooMS through PMF using MALDI, it effectively complicates LC-MSMS data interpretation. Fortunately, probabilistic search engines like Mascot and MaxQuant are still suited for sparse peptide identification and are therefore most used in LC-MSMS species classification. Still, these peptides don't derive from functionally different proteins from one given organism, for which most scoring algorithms are developed. Rather, the data consists of often low quality MSMS spectra of a few homologous protein targets that could be derived from a plethora of organisms, i.e. a long list of very similar orthologue sequences. The net effect is that basic search results display huge amounts of ambiguity, in turn impairing accurate post-processing tools like Unipept. Therefore, we suggest to embrace and even extend the ambiguity using a newly developed "isoBLAST" approach in order to obtain an exhaustive set of potential peptide candidates (PPCs) for each spectrum. Using only the collagen PPCs, we then walk down the taxonomic tree retaining unique peptides at every bifurcation and plot the complete set of considered peptides in a sunburst plot color-coded by an adapted Bray Curtis dissimilarity score. Using public and in-house data, we show the performance of the approach on different instruments.

*First authors
**Last authors

## November 21

## Fifth Session: ZooMS Applied to Archaeological Fish Exploitation

### Plenty of fish: using palaeoproteomics for marine historical ecology and ecological baselines

Rachel M. Winter[1, 2], Elena Desiderà[3, 4], Paolo Guidetti[3, 4], Matthew J. Collins[5,6], Alberto J. Taurozzi[5], Canan Çakirlar[1]

*1 : Groningen Institute of Archaeology, Rijksuniversiteit Groningen*
*2 : Centre de Recherche sur la Biodiversité et l'Environnement (CNRS), Université Toulouse III - Paul Sabatier BioEco team*
*3 : Stazione Zoologica Anton Dohrn, Department of Integrative Marine Ecology (EMI)*
*4 : National Biodiversity Future Center*
*5 : Globe Institute, University of Copenhagen*
*6 : McDonald Institute for Archaeological Research, Cambridge University*

In the past 15 years, since the introduction of Zooarchaeology by Mass Spectrometry (ZooMS), a lot of the applications have been to mammalian taxa with less work to date done on birds, reptiles, and fish. This talk will provide a short discussion of the enormous potential for applying ZooMS to fish remains before presenting a couple of case studies for the ways in which a multidisciplinary approach including ZooMS can provide ecological baselines, contribute to marine historical ecology, and inform modern conservation strategies. The first case study will cover the application of ZooMS to groupers (Epinephelidae) in the eastern Mediterranean. The Mediterranean basin is a biodiversity hotspot which has been exploited for millennia, with groupers remaining a popular target, and is facing drastic ecological changes as a result of Lessepsian invasions (non-indigenous species from the Red Sea and Indo-Pacific entering the Mediterranean Sea via the Suez Canal). The second case study will focus on marine shagreen, a luxury leather made from ray or shark skin which peaked in popularity in northwestern Europe between the late 17th and early 19th centuries. Currently, one-third of elasmobranch (sharks, rays, and skates) species are at risk of extinction according to the IUCN with the scarcity of historical data and ecological baselines hampering conservation efforts. Between these two case studies, this talk will showcase how the incorporation of ZooMS and long-term perspectives delving into the Holocene can contribute to questions in marine historical ecology and inform marine resource management.

## Exploring the Possibilities of Sturgeon Identification via ZooMS

Teodora Mladenović[1], Samantha Presslee[2], Giulia Zampirolo[3], Mikkel Winther Pedersen[3], Dimitrije Marković[1], Ivana Živaljević[4], David Orton[2]

*1 : University of Belgrade, Faculty of Philosophy, Department of Archaeology, Belgrade, Serbia*
*2 : University of York, Department of Archaeology, York, United Kingdom*
*3 : University of Copenhagen, The Globe Institute, Copenhagen, Denmark*
*4 : University of Novi Sad, Faculty of Philosophy, Department of History, Novi Sad, Serbia*

Sturgeons were among the most intriguing creatures that inhabited the waters of Europe in the past, and are among the most endangered species on earth today. There is considerable historical data on sturgeon fishing, trade, and consumption in the Middle Danube region, particularly during the medieval and modern periods, but archaeological evidence is scarce and hindered by preservation and identification problems. Species identification is crucial for reconstructing the historical ecology of sturgeons, fishing practices, and comprehending the particular species-specific characteristics – such as size, appearance, migratory behaviour, as well as their conservation status. However, the morphological differentiation of commonly recovered skeletal elements is highly challenging. Overfishing and the construction of dams on the Danube have intensified sturgeons' endangered status, and comparative collections in the region and beyond are scarce as a result. With DNA analysis being expensive, especially considering sturgeons' unusually large genomes, and often proving ineffective, ZooMS may present a solution. This paper presents the results of an initial study, aimed at developing markers to enable precise and reliable identification of archaeological specimens of five sturgeon species – the beluga sturgeon, Russian sturgeon, fringebarbel sturgeon, starry sturgeon, and sterlet. A combination of modern and genetically-identified archaeological specimens were used as reference specimens for marker development using LC-MS/MS, revealing distinct markers for all five species. These were then applied to a sample of 30 sturgeon specimens from various historical sites in Serbia using standard ZooMS protocols, demonstrating their ability to refine and in some cases correct the original morphological identifications.

## Fish in the Oasis: Zooarchaeological and ZooMS insights into fish exploitation along the Ancient Silk Road in Central Asia

Carli Peters[1], Noel Amano[1], Robert Spengler[1]

*1 : Max Planck Institute of Geoanthropology*

The identification of fish remains in the zooarchaeological record mostly depends on the presence of a few diagnostic elements, while many other elements, such as ribs and spines, are undiagnostic and thus not useful for making taxonomic identifications. To overcome these issues, we used a combination of zooarchaeology and ZooMS to reconstruct fish diversity and exploitation at three Oasis sites in Central Asia, Bukhara, Paykend, and Panjakent, that are located along the ancient Silk Road. These sites range in age from the third century BC to the Medieval Period. At the same time, we aimed to gain more insight the ecology of the fish present at these sites and the nature of the water bodies (riverine or lacustrine environments) that were exploited by humans in the past. Initially, all fish remains were analysed morphologically, and taxonomic and taphonomic information was noted down. Then, ZooMS was used to identify fish remains that could not be taxonomically identified during zooarchaeological analysis. Measurements of fish vertebrae were used to reconstruct the size range of the identified fish. Here, we present the preliminary results of these analyses and discuss the utility and challenges of combining zooarchaeological and ZooMS approaches when dealing with non-mammal material.

## Sixth Session: Exploring Biodiversity with ZooMS

### Establishing Taxonomic Accuracy in Ichthyoarchaeology: A DNA-Barcoded Collagen Reference Collection for Indo Pacific Neolithic Fish

Nayeli G. Jiménez Cano[1], Kristine Korzow Ritcher[2], Séverine Zirah[3], Agnès Dettaï[4], Elise Dufour[5], Matthew J. Collins[6, 7], Philippe Béarez[8]

*1 : Archéozoologie, archéobotanique : sociétés, pratiques et environnements (UMR 7209) Muséum National d'Histoire Naturelle (MNHN)*
*2 : Department of Anthropology, Harvard University*
*3 : Molécules de Communication et Adaptation des Micro-organismes (UMR 7245) Muséum National d'Histoire Naturelle (MNHN), Centre National de la Recherche Scientifique – CNRS*
*4 : Institut de Systématique, Evolution, Biodiversité (UMR 7205) Muséum National d'Histoire Naturelle (MNHN)*
*5 : Archéozoologie, archéobotanique : sociétés, pratiques et environnements (UMR 7209) Muséum National d'Histoire Naturelle (MNHN), Centre National de la Recherche Scientifique – CNRS*
*6 : Section for Evolutionary Genomics, The Globe Institute, University of Copenhagen*
*7 : McDonald Institute for Archaeological Research, Department of Archaeology, University of Cambridge*
*8 : Archéozoologie, Archéobotanique : sociétés, pratiques et environnements (UMR 7209) Muséum National d'Histoire Naturelle (MNHN), Centre National de la Recherche Scientifique - CNRS*

An important aim of ichthyoarchaeological research is to provide insights into past environments to inform modern conservation efforts by achieving reliable species-level identification in analyses. Identifications based on osteological characters often present difficulties, leading to the search for new objective methods such as paleoproteomics. However, ZooMS markers should rely on material from taxonomically secure samples, a process that is not always achieved when creating fish reference collections, as species determination is most often based on the use of morphological identification keys. In this presentation, we share the results of creating a collagen reference collection composed of 58 DNA-barcoded fish species, including 52 teleosts and 6 elasmobranchs, designed to aid in the taxonomic identification of Neolithic fish from Indo-Pacific archaeological sites. We will present biomarkers to aid ichthyoarchaeological identification at the family, genus, and species levels, as well as the methodological challenges involved in assigning underrepresented fish species and highly diverse tropical fish families. The inclusion of a reference collagen database in osteological reference collections can provide accurate, efficient, and low-cost identification of fish remains. Combined with traditional ichthyoarchaeological methods, this approach has the potential to provide sound ecological baselines for the reconstruction of ancient fish exploitation.

# ZooMS and Zooarchaeology to identify avian remains: Integrating methodologies for examining past environments, prior avian distribution patterns and the role of wetlands in crucial moments in the human past

Maria Codlin[1], Lisa Yeomans[2], Beatrice Demarchi[3]

*1 : Department of Life Sciences and Systems Biology, University of Turin*
*2 : University of Copenhagen = Københavns Universitet*
*3 : Department of Life Sciences and Systems Biology, University of Turin*

Compared to zooarchaeological and paleoproteomic studies involving mammalian remains, those involving bird remains are relatively scarce. Those studies that can accurately identify avian taxa below the family level are even rarer. Birds are hard to identify from their morphological characteristics, especially when faced with fragmentary remains. Yet both avian bone and egg remains provide rich avenues for examining past environments and seasonality, based on breeding and feeding requirements and migratory patterns, evidence for human engagement with domestication, subsistence and ritual practices, as well as as direct evidence for past migration patterns of migratory birds. Moreover, proteins within the mineral matrix of eggshells can survive into deep time, far longer than proteins can survive within animal bones. On their own, both proteomics approaches and morphological approaches have some limitations in taxonomic discrimination, but when integrated, can improve taxonomic resolution and our understanding of species diversity. We present new methodological developments from the ArchaeoBiomics laboratory at the University of Turin to improve taxonomic identification of aquatic birds through the study of proteins recovered from avian bone and eggshell from archaeological sites. We integrate protein-based identifications with zooarchaeological analysis from two wetland sites to explore seasonal patterns of wetland exploitation in the context of a late Pleistocene to Holocene transition site Southwest Asia, and identify evidence for the importance of wetland exploitation at Teotihuacan, Mexico, one of the earliest cities in the Americas.

## ZooMS and Palaeoproteomics on Two Charismatic Taxonomic Groups: Whales (collagen) and South American Camelids (keratin)

Youri van den Hurk[1,2], Danny Buss[2], Martin Seiler[2], Bente Philippsen[2], James Barrett[2], Sam Presslee[3], Jessica Hendy[3], Elise Dufour[1], Nicolas Goepfert[4], and Séverine Zirah[5]

*1 : UMR 7209 Archéozoologie, Archéobotanique - Sociétés, Pratiques et Environnements (AASPE), Centre national de la recherche scientifique (CNRS), Muséum national d'Histoire naturelle (MNHN) –* *yourivandenhurk@gmail.com*
*2 : University Museum - Norwegian University of Science and Technology*
*3 : BioArCh, University of York*
*4 : UMR 8096 Archaeology of the Americas (ArchAm), Centre national de la recherche scientifique (CNRS), University Paris 1 – Panthéon-Sorbonne*
*5 : UMR 7245 Molécules de Communication et Adaptation des Micro-organismes (MCAM), Centre national de la recherche scientifique (CNRS), Muséum national d'Histoire naturelle (MNHN)*

The morphological identification of zooarchaeological material is often faced with difficulties due to fragmentation and numerous other taphonomic conditions. Here we discuss the potential ZooMS and palaeoproteomic analyses can have on our deep-time interaction with two charismatic taxonomic groups. Whale bones are infamously hard to identify to the species level. ZooMS collagen reference spectra have been created for a number of whale taxa, but others have not been previously studied. This not only limits our understanding of the exploitation of these other taxa, but ZooMS-based species identifications of archaeological specimens might be wrong due to the limited number of reference spectra available. We reconstructed the ZooMS reference spectra for the remaining large whale taxa and assessed their potential for archaeological research on a European level. In particular we focus on the grey whale, the only whale species to have been extirpated from the North Atlantic in historical times. Pre-Hispanic Andean textile specimens have been recovered during excavations in the Mochica culture (CE 100-850) area in modern Peru. These textiles represent a unique biocultural archive with the potential to provide deep-time data regarding the historical exploitation of camelids by past Mochica society. For the creation of these textiles, camelid fibers where used, which can either derive from the domesticated llama (*Lama glama*) or alpaca (*Vicugna pacos*), or their wild ancestors, the vicuña (*Vicugna vicugna*) or guanaco (*Lama guanicoe*). We performed keratin-based palaeoproteomic analysis in order to reconstruct which taxa were used for textile creation by the Mochica culture.

# NOTES

# DISCUSSION SESSIONS

## Discussion Session 1: ZooMS Current Practices and Methodological Advances

## Discussion Session 2: ZooMS and Zooarchaeology

# PARTICIPANTS and ORGANIZERS

| Last Name | First Name | Email | Affiliations |
|---|---|---|---|
| Archer | Will | will.archer@nasmus.co.za | National Museum, Bloemfontein |
| Artizzu | Aurélie | aureatz@outlook.fr | Université Paris 1 Panthéon-Sorbonne |
| Baltazart | Karl | karl.baltazart@univ-lille.fr | Université de Lille |
| Barakat | Sarah | sarah.barakat@abdn.ac.uk | University of Aberdeen |
| Binois-Roman | Annelise | annelise.binois-roman@univ-paris1.fr | Université Paris 1 Panthéon-Sorbonne |
| Brandt | Luise Ørsted | luise.brandt@sund.ku.dk | University of Copenhagen |
| Burnett | Alexandra | alexandra.burnett@ugent.be | Ghent University |
| Codlin | Maria | maria.codlin@gmail.com | University of Turin |
| Collins | Matthew | matthew@palaeome.org | University of Copenhagen; University of Cambridge |
| Daled | Simon | simon.daled@ugent.be | Ghent University |
| De Almeida | Paul | dealmeidapaul@hotmail.fr | Aix-Marseille Université |
| Dekker | Joannes | jan@palaeome.org | University of Copenhagen |
| Dhaenens | Maarten | maarten.dhaenens@ugent.be | Ghent University |
| Engels | Ian | ian.engels@ugent.be | Ghent University |
| Gallo | Giulia | giulia.gallo@college-de-france.fr | Collège de France;<br>Université Toulouse Jean Jaurès |
| Hansen | Jakob | jakob.thestrup.hansen@regionh.dk | University of Copenhagen |
| Heikkilä | Raija Katarina | raija-katarina.heikkila@college-de-france.fr | Collège de France |
| Jean-Jacques | Hublin | jean-jacques.hublin@college-de-france.fr | Collège de France;<br>Max Planck Institute for Evolutionary Anthropology |
| Jiménez Cano | Nayeli | njimenezcano@gmail.com | Muséum national d'Histoire naturelle |
| Jono | Marine | Marine.Jono@etu.univ-paris1.fr | Université Paris 1 Panthéon-Sorbonne |
| Just | Romane | romane.melyssandre@gmail.com | Université Paris 1 Panthéon-Sorbonne |
| Kaesmann | Brieuc | brieuc.kaesmann@ens.psl.eu | Collège de France |
| Kask | Silvia-Kristiin | silviakristiin@gmail.com | University of Tartu |
| Lévêque | Élodie | elodie.leveque@palaeome.org | Université Paris 1 Panthéon-Sorbonne |
| Mladenovic | Teodora | teodoramladenovic25@gmail.com | University of Belgrade |
| Mylopotamitaki | Dorothea | dorothea.mylopotamitaki@palaeoproteomics.org | Collège de France |
| Nikolakopoulou | Nikolitsa | nicole-nik@live.com | University of Tübingen |
| Orton | David | david.orton@york.ac.uk | University of York |
| Peters | Carli | peters@gea.mpg.de | Max Planck Institute of Geoanthropology |
| Presslee | Samantha | sam.presslee@york.ac.uk | University of York |
| Raymond | Pauline | pauline.raymond@college-de-france.fr | Collège de France |
| Rendu | William | William.Rendu@cnrs.fr | International Research Laboratory ZooStan CNRS; Al-Farabi Kazakh National University |
| Ruebens | Karen | k.j.ruebens@reading.ac.uk | University of Reading |
| Sinet-Mathiot | Virginie | virginie.sinetmathiot@palaeome.org | University of Bordeaux |
| Speller | Camilla | camilla.speller@ubc.ca | University of British Columbia |
| Touzet | Helene | Helene.Touzet@univ-lille.fr | Université de Lille; CNRS |
| van der Hurk | Youri | yourivandenhurk@gmail.com | Muséum national d'Histoire naturelle |
| Vidas | Lia | lia.vidas@inantro.hr | University of Zagreb; Institute for Anthropological Research |
| Wagner | Anna | wagner.anna98@gmail.com | University of Reading |
| Welker | Frido | frido.welker@sund.ku.dk | University of Copenhagen |
| Winter | Rachel | rachelwinter@palaeome.org | Université Toulouse III - Paul Sabatier |
| Xia | Huan | xiah@lzu.edu.cn | Lanzhou University |
| Yang | Fei | fy262@outlook.com | University of Tübingen |

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