DISSERTATIONES
BIOLOGICAE
UNIVERSITATIS
TARTUENSIS
462

BIANCAMARIA BONUCCI

Reading the archaeological record through ancient biomolecules: preservation, disease landscapes, and human-microbe interactions in the past

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
462

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
462

BIANCAMARIA BONUCCI

Reading the archaeological record through ancient biomolecules: preservation, disease landscapes, and human-microbe interactions in the past

UNIVERSITY OF TARTU
Press
1632

Institute of Molecular and Cell Biology, Institute of Genomics, University of Tartu, Estonia

The dissertation was accepted for the commencement of the degree of Doctor of Philosophy in Gene Technology on the 19th of June, 2026 by the Council of the Institute of Molecular and Cell Biology, University of Tartu.

Supervisors: Christiana Lyn Scheib, PhD; Research Fellow, Department of Zoology, University of Cambridge, Cambridge, UK

Kristiina Tambets, PhD; Professor of Archaeogenomics, Estonian Biocentre, Institute of Genomics, University of Tartu, Tartu, Estonia

Antonio de Dios Martinez, PhD; Research Fellow in Ancient Metagenomics, Estonian Biocentre, Institute of Genomics, University of Tartu, Tartu, Estonia

Opponent: Camilla Speller, PhD; Associate Professor, Department of Anthropology, University of British Columbia, Vancouver, Canada

Commencement: Room No 105, 23B Riia St, Tartu, on 14th of August, 2026, at 10.15.

Publication of this thesis is granted by the Institute of Molecular and Cell Biology and the Institute of Genomics, University of Tartu.

This research was funded by the European Research Council Advanced Grant “Making Ancestors: The Politics of Death in European Prehistory” (no. 885137); the Ministry of Education and Research Centres of Excellence grant TK215 Estonian Roots: Centre of Excellence for transdisciplinary studies on ethnogenesis and cultural diversity; the European Union though the European Regional Development Fund (Project No. 2014–2020.4.01.16-0030). Data analyses were in part carried out at the High-Performance Computer Center of the University of Tartu.

HARIDUS- JA TEADUSMINISTEERIUM

ISSN 1024-6479 (print)
ISBN 978-9908-57-275-8 (print)
ISSN 2806-2140 (pdf)
ISBN 978-9908-57-276-5 (pdf)

University of Tartu Press
www.tyk.ee

To my parents,
for their sacrifices,
for nurturing my curiosity,
and for always encouraging me
to pursue my dreams.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

Figures

Tables

LIST OF ORIGINAL PUBLICATIONS

I. **Bonucci, B**., de-Dios, T., Barbieri, R., Thompson, J. E., Panella, S., Radina, F., Sivilli, S., Kabral, H., Solnik, A., Tafuri, M. A., Robb, J., & Scheib, C. L. (2025). Ancient microbial DNA and proteins preserve in concretions covering human remains. iScience, 28(8), 113182. https://doi.org/10.1016/j.isci.2025.113182.

II. de-Dios, T., **Bonucci, B.**, Barbieri, R., Kushniarevich, A., D'Atanasio, E., Dittmar, J., Cessford, C., Solnik, A., Robb, J. E., Warinner, C., Oras, E., & Scheib, C. L. (2025). Bone adhered sediments as a source of target and environmental DNA and proteins. Molecular Biology and Evolution, 42(9), msaf202. https://doi.org/10.1093/molbev/msaf202.

III. Beneker, O., Molinaro, L., Guellil, M., Sasso, S., Kabral, H., **Bonucci, B.**, Gaens, N., D'Atanasio, E., Mezzavilla, M., Delbrassine, H., Braet, L., Lambert, B., Deckers, P., Biagini, S. A., Hui, R., Becelaere, S., Geypen, J., Hoebreckx, M., Berk, B., Driesen, P., Pijpelink, A., van Damme, P., Vanhoutte, S., De Winter, N., Saag, L., Pagani, L., Tambets, K., Scheib, C. L., Larmuseau, M. H. D., & Kivisild, T. (2025). Urbanization and genetic homogenization in the medieval Low Countries revealed through a ten-century paleogenomic study of the city of Sint-Truiden. Genome Biology, 26(1), 127. https://doi.org/10.1186/s13059-025-03580-z

IV. de-Dios T.*, **Bonucci B**.*, Thompson J. E., Panella S., Barbieri R., Keller M., Saupe T., Bernardini S., Sasso S., Thirolle M., Solnik A., Kabral H., Aranguren B., Sanz M., Daura J., Lalueza-Fox C., Kivisild T., Metspalu M., Guellil M., Tafuri M. A., Robb J., Scheib C. L. Co-occurrence of *Yersinia pestis* and other zoonoses during European prehistory. bioRxiv doi: https://doi.org/10.64898/2026.02.20.707102.

*These authors contributed equally.

AUTHOR'S CONTRIBUTION TO THE LISTED ARTICLES

The author's contributions to the listed publications are as follows:

REF I: I was part of conceiving the study, performed laboratory work, participated in all data analyses, interpreted results and co-wrote the manuscript.

REF II: I generated figures, participated in the microbial analysis, performed the Dstat, interpreted the results and co-wrote the manuscript.

REF III: I performed laboratory work.

REF IV: I performed laboratory work, participated in all data analyses, generated figures, interpreted results and co-wrote the manuscript.

ABBREVIATIONS

1240k	1240k single nucleotide polymorphism capture panel
AADR	Allen Ancient DNA Resource
aDNA	Ancient DNA
ANGSD	Analysis of Next Generation Sequencing Data
ANI	Average Nucleotide Identity
ANOVA	Analysis of Variance
BAM	Binary Alignment Map
BCE	Before Common Era
BLAST	Basic Local Alignment Search Tool
BLASTP	Basic Local Alignment Search Tool for proteins
BP	Before Present
bp	base pairs
BWA	Burrows-Wheeler Aligner
CAA	Chloroacetamide
cal	calibrated years
CE	Common Era
clr	Centered Log-Ratio
CpG	Cytosine-phosphate-guanine dinucleotide
cRAP	common Repository of Adventitious Proteins
DNA	Deoxyribonucleic acid
DDR	DNA damage response
dsDNA	double-stranded DNA
EDTA	Ethylenediaminetetraacetic acid
eHOMD	expanded Human Oral Microbiome Database
FASP	Filter-aided sample preparation
FDR	False discovery rate
Fst	fixation index
GASP	Gel-aided sample preparation
GATK	Genome Analysis Toolkit
HBV	Hepatitis B virus
HCl	Hydrochloric acid
HHV	Human herpesvirus
HOM	Human Oral Microbiome
HO	Human Origins array
HRC	Haplotype Reference Consortium
HSV-1	Herpes simplex virus type 1
IBD	Identity by descent
IGV	Integrative Genomics Viewer
LC-MS/MS	Liquid chromatography-tandem mass spectrometry

LNBA	Late Neolithic and Bronze Age
MAF	Minor Allele Frequency
MAGs	Metagenome-assembled genomes
MALT	MEGAN Alignment Tool
MAPQ	Mapping quality
MEGAN	MEtaGenome ANalyzer
ML	Maximum likelihood
MTBC	*Mycobacterium tuberculosis* complex
NCBI	National Center for Biotechnology Information
NGS	Next-generation sequencing
nMDS	non-metric multidimensional scaling
PAHs	Polycyclic aromatic hydrocarbons
PCA	Principal Component Analysis
pCD1	Plasmid calcium dependence 1
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
pH	potential of hydrogen
pla	plasminogen activator
PMD	post-mortem damage
pMT1	Plasmid murine toxin 1
PNK	Polynucleotide kinase
pPCP1	Plasmid coagulase/plasminogen activator 1
PSM	Peptide-spectrum match
qPCR	quantitative Polymerase Chain Reaction
RNA	Ribonucleic acid
rRNA	ribosomal RNA
sedaDNA	sedimentary ancient DNA
SMAG	Species-level Metagenome-Assembled Genome catalog
SNP	Single Nucleotide Polymorphism
SP3	Single-pot, solid-phase-enhanced sample preparation
TBE	Transfer Bootstrap Expectation
TCEP	Tris(2-carboxyethyl)phosphine
TEAB	Triethylammonium bicarbonate
TFA	Trifluoroacetic acid
tRNA	transfer RNA
UDG	Uracil-DNA glycosylase
UK10K	United Kingdom 10,000
USER	Uracil-Specific Excision Reagent
UV	Ultraviolet
WHO	World Health Organization
ymt	Yersinia murine toxin

1. INTRODUCTION

Ancient biomolecular research has transformed the way archaeological remains can be studied. Human bones, teeth, sediments, dental calculus, and mineralized deposits are no longer viewed only as anatomical or environmental materials, but archives that may preserve traces of human genomes, microbial communities, pathogens, proteins, dietary evidence, and post-mortem processes (Der Sarkissian et al., 2021; Warinner, 2022; Warinner et al., 2017a). The rise of ancient metagenomics has been particularly important within this framework because it has expanded ancient DNA (aDNA) research beyond host genomes and single target organisms, allowing mixed biological assemblages to be interpreted and studied (Der Sarkissian et al., 2021; Warinner et al., 2017a). At the same time, ancient proteins are a complementary molecular archive, because they may survive in archaeological and palaeontological materials where DNA is poorly preserved (Hendy, 2021; Hendy, Welker, et al., 2018a; Warinner et al., 2022). Together, ancient metagenomics and palaeoproteomics make it possible to move towards a better understanding of past biological environments, human-microbe relationships, diet, disease, and preservation.

This thesis explores the potential and limitations of ancient metagenomics and palaeoproteomics for the study of archaeological materials. In particular, it focuses on how different substrates preserve biomolecular information and how this information can be used to reconstruct past interactions between humans, microbes, animals, and environments. Teeth are especially valuable in the study of the past because a single tooth contains multiple microhabitats that retain different information. For example, dental calculus, a mineralized form of dental plaque, is the richest known source of ancient DNA in the archaeological record (Mann et al., 2018), and it is the only part of the human body that routinely fossilizes during life. It is primarily composed of oral microbes, but can preserve entrapped materials such as dietary microfossils, airborne and waterborne pollutants, host DNA, proteins derived from food consumption, and in general acts as a sink for a range of oral debris (Hendy, Warinner, et al., 2018; Radini et al., 2017, 2019; Warinner et al., 2015; Weyrich et al., 2015). In addition, some pathogens have been found in dental calculus, including *Mycobacterium leprae*, the causative agent of leprosy (Fotakis et al., 2020). Dentine, instead, and especially the inner surface of the dental pulp chamber, is one of the best sources for the retrieval of host and pathogen DNA. Teeth are vascularized during life, with the dental pulp being supplied with blood and nutrients via the root canal. Chronic blood-borne infections and acute events can introduce pathogens into the dental pulp, and pathogen DNA may remain preserved within the pulp cavity after death (Bos et al., 2019; Spyrou et al., 2019; Warinner, 2022). Also, after death oral bacteria contribute to dental decomposition (Mann et al., 2018) and their proteins and DNA can also be found in samples obtained from dentine.

This thesis also aims to investigate less conventional substrates, including concretions adhering to human remains and bone-adhered sediments, showing

that overlooked, and for many years discarded, materials can retain ancient DNA and proteins. Those can provide information on human-associated microbes, human and non-human animals' DNA, and information on depositional environments (REF I; REF II). The four studies included in this dissertation approach ancient metagenomics from different perspectives. The first study evaluates sediment concretions adhering to human remains as potential reservoirs of ancient DNA and proteins, showing that, despite poor human DNA preservation, concretions can preserve oral microbial DNA and human and bacterial peptides (REF I). The second study investigates bone-adhered sediments as a minimally destructive source of genomic and proteomic information, demonstrating that such sediments can preserve host DNA, gut-associated microbial signals, and even environmental biomolecular evidence, including a partial genome and proteome of a medieval black rat, or *Rattus rattus* (REF II). These studies contribute to the growing recognition that archaeological materials are not homogeneous archives, but they are constituted of microenvironments whose biomolecular content depends on substrate formation, mineralization, degradation, contamination, and depositional history (Der Sarkissian et al., 2021; Hendy, Welker, et al., 2018a; Warinner, 2022). Because archaeological samples are finite, these studies open up the possibility of using alternative and less destructive materials to retrieve information about the past, while helping to safeguard our archaeological heritage.

The second part of the thesis applies ancient metagenomics to the study of infectious diseases and zoonotic history. In the medieval cemetery of Sint-Truiden (city in what is now Belgium), metagenomic screening revealed multiple pathogen signals, including hepatitis B virus (HBV), Borrelia recurrentis, Herpes simplex virus (HSV-1), and *Yersinia pestis* (REF III). The detection of *Y. pestis* in fourteenth-century individuals is especially significant because written sources do not record plague in Sint-Truiden at that time, demonstrating that the absence of historical documentation should not automatically be interpreted as the absence of disease (REF III). The final study investigates the co-occurrence of *Yersinia pestis* and *Erysipelothrix rhusiopathiae* in prehistoric Europe, contributing to current discussions on the diversity of Late Neolithic and Bronze Age *Y. pestis*, and the role of zoonotic pathogens in contexts of increasing human-animal contact (REF IV). Together, these studies show that ancient metagenomics is not only a tool for identifying individual infections, but also a way to reconstruct broader infectious landscapes and ecological relationships among humans, animals, microbes, and environments.

By combining ancient metagenomics and proteomics, and inserting them in the archaeological context, this work highlights both the promise and the interpretive challenges of studying complex biomolecular archives. As shown by the studies presented here, these approaches can recover information from both traditional and overlooked substrates, while also revealing biological processes that may remain invisible in skeletal evidence or written sources (REF I–IV).

2. LITERATURE OVERVIEW

Humans have always had a complex relationship with microbes. From coopting them to release nutrients from food or unlock psychoactive chemicals, to coping with the diseases caused by falling out of balance with them, microbes are invisible figures that have profoundly shaped human existence. This literature overview highlights the state of the art in microbial archaeology and reflects upon the question 'what can archaeological remains tell us about our microbial past?'

2.1 A microbial world

Microorganisms, or microbes, are a diverse range of organisms that are not visible to the naked eye. The existence of unseen life was suspected for centuries: Marcus Terentius Varro in a 1st-century BCE book entitled *De Re Rustica* (On Agriculture) indicated the possibility of diseases spreading by unseen organisms, which he called *animalia minuta* (Varro, 1934). It was the Dutch merchant Antonie van Leeuwenhoek who was the first to observe and describe microorganisms, or "little animals", using a single-lensed microscope of his own design in 1677 (Corliss, 2002). But the importance of microorganisms to human health was not fully appreciated until almost 200 years later, when Pasteur and Koch first cultured bacteria from diseased tissues (Opal, 2009).

Now we know that microbes include bacteria, archaea, fungi, protists, microscopic animals, and microscopic plants. Microbes occupy every imaginable ecological niche across diverse environments, including soil, air, shallow waters, and deep seas. They recycle nutrients, degrade pollutants and perform photosynthesis. Despite being microscopic and easy to overlook, microorganisms influence a lot of aspects of our daily lives. More than half of the cells in the human body are, in fact, bacterial ones (Sender et al., 2016a, 2016b). Our gut microbiome, composed of 10^{13} to 10^{14} bacterial and archaeal cells, helps us process food, some of the microbes that constitute it are fundamental for the production of B and K vitamins (LeBlanc et al., 2013; Warinner, 2022). Some microbes, such as S*accharomyces cerevisiae* or *Lactobacillus delbrueckii*, are also key actors in the fermentation processes of what we eat and drink, they are the ones that convert grapes to wine, milk to yogurt and more (Bourdichon et al., 2012, 2021).

Humans and animals have always had a complex relationship with microbes. They have been important in shaping who we are, our evolution, and have been key actors in historical and prehistoric events. By focusing on microbes and the biomolecular traces they left, we can get a better understanding of our shared past.

2.2 Ancient DNA as the foundation of biomolecular archaeology

2.2.1 Ancient DNA, from the first steps to high-throughput sequencing

Ancient DNA is genetic material that can be extracted from ancient remains dating from decades to tens of thousands of years.

The source of aDNA can be as varied as the traces that living organisms leave behind, from skeletal remains and mummified tissues to cave sediments, dental plaque, and fossilized feces. The advancement that aDNA has made in the past few decades has opened doors to countless new research questions concerning our history.

In 1984, two short fragments of mitochondrial DNA, comprising 229 nucleotide pairs, were sequenced from a quagga, a now extinct zebra-like species (*Equus quagga*) from a museum specimen by Higuchi and colleagues (Higuchi et al., 1984). In this study, the extracted quagga DNA was cloned through bacterial plasmids (*E. coli*), inserts that were then sequenced by the Sanger sequencing method (Higuchi et al., 1984), which itself was a relatively new technology, developed by Fredrick Sanger and colleagues in 1977 (Sanger et al., 1977). The extraction and sequencing of this extinct species' DNA is considered the first retrieval and sequencing of an aDNA molecule (Orlando et al., 2021). Over the next few years, through investigations of mummified specimens, Svante Pääbo showed that the preservation of DNA was not possible only in museum specimens but could be replicated in different mummified human samples dated to several thousand years (Pääbo, 1985a, 1985b, 1986). Although it later turned out that these very first DNA sequences he retrieved were surely contamination from present-day people (Zhao, 2024), these first works opened the doors to a revolution.

The development of next-generation sequencing (NGS) transformed the situation. Unlike Sanger sequencing or PCR-based assays, NGS made it possible to sequence millions of short DNA fragments in parallel, an approach particularly well suited to ancient DNA because ancient molecules are usually highly fragmented and chemically damaged (Orlando et al., 2021). In combination with improved library preparation, indexing, capture enrichment, and bioinformatic methods, high-throughput sequencing enabled researchers to move from the detection of single diagnostic fragments to genome-scale reconstruction.

This shift also changed the scale of ancient microbiome research. Early microbiome studies often relied on PCR amplification of marker genes such as 16S rRNA, which provided broad taxonomic inventories but limited information on strain-level diversity or function. It was also demonstrated that extensive length polymorphisms in the V3 region were a cause of differential amplification and lead to taxonomic bias in ancient microbiome reconstructions (Ziesemer et al., 2015). Shotgun metagenomics made it possible to analyse all DNA preserved in a sample, allowing researchers to reconstruct microbial community composition, functional potential, and sometimes individual microbial genomes from substrates

such as dental calculus, palaeofaeces, skeletal remains, and sediments (Orlando et al., 2021; Warinner et al., 2015; Warinner, Rodrigues, et al., 2014; Ziesemer et al., 2015). This has moved ancient DNA research from the study of isolated short sequences toward genome-wide and metagenomic approaches capable of investigating DNA from different sources within the same methodological framework.

2.2.2 How does DNA degrade

Every cell of the human body is affected by thousands of DNA lesions each day (Lindahl & Barnes, 2000). Some DNA errors are caused by physiological processes, such as DNA mismatches that can occur during DNA replication or DNA strand breaks caused by abortive topoisomerase activity (Jackson & Bartek, 2009). In addition to this, hydrolytic reactions and non-enzymatic methylations cause lesions to the DNA in the cells every day. DNA damage is also produced by reactive-oxygen compounds that are by-products of oxidative respiration or through redox-cycling events that involve environmental toxic agents (Valko et al., 2006). Reactive oxygen and nitrogen compounds are also produced by macrophages and neutrophils at sites of inflammation and infections (Kawanishi et al., 2006). Also environmental factors such as ultraviolet (UV) light, mycotoxins, polycyclic aromatic hydrocarbons (PAHs) and ionizing radiation can cause DNA strand breaks. In mammalian cells, DNA damage activates mechanisms that arrest the cell cycle in order to allow the repair of the DNA, or if that is too severe to induce cell death, most commonly by apoptosis(Plesca et al., 2008). These mechanisms evolved to fight the threats posed to DNA, are collectively called DNA-damage response (DDR) that detect DNA lesions, signal their presence and promote their repair (Harper & Elledge, 2007).

However, as an organism dies, these enzymatic repair mechanisms that maintain genomic integrity during life cease to function. Already in 1993 Lindahl examined postmortem damage in detail (Lindahl, 1993). He presented a theoretical model of DNA degradation based on the chemical properties of DNA, identifying hydrolytic and oxidative decomposition as the most likely types of damage that accumulate in aDNA. A few years later, Hofreiter and colleagues (Hofreiter et al., 2001) noted that the excess of C to T and G to A miscoding lesions in PCR-amplified DNA was likely a result of deamination of cytosine residues in aDNA template molecules. The first step of DNA damage is usually a hydrolytic attack of the bond that attaches the nitrogenous base of a purine to the nucleotide's deoxyribose sugar. This process is called depurination, as this step leads to the removal of G and A bases, producing abasic sites along the DNA molecule. These abasic sites make the phosphate backbone more exposed and thus susceptible to attack. Next, cleavage of the phosphate bone occurs and usually this happens at the 3' abasic sites, a process called nicking. Because they only affect one strand at any given position, these are called single-stranded nicks. As these nicks accumulate along the DNA molecules, the DNA becomes destabilised and the hydrogen bonds between the complementary bases become the primary force

holding the DNA together. When two nicks form close to each other, the hydrogen bonds between the nicks may not be strong enough to hold the DNA together and the helix may separate in a process known as melting (Fellows Yates et al., 2025).

Over time, the effect of nicking over the DNA molecule causes a high degree of fragmentation. Once the DNA is fragmented, nucleotides within the single stranded overhangs become more exposed to further chemical attacks. The amine group of the cytosine nitrogenous base is particularly vulnerable to hydrolytic attack when not hydrogen-bonded to its complementary guanine, and it undergoes cytosine deamination. During this process, the ammine group (NH2) is lost and the cytosine base is turned into uracil, which is usually found in RNA, not DNA.

Although cytosine deamination may occur at any time in DNA, the rate of its formation is higher in single-stranded DNA (Frederico et al., 1993; Lindahl & Nyberg, 1974). Consequently, aDNA molecules tend to accumulate uracils at the fragment ends (Bokelmann et al., 2020). However, as most DNA polymerases are not able to correctly recognize uracils, they misread uracils as adenines and incorporate a thymine on the opposite strand during DNA amplification. During each subsequent amplification cycle, the misincorporated T will propagate across the subsequent copies of the original DNA molecule. This process produces the characteristic patterns of C to T transitions observed in aDNA datasets, as well as the corresponding G to A transitions in the complementary strand (Fellows Yates et al., 2025).

One important variant of the cytosine deamination process occurs when the cytosine is methylated, as is common in CpG islands involved in epigenetic DNA regulation. Methylated cytosines can also undergo deamination, and when that occurs the cytosine turns into a thymine, instead of a uracil.

2.2.3 Characterising and authenticating aDNA damage

Although aDNA was long known to be degraded, its true length distribution and damage structure remained poorly understood before the rise of NGS. Early PCR-based studies typically targeted fragments of approximately 100–500 bp, partly because shorter molecules were difficult to extract, visualize, and amplify with the methods then available. This created the misleading impression that aDNA was present in low abundance but often retained moderate fragment length. Dabney, Meyer, and Pääbo (Dabney et al., 2013) later emphasized that aDNA is usually much shorter and that earlier laboratory protocols systematically failed to recover a large proportion of the shortest endogenous molecules.

The field changed decisively with the adoption of high-throughput sequencing, because short ancient molecules could now be sequenced directly after adapter ligation without requiring long intact PCR targets. Briggs and colleagues, working on Neanderthal DNA, showed that purines were overrepresented immediately adjacent to strand breaks, consistent with depurination-driven fragmentation (Briggs et al., 2007). They also demonstrated the now well known excess of

C to T substitutions at the 5′ ends of fragments and complementary G to A substitutions at the 3′ ends. This pattern, often visualized as the "smile plot" (Figure 1), provided one of the clearest empirical demonstrations that postmortem damage follows recognizable and interpretable molecular rules (Briggs et al., 2007).

The recognition that damage is predictable transformed it from a problem into a tool. In modern aDNA research, damage profiles are central to authentication because they help researchers distinguish genuine ancient molecules from modern contamination. This principle underlies software such as mapDamage 2.0 and PMDtools, which quantify terminal substitutions and fragmentation patterns in order to estimate authenticity and preservation state (Jónsson et al., 2013; Skoglund et al., 2014). Damage is therefore not simply a consequence of decay; it is one of the most important criteria by which aDNA is identified as ancient.
At the same time, damage is not a straightforward molecular clock. (Allentoft et al., 2012) showed that DNA decay rates are highly temperature-dependent, and this means that a younger sample from a warm environment may be more damaged than an older sample from a cold one. Damage patterns are therefore most useful in relative rather than absolute terms: they help establish authenticity and compare preservation, but they cannot on their own provide a simple measure of sample age across different depositional contexts (Allentoft et al., 2012).

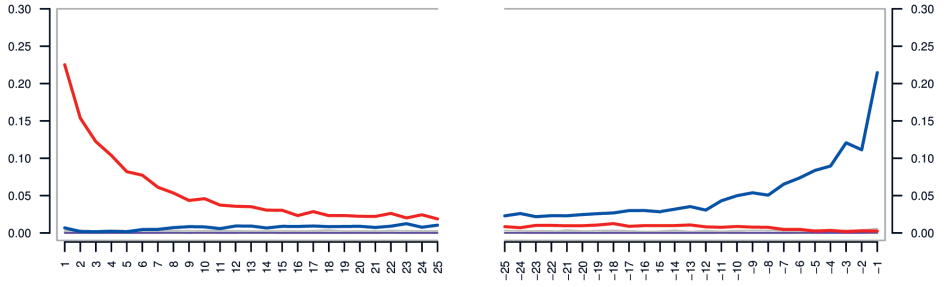

Figure 1: Characteristic aDNA damage pattern.

Colloquially known as the "smiley plot", it represents the output of the MapDamage2 program. The red line represents the C to T misincorporation rate from the 5' end on the forward read, while the blue line represents the G to A misincorporation rate from the 3' end on the reverse read. Adapted from MapDamage plots (Jónsson et al., 2013).

2.3 The ancient metagenomics revolution: from single genomes to mixed DNA assemblages

Ancient metagenomics can be defined as the study of the total DNA content of samples that have degraded over time (Fellows Yates, Andrades Valtueña, et al., 2021; Warinner et al., 2017a). Ancient metagenomics has emerged as one of the most important extensions of biomolecular archaeology, because it expands the focus of aDNA research beyond host genomes and single target organisms. In recent years, the field of aDNA has undergone a shift. In fact, rather than treating

microbial sequences as incidental background to human aDNA, in the last 10–15 years researchers have argued that they should be understood as part of the archaeological framework in which biomolecular data are interpreted through context, taphonomy, preservation, and depositional history (Bozzi et al., 2024; Der Sarkissian et al., 2021; Warinner et al., 2017a).

In this respect, ancient metagenomics occupies an intermediate space between environmental genomics, paleopathology, forensics and archaeological sciences. It is at the same time a study of past microbes, of preservation environments, and of the histories of the sources from which biomolecules are recovered. Most archaeological samples preserve mixtures of host biomolecules, commensal microbes, environmental organisms, post-mortem colonizers, and modern contaminants. The interpretive challenge is therefore not only taxonomic identification, but the disentangling of overlapping biological and depositional processes. This problem is precisely why ancient metagenomics has become so relevant to archaeology: it forces explicitly contextual reasoning about burial, preservation, material association, and post-depositional change (Der Sarkissian et al., 2021).

2.4 Proteins as a complementary data source

Proteins provide an important complementary archive to aDNA because they can persist in archaeological and palaeontological materials under conditions where DNA is poorly preserved. Since the first reports of amino acids in fossils, ancient protein research has shown that proteins are not immune to degradation, but that their chemical structure, abundance, tissue context, and association with mineral matrices can allow some peptides to survive over long timescales (Abelson, 1954; Goodfriend & Weidman, 2001; Hendy, Warinner, et al., 2018; Miller & Wyckoff, 1968; Paterson et al., 2025; Warinner, 2022). Peggy Ostrom was the first to improve the sequence coverage of ancient proteins peptide mass fingerprinting and liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Ostrom et al., 2006). With the development use of high-sensitivity mass spectrometry, palaeoproteomics has become increasingly important for archaeology and evolutionary biology, enabling the study not only of individual proteins, but also of complex proteomes and metaproteomes preserved in archaeological materials (Hendy, 2021; Hendy, Welker, et al., 2018b).

Protein preservation is highly selective and depends on both molecular properties and depositional context. Mineralized substrates are particularly important because mineral matrices can protect proteins from rapid degradation and facilitate their long-term survival. This has been demonstrated in ostrich eggshell, where mineral-bound proteins have survived for millions of years, and in dental enamel, which can preserve phylogenetically informative proteins beyond the temporal range usually accessible to aDNA (Cappellini et al., 2019; Demarchi et al., 2016).

In archaeological contexts, proteins have been recovered from bone, dentine, enamel, dental calculus, parchment, ceramics, palaeofaeces, and eggshell, but each

substrate preserves different classes of proteins and therefore supports different kinds of interpretation (Hendy 2021; Warinner et al. 2022). Collagen remains one of the most commonly recovered ancient proteins because of its abundance, structure, and association with bone mineral, while enamel proteins and dental calculus proteins can provide information on taxonomy, diet, host biology, oral microbiomes, and immune responses (Welker et al. 2015; Mackie et al. 2017; Cappellini et al. 2019; Hendy 2021).

Like aDNA, ancient proteins are affected by diagenesis. They are progressively fragmented and chemically modified through processes such as hydrolysis, oxidation, deamidation, and backbone cleavage, and these modifications vary according to protein structure, burial environment, temperature, pH, water availability, and microbial activity (Hendy, Welker, et al., 2018a; Warinner et al., 2022). Deamidation of glutamine and asparagine is often used as one line of evidence for protein degradation and antiquity, but it cannot be treated as a simple molecular clock because its rate depends on sequence context, three-dimensional structure, and environmental conditions (Ásmundsdóttir et al., 2026; Hendy, Welker, et al., 2018a; Warinner et al., 2022). For this reason, palaeoproteomic interpretation requires careful attention to substrate formation, contamination, peptide specificity, degradation patterns, and the archaeological context in which proteins are recovered.

2.5 Methods in ancient metagenomics and ancient proteomics

2.5.1 From archaeological material to sequencing

Ancient metagenomic work should begin in physically isolated clean-room facilities, because ancient substrates usually contain low quantities of DNA mixed with environmental, handling-derived, and laboratory-derived microbial DNA (Hendy et al., 2018; Hübler et al., 2019). Sampling is thus performed using protective clothing, surface cleaning, UV irradiation or chemical decontamination where appropriate, and extraction and library blanks to monitor contamination introduced during laboratory processing (Hendy et al., 2018; Hübler et al., 2019). Sampling protocols and sample quantities highly depend on the material under study, but all the protocols for DNA extraction from archaeological bones, teeth, and sediment are optimized for highly fragmented molecules, often using decalcification followed by silica-based purification to recover short or ultrashort DNA fragments (Rohland et al., 2018; Rohland & Hofreiter, 2007).

After extraction, aDNA molecules are converted into sequencing libraries, most commonly using double-stranded library preparation or, for highly degraded material, single-stranded library preparation that can improve recovery of short and damaged molecules (Gansauge & Meyer, 2013; Meyer & Kircher, 2010). In 2010, Briggs and colleagues developed the first enzymatic process to remove damage signatures from aDNA fragments, focusing initially on aDNA from

mammoths and Neanderthals (Briggs et al., 2010). With this method, aDNA fragments are phosphorylated using polynucleotide kinase (PNK) and then treated with the Uracil-Specific Excision Reagent (USER), a mixture of two enzymes. The first enzyme, uracil-DNA glycosylase (UDG), locates and removes uracils from the DNA fragments, leaving abasic sites. The second enzyme, endonuclease-VIII, then clips the phosphate backbone at these sites. USER treatment can be very useful because it removes all of the uracils from a DNA extract, but it also shortens the DNA molecules. UDG or partial-UDG treatment may be used to reduce cytosine-deamination-derived sequencing errors, while half-UDG treatment preserves terminal damage patterns that are useful for authenticating aDNA molecules (Briggs et al., 2010; Rohland et al., 2015).

Shotgun sequencing is useful when the aim is broad microbial or metagenomic screening, whereas deeper sequencing or targeted enrichment can be used when a specific pathogen or low-abundance taxon is suspected (Hübler et al., 2019; Pochon et al., 2023). Targeted enrichment is a laboratory approach used when a particular taxon is suspected but present at low abundance. After sequencing libraries are prepared, synthetic DNA or RNA probes complementary to the target sequences are hybridized to the library molecules; the bound target molecules are then pulled down, amplified, and sequenced at higher relative abundance. This approach can substantially increase the recovery of low-endogenous or low-abundance ancient DNA and has been used for both human and ancient pathogen studies (Carpenter et al., 2013; Maricic et al., 2010; Schuenemann et al., 2011). To note is that targeted enrichment depends on probe design and prior knowledge of the target, it is not suitable for metagenomic community profiling, and needs careful evaluation of time and temperature of hybridization (Carpenter et al., 2013; Warinner et al., 2017b).

2.5.2 Bioinformatics methods in ancient metagenomics

Bioinformatic analysis of ancient metagenomic data begins with preprocessing steps to reduce technical noise and keep informative sequences for downstream analysis. These steps commonly include demultiplexing, adapter trimming, merging of overlapping reads, filtering for quality and length, duplicate removal, mapping, and taxonomic classification. Because ancient DNA molecules are usually short, damaged, low in abundance, and mixed with DNA from many biological sources, preprocessing choices can strongly influence the final composition of the dataset. For this reason, reproducible workflows such as PALEOMIX and nf-core/eager (Figure 2) are commonly used in ancient DNA and ancient metagenomic studies, because they allow standardized processing and reproducibility across datasets (Fellows Yates, Lamnidis, et al., 2021; Schubert et al., 2014).

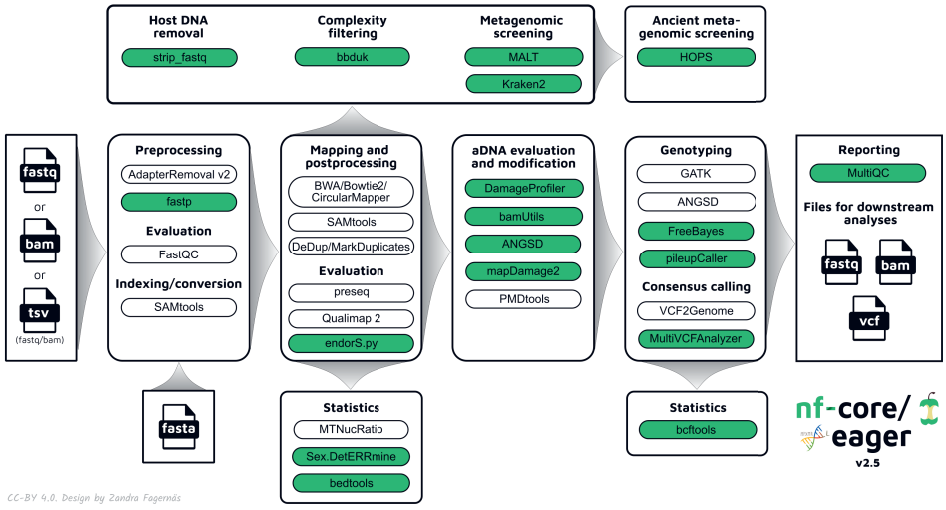

Figure 2: Overview diagram of the main steps and tools of the nf-core/eager pipeline.

The pipeline can take sequencing data in FASTQ or BAM format, or a TSV file specifying FASTQ/BAM inputs. Raw reads can first undergo preprocessing, including adapter removal and quality trimming, followed by quality evaluation with and indexing or file conversion with SAMtools. Upstream steps include host DNA removal with strip_fastq, complexity filtering with bbduk, and metagenomic screening with MALT or Kraken2, which can be followed by ancient metagenomic screening with HOPS. Processed reads are then mapped to a reference genome using BWA, Bowtie2 or CircularMapper, followed by post-processing with SAMtools and duplicate removal with DeDup or MarkDuplicates. Mapping quality and library complexity can be assessed using preseq, Qualimap 2 and endoR.py, while additional statistics can be generated with MTNucRatio, Sex.DetERRmine and bedtools. Ancient DNA authenticity and damage patterns can be evaluated and modified using DamageProfiler, bamUtils, ANGSD, mapDamage2 and PMDtools. Downstream analyses include genotyping with GATK, ANGSD, FreeBayes and pileupCaller, consensus calling with VCF2Genome and MultiVCFAnalyzer, and variant statistics with bcftools. Final quality-control summaries are collected in MultiQC, and the pipeline produces files for downstream analyses, including FASTQ, BAM and VCF outputs. Licensed under CC BY 4.0, designed by Zandra Fagernäs.

Taxonomic classification is then used to answer the question ‘what is there’, and identify the organisms represented in a sample.This can rely on nucleotide-level k-mer-based tools such as Kraken2 (D. E. Wood et al., 2019) and KrakenUniq (Breitwieser et al., 2018), or on alignment-based approaches such as BLAST (Altschul et al., 1990), MALT (Herbig et al., 2016) and MEGAN (Huson et al., 2007). Taxonomic profiling can also use amino-acid-level classifiers, such as Kaiju, which compares translated reads against protein databases (Menzel et al., 2016) , or DIAMOND, which allows fast protein-level searches and can be

useful for functional or distant-homology analyses (Buchfink et al., 2015). Marker-gene-based approaches, such as MetaPhlAn, estimates microbial community composition from clade-specific marker genes (Segata et al., 2012). In ancient metagenomics all taxonomic assignments should be treated as preliminary and need further validation, because they can vary depending on the taxonomic classifier, and because short and damaged reads may be misassigned, especially when closely related taxa share conserved genomic regions or when the true source organism is absent from the reference database (Pochon et al., 2023; Warinner et al., 2017b)

After taxonomic profiling, microbial diversity analyses can be used to compare community composition between samples. Alpha diversity describes diversity within a sample, while beta diversity describes differences between samples, often using measures such as Bray-Curtis, Jaccard, or Euclidean distance, and ordination methods such as Principal Component Analysis (PCA), Principal Coordinate Analysis (PCoA), or non-Metric Multidimensional Scaling (nMDS) (McMurdie & Holmes, 2013). In ancient metagenomics, abundance tables need to be interpreted cautiously because read counts are compositional and affected by sequencing depth, genome size, mappability, database composition, preservation, contamination, and laboratory processing. Therefore, low-abundance and low-prevalence taxa should be filtered, negative controls should be assessed, and transformations such as centred log-ratio (clr) transformation can be used when compositional assumptions are relevant (Aitchison, 1986).

In ancient metagenomics it is important for abundance tables to be filtered, negative controls should be assessed, and contaminant-detection tools such as decontam (Davis et al., 2018) and source-tracking tools as SourceTracker2 (McGhee et al., 2020) can be used to evaluate the possible origin of microbial signals and whether it is a true or a false positive hit.

For candidate microbial taxa, taxonomic classification alone is not sufficient. Candidate hits are usually validated by remapping reads to one or more reference genomes using short-read aligners such as Burrows-Wheeler Aligner (BWA) (Li & Durbin, 2009), Bowtie2 (Langmead & Salzberg, 2012), and many others. Mapper choice and parameter settings are especially important because short fragment length, post-mortem damage, and reference divergence can affect read alignment and downstream interpretation (Key et al., 2017; Warinner et al., 2017a). This allows researchers to assess number of reads, duplication, edit distance, fragment length distribution, breadth and depth of coverage, evenness of coverage, edit distance decay, Average Nucleotide Identity (ANI) (Hübler et al., 2019; Pochon et al., 2023). Authentication also relies on aDNA damage patterns, especially terminal C to T and G to A substitutions, which can be assessed with tools such as mapDamage (Jónsson et al., 2013), PMDtools (Skoglund et al., 2014), PyDamage (Borry et al., 2021), and MetaDamage(Everett & Cribdon, 2023) specifically for sedimentary ancient DNA (sedaDNA). These tools help assess whether the organism is, indeed, ancient and present in the sample. Subsequently, the genomes can be assessed for their gene content, in order to perform comparative

identification of virulence genes over their evolutionary time frames (Spyrou et al., 2019; Vågene et al., 2018).

When sufficient data are recovered, microbial genome reconstruction and comparative genomic analysis can be performed. This may include consensus genome calling, single-nucleotide polymorphisms (SNPs) identification, gene-content analysis, and screening for virulence-associated genes, plasmids, anti-microbial resistance markers, or other functional elements. In ancient pathogen studies, gene-content analysis can be important for understanding how virulence-associated traits changed through time, as shown in studies of *Y.pestis* (Spyrou et al., 2019), among others. However, the absence of a gene in a low-coverage ancient genome must be interpreted cautiously, because it may reflect poor preservation, or reference bias instead of a true absence.

Phylogenetic analysis can place ancient strains in an evolutionary context. However, reliable phylogenetic inference requires enough authentic DNA to recover informative genomic positions, sufficient breadth and evenness of coverage, limited contamination, and confident distinction from closely related taxa. For suitable datasets, tree reconstruction is commonly based on SNP alignments, with maximum-likelihood (ML) tools such as IQ-TREE2 (Minh et al., 2020) and RAxML-NG (Kozlov et al., 2019) often used as tools for tree building. BEAST2 (Bouckaert et al., 2019) is a tool commonly used for Bayesian time calibration and evolutionary rate estimation. Microbial phylogenetics also requires attention to recombination, because horizontally transferred regions and mobile elements can distort branch lengths and tree topology. Therefore, analyses often require filtering out recombinant regions, repetitive or poorly mappable regions, low-quality SNPs, homoplasy, and sites affected by excessive missingness, and of course post-mortem damage, using tools such as bcftools (Danecek et al., 2021) , Gubbins (Croucher et al., 2015) and ClonalFrameML (Didelot & Wilson, 2015).

In addition to microbial phylogenetic and comparative genomic approaches, population-genetic analyses can be useful when ancient metagenomic data contain sufficient host, or any other eukaryotic DNA. This may be relevant, for example, when non-human animal taxa are identified in archaeological sediments, dental calculus, coprolites, or other ancient metagenomes. In such cases, taxonomic identification can be followed by mapping reads to an appropriate reference genome and assessing whether enough informative genomic positions are covered to support population-level analysis. Depending on data quality and reference availability, analyses may include sex determination, PCA, outgroup $f3$ statistics, D-statistics, or related methods originally developed for ancient human population genomics (Patterson et al., 2006, 2012; Skoglund et al., 2013). PCA can be used to project low-coverage ancient individuals onto variation described by modern and ancient reference populations, helping to evaluate broad genetic affinities. D-statistics can test whether a sample shares more alleles with one reference population than another. Genotype imputation can also be used in some cases to increase the number of analysable variants in low-coverage ancient genomic data using tools such as Beagle (Browning et al., 2018) or GLIMPSE (Rubinacci et al.,

2021). This can improve the resolution of downstream population-genetic analyses and has been evaluated specifically for low-coverage ancient genomes.

In general, ancient metagenomic bioinformatics workflows proceed from broad screening to increasingly strict validation.

2.5.3 Ancient protein extraction

Similar to aDNA analysis, the extraction and authentication of ancient proteins are challenging. Usually there are two approaches to extract proteins from ancient specimens: bottom-up and top-down. Top-down proteomics is used very little to analyse ancient proteins because of analytical difficulties (Cleland et al., 2021). Bottom-up proteomics, also called 'shotgun', involves ancient proteins being digested by enzymes, of which the most common one is trypsin.

In order to release the bound protein in some archeological material such as bones, dental calculus, eggshells and teeth, a demineralisation step is required (Warinner et al., 2022). This is usually achieved by using a weak acid such as EDTA or cold hydrochloric acid (HCl) (Palmer et al., 2021). This allows the proteins to be released from the mineral matrices. To make ancient proteins soluble, detergents, sonication, chaotropic agents, and other buffers are used (Warinner et al., 2022). After demineralisation and protein solubilisation, there is the need to perform buffer exchange so that extracts can be compatible with downstream analysis. There are three main ways in which this is done for ancient proteins, the GASP protocol uses gels, the FASP uses filters and SP3 uses magnetic beads.

Afterwards, extracts are incubated with digestion enzymes (usually trypsin) to generate peptides suitable for LC-MS/MS analysis (Hendy, Welker, et al., 2018a; Warinner et al., 2022). Peptides are then purified, desalted, separated by liquid chromatography, and introduced into the mass spectrometer, where precursor ions and their fragment ions are measured (Hendy, Welker, et al., 2018a; Warinner et al., 2022). The resulting spectra provide the basis for peptide and protein identification, but they do not by themselves identify proteins without computational comparison against reference databases (Hendy, Welker, et al., 2018a; Warinner et al., 2022). For non-mineralised archaeological materials demineralisation is not necessary, and the protocols are changed based on sampling size and sample preservation (Fiddyment et al., 2019).

2.5.4 Ancient protein identification and data analysis

Contamination control is essential throughout ancient protein workflows because proteins found may derive from handling, laboratory reagents, conservation treatments, or carry-over between mass-spectrometry runs (Hendy, Welker, et al., 2018a; Ramsøe et al., 2020; Warinner et al., 2022). For this reason, extraction blanks, instrument blanks, contaminant databases, careful documentation of sampling and laboratory protocols are important components of palaeoproteomic analysis (Hendy, Welker, et al., 2018a; Ramsøe et al., 2020; Warinner et al., 2022).

After LC-MS/MS acquisition, ancient proteomic data are analysed by matching experimental spectra to theoretical peptide spectra generated from protein sequence databases (Hendy, Welker, et al., 2018a; Warinner et al., 2022). This process is usually performed with database-search engines such as Mascot, MaxQuant, PEAKS, pFind, MetaMorpheus, FragPipe, or related tools (Rodriguez Palomo et al., 2024). Different search engines can produce different identifications from the same ancient dataset, so search strategy, parameter choice, and reporting transparency are particularly important in the field (Hendy, Welker, et al., 2018a; Rodriguez Palomo et al., 2024).

Peptide-spectrum matches (PSMs) are usually filtered using false-discovery-rate (FDR) approaches, often based on target-decoy database searching (Elias & Gygi, 2007; Hendy, Welker, et al., 2018a; Warinner et al., 2022). FDR control is necessary because mass-spectrometry database searches can generate good looking but incorrect matches, particularly when used for low-abundance, degraded, and taxonomically complex archaeological samples(Elias & Gygi, 2007; Hendy, Welker, et al., 2018a; Warinner et al., 2022).

Another central problem in palaeoproteomics and metaproteomics derives from the fact that many peptides are shared across homologous proteins, conserved protein families, and closely related taxa (Warinner et al., 2022). This means that the identification of a peptide does not always allow confident assignment to a single protein, species, or biological source (Aebersold et al., 2005; Hendy, Welker, et al., 2018a; Mesuere et al., 2018). The problem is particularly important in ancient metaproteomic samples, where proteins from humans, microbes, animals, plants, diet, soil, and contaminants may be present in the same dataset (Hendy, 2021; Hendy, Welker, et al., 2018a; Warinner et al., 2022). The choice of reference database strongly influences ancient protein identification (Hendy, Welker, et al., 2018a; Warinner et al., 2022). Narrow databases can miss proteins from unsampled or extinct organisms, whereas overly broad databases can increase search time, taxonomic ambiguity, and false-discovery problems (Hendy, Welker, et al., 2018a; Rodriguez Palomo et al., 2024; Warinner et al., 2022). For this reason, archaeological proteomic studies often use combinations of curated protein databases, taxon-specific databases, contaminant databases such as cRAP, and specialized databases such as the Human Oral Microbiome Database when analysing dental calculus or oral-associated samples (Hendy, Welker, et al., 2018a; Warinner et al., 2022).

After peptide identification, taxonomic and functional interpretation happen. Peptides are evaluated for sequence specificity, spectral quality, taxonomic uniqueness, reproducibility, absence or low abundance in blanks, and consistency with the archaeological substrate (Hendy, Welker, et al., 2018a; Ramsøe et al., 2020; Warinner et al., 2022). In metaproteomic datasets, tools such as Unipept can support peptide-based taxonomic and functional annotation, but these assignments remain dependent on database completeness and on the presence of diagnostic peptides(Mesuere et al., 2018; Warinner et al., 2022). Authentication of ancient proteins depends on multiple lines of evidence rather than on a single criterion (Hendy, Welker, et al., 2018a; Ramsøe et al., 2020; Warinner et al., 2022).

Degradation patterns such as glutamine and asparagine deamidation can support the antiquity of a protein signal, but deamidation should not be treated as a simple molecular clock because rates vary according to protein structure, peptide position, burial environment, temperature, pH, and laboratory treatment (Ramsøe et al., 2020, 2021; Warinner et al., 2022). Reliable interpretation should therefore combine peptide-spectrum quality, taxonomic specificity, reproducibility, blank controls, degradation patterns, and archaeological plausibility (Hendy, Welker, et al., 2018a; Ramsøe et al., 2020; Warinner et al., 2022).

2.5.5 Metadata, databases, reproducibility

In ancient metagenomics and ancient proteomics, metadata, databases, and reproducibility are particularly important topics because results are highly dependent on technical choices made during laboratory processing, bioinformatic analysis, and database searching. As a result of the aforementioned characteristics of ancient metagenomics, taxonomic assignments can change depending on the reference database, classifier, alignment parameters, filters, abundance thresholds, and contaminant-screening strategy used. Studies evaluating ancient metagenomic classification have shown that ancient DNA characteristics can strongly affect taxonomic assignment, making transparent reporting of analytical parameters essential (Eisenhofer & Weyrich, 2019). Community resources such as AncientMetagenomeDir were developed to standardise published ancient metagenomic sample metadata, making datasets easier to find, compare, and reuse (Fellows Yates, Andrades Valtueña, et al., 2021). Platforms such as protocols.io help address the limitation of reproducibility by providing a citable and versioned space for sharing step-by-step experimental and computational methods (Teytelman et al., 2016).

For ancient proteomics, reproducibility similarly depends on reporting sample preparation, digestion protocols, LC-MS/MS settings, search engines, protein reference databases, peptide-spectrum matching criteria, false discovery rate thresholds, and post-translational or diagenetic modifications. Public repositories such as PRIDE and the ProteomeXchange consortium provide infrastructure for depositing raw and processed mass spectrometry data, supporting transparency, reanalysis, and comparison across studies (Deutsch et al., 2017; Perez-Riverol et al., 2022).

In both fields, the publication of raw data, controls, metadata tables, code, software versions, protocols and reference databases used is therefore not only a technical requirement, but also an ethical one, because destructive sampling of ancient material should produce data that remains reusable beyond a single study. Recent discussions in ancient genomics and palaeoproteomics have stressed that incomplete archiving and poor metadata can limit replication and long-term reuse, highlighting the need for standardised reporting and open, well-documented workflows (Bergström, 2024; Dekker et al., 2025).

2.6 Host associated biomolecular archives

As we have already extensively seen in this literature overview, humans are not autonomous organisms, but more biological units that also include microbial symbionts and their genomes (Bordenstein & Theis, 2015). Therefore, humans can be considered a superorganism, also called 'holobiont', together with their microbial component. But once an organism dies, its associated microbes can still be retrieved, as some tissues become real biomolecular archives.

2.6.1 Teeth as microbial archives

Teeth are among the most informative substrates in ancient biomolecular research because they preserve several distinct microbial signals within a single anatomical structure (Figure 3). A tooth is not a single archive, but a composite one. Dental calculus preserves the oral microbiome and associated host, dietary, and environmental debris; the pulp chamber and dentine may retain traces of blood-borne pathogens present during death; and tooth tissues can also be colonized by postmortem microbial communities during decomposition (Bos et al., 2019; Mann et al., 2018; Margaryan et al., 2018; Spyrou et al., 2019; Warinner, 2022). This makes teeth especially valuable for ancient metagenomics, because they allow the investigation of oral ecology, infectious disease, and postmortem microbial processes within the same broader substrate.

The importance of teeth also lies in their preservation. Their dense mineralized structure provides physical protection to biomolecules, while the distinct microenvironments within the tooth preserve different fractions of the ancient biological signal. Teeth therefore occupy a central place in ancient microbiome and pathogen research, while also illustrating a broader methodological point: archaeological biomolecular archives are not homogeneous, and their interpretive value depends on understanding how different anatomical compartments form, preserve, and transform over time (Warinner, 2022).

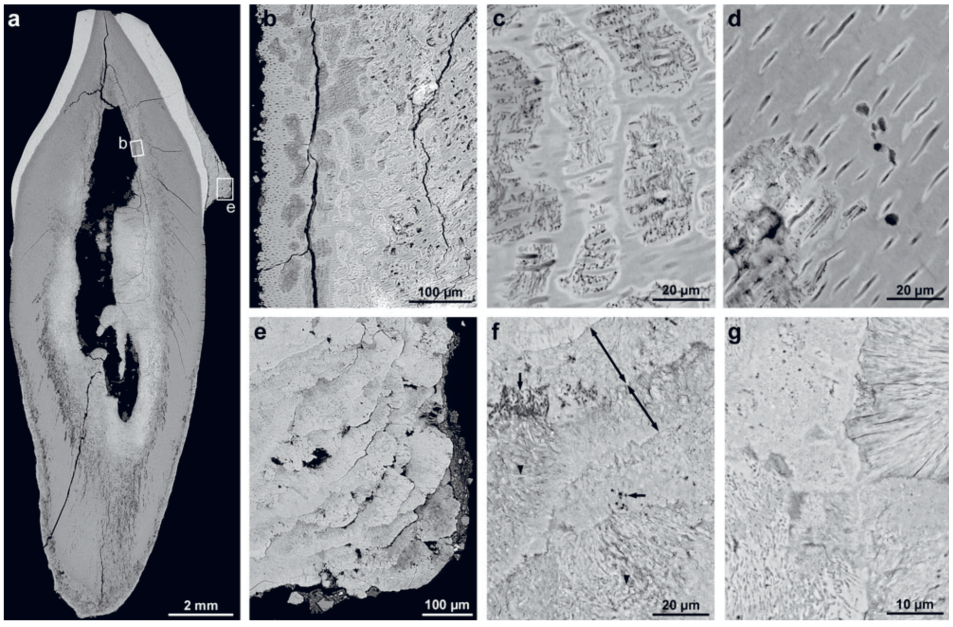

Figure 3: SEM images of a cross-section of an archaeological human tooth with a dental calculus deposit.

a) Labio-lingual section of a lower incisor with dental calculus on the labial crown surface. b) Detail of the dentine at the border of the pulp cavity showing areas of altered mineral density (left) and pitting (right) caused by acid-producing environmental microbes that invaded the pulp cavity post-mortem; c) Detail of zones of hypomineralized (dark gray) and hypermineralized (white) dentine; d) Detail of bacteria infiltrating the dentine tubules arrows. (e–f) Details of the dental calculus which exhibits a layered structure. g) Detail of microorganisms within dental calculus showing large crystal structures and high bacterial cell density. Reproduced from Supplementary figure 6 (Warinner, Rodrigues, et al., 2014) with permission from Springer Nature.

2.6.1.1 Dental calculus as the primary host-associated archive

Among host-associated substrates, dental calculus has emerged as one of the most important archives in ancient metagenomics. As a mineralized form of dental plaque, it routinely fossilizes during life and preserves biomolecular evidence with remarkable stability. At least since the 1980s it was clear to researchers that dental calculus played an important role in the reconstruction of past human diet and oral ecology (Dobney & Brothwell, 1986, 1988). It is now widely regarded as one of the richest known sources of ancient microbial DNA in the archaeological record, and it has provided some of the clearest insights into the evolution of the oral microbiome, long-term host-microbe relationships, and the interactions among oral health, diet, and environment (Adler et al., 2013; Fellows Yates, Velsko, et al., 2021; Velsko et al., 2019; Warinner, Rodrigues, et al., 2014; Weyrich et al., 2017).

What makes dental calculus especially powerful is that it is more than a simple microbial archive. In addition to preserving oral bacterial communities, it can entrap host biomolecules, dietary particles, inhaled debris, and environmental material (Radini et al., 2017, 2019). This means that calculus can be used not only to reconstruct oral microbial composition, but also to investigate broader questions of diet, inflammation, health, and microbial adaptation (Hendy, Warinner, et al., 2018; Warinner, Hendy, et al., 2014). For this reason, dental calculus has become a key substrate in both ancient metagenomics and ancient metaproteomics, and it remains one of the strongest examples of how host-associated biomolecular archives can retain complex multisource evidence over archaeological timescales.

2.6.1.2 Dentine and the pulp chamber for pathogen detection

Whereas calculus primarily reflects the oral environment, dentine and especially the pulp chamber are important for the detection of blood-borne pathogens. Because the pulp cavity is vascularized during life, pathogens circulating in the bloodstream can reach the dental pulp and, if death occurs during active infection, traces of those pathogens may remain within the tooth. This principle was already central to early paleomicrobiological studies, since the first PCR-based works on historical plague victims, where *Y. pestis* DNA was detected from dental pulp and used to support the diagnosis of ancient septicemic infection(Drancourt et al., 1998; Raoult et al., 2000; Wiechmann & Grupe, 2005). This principle has remained central to ancient pathogen genomics, where teeth have repeatedly been used to identify and reconstruct genomes of pathogens associated with systemic infection (Bos et al., 2011; Rascovan et al., 2019; Rasmussen et al., 2015; Vågene et al., 2018). Teeth clearly illustrate the analytical advantage of working with anatomically structured substrates: different dental tissues allow different questions to be asked. Calculus is particularly informative for oral microbial ecology, whereas pulp and dentine are often more directly relevant to systemic infection and pathogen detection near the time of death (Warinner, 2022).

2.6.1.3 Caries, the necrobiome, and postmortem tooth microbiology

Teeth can also preserve evidence of disease-associated and postmortem microbial processes. Carious lesions are of particular interest because they can trap and preserve bacteria associated with oral dysbiosis and dental decay, including cariogenic taxa such as *Streptococcus mutans* (Simón et al., 2014). Molecular analysis of archaeological caries has therefore been used to investigate the long-term history of oral disease directly from diseased tissue rather than from generalized oral biofilm alone. At the same time, tooth tissues may also be colonized after death by microbes involved in decomposition. This postmortem community, often considered as part of the necrobiome or thanatomicrobiome, is typically a mixture of environmental organisms and microbes translocating from host-

associated niches during decomposition (Javan et al., 2016; Metcalf et al., 2016). Applied to teeth, this means that not all microbial DNA recovered from dentine or internal tooth structures necessarily reflects ante-mortem biology, some may derive from post-mortem colonization and decomposition. This distinction is methodologically important for ancient metagenomics, because it requires careful interpretation of mixed microbial signals preserved in dental tissues (Mann et al., 2018; Warinner, 2022).

2.6.2 Palaeofaeces as gut-associated biomolecular archives

Palaeofaeces are another major host-associated archive in ancient metagenomics because they preserve direct evidence of the gut microbiome, dietary residues, and intestinal pathogens. Their archaeological importance lies in the fact that they provide access to a host-associated microbial community that is otherwise largely inaccessible in the archaeological record. Recent work has shown that palaeofaeces can support both community-level and genome-resolved analyses of ancient gut microbiota, including the reconstruction of previously undescribed microbial lineages and the investigation of long-term evolutionary histories within the human gut ecosystem (Borry et al., 2020; Maixner et al., 2021; Tett et al., 2019; Wibowo et al., 2021).

At the same time, palaeofaeces are analytically challenging. Their morphology may be distorted, their source species may be uncertain, and their preservation depends heavily on environmental conditions such as desiccation, freezing, salinity, or mineralization. Reviews of coprolite and palaeofaeces research therefore stress the importance of multiproxy approaches and careful source attribution (Shillito & Mackay, 2020). More broadly, palaeofaeces are best interpreted as integrated archives of diet, health, microbiome composition, and local environment rather than as isolated gut samples (Warinner, 2022).

2.6.3 Bones

Bones can yield very important microbial information (Warinner, 2022). In particular, skeletal lesions can act as a signpost that a pathogen was present at the time of death. For example, aDNA testing of spine, ribs and other bone lesions has led to positive diagnosis of tuberculosis, and the identification of the *Mycobacterium tuberculosis complex* (MTBC) strains which caused the disease (Bos et al., 2014). Other pathogens identified by analysing aDNA from pathological bone lesions are *Mycobacterium leprae*, the causative agent of leprosy (Schuenemann, Avanzi, et al., 2018; Schuenemann et al., 2013) and *Treponema pallidum subspecies pallidum*, the causative agent of syphilis (Schuenemann, Kumar Lankapalli, et al., 2018). However, when analysing the microbial composition of bones, it is important to distinguish pathogens from close relatives which are present in soil and colonize the skeleton after death, as for example some *Mycobacteria species*, *Clostridium tetani* and *Clostridium botulinum* (Warinner et al., 2017a).

The petrous bone, which is a portion of the skull that contains the middle and inner ear, is usually used to retrieve host DNA. Its dense structure preserves DNA very well. Even if pathogens are usually not recovered from this source, there have been a few cases in which this has happened. This is the case for *Treponema pallidum* from a human infant and *Brucella melitensis* from an adult sheep in the case of bacteria (L'Hôte et al., 2024; Majander et al., 2020). Hepatitis B virus and Human Betaherpesvirus 6A and 6B have also been retrieved from petrous bones (Guellil et al., 2026; Sun et al., 2024).

2.7 Environmental DNA and ancient sediments

Environmental DNA, or eDNA, refers to genetic material recovered directly from environmental samples without the need to isolate or physically observe the target organism (Taberlet et al. 2018). This DNA can originate from cellular and extracellular material shed by organisms into their surroundings, including skin cells, hair, mucus, feces, urine, reproductive material, or decomposing tissue. When preserved in sedimentary archives, this material is commonly referred to as sedaDNA. Sediments are particularly important because they can accumulate biological traces over long periods of time, creating archives that may preserve information on past ecosystems, environments, human activities, and species that are otherwise absent from the visible archaeological or palaeontological record.

The use of sediments as biomolecular archives developed from early studies showing that DNA could persist outside conventional skeletal or tissue remains. One of the first demonstrations came from lake sediments, where Coolen and Overmann (Coolen & Overmann, 1998) recovered bacterial DNA from Holocene deposits associated with purple sulphur bacteria.

Shortly after, Willerslev and colleagues (Willerslev et al., 2003) showed that both plant and animal DNA could be recovered from Siberian permafrost deposits and temperate cave sediments from New Zealand, even in the absence of visible macrofossils. This study was especially important because it demonstrated that sediments could preserve signals of extinct megafauna and plants, and not only microbial DNA. Around the same period, (Hofreiter et al., 2003) introduced the potential of "molecular caving", showing that cave sediments could be used to identify molecularly extinct.

At the same time, early research also made clear that sedaDNA records require careful interpretation. A work on New Zealand cave sediments, showed that DNA from recently introduced sheep could be detected in older pre-European layers, suggesting that vertical movement of DNA through some sediment profiles may occur (Haile et al., 2007). However, vertical migration appears to be less likely in compacted lake sediments or permanently frozen deposits, where DNA may become immobilised within the sediment matrix (Anderson-Carpenter et al., 2011).

The development of high-throughput sequencing further expanded the potential of sedaDNA. Pedersen and colleagues, for example, used lake sediment DNA together with other palaeoecological evidence to investigate the postglacial

viability of the North American ice-free corridor (Pedersen et al., 2016). More recently, environmental DNA from the Kap København Formation in northern Greenland revealed a two million years old ecosystem containing a mixture of taxa, including plants and animals that were not fully represented by macrofossil or pollen evidence alone (Kjær et al., 2022). This study is particularly relevant because it suggested that ancient DNA may survive over exceptional timescales under favourable conditions, especially in cold, mineral-rich sediments where DNA can bind to clay and other mineral surfaces.

In archaeological contexts, sedaDNA has become increasingly important for detecting human activity, animal exploitation, and site use. Seersholm and colleagues analysed Greenlandic midden sediments and identified DNA evidence for bowhead whale exploitation by Paleo-Inuit groups around 4,000 years ago, showing that archaeological sediments can preserve evidence of subsistence practices that may be invisible in zooarchaeological assemblages (Seersholm et al., 2016). Cave sediments have also become central to ancient DNA research. A key study in 2017 recovered Neanderthal and Denisovan mitochondrial DNA from Pleistocene cave sediments, demonstrating that hominin presence can be detected even in the absence of skeletal remains (Slon et al., 2017). Subsequent studies extended this approach to Denisovan DNA from Baishiya Karst Cave (Zhang et al., 2020), hominin and faunal turnovers at Denisova Cave (Zavala et al., 2021), and Neanderthal population history using nuclear and mitochondrial DNA from cave sediments (Vernot et al., 2021). Microstratigraphic studies have further improved our understanding of how DNA is preserved within sediments. A work from Massilani and colleagues (Massilani et al., 2022) showed that ancient faunal and hominin DNA can be concentrated in small micro-contexts, such as microscopic bone or coprolitic particles, and that resin-impregnated sediment blocks can preserve DNA at fine spatial scales.

Although many sedaDNA studies have focused on lakes, caves, rockshelters, and permafrost, open-air archaeological deposits are now also being explored. These contexts are more challenging because they are more exposed to temperature fluctuations, water movement, erosion, and redeposition. Nevertheless, recent work suggests that open-air sediments can preserve useful genetic information. Ancient human mitochondrial DNA from sediments was retrieved from at open-air archaeological sites in Japan, particularly from sediments associated with human bones (Sawafuji et al., 2026). Similarly, Zampirolo and colleagues investigated open-air archaeological deposits and palaeo-meanders in Serbia, showing that sedaDNA can preserve evidence of terrestrial and freshwater ecosystems in unsheltered deposits, while also highlighting the problems created by erosion, redeposition, complex chronology, and uncertain DNA movement in dynamic open-air environments (Zampirolo et al., 2026).

2.8 Beyond human remains: other biomolecular archives

Beyond all the previously mentioned sources, microbial DNA and biomolecular traces can also be recovered and investigated from a range of less conventional archaeological, and historical materials. Ceramic vessels, amphorae, jars, and other food containers are particularly important for the study of ancient fermentation, because absorbed residues and surface deposits may preserve molecular, chemical, microscopic, or microbial evidence of wine, beer, dairy, and mixed fermented beverages (Aouizerat et al., 2019; Cavalieri et al., 2003; Drieu et al., 2020; McGovern et al., 2004). However, the identification of ancient wine or beer from vessels remains methodologically difficult, and biomolecular interpretations should be supported by other analyses, such as residue chemistry, archaeobotanical or microfossil data, and controls (Drieu et al., 2020; J. Wang et al., 2021). Chewed birch pitch, also called ancient "chewing-gums" is another unusual but highly informative archive, because saliva and oral material can become trapped in the hydrophobic pitch matrix. A 5700-year-old piece of chewed birch pitch from Syltholm, Denmark, yielded a complete human genome, oral microbial DNA, bacterial and viral taxa including Epstein-Barr virus, and plant and animal DNA probably linked to recent diet of studied individuals (Jensen et al., 2019) . Mesolithic chewed pitch from Huseby Klev, Sweden, has also been used to reconstruct oral microbiome profiles and identify oral-health-associated microbial signals, showing that pitch can preserve information on human biology, oral ecology, diet, and material use even in the absence of skeletal remains (Kırdök et al., 2024).

Other materials can also preserve microbial signatures. Parchments and manuscripts can retain animal DNA together with bacterial, fungal, viral, and human-associated traces, providing information on production, handling, conservation state, and deterioration processes (Fiddyment et al., 2019; Perini et al., 2020; Piñar, Tafer, et al., 2020). Painted surfaces, paintings, drawings, murals, and other artworks may preserve bacterial and fungal communities, which can help reconstruct biodeterioration processes, storage environments, handling histories, and conservation needs (López-Miras et al., 2013; Piñar, Sclocchi, et al., 2020; Rosado et al., 2014; Torralba et al., 2021).

Historical medical and museum collections form another important archive for microbial and pathogen DNA. Formalin-fixed and paraffin-embedded tissues, microscopy slides, alcohol-preserved specimens, pathological tissues, and parchment can all retain microbial or pathogen genetic material, although preservation is often affected by fixation artefacts, fragmentation, contamination, and storage history (Blevins et al., 2026; Campos & Gilbert, 2012; Raxworthy & Smith, 2021). Historical blood slides, for example, have been used to recover DNA from eradicated European malaria parasites, including *Plasmodium vivax* and *P. falciparum* (de-Dios et al., 2019; Gelabert et al., 2016; van Dorp et al., 2020). Similarly, archived pathological tissues have yielded a near full-length HIV-1 genome from formalin-fixed tissue from 1966 and genomic data from nineteenth-century *Vibrio cholerae* preserved in intestinal material from the 19th century

Philadelphia cholera outbreak (Devault, Golding, et al., 2014; Devault, McLoughlin, et al., 2014; Gryseels et al., 2020). Exceptionally preserved animal remains can also retain host-associated microbial DNA, as shown by the analysis of mammoth remains from which researchers identified microbial taxa associated with mammoth tissues and reconstructed partial genomes from host-associated bacterial genera including *Actinobacillus*, *Pasteurella*, *Streptococcus*, and *Erysipelothrix* (Guinet et al., 2025). Together, these examples show that microbial archaeology is at a stage in which it can extend beyond the usual substrates, broadening the archaeological record to include traces of food production, craft practices, object use, conservation history, disease, and host-microbe-environment interactions.

2.9 Ancient pathogen genomics

2.9.1 From the first steps of palaeomicrobiology to ancient pathogen genomics

Ancient pathogen genomics is one of the major subfields of archaeogenomics because it allows infectious diseases to be investigated molecularly and directly, rather than only through skeletal lesions, historical descriptions, or modern pathogen diversity. From the early 1990s onward, PCR-based studies were already used to detect pathogen DNA in archaeological remains, especially for diseases such as tuberculosis and plague (Drancourt et al., 1998; Drancourt & Raoult, 2005; Spigelman & Lemma, 1993). These early studies established the foundations of palaeomicrobiology, a field focused on the detection and identification of ancient microorganisms from preserved biological material (Drancourt & Raoult, 2005). These studies were important because they showed that pathogen DNA could survive in archaeological material and that the diagnosis of past infectious diseases was possible. However, at the same time, early PCR-based work was often difficult to authenticate, because pathogen DNA was present in low quantities and results could be affected by contamination (Drancourt & Raoult, 2005; Gilbert et al., 2004) .

The development of high-throughput sequencing, metagenomic screening, and targeted enrichment changed the scale of ancient pathogen research (Bos et al., 2011; Schuenemann et al., 2011). As a result, ancient pathogen research moved from retrospective diagnosis toward evolutionary and epidemiological questions. Ancient pathogen genomes now provide a molecular fossil record of infection, allowing researchers to investigate pathogen emergence, extinct lineages, dispersal, virulence evolution, and the relationship between epidemics and human history (Bos et al., 2019; Duchêne et al., 2020; Spyrou et al., 2019)

2.9.2 *Yersinia pestis* as a model pathogen in ancient pathogen genomics

Among ancient pathogens, *Y. pestis* has played a particularly important role in the development of the field. *Y. pestis*, the causative agent of plague, evolved from the closely related enteric pathogen *Yersinia pseudotuberculosis* and is historically associated with three major pandemics: the First Pandemic, beginning with the Plague of Justinian in the 6th century CE; the Second Pandemic, beginning with the Black Death in the 14th century; and the Third Pandemic, which began in the 19th century and gave rise to much of the modern global distribution of plague (Achtman et al., 1999; Barbieri et al., 2020; Parkhill et al., 2001; Perry & Fetherston, 1997; Stenseth et al., 2008).

Y. pestis is maintained today as a zoonotic pathogen in natural reservoirs, mainly through cycles involving wild rodents and their fleas. Humans are incidental hosts and are usually infected through the bite of infected fleas, direct contact with infected animals or tissues, or inhalation of infectious droplets in cases of pneumonic plague (Barbieri et al., 2020; Perry & Fetherston, 1997; Stenseth et al., 2008). Modern plague can present as bubonic, septicaemic, or pneumonic disease, with pneumonic plague being especially relevant epidemiologically because it can allow human-to-human transmission through respiratory droplets if left untreated (Perry & Fetherston, 1997).Genomically, the reference strain CO92 contains a 4.65 Mb chromosome and three virulence-associated plasmids, pCD1, pMT1, and pPCP1 (Parkhill et al., 2001). These plasmids encode key traits involved in mammalian infection and vector-borne transmission: pCD1 carries the type III secretion system and *Yersinia* outer proteins, pMT1 includes factors such as the F1 capsule antigen and *ymt* gene, which is important for flea-borne transmission, and pPCP1 carries the plasminogen activator gene *pla*, which promotes invasion from the site of inoculation(Demeure et al., 2019; Parkhill et al., 2001; Perry & Fetherston, 1997).

As seen in this literature overview, plague was a central topic in ancient pathogen research before whole-genome sequencing. Works from Drancourt and colleagues (Drancourt et al., 1998) and Raoult and colleagues (Raoult et al., 2000) were foundational, but also controversial (Gilbert et al., 2004). The transition to genome-scale plague research occurred with targeted enrichment and high-throughput sequencing: Haensch et al. (Haensch et al., 2010) provided molecular evidence for distinct *Y. pestis* clones during the Black Death and later outbreaks, and in 2011 Schuenemann and colleagues (Schuenemann et al., 2011) enriched the *Y. pestis* pPCP1 plasmid, and Bos et al. (Bos et al., 2011) reconstructed a draft genome from Black Death victims in London.

As of today, more than 200 ancient genomes of *Y.pestis* have been retrieved (Fellows Yates, Andrades Valtueña, et al., 2021). Genome-scale studies have refined the history of the historically documented pandemics. For the First Pandemic, earlier detections from sixth-century remains in Bavaria (Wiechmann & Grupe, 2005) were followed by genome-scale studies on the Justinianic Plague strains (Harbeck et al., 2013; Wagner et al., 2014). Later on, Keller and colleagues

(Keller et al., 2019) showed that *Y. pestis* was geographically widespread and genetically diverse in western Europe during the First Pandemic. More recent data from Jerash in Jordan have extended genomic evidence closer to the historically described eastern Mediterranean context of the Plague of Justinian (Adapa et al., 2025).

For the Second Pandemic, ancient genomes have clarified the relationship between the Black Death, later European outbreaks, and broader Eurasian plague diversity (Bos et al., 2011; Spyrou et al., 2016, 2019, 2022). Although the Third Pandemic is mostly studied through modern and historical isolates rather than ancient DNA, it remains important as the source of many extant *Y. pestis* lineages and as a comparative framework for understanding plague phylogeography, reservoir ecology, and global dispersal (Barbieri et al., 2020; Stenseth et al., 2008).

Ancient genomes have also pushed the history of *Y. pestis* far earlier than the documented pandemics. Late Neolithic and Bronze Age genomes showed that *Y. pestis* lineages were present in Eurasia by approximately 5,000 years before present, although many early strains lacked genetic features associated with efficient flea-borne bubonic transmission (Andrades Valtueña et al., 2017; Rasmussen et al., 2015; Spyrou et al., 2018). Subsequent studies have expanded the geographic and ecological range of prehistoric plague, including evidence from Late Neolithic/Bronze Age Central Europe, Britain, Scandinavia, the Baltic region, and eastern Eurasia (Andrades Valtueña et al., 2022; Rascovan et al., 2019; Seersholm et al., 2024; Susat et al., 2021; Swali et al., 2023).

Specifically, in 2021 Susat and colleagues (Susat et al., 2021) reconstructed a ca. 5,000-year-old *Y. pesti*s genome from a hunter-fisher-gatherer buried at Riņņukalns in Latvia and argued that this very early strain probably represented an isolated zoonotic event rather than evidence for a large prehistoric pandemic. In a later study, they identified two *Y. pestis*-positive individuals among 133 tested individuals from the Late Neolithic Warburg necropolis in Germany and showed that the two infections belonged to distinct strains, suggesting the possibility of independent infection events rather than a single local outbreak (Susat et al., 2024). As the title of the study clearly states, they also reported *Y. pestis* DNA in a Neolithic dog from Sweden, raising the possibility that dogs or other domestic and wild animal interactions may have contributed to human being exposed to early *Y. pestis* reservoirs (Susat et al., 2024).

Seersholm et al. (Seersholm et al., 2024) reported an extensive signal of plague infection in Neolithic Scandinavia. They detected *Y. pestis* in at least 17% of the sampled individuals and inferred three distinct infection events over approximately 120 years. This study is especially interesting because it shows plague infections across a multi-generation patrilineal kin group. However, the authors also note that the detected rate reflects the sampled and preserved individuals, and should not be treated as an estimate of the prevalence of the disease in the population (Seersholm et al., 2024). New data from eastern Eurasia further extend the geographic and chronological range of early plague. Macleod and colleagues (Macleod et al., 2024) reported *Y. pestis* infections in hunter-gatherer cemeteries

from the Lake Baikal region beginning around 5,600–5,400 cal BP, including a high reported detection rate at Ust'-Ida I.

In general, it is important to say that large-scale screening studies are now moving the field away from isolated case studies as the one from (Sikora et al., 2025). Furthermore, studies of animal hosts suggest that prehistoric plague should be interpreted in relation to human-animal interactions, domesticates, wild reservoirs (Light-Maka et al., 2025; Susat et al., 2024). *Y. pestis* remains a model organism in ancient pathogen genomics: it illustrates the methodological transition from debated PCR-based diagnosis to genome-scale analysis, while also showing how ancient DNA can transform interpretations of pandemic history, pathogen evolution and zoonotic ecology.

2.9.3 Zoonoses and disease landscapes

The term "Zoonoses" comes from the Greek words "Zoon", which means animal, and "nosos", which means disease. Any infection or disease that is naturally transmissible between vertebrate animals and humans is classified as a zoonosis according to the World Health Organization (WHO) (WHO, 2020). Zoonoses are widespread, with around 61% of all infectious diseases that affect humans and 75% of new or reemerging infections considered zoonotic (Morse et al., 2012; Taylor et al., 2001). Zoonotic infections can be caused by different pathogens, including viruses, fungi, parasites and bacteria (Morse et al., 2012). The financial costs and economic losses associated with zoonotic diseases are incredibly heavy. The most recent and important example of the worldwide economic impact of a zoonosis is the COVID-19 pandemic, which affected the global economy in what was described as "the most serious challenge of the post-war era due to the sudden halt in economic activity in both advanced and developing countries"(UNIDO, 2020). Their burden on human lives is also substantial: neglected zoonotic diseases alone have been estimated to cause approximately 2.4 billion human illnesses and 2.2 million deaths every year, with the greatest impact falling on low- and middle-income regions and mostly livestock-dependent populations (Grace et al., 2012; Rahman et al., 2020). For their impact on both animals and humans at local and global scale, zoonoses are central to initiatives such as the 'One Health' approach, which aims to bring together human, animal and environmental health (Ronald M. Atlas, 2012).

The archaeological importance of zoonoses was recognized before aDNA studies focused on them. Epidemiological and evolutionary studies linked major changes in disease ecology to the Neolithic transition, when stabilization, population growth, animal domestication, herding, storage of food, accumulation of waste, and closer contact with animals changed the conditions for pathogen transmission (Armelagos et al., 2005; Barrett et al., 1998; Pearce-Duvet, 2006). In this framework, domestication was seen as a process that altered the conditions under which existing pathogens, vectors, and livestock-associated infections could circulate. Pearce-Duvet (Pearce-Duvet, 2006), for example, argued that agriculture and animal domestication may have changed the transmission ecology of pre-

existing pathogens, increased the success of vectors, created new forms of human-wildlife contact, and provided more stable routes for infections to move between animals and humans. Molecular-clock and comparative genetic studies debated whether the expansion of *Plasmodium falciparum* was linked to Holocene demographic and ecological change, including agriculture, mosquito habitats, and human population growth (Joy et al., 2003; Tanabe et al., 2010).

Ancient DNA has made it possible to test some of these long-standing hypotheses directly. A key example is *Salmonella enterica*: Key and colleagues (Key et al., 2020) recovered eight ancient *S. enterica* genomes, up to 6,500 years old, from western Eurasian foragers, pastoralists, and agro-pastoralists, and linked the emergence of human-adapted lineages to Neolithization and changing human-animal relationships. This is particularly relevant because *Salmonella* is a food-related pathogen and this study helps to connect pathogen evolution to animal management, diet, hygiene and settlement practices. More recently, the recovery of the genome of an 8,000-year-old *Brucella melitensis* from a sheep and its phylogenetic placement suggests an increase in the potential for pathogen transmission within the herd (L'Hôte et al., 2024).

Other livestock-associated pathogens further broaden this picture. Recent zooarchaeological work has identified ancient pathogen signatures in domesticated and wild animal remains, including *E. rhusiopathiae* and *Streptococcus lutetiensis*, and has shown that animal bones can be used to investigate ancient reservoirs, host ranges, and possible spillover pathways (W Runge et al., 2026). *E. rhusiopathiae*, the causative agent of swine erysipelas and a zoonotic occupational pathogen in humans, is an especially useful case because it shifts attention toward diseases connected to animal management and animal products.

Finally, large-scale metagenomic screening studies represent an important stage in this field. In a recent paper, Sikora and colleagues (Sikora et al., 2025) screened shotgun sequencing data from 1,313 ancient humans across 37,000 years of Eurasian history and identified bacterial, viral, and parasite DNA. Their study found that zoonotic pathogen signals became detectable from around 6,500 years ago and peaked around 5,000 years ago, broadly overlapping with the spread of livestock domestication and pastoralist lifeways. This should be interpreted carefully: it does not mean that zoonoses were absent before this period, because preservation, sampling, detection, and pathogen biology all affect visibility. Rather, it provides large-scale genomic support for older hypotheses that the change in human-animal relationships could have altered exposure, transmission, and reservoir dynamics.

2.9.4 Limitations in ancient pathogen genomics

Despite its potential, ancient pathogen genomics has important methodological and interpretative limitations. Authentication remains central because pathogen DNA is often present in low abundance, and mixed with host DNA, environmental microorganisms, and modern contaminants (Duchêne et al., 2020; Key et al., 2017; Spyrou et al., 2019). Pathogen assignments should therefore be evaluated using

multiple lines of evidence, including short fragment lengths, cytosine deamination patterns, evenness of coverage, negative controls, competitive mapping against related organisms, and phylogenetic placement (Key et al., 2017; Spyrou et al., 2019).

Pathogen detection also depends strongly on pathogen biology, tissue tropism, and sampling strategy. Different pathogens are more likely to be preserved in different anatomical substrates depending on whether they caused systemic infection, chronic disease, respiratory infection, or gastrointestinal disease (Blevins et al., 2026). As explained before in this literature, teeth and dental pulp are useful for detecting blood-borne pathogens such as *Y. pestis*, because pathogens circulating in the bloodstream may enter the pulp chamber before death (Drancourt et al., 1998; Drancourt & Raoult, 2005; Raoult et al., 2000). However, sometimes when studying both the host and the infectious agent, it's important to remember that the best substrate for host DNA might not always be the best substrate for pathogen DNA.

Finally, detection of pathogen DNA does not automatically prove disease symptoms, cause of death, or epidemic impact. Some microorganisms may represent asymptomatic carriage, opportunistic infection, oral or environmental colonisation, post-mortem microbial activity, or contamination (Key et al., 2017; Spyrou et al., 2019). Conversely, failure to detect a pathogen does not prove that an individual or population was uninfected, because pathogen load, tissue preservation, sampling choice, and analytical methods all affect recovery (Duchêne et al., 2020; Spyrou et al., 2019). For this reason, ancient pathogen results are strongest when interpreted together with archaeological context, skeletal pathology, radiocarbon chronology, historical evidence, host genomic data, proteomics (Blevins et al., 2026; Spyrou et al., 2019).

2.10 Ethics in ancient metagenomics research: from sampling to long-term responsibility, to community involvement

Ethical ancient DNA research should be treated as a form of stewardship rather than only as a technical procedure. For human remains, recent global guidance (Alpaslan-Roodenberg et al., 2021) emphasizes that researchers should comply with relevant laws and permissions, prepare a detailed research plan before sampling, minimize physical damage, make data available in ways that permit scientific scrutiny, and engage stakeholders from the beginning of the project. Fleskes et al. (Fleskes et al., 2022) emphasize that aDNA research involves unresolved tensions around consent, destructive sampling, data access, data management, and accountability to stakeholder communities, especially descendant and Indigenous communities.

Some recent works also caution that open data policies cannot be treated as universally neutral because they may conflict with Indigenous Data Sovereignty

and with community authority over decisions about publication, access, and reuse (Kowal et al., 2023).

Best practice begins before sampling. Researchers should ask whether aDNA analysis is necessary for the research question, and how much material is strictly required. Sampling should be targeted, minimal, well documented, and justified in advance, following the emphasis on prior planning and damage minimization in (Alpaslan-Roodenberg et al., 2021). Documentation should include the sampled element or matrix, the amount removed, contamination-control procedures, the fate of the remaining extracts and libraries, and whether future use of those materials would require renewed permission. This is particularly important because, as Gibbon and colleagues (Gibbon et al., 2024) argue, aDNA data will persist indefinitely in sequenced libraries and may affect not only the sampled individual, but also living communities and future generations. It is thus important to discuss with the community involved or stakeholders (museum curators, for example) the future storage of the physical material itself but of the extracts, libraries, and sequences derived from it.

Kowal and colleagues (Kowal et al., 2023) argue that aDNA researchers should not treat the legal minimum as the ethical standard, and that excluding community perspectives from decisions about publication and data sharing is itself ethically problematic, particularly in relation to Indigenous Data Sovereignty. It is important that aDNA research, especially in the Global South, addresses histories of scientific colonialism, extractivism, unequal access to resources, and limited participation of local researchers and communities (Ávila-Arcos et al., 2022).

Consent should also be understood relationally. Because ancient individuals cannot consent for themselves, Gibbon and colleagues in 2024 proposed informed proxy or relational autonomy consent, in which living people or groups affected by the research may provide permission on behalf of the deceased (Gibbon et al., 2024).

These ethical concerns should not be restricted to visible human remains. Johnson and colleagues define “composites” as archaeological materials with multiple genetic sources, including sediment, coprolites, birch pitch, and dental calculus, and argue that ethical principles developed for human remains can also guide research on these mixed materials (Johnson et al., 2024). This is important because composite samples may contain human, animal, plant, microbial, dietary, and environmental DNA. They can therefore reveal information about human presence, diet, disease, mobility, land use, and relationships between people and place, even when the material being sampled is not a bone or tooth. Johnson and colleagues explicitly warn that composite samples should not become a way to avoid collaboration, consultation, or legal and ethical responsibilities (Johnson et al., 2024).

Sedimentary aDNA requires particular ethical care. A work by Lewis and colleagues argue that sediments and soils are often treated as excavation by-products, yet sedaDNA can contain genetic information from past fauna, flora, microbial communities, and human ancestors, which may be culturally significant

for Indigenous peoples (Lewis et al., 2025). For this reason, sediment should not be treated as ethically neutral "dirt."

Microbial aDNA raises related issues. Dental calculus, coprolites, sediments, and pathological tissues can preserve microbial DNA that informs on past diet, health, disease, migration, and human-environment interactions. For example, recently, a work by Velsko and colleagues (Velsko et al., 2024) showed that archaeological dental calculus can preserve high levels of endogenous oral bacterial DNA from Oceania, making it a valuable archive for potentially reconstructing past migration histories. At the same time, microbial data can be sensitive because they may reveal information about disease, diet, lifestyle, ancestry, or community-specific biological histories. Handsley-Davis and colleagues for instance, found a distinctive oral microbiota in Indigenous Australian dental calculus and argued that oral microbiota may be shaped by long relationships with Country (Ancestral homeland), showing that microbial data can intersect with Indigenous heritage, health, and identity (Handsley-Davis et al., 2022). All this evidence shows how carefully researchers have to produce, handle and communicate the results.

Overall, ethical aDNA research requires a precautionary and relational approach: a sample does not need to be a human bone to be culturally, biologically, or politically sensitive.

2.11 Future and perspectives in the field of ancient metagenomics: about MAGS, functional studies, multidisciplinarity

Until a few years ago, the only option for aDNA analysis was to take the short-read sequencing data and align them against some known reference genomes. However, this is problematic, because this approach relies on the organisms whose genomes are present in the reference database. A major future direction in ancient metagenomics is the transition from taxonomic profiling towards genome reconstruction of ancient microbial communities through metagenome-assembled genomes, or MAGs (Wibowo et al., 2021). This shift is important because de novo assembly and binning can recover previously undescribed species-level genome bins from ancient material (Wibowo et al., 2021), thus allowing researchers to avoid databases. For example, Wibowo et al. reconstructed 498 medium- and high-quality microbial genomes from ancient palaeofaeces, and 39% of the 181 most confidently ancient gut genomes represented previously undescribed species-level genome bins (Wibowo et al., 2021). Similarly, ancient dental calculus has been used for de novo metagenomic assembly, with Granehäll et al. reconstructing 117 MAGs from 20 calculus samples and identifying ancient archaeal diversity within *Methanobrevibacter* that was poorly represented in modern reference databases (Granehäll et al., 2021). However, MAG recovery from ancient samples remains technically challenging because aDNA is fragmented, damaged, low in

abundance, and often mixed with environmental or modern contaminant DNA (Borry et al., 2021; Wibowo et al., 2021). Future work will therefore need continuous aDNA-aware pipelines for MAGs, rather than direct transfer of pipelines developed for modern high-quality metagenomic data (Borry et al., 2021; Pochon et al., 2023).

A second major perspective is the development of functional ancient metagenomics, in which the field moves beyond "which taxa were present?" toward "what genes, pathways, and functional capacities were present?" (Gancz et al., 2023; Wibowo et al., 2021). Functional profiling of ancient palaeofaeces has already shown differences between ancient and industrialized gut microbiomes, including lower abundance of antibiotic-resistance and mucin-degrading genes and enrichment of mobile genetic elements in ancient samples (Wibowo et al., 2021). Ancient dental calculus studies have also linked microbial functional profiles to diet and lifestyle, with Gancz et al. reporting that microbial functions reflected differences in dairy and carbohydrate consumption across British archaeological populations (Gancz et al., 2023). Functional approaches are especially promising because they can connect microbial communities to host diet, oral hygiene, inflammation, pathogen exposure, antibiotic resistance, carbohydrate metabolism, and biosynthetic potential (Gancz et al., 2023; Klapper et al., 2023; Wibowo et al., 2021). One particularly striking example is the recovery of ancient biosynthetic gene clusters from dental calculus MAGs, which allowed Klapper and colleagues to investigate natural-product biosynthesis in Middle and Upper Palaeolithic oral bacteria (Klapper et al., 2023). This type of work opens a new perspective in which ancient metagenomics can even contribute to biotechnology, because ancient genomes may preserve biosynthetic pathways that are absent or rare in modern microbiomes which have been sampled (Klapper et al., 2023). At the same time, functional interpretation must remain cautious because of short reads, and incomplete MAGs.

A third future direction is deeper multidisciplinarity, because ancient metagenomic data are most informative when interpreted together with archaeology, bioarchaeology, palaeopathology, palaeoproteomics, microremains recovery, isotopes data, and environmental evidence (Modi et al., 2023; Quagliariello et al., 2022) . Especially dental calculus is valuable for this kind of integrated work, because it can preserve microbial DNA, host molecules, dietary residues, plant microremains, proteins, and environmental particles within the same biofilm matrix (Jersie-Christensen et al., 2018; Warinner et al., 2015). For example, Quagliariello et al. integrated oral microbiome data from dental calculus with embedded plant remains to investigate how the Neolithic transition toward agriculture shaped oral microbiome composition in Italy (Quagliariello et al., 2022). Future studies should therefore be designed from the beginning as integrated archaeological-biological projects, rather than as purely sequencing-based surveys, because contextual information is essential for distinguishing biological signal from taphonomy, contamination, sampling bias, and post-excavation history (Fellows Yates, Andrades Valtueña, et al., 2021; Warinner et al., 2017a). Such multidisciplinarity would also help tackle some of the challenges that were

already highlighted by Green and Speller in 2017, in their discussion of ancient DNA extraction from novel sources (Green & Speller, 2017). These include the need for a more detailed understanding of substrate-specific DNA preservation and degradation processes, as well as the development of multi-proxy approaches capable of supporting the authentication of ancient genetic signals.

Overall, the future of ancient metagenomics will likely be towards genome reconstruction, functional studies and a better dialogue between disciplines, with a multi-omic archaeology grounding molecular results in human evolution, environment, health, and material culture.

3. AIMS OF THE STUDY

The overall aim of this thesis was to evaluate the potential of ancient metagenomics and palaeoproteomics for reconstructing past human-environment, human-animal, and human-microbe interactions using archaeological materials. In particular, the thesis aimed to assess how both conventional and overlooked or usually discarded archaeological substrates preserve ancient biomolecular information, and how such information can be used to investigate microbial ecology, biomolecular preservation, infectious diseases, and zoonotic transmission in past populations.

3.1 Specific aims of the first study (REF I) : Biomolecular preservation in sediment concretions

Archaeological remains covered with concretions, including human bones, are commonly found in certain areas and time periods of interest for understanding the past, but had not been investigated for potential ancient DNA (aDNA) and protein content. In this study, we aimed to extract aDNA and proteins in tandem from human dental remains and their surrounding concretions, in order to have the opportunity to investigate this unexplored source.

3.2 Specific aims of the second study (REF II): Bone-adhered sediments as sources of ancient biomolecules

Sediments adhered to human skeletal remains are an abundant material that is often ignored or discarded during excavation, postexcavation cleaning, or laboratory processing (Damgaard et al. 2015; Pinhasi et al. 2019). This source is persistent in skeletal collections and can be found even after years of storage. In some cases, these sediments could be the last remaining material that provides environmental data of a site years after its excavation. However, the role of bone-adhered sediments as a reliable source of ancient biomolecules is poorly understood. In this study, we aimed at exploring the biomolecular content of sediments adhered to different human skeletal elements. In doing so, we explored human, animal and microbial content both from a genomics and from a proteomics perspective. We also wanted to understand if the human DNA found in the sediments was comparable with the original skeletal elements.

3.3 Specific aims of the third study (REF III): Infectious disease landscapes in medieval Sint-Truiden

In addition to reconstructing human genetic ancestry and population structure, the study aimed to explore the infectious disease landscape of medieval and post-medieval Sint-Truiden through metagenomic screening of shotgun sequencing data from dental remains. Teeth were selected instead of petrous bones partly because they allowed the recovery of both human and pathogen DNA. The metagenomic investigation aimed to detect human-associated pathogens and pathobionts, evaluate their temporal distribution across the eighth to eighteenth centuries, and test whether infections such as plague could be identified even where written historical sources were silent.

3.4 Specific aims of the fourth study (REF IV): Co-occurrence of zoonotic pathogens in prehistoric Europe

The study aimed to use metagenomic screening of ancient DNA libraries from Copper Age individuals at *Grotta della Spinosa* to investigate the presence of pathogens and the infectious disease burden in the prehistoric community. In particular, we sought to identify and authenticate pathogen DNA, reconstruct microbial genomes where possible, and assess whether multiple pathogens could co-occur within the same individuals or archaeological contexts. The study was especially focused on the detection of *Yersinia pestis* and *Erysipelothrix* spp., in order to explore prehistoric disease burden, pathogen co-occurrence, and the possible role of zoonotic transmission during the Late Neolithic/Bronze Age period in Southern Europe.

4. MATERIALS AND METHODS

This chapter of the thesis contains an overview of the methods used in each publication that constitutes the dissertation work. All the ancient DNA laboratory work that is presented in this thesis was carried out in the dedicated ancient DNA facilities at the Institute of Genomics, University of Tartu. Library quantification and sequencing were performed at the Estonian Biocentre Core Laboratory. For further details, please refer to the original publications and supplementary materials.

4.1 Ref I

In this study, DNA was extracted from 62 skeletal samples from *Grotta Scaloria* and *Titolo*, with all necessary permissions obtained from The Soprintendenza Archeologia, Belle Arti e Paesaggio per le province di Barletta-Andria-Trani e Foggia and the Soprintendenza Archeologia, Belle Arti e Paesaggio per la Città Metropolitana di Bari.

Samples included tooth roots, concretion-covered tissues, concretion powder, and dental calculus. Sampling tools were sterilised between samples, and materials were decalcified in EDTA before DNA purification. Tooth-root extracts were concentrated with Vivaspin Turbo 15 columns, and all extracts were purified using Roche High Pure Viral Nucleic Acid Large Volume columns. DNA was eluted in EB buffer and stored at −20 °C. One extraction was performed per sample, and 30 μL of extract were used for library preparation.

Sequencing libraries were prepared following established protocols (Keller et al., n.d.) and quantified using Qubit, TapeStation, and qPCR. Libraries were sequenced on an Illumina NextSeq 500/550 using paired-end 150-cycle chemistry.

Before mapping, the raw sequences were trimmed with AdapterRemoval v.2.3.3 (Schubert et al., 2016), the sequences shorter than 30 bp and with quality below 20 were discarded, we removed low complexity sequences using prinseq v.0.20.4 and a dust value of 7 (Schmieder & Edwards, 2011). At the end, we collapsed all duplicated reads using bbmap v.38.18 tool suite (Bushnell, 2014).

Reads were mapped to the human reference genome hg19 using bwa backtrack (Li & Durbin, 2009) with edit distance and seeding modified to account for aDNA damage (–n 0.01 and –l 16000). Alignments were processed with SAMTools (Li et al., 2009) and Picard (Broad Institute, 2019.), retaining non duplicated reads with mapping quality ≥30. Mapping statistics, genetic sex estimation, and post-mortem damage profiles were generated using Qualimap2 (Okonechnikov et al., 2016), bedtools (Quinlan & Hall, 2010), sexing.py script (Skoglund et al., 2013), and mapDamage2.2.1 (Jónsson et al., 2013).

After preprocessing, all the reads that didn't map to the human genome were processed with KrakenUniq (Breitwieser et al., 2018) and the standard MicrobialDB. We have filtered our raw KrakenUniq reports considering valid

microbial species with a minimum E-score (proportion of unique kmers by read per genome coverage) of 7. Microbial profiles were analysed with SourceTracker2 (McGhee et al., 2020) using reference datasets representing oral, skin, soil, and gut microbiomes (Bissett et al., 2016; Human Microbiome Project Consortium, 2012; Oh et al., 2016; Rampelli et al., 2015). Oral-associated microbial species were selected from eHOMD and SourceTracker2 results. We extracted these species from our samples' raw reports and combined them with published ancient and modern oral microbiome datasets. After merging these, we have normalised each sample for library size, discarded species with a representation below 0.02%, clr-transformed the dataset, and a PCA was computed using mixOmics R package (Rohart et al., 2017). Selected samples were mapped against reference genomes of oral bacterial taxa, including Anaerolineaceae bacterium oral taxon 439, Actinomyces sp. oral taxon 414, and Olsenella sp. oral taxon 807.

Ancient DNA authenticity was assessed through genome coverage, mappability, GC content, and deamination patterns. For phylogenetic analysis, published ancient and modern Anaerolineaceae genomes were combined with our five newly generated samples with >3× coverage. Variants were called with GATK (McKenna et al., 2010), filtered for quality, depth, missingness, and proximity, and used to build a maximum-likelihood tree with RAxML (Stamatakis, 2014), rooted with a Neanderthal strain (Stamatakis, 2014; Weyrich et al., 2017) and visualised with ggtree (G. Yu et al., 2017).

For proteomic analysis, the pellets and 50 µL of supernatant were retained. In order to perform protein extraction, 150 µL of 2M guanidine HCl were added, then 20 µL each of 100 mM CAA and TCEP. Samples were incubated at 99C for 10 min then allowed to cool. 10 µL of a mix of Sera Mag beads was added along with 230 µL of 100% ethanol and incubated at 24 °C for 5 min on a shaker. Beads were pelleted using a magnetic rack and the supernatant removed. Beads were washed three times with 80% ethanol then 150 µL of TEAB and 1 µL trypsin were added to each tube. The tubes were incubated for overnight digestion at 37 °C, with light shaking. The peptides were then immobilised on Pierce™ C18 Tips, which were prepared with methanol, AT80 and 0.1% TFA. Samples were run through the tips twice and followed by two rounds of 150 µL 0.1% TFA. Peptides were analysed by LC-MS/MS on a Q Exactive HF mass spectrometer.

Raw spectra were searched with novor.cloud (Ma, 2015) and pFind (Chi et al., 2018) SwissProt (Release 2017/01), cRAP (as for 04/03/2019) (https://www.thegpm.org/crap/) and the extended Human Oral Microbiome Database (eHOMD). Peptide identifications were filtered using a ≤1% false-discovery rate, and proteins were accepted only when supported by at least two unique PSMs. Contaminants were removed using extraction blanks and the cRAP database, except for human collagen. Peptide preservation was assessed through post-translational modifications, especially glutamine and asparagine deamidation, and peptide length distributions. We compared bacterial peptides against an in-house database, including the eHOMD (version 3.1), the SMAG catalog (Ma et al., 2023), RefSoil (Choi et al., 2017), and the European Soil Diversity database, to classify bacterial peptides. Non-modified bacterial peptides were

additionally searched against NCBI nr using BLASTP, retaining only matches with 100% identity and coverage, and results were parsed with MEGAN (Huson et al., 2007).

4.2 Ref II

In this work, between 3.43 and 15.58 mg of sediment samples were collected from the cavities of ribs, vertebrae, and petrous bones using a dental scraper cleaned between samples with 6% NaOCl, double-distilled water, and 70% ethanol. Each sample was incubated overnight at room temperature in 500 µL of 0.5 M EDTA. Supernatants were then purified, following a published protocol (Keller & L Scheib, 2023), with the difference that there was no concentration of the extracts and 4.5 mL of PB buffer were added at the beginning, and the final elution volume was of 60 µL EB buffer. One extraction was performed per sample for screening, and 30 µL of extract were used for library preparation.

Sequencing libraries were prepared using a modified NEBNext Library Preparation Kit protocol. Libraries were single-indexed and PCR-amplified for 18 cycles. Amplified libraries were purified with MinElute columns and quantified using Qubit, TapeStation, and qPCR. Samples were shotgun-sequenced to approximately 20 million reads on an Illumina NextSeq 500/550 using a High-Output single-end 75 bp kit.

Raw FASTQ files were processed with AdapterRemoval2 (Schubert et al., 2016) to remove adapters, indexes, and poly-G tails. Reads shorter than 30 bp and with quality below 20 were discarded. Low-complexity reads were removed with prinseq (Schmieder & Edwards, 2011) using a dust value of 7, and duplicate reads were collapsed with the bbmap tool suite (Bushnell, 2014). For microbial analysis, first human reads were removed by mapping against the human reference genome build 37 using BWA backtrack (Li & Durbin, 2009) with parameters adjusted for ancient DNA damage.

Non-human reads were then classified with KrakenUniq (Breitwieser et al., 2018) using the standard MicrobialDB. A second KrakenUniq screening with a custom mitochondrial and plastid genome database was used to improve detection of eukaryotic taxa. Candidate species were further checked through competitive mapping to reduce cross-mapping between closely related species.

KrakenUniq reports were filtered and microbial species were considered valid when they had an E-score of at least 7. SourceTracker2 (McGhee et al., 2020) was used to estimate the likely origin of microbial reads using reference datasets for oral, skin, soil, gut, and domestic animal gut microbiomes. Species were retained when they had at least 200 reads in the reference dataset, 50 reads in the target sample, and an abundance above 0.02% across the full dataset. Microbial community structure was explored using Bray–Curtis distances and nMDS in R. Potential contaminants were identified from extraction blanks using decontam with a threshold of 0.7 and removed from downstream analyses.

Ancient DNA authenticity of microbial reads was assessed by mapping reads from source-associated taxa to reference genomes and estimating terminal cytosine deamination with mapDamage2 (Jónsson et al., 2013). GC content was also evaluated, and differences in damage between microbial sources were tested using ANOVA. Human- and ruminant-gut-associated species were extracted from KrakenUniq reports, filtered using an E-score ≥7 and at least 10 reads, normalised by library size, and filtered for minimum abundance. The resulting dataset was clr-transformed and analysed by PCA using the mixOmics R package (Rohart et al., 2017). Microbial taxa of interest were further validated using krakentools (https://bio.tools/krakentools), megablast (Chen et al., 2015), and MEGAN6 (Huson et al., 2007).

Modern human contamination was estimated using schmutzi (Renaud et al., 2015) on mitochondrial and nuclear human reads. For selected samples, mitochondrial contamination was also checked manually in IGV (Robinson et al., 2011) by comparing allele depths at informative positions. Human mitochondrial haplogroups were assigned using GATK (McKenna et al., 2010), bcftools (Danecek et al., 2021), and HaploGrep3 (Schönherr et al., 2023). Genetic sex was estimated using ry_compute, with established ratio thresholds for male, female, and undetermined classifications (Skoglund et al., 2013) .

To analyse black rat sequences from sample NMS022B, published *R. rattus* and *R. tanezumi* (Massini Espino et al., n.d.; Puckett et al., 2016; Teng et al., 2016; H. Yu et al., 2022) genomic data were downloaded and processed together with the ancient sample. Modern data were mapped with BWA mem, while ancient data were mapped with BWA backtrack. Individuals with more than 0.01× genomic coverage were included in population genetic analyses. Genotype likelihoods were generated with ANGSD (Korneliussen et al., 2014) and used for PCA with PCAngsd (Meisner & Albrechtsen, 2018). A pseudohaploid dataset excluding transitions was also generated for f4-statistics using qpDstat in ADMIXTOOLS (Patterson et al., 2012). Statistical significance was assessed using block jackknife resampling. For mitochondrial phylogeny, SNPs were called with GATK UnifiedGenotyper and filtered by depth and genotype quality. Consensus mitochondrial sequences were generated with bcftools, and poorly covered positions were masked. A maximum-likelihood phylogenetic tree was built with RAxML (Stamatakis, 2014) using the GTR-GAMMA model, 100 bootstraps, and *R. tanezumi* as the outgroup. Trees were visualised with ggtree (G. Yu et al., 2017).

Published human data from Cherry Hinton and sediment-derived human reads were mapped and processed using the same aDNA pipeline. Pseudohaploid calls were generated with pileupcaller (available at https://github.com/stschiff/sequenceTools) using the HO dataset as a calling panel (Lazaridis et al., 2014). Terminal deamination patterns were compared between sediment and skeletal samples using mapDamage2 (Jónsson et al., 2013) and a Welch two-sample t-test. Low-coverage genomes from CHRY038B and CHRY038 were imputed with QUILT (Davies et al., 2021) using the HRC reference panel (McCarthy et al., 2016). Post-imputation filtering retained high-

confidence genotypes, although results were treated as suggestive because sequence coverage was below the recommended level for imputation. Human population affinities were explored by projecting the samples onto a PCA of modern European individuals from the HO dataset using smartpca. Relatedness between CHRY038 and CHRY038B was assessed with READv2 (Alaçamlı et al., 2024). Phenotype-associated SNPs were extracted from the imputed genomes, including HIrisPlex-S (Chaitanya et al., 2018) pigmentation markers and selected diet- and disease-related variants. Pigmentation probabilities were estimated using the HIrisPlex-S webtool (https://hirisplex.erasmusmc.nl/).

During DNA extraction, the remaining pellet and 50 µL of supernatant were retained for protein analysis. Proteins were extracted using a modified SP3 protocol with guanidine HCl, CAA, TCEP, Sera-Mag beads, ethanol washes, and overnight trypsin digestion. Peptides were purified using C18 tips and stored at −20 °C before analysis. Peptides were analysed by LC-MS/MS at the Proteomics Core Facility, University of Tartu. Samples were separated by nanoLC and analysed on a Q Exactive Plus mass spectrometer using data-dependent acquisition. Raw spectra were used for downstream protein identification and preservation analysis. Raw mass spectrometry data were searched with pFind (Chi et al., 2018) against SwissProt (Release 2017/01) and cRAP (as for 04/03/2019) (https://www.thegpm.org/crap/), and results were compared with novor.cloud (Ma, 2015). Searches included carbamidomethylation as a fixed modification and several variable modifications. PSMs were filtered at ≤1% false discovery rate, and only peptides with pFind scores ≤0.01 and at least two PSMs were retained. Contaminants identified in extraction blanks and the cRAP database were removed. Peptides were further checked using BLASTP, MEGAN (Huson et al., 2007), and Unipept (Vande Moortele et al., 2025). For rat peptide identification, a custom Rattus protein database was built using *R. rattus* and *R. norvegicus* reference protein sequences. Filtered peptides were searched against this database and NCBI nr. Species-specific peptides with 100% identity and coverage were parsed with MEGAN (Huson et al., 2007).

4.3 Ref III

In this study, teeth of 404 individuals from Sint-Truiden and 15 from Ypres were sampled for the analyses. Tooth roots were sampled with a sterile drill wheel and briefly brushed to remove surface dirt using full-strength household bleach, 6% w/v NaOCl, and a disposable toothbrush soaked in bleach before use. Decontamination, extraction, purification, and library preparation were performed following published aDNA laboratory protocols (Keller & Scheib, 2023). Shotgun sequencing of pooled libraries was performed in two phases: an initial screening phase to assess endogenous DNA content, contamination, and sample complexity, followed by deeper sequencing of selected samples to reach a target autosomal depth of >0.1× for genotype imputation. Libraries were sequenced at the KU

Leuven Genomics Core facilities using 100 bp paired-end kits on Illumina NovaSeq S4 flow cells for 200 cycles.

All downstream analyses of human DNA were performed using established pipelines on the University of Tartu cluster. Adapters, low-quality 3′ ends, and flanking N bases were removed with CutAdapt-2.10 (Martin, 2011), keeping read pairs only when both reads were at least 28 bp long. Paired-end reads were merged with FLASH-1.2.11 (Magoč & Salzberg, 2011) and mapped to the hs37d5 human reference genome using BWA-backtrack (Li & Durbin, 2009), chosen because of the short read length. BAM files were generated with SAMTools-1.9 (Li et al., 2009), unmapped reads were discarded, duplicates were removed with Picard MarkDuplicates (Broad Institute, 2019.), and local realignment around indels was performed with GATK (McKenna et al., 2010). Reads with MAPQ <10 were removed, and mapping statistics were generated with SAMTools (Li et al., 2009).

Endogenous DNA content was calculated as the proportion of mapped reads, including duplicates, out of the total number of reads. Ancient DNA authenticity was assessed with mapDamage2.0.6 (Jónsson et al., 2013) by measuring C to T substitutions at read ends. Autosomal contamination was estimated with ANGSD-0.917 (Korneliussen et al., 2014) using the X-chromosome contamination module, while mitochondrial contamination was calculated from the proportion of non-consensus bases at haplogroup-defining positions. All samples were mapped against the hs37d5 human reference genome and checked against sample-specific mitochondrial haplogroup-defining sites. After filtering alignments with MAPQ >20, we estimated genetic sex using ry_compute (Skoglund et al., 2013) and karyo_RxRy (Anastasiadou et al., 2024).

Mitochondrial variants were called with bcftools v1.14 (Danecek et al., 2021) using the hs37d5 mitochondrial sequence as reference. Mitochondrial haplogroups were assigned with HaploGrep v2.1.21 (Weissensteiner et al., 2016) using PhyloTree Build 17 (van Oven, 2015). Binary Y-chromosome variants were called at approximately 273,000 previously identified polymorphic sites using ANGSD haploid calling. The output was converted to PLINK, VCF, and BED formats, filtered against reference and alternative alleles, converted to haploid format, and annotated with bedtools (Quinlan & Hall, 2010). Y-chromosome sub-haplogroups were assigned by comparing derived allele calls to the YFull tree (available at https://www.yfull.com/), requiring at least two variants to support terminal branch assignment.

For genetic relatedness analyses, pseudohaploid genotypes were generated from 382 individuals with coverage above 0.01×. One randomly sampled allele was called with ANGSD at common variants from the UK10K subset of the HRC panel (McCarthy et al., 2016), filtered for Minor Allel Frequency (MAF) >5%. The output was converted to PLINK, VCF, and BED formats for downstream analyses. Close biological relationships from the 1st to 3rd degree were screened using KIN (Popli et al., 2023) and READv2 (Alaçamlı et al., 2024) on 372 individuals with coverage >0.01×. Genotypes of Sint-Truiden and Ypres individuals were imputed with QUILT (Davies et al., 2021) using the HRC v1.1 reference panel. After filtering and removal of individuals with coverage below 0.1×,

338 Sint-Truiden and 8 Ypres individuals were retained. The final imputed dataset was merged with published genomes and used for downstream analyses.

A PCA was performed after merging the imputed Sint-Truiden data with Early Medieval, Iron Age, and modern European reference genomes. After LD pruning and exclusion of likely non-neutral regions, PCA was calculated with FlashPCA 2.0 (Abraham et al., 2017). Population structure analyses were performed using imputed genotype data downsampled to the 600K SNPs available in the Allen Ancient DNA Resource (AADR) (Mallick et al., 2024). Supervised ADMIXTURE (Alexander et al., 2009) was used to model target groups. qpWave and qpAdm analyses were performed with AdmixTools (Patterson et al., 2012). HapNe-LD (Fournier et al., 2023) was used to estimate effective population size through time after removing closely related individuals. IBD segments and kinship coefficients were estimated with IBIS (Seidman et al., 2020) using imputed ancient and modern reference genomes. Individual connectedness was estimated with the PiC score (the proportion of individuals in a reference group sharing IBD with each target individual). Runs of homozygosity were detected with hapROH (Ringbauer et al., 2021) in imputed genomes and compared with estimates from non-imputed pseudo-haploid data. Genome-wide heterozygosity was estimated with PLINK.

To test for enrichment of allele-frequency differences in immune genes before and after the start of the second plague pandemic, Fst (Fixation index) analyses were performed with PLINK using neutral, innate immunity, adaptive immunity, and HLA variants. Immune variants above the 99th percentile of neutral Fst values were considered highly differentiated. Genome-wide Fst scans were also performed to identify possible selection signals between earlier and later time groups, and selected variants were annotated with VEP (McLaren et al., 2016). Variants with strong phenotypic effects were analysed after filtering imputed genotypes by dosage. Genotypes were extracted for 112 SNPs associated with diet, disease, immunity, and pigmentation, including HIrisPlex-S (Chaitanya et al., 2018) markers. Pigmentation probabilities were estimated with the HIrisPlex-S webtool (https://hirisplex.erasmusmc.nl/). Allele frequencies were compared between earlier and later Sint-Truiden cohorts and with modern Flemish genomes using ANOVA with Bonferroni correction.

For metagenomic analyses, raw sequencing data were merged by sample, quality-checked, trimmed, merged, and then screened with KrakenUniq (Breitwieser et al., 2018) for human-associated pathogens and pathobionts. The database used for analysis included complete genomes and chromosome-level assemblies of bacteria, viruses, archaea, and protozoa, the human genome, the NCBI Viral Neighbor database, and the contaminant databases UniVec and EmVec. E-values were calculated as described in Guellil et al. (Guellil et al., 2022). Reported taxa were validated by mapping reads to reference sequences, removing duplicates, and assessing damage patterns with mapDamage2 (Jónsson et al., 2013). Mapping statistics and visualisations were generated with Qualimap (Okonechnikov et al., 2016), aDNA-BAMPlotter (Guellil, 2021), and Python modules. Sample ST1516 was the best candidate for *Yersinia pestis* and was

enriched using a custom *Y. pestis/Y. pseudotuberculosis* myBaits target enrichment kit (Keller et al., 2023). Enrichment followed the myBaits protocol using half a bait aliquot, followed by amplification and sequencing on an Illumina NextSeq500. Additional samples were also enriched but with lower success.

4.4 Ref IV

For this study, we collected 9 samples from different areas of *Grotta della Spinosa*, Grosseto, Tuscany, Italy. Portions of tooth crowns, tooth roots, petrous bones, and one incus were sampled with a sterile drill wheel and briefly brushed to remove surface dirt using full-strength household bleach, 6% w/v NaOCl, and a disposable toothbrush soaked in bleach before use. Decontamination, extraction, purification, and library preparation were performed following published aDNA laboratory protocols (Keller & Scheib, 2023).

Libraries were double-stranded, double-indexed, and split into two amplifications. Library preparation success and DNA concentration were assessed using QubitKrakenUniq, TapeStation, and qPCR (KAPA Library Quantification Kit – Illumina® platforms). DNA was initially shotgun-sequenced on an Illumina NextSeq500/550 High-Output paired-end 150-cycle kit at the University of Tartu Institute of Genomics Core Facility. Deeper sequencing was performed in the same facility using the Illumina NextSeq2000 platform. The dsDNA library of sample GSP013 was enriched using a custom *Y. pestis/Y. pseudotuberculosis* myBaits target enrichment kit from Daicel Arbor Biosciences (Keller et al., 2023). Hybridization was carried out for 35 h at 62 °C, and the enriched library was sequenced on the Illumina NextSeq2000 platform.

Raw FASTQ files were processed by removing adapters, indexes, and poly-G tails using cutadapt (Martin, 2011).Sequences shorter than 30 bp were discarded. Trimmed sequences were aligned to the human reference genome GRCh37, using BWA mem (Li & Durbin, 2009) with re-seeding disabled and converted to BAM format with SAMTools (Li et al., 2009). For paired-end sequencing, different flow-cell lanes were merged, duplicates were removed using Picard MarkDuplicates (Broad Institute, 2019.) and SAMTools (Li et al., 2009), and indels were realigned using GATK(McKenna et al., 2010). Reads with mapping quality below 10 were filtered out. Different runs and extractions from the same sample were then merged. Ancient DNA damage patterns were estimated with mapDamage (Broad Institute, 2019.; Jónsson et al., 2013). Modern human contamination was estimated for mitochondrial DNA using ContamMix, and for male individuals on the X chromosome using ANGSD (Korneliussen et al., 2014). Possible genetic relationships between individuals were estimated using READv2 (Alaçamlı et al., 2024).

Autosomal variants were called with ANGSD using the 1240k positions from the AADR dataset. ANGSD output was converted to PLINK format, and autosomal SNP counts were estimated. Six individuals with more than 20,000 autosomal 1240k SNPs were selected for genome-wide analysis. The newly generated

data were merged with the AADR dataset (v62.0, https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/FFIDCW, version: January 2025), subsetted to the HO array, and filtered for positions with MAF > 0.1. The dataset was converted to EIGENSTRAT format using EIGENSOFT, and a PCA was performed with smartpca by projecting the new and published ancient individuals onto present-day Western Eurasian genetic variation.

For metagenomic analyses, raw sequences were pre-processed using AdapterRemovalv2 (Schubert et al., 2016) to trim adapters and collapse paired-end reads when necessary. Reads shorter than 30 bp and bases with quality below 20 were discarded. Low-complexity reads were removed using prinseq-lite with a dust value of 7, and duplicated sequences were removed with BBmap (Bushnell, 2014). High-complexity deduplicated sequences were screened with KrakenUniq (Breitwieser et al., 2018) against the standard MicrobialDB. Reports were filtered using a minimum E-score of 7 and a minimum of 100 assigned reads, except for viruses.

Ancient published *Yersinia pestis* genomes were mapped against the *Y. pestis* CO92 reference genome using BWA backtrack (Li & Durbin, 2009) with ancient-DNA-sensitive parameters. Duplicates were removed with Picard MarkDuplicates (Broad Institute, 2019.) and reads with mapping quality ≥30 were retained. Variants were called using GATK UnifiedGenotyper with the - *-emit-all-sites* option (McKenna et al., 2010). A high-quality biallelic SNP dataset was created with vcftools (Danecek et al., 2011) by filtering for minimum depth of 3×, genotype quality of 30, removal of indels and heterozygous positions, maximum missingness of 40%, allele depth ratio of 90%, and a minimum distance of 3 bp between SNPs. tRNA, rRNA, and repetitive regions were also removed. The final dataset contained 2,026 SNPs across 36 ancient *Y. pestis* genomes, *Y. pestis* CO92, and *Y. pseudotuberculosis*. A maximum-likelihood phylogeny was built using raxml-ng with the GTR+G model and 1,000 bootstraps (Kozlov et al., 2019). Tree support was assessed using FBP and TBE.

Known virulence genes were screened in *Y. pestis* on the chromosome and plasmids pCD1, pMT1, and pPCP1 using a mapping quality filter of 1. Gene coverage was analysed with BEDTools (Quinlan & Hall, 2010), and selected genes were manually inspected in IGV to assess the presence of functional SNPs or indels.

E. rhusiopathiae genomes were processed with AdapterRemoval(Schubert et al., 2016) using the same parameters. Modern genomes were mapped against the *E. rhusiopathiae* NCTC8163 reference genome using BWA mem (Li & Durbin, 2009), while ancient genomes were processed following the same steps used for *Y. pestis*. VCF files were generated with GATK UnifiedGenotyper (McKenna et al., 2010) for 196 modern and 8 high-coverage ancient genomes. Positions were filtered for minimum coverage of 5×, genotype quality of 20, and removal of indels. Heterozygous positions were reassigned when the majority allele was supported by at least 90% of reads; otherwise, they were discarded. Repetitive regions, tRNA, and rRNA genes were also masked. Filtered VCF files were converted into consensus sequences using bcftools (Danecek et al., 2021), masking low-quality positions with N.

The genomes were concatenated, and an initial maximum-likelihood tree was built with FastTree (Price et al., 2009). This tree and alignment were used to detect recombination with ClonalFrameML (Didelot & Wilson, 2015). After removing recombination tracks and filtering for missingness, SNP distance, and non-variant positions, a high-quality dataset of 6,809 SNPs was obtained. This dataset was used to include modern *E. tonsillarum*, Es2 genomes, and intermediate-coverage *E. rhusiopathiae* genomes. A maximum-likelihood tree was built with raxml-ng using the GTR+G model and 1,000 bootstraps (Kozlov et al., 2019), with node support assessed by TBE and FBP. Low-coverage samples were called pseudo-haploid with PileupCaller and projected onto the tree using epa-ng (Barbera et al., 2019). Samples with first placement LWR above 70% were grafted using gappa, and the final tree was midpoint-rooted.

The same approach followed for *Y. pestis* was used to screen known virulence genes in *E. rhusiopathiae*. A PCA of *E. rhusiopathiae* virulence genes was created from a SNP dataset of these regions in 170 modern and ancient high-quality genomes. Lower coverage genomes were projected using pseudohaploid calls. Since most ancient *E. rhusiopathiae* samples showed genetic affinity with Clade 2, a genome-wide Fst analysis was performed between ancient genomes and modern Clade 2 genomes, excluding tRNA and rRNA genes. The Weir and Cockerham estimator in vcftools (Danecek et al., 2011) was used, and the top 1% genes with high Fst were selected. To identify genes with different coverage between modern and ancient strains, genome-wide normalised coverage was calculated for each genome. A gene was considered present in ancient *E. rhusiopathiae* genomes from human individuals when at least 75% of individuals in a group showed at least 75% breadth of coverage. If more than 75% of individuals had less than 75% breadth of coverage, the gene was considered absent.

5. RESULTS AND DISCUSSION

This section summarises the main results and discussions of the four scientific publications included in this dissertation. The aim of the section is to provide an overview of the main findings of the works. More detailed information can be found in the original articles and their respective supplementary material.

5.1 Concretions covering human remains preserve microbial ancient DNA and proteins (REF I)

In this study, we have explored aDNA and protein content and preservation of concretions covering human remains. In order to do that, we extracted aDNA and proteins in tandem from human dental elements and their surrounding concretions and compared them to non-concreted human dental elements from the same site. We produced 24 libraries from 24 total samples from 5 individuals from the site of Titolo (Figure 4). The analysis of samples from *Grotta della Scaloria*, a nearby site, were unsuccessful, so they are reported only in the original manuscript.

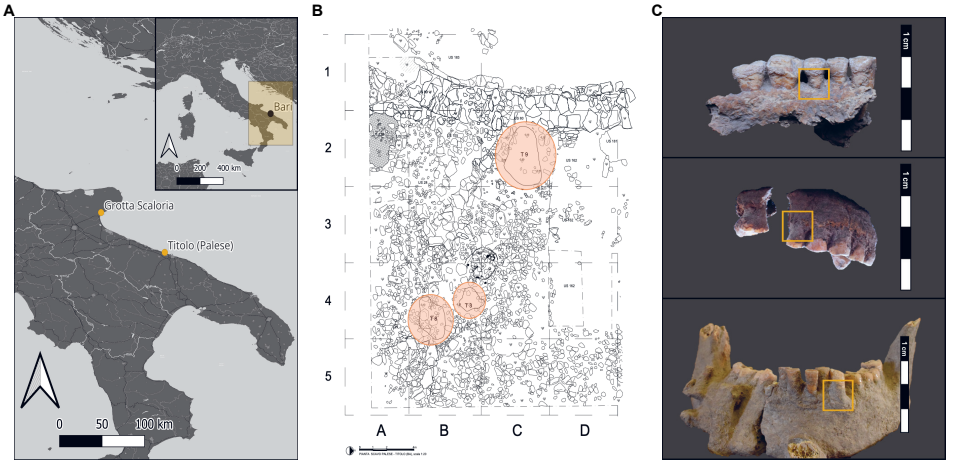

Figure 4: Map of Italy, with the sites highlighted, site map, and archaeological material.

A) Map of Italy and detail of Puglia, displaying the sites named in text. B) Layout of burials at Titolo (Palese, BA, Puglia, Italy) with the location of the three burials with skeletal remains covered by sediment concretions highlighted in orange. C) Concretion-covered dentitions of the three individuals under study: PAL002, burial T3 (top), PAL005, burial T8 (middle), PAL006, burial T9 (bottom), with sampled region in yellow. Reprinted from Figure 1 (Bonucci et al., 2025), licensed under Creative Commons CC BY license. Copyright © 2025 The Author(s). Published by Elsevier Inc.

5.1.1 Low ancient human DNA content in concretions and corresponding tissues

Across the samples from Titolo, initial analysis showed a very low preservation of human DNA (<0.03% of human DNA content on average) in the test set and 375-26,502 reads (0.01%–0.30% human DNA content) in the control set. Moreover, the low deamination rates detected in most of the samples may indicate modern contamination.

Critically, the source material did not reach the minimum amount of unique human reads to perform population genetic analyses. This is a remarkable finding which should be considered in future biomolecular studies, and we caution that the utility of human remains covered in concretions may be limited. Nevertheless, it is possible that further studies of concretions surrounding human skeletal or dental remains, from other archaeological periods or environments, could yield different results. It is important to note that we used a screening sequencing depth for the analysis, and thus, higher coverage values for human data could be achieved either with deeper sequencing or capture.

5.1.2 Ancient oral microbial genomes can be retrieved from concretions

The remaining reads were identified by KrakenUniq as belonging to microbial (2%–6.8% test set; 0.92%–4.8% control set) or were unclassified (93%–98% test set; 95.16%–98.82% control set). We filtered our data to include only the species assigned with an E-score above 7, which is the minimum threshold we used to trust that the assignment of a species is true (see Methods section).

The SourceTracker2 (McGhee et al., 2020) analysis of the 9 dental calculus samples from Titolo showed that 55% to 95% of the classified reads originated from oral taxa, while very few stemmed from the skin, soil, or gut microbiome. From the test samples, PAL002G and PAL005F showed little presence of oral microbes, while much of the microbial species present in PAL005E, PAL006C, and PAL006D (from 50% to 80%) belonged to a human oral source (Figure 5A). We used a PCA to place our newly generated ancient samples in relation to modern and ancient published samples (Figure 5B). We could compare all the dental calculus from this study and samples PAL005E, PAL006C, and PAL006 to other ancient and modern published dental calculus samples. The PCA loadings indicate that Anaerolineaceae bacterium oral taxon 439 (henceforth indicated as Abot439), Olsenella sp. oral taxon 807 (Osot807) and *Desulfomicrobium orale* are the species that most contributed to the distribution of the samples in the left quadrant of the PCA plot.

Despite the fact that the concretions seem to show generally lower abundances of oral bacteria, PAL005E and PAL006C yielded a substantial number of reads for 33 and 46 oral species, respectively (Figure 5C). In particular, we found a high abundance of two sulfate-reducing bacteria, *Desulfobulbus oralis* and *Desulfomicrobium orale*, as well as other common oral species such as Abot439, Actinomyces sp. oral taxon 414 (Asot414) and Osot807.

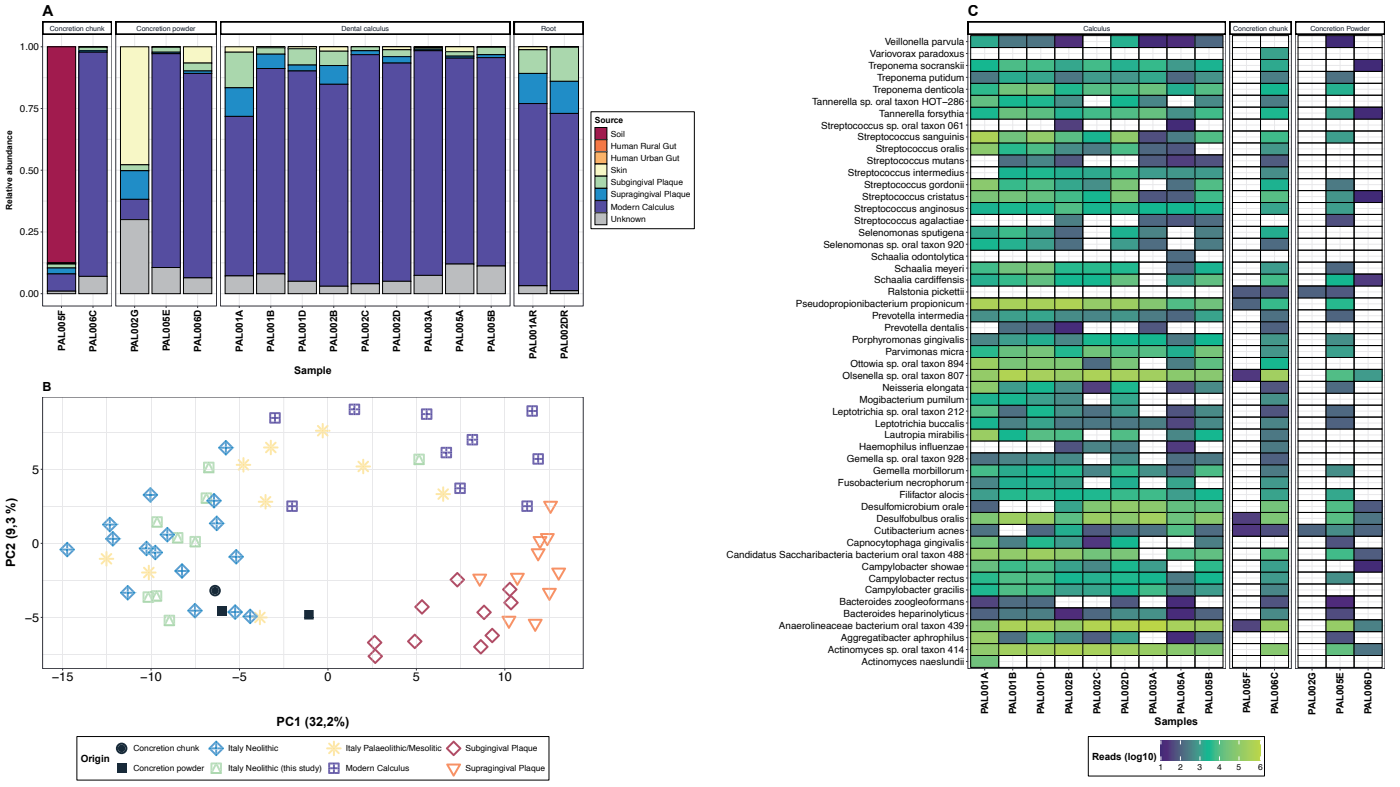

Figure 5: Microbial ecology background of the samples.
A) Stacked bar plot of Sourcetracker analysis showing the proportion of KrakenUniq classified reads at the species level stemming from modern dental calculus, modern subgingival and supragingival plaque, skin, soil, and gut microbiome (both rural and urban) in the samples taken into consideration in our study. B) PCA of samples in context of modern and ancient published oral metagenomes. C) Heatmap of oral species detected. The number of reads is displayed in log10 scale. All E-scores are over 7. Reprinted from Figure 3 (Bonucci et al., 2025), licensed under Creative Commons CC BY license. Copyright © 2025 The Author(s). Published by Elsevier Inc.

We further explored in more detail the most prevalent species in PAL005E and PAL006C, by aligning the sequences from PAL005E against the references of Abot439 (Figure 6A). We retrieved 192,093 reads unique to Abot439 from PAL005E, with 2.87X of average coverage, and 161,658 reads unique to Abot439 from PAL006C, with 2.63X of average coverage. Both samples displayed C to T terminal substitutions. A maximum likelihood tree made with 16,469 SNPs with a depth ≥3X found across 5 Titolo samples and 34 published strains reveals close affinity with other Neolithic and Bronze age strains of the bacterium (Figure 6B). The Titolo strains in particular clustered by individual, regardless of the source from which the DNA was extracted from (calculus or concretions). However, node support was low across all Neolithic Italian strains of Abot439 (including the published ones), indicating insufficient resolution to adequately distinguish between these genetically similar strains, due to low coverage.

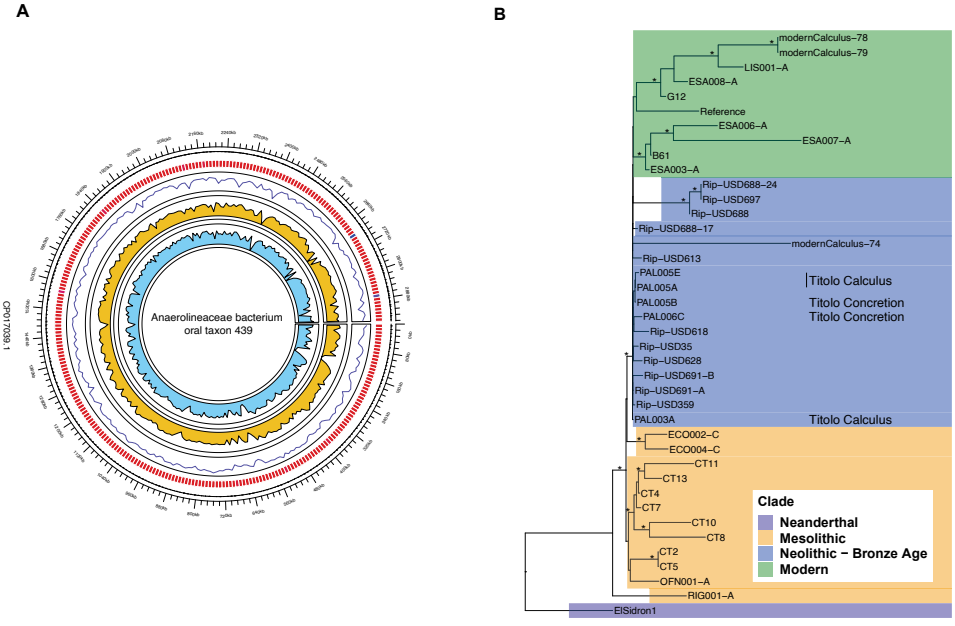

Figure 6: Circos plot of the mapping of samples PAL005E and PAL006C and phylogenetic tree of Abot439.

A) Circos coverage plot mapping samples to Abot439 reference genome. From the outer to the inner ring: mappability, GC content, depth of coverage for sample PAL005E, depth of coverage for PAL006C. B) Maximum-likelihood tree created with RAxMl and 1,000 bootstraps. The tree includes 16,469 biallelic positions and 39 modern and ancient Abot439 strains. Clades are highlighted in colour. Nodes with a support over 90% are marked with an asterisk (∗). Reprinted from Figure 4 (Bonucci et al., 2025), licensed under Creative Commons CC BY license. Copyright © 2025 The Author(s). Published by Elsevier Inc.

5.1.3 Preservation of human and bacterial peptides

All extractions were processed in tandem for ancient peptide data (see Methods for further details). RAW files were searched against SwissProt, eHOMD, and the cRAP database with pFind. Using this tool, we identified 13,657 unique peptides corresponding to 2,938 proteins (Figure 7A). Peptides from *Homo sapiens* were present in all samples, with different percentages. Teeth had the lowest average number of human peptides (Average = 150). We identified 45 protein species common to all individuals (Figure 7C), with 16 of these assigned to human oral microbes, 24 to non-human vertebrates, 4 to other bacteria and one to *Homo sapiens*. Of the four individuals from Titolo, only PAL005 appears to have a unique protein community. In fact, 114 bacterial species were identified as unique to this individual, with very few peptides being poorly identified. Individual PAL005 hosts the highest number of human oral microbiome (HOM) specific species, with 88 bacterial species identified, followed by individuals PAL006 (n = 31) and PAL001 (n = 23).

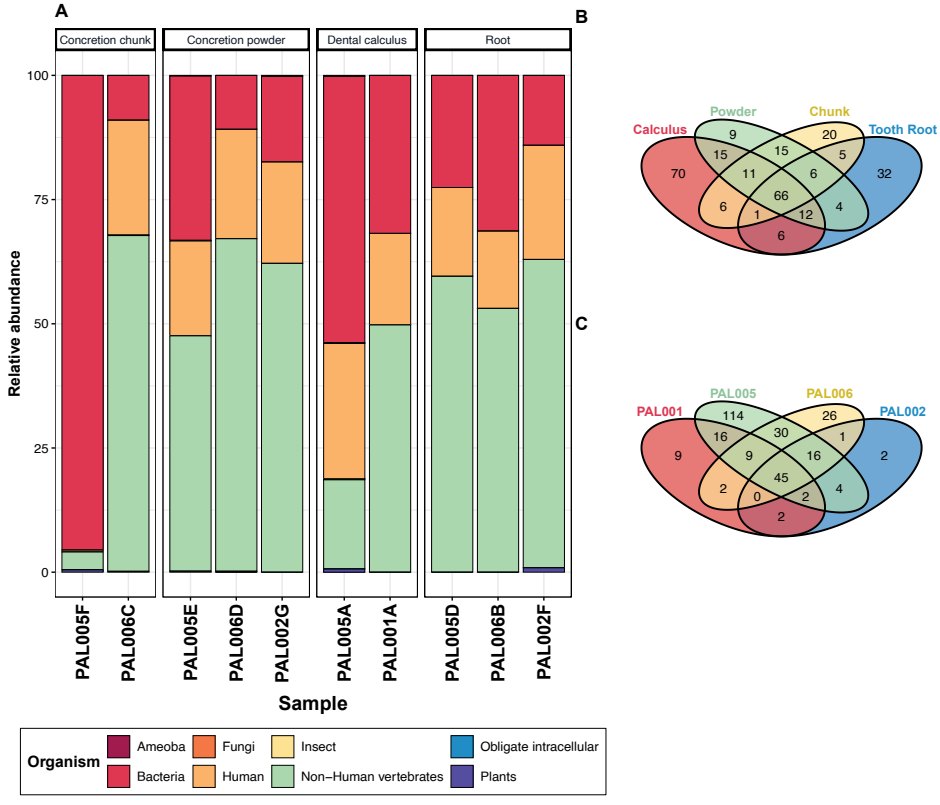

Figure 7: Relative proportions of peptides by organism and Venn diagrams showing number of species per sample type and individual.

A) Stacked bar plot showing the relative abundance for peptide origin based on pFind output. B) Venn diagram of species identified by pFind, categorized by sample type. C) Venn diagram of species identified by pFind, categorized by individual. Reprinted from Figure 5 (Bonucci et al., 2025), licensed under Creative Commons CC BY license. Copyright © 2025 The Author(s). Published by Elsevier Inc.

Moreover, we identified HOM peptides specific to each sample type. As expected, calculus contains the highest number of bacteria from the human oral microbiome (n = 90), followed by teeth (n = 54), chunks of concretions (n = 50) and concretions powder (n = 43) (Figure 7B).

Finally, to confirm the specificity of all the HOM candidate bacteria identified, we merged peptides whenever possible and performed a BLAST search against the NCBI nr database. We found specific peptides associated with HOM bacteria. Nevertheless, the ion coverage of these specific peptides remains relatively low. However, when considering the large number of peptides linked to these bacteria, it suggests that recovering human-associated bacteria, along with human-specific peptides, from bone-adhered concretions may be possible.

Interestingly, palaeoproteomics analyses of some samples revealed the presence of proteins from the same oral microbiome species for which DNA was retrieved, highlighting the advantages of combining these approaches for a more comprehensive understanding of substrates.

5.2 Obtaining ancient genomic and proteomic data in a nondestructive manner from bone-adhered sediments. (REF II)

In this study, we performed metagenomic and metaproteomic analysis on sediments found inside several human skeletal elements (temporal bone, rib, and vertebrae), originating from 11 individuals from 7 Neolithic to Medieval sites in the Cambridgeshire region, England (Figure 8).

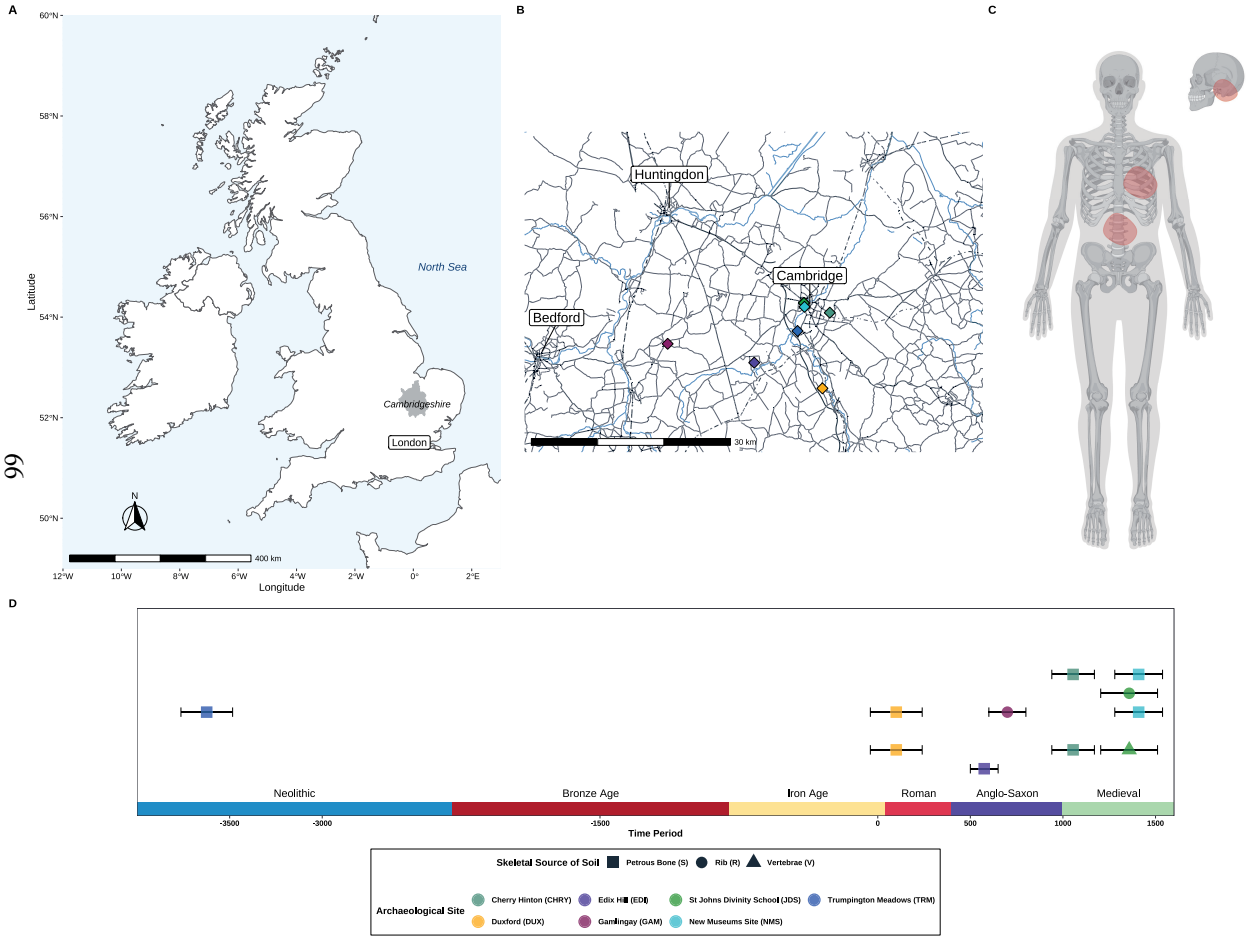

Figure 8: Geographical, chronological, and osteological sampling areas used in this study. a) Map of the United Kingdom with the Cambridgeshire area highlighted. b) Map of the sites used in this study. c) Highlighted with circles, skeletal elements from which the sediments were sampled for this study (Created in BioRender. Bonucci, B. [2025] https://BioRender.com/xok2pd2). d) Timeline of the chronological order of the sites used in this study (legend below). Skeletal elements indicated by shape. Reprinted from Figure 1 (de-Dios et al., 2025), licensed under Creative Commons CC BY license. Copyright © 2025, © The Author(s) 2025. Published by Oxford University Press on behalf of Society for Molecular Biology and Evolution.

5.2.1 Endogenous Human aDNA and Proteins

Human aDNA was recovered from all sediment samples except those from Duxford. Endogenous human DNA abundance in sediments did not correlate with that of the original bone, although sediment-derived sequences showed significantly higher terminal C to T deamination than bone-derived DNA (Welch's t-test, P = 0.01547). Genetic sex estimates from sediment-derived human DNA were generally consistent with those obtained from the original skeletal elements, although low endogenous content in some samples limits confidence. A special case is sample CHRY038B (Petrous bone sediment), which yielded a human genome at an average depth of roughly 0.01× (3% of endogenous DNA). The genetic sex, mitochondrial haplotype (XX, U5b3), matched those of the original bone sample. READv2 (Alaçamlı et al., 2024) classified the sediment and bone libraries as 'IDENTICAL' and PCA placed the sediment-derived genome close to the original skeletal sample, with overlapping 95% confidence intervals. We then pulled imputed phenotypes to check the concordance between information in the original bone sample and bone-adhered sediments. We applied a genotype dosage (DS) of 0.1 and observed a concordance of ~73.4% with 29 genotypes of the total 109 phenotypic-informative markers differing between the two sample sources.

From a proteomics perspective, peptides were assigned to *Homo sapiens* by pFind in each sample, but a more detailed manual verification with BLASTp, revealed that only five samples preserved peptides with 100% sequence coverage and specificity only to *Homo sapiens*. These peptides correspond to the proteins alpha-2-HS-glycoprotein, Serpin family F member 1, and Hornerin.

5.2.2 Detection of microbial DNA and peptides

An initial metagenomic screening of the samples using Kraken2 (D. E. Wood et al., 2019), with the NCBI nt database classified between 0.98% and 5.2% of the DNA sequences. Subsequently, we performed a second screening using KrakenUniq (Breitwieser et al., 2018) and generated a microbial assignment dataset based on hits with an E-score above 7 (see Methods). The microbial source tracking (MST) of the samples using SourceTracker2 (McGhee et al., 2020) revealed no clear pattern consistent with anatomical sampling location (Figure 9a).

A nonmetric multidimensional scaling (nMDS) from the same dataset using Bray–Curtis distances shows that most of the samples fall in between the diversity of the reference sources, apart from TRM003B and GAM042B, which fall close to the blanks. We excluded potential contaminant taxa as found in extraction blanks using decontam with a threshold of 0.7 (Davis et al., 2018). Those included 121 microbial species.

A closer inspection of the KrakenUniq screened data using source-specific species assigned by SourceTracker2 per_sink_feature_assignments mode reveals that most of the PbS samples (CHRY, DUX, and NMS) contain traces of human gut and/or ruminant gut microbial species (Figure 9b).

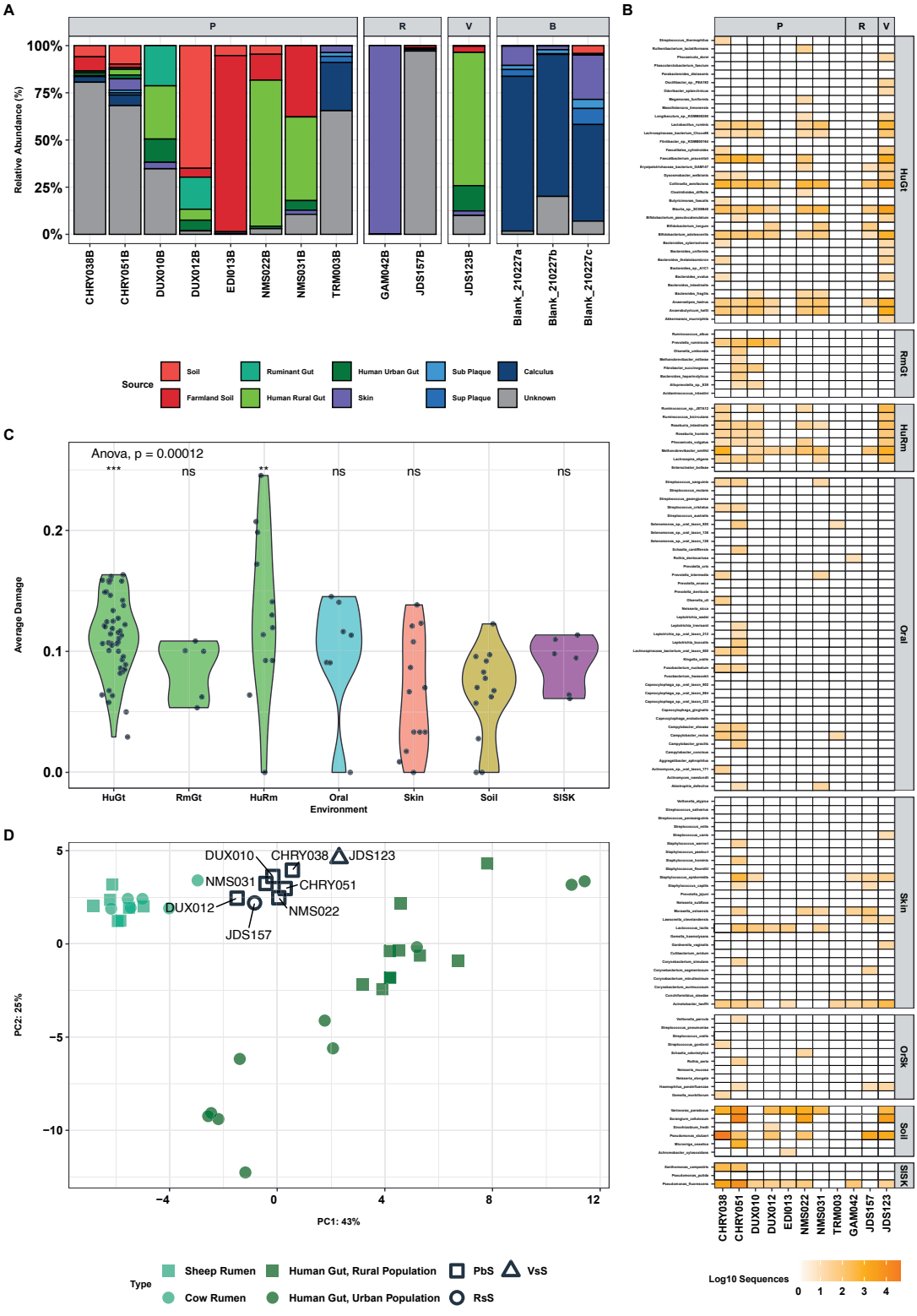

Figure 9: Bacterial composition of the samples in this study.

a) Stacked bar chart of MST results estimated by SourceTracker2. b) Presence of source characteristic bacteria per sample inferred by mapping. c) Average terminal damage by each source. Source means were compared using an ANOVA test. Statistical significance is marked using * ($P \leq 0.05$), ** ($P \leq 0.01$), and *** ($P \leq 0.001$). d) Principal component analysis of gut-associated bacteria in the analysed samples and published gut microbiome data from humans and domesticated ruminants (cow and sheep). Reprinted from Figure 3 (de-Dios et al., 2025), licensed under Creative Commons CC BY license. Copyright © 2025, © The Author(s) 2025. Published by Oxford University Press on behalf of Society for Molecular Biology and Evolution.

Mapping of the sequences assigned to the source-characteristic species and subsequent aDNA damage pattern characterization reveals the presence of deamination levels consistent with those expected for authentic aDNA sequences (Figure 9c).

Given the abundance of gut-associated microbial sequences in the samples, we decided to further explore the microbial composition by performing a PCA, which included 79 microbial species found across 33 of the included reference metagenomes (Rampelli et al. 2015; Stewart et al. 2019; Tisza and Buck 2021; Su et al. 2022) and eight of our sediment samples (Fig. 9d). All sediment samples fall within the PCA space defined by the reference gut metagenomes, forming a cline between samples with higher levels of inferred rumen microbes falling closer to the ruminant gut and samples more closely the gut microbial taxa typical of nonindustrialized populations. This suggests that some sediment samples contain microbial abundance profiles that overlap compositionally with both human- and domestic animal-associated gut microbiomes.

Regarding potential bacterial proteins, none of the bacterial peptides identified in the samples could be exclusively linked to a pathogen, or to microbes associated with human microbiota.

5.2.3 Black rat (*Rattus rattus*) DNA and proteins retrieved from one sample

After whole genome mapping, we recovered sequences belonging to *Homo* (1,744 to 545,112), *Ovis* (35,913 to 4,617), *Bos* (15,969), *Canis* (8,369 to 4,876), *Trichuris* (20,396), *Ascaris* (22,978 to 463), *Apodemus* (6,571), and *Rattus* (7,698,244) genera.

Most of the sequences display features such as length, terminal deamination, and edit distance distribution compatible with ancient genomes. In the vertebral sample (JDS123B), we have found sequences assigned to parasitic species (*Ascaris lumbricoides*, roundworm; *Trichuris* spp., whipworm). Unfortunately, we could not discern the anatomical origin of vertebral remains due to the conservation state of the bone.

Perhaps more interesting, we found a large amount of sequences assigned to the black rat (*Rattus rattus*) in sample NMS0022B, with an average depth of coverage of 0.1877× for the nuclear and 8.09× for the mitochondrial genomes. By analysing the heterozygosity level of the mitochondrial genome (0 after filtering for potential aDNA damage by removing transitions), we conclude that the

sequences most likely originate from a single male rat, given the ratio of X and Y chromosome sequences (1/1, 1 to 2 autosomes). We created a genome-wide SNP dataset of historical (H. Yu et al., 2022) and modern (Puckett et al., 2016) *R. rattus* and then performed a PCA and mitochondrial ML tree, including our Medieval genome (Figure 10a,b). The NMS022B rat genome displays affinities to other Western and Northern European Medieval and Early Modern rats. We then formally validated this result, conducting an f4 analysis (D statistics) with AdmixTools (Patterson et al., 2012), concluding that NMS022B is genetically closer to other medieval black rats (Figure 10c).

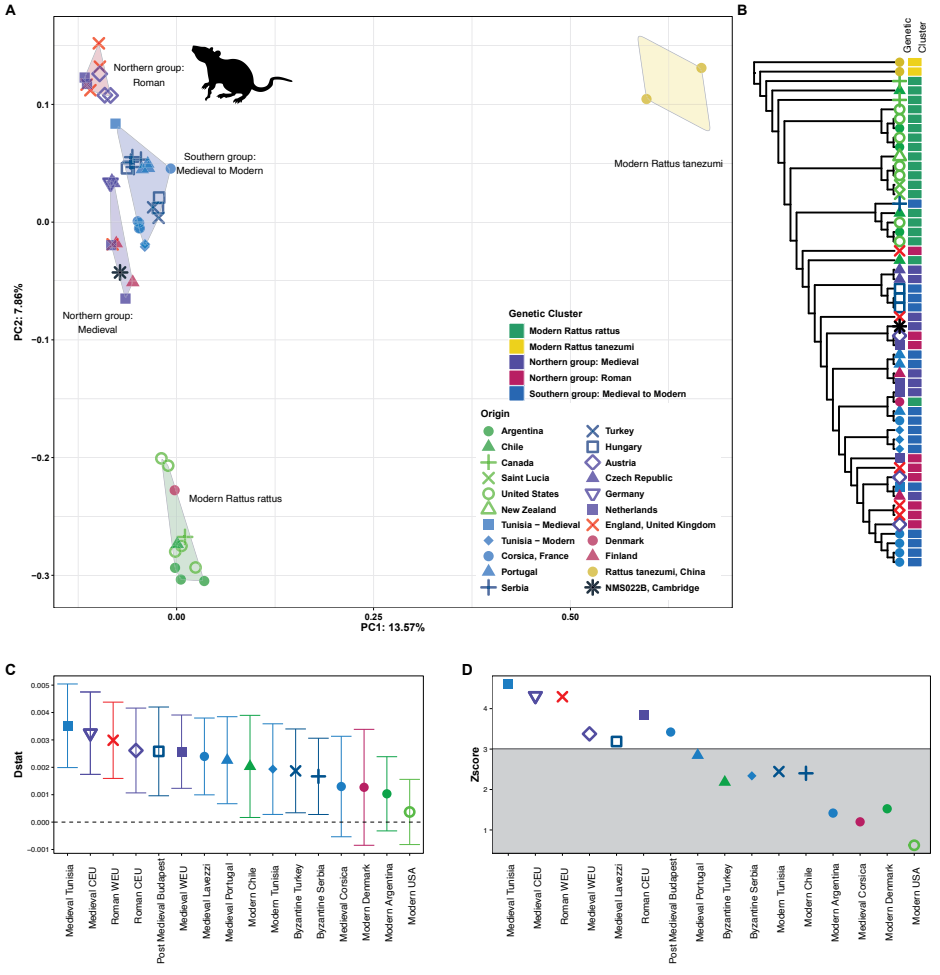

Figure 10: Population genomic analyses of the ancient *R. rattus* genome recovered from NMS022B.

a) PCA created by PCAngsd using 513,655 variable positions found in NMS022B and 51 modern and ancient *R. rattus*. Cluster names are annotated in the figure. PC1 and PC2 account for 13.57% and 7.86% of the described variance respectively. b) ML Tree created using RAxML, a dataset of 3,467 SNPs found across 53 modern and ancient *R. rattus* and

NMS022B. Clusters are annotated in colour-coded format. c) f4-statistics (Dstat) under the test relationship f4 (R. tanemuzi, NMS022B, Modern_Canada, X) of NMS022B sample and other historical and modern *R. rattus* populations. d) Z-scores under the test relationship assessed through block jack-knife resampling. Reprinted from Figure 5 (de-Dios et al., 2025), licensed under Creative Commons CC BY license. Copyright © 2025, © The Author(s) 2025. Published by Oxford University Press on behalf of Society for Molecular Biology and Evolution.

These findings are also supported by the palaeoproteomics data. Indeed, NMS022B contains the highest number of total unique peptides and proteins among all the samples. It also has the highest number of peptides assigned to rodents. However, because the SwissProt database contains protein sequences for *R. norvegicus* but not *R. rattus*, we created a database incorporating all published protein sequences from both species on NCBI.

Analysing the data using this database, we identified a total of 2,758 peptides; with 2,719 assigned to *R. rattus*, and 39 to *R. norvegicus* (Figure 11a). This supports *R. rattus* as the most probable source of Rattus genus peptides found in NMS022B.

To confirm that the peptides come from *R. rattus*, we focused on the GPSGAAGPDGNKGEAGAVGAPGSAG (PSMs count = 26) peptide sequence from collagen alpha-2 (1) chain, and the RPFGEVYELEIDTLETICH (PSMs count = 2) peptide sequence from the alpha-2-HS-glycoprotein isoform XI protein. This scrutiny pinpoints differences at two amino acid positions that allow the differentiation of *R. rattus* from *R. norvegicus* (Figure 11b, c). Overall, we successfully identified 44 partial proteins (≥5% of coverage) belonging to *R. rattus*.

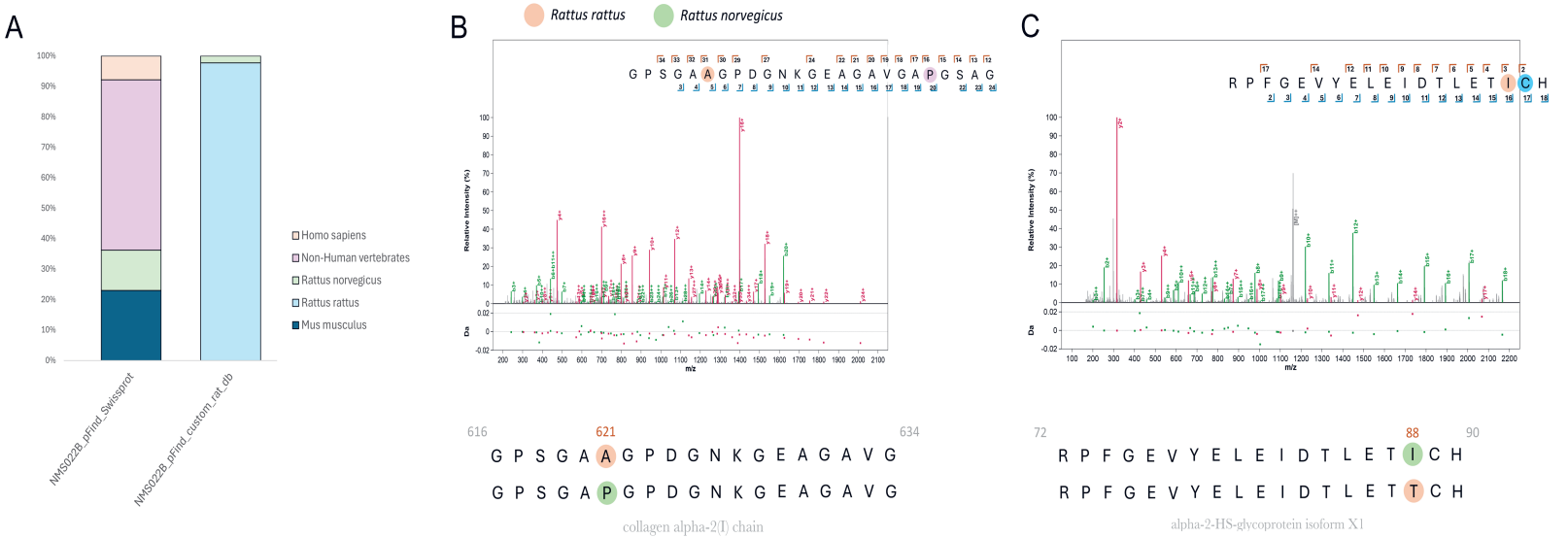

Figure 11: *R. rattus* specific peptide identification from sample NMS022B.

a) Stacked bar plot showed the relative abundance of peptide origins based on SwissProt identification from pFind output and our custom *R. rattus* database. b) Spectrum from GPSGAAGPDGNKGEAGAVGAPGSAG (PSM count = 26) (collagen alpha-2 [I] chain) identified with pFind specific to *R. rattus*. Amino acids shown in parentheses indicate hydroxylated proline residues. Sequence variation at position 621 of the protein (highlighted by a circle for *R. rattus* and by a square for *R. norvegicus*) was used to distinguish between the two species. c) Spectrum of the peptide RPFGEVYELEIDTLETICH (PSM count = 2) (alpha-2-HS-glycoprotein isoform X1) identified with pFind as specific to *R. rattus*. Amino acids shown in parentheses represent carbamidomethylation of cysteine residue. Sequence variation at position 88 of the peptide (highlighted by a circle for *R. rattus* and a square for *R. norvegicus*) was used to distinguish between the two species. Reprinted from Figure 6 (de-Dios et al., 2025), licensed under Creative Commons CC BY license. Copyright © 2025, © The Author(s) 2025. Published by Oxford University Press on behalf of Society for Molecular Biology and Evolution.

5.3 Infectious landscape in a long-used medieval cemetery in Flanders (REF III)

In our study, we generated whole-genome shotgun data from 404 medieval human skeletal remains from Sint-Truiden, a city in nowadays Belgium. After quality control, we retained 372 genomes for downstream analyses, including 332 individuals with coverage above 0.1× that were used for imputation-based analyses. Based on radiocarbon dating and archaeological context, we assigned the burials to four broad chronological phases spanning approximately the 8th to the 18th centuries.

5.3.1 Population genetics of Sint-Truiden through time

From a population genetic point of view, our results indicate that the population of Sint-Truiden was more heterogeneous in the Early and High Middle Ages than in the later medieval and post-medieval periods. Through time, this variation became smaller, and the individuals became genetically closer to the present-day population of Limburg, the easternmost province in Flanders. At the same time, the main ancestry profile of the site remained largely stable, with a dominant Gaulish-related component and a smaller Germanic-related component. For the present thesis, however, these human genomic results are mainly important because they provide the population background for the metagenomics analyses. In fact, they come from a well-contextualized urban population followed across a long time period.

5.3.2 Metagenomic findings and broader infectious landscape

The metagenomic component is one of the most important aspects of our study because it allowed us to directly investigate the infectious landscape of medieval Sint-Truiden. In fact, since we performed shotgun sequencing on teeth, we could recover not only human DNA but also microbial DNA from the same individuals. This made it possible to study pathogen presence within the same framework used for ancestry, chronology, and burial context.

Our metagenomic screening of 372 individuals identified probable pathogen signals in 35 cases. These included 10 likely cases of hepatitis B virus (HBV), 6 of *Borrelia recurrentis*, 5 of *Yersinia pestis*, 3 of human betaherpesvirus 6A (HHV-6A), 1 of human betaherpesvirus 6B (HHV-6B), 3 of *Leptospira interrogans*, and 2 of herpes simplex virus 1 (HSV-1), together with several other findings. One individual showed evidence of coinfection of *Yersinia pestis* and HBV. This is in line with previous studies reporting the presence of *Y. pestis* with a chronic infection.

These results show clearly that plague was only one part of a broader infectious environment. In particular, radiocarbon dating of 34 individuals with pathogen findings showed a wide temporal range for most pathogens, except for *Y. pestis* findings, all of which dated to the fourteenth centoury. (Figure 12).

At the same time, these findings must be interpreted carefully. In many cases, pathogen detection was based on low levels of DNA, and not all signals could be fully validated. Therefore, the metagenomic profile we recovered should not be understood as a complete picture of infection in the cemetery. False negatives are likely, and some pathogens would remain undetectable because of preservation limits or tissue tropism. Even with these limitations, the metagenomic approach substantially improves our understanding of medieval health, because it can detect infections that do not leave skeletal lesions and that may also remain unmentioned in historical sources.

5.3.3 Plague evidence despite historical absence

Among all metagenomic findings, the detection of *Yersinia pestis* is historically the most important, because it intervenes directly in the historical discourse on the second plague pandemic in the Low Countries. We identified *Y. pestis* in five of the late 58 medieval burials, and all radiocarbon-dated plague-positive cases fell in the fourteenth century. This result is particularly significant because the surviving written record from Sint-Truiden does not mention plague in the town during that century. Although the abbey chronicle includes later mentions about the Black Death in Europe, it does not describe plague circulating in Sint-Truiden itself. The surviving fourteenth-century administrative records are also silent on this point, while more convincing documentary evidence of excess mortality appears only in the early fifteenth century.

For this reason, our findings are important not only at the local level, but also for plague discourse more generally. They show that the absence of written evidence should not automatically be interpreted as evidence for the absence of disease. This demonstrates how metagenomics can correct the limits of documented history and reveal epidemic events that remain invisible in local archives.

The burial context makes this point even stronger. The plague-positive individuals were buried in single graves distributed across the cemetery and not in mass burials or other exceptional funerary contexts. This means that plague mortality in Sint-Truiden did not produce an archaeologically dramatic signal. We also observed that almost 10% of the metagenomically screened individuals from the late medieval group carried *Y. pestis* reads. However, the true number of plague-related burials may have been higher.

The strain-level interpretation remained limited because target enrichment did not produce enough coverage for full assignment in most plague-positive individuals. However, in one individual, ST1516, we identified two substitutions that are absent from Black Death strains but present in the main Second Pandemic lineage. This makes it unlikely that the individual died during the initial 1346–1352 Black Death outbreak and instead suggests a later fourteenth-century case.

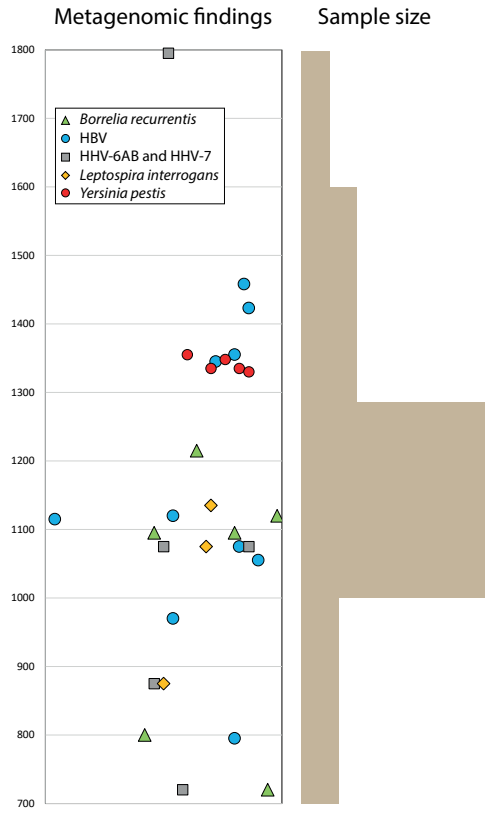

Figure 12: Temporal distribution of the metagenomic findings.

Individuals with pathogen findings and radiocarbon dates are presented for the subset of pathogens detected at least twice. HBV—hepatitis B virus, HHV—human betaherpesviruses. The mean value of the 95% range of the radiocarbon date of each individual is shown on the y axis, x axis values showing δ15N values. Reprinted from Figure 8 (Beneker et al., 2025), licensed under Creative Commons CC BY license. Copyright © 2025, © The Author(s) 2025.

5.3.4 Immunity-related analyses

Because we found direct evidence of plague in the cemetery, we also investigated whether the second plague pandemic was associated with detectable changes in immune-related human genetic variation. In our analyses, we compared 239 early-phase genomes with 46 later-phase genomes and examined differentiation in innate and adaptive immunity loci. However, despite the mentioned evidence of plague-related mortality in Sint-Truiden, we did not observe increased Fst of variants in the innate and adaptive immunity genes when comparing the Sint-Truiden burial cohorts that pre- and post-date the start of the second pandemic. Our results, thus, do not offer support for Klunk et al. (Klunk et al., 2022) observation of a significantly higher differentiation of innate immune genes in their London and Danish cohorts dating before and after the Black Death.

On the one hand, our dataset may not be ideally suited for testing such effects. The radiocarbon dates of the Sint-Truiden plague victims are also too wide to determine whether any one of them died during the Black Death outbreak, and our metagenomic data did not allow us to determine which strain the Sint-Truiden plague victims had.

Nonetheless, target enrichment gave us the opportunity to check the presence of diagnostic SNPs at low coverage in our best sample, ST1516. The detection of two post-Black Death variants makes it unlikely the individual died during the Black Death epidemic. The plague victims are therefore likely associated with post-Black Death epidemics. Later outbreaks of plague during the second pandemic would have been expected to exert similar selective pressures on immune genes, and yet we do not find evidence of concerted change in the immune genes.

Although we don't observe genome-scale changes in response to the second pandemic in the form of allele frequency changes in the majority of the immune genes, we cannot rule out the case of natural selection affecting individual immunity genes in relation to the vulnerability to plague or other infectious diseases that would have affected the health of the Sint-Truiden population.

5.4 Evidence for the co-occurrence of *Yersinia pestis* and the zoonotic pathogen *Erysipelothrix rhusiopathiae* in prehistoric Europe (REF IV)

To investigate the possible presence of pathogens in our samples, as well as the genetic ancestries and biological relatedness of the human individuals buried at Grotta della Spinosa (GSP), we initially selected teeth (n = 3) and skeletal elements (auditory ossicle = 1, petrous portion of temporal bone = 6) from what we assumed were nine different individuals. The remains were entirely disarticulated and commingled. We extracted DNA from these skeletal remains and built double-indexed double-stranded DNA (dsDNA) libraries at the University of Tartu, Institute of Genomics' aDNA facility. We sequenced the skeletal samples, generating a total of 176,415,256 reads.

5.4.1 Genetic ancestries of *Grotta della Spinosa*

READv2 (Alaçamlı et al., 2024) confirmed samples GSP005 (a petrous portion) and GSP013 (a tooth) to be identical, suggesting they originated from the same individual (referred to as GSP013 in the text), leaving eight unique individuals with a final average human coverage of 0.26× (range: 0.0016×–0.834× , median = 0.15×) and modern human mtDNA contamination between 0.1–7.3%.

To prepare the selected datasets for genome-wide analysis, we called the SNPs on the '1240k' panel (Rohland et al., 2022) retaining an average 156,000 SNPs per sample. All individuals with > 20,000 overlapping SNPs, were merged with publicly available datasets from West Eurasia using the AADR data (version v62.0). To investigate the genetic ancestries of the human individuals from *Grotta*

della Spinosa, we projected the newly generated and the publicly available ancient individuals onto the genetic variation of present-day Eurasian populations. We found that all newly generated individuals from the site fall into the so-called European Neolithic cluster, presenting a homogenous ancestral composition to be expected from a Copper Age Italian population.

5.4.2 *Y. pestis* presence in Southern Europe earlier than previously thought

After metagenomic screening of the libraries using KrakenUniq, we observed sequences potentially associated with *Y.pestis* and *E. rhusiopathiae* in the GSP013 libraries. Additionally, we found evidence of Hepatitis B virus (HBV) infection in 4 of the 8 analysed individuals (GSP004, GSP011, GSP013, GSP014) (Table 1). After target enrichment and deeper sequencing, from GSP013, we retrieved a complete *Y. pestis* genome at an average depth of 3.26× coverage, a partial *E. rhusiopathiae* genome at 0.515×, and a partial HBV sequence at 0.756× (Table 1).

Table 1: Mapping statistics for putative pathogens detected in the samples.

Individual	Organism	Q1 Reads	Unique Q30 Reads	Average Depth	Breadth of Coverage
GSP013	*Y. pestis CO92* (Chromosome)	4,635,707	295,257	3.232×	81.14%
GSP013	*Y. pestis CO92 (pCD1)*	120,328	7,460	5.539×	86.16%
GSP013	*Y. pestis CO92 (pMT1)*	45,999	3,708	1.932×	60.11%
GSP013	*Y. pestis CO92 (pPCP1)*	39,592	2,502	14.143×	79.12%
GSP013	*E. rhusiopathiae*	28,245	17,663	0.515×	38.73%
GSP013	Hepatitis B virus	173	36	0.756×	28.63%
GSP014	Hepatitis B virus	179	121	1.846×	55.69%
GSP004	Hepatitis B virus	1	1	0.0116×	1.16%
GSP011	Hepatitis B virus	5	3	0.0374×	3.74%

Basic mapping statistics for the analysed samples against *Y. pestis* CO92, *E. rhusiopathiae* NCTC8163 (GCF_900637845) and HBV ayw reference genomes.

We then built a genome-wide, high-coverage SNP dataset. The final dataset consisted of 37 high-quality (≥ 3-fold average depth) ancient *Y. pestis* genomes found in human individuals, GSP013, all mapped against *Y. pestis* strain CO92 (Capestany et al., 2006; Sabet et al., 2008) and with *Y. pseudotuberculosis* IP32881 (Chain et al., 2004) as root. We created a Maximum Likelihood (ML) phylogenetic tree using raxml-ng(Kozlov et al., 2019) and 1,042 variant sites (Figure 13A–B). We observed that GSP013 falls within the diversity of the LNBA-clade of *Y. pestis* (Transfer Bootstrap Expectation (TBE) value=0.9998; average tree TBE=0.9771), basal to all other genomes with the exception of the

Northern Caucasus strain RK1001 (4828–4622 cal BP) and a strain from the Altai region, RISE509 (4837–4627 cal BP). This placement represents the oldest currently attested evidence of LNBA-*Y. pestis* infection in Europe. Its phylogenetic position, basal to most other European LNBA genomes and only slightly more derived than strains from the Northern Caucasus and the Altai region, suggests that *Y. pestis* diversity was already present across a wide geographic area by this period. This is consistent with the hypothesis that LNBA- plague was not restricted to the Eurasian steppe but may have been maintained in broader animal reservoirs.

Next, we evaluated the presence or absence of known *Y. pestis* virulence factors in the reference genome (CO92) for the sample reported here and compared the results with other samples from previous studies (Andrades Valtueña et al., 2017, 2022; Neumann et al., 2022; Parkhill et al., 2001; Rascovan et al., 2019; Rasmussen et al., 2015; Spyrou et al., 2018; Susat et al., 2021) (Figure 13C). We observed the absence of the filamentous prophage, which is missing in all published Neolithic and LNBA genomes, and today is mostly found in 1.ORI genomes (Derbise & Carniel, 2014). As expected, GSP013 also lacks both the *ymt* gene, which enables *Y. pestis* to survive within the midgut of the flea (Hinnebusch et al., 2017), which is crucial for flea infection in a broad range of hosts (Bland et al., 2021) and YPMT1.66c, which is involved in resistance to mammalian innate immunity (Pradel et al., 2014). Previous works have shown that *ymt*(–) *Y. pestis* can be transmitted by human ectoparasites, which implies that bubonic forms of the disease might have already been present in prehistoric times. However, the absence of YPMT1.66c would reduce the ability of *Y. pestis* to survive within macrophages and replicate efficiently at the flea bite site, which are essential steps for the establishment of bubonic plague. Consequently, the emergence of fully developed bubonic forms during the LNBA period remains unlikely. The loss of the filamentous prophage has been associated with reduced bacterial virulence and fitness during mammalian infection, raising questions about the pathogenic potential of prehistoric *Y. pestis* genomes (Carniel, 2003; Macleod et al., 2024).

We then manually explored the *pla* genotype of GSP013, and found that it exhibited the ancestral allele, which means that *pla* carries a single isoleucine substitution at position 259 (I259), located on the surface loop 5, whereas modern lineages have a conserved threonine at this residue (T259). Experimental work suggests that forms of the bacterium with the ancestral variant are less efficient in colonizing tissues but can cause the pneumonic form of plague (Andrades Valtueña et al., 2017).

This distinction might suggest that early *Y. pestis* genomes, such as the one carried by GSP013, could have caused deadly respiratory infections. When considered together with the lack of evidence for plague-related mass mortality at *Grotta della Spinosa*, these findings suggest that non-bubonic manifestations of plague may have predominated. Such forms are generally less likely to cause a major epidemic than the bubonic one, but the epidemiological impact still warrants further investigation.

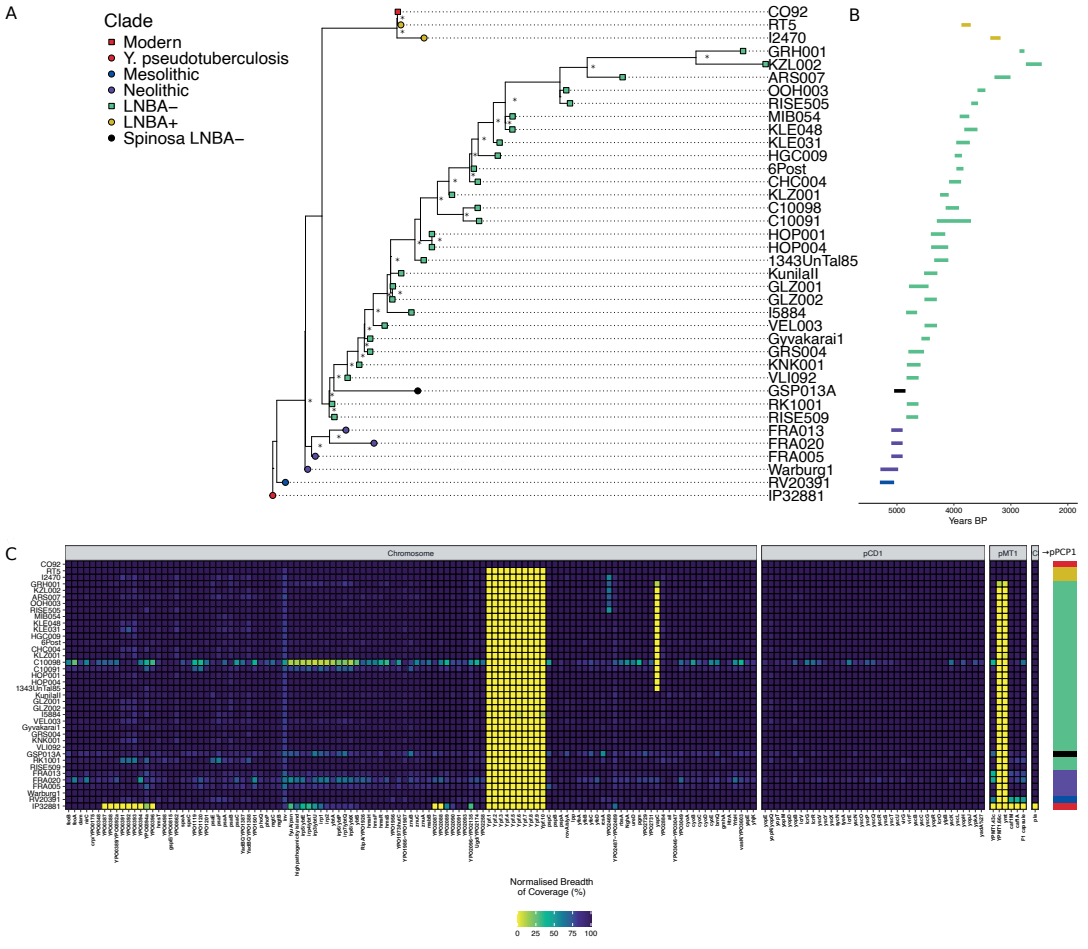

Figure 13: Phylogenetic relationships and gene content of GSP013.

(A) Maximum Likelihood tree of GSP013 strain and 37 published ancient and modern *Y. pestis* genomes. The tree is rooted to Y. pseudotuberculosis strain IP32881. We have used GTR+G and 1,000 bootstraps. Nodes with a TBE bootstrap support ≥ 0.9 are marked with (*) at the base of the node. GSP013A has been colour-highlated inside clade LNBA-. (B) Dating estimates for each sample in years before present (BP). (C) Heatmap of inferred presence/absence of virulence-associated genes assessed by normalised depth-of-coverage across the *Yersinia pestis* Chromosome and plasmids pCD1, pMT1 and pPCP1 with the colour bar denoting the Clade. Reprinted from Figure 2 of the original pre-print.

5.4.3 *Erysipelothrix* as a possible marker of zoonotic contacts

The presence of *E. rhusiopathiae* in GSP013 suggests possible contact with infected animals or animal-associated environments close to the individual's death. *E. rhusiopathiae* is a zoonotic bacterium primarily associated with animals, especially pigs, birds, fish, and wild mammals, and human infection is usually linked to direct contact with infected animals or contaminated animal products (Q. Wang & Riley, 2015; Wu et al., 2021) . Although the organism does not actively grow in soil, it can survive in contaminated environments for several weeks (R. L. Wood, 1973), meaning that its detection in archaeological material may reflect either infection during life or post-mortem contact with animal-associated material.For this reason, the biological interpretation of *Erysipelothrix* reads requires caution and must rely on authentication, genomic placement, and archaeological context.

We screened 2,495 individuals from published ancient and historical datasets for the presence of *Erysipelothrix* spp, and we mapped all collected data against a set of 12 different *Erysipelothrix* spp. genome assemblies. Following this initial mapping, we considered as positive hits the ones that simultaneously displayed a depth of coverage above 0.01× and a breadth of 1%, while also showing decreasing edit distance values with an average edit distance below 1.5. Other factors such as terminal deamination, PMD scores, and read length distribution (for samples with UDG treatment) were taken into account. A final species confirmation was accomplished by projecting with epa-ng the retrieved genome to a core genome ML phylogeny, built from 636 core genes shared between *E. tonsillarum* and *E. rhusiopathiae*.

We validated the presence of 60 previously unreported *Erysipelothrix* spp. genomes in published data (45 *E. rhusiopathiae* and 15 *E. tonsillarum*) (Figure 14), with depths of coverage ranging from 0.01× to 65.38× (Average of 3.998×, Median of 0.15×). We found *Erysipelothrix* spp. sequences in data from different library construction approaches, such as shotgun libraries and *Y. pestis, S. enterica,* and 1240k captures. In downstream analysis, we then discarded those regions of *E. rhusiopathiae* that display affinity to *Y. pestis* and *S. enterica* sequences.

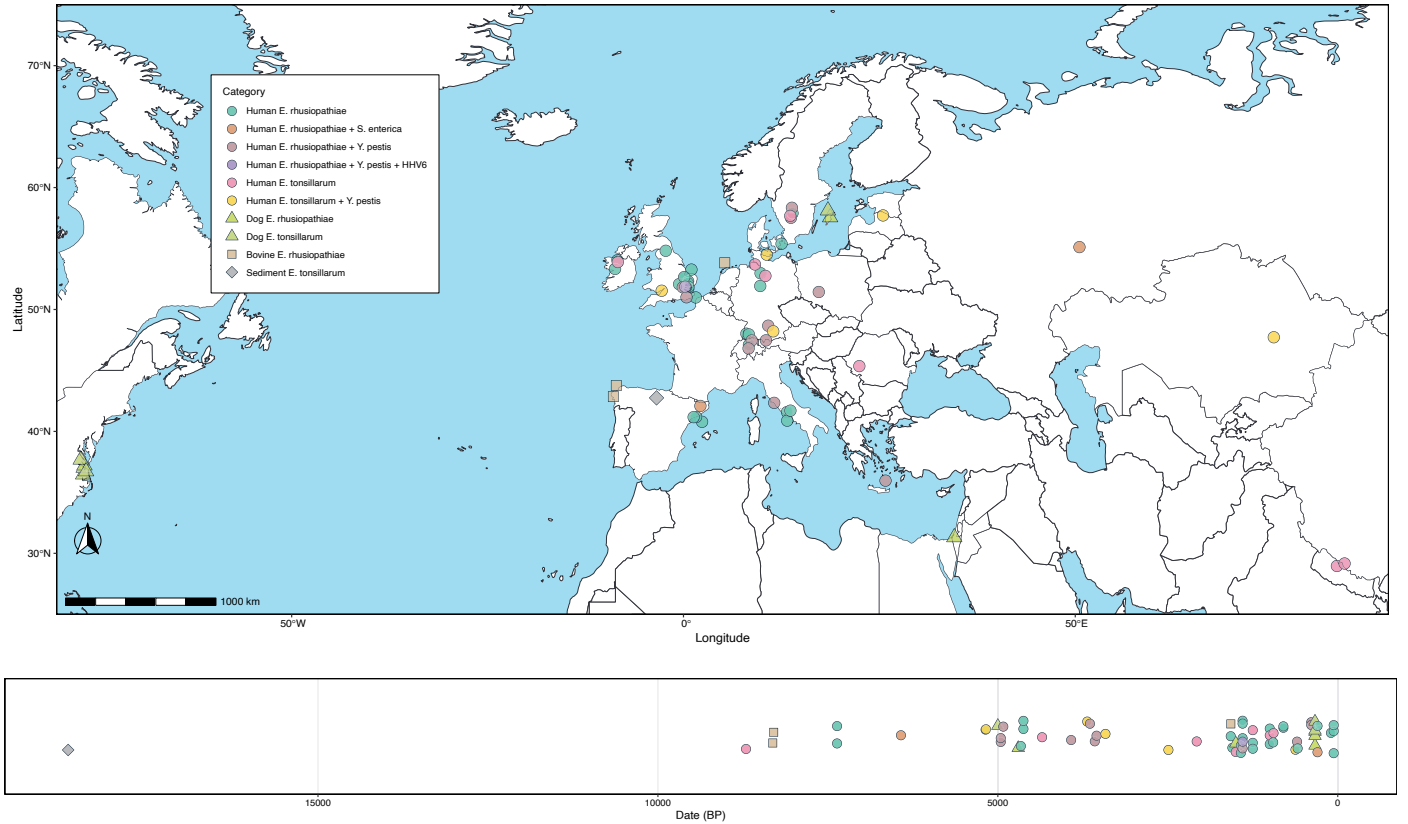

Figure 14: Geographic and temporal distribution of *Erysipelothrix* spp. genomes reported in this study.

Map with the geographical origin of the retrieved genomes, one dimensional transect with the estimated age of the genomes. Reprinted from Figure 3 of the original pre-print.

For the 184 previously published ancient *Y. pestis* samples, we compared the presence of *Erysipelothrix* spp. at different points in time. We found that *Erysipelothrix* spp. were present in 4 of 93 samples (4.3%) from the Second Pandemic (SP) and 1 of 35 samples (2.9%) from the First Pandemic (FP), whereas prevalence was markedly higher in Prehistoric (Pr) samples (10/46; 21.7%). Pairwise Fisher's exact tests showed that prevalence in Pr was significantly higher than in SP ($p = 0.0024$) and FP ($p = 0.0196$), while no difference was observed between SP and FP ($p = 1.00$). These results could be affected by the higher number of captured genomes of historical *Y. pestis*, in contrast to prehistoric genomes that originate from shotgun libraries in most cases, hindering our ability to detect *Erysipelothrix spp*. bacteria. Given the low sample sizes, these results should be taken with a grain of salt.

Subsequently, we built a phylogenetic SNPs dataset, excluding repetitive regions, conserved genes (t/rRNAs), low-quality SNPs, and recombinant tracks that could potentially affect the tree topology. This high-quality dataset included 6,809 SNPs identified in the previous 170 modern and ancient *E. rhusiopathiae* genomes. We built a ML phylogeny using raxml-ng and this high-quality dataset, with 1,000 bootstraps and the GTR+G model. We used epa-ng to project low-coverage genomes (between 1× and 0.05×) onto the tree, with an average first placing Likelihood Weight Ratio (fpLWR) of 80.96%, only displaying those genomes with a fpLWR above 70% (Figure 15). Most of the ancient samples cluster at the base of the tree in Clade 2, but without a clear pattern regarding geographical order nor host. GSP013 was robustly placed among other Neolithic and Bronze Age genomes within Clade 2, supporting the authenticity of the signal and suggesting that prehistoric *E. rhusiopathiae* diversity was related to, but not identical with, modern clade diversity.

To identify variation in gene content between ancient and modern genomes of *E. rhusiopathiae*, we investigated the coverage of a set of 45 previously described virulence genes (Ogawa et al., 2011; Seru et al., 2024). We additionally decided to explore regions showing different coverage patterns between ancient and modern genomes.

In general, ancient genomes seem to have comparable gene content, to present day strains from Clade 2 and Intermedia. An exception to this is the presence of genes WcaJ, DAPDC, RmlB, which are involved in the synthesis of the peptidoglycan wall (Allard et al., 2002; Patel et al., 2012; Pavelka & Jacobs, 1996; Peverelli et al., 2016; Sen et al., 2011), as well as InlJ, involved in cell adhesion and biofilm formation (Capestany et al., 2006; Sabet et al., 2008). EEP, associated with immune system evasion (Yamamoto et al., 2017), is absent in most modern genomes. These evidences seem to point to differences of infectivity between ancient and modern genomes. Conversely, ancient genomes do not present coverage of genes macB, DrrA, YkoD, and an unnamed ABC transporter permease (NCTC8163_01354), all of them linked to antibiotic drug resistance (Greene et al., 2018; Kaur, 1997; Reizer et al., 1992; Schyns et al., 2005), fact that could be explained by the lack of selective pressure created by the use of antibiotics (Söderlund et al., 2020). However, more studies involving ancient and modern genomes of *E. rhusiopathiae* are necessary to have a better understanding of its adaptation and diversification as a pathogen

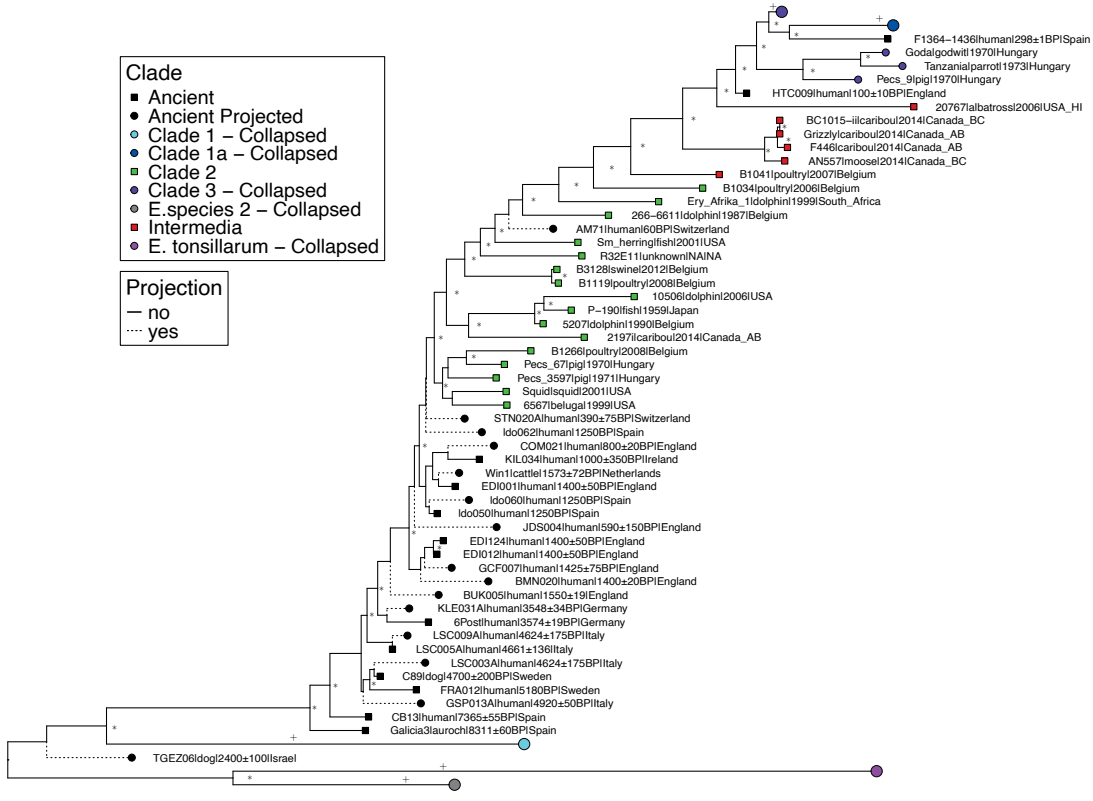

Figure 15: Phylogeny of modern and ancient *Erysipelothrix rhusiopathiae*.

ML tree of 6,809 recombination free SNPs found in 224 genomes of modern and ancient *E. rhusiopathiae*. Low coverage samples projected with epa-ng and with a first placement LWR≥70% are displayed with a dotted line. Nodes with a TBE≥0.8 are marked with a *. Collapsed nodes with a TBE≥0.8 are marked with a +. Reprinted from Figure 4 of the original pre-print.

6. CONCLUSIONS

The four studies included in this thesis contribute to a deeper understanding of how ancient metagenomics and palaeoproteomics can be powerful tools to investigate the archaeological record. Together, they show that archaeological materials should not be seen as homogeneous biomolecular archives, but rather as complex and heterogeneous substrates composed of different microenvironments, each with its own preservation dynamics. DNA and proteins do not survive in the same way across all materials, nor do they necessarily reflect the same biological, environmental, or depositional processes. Instead, their recovery and interpretation depend on the specific properties of the sampled substrate, and the methodological approaches used to analyse it. The results also demonstrate that both conventional and overlooked materials can provide information on human-associated microbial communities, depositional environments, infectious disease, and past human–animal interactions.

Ref I – In this study, we evaluated the preservation of ancient biomolecules in a previously overlooked material: sediment concretions tightly adhered to archaeological human remains. During the Neolithic, fluctuations in climate, characterized by alternating arid and wet periods, may have influenced biomolecule preservation by affecting environmental conditions. Results of our analysis show that the concreted sediment attached to the remains seems to be comparable to the underlying dental tissue. Despite poor preservation of human aDNA, high-quality ancient microbial genomes can be retrieved from concreted tissue. Overall, although dental calculus remains the best source for investigating ancient oral communities, we showed that concreted elements and sediments can still be used to recover human oral species when other human remains are not available. Furthermore, human peptides were identified in archaeological sediment concretions. Interestingly, metagenomics and palaeoproteomics analyses of some samples revealed both ancient DNA and proteins from the same oral microbiome species, highlighting the advantages of combining these approaches for a more comprehensive understanding of substrates.

Our study indicates that bacterial and human peptides are not uniformly preserved across different sample types, supporting the idea that different microenvironments may favour distinct preservation patterns. For instance, differences in mineralization, microbial activity, or exposure to environmental conditions may influence molecular stability and degradation. Future research involving larger sample sizes, geochemical methods and, possibly, freshly excavated remains, is necessary to validate or challenge our initial findings.

Ref II – Bone-adhered sediments are a unique resource for the study of ancient genomic and proteomic data recovered nondestructively. In our study, we reconstructed a partial human genome, the genetic profile of which matched the one recovered from the original bone. Additionally, sequences matching the genomes of endogenous gut microbiome bacteria were identified. Above all, we managed to retrieve the partial genome and proteome of a Black Rat (*Rattus rattus*), sharing close genetic affinities to medieval *Rattus rattus*. These results are remarkable

since they demonstrate that material that is usually discarded or overlooked, can be used to reveal different information about a deceased individual and the environmental conditions at the time of their death. Although endogenous human biomolecules retrieved using this method are not as abundant as those recovered from traditional skeletal sampling, bone-adhered sediment can be used in a complementary fashion, providing other types of supporting information for the researcher. In addition, this material could provide an alternative source of biomolecules while avoiding destructive sampling of human remains. However, more studies using freshly excavated remains are needed. Also, aDNA recovery across different archaeological contexts differs greatly, and more investigations have yet to be conducted in different environments and archaeological contexts.

Ref III – Our metagenomic screens revealed the presence of multiple infections in the Sint-Truiden individuals, including cases of HBV, *B. recurrentis*, roseoloviruses, HSV-1, and *Y. pestis*. The time range of HBV and *B. recurrentis* cases is very broad. The latter pathogen is the causative agent for louse-borne relapsing fever. Being transmitted by the human body louse, this pathogen can be indicative of poor hygiene in medieval Sint-Truiden.

The presence of *Y. pestis* in individual ST1516 and the discovery of more putative cases in the fourteenth c. is extremely important because there are no written records of plague cases at the time in Sint-Truiden. These findings thus highlight the fact that the absence of historical records referring to diseases should not automatically be taken as proof of the absence of the disease. This is indeed a further confirmation of the importance of metagenomic screening followed by targeted capture for the study of infectious diseases in the past. Further conclusions on the human population genetics analyses carried out in the study can be found in the original Ref III article.

Ref IV – Our data confirms the presence of LNBA-*Y.pestis* in Western Europe 300 to 400 years before other LNBA-published genomes from Central Europe. This early case of *Y. pestis* in Southern Europe, far from the predicted focus of the disease in the Eurasian steppe, together with its gene content, aligns with the previous hypothesis that *Y. pestis* diversity was already established on the continent. The individual GSP013 also carried signs for *E. rhusiopathiae*. By analysing published data, we also found 44 additional cases of ancient *E. rhusiopathiae*, pushing back the earliest evidence for a possible human infection to a Neolithic human individual from Spain. In the study, we speculate that there is an overrepresentation of *Y. pestis* and *Erysipelothrix* co-occurrence during prehistory, compared to historical times. We think this could be associated with the Neolithic transition, a period in which humans experienced an increased close contact with animals due to the introduction of husbandry, a more sedentary lifestyle and agriculture. Because of the different library strategies used in published studies, additional research is needed to prove or challenge our hypothesis. However, these are exciting new results, because they provide a broader look into the spatiotemporal boundaries of *Y. pestis* in prehistoric Europe. They highlight the importance of zoonotic pathogens such as *E. rhusiopathiae*, whose role in human diseases has been neglected.

The studies presented in this thesis highlight the importance of ancient metagenomics and ancient omics as tools to investigate the archaeological record. Despite more than 40 years since the first extraction of DNA from ancient remains, much work remains to fully understand the attachment of DNA and other biomolecules to surfaces and thus to determine the best protocols to use for different substrates, both in terms of preservation and in terms of minimal destruction, when possible. Ancient metagenomics, far from being just an archival science, is the starting ground to study the relationship between humans and microbes, how this relationship has changed and evolved over time. Ancient metagenomics opens a window into the understanding of how pathogens and commensals have shaped our biology, how we have influenced the changing of the microbes we encountered, and how these mutual influences exist today.

SUMMARY IN ESTONIAN

Arheoloogilise leiuainese tõlgendamine iidsete biomolekulide kaudu: DNA ja valkude säilimine, haigused ning inimese ja mikroobide vastasmõjud minevikus

Muistsete biomolekulide uurimine on oluliselt muutnud seda, millist teavet arheoloogilised säilmed meile annavad. Neid ei käsitleta enam üksnes anatoomiliste või keskkondlike materjalidena, vaid looduslike arhiividena, milles on säilinud jäljed inimese ja teiste organismide genoomidest, mikroobikooslustest, patogeenidest, valkudest, toitumisega seotud leidudest ja surmajärgsetest protsessidest üldisemalt (Warinner et al. 2017a; Warinner 2022; Der Sarkissian et al. 2021). Iidse metagenoomika esiletõus on olnud eriti oluline, sest see on laiendanud vana DNA uuringuid väljapoole peremeesorganismi genoome, võimaldades tõlgendada ja uurida segakoosseisulisi bioloogilisi kooslusi (Warinner et al. 2017a; Der Sarkissian et al. 2021). Samal ajal moodustavad muistsed valgud täiendava molekulaarse arhiivi, sest need võivad säilida tingimustes, kus DNA ei säili (Jessica Hendy, Welker, et al. 2018; Hendy 2021; Warinner et al. 2022).

Käesolev väitekiri uurib vana DNA metagenoomika ja paleoproteoomika võimalusi ja piiranguid arheoloogiliste materjalide uurimisel. Eelkõige keskendub see sellele, kuidas erinevad substraadid säilitavad biomolekulaarset informatsiooni ning kuidas seda informatsiooni saab kasutada minevikus toimunud inimeste, mikroobide, loomade ja keskkondade vaheliste vastastikmõjude rekonstrueerimiseks. Hambad on mineviku uurimisel eriti väärtuslikud, sest üksainus hammas sisaldab mitut mikroelupaika, mis säilitavad erinevat tüüpi informatsiooni. Näiteks hambakivi, mis on mineraliseerunud hambakatu vorm, on teadaolevalt üks rikkalikumaid vana DNA allikaid arheoloogilises materjalis (Mann et al. 2018) ning see on ainus materjal inimkehas, mis tavapäraselt kivistub juba eluajal. Hambakivi koosneb peamiselt suumikroobidest, kuid võib säilitada ka muid materjale, nagu näiteks toiduga seotud mikrofossiile, õhu- ja veesaasteaineid, peremeesorganismi DNA-d, toidutarbimisest pärinevaid valke, kuid toimib üldisemalt ka mitmesuguse suuõõnde sattunud "prahi" kogumiskohana (Warinner et al. 2015; Weyrich et al. 2015; Radini et al. 2017, 2019; Jessica Hendy, Warinner, et al. 2018). Dentiin, seevastu, ning eriti hambaõõne sisepind, on üks parimaid allikaid peremeesorganismi ja patogeenide DNA eraldamiseks. Hambad on eluajal vaskulariseeritud ning hambapulp on vere ja toitainetega varustatud juurekanali kaudu. Kroonilised vere kaudu levivad infektsioonid viivad patogeenid hambapulpi ning surma hetkel jäävad hambaõõnes olevad patogeenid sinna püsima ja lagunevad koos koega (Spyrou et al. 2019; Bos et al. 2019; Warinner 2022).

Käesoleva väitekirja eesmärk on uurida ka vähem tavapäraseid substraate, sealhulgas inimjäänustele kinnitunud konkretsioone ja luudele kinnitunud setteid, näidates, et aastaid tähelepanuta jäänud bioarheoloogilised materjalid võivad säilitada vana DNA-d ja valke.

Selles väitekirjas sisalduvad neli uurimust lähenevad iidsele metagenoomikale erinevatest vaatenurkadest. Esimese uurimuse (REF I) eesmärk oli uurida bio-

molekulide säilimist arheoloogilisi säilmeid katvates konkretsioonides. Selliseid konkretsioone leidub sageli piirkondades ja ajaperioodidel, mis on mineviku mõistmise seisukohalt olulised, kuid nendes säilinud DNA ja valkude sisaldust ei ole varem uuritud. Selles uurimuses püüdsime eraldada vana DNA-d ja valke paralleelselt inimese hambajäänustest ning neid ümbritsevatest konkretsioonidest, et uurida seda seni vähe uuritud allikat. Meie analüüsi tulemused näitavad, et säilmetele kinnitunud konkretiseerunud sete näib olevat võrreldav selle all paikneva hambakoega. Hoolimata inimese vana DNA halvast säilivusest on konkretiseerunud koest võimalik eraldada kvaliteetseid vanu mikroobigenoome. Kuigi hambakivi jääb üldiselt parimaks allikaks muistsete suumikroobikoosluste uurimiseks, näitasime, et konkretsioone saab siiski kasutada inimese suuõõne liikide tuvastamiseks juhul, kui teisi inimsäilmeid ei ole arheoloogilises materjalis saadaval. Lisaks tuvastasime arheoloogilistes settekonkretsioonides inimese peptiide. Huvitaval kombel näitasid mõnede proovide metagenoomika ja paleoproteoomika analüüsid nii vana DNA kui ka valkude olemasolu samadest suu mikrobioomi liikidest, rõhutades meie lähenemisviiside kombineerimise eeliseid substraatide terviklikumaks mõistmiseks. Meie uurimus näitab, et bakteriaalsed ja inimese peptiidid ei säili eri proovitüüpides ühtlaselt, toetades ideed, et erinevad mikrokeskkonnad võivad soodustada erinevaid biomolekulide säilimise mustreid. Näiteks võivad erinevused mineraliseerumises, mikroobses aktiivsuses või kokkupuutes keskkonnatingimustega mõjutada molekulide stabiilsust ja lagunemist. Tulevased uuringud suuremate valimite, geokeemiliste meetodite ja võimaluse korral vahetult väljakaevatud säilmetega on vajalikud meie esialgsete tulemuste kinnitamiseks või ümberhindamiseks.

Teise uurimuse eesmärk oli uurida erinevatele inimese skeletielementidele kinnitunud setete biomolekulide sisaldust (REF II). Inimese skeletijäänustele kinnitunud setted on materjal, mida sageli ignoreeritakse või eemaldatakse väljakaevamiste, väljakaevamisjärgse puhastamise või laboritöötluse käigus edasisest analüüsist (Damgaard et al. 2015; Pinhasi et al. 2019). Ometi püsib see allikas skeletikogudes kaua ja seda võib leida isegi pärast aastatepikkust säilitamist. Mõnel juhul võivad sellised setted olla ainus allesjäänud materjal, mis annab teavet leiukoha keskkonna kohta aastaid pärast väljakaevamisi. Uurisime inimese, looma ja mikroobide päritolu materjali nii genoomika kui ka proteoomika vaatenurgast ning võrdlesime setetest saadud tulemusi skeletielementide tulemustega. Huvitav on, et meil õnnestus ühest proovist rekonstrueerida osaline inimese genoom, mille geneetiline profiil vastas luuproovist saadud genoomile. Lisaks tuvastasime järjestusi, mis vastasid endogeensetele soolestiku mikrobioomi bakterigenoomide järjestustele, aga ka koduroti (*Rattus rattus*) osaline genoom ja proteoom, millel olid lähedased geneetilised seosed keskaegse *Rattus rattus*'ega. Need tulemused on märkimisväärsed, sest need näitavad, et materjali, mis tavaliselt kõrvale heidetakse või tähelepanuta jäetakse, saab kasutada erinevat tüüpi informatsiooni saamiseks surnud indiviidi ja tema surmahetke keskkonnatingimuste kohta. Kuigi selle meetodiga saadud endogeenseid inimese biomolekule ei ole nii rohkelt kui traditsioonilisest skeletiproovist saadud materjalis, saab luule kinnitunud setet kasutada täiendava infoallikana. Lisaks võib see materjal

olla alternatiivne biomolekulide allikas, mis aitab vältida inimsäilmete destruktiivset proovistamist. Siiski erineb vana DNA saagikus erinevates materjalides oluliselt ning edaspidi on vaja täiendavaid uuringuid erinevates keskkondades ja arheoloogilistes kontekstides.

Väitekirja teine osa rakendab vana DNA metagenoomikat nakkus- ja zoonootilise päritoluga haiguste ajaloo uurimisel. Kolmanda uurimuse (REF III) eesmärk oli lisaks inimese geneetilise päritolu ja populatsiooni struktuuri rekonstrueerimisele uurida tänapäeva Belgia alal paiknenud keskaegse ja varauusaegse linna – Sint Truideni – nakkushaigusi, kasutades hambajäänustest saadud pärilikkusaine metagenoomset analüüsi, eesmärgiga tuvastada inimesega seotud patogeene ja patobionte, hinnata nende ajalist jaotust 8.–18. sajandini ning testida, kas selliseid nakkusi nagu nt katk on võimalik tuvastada ka juhul, kus kirjalikke ajalooallikaid haiguspuhamgute kohta teada ei ole. Meie sõeluuringud näitasid Sint-Truideni indiviididel mitme nakkuse olemasolu, sealhulgas B-hepatiidi viiruse (HBV), *Borrelia recurrentis*'e, roseololi ja herpest põhjustavate viiruste, (HHV6, HSV-1) ja katkutekitaja *Yersinia pestis*'e juhtumeid. HBV ja *B. recurrentis*'e juhtumite ajaline ulatus oli väga lai. Viimane neist põhjustab täide kaudu levivat taastuvat palavikku. Kuna see patogeen levib inimese kehatäi kaudu, võib see viidata halbadele hügieenitingimustele keskaegses Sint-Truidenis. *Y. pestis*'e olemasolu ühel indiviidil (ST1516) ning täiendavate võimalike 14. sajandi katkujuhtumite avastamine on äärmiselt oluline, sest Sint-Truidenis puudusid selle perioodi haiguspuhangute kohta kirjalikud allikad. Need leiud rõhutavad seega, et haigustele viitavate ajalooliste ülestähenduste puudumist ei tohiks vaikimisi käsitleda tõendina haiguse puudumise kohta. See kinnitab veel kord metagenoomse analüüsi olulisust mineviku nakkushaiguste uurimisel.

Neljanda uurimuse eesmärk oli kasutada Grotta della Spinosa (Itaalia) vaseaja indiviidide vana DNA raamatukogude metagenoomseid sõeluuringuid, et uurida eelajaloolise kogukonna nakkushaiguste koormust (REF IV). Eelkõige püüdsime tuvastada patogeenide DNA-d, võimaluse korral ka rekonstrueerida mikroobigenoome, ning hinnata, kas mitu patogeeni võisid esineda samaaegselt ühes ja samas indiviidis või arheoloogilises konteksti. Meie tulemused kinnitavad hilise neoliitikumi/pronksiaja katku, nn LNBA-*Y. pestis*'e, olemasolu Lääne-Euroopas ligikaudu 300 aastat enne teisi Kesk-Euroopast avaldatud LNBA-*Y. pestis*'e genoome. See varajane *Y. pestis*'e juhtum Itaalias, kaugel haiguse prognoositud lähtekoldest Euraasia stepis, on kooskõlas varasema hüpoteesiga, et *Y. pestis*'e mitmekesisus oli Euroopas juba ammu välja kujunenud.

Indiviidil GSP013 esinesid ka *Erysipelothrix rhusiopathiae*'le viitavad tunnused. Avaldatud andmeid analüüsides leidsime lisaks 44 muistset *E. rhusiopathiae* juhtumit. Uurimuses oletame, et eelajaloos esineb *Y. pestis*'e ja *Erysipelothrix*'i koosesinemist võrreldes ajalooliste perioodidega palju enam. Arvame, et see võib olla seotud neoliitilise üleminekuga – ajajärguga, mil inimesed puutusid loomadega tihedamalt kokku loomakasvatuse kasutuselevõtu, paiksema eluviisi ja põllumajanduse tõttu. Avaldatud uurimustes kasutatud erinevate genoomsete raamatukogude koostamise strateegiate tõttu on meie hüpoteesi kinnitamiseks või ümberlükkamiseks vaja täiendavaid uuringuid. Sellegipoolest on need põnevad uued tulemused, sest need pakuvad laiemat vaadet katku leviku ajalis-ruumilistele

piiridele eelajaloolises Euroopas. Need rõhutavad selliste zoonootiliste patogeenide nagu *E. rhusiopathiae* olulisust, mille rolli inimese haigustes on seni alahinnatud. Üheskoos näitavad need uurimused, et vana DNA metagenoomika ei ole üksnes vahend üksikute infektsioonide tuvastamiseks, vaid ka viis rekonstrueerida laiemaid nakkushaiguste maastikke ning ökoloogilisi suhteid inimeste, loomade, mikroobide ja keskkondade vahel (REF III–IV).

Kombineerides vana DNA metagenoomikat ja proteoomikat ning asetades need arheoloogilisse konteksti, toob käesolev töö esile nii keerukate biomolekulaarsete arhiivide uurimise võimalused kui ka tõlgenduslikud väljakutsed. Nagu siin esitatud uurimused näitavad, võivad need lähenemisviisid anda informatsiooni nii traditsioonilistest kui ka tähelepanuta jäetud materjalidest, tuues samal ajal nähtavale bioloogilisi protsesse, mis võivad jääda luulises materjalis või kirjalikes allikates märkamatuks (REF I–IV).

REFERENCES

Abelson, P. H. (1954). Amino acids in fossils / P.H. Abelson. *Science (New York, N.Y.)*, *119*, 576.

Abraham, G., Qiu, Y., & Inouye, M. (2017). FlashPCA2: principal component analysis of Biobank-scale genotype datasets. *Bioinformatics (Oxford, England)*, *33*(17), 2776–2778. https://doi.org/10.1093/bioinformatics/btx299

Achtman, M., Zurth, K., Morelli, G., Torrea, G., Guiyoule, A., & Carniel, E. (1999). *Yersinia pestis*, the cause of plague, is a recently emerged clone of *Yersinia pseudotuberculosis*. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(24), 14043–14048. https://doi.org/10.1073/pnas.96.24.14043

Adapa, S. R., Hendrix, K., Upadhyay, A., Dutta, S., Vianello, A., O'Corry-Crowe, G., Monroy, J., Ferrer, T., Remily-Wood, E., Ferreira, G. C., Decker, M., Tykot, R. H., Tripathy, S., & Jiang, R. H. Y. (2025). Genetic Evidence of *Yersinia pestis* from the First Pandemic. *Genes*, *16*(8), 926. https://doi.org/10.3390/genes16080926

Adler, C. J., Dobney, K., Weyrich, L. S., Kaidonis, J., Walker, A. W., Haak, W., Bradshaw, C. J. A., Townsend, G., Sołtysiak, A., Alt, K. W., Parkhill, J., & Cooper, A. (2013). Sequencing ancient calcified dental plaque shows changes in oral microbiota with dietary shifts of the Neolithic and Industrial revolutions. *Nature Genetics*, *45*(4), 450–455, 455e1. https://doi.org/10.1038/ng.2536

Aebersold, R., Nesvizhskii, A., Keller, A., Eng, J., & Li, X.-J. (2005). Computational tools for tandem mass spectrometry–based high-throughput quantitative proteomics. In *Informatics In Proteomics* (pp. 335–352). CRC Press. https://doi.org/10.1201/9781420027624.ch16

Aitchison, J. (1986). *The statistical analysis of compositional data*. Springer My Copy UK.

Alaçamlı, E., Naidoo, T., Güler, M. N., Sağlıcan, E., Aktürk, Ş., Mapelli, I., Vural, K. B., Somel, M., Malmström, H., & Günther, T. (2024). READv2: advanced and user-friendly detection of biological relatedness in archaeogenomics. *Genome Biology*, *25*(1), 216. https://doi.org/10.1186/s13059-024-03350-3

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, *19*(9), 1655–1664. https://doi.org/10.1101/gr.094052.109

Allard, S. T. M., Beis, K., Giraud, M. F., Hegeman, A. D., Gross, J. W., Wilmouth, R. C., Whitfield, C., Graninger, M., Messner, P., Allen, A. G., Maskell, D. J., & Naismith, J. H. (2002). Toward a structural understanding of the dehydratase mechanism. *Structure (London, England: 1993)*, *10*(1), 81–92. https://doi.org/10.1016/s0969-2126(01)00694-3

Allentoft, M. E., Collins, M., Harker, D., Haile, J., Oskam, C. L., Hale, M. L., Campos, P. F., Samaniego, J. A., Gilbert, M. T. P., Willerslev, E., Zhang, G., Scofield, R. P., Holdaway, R. N., & Bunce, M. (2012). The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils. *Proceedings. Biological Sciences*, *279*(1748), 4724–4733. https://doi.org/10.1098/rspb.2012.1745

Alpaslan-Roodenberg, S., Anthony, D., Babiker, H., Bánffy, E., Booth, T., Capone, P., Deshpande-Mukherjee, A., Eisenmann, S., Fehren-Schmitz, L., Frachetti, M., Fujita, R., Frieman, C. J., Fu, Q., Gibbon, V., Haak, W., Hajdinjak, M., Hofmann, K. P., Holguin, B., Inomata, T., … Zahir, M. (2021). Ethics of DNA research on human remains: five globally applicable guidelines. *Nature*, *599*(7883), 41–46. https://doi.org/10.1038/s41586-021-04008-x

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2

Anastasiadou, K., Silva, M., Booth, T., Speidel, L., Audsley, T., Barrington, C., Buckberry, J., Fernandes, D., Ford, B., Gibson, M., Gilardet, A., Glocke, I., Keefe, K., Kelly, M., Masters, M., McCabe, J., McIntyre, L., Ponce, P., Rowland, S., … Skoglund, P. (2024). Detection of chromosomal aneuploidy in ancient genomes. *Communications Biology*, *7*(1), 14. https://doi.org/10.1038/s42003-023-05642-z

Anderson-Carpenter, L. L., McLachlan, J. S., Jackson, S. T., Kuch, M., Lumibao, C. Y., & Poinar, H. N. (2011). Ancient DNA from lake sediments: bridging the gap between paleoecology and genetics. *BMC Evolutionary Biology*, *11*(1), 30. https://doi.org/10.1186/1471-2148-11-30

Andrades Valtueña, A., Mittnik, A., Key, F. M., Haak, W., Allmäe, R., Belinskij, A., Daubaras, M., Feldman, M., Jankauskas, R., Janković, I., Massy, K., Novak, M., Pfrengle, S., Reinhold, S., Šlaus, M., Spyrou, M. A., Szécsényi-Nagy, A., Tõrv, M., Hansen, S., … Krause, J. (2017). The stone age plague and its persistence in Eurasia. *Current Biology*, *27*(23), 3683–3691.e8. https://doi.org/10.1016/j.cub.2017.10.025

Andrades Valtueña, A., Neumann, G. U., Spyrou, M. A., Musralina, L., Aron, F., Beisenov, A., Belinskiy, A. B., Bos, K. I., Buzhilova, A., Conrad, M., Djansugurova, L. B., Dobeš, M., Ernée, M., Fernández-Eraso, J., Frohlich, B., Furmanek, M., Hałuszko, A., Hansen, S., Harney, É., … Herbig, A. (2022). Stone Age *Yersinia pestis* genomes shed light on the early evolution, diversity, and ecology of plague. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(17), e2116722119. https://doi.org/10.1073/pnas.2116722119

Aouizerat, T., Gutman, I., Paz, Y., Maeir, A. M., Gadot, Y., Gelman, D., Szitenberg, A., Drori, E., Pinkus, A., Schoemann, M., Kaplan, R., Ben-Gedalya, T., Coppenhagen-Glazer, S., Reich, E., Saragovi, A., Lipschits, O., Klutstein, M., & Hazan, R. (2019). Isolation and characterization of live yeast cells from ancient vessels as a tool in bio-archaeology. *mBio*, *10*(2). https://doi.org/10.1128/mbio.00388-19

Armelagos, G. J., Brown, P. J., & Turner, B. (2005). Evolutionary, historical and political economic perspectives on health and disease. *Social Science & Medicine (1982)*, *61*(4), 755–765. https://doi.org/10.1016/j.socscimed.2004.08.066

Ásmundsdóttir, R. D., Troché, G., Olsen, J. V., Schrader, S., & Welker, F. (2026). Composition variations in archaeological human bone proteomes. *Journal of Archaeological Science*, *190*(106560), 106560. https://doi.org/10.1016/j.jas.2026.106560

Ávila-Arcos, M. C., de la Fuente Castro, C., Nieves-Colón, M. A., & Raghavan, M. (2022). Recommendations for sustainable ancient DNA research in the Global South: Voices from a new generation of paleogenomicists. *Frontiers in Genetics*, *13*, 880170. https://doi.org/10.3389/fgene.2022.880170

Barbera, P., Kozlov, A. M., Czech, L., Morel, B., Darriba, D., Flouri, T., & Stamatakis, A. (2019). EPA-ng: Massively Parallel Evolutionary Placement of Genetic Sequences. *Systematic Biology*, *68*(2), 365–369. https://doi.org/10.1093/sysbio/syy054

Barbieri, R., Signoli, M., Chevé, D., Costedoat, C., Tzortzis, S., Aboudharam, G., Raoult, D., & Drancourt, M. (2020). *Yersinia pestis*: the Natural History of Plague. *Clinical Microbiology Reviews*, *34*(1), e00044–19. https://doi.org/10.1128/CMR.00044-19

Barrett, R., Kuzawa, C. W., McDade, T., & Armelagos, G. J. (1998). EMERGING AND RE-EMERGING INFECTIOUS DISEASES: The third epidemiologic transition. *Annual Review of Anthropology*, *27*(1), 247–271. https://doi.org/10.1146/annurev.anthro.27.1.247

Bergström, A. (2024). Improving data archiving practices in ancient genomics. *Scientific Data*, *11*(1), 754. https://doi.org/10.1038/s41597-024-03563-y

Bissett, A., Fitzgerald, A., Court, L., Meintjes, T., Mele, P. M., Reith, F., Dennis, P. G., Breed, M. F., Brown, B., Brown, M. V., Brugger, J., Byrne, M., Caddy-Retalic, S., Carmody, B., Coates, D. J., Correa, C., Ferrari, B. C., Gupta, V. V. S. R., Hamonts, K., … Young, A. (2016). Introducing BASE: the Biomes of Australian Soil Environments soil microbial diversity database. *GigaScience*, *5*, 21. https://doi.org/10.1186/s13742-016-0126-5

Bland, D. M., Miarinjara, A., Bosio, C. F., Calarco, J., & Hinnebusch, B. J. (2021). Acquisition of yersinia murine toxin enabled *Yersinia pestis* to expand the range of mammalian hosts that sustain flea-borne plague. *PLoS Pathogens*, *17*(10), e1009995. https://doi.org/10.1371/journal.ppat.1009995

Blevins, K. E., Ávila-Arcos, M. C., Schuenemann, V. J., & Stone, A. C. (2026). Ancient DNA insights into diverse pathogens and their hosts. *Nature Reviews. Genetics*, *27*(1), 96–111. https://doi.org/10.1038/s41576-025-00912-4

Bokelmann, L., Glocke, I., & Meyer, M. (2020). Reconstructing double-stranded DNA fragments on a single-molecule level reveals patterns of degradation in ancient samples. *Genome Research*, *30*(10), 1449–1457. https://doi.org/10.1101/gr.263863.120

Bordenstein, S. R., & Theis, K. R. (2015). Host biology in light of the microbiome: Ten principles of holobionts and hologenomes. *PLoS Biology*, *13*(8), e1002226. https://doi.org/10.1371/journal.pbio.1002226

Borry, M., Cordova, B., Perri, A., Wibowo, M., Prasad Honap, T., Ko, J., Yu, J., Britton, K., Girdland-Flink, L., Power, R. C., Stuijts, I., Salazar-García, D. C., Hofman, C., Hagan, R., Samdapawindé Kagoné, T., Meda, N., Carabin, H., Jacobson, D., Reinhard, K., … Warinner, C. (2020). CoproID predicts the source of coprolites and paleofeces using microbiome composition and host DNA content. *PeerJ*, *8*(e9001), e9001. https://doi.org/10.7717/peerj.9001

Borry, M., Hübner, A., Rohrlach, A. B., & Warinner, C. (2021). PyDamage: automated ancient damage identification and estimation for contigs in ancient DNA de novo assembly. *PeerJ*, *9*(e11845), e11845. https://doi.org/10.7717/peerj.11845

Bos, K. I., Harkins, K. M., Herbig, A., Coscolla, M., Weber, N., Comas, I., Forrest, S. A., Bryant, J. M., Harris, S. R., Schuenemann, V. J., Campbell, T. J., Majander, K., Wilbur, A. K., Guichon, R. A., Wolfe Steadman, D. L., Cook, D. C., Niemann, S., Behr, M. A., Zumarraga, M., … Krause, J. (2014). Pre-Columbian mycobacterial genomes reveal seals as a source of New World human tuberculosis. *Nature*, *514*(7523), 494–497. https://doi.org/10.1038/nature13591

Bos, K. I., Kühnert, D., Herbig, A., Esquivel-Gomez, L. R., Andrades Valtueña, A., Barquera, R., Giffin, K., Kumar Lankapalli, A., Nelson, E. A., Sabin, S., Spyrou, M. A., & Krause, J. (2019). Paleomicrobiology: Diagnosis and evolution of ancient pathogens. *Annual Review of Microbiology*, *73*(1), 639–666. https://doi.org/10.1146/annurev-micro-090817-062436

Bos, K. I., Schuenemann, V. J., Golding, G. B., Burbano, H. A., Waglechner, N., Coombes, B. K., McPhee, J. B., DeWitte, S. N., Meyer, M., Schmedes, S., Wood, J., Earn, D. J. D., Herring, D. A., Bauer, P., Poinar, H. N., & Krause, J. (2011). A draft genome of *Yersinia pestis* from victims of the Black Death. *Nature*, *478*(7370), 506–510. https://doi.org/10.1038/nature10549

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., De Maio, N., Matschiner, M., Mendes, F. K., Müller, N. F., Ogilvie, H. A., du Plessis, L., Popinga, A., Rambaut, A.,

Rasmussen, D., Siveroni, I., … Drummond, A. J. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *15*(4), e1006650. https://doi.org/10.1371/journal.pcbi.1006650

Bourdichon, F., Arias, E., Babuchowski, A., Bückle, A., Bello, F. D., Dubois, A., Fontana, A., Fritz, D., Kemperman, R., Laulund, S., McAuliffe, O., Miks, M. H., Papademas, P., Patrone, V., Sharma, D. K., Sliwinski, E., Stanton, C., Von Ah, U., Yao, S., & Morelli, L. (2021). The forgotten role of food cultures. *FEMS Microbiology Letters*, *368*(14). https://doi.org/10.1093/femsle/fnab085

Bourdichon, F., Casaregola, S., Farrokh, C., Frisvad, J. C., Gerds, M. L., Hammes, W. P., Harnett, J., Huys, G., Laulund, S., Ouwehand, A., Powell, I. B., Prajapati, J. B., Seto, Y., Ter Schure, E., Van Boven, A., Vankerckhoven, V., Zgoda, A., Tuijtelaars, S., & Hansen, E. B. (2012). Food fermentations: microorganisms with technological beneficial use. *International Journal of Food Microbiology*, *154*(3), 87–97. https://doi.org/10.1016/j.ijfoodmicro.2011.12.030

Bozzi, D., Neuenschwander, S., Cruz Dávalos, D. I., Sousa da Mota, B., Schroeder, H., Moreno-Mayar, J. V., Allentoft, M. E., & Malaspinas, A.-S. (2024). Towards predicting the geographical origin of ancient samples with metagenomic data. *Scientific Reports*, *14*(1), 21794. https://doi.org/10.1038/s41598-023-40246-x

Breitwieser, F. P., Baker, D. N., & Salzberg, S. L. (2018). KrakenUniq: confident and fast metagenomics classification using unique k-mer counts. *Genome Biology*, *19*(1), 198. https://doi.org/10.1186/s13059-018-1568-0

Briggs, A. W., Stenzel, U., Johnson, P. L. F., Green, R. E., Kelso, J., Prüfer, K., Meyer, M., Krause, J., Ronan, M. T., Lachmann, M., & Pääbo, S. (2007). Patterns of damage in genomic DNA sequences from a Neandertal. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(37), 14616–14621. https://doi.org/10.1073/pnas.0704665104

Briggs, A. W., Stenzel, U., Meyer, M., Krause, J., Kircher, M., & Pääbo, S. (2010). Removal of deaminated cytosines and detection of in vivo methylation in ancient DNA. *Nucleic Acids Research*, *38*(6), e87. https://doi.org/10.1093/nar/gkp1163

Broad Institute. (2019.). *"Picard Toolkit."* https://broadinstitute.github.io/picard/

Browning, B. L., Zhou, Y., & Browning, S. R. (2018). A one-penny imputed genome from next-generation reference panels. *American Journal of Human Genetics*, *103*(3), 338–348. https://doi.org/10.1016/j.ajhg.2018.07.015

Buchfink, B., Xie, C., & Huson, D. H. (2015). Fast and sensitive protein alignment using DIAMOND. *Nature Methods*, *12*(1), 59–60. https://doi.org/10.1038/nmeth.3176

Bushnell, B. (2014). *BBMap: A Fast, Accurate, Splice-Aware Aligner*. https://escholarship.org/uc/item/1h3515gn

Campos, P. F., & Gilbert, T. M. P. (2012). DNA extraction from keratin and chitin. *Methods in Molecular Biology (Clifton, N.J.)*, *840*, 43–49. https://doi.org/10.1007/978-1-61779-516-9_6

Capestany, C. A., Kuboniwa, M., Jung, I.-Y., Park, Y., Tribble, G. D., & Lamont, R. J. (2006). Role of the Porphyromonas gingivalis InlJ protein in homotypic and heterotypic biofilm development. *Infection and Immunity*, *74*(5), 3002–3005. https://doi.org/10.1128/IAI.74.5.3002-3005.2006

Cappellini, E., Welker, F., Pandolfi, L., Ramos-Madrigal, J., Samodova, D., & Rüther, P. L. (2019). Early Pleistocene enamel proteome from Dmanisi resolves Stephanorhinus phylogeny. *Bibcode:2019Natur.574..103C*, *574*, 103–107. https://doi.org/10.1038/s41586-019-1555-y.PMC6894936

Carniel, E. (2003). Evolution of pathogenic Yersinia, some lights in the dark. *Advances in Experimental Medicine and Biology*, *529*, 3–12. https://doi.org/10.1007/0-306-48416-1_1

Carpenter, M. L., Buenrostro, J. D., Valdiosera, C., Schroeder, H., Allentoft, M. E., Sikora, M., Rasmussen, M., Gravel, S., Guillén, S., Nekhrizov, G., Leshtakov, K., Dimitrova, D., Theodossiev, N., Pettener, D., Luiselli, D., Sandoval, K., Moreno-Estrada, A., Li, Y., Wang, J., … Bustamante, C. D. (2013). Pulling out the 1%: whole-genome capture for the targeted enrichment of ancient DNA sequencing libraries. *American Journal of Human Genetics*, *93*(5), 852–864. https://doi.org/10.1016/j.ajhg.2013.10.002

Cavalieri, D., McGovern, P. E., Hartl, D. L., Mortimer, R., & Polsinelli, M. (2003). Evidence for S. cerevisiae Fermentation in Ancient Wine. *Journal of Molecular Evolution*, *57*(0), S226–S232. https://doi.org/10.1007/s00239-003-0031-2

Chain, P. S. G., Carniel, E., Larimer, F. W., Lamerdin, J., Stoutland, P. O., Regala, W. M., Georgescu, A. M., Vergez, L. M., Land, M. L., Motin, V. L., Brubaker, R. R., Fowler, J., Hinnebusch, J., Marceau, M., Medigue, C., Simonet, M., Chenal-Francisque, V., Souza, B., Dacheux, D., … Garcia, E. (2004). Insights into the evolution of *Yersinia pestis* through whole-genome comparison with Yersinia pseudotuberculosis. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(38), 13826–13831. https://doi.org/10.1073/pnas.0404012101

Chaitanya, L., Breslin, K., Zuñiga, S., Wirken, L., Pośpiech, E., Kukla-Bartoszek, M., Sijen, T., Knijff, P. de, Liu, F., Branicki, W., Kayser, M., & Walsh, S. (2018). The HIrisPlex-S system for eye, hair and skin colour prediction from DNA: Introduction and forensic developmental validation. *Forensic Science International. Genetics*, *35*, 123–135. https://doi.org/10.1016/j.fsigen.2018.04.004

Chen, Y., Ye, W., Zhang, Y., & Xu, Y. (2015). High speed BLASTN: an accelerated MegaBLAST search tool. *Nucleic Acids Research*, *43*(16), 7762–7768. https://doi.org/10.1093/nar/gkv784

Chi, H., Liu, C., Yang, H., Zeng, W.-F., Wu, L., Zhou, W.-J., Wang, R.-M., Niu, X.-N., Ding, Y.-H., Zhang, Y., Wang, Z.-W., Chen, Z.-L., Sun, R.-X., Liu, T., Tan, G.-M., Dong, M.-Q., Xu, P., Zhang, P.-H., & He, S.-M. (2018). Comprehensive identification of peptides in tandem mass spectra using an efficient open search engine. *Nature Biotechnology*. https://doi.org/10.1038/nbt.4236

Choi, J., Yang, F., Stepanauskas, R., Cardenas, E., Garoutte, A., Williams, R., Flater, J., Tiedje, J. M., Hofmockel, K. S., Gelder, B., & Howe, A. (2017). Strategies to improve reference databases for soil microbiomes. *The ISME Journal*, *11*(4), 829–834. https://doi.org/10.1038/ismej.2016.168

Cleland, T. P., Schroeter, E. R., & Colleary, C. (2021). Diagenetiforms: A new term to explain protein changes as a result of diagenesis in paleoproteomics. *Journal of Proteomics*, *230*(103992), 103992. https://doi.org/10.1016/j.jprot.2020.103992

Coolen, M. J., & Overmann, J. (1998). Analysis of subfossil molecular remains of purple sulfur bacteria in a lake sediment. *Applied and Environmental Microbiology*, *64*(11), 4513–4521. https://doi.org/10.1128/AEM.64.11.4513-4521.1998

Corliss, J. O. (2002). A salute to Antony van Leeuwenhoek of delft, most versatile 17th century founding father of protistology. *Protist*, *153*(2), 177–190. https://doi.org/10.1078/1434-4610-00096

Croucher, N. J., Page, A. J., Connor, T. R., Delaney, A. J., Keane, J. A., Bentley, S. D., Parkhill, J., & Harris, S. R. (2015). Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Research*, *43*(3), e15. https://doi.org/10.1093/nar/gku1196

Dabney, J., Meyer, M., & Pääbo, S. (2013). Ancient DNA damage. *Cold Spring Harbor Perspectives in Biology*, *5*(7), a012567. https://doi.org/10.1101/cshperspect.a012567

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., Durbin, R., & 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics (Oxford, England)*, *27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Danecek, P., Bonfield, J. K., Liddle, J., Marshall, J., Ohan, V., Pollard, M. O., Whitwham, A., Keane, T., McCarthy, S. A., Davies, R. M., & Li, H. (2021). Twelve years of SAMtools and BCFtools. *GigaScience*, *10*(2). https://doi.org/10.1093/gigascience/giab008

Davies, R. W., Kucka, M., Su, D., Shi, S., Flanagan, M., Cunniff, C. M., Chan, Y. F., & Myers, S. (2021). Rapid genotype imputation from sequence with reference panels. *Nature Genetics*, *53*(7), 1104–1111. https://doi.org/10.1038/s41588-021-00877-0

Davis, N. M., Proctor, D. M., Holmes, S. P., Relman, D. A., & Callahan, B. J. (2018). Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*, *6*(1), 226. https://doi.org/10.1186/s40168-018-0605-2

de-Dios, T., van Dorp, L., Gelabert, P., Carøe, C., Sandoval-Velasco, M., Fregel, R., Escosa, R., Aranda, C., Huijben, S., Balloux, F., Gilbert, M. T. P., & Lalueza-Fox, C. (2019). Genetic affinities of an eradicated European Plasmodium falciparum strain. *Microbial Genomics*, *5*(9). https://doi.org/10.1099/mgen.0.000289

Dekker, J. A. A., Peters, C., Winter, R. M., Collins, M. J., Dickinson, M. R., Harvey, V. L., Hill, E., Nair, B., Tsutaya, T., Viñas-Caron, L. C., Warinner, C., Welker, F., & Fagernäs, Z. (2025). Open science, communication, and collaboration for the future of palaeoproteomics. *Peer Community Journal*, *5*(XX). https://doi.org/10.24072/pcjournal.576

Demarchi, B., Hall, S., Roncal-Herrero, T., Freeman, C. L., Woolley, J., Crisp, M. K., Wilson, J., Fotakis, A., Fischer, R., Kessler, B. M., Rakownikow Jersie-Christensen, R., Olsen, J. V., Haile, J., Thomas, J., Marean, C. W., Parkington, J., Presslee, S., Lee-Thorp, J., Ditchfield, P., … Collins, M. J. (2016). Protein sequences bound to mineral surfaces persist into deep time. *eLife*, *5*, e17092. https://doi.org/10.7554/eLife.17092

Demeure, C., Dussurget, O., Fiol, G. M., Le Guern, A.-S., Savin, C., & Pizarro-Cerdá, J. (2019). *Yersinia pestis* and plague: an updated view on evolution, virulence determinants, immune subversion, vaccination and diagnostics. *Microbes and Infection*, *21*(5-6), 202–212. https://doi.org/10.1016/j.micinf.2019.06.007

Derbise, A., & Carniel, E. (2014). YpfÎ¦: a filamentous phage acquired by Yersinia pesti. *Frontiers in Microbiology*, *5*, 701. https://doi.org/10.3389/fmicb.2014.00701

Der Sarkissian, C., Velsko, I. M., Fotakis, A. K., Vågene, Å. J., Hübner, A., & Fellows Yates, J. A. (2021). Ancient metagenomic studies: Considerations for the wider scientific community. *mSystems*, *6*(6), e0131521. https://doi.org/10.1128/msystems.01315-21

Deutsch, E. W., Csordas, A., Sun, Z., Jarnuczak, A., Perez-Riverol, Y., Ternent, T., Campbell, D. S., Bernal-Llinares, M., Okuda, S., Kawano, S., Moritz, R. L., Carver, J. J., Wang, M., Ishihama, Y., Bandeira, N., Hermjakob, H., & Vizcaíno, J. A. (2017). The ProteomeXchange consortium in 2017: supporting the cultural change in proteomics public data deposition. *Nucleic Acids Research*, *45*(D1), D1100–D1106. https://doi.org/10.1093/nar/gkw936

Devault, A. M., Golding, G. B., Waglechner, N., Enk, J. M., Kuch, M., Tien, J. H., Shi, M., Fisman, D. N., Dhody, A. N., Forrest, S., Bos, K. I., Earn, D. J. D., Holmes, E. C., &

Poinar, H. N. (2014). Second-pandemic strain of Vibrio cholerae from the Philadelphia cholera outbreak of 1849. *The New England Journal of Medicine*, *370*(4), 334–340. https://doi.org/10.1056/NEJMoa1308663

Devault, A. M., McLoughlin, K., Jaing, C., Gardner, S., Porter, T. M., Enk, J. M., Thissen, J., Allen, J., Borucki, M., DeWitte, S. N., Dhody, A. N., & Poinar, H. N. (2014). Ancient pathogen DNA in archaeological samples detected with a Microbial Detection Array. *Scientific Reports*, *4*(1), 4245. https://doi.org/10.1038/srep04245

Didelot, X., & Wilson, D. J. (2015). ClonalFrameML: efficient inference of recombination in whole bacterial genomes. *PLoS Computational Biology*, *11*(2), e1004041. https://doi.org/10.1371/journal.pcbi.1004041

Dobney, K., & Brothwell, D. (1986). Dental calculus: Its relevance to ancient diet and oral ecology. *Teeth Anthropol BAR Int Ser*, *291*, 55–81.

Dobney, K., & Brothwell, D. (1988). A scanning electron microscope study of archaeological dental calculus. *Scanning Electron Microscopy in Archaeology. BAR International Series*, *452*, 372–385.

Drancourt, M., Aboudharam, G., Signoli, M., Dutour, O., & Raoult, D. (1998). Detection of 400-year-old Yersinia pestis DNA in human dental pulp: an approach to the diagnosis of ancient septicemia. *Proceedings of the National Academy of Sciences of the United States of America*, *95*(21), 12637–12640. https://doi.org/10.1073/pnas.95.21.12637

Drancourt, M., & Raoult, D. (2005). Palaeomicrobiology: current issues and perspectives. *Nature Reviews. Microbiology*, *3*(1), 23–35. https://doi.org/10.1038/nrmicro1063

Drieu, L., Rageot, M., Wales, N., Stern, B., Lundy, J., Zerrer, M., Gaffney, I., Bondetti, M., Spiteri, C., Thomas-Oates, J., & Craig, O. E. (2020). Is it possible to identify ancient wine production using biomolecular approaches? *Science and Technology of Archaeological Research*, *6*(1), 16–29. https://doi.org/10.1080/20548923.2020.1738728

Duchêne, S., Ho, S. Y. W., Carmichael, A. G., Holmes, E. C., & Poinar, H. (2020). The recovery, interpretation and use of ancient pathogen genomes. *Current Biology*, *30*(19), R1215–R1231. https://doi.org/10.1016/j.cub.2020.08.081

Eisenhofer, R., & Weyrich, L. S. (2019). Assessing alignment-based taxonomic classification of ancient microbial DNA. *PeerJ*, *7*(e6594), e6594. https://doi.org/10.7717/peerj.6594

Elias, J. E., & Gygi, S. P. (2007). Target-decoy search strategy for increased confidence in large-scale protein identifications by mass spectrometry. *Nature Methods*, *4*(3), 207–214. https://doi.org/10.1038/nmeth1019

Everett, R., & Cribdon, B. (2023). MetaDamage tool: Examining post-mortem damage in sedaDNA on a metagenomic scale. *Frontiers in Ecology and Evolution*, *10*(888421). https://doi.org/10.3389/fevo.2022.888421

Fellows Yates, J. A., Andrades Valtueña, A., Vågene, Å. J., Cribdon, B., Velsko, I. M., Borry, M., Bravo-Lopez, M. J., Fernandez-Guerra, A., Green, E. J., Ramachandran, S. L., Heintzman, P. D., Spyrou, M. A., Hübner, A., Gancz, A. S., Hider, J., Allshouse, A. F., Zaro, V., & Warinner, C. (2021). Community-curated and standardised metadata of published ancient metagenomic samples with AncientMetagenomeDir. *Scientific Data*, *8*(1), 31. https://doi.org/10.1038/s41597-021-00816-y

Fellows Yates, J. A., Lamnidis, T. C., Borry, M., Andrades Valtueña, A., Fagernäs, Z., Clayton, S., Garcia, M. U., Neukamm, J., & Peltzer, A. (2021). Reproducible, portable, and efficient ancient genome reconstruction with nf-core/eager. *PeerJ*, *9*(e10947), e10947. https://doi.org/10.7717/peerj.10947

Fellows Yates, J. A., Velsko, I. M., Aron, F., Posth, C., Hofman, C. A., Austin, R. M., Parker, C. E., Mann, A. E., Nägele, K., Arthur, K. W., Arthur, J. W., Bauer, C. C., Crevecoeur, I., Cupillard, C., Curtis, M. C., Dalén, L., Díaz-Zorita Bonilla, M., Díez Fernández-Lomana, J. C., Drucker, D. G., … Warinner, C. (2021). The evolution and changing ecology of the African hominid oral microbiome. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(20), e2021655118. https://doi.org/10.1073/pnas.2021655118

Fellows Yates, J. A., Warinner, C., Andrades Valtueña, A., Borry, M., Denise, R., Guellil, M., Herbig, A., Hiß, A., Hübner, A., Kocher, A., Lamnidis, T. C., Michel, M. E., Nota, K., Oskolkov, N., Pach, A. L., Pérez, V., Schmid, C., Velsko, I. M., Warner, R., … Zeibig, T. (2025). *Introduction to Ancient Metagenomics*. Zenodo. https://doi.org/10.5281/ZENODO.17736308

Fiddyment, S., Teasdale, M. D., Vnouček, J., Lévêque, É., Binois, A., & Collins, M. J. (2019). So you want to do biocodicology? A field guide to the biological analysis of parchment. *Heritage Science*, *7*(1). https://doi.org/10.1186/s40494-019-0278-6

Fleskes, R. E., Bader, A. C., Tsosie, K. S., Wagner, J. K., Claw, K. G., & Garrison, N. A. (2022). Ethical guidance in human paleogenomics: New and ongoing perspectives. *Annual Review of Genomics and Human Genetics*, *23*(1), 627–652. https://doi.org/10.1146/annurev-genom-120621-090239

Fotakis, A. K., Denham, S. D., Mackie, M., Orbegozo, M. I., Mylopotamitaki, D., Gopalakrishnan, S., Sicheritz-Pontén, T., Olsen, J. V., Cappellini, E., Zhang, G., Christophersen, A., Gilbert, M. T. P., & Vågene, Å. J. (2020). Multi-omic detection of Mycobacterium leprae in archaeological human dental calculus. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *375*(1812), 20190584. https://doi.org/10.1098/rstb.2019.0584

Fournier, R., Tsangalidou, Z., Reich, D., & Palamara, P. F. (2023). Haplotype-based inference of recent effective population size in modern and ancient DNA samples. *Nature Communications*, *14*(1), 7945. https://doi.org/10.1038/s41467-023-43522-6

Frederico, L. A., Kunkel, T. A., & Shaw, B. R. (1993). Cytosine deamination in mismatched base pairs. *Biochemistry*, *32*(26), 6523–6530. https://doi.org/10.1021/bi00077a005

Gancz, A. S., Farrer, A. G., Nixon, M. P., Wright, S., Arriola, L., Adler, C., Davenport, E. R., Gully, N., Cooper, A., Britton, K., Dobney, K., Silverman, J. D., & Weyrich, L. S. (2023). Ancient dental calculus reveals oral microbiome shifts associated with lifestyle and disease in Great Britain. *Nature Microbiology*, *8*(12), 2315–2325. https://doi.org/10.1038/s41564-023-01527-3

Gansauge, M.-T., & Meyer, M. (2013). Single-stranded DNA library preparation for the sequencing of ancient or damaged DNA. *Nature Protocols*, *8*(4), 737–748. https://doi.org/10.1038/nprot.2013.038

Gelabert, P., Sandoval-Velasco, M., Olalde, I., Fregel, R., Rieux, A., Escosa, R., Aranda, C., Paaijmans, K., Mueller, I., Gilbert, M. T. P., & Lalueza-Fox, C. (2016). Mitochondrial DNA from the eradicated European Plasmodium vivax and P. falciparum from 70-year-old slides from the Ebro Delta in Spain. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(41), 11495–11500. https://doi.org/10.1073/pnas.1611017113

Gibbon, V. E., Thompson, J. C., & Alves, S. (2024). Informed proxy consent for ancient DNA research. *Communications Biology*, *7*(1), 815. https://doi.org/10.1038/s42003-024-06413-0

Gilbert, M. T. P., Cuccui, J., White, W., Lynnerup, N., Titball, R. W., Cooper, A., & Prentice, M. B. (2004). Absence of *Yersinia pestis*-specific DNA in human teeth from five European excavations of putative plague victims. *Microbiology (Reading, England)*, *150*(Pt 2), 341–354. https://doi.org/10.1099/mic.0.26594-0

Goodfriend, G. A., & Weidman, C. R. (2001). Ontogenetic trends in aspartic acid racemization and amino acid composition within modern and fossil shells of the bivalve Arctica. *Geochimica et Cosmochimica Acta*, *65*(12), 1921–1932. https://doi.org/10.1016/s0016-7037(01)00564-6

Grace, D., Mutua, F., Ochungo, P., Kruska, R., Jones, K., Brierley, L., Lapar, M. L., Said, M. Y., Herrero, M., Phuc, P. M., Thao, N. B., Akuku, I., & Ogutu, F. (2012). *Mapping of poverty and likely zoonoses hotspots. Nairobi: International Livestock Research Institute*.

Granehäll, L., Huang, K. D., Tett, A., Manghi, P., Paladin, A., O'Sullivan, N., Rota-Stabelli, O., Segata, N., Zink, A., & Maixner, F. (2021). Metagenomic analysis of ancient dental calculus reveals unexplored diversity of oral archaeal Methano-brevibacter. *Microbiome*, *9*(1), 197. https://doi.org/10.1186/s40168-021-01132-8

Green, E. J., & Speller, C. F. (2017). Novel Substrates as Sources of Ancient DNA: Prospects and Hurdles. *Genes*, *8*(7). https://doi.org/10.3390/genes8070180

Greene, N. P., Kaplan, E., Crow, A., & Koronakis, V. (2018). Corrigendum: Antibiotic resistance mediated by the MacB ABC transporter family: A structural and functional perspective. *Frontiers in Microbiology*, *9*, 2318. https://doi.org/10.3389/fmicb.2018.02318

Gryseels, S., Watts, T. D., Kabongo Mpolesha, J.-M., Larsen, B. B., Lemey, P., Muyembe-Tamfum, J.-J., Teuwen, D. E., & Worobey, M. (2020). A near full-length HIV-1 genome from 1966 recovered from formalin-fixed paraffin-embedded tissue. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(22), 12222–12229. https://doi.org/10.1073/pnas.1913682117

Guellil, M. (2021). *MeriamGuellil/aDNA-BAMPlotter: aDNA-BAMPlotter*. https://doi.org/10.5281/zenodo.5676092

Guellil, M., Keller, M., Dittmar, J. M., Inskip, S. A., Cessford, C., Solnik, A., Kivisild, T., Metspalu, M., Robb, J. E., & Scheib, C. L. (2022). An invasive Haemophilus influenzae serotype b infection in an Anglo-Saxon plague victim. *Genome Biology*, *23*(1), 22. https://doi.org/10.1186/s13059-021-02580-z

Guellil, M., van Dorp, L., Saag, L., Beneker, O., Bonucci, B., Sasso, S., Saupe, T., Solnik, A., Kabral, H., Allmäe, R., Bates, J., Dittmar, J. M., Ge, X. J., Inskip, S., Jonuks, T., Karmanov, V. N., Khartanovich, V. I., Larmuseau, M. H. D., Aneli, S., … Tambets, K. (2026). Tracing 2500 years of human betaherpesvirus 6A and 6B diversity through ancient DNA. *Science Advances*, *12*(1), eadx5460. https://doi.org/10.1126/sciadv.adx5460

Guinet, B., Oskolkov, N., Moreland, K., Dehasque, M., Chacón-Duque, J. C., Anger-björn, A., Arsuaga, J. L., Danilov, G., Kanellidou, F., Kitchener, A. C., Muller, H., Plotnikov, V., Protopopov, A., Tikhonov, A., Termes, L., Zazula, G., Mortensen, P., Grigorieva, L., Richards, M., … van der Valk, T. (2025). Ancient host-associated microbes obtained from mammoth remains. *Cell*, *188*(23), 6606–6619.e24. https://doi.org/10.1016/j.cell.2025.08.003

Haensch, S., Bianucci, R., Signoli, M., Rajerison, M., Schultz, M., Kacki, S., Vermunt, M., Weston, D. A., Hurst, D., Achtman, M., Carniel, E., & Bramanti, B. (2010). Distinct clones of *Yersinia pestis* caused the black death. *PLoS Pathogens*, *6*(10), e1001134. https://doi.org/10.1371/journal.ppat.1001134

Haile, J., Holdaway, R., Oliver, K., Bunce, M., Gilbert, M. T. P., Nielsen, R., Munch, K., Ho, S. Y. W., Shapiro, B., & Willerslev, E. (2007). Ancient DNA chronology within sediment deposits: are paleobiological reconstructions possible and is DNA leaching a factor? *Molecular Biology and Evolution*, *24*(4), 982–989. https://doi.org/10.1093/molbev/msm016

Handsley-Davis, M., Kapellas, K., Jamieson, L. M., Hedges, J., Skelly, E., Kaidonis, J., Anastassiadis, P., & Weyrich, L. S. (2022). Heritage-specific oral microbiota in Indigenous Australian dental calculus. *Evolution, Medicine, and Public Health*, *10*(1), 352–362. https://doi.org/10.1093/emph/eoac024

Harbeck, M., Seifert, L., Hänsch, S., Wagner, D. M., Birdsell, D., Parise, K. L., Wiechmann, I., Grupe, G., Thomas, A., Keim, P., Zöller, L., Bramanti, B., Riehm, J. M., & Scholz, H. C. (2013). *Yersinia pestis* DNA from skeletal remains from the 6(th) century AD reveals insights into Justinianic Plague. *PLoS Pathogens*, *9*(5), e1003349. https://doi.org/10.1371/journal.ppat.1003349

Harper, J. W., & Elledge, S. J. (2007). The DNA damage response: ten years after. *Molecular Cell*, *28*(5), 739–745. https://doi.org/10.1016/j.molcel.2007.11.015

Hendy, J. (2021). Ancient protein analysis in archaeology. *Science Advances*, *7*(3), eabb9314. https://doi.org/10.1126/sciadv.abb9314

Hendy, J., Warinner, C., Bouwman, A., Collins, M. J., Fiddyment, S., Fischer, R., Hagan, R., Hofman, C. A., Holst, M., Chaves, E., Klaus, L., Larson, G., Mackie, M., McGrath, K., Mundorff, A. Z., Radini, A., Rao, H., Trachsel, C., Velsko, I. M., & Speller, C. F. (2018). Proteomic evidence of dietary sources in ancient dental calculus. *Proceedings. Biological Sciences*, *285*(1883), 20180977. https://doi.org/10.1098/rspb.2018.0977

Hendy, J., Welker, F., Demarchi, B., Speller, C., Warinner, C., & Collins, M. J. (2018a). A guide to ancient protein studies. *Nature Ecology & Evolution*, *2*(5), 791–799. https://doi.org/10.1038/s41559-018-0510-x

Hendy, J., Welker, F., Demarchi, B., Speller, C., Warinner, C., & Collins, M. J. (2018b). A guide to ancient protein studies (PDF). *Nature Ecology & Evolution*, *2*(5), 791–799.

Herbig, A., Maixner, F., Bos, K., Zink, A., Krause, J., & Huson, D. (2016). MALT: Fast alignment and analysis of metagenomic DNA sequence data applied to the Tyrolean Iceman. In *bioRxiv*. bioRxiv. https://doi.org/10.1101/050559

Higuchi, R., Bowman, B., Freiberger, M., Ryder, O. A., & Wilson, A. C. (1984). DNA sequences from the quagga, an extinct member of the horse family. *Nature*, *312*(5991), 282–284. https://doi.org/10.1038/312282a0

Hinnebusch, B. J., Jarrett, C. O., & Bland, D. M. (2017). "fleaing" the plague: Adaptations of *Yersinia pestis* to its insect vector that lead to transmission. *Annu. Rev. Microbio*, *8*(71), 215–232.

Hofreiter, M., Jaenicke, V., Serre, D., von Haeseler, A., & Pääbo, S. (2001). DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA. *Nucleic Acids Research*, *29*(23), 4793–4799. https://doi.org/10.1093/nar/29.23.4793

Hofreiter, M., Mead, J. I., Martin, P., & Poinar, H. N. (2003). Molecular caving. *Current Biology*, *13*(18), R693–R695. https://doi.org/10.1016/j.cub.2003.08.039

Hübler, R., Key, F. M., Warinner, C., Bos, K. I., Krause, J., & Herbig, A. (2019). HOPS: automated detection and authentication of pathogen DNA in archaeological remains. *Genome Biology*, *20*(1), 280. https://doi.org/10.1186/s13059-019-1903-0

Human Microbiome Project Consortium. (2012). Structure, function and diversity of the healthy human microbiome. *Nature*, *486*(7402), 207–214. https://doi.org/10.1038/nature11234

Huson, D. H., Auch, A. F., Qi, J., & Schuster, S. C. (2007). MEGAN analysis of metagenomic data. *Genome Research*, *17*(3), 377–386. https://doi.org/10.1101/gr.5969107

Jackson, S. P., & Bartek, J. (2009). The DNA-damage response in human biology and disease. *Nature*, *461*(7267), 1071–1078. https://doi.org/10.1038/nature08467

Javan, G. T., Finley, S. J., Can, I., Wilkinson, J. E., Hanson, J. D., & Tarone, A. M. (2016). Human thanatomicrobiome succession and time since death. *Scientific Reports*, *6*(1), 29598. https://doi.org/10.1038/srep29598

Jensen, T. Z. T., Niemann, J., Iversen, K. H., Fotakis, A. K., Gopalakrishnan, S., Vågene, Å. J., Pedersen, M. W., Sinding, M.-H. S., Ellegaard, M. R., Allentoft, M. E., Lanigan, L. T., Taurozzi, A. J., Nielsen, S. H., Dee, M. W., Mortensen, M. N., Christensen, M. C., Sørensen, S. A., Collins, M. J., Gilbert, M. T. P., … Schroeder, H. (2019). A 5700 year-old human genome and oral microbiome from chewed birch pitch. *Nature Communications*, *10*(1), 5520. https://doi.org/10.1038/s41467-019-13549-9

Jersie-Christensen, R. R., Lanigan, L. T., Lyon, D., Mackie, M., Belstrøm, D., Kelstrup, C. D., Fotakis, A. K., Willerslev, E., Lynnerup, N., Jensen, L. J., Cappellini, E., & Olsen, J. V. (2018). Quantitative metaproteomics of medieval dental calculus reveals individual oral health status. *Nature Communications*, *9*(1), 4744. https://doi.org/10.1038/s41467-018-07148-3

Johnson, T., Thakar, H. B., Watkins, J., & Linderholm, A. (2024). *Working Ethically with Ancient DNA from Composites in the United States. Advances in Archaeological Practice*. *12*, 86–97. https://doi.org/10.1017/aap.2023.32

Jónsson, H., Ginolhac, A., Schubert, M., Johnson, P. L. F., & Orlando, L. (2013). mapDamage2.0: fast approximate Bayesian estimates of ancient DNA damage parameters. *Bioinformatics (Oxford, England)*, *29*(13), 1682–1684. https://doi.org/10.1093/bioinformatics/btt193

Joy, D. A., Feng, X., Mu, J., Furuya, T., Chotivanich, K., Krettli, A. U., Ho, M., Wang, A., White, N. J., Suh, E., Beerli, P., & Su, X.-Z. (2003). Early origin and recent expansion of Plasmodium falciparum. *Science (New York, N.Y.)*, *300*(5617), 318–321. https://doi.org/10.1126/science.1081449

Kaur, P. (1997). Expression and characterization of DrrA and DrrB proteins of Streptomyces peucetius in Escherichia coli: DrrA is an ATP binding protein. *Journal of Bacteriology*, *179*(3), 569–575. https://doi.org/10.1128/jb.179.3.569-575.1997

Kawanishi, S., Hiraku, Y., Pinlaor, S., & Ma, N. (2006). Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. *Biological Chemistry*, *387*(4), 365–372. https://doi.org/10.1515/BC.2006.049

Keller, M., Guellil, M., Slavin, P., Saag, L., Irdt, K., Niinemäe, H., Solnik, A., Malve, M., Valk, H., Kriiska, A., Cessford, C., Inskip, S., Robb, J., Cooper, C., von Planta, C., Seifert, M., Reitmaier, T., Baetsen, W., Walker, D., … Scheib, C. (2023). A refined phylochronology of the Second Plague Pandemic in western Eurasia. In *bioRxiv*. https://doi.org/10.1101/2023.07.18.549544

Keller, M., & L Scheib, C. (2023). *Ancient DNA extract purification (chunk samples/high volume) v1*. https://doi.org/10.17504/protocols.io.j8nlkwje6l5r/v1

Keller, M., & Scheib, C. L. (2023). *Ancient DNA protocol collection – University of Tartu, Institute of Genomics*. Protocols.io. https://dx.doi.org/10.17504/protocols.io.n92ldzbd8v5b/v1

Keller, M., Scheib, C. L., & Bonucci, B. (n.d.). *Library preparation (dsDNA double indexing, non-UDG, 2x split)*. protocols.io https://dx.doi.org/10.17504/protocols.io.n2bvj6xqxlk5/v1

Keller, M., Spyrou, M. A., Scheib, C. L., Neumann, G. U., Kröpelin, A., Haas-Gebhard, B., Päffgen, B., Haberstroh, J., Ribera I Lacomba, A., Raynaud, C., Cessford, C., Durand, R., Stadler, P., Nägele, K., Bates, J. S., Trautmann, B., Inskip, S. A., Peters, J., Robb, J. E., … Krause, J. (2019). Ancient *Yersinia pestis* genomes from across Western Europe reveal early diversification during the First Pandemic (541-750). *Proceedings of the National Academy of Sciences of the United States of America*, *116*(25), 12363–12372. https://doi.org/10.1073/pnas.1820447116

Key, F. M., Posth, C., Esquivel-Gomez, L. R., Hübler, R., Spyrou, M. A., Neumann, G. U., Furtwängler, A., Sabin, S., Burri, M., Wissgott, A., Lankapalli, A. K., Vågene, Å. J., Meyer, M., Nagel, S., Tukhbatova, R., Khokhlov, A., Chizhevsky, A., Hansen, S., Belinsky, A. B., … Krause, J. (2020). Emergence of human-adapted Salmonella enterica is linked to the Neolithization process. *Nature Ecology & Evolution*, *4*(3), 324–333. https://doi.org/10.1038/s41559-020-1106-9

Key, F. M., Posth, C., Krause, J., Herbig, A., & Bos, K. I. (2017). Mining metagenomic data sets for ancient DNA: Recommended protocols for authentication. *Trends in Genetics: TIG*, *33*(8), 508–520. https://doi.org/10.1016/j.tig.2017.05.005

Kırdök, E., Kashuba, N., Damlien, H., Manninen, M. A., Nordqvist, B., Kjellström, A., Jakobsson, M., Lindberg, A. M., Storå, J., Persson, P., Andersson, B., Aravena, A., & Götherström, A. (2024). Metagenomic analysis of Mesolithic chewed pitch reveals poor oral health among stone age individuals. *Scientific Reports*, *13*(1), 22125. https://doi.org/10.1038/s41598-023-48762-6

Kjær, K. H., Winther Pedersen, M., De Sanctis, B., De Cahsan, B., Korneliussen, T. S., Michelsen, C. S., Sand, K. K., Jelavić, S., Ruter, A. H., Schmidt, A. M. A., Kjeldsen, K. K., Tesakov, A. S., Snowball, I., Gosse, J. C., Alsos, I. G., Wang, Y., Dockter, C., Rasmussen, M., Jørgensen, M. E., … Willerslev, E. (2022). A 2-million-year-old ecosystem in Greenland uncovered by environmental DNA. *Nature*, *612*(7939), 283–291. https://doi.org/10.1038/s41586-022-05453-y

Klapper, M., Hübner, A., Ibrahim, A., Wasmuth, I., Borry, M., Haensch, V. G., Zhang, S., Al-Jammal, W. K., Suma, H., Fellows Yates, J. A., Frangenberg, J., Velsko, I. M., Chowdhury, S., Herbst, R., Bratovanov, E. V., Dahse, H.-M., Horch, T., Hertweck, C., González Morales, M. R., … Stallforth, P. (2023). Natural products from reconstructed bacterial genomes of the Middle and Upper Paleolithic. *Science (New York, N.Y.)*, *380*(6645), 619–624. https://doi.org/10.1126/science.adf5300

Klunk, J., Vilgalys, T. P., Demeure, C. E., Cheng, X., Shiratori, M., Madej, J., Beau, R., Elli, D., Patino, M. I., Redfern, R., DeWitte, S. N., Gamble, J. A., Boldsen, J. L., Carmichael, A., Varlik, N., Eaton, K., Grenier, J.-C., Golding, G. B., Devault, A., … Barreiro, L. B. (2022). Evolution of immune genes is associated with the Black Death. *Nature*, *611*(7935), 312–319. https://doi.org/10.1038/s41586-022-05349-x

Korneliussen, T. S., Albrechtsen, A., & Nielsen, R. (2014). ANGSD: Analysis of Next Generation Sequencing Data. *BMC Bioinformatics*, *15*(1), 356. https://doi.org/10.1186/s12859-014-0356-4

Kowal, E., Weyrich, L. S., Argüelles, J. M., Bader, A. C., Colwell, C., Cortez, A. D., Davis, J. L., Figueiro, G., Fox, K., Malhi, R. S., Matisoo-Smith, E., Nayak, A., Nelson, E. A., Nicholas, G., Nieves-Colón, M. A., Russell, L., Ulm, S., Vergara-Silva, F., Villanea, F. A., … Tsosie, K. S. (2023). Community partnerships are fundamental to ethical ancient DNA research. *HGG Advances*, *4*(2), 100161. https://doi.org/10.1016/j.xhgg.2022.100161

Kozlov, A. M., Darriba, D., Flouri, T., Morel, B., & Stamatakis, A. (2019). RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics (Oxford, England)*, *35*(21), 4453–4455. https://doi.org/10.1093/bioinformatics/btz305

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. *Nature Methods*, *9*(4), 357–359. https://doi.org/10.1038/nmeth.1923

Lazaridis, I., Patterson, N., Mittnik, A., Renaud, G., Mallick, S., Kirsanow, K., Sudmant, P. H., Schraiber, J. G., Castellano, S., Lipson, M., Berger, B., Economou, C., Bollongino, R., Fu, Q., Bos, K. I., Nordenfelt, S., Li, H., de Filippo, C., Prüfer, K., … Krause, J. (2014). Ancient human genomes suggest three ancestral populations for present-day Europeans. *Nature*, *513*(7518), 409–413. https://doi.org/10.1038/nature13673

LeBlanc, J. G., Milani, C., de Giori, G. S., Sesma, F., van Sinderen, D., & Ventura, M. (2013). Bacteria as vitamin suppliers to their host: a gut microbiota perspective. *Current Opinion in Biotechnology*, *24*(2), 160–168. https://doi.org/10.1016/j.copbio.2012.08.005

Lewis, D. A., Simpson, R., Hermes, A., Brown, A., & Llamas, B. (2025). More than dirt: Sedimentary ancient DNA and Indigenous Australia. *Molecular Ecology Resources*, *25*(2), e13835. https://doi.org/10.1111/1755-0998.13835

L'Hôte, L., Light, I., Mattiangeli, V., Teasdale, M. D., Halpin, Á., Gourichon, L., Key, F. M., & Daly, K. G. (2024). An 8000 years old genome reveals the Neolithic origin of the zoonosis Brucella melitensis. *Nature Communications*, *15*(1), 6132. https://doi.org/10.1038/s41467-024-50536-1

Light-Maka, I., Hermes, T. R., Bianco, R. A., Semerau, L., Kosintsev, P., Alekseeva, V., Kim, D., Hanage, W. P., Herbig, A., Jeong, C., Warinner, C., & Key, F. M. (2025). Bronze Age *Yersinia pestis* genome from sheep sheds light on hosts and evolution of a prehistoric plague lineage. *Cell*, *188*(20), 5748–5762.e18. https://doi.org/10.1016/j.cell.2025.07.029

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics (Oxford, England)*, *25*(14), 1754–1760. https://doi.org/10.1093/bioinformatics/btp324

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics (Oxford, England)*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Lindahl, T. (1993). Instability and decay of the primary structure of DNA. *Nature*, *362*(6422), 709–715. https://doi.org/10.1038/362709a0

Lindahl, T., & Barnes, D. E. (2000). An excellent overview of the extent of endogenous DNA damage, the types of DNA lesions arising from cell autonomous sources. *Cold Spring Harb. Symp. Quant. Biol*, *65*, 127–134.

Lindahl, T., & Nyberg, B. (1974). Heat-induced deamination of cytosine residues in deoxyribonucleic acid. *Biochemistry*, *13*(16), 3405–3410. https://doi.org/10.1021/bi00713a035

López-Miras, M. D. M., Martín-Sánchez, I., Yebra-Rodríguez, A., Romero-Noguera, J., Bolívar-Galiano, F., Ettenauer, J., Sterflinger, K., & Piñar, G. (2013). Contribution of the microbial communities detected on an oil painting on canvas to its biodeterioration. *PloS One*, *8*(11), e80198. https://doi.org/10.1371/journal.pone.0080198

Ma, B. (2015). Novor: real-time peptide de novo sequencing software. *Journal of the American Society for Mass Spectrometry*, *26*(11), 1885–1894. https://doi.org/10.1007/s13361-015-1204-0

Ma, B., Lu, C., Wang, Y., Yu, J., Zhao, K., Xue, R., Ren, H., Lv, X., Pan, R., Zhang, J., Zhu, Y., & Xu, J. (2023). Author Correction: A genomic catalogue of soil microbiomes boosts mining of biodiversity and genetic resources. *Nature Communications*, *14*(1), 8079. https://doi.org/10.1038/s41467-023-44072-7

Macleod, R., Seersholm, F., de Sanctis, B., Lieverse, A., Timpson, A., Stenderup, J., Gaunitz, C., Vinner, L., Schulting, R., Goriunova, O. I., Bazaliiskii, V. I., Vasiliev, S., Jessup, E., Wang, Y., Thomas, M., Corbett-Detig, R., Iversen, A. K. N., Weber, A., Sikora, M., & Willerslev, E. (2024). Lethal plague outbreaks in Lake Baikal Hunter–gatherers 5500 years ago. In *bioRxiv*. https://doi.org/10.1101/2024.11.13.623490

Magoč, T., & Salzberg, S. L. (2011). FLASH: fast length adjustment of short reads to improve genome assemblies. *Bioinformatics (Oxford, England)*, *27*(21), 2957–2963. https://doi.org/10.1093/bioinformatics/btr507

Maixner, F., Sarhan, M. S., Huang, K. D., Tett, A., Schoenafinger, A., Zingale, S., Blanco-Míguez, A., Manghi, P., Cemper-Kiesslich, J., Rosendahl, W., Kusebauch, U., Morrone, S. R., Hoopmann, M. R., Rota-Stabelli, O., Rattei, T., Moritz, R. L., Oeggl, K., Segata, N., Zink, A., … Kowarik, K. (2021). Hallstatt miners consumed blue cheese and beer during the Iron Age and retained a non-Westernized gut microbiome until the Baroque period. *Current Biology*, *31*(23), 5149–5162.e6. https://doi.org/10.1016/j.cub.2021.09.031

Majander, K., Pfrengle, S., Kocher, A., Neukamm, J., du Plessis, L., Pla-Díaz, M., Arora, N., Akgül, G., Salo, K., Schats, R., Inskip, S., Oinonen, M., Valk, H., Malve, M., Kriiska, A., Onkamo, P., González-Candelas, F., Kühnert, D., Krause, J., & Schuenemann, V. J. (2020). Ancient bacterial genomes reveal a high diversity of Treponema pallidum strains in early modern Europe. *Current Biology*, *30*(19), 3788–3803.e10. https://doi.org/10.1016/j.cub.2020.07.058

Mallick, S., Micco, A., Mah, M., Ringbauer, H., Lazaridis, I., Olalde, I., Patterson, N., & Reich, D. (2024). The Allen Ancient DNA Resource (AADR) a curated compendium of ancient human genomes. *Scientific Data*, *11*(1), 182. https://doi.org/10.1038/s41597-024-03031-7

Mann, A. E., Sabin, S., Ziesemer, K., Vågene, Å. J., Schroeder, H., Ozga, A. T., Sankaranarayanan, K., Hofman, C. A., Fellows Yates, J. A., Salazar-García, D. C., Frohlich, B., Aldenderfer, M., Hoogland, M., Read, C., Milner, G. R., Stone, A. C., Lewis, C. M., Jr, Krause, J., Hofman, C., … Warinner, C. (2018). Differential preservation of endogenous human and microbial DNA in dental calculus and dentin. *Scientific Reports*, *8*(1), 9822. https://doi.org/10.1038/s41598-018-28091-9

Margaryan, A., Hansen, H. B., Rasmussen, S., Sikora, M., Moiseyev, V., Khoklov, A., Epimakhov, A., Yepiskoposyan, L., Kriiska, A., Varul, L., Saag, L., Lynnerup, N., Willerslev, E., & Allentoft, M. E. (2018). Ancient pathogen DNA in human teeth and petrous bones. *Ecology and Evolution*, *8*(6), 3534–3542. https://doi.org/10.1002/ece3.3924

Maricic, T., Whitten, M., & Pääbo, S. (2010). Multiplexed DNA sequence capture of mitochondrial genomes using PCR products. *PloS One*, *5*(11), e14004. https://doi.org/10.1371/journal.pone.0014004

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal*, *17*(1). https://doi.org/10.14806/ej.17.1.200

Massilani, D., Morley, M. W., Mentzer, S. M., Aldeias, V., Vernot, B., Miller, C., Stahlschmidt, M., Kozlikin, M. B., Shunkov, M. V., Derevianko, A. P., Conard, N. J., Wurz, S., Henshilwood, C. S., Vasquez, J., Essel, E., Nagel, S., Richter, J., Nickel, B., Roberts, R. G., … Meyer, M. (2022). Microstratigraphic preservation of ancient faunal and hominin DNA in Pleistocene cave sediments. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(1), e2113666118. https://doi.org/10.1073/pnas.2113666118

Massini Espino, M., Mychajliw, A. M., Almonte, J. N., & Allentoft, M. E. (n.d.). Van Dam AR. Raptor roosts as invasion archives: insights from the first black rat mitochondrial genome sequenced from the Caribbean. *Biol Invasions*, *2022*(1), 17–25. https://doi.org/10.1007/s10530-021-02636-y.)

McCarthy, S., Das, S., Kretzschmar, W., Delaneau, O., Wood, A. R., Teumer, A., Kang, H. M., Fuchsberger, C., Danecek, P., Sharp, K., Luo, Y., Sidore, C., Kwong, A., Timpson, N., Koskinen, S., Vrieze, S., Scott, L. J., Zhang, H., Mahajan, A., … Haplotype Reference Consortium. (2016). A reference panel of 64,976 haplotypes for genotype imputation. *Nature Genetics*, *48*(10), 1279–1283. https://doi.org/10.1038/ng.3643

McGhee, J. J., Rawson, N., Bailey, B. A., Fernandez-Guerra, A., Sisk-Hackworth, L., & Kelley, S. T. (2020). Meta-SourceTracker: application of Bayesian source tracking to shotgun metagenomics. *PeerJ*, *8*, e8783. https://doi.org/10.7717/peerj.8783

McGovern, P. E., Zhang, J., Tang, J., Zhang, Z., Hall, G. R., Moreau, R. A., Nuñez, A., Butrym, E. D., Richards, M. P., Wang, C.-S., Cheng, G., Zhao, Z., & Wang, C. (2004). Fermented beverages of pre- and proto-historic China. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(51), 17593–17598. https://doi.org/10.1073/pnas.0407921102

McKenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., & DePristo, M. A. (2010). The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*, *20*(9), 1297–1303. https://doi.org/10.1101/gr.107524.110

McLaren, W., Gil, L., Hunt, S. E., Riat, H. S., Ritchie, G. R. S., Thormann, A., Flicek, P., & Cunningham, F. (2016). The Ensembl Variant Effect Predictor. *Genome Biology*, *17*(1), 122. https://doi.org/10.1186/s13059-016-0974-4

McMurdie, P. J., & Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PloS One*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Meisner, J., & Albrechtsen, A. (2018). Inferring Population Structure and Admixture Proportions in Low-Depth NGS Data. *Genetics*, *210*(2), 719–731. https://doi.org/10.1534/genetics.118.301336

Menzel, P., Ng, K. L., & Krogh, A. (2016). Fast and sensitive taxonomic classification for metagenomics with Kaiju. *Nature Communications*, *7*(1), 11257. https://doi.org/10.1038/ncomms11257

Mesuere, B., Van der Jeugt, F., Willems, T., Naessens, T., Devreese, B., Martens, L., & Dawyndt, P. (2018). High-throughput metaproteomics data analysis with Unipept: A tutorial. *Journal of Proteomics*, *171*, 11–22. https://doi.org/10.1016/j.jprot.2017.05.022

Metcalf, J. L., Carter, D. O., & Knight, R. (2016). Microbiology of death. *Current Biology*, *26*(13), R561–R563. https://doi.org/10.1016/j.cub.2016.03.042

Meyer, M., & Kircher, M. (2010). Illumina sequencing library preparation for highly multiplexed target capture and sequencing. *Cold Spring Harbor Protocols*, *2010*(6), db.prot5448. https://doi.org/10.1101/pdb.prot5448

Miller, M. F., 2nd, & Wyckoff, R. W. (1968). Proteins in dinosaur bones. *Proceedings of the National Academy of Sciences of the United States of America*, *60*(1), 176–178. https://doi.org/10.1073/pnas.60.1.176

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, *37*(5), 1530–1534. https://doi.org/10.1093/molbev/msaa015

Modi, A., Attolini, D., Zaro, V., Pisaneschi, L., Innocenti, G., Vai, S., Caramelli, D., Moggi Cecchi, J., Quagliariello, A., Mariotti Lippi, M., & Lari, M. (2023). Combined metagenomic and archaeobotanical analyses on human dental calculus: A cross-section of lifestyle conditions in a Copper Age population of central Italy. *Quaternary International: The Journal of the International Union for Quaternary Research*, *653-654*, 69–81. https://doi.org/10.1016/j.quaint.2021.12.003

Morse, S. S., Mazet, J. A. K., Woolhouse, M., Parrish, C. R., Carroll, D., Karesh, W. B., Zambrana-Torrelio, C., Lipkin, W. I., & Daszak, P. (2012). Prediction and prevention of the next pandemic zoonosis. *Lancet*, *380*(9857), 1956–1965. https://doi.org/10.1016/S0140-6736(12)61684-5

Neumann, G. U., Skourtanioti, E., Burri, M., Nelson, E. A., Michel, M., Hiss, A. N., McGeorge, P. J. P., Betancourt, P. P., Spyrou, M. A., Krause, J., & Stockhammer, P. W. (2022). Ancient *Yersinia pestis* and Salmonella enterica genomes from Bronze Age Crete. *Current Biology*, *32*(16), 3641–3649.e8. https://doi.org/10.1016/j.cub.2022.06.094

Ogawa, Y., Ooka, T., Shi, F., Ogura, Y., Nakayama, K., Hayashi, T., & Shimoji, Y. (2011). The genome of *Erysipelothrix rhusiopathiae*, the causative agent of swine erysipelas, reveals new insights into the evolution of firmicutes and the organism's intracellular adaptations. *Journal of Bacteriology*, *193*(12), 2959–2971. https://doi.org/10.1128/JB.01500-10

Oh, J., Byrd, A. L., Park, M., NISC Comparative Sequencing Program, Kong, H. H., & Segre, J. A. (2016). Temporal Stability of the Human Skin Microbiome. *Cell*, *165*(4), 854–866. https://doi.org/10.1016/j.cell.2016.04.008

Okonechnikov, K., Conesa, A., & García-Alcalde, F. (2016). Qualimap 2: advanced multi-sample quality control for high-throughput sequencing data. *Bioinformatics (Oxford, England)*, *32*(2), 292–294. https://doi.org/10.1093/bioinformatics/btv566

Opal, S. M. (2009). A brief history of microbiology and immunology. *Vaccines: A Biography*, 31–56.

Orlando, L., Allaby, R., Skoglund, P., Der Sarkissian, C., Stockhammer, P. W., Ávila-Arcos, M. C., Fu, Q., Krause, J., Willerslev, E., Stone, A. C., & Warinner, C. (2021). Ancient DNA analysis. *Nature Reviews. Methods Primers*, *1*(1). https://doi.org/10.1038/s43586-020-00011-0

Ostrom, P. H., Gandhi, H., Strahler, J. R., Walker, A. K., Andrews, P. C., Leykam, J., Stafford, T. W., Kelly, R. L., Walker, D. N., Buckley, M., & Humpula, J. (2006). Unraveling the sequence and structure of the protein osteocalcin from a 42ka fossil horse. *Geochimica et Cosmochimica Acta*, *70*(8), 2034–2044. https://doi.org/10.1016/j.gca.2006.01.004

Pääbo, S. (1985a). Molecular cloning of Ancient Egyptian mummy DNA. *Nature*, *314*(6012), 644–645. https://doi.org/10.1038/314644a0

Pääbo, S. (1985b). Preservation of DNA in ancient Egyptian mummies. *Journal of Archaeological Science*, *12*(6), 411–417. https://doi.org/10.1016/0305-4403(85)90002-0

Pääbo, S. (1986). Molecular genetic investigations of ancient human remains. *Cold Spring Harbor Symposia on Quantitative Biology*, *51*(0), 441–446. https://doi.org/10.1101/sqb.1986.051.01.053

Palmer, K. S., Makarewicz, C. A., Tishkin, A. A., Tur, S. S., Chunag, A., & Diimajav, E. (2021). Comparing the Use of Magnetic Beads with Ultrafiltration for Ancient Dental Calculus Proteomics (PDF). *Journal of Proteome Research*, *20*(3), 1689–1704. https://doi.org/10.1021/acs.jproteome.0c00862

Parkhill, J., Wren, B. W., Thomson, N. R., Titball, R. W., Holden, M. T., Prentice, M. B., Sebaihia, M., James, K. D., Churcher, C., Mungall, K. L., Baker, S., Basham, D., Bentley, S. D., Brooks, K., Cerdeño-Tárraga, A. M., Chillingworth, T., Cronin, A., Davies, R. M., Davis, P., … Barrell, B. G. (2001). Genome sequence of *Yersinia pestis*, the causative agent of plague. *Nature*, *413*(6855), 523–527. https://doi.org/10.1038/35097083

Patel, K. B., Toh, E., Fernandez, X. B., Hanuszkiewicz, A., Hardy, G. G., Brun, Y. V., Bernards, M. A., & Valvano, M. A. (2012). Functional characterization of UDP-glucose:undecaprenyl-phosphate glucose-1-phosphate transferases of Escherichia coli and Caulobacter crescentus. *Journal of Bacteriology*, *194*(10), 2646–2657. https://doi.org/10.1128/JB.06052-11

Paterson, R. S., Mackie, M., Capobianco, A., Heckeberg, N. S., Fraser, D., Demarchi, B., Munir, F., Patramanis, I., Ramos-Madrigal, J., Liu, S., Ramsøe, A. D., Dickinson, M. R., Baldreki, C., Gilbert, M., Sardella, R., Bellucci, L., Scorrano, G., Leonardi, M., Manica, A., … Cappellini, E. (2025). Phylogenetically informative proteins from an Early Miocene rhinocerotid. *Nature*, *643*(8072), 719–724. https://doi.org/10.1038/s41586-025-09231-4

Patterson, N., Moorjani, P., Luo, Y., Mallick, S., Rohland, N., Zhan, Y., Genschoreck, T., Webster, T., & Reich, D. (2012). Ancient admixture in human history. *Genetics*, *192*(3), 1065–1093. https://doi.org/10.1534/genetics.112.145037

Patterson, N., Price, A. L., & Reich, D. (2006). Population structure and eigenanalysis. *PLoS Genetics*, *2*(12), e190. https://doi.org/10.1371/journal.pgen.0020190

Pavelka, M. S., Jr, & Jacobs, W. R., Jr. (1996). Biosynthesis of diaminopimelate, the precursor of lysine and a component of peptidoglycan, is an essential function of Mycobacterium smegmatis. *Journal of Bacteriology*, *178*(22), 6496–6507. https://doi.org/10.1128/jb.178.22.6496-6507.1996

Pearce-Duvet, J. M. C. (2006). The origin of human pathogens: evaluating the role of agriculture and domestic animals in the evolution of human disease. *Biological Reviews of the Cambridge Philosophical Society*, *81*(3), 369–382. https://doi.org/10.1017/S1464793106007020

Pedersen, M. W., Ruter, A., Schweger, C., Friebe, H., Staff, R. A., Kjeldsen, K. K., Mendoza, M. L. Z., Beaudoin, A. B., Zutter, C., Larsen, N. K., Potter, B. A., Nielsen, R., Rainville, R. A., Orlando, L., Meltzer, D. J., Kjær, K. H., & Willerslev, E. (2016). Postglacial viability and colonization in North America's ice-free corridor. *Nature*, *537*(7618), 45–49. https://doi.org/10.1038/nature19085

Perez-Riverol, Y., Bai, J., Bandla, C., García-Seisdedos, D., Hewapathirana, S., Kamatchinathan, S., Kundu, D. J., Prakash, A., Frericks-Zipper, A., Eisenacher, M., Walzer, M., Wang, S., Brazma, A., & Vizcaíno, J. A. (2022). The PRIDE database resources in 2022: a hub for mass spectrometry-based proteomics evidences. *Nucleic Acids Research*, *50*(D1), D543–D552. https://doi.org/10.1093/nar/gkab1038

Perini, N., Mercuri, F., Orlanducci, S., Thaller, M. C., & Migliore, L. (2020). The integration of metagenomics and chemical physical techniques biodecoded the buried traces of the biodeteriogens of parchment purple spots. *Frontiers in Microbiology*, *11*, 598945. https://doi.org/10.3389/fmicb.2020.598945

Perry, R. D., & Fetherston, J. D. (1997). *Yersinia pestis*–etiologic agent of plague. *Clinical Microbiology Reviews*, *10*(1), 35–66. https://doi.org/10.1128/CMR.10.1.35

Peverelli, M. G., Soares da Costa, T. P., Kirby, N., & Perugini, M. A. (2016). Dimerization of bacterial diaminopimelate decarboxylase is essential for catalysis. *The Journal of Biological Chemistry*, *291*(18), 9785–9795. https://doi.org/10.1074/jbc.M115.696591

Piñar, G., Sclocchi, M. C., Pinzari, F., Colaizzi, P., Graf, A., Sebastiani, M. L., & Sterflinger, K. (2020). The microbiome of Leonardo da Vinci's drawings: A bioarchive of their history. *Frontiers in Microbiology*, *11*, 593401. https://doi.org/10.3389/fmicb.2020.593401

Piñar, G., Tafer, H., Schreiner, M., Miklas, H., & Sterflinger, K. (2020). Decoding the biological information contained in two ancient Slavonic parchment codices: an added historical value. *Environmental Microbiology*, *22*(8), 3218–3233. https://doi.org/10.1111/1462-2920.15064

Plesca, D., Mazumder, S., & Almasan, A. (2008). DNA damage response and apoptosis. *Methods in Enzymology*, *446*, 107–122. https://doi.org/10.1016/S0076-6879(08)01606-6

Pochon, Z., Bergfeldt, N., Kırdök, E., Vicente, M., Naidoo, T., van der Valk, T., Altınışık, N. E., Krzewińska, M., Dalén, L., Götherström, A., Mirabello, C., Unneberg, P., & Oskolkov, N. (2023). aMeta: an accurate and memory-efficient ancient metagenomic profiling workflow. *Genome Biology*, *24*(1), 242. https://doi.org/10.1186/s13059-023-03083-9

Popli, D., Peyrégne, S., & Peter, B. M. (2023). KIN: a method to infer relatedness from low-coverage ancient DNA. *Genome Biology*, *24*(1), 10. https://doi.org/10.1186/s13059-023-02847-7

Pradel, E., Lemaître, N., Merchez, M., Ricard, I., Reboul, A., Dewitte, A., & Sebbane, F. (2014). New insights into how *Yersinia pestis* adapts to its mammalian host during bubonic plague. *PLoS Pathogens*, *10*(3), e1004029. https://doi.org/10.1371/journal.ppat.1004029

Price, M. N., Dehal, P. S., & Arkin, A. P. (2009). FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. *Molecular Biology and Evolution*, *26*(7), 1641–1650. https://doi.org/10.1093/molbev/msp077

Puckett, E. E., Park, J., Combs, M., Blum, M. J., Bryant, J. E., Caccone, A., Costa, F., Deinum, E. E., Esther, A., Himsworth, C. G., Keightley, P. D., Ko, A., Lundkvist, Å., McElhinney, L. M., Morand, S., Robins, J., Russell, J., Strand, T. M., Suarez, O., … Munshi-South, J. (2016). Global population divergence and admixture of the brown rat (Rattus norvegicus). *Proceedings. Biological Sciences*, *283*(1841). https://doi.org/10.1098/rspb.2016.1762

Quagliariello, A., Modi, A., Innocenti, G., Zaro, V., Conati Barbaro, C., Ronchitelli, A., Boschin, F., Cavazzuti, C., Dellù, E., Radina, F., Sperduti, A., Bondioli, L., Ricci, S., Lognoli, M., Belcastro, M. G., Mariotti, V., Caramelli, D., Mariotti Lippi, M., Cristiani, E., … Lari, M. (2022). Ancient oral microbiomes support gradual Neolithic dietary shifts towards agriculture. *Nature Communications*, *13* (1), 6927. https://doi.org/10.1038/s41467-022-34416-0

Quinlan, A. R., & Hall, I. M. (2010). BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics (Oxford, England)*, *26*(6), 841–842. https://doi.org/10.1093/bioinformatics/btq033

Radini, A., Nikita, E., Buckley, S., Copeland, L., & Hardy, K. (2017). Beyond food: The multiple pathways for inclusion of materials into ancient dental calculus. *American Journal of Physical Anthropology*, *162 Suppl 63*(S63), 71–83. https://doi.org/10.1002/ajpa.23147

Radini, A., Tromp, M., Beach, A., Tong, E., Speller, C., McCormick, M., Dudgeon, J. V., Collins, M. J., Rühli, F., Kröger, R., & Warinner, C. (2019). Medieval women's early involvement in manuscript production suggested by lapis lazuli identification in dental calculus. *Science Advances*, *5*(1), eaau7126. https://doi.org/10.1126/sciadv.aau7126

Rahman, M. T., Sobur, M. A., Islam, M. S., Ievy, S., Hossain, M. J., El Zowalaty, M. E., Rahman, A. T., & Ashour, H. M. (2020). Zoonotic diseases: Etiology, impact, and control. *Microorganisms*, *8*–(9), E1405. https://doi.org/10.3390/microorganisms8091405

Rampelli, S., Schnorr, S. L., Consolandi, C., Turroni, S., Severgnini, M., Peano, C., Brigidi, P., Crittenden, A. N., Henry, A. G., & Candela, M. (2015). Metagenome Sequencing of the Hadza Hunter-Gatherer Gut Microbiota. *Current Biology : CB*, *25*(13), 1682–1693. https://doi.org/10.1016/j.cub.2015.04.055

Ramsøe, A., Crispin, M., Mackie, M., McGrath, K., Fischer, R., Demarchi, B., Collins, M. J., Hendy, J., & Speller, C. (2021). Assessing the degradation of ancient milk proteins through site-specific deamidation patterns. *Scientific Reports*, *11*(1), 7795. https://doi.org/10.1038/s41598-021-87125-x

Ramsøe, A., van Heekeren, V., Ponce, P., Fischer, R., Barnes, I., Speller, C., & Collins, M. J. (2020). DeamiDATE 1.0: Site-specific deamidation as a tool to assess authenticity of members of ancient proteomes. *Journal of Archaeological Science*, *115*(105080), 105080. https://doi.org/10.1016/j.jas.2020.105080

Raoult, D., Aboudharam, G., Crubézy, E., Larrouy, G., Ludes, B., & Drancourt, M. (2000). Molecular identification by "suicide PCR" of *Yersinia pestis* as the agent of medieval black death. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(23), 12800–12803. https://doi.org/10.1073/pnas.220225197

Rascovan, N., Sjögren, K.-G., Kristiansen, K., Nielsen, R., Willerslev, E., Desnues, C., & Rasmussen, S. (2019). Emergence and Spread of Basal Lineages of *Yersinia pestis* during the Neolithic Decline. *Cell*, *176*(1-2), 295–305.e10. https://doi.org/10.1016/j.cell.2018.11.005

Rasmussen, S., Allentoft, M. E., Nielsen, K., Orlando, L., Sikora, M., Sjögren, K.-G., Pedersen, A. G., Schubert, M., Van Dam, A., Kapel, C. M. O., Nielsen, H. B., Brunak, S., Avetisyan, P., Epimakhov, A., Khalyapin, M. V., Gnuni, A., Kriiska, A., Lasak, I., Metspalu, M., … Willerslev, E. (2015). Early divergent strains of *Yersinia pestis* in Eurasia 5,000 years ago. *Cell*, *163*(3), 571–582. https://doi.org/10.1016/j.cell.2015.10.009

Raxworthy, C. J., & Smith, B. T. (2021). Mining museums for historical DNA: advances and challenges in museomics. *Trends in Ecology & Evolution*, *36*(11), 1049–1060. https://doi.org/10.1016/j.tree.2021.07.009

Reizer, J., Reizer, A., & Saier, M. H., Jr. (1992). A new subfamily of bacterial ABC-type transport systems catalyzing export of drugs and carbohydrates. *Protein Science*, *1*(10), 1326–1332. https://doi.org/10.1002/pro.5560011012

Renaud, G., Slon, V., Duggan, A. T., & Kelso, J. (2015). Schmutzi: estimation of contamination and endogenous mitochondrial consensus calling for ancient DNA. *Genome Biology*, *16*, 224. https://doi.org/10.1186/s13059-015-0776-0

Ringbauer, H., Novembre, J., & Steinrücken, M. (2021). Parental relatedness through time revealed by runs of homozygosity in ancient DNA. *Nature Communications*, *12*(1), 5425. https://doi.org/10.1038/s41467-021-25289-w

Robinson, J. T., Thorvaldsdóttir, H., Winckler, W., Guttman, M., Lander, E. S., Getz, G., & Mesirov, J. P. (2011). Integrative genomics viewer. *Nature Biotechnology*, *29*(1), 24–26. https://doi.org/10.1038/nbt.1754

Rodriguez Palomo, I., Nair, B., Chiang, Y., Dekker, J., Dartigues, B., Mackie, M., Evans, M., Macleod, R., Olsen, J. V., & Collins, M. J. (2024). Benchmarking the identification of a single degraded protein to explore optimal search strategies for ancient proteins. *Peer Community Journal*, *4*(e107). https://doi.org/10.24072/pcjournal.491

Rohart, F., Gautier, B., Singh, A., & Lê Cao, K.-A. (2017). mixOmics: An R package for 'omics feature selection and multiple data integration. *PLoS Computational Biology*, *13*(11), e1005752. https://doi.org/10.1371/journal.pcbi.1005752

Rohland, N., Glocke, I., Aximu-Petri, A., & Meyer, M. (2018). Extraction of highly degraded DNA from ancient bones, teeth and sediments for high-throughput sequencing. *Nature Protocols*, *13*(11), 2447–2461. https://doi.org/10.1038/s41596-018-0050-5

Rohland, N., Harney, E., Mallick, S., Nordenfelt, S., & Reich, D. (2015). Partial uracil-DNA-glycosylase treatment for screening of ancient DNA. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *370*(1660), 20130624. https://doi.org/10.1098/rstb.2013.0624

Rohland, N., & Hofreiter, M. (2007). Comparison and optimization of ancient DNA extraction. *BioTechniques*, *42*(3), 343–352. https://doi.org/10.2144/000112383

Rohland, N., Mallick, S., Mah, M., Maier, R., Patterson, N., & Reich, D. (2022). Three assays for in-solution enrichment of ancient human DNA at more than a million SNPs. *Genome Research*, *32*(11-12), 2068–2078. https://doi.org/10.1101/gr.276728.122

Ronald M. Atlas. (2012). One health: Its origins and future. In *Current Topics in Microbiology and Immunology* (pp. 1–13). Springer Berlin Heidelberg. https://doi.org/10.1007/82_2012_223

Rosado, T., Mirão, J., Candeias, A., & Caldeira, A. T. (2014). Microbial communities analysis assessed by pyrosequencing: a new approach applied to conservation state studies of mural paintings. *Analytical and Bioanalytical Chemistry*, *406*, 887–895.

Rubinacci, S., Ribeiro, D. M., Hofmeister, R. J., & Delaneau, O. (2021). Efficient phasing and imputation of low-coverage sequencing data using large reference panels. *Nature Genetics*, *53*(1), 120–126. https://doi.org/10.1038/s41588-020-00756-0

Sabet, C., Toledo-Arana, A., Personnic, N., Lecuit, M., Dubrac, S., Poupel, O., Gouin, E., Nahori, M.-A., Cossart, P., & Bierne, H. (2008). The Listeria monocytogenes virulence factor InlJ is specifically expressed in vivo and behaves as an adhesin. *Infection and Immunity*, *76*(4), 1368–1378. https://doi.org/10.1128/IAI.01519-07

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America*, *74*(12), 5463–5467. https://doi.org/10.1073/pnas.74.12.5463

Sawafuji, R., Sawaura, R., Yokoo, M., Kumaki, T., Thomasen, N. A., Tsutaya, T., & Pedersen, M. W. (2026). From bones to sediments: Ancient human DNA from open-air archaeological sites. *Journal of Archaeological Science*, *189*(106537), 106537. https://doi.org/10.1016/j.jas.2026.106537

Schmieder, R., & Edwards, R. (2011). Quality control and preprocessing of metagenomic datasets. *Bioinformatics (Oxford, England)*, *27*(6), 863–864. https://doi.org/10.1093/bioinformatics/btr026

Schönherr, S., Weissensteiner, H., Kronenberg, F., & Forer, L. (2023). Haplogrep 3 - an interactive haplogroup classification and analysis platform. *Nucleic Acids Research*, *51*(W1), W263–W268. https://doi.org/10.1093/nar/gkad284

Schubert, M., Ermini, L., Der Sarkissian, C., Jónsson, H., Ginolhac, A., Schaefer, R., Martin, M. D., Fernández, R., Kircher, M., McCue, M., Willerslev, E., & Orlando, L. (2014). Characterization of ancient and modern genomes by SNP detection and phylogenomic and metagenomic analysis using PALEOMIX. *Nature Protocols*, *9*(5), 1056–1082. https://doi.org/10.1038/nprot.2014.063

Schubert, M., Lindgreen, S., & Orlando, L. (2016). AdapterRemoval v2: rapid adapter trimming, identification, and read merging. *BMC Research Notes*, *9*, 88. https://doi.org/10.1186/s13104-016-1900-2

Schuenemann, V. J., Avanzi, C., Krause-Kyora, B., Seitz, A., Herbig, A., & Inskip, S. (2018). *Ancient genomes reveal a high diversity of Mycobacte- rium leprae in medieval Europe*.

Schuenemann, V. J., Bos, K., DeWitte, S., Schmedes, S., Jamieson, J., Mittnik, A., Forrest, S., Coombes, B. K., Wood, J. W., Earn, D. J. D., White, W., Krause, J., & Poinar, H. N. (2011). Targeted enrichment of ancient pathogens yielding the pPCP1 plasmid of *Yersinia pestis* from victims of the Black Death. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(38), E746–E752. https://doi.org/10.1073/pnas.1105107108

Schuenemann, V. J., Kumar Lankapalli, A., Barquera, R., Nelson, E. A., Iraíz Hernández, D., Acuña Alonzo, V., Bos, K. I., Márquez Morfín, L., Herbig, A., & Krause, J. (2018). Historic Treponema pallidum genomes from Colonial Mexico retrieved from archaeological remains. *PLoS Neglected Tropical Diseases*, *12*(6), e0006447. https://doi.org/10.1371/journal.pntd.0006447

Schuenemann, V. J., Singh, P., Mendum, T. A., Krause-Kyora, B., Jäger, G., Bos, K. I., Herbig, A., Economou, C., Benjak, A., Busso, P., Nebel, A., Boldsen, J. L., Kjellström, A., Wu, H., Stewart, G. R., Taylor, G. M., Bauer, P., Lee, O. Y.-C., Wu, H. H. T., … Krause, J. (2013). Genome-wide comparison of medieval and modern Mycobacterium leprae. *Science (New York, N.Y.)*, *341*(6142), 179–183. https://doi.org/10.1126/science.1238286

Schyns, G., Potot, S., Geng, Y., Barbosa, T. M., Henriques, A., & Perkins, J. B. (2005). Isolation and characterization of new thiamine-deregulated mutants of Bacillus subtilis. *Journal of Bacteriology*, *187*(23), 8127–8136. https://doi.org/10.1128/JB.187.23.8127-8136.2005

Seersholm, F. V., Pedersen, M. W., Søe, M. J., Shokry, H., Mak, S. S. T., Ruter, A., Raghavan, M., Fitzhugh, W., Kjær, K. H., Willerslev, E., Meldgaard, M., Kapel, C. M. O., & Hansen, A. J. (2016). DNA evidence of bowhead whale exploitation by Greenlandic Paleo-Inuit 4,000 years ago. *Nature Communications*, *7*(1), 13389. https://doi.org/10.1038/ncomms13389

Seersholm, F. V., Sjögren, K.-G., Koelman, J., Blank, M., Svensson, E. M., Staring, J., Fraser, M., Pinotti, T., McColl, H., Gaunitz, C., Ruiz-Bedoya, T., Granehäll, L., Villegas-Ramirez, B., Fischer, A., Price, T. D., Allentoft, M. E., Iversen, A. K. N., Axelsson, T., Ahlström, T., … Sikora, M. (2024). Repeated plague infections across six generations of Neolithic Farmers. *Nature*, *632*(8023), 114–121. https://doi.org/10.1038/s41586-024-07651-2

Segata, N., Waldron, L., Ballarini, A., Narasimhan, V., Jousson, O., & Huttenhower, C. (2012). Metagenomic microbial community profiling using unique clade-specific marker genes. *Nature Methods*, *9*(8), 811–814. https://doi.org/10.1038/nmeth.2066

Seidman, D. N., Shenoy, S. A., Kim, M., Babu, R., Woods, I. G., Dyer, T. D., Lehman, D. M., Curran, J. E., Duggirala, R., Blangero, J., & Williams, A. L. (2020). Rapid, Phase-free Detection of Long Identity-by-Descent Segments Enables Effective Relationship Classification. *American Journal of Human Genetics*, *106*(4), 453–466. https://doi.org/10.1016/j.ajhg.2020.02.012

Sender, R., Fuchs, S., & Milo, R. (2016a). Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. *Cell*, *164*(3), 337–340. https://doi.org/10.1016/j.cell.2016.01.013

Sender, R., Fuchs, S., & Milo, R. (2016b). Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biology*, *14*(8), e1002533. https://doi.org/10.1371/journal.pbio.1002533

Sen, M., Shah, B., Rakshit, S., Singh, V., Padmanabhan, B., Ponnusamy, M., Pari, K., Vishwakarma, R., Nandi, D., & Sadhale, P. P. (2011). UDP-glucose 4, 6-dehydratase activity plays an important role in maintaining cell wall integrity and virulence of Candida albicans. *PLoS Pathogens*, *7*(11), e1002384. https://doi.org/10.1371/journal.ppat.1002384

Seru, L. V., Forde, T. L., Roberto-Charron, A., Mavrot, F., Niu, Y. D., & Kutz, S. J. (2024). Genomic characterization and virulence gene profiling of *Erysipelothrix rhusiopathiae* isolated from widespread muskox mortalities in the Canadian Arctic Archipelago. *BMC Genomics*, *25*(1), 691. https://doi.org/10.1186/s12864-024-10592-9

Shillito, L.-M., & Mackay, H. (2020). Middens, waste disposal, and health at Çatalhöyük. *Near Eastern Archaeology*, *83*(3), 168–174. https://doi.org/10.1086/710134

Sikora, M., Canteri, E., Fernandez-Guerra, A., Oskolkov, N., Ågren, R., Hansson, L., Irving-Pease, E. K., Mühlemann, B., Holtsmark Nielsen, S., Scorrano, G., Allentoft, M. E., Valeur Seersholm, F., Schroeder, H., Gaunitz, C., Stenderup, J., Vinner, L., Jones, T. C., Nystedt, B., Sjögren, K.-G., … Willerslev, E. (2025). The spatiotemporal distribution of human pathogens in ancient Eurasia. *Nature*, *643*(8073), 1011–1019. https://doi.org/10.1038/s41586-025-09192-8

Simón, M., Montiel, R., Smerling, A., Solórzano, E., Díaz, N., Álvarez-Sandoval, B. A., Jiménez-Marín, A. R., & Malgosa, A. (2014). Molecular analysis of ancient caries. *Proceedings. Biological Sciences*, *281*(1790), 20140586. https://doi.org/10.1098/rspb.2014.0586

Skoglund, P., Northoff, B. H., Shunkov, M. V., Derevianko, A. P., Pääbo, S., Krause, J., & Jakobsson, M. (2014). Separating endogenous ancient DNA from modern day contamination in a Siberian Neandertal. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(6), 2229–2234. https://doi.org/10.1073/pnas.1318934111

Skoglund, P., Storå, J., Götherström, A., & Jakobsson, M. (2013). Accurate sex identification of ancient human remains using DNA shotgun sequencing. *Journal of Archaeological Science*, *40*(12), 4477–4482. https://doi.org/10.1016/j.jas.2013.07.004

Slon, V., Hopfe, C., Weiß, C. L., Mafessoni, F., de la Rasilla, M., Lalueza-Fox, C., Rosas, A., Soressi, M., Knul, M. V., Miller, R., Stewart, J. R., Derevianko, A. P., Jacobs, Z., Li, B., Roberts, R. G., Shunkov, M. V., de Lumley, H., Perrenoud, C., Gušić, I., … Meyer, M. (2017). Neandertal and Denisovan DNA from Pleistocene sediments. *Science (New York, N.Y.)*, *356*(6338), 605–608. https://doi.org/10.1126/science.aam9695

Söderlund, R., Formenti, N., Caló, S., Chiari, M., Zoric, M., Alborali, G. L., Sørensen Dalgaard, T., Wattrang, E., & Eriksson, H. (2020). Comparative genome analysis of *Erysipelothrix rhusiopathiae* isolated from domestic pigs and wild boars suggests host adaptation and selective pressure from the use of antibiotics. *Microbial Genomics*, *6*(8). https://doi.org/10.1099/mgen.0.000412

Spigelman, M., & Lemma, E. (1993). The use of the polymerase chain reaction (PCR) to detect *Mycobacterium tuberculosis* in ancient skeletons. *International Journal of Osteoarchaeology*, *3*(2), 137–143. https://doi.org/10.1002/oa.1390030211

Spyrou, M. A., Bos, K. I., Herbig, A., & Krause, J. (2019). Ancient pathogen genomics as an emerging tool for infectious disease research. *Nature Reviews. Genetics*, *20*(6), 323–340. https://doi.org/10.1038/s41576-019-0119-1

Spyrou, M. A., Musralina, L., Gnecchi Ruscone, G. A., Kocher, A., Borbone, P.-G., Khartanovich, V. I., Buzhilova, A., Djansugurova, L., Bos, K. I., Kühnert, D., Haak, W., Slavin, P., & Krause, J. (2022). The source of the Black Death in fourteenth-century central Eurasia. *Nature*, *606*(7915), 718–724. https://doi.org/10.1038/s41586-022-04800-3

Spyrou, M. A., Tukhbatova, R. I., Feldman, M., Drath, J., Kacki, S., Beltrán de Heredia, J., Arnold, S., Sitdikov, A. G., Castex, D., Wahl, J., Gazimzyanov, I. R., Nurgaliev, D. K., Herbig, A., Bos, K. I., & Krause, J. (2016). Historical *Y. pestis* genomes reveal the European Black Death as the source of ancient and modern plague pandemics. *Cell Host & Microbe*, *19*(6), 874–881. https://doi.org/10.1016/j.chom.2016.05.012

Spyrou, M. A., Tukhbatova, R. I., Wang, C.-C., Valtueña, A. A., Lankapalli, A. K., Kondrashin, V. V., Tsybin, V. A., Khokhlov, A., Kühnert, D., Herbig, A., Bos, K. I., & Krause, J. (2018). Analysis of 3800-year-old *Yersinia pestis* genomes suggests Bronze Age origin for bubonic plague. *Nature Communications*, *9*(1), 2234. https://doi.org/10.1038/s41467-018-04550-9

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics (Oxford, England)*, *30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stenseth, N. C., Atshabar, B. B., Begon, M., Belmain, S. R., Bertherat, E., Carniel, E., Gage, K. L., Leirs, H., & Rahalison, L. (2008). Plague: past, present, and future. *PLoS Medicine*, *5*(1), e3. https://doi.org/10.1371/journal.pmed.0050003

Sun, B., Andrades Valtueña, A., Kocher, A., Gao, S., Li, C., Fu, S., Zhang, F., Ma, P., Yang, X., Qiu, Y., Zhang, Q., Ma, J., Chen, S., Xiao, X., Damchaabadgar, S., Li, F., Kovalev, A., Hu, C., Chen, X., … Cui, Y. (2024). Origin and dispersal history of Hepatitis B virus in Eastern Eurasia. *Nature Communications*, *15*(1), 2951. https://doi.org/10.1038/s41467-024-47358-6

Susat, J., Haller-Caskie, M., Bonczarowska, J. H., da Silva, N. A., Schierhold, K., Rind, M. M., Schmölcke, U., Kirleis, W., Sondermann, H., Rinne, C., Müller, J., Nebel, A., & Krause-Kyora, B. (2024). Neolithic *Yersinia pestis* infections in humans and a dog. *Communications Biology*, *7*(1), 1013. https://doi.org/10.1038/s42003-024-06676-7

Susat, J., Lübke, H., Immel, A., Brinker, U., Macāne, A., Meadows, J., Steer, B., Tholey, A., Zagorska, I., Gerhards, G., Schmölcke, U., Kalniņš, M., Franke, A., Pētersone-Gordina, E., Teßman, B., Tõrv, M., Schreiber, S., Andree, C., Bērziņš, V., … Krause-Kyora, B. (2021). A 5,000-year-old hunter-gatherer already plagued by *Yersinia pestis*. *Cell Reports*, *35*(13), 109278. https://doi.org/10.1016/j.celrep.2021.109278

Swali, P., Schulting, R., Gilardet, A., Kelly, M., Anastasiadou, K., Glocke, I., McCabe, J., Williams, M., Audsley, T., Loe, L., Fernández-Crespo, T., Ordoño, J., Walker, D., Clare, T., Cook, G., Hodkinson, I., Simpson, M., Read, S., Davy, T., … Skoglund, P. (2023). *Yersinia pestis* genomes reveal plague in Britain 4000 years ago. *Nature Communications*, *14*(1), 2930. https://doi.org/10.1038/s41467-023-38393-w

Tanabe, K., Mita, T., Jombart, T., Eriksson, A., Horibe, S., Palacpac, N., Ranford-Cartwright, L., Sawai, H., Sakihama, N., Ohmae, H., Nakamura, M., Ferreira, M. U., Escalante, A. A., Prugnolle, F., Björkman, A., Färnert, A., Kaneko, A., Horii, T., Manica, A., … Balloux, F. (2010). Plasmodium falciparum accompanied the human expansion out of Africa. *Current Biology*, *20*(14), 1283–1289. https://doi.org/10.1016/j.cub.2010.05.053

Taylor, L. H., Latham, S. M., & Woolhouse, M. E. J. (2001). Risk factors for human disease emergence. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *356*(1411), 983–989. https://doi.org/10.1098/rstb.2001.0888

Teng, H., Zhang, Y., Shi, C., Mao, F., Hou, L., Guo, H., Sun, Z., & Zhang, J. (2016). Whole-Genome Sequencing Reveals Genetic Variation in the Asian House Rat. *G3 (Bethesda, Md.)*, *6*(7), 1969–1977. https://doi.org/10.1534/g3.116.029504

Tett, A., Huang, K. D., Asnicar, F., Fehlner-Peach, H., Pasolli, E., Karcher, N., Armanini, F., Manghi, P., Bonham, K., Zolfo, M., De Filippis, F., Magnabosco, C., Bonneau, R., Lusingu, J., Amuasi, J., Reinhard, K., Rattei, T., Boulund, F., Engstrand, L., … Segata, N. (2019). The Prevotella copri complex comprises four distinct clades underrepresented in Westernized populations. *Cell Host & Microbe*, *26*(5), 666–679.e7. https://doi.org/10.1016/j.chom.2019.08.018

Teytelman, L., Stoliartchouk, A., Kindler, L., & Hurwitz, B. L. (2016). Protocols.Io: Virtual communities for protocol development and discussion. *PLoS Biology*, *14*(8), e1002538. https://doi.org/10.1371/journal.pbio.1002538

Torralba, M. G., Kuelbs, C., Moncera, K. J., Roby, R., & Nelson, K. E. (2021). Characterizing microbial signatures on sculptures and paintings of similar provenance. *Microbial Ecology*, *81*(4), 1098–1105. https://doi.org/10.1007/s00248-020-01504-x

UNIDO. (2020). *Coronavirus: the economic impact – 26 May 2020*. United Nations Industrial Development Organization. https://www.unido.org/stories/coronavirus-economic-impact-26-may-2020

Vågene, Å. J., Herbig, A., Campana, M. G., Robles García, N. M., Warinner, C., Sabin, S., Spyrou, M. A., Andrades Valtueña, A., Huson, D., Tuross, N., Bos, K. I., & Krause, J. (2018). Salmonella enterica genomes from victims of a major sixteenth-century epidemic in Mexico. *Nature Ecology & Evolution*, *2*(3), 520–528. https://doi.org/10.1038/s41559-017-0446-6

Valko, M., Rhodes, C. J., Moncol, J., Izakovic, M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions*, *160*(1), 1–40. https://doi.org/10.1016/j.cbi.2005.12.009

Vande Moortele, T., Devlaminck, B., Van de Vyver, S., Van Den Bossche, T., Martens, L., Dawyndt, P., Mesuere, B., & Verschaffelt, P. (2025). Unipept in 2024: Expanding Metaproteomics Analysis with Support for Missed Cleavages and Semitryptic and Nontryptic Peptides. *Journal of Proteome Research*, *24*(2), 949–954. https://doi.org/10.1021/acs.jproteome.4c00848

van Dorp, L., Gelabert, P., Rieux, A., de Manuel, M., de-Dios, T., Gopalakrishnan, S., Carøe, C., Sandoval-Velasco, M., Fregel, R., Olalde, I., Escosa, R., Aranda, C., Huijben, S., Mueller, I., Marquès-Bonet, T., Balloux, F., Gilbert, M. T. P., & Lalueza-Fox, C. (2020). Plasmodium vivax malaria viewed through the lens of an eradicated European strain. *Molecular Biology and Evolution*, *37*(3), 773–785. https://doi.org/10.1093/molbev/msz264

van Oven, M. (2015). PhyloTree Build 17: Growing the human mitochondrial DNA tree. *Forensic Science International. Genetics Supplement Series*, *5*, e392–e394. https://doi.org/10.1016/j.fsigss.2015.09.155

Varro, M. T. (1934). On Agriculture (H. B. A. W. D. Hooper, Trans.). In *Cato and Varro: On Agriculture* (Vol. 283). Harvard University Press.

Velsko, I. M., Fagernäs, Z., Tromp, M., Bedford, S., Buckley, H. R., Clark, G., Dudgeon, J., Flexner, J., Galipaud, J.-C., Kinaston, R., Lewis, C. M., Jr, Matisoo-Smith, E., Nägele, K., Ozga, A. T., Posth, C., Rohrlach, A. B., Shing, R., Simanjuntak, T., Spriggs, M., … Warinner, C. (2024). Exploring the potential of dental calculus to shed light on past human migrations in Oceania. *Nature Communications*, *15*(1), 10191. https://doi.org/10.1038/s41467-024-53920-z

Velsko, I. M., Fellows Yates, J. A., Aron, F., Hagan, R. W., Frantz, L. A. F., Loe, L., Martinez, J. B. R., Chaves, E., Gosden, C., Larson, G., & Warinner, C. (2019). Microbial differences between dental plaque and historic dental calculus are related to oral biofilm maturation stage. *Microbiome*, *7*(1), 102. https://doi.org/10.1186/s40168-019-0717-3

Vernot, B., Zavala, E. I., Gómez-Olivencia, A., Jacobs, Z., Slon, V., Mafessoni, F., Romagné, F., Pearson, A., Petr, M., Sala, N., Pablos, A., Aranburu, A., de Castro, J. M. B., Carbonell, E., Li, B., Krajcarz, M. T., Krivoshapkin, A. I., Kolobova, K. A., Kozlikin, M. B., … Meyer, M. (2021). Unearthing Neanderthal population history using nuclear and mitochondrial DNA from cave sediments. *Science (New York, N.Y.)*, *372*(6542), eabf1667. https://doi.org/10.1126/science.abf1667

Wagner, D. M., Klunk, J., Harbeck, M., Devault, A., Waglechner, N., Sahl, J. W., Enk, J., Birdsell, D. N., Kuch, M., Lumibao, C., Poinar, D., Pearson, T., Fourment, M., Golding, B., Riehm, J. M., Earn, D. J. D., Dewitte, S., Rouillard, J.-M., Grupe, G., … Poinar, H. (2014). *Yersinia pestis* and the plague of Justinian 541-543 AD: a genomic analysis. *The Lancet Infectious Diseases*, *14*(4), 319–326. https://doi.org/10.1016/S1473-3099(13)70323-2

Wang, J., Jiang, L., & Sun, H. (2021). Early evidence for beer drinking in a 9000-year-old platform mound in southern China. *PloS One*, *16*(8), e0255833. https://doi.org/10.1371/journal.pone.0255833

Wang, Q., & Riley, T. V. (2015). *Erysipelothrix rhusiopathiae*. In *Molecular Medical Microbiology* (pp. 859–872). Elsevier. https://doi.org/10.1016/b978-0-12-397169-2.00047-0

Warinner, C. (2022). An archaeology of microbes. *Journal of Anthropological Research*, 000–000. https://doi.org/10.1086/721976

Warinner, C., Hendy, J., Speller, C., Cappellini, E., Fischer, R., Trachsel, C., Arneborg, J., Lynnerup, N., Craig, O. E., Swallow, D. M., Fotakis, A., Christensen, R. J., Olsen, J. V., Liebert, A., Montalva, N., Fiddyment, S., Charlton, S., Mackie, M., Canci, A., … Collins, M. J. (2014). Direct evidence of milk consumption from ancient human dental calculus. *Scientific Reports*, *4*(1), 7104. https://doi.org/10.1038/srep07104

Warinner, C., Herbig, A., Mann, A., Fellows Yates, J. A., Weiß, C. L., Burbano, H. A., Orlando, L., & Krause, J. (2017a). A robust framework for microbial archaeology. *Annual Review of Genomics and Human Genetics*, *18*(1), 321–356. https://doi.org/10.1146/annurev-genom-091416-035526

Warinner, C., Herbig, A., Mann, A., Fellows Yates, J. A., Weiß, C. L., Burbano, H. A., Orlando, L., & Krause, J. (2017b). A robust framework for microbial archaeology. *Annual Review of Genomics and Human Genetics*, *18*(1), 321–356. https://doi.org/10.1146/annurev-genom-091416-035526

Warinner, C., Korzow Richter, K., & Collins, M. J. (2022). Paleoproteomics. *Chemical Reviews*, *122*(16), 13401–13446. https://doi.org/10.1021/acs.chemrev.1c00703

Warinner, C., Rodrigues, J. F. M., Vyas, R., Trachsel, C., Shved, N., Grossmann, J., Radini, A., Hancock, Y., Tito, R. Y., Fiddyment, S., Speller, C., Hendy, J., Charlton, S., Luder, H. U., Salazar-García, D. C., Eppler, E., Seiler, R., Hansen, L. H., Castruita, J. A. S., … Cappellini, E. (2014). Pathogens and host immunity in the ancient human oral cavity. *Nature Genetics*, *46*(4), 336–344. https://doi.org/10.1038/ng.2906

Warinner, C., Speller, C., & Collins, M. J. (2015). A new era in palaeomicrobiology: prospects for ancient dental calculus as a long-term record of the human oral microbiome. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *370*(1660), 20130376. https://doi.org/10.1098/rstb.2013.0376

Weissensteiner, H., Pacher, D., Kloss-Brandstätter, A., Forer, L., Specht, G., Bandelt, H.-J., Kronenberg, F., Salas, A., & Schönherr, S. (2016). HaploGrep 2: mitochondrial haplogroup classification in the era of high-throughput sequencing. *Nucleic Acids Research*, *44*(W1), W58–W63. https://doi.org/10.1093/nar/gkw233

Weyrich, L. S., Dobney, K., & Cooper, A. (2015). Ancient DNA analysis of dental calculus. *Journal of Human Evolution*, *79*, 119–124. https://doi.org/10.1016/j.jhevol.2014.06.018

Weyrich, L. S., Duchene, S., Soubrier, J., Arriola, L., Llamas, B., Breen, J., Morris, A. G., Alt, K. W., Caramelli, D., Dresely, V., Farrell, M., Farrer, A. G., Francken, M., Gully, N., Haak, W., Hardy, K., Harvati, K., Held, P., Holmes, E. C., … Cooper, A. (2017). Neanderthal behaviour, diet, and disease inferred from ancient DNA in dental calculus. *Nature*, *544*(7650), 357–361. https://doi.org/10.1038/nature21674

WHO. (2020). *Zoonoses*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/zoonoses

Wibowo, M. C., Yang, Z., Borry, M., Hübner, A., Huang, K. D., Tierney, B. T., Zimmerman, S., Barajas-Olmos, F., Contreras-Cubas, C., García-Ortiz, H., Martínez-Hernández, A., Luber, J. M., Kirstahler, P., Blohm, T., Smiley, F. E., Arnold, R., Ballal, S. A., Pamp, S. J., Russ, J., … Kostic, A. D. (2021). Reconstruction of ancient microbial genomes from the human gut. *Nature*, *594*(7862), 234–239. https://doi.org/10.1038/s41586-021-03532-0

Wiechmann, I., & Grupe, G. (2005). Detection of *Yersinia pestis* DNA in two early medieval skeletal finds from Aschheim, Upper Bavaria, 6th century A.D. *American Journal of Physical Anthropology*, *126*(1), 48–55.

Willerslev, E., Hansen, A. J., Binladen, J., Brand, T. B., Gilbert, M. T. P., Shapiro, B., Bunce, M., Wiuf, C., Gilichinsky, D. A., & Cooper, A. (2003). Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science (New York, N.Y.)*, *300*(5620), 791–795. https://doi.org/10.1126/science.1084114

Wood, D. E., Lu, J., & Langmead, B. (2019). Improved metagenomic analysis with Kraken 2. *Genome Biology*, *20*(1), 257. https://doi.org/10.1186/s13059-019-1891-0

Wood, R. L. (1973). Survival of *Erysipelothrix rhusiopathiae* in soil under various environmental conditions. *The Cornell Veterinarian*, *63*(3), 390–410. https://www.ncbi.nlm.nih.gov/pubmed/4592996

W Runge, A. K., Light-Maka, I., Massy, K., Keller, M., Trixl, S., Kabral, H., Kirkpatrick, C. L., Bos, K., Eger, J., Ernée, M., Kyselý, R., Hochmuth, M., Poradowski, D., Chrószcz, A., Benecke, N., Daněček, D., Klementová, J., Nagler, A., Kalmykov, A. A., … Key, F. M. (2026). Probing the zooarchaeological record across time and space for ancient pathogen DNA. *Nature Communications*, *17*(1). https://doi.org/10.1038/s41467-026-71543-4

Wu, C., Lv, C., Zhao, Y., Zhu, W., Liu, L., Wang, T., Kang, C., Yang, Y., Sun, X., Zhang, Q., & Jin, M. (2021). Characterization of *Erysipelothrix rhusiopathiae* Isolates from Diseased Pigs in 15 Chinese Provinces from 2012 to 2018. *Microorganisms*, *9*(12), 2615. https://doi.org/10.3390/microorganisms9122615

Yamamoto, T., Kida, Y., Sakamoto, Y., & Kuwano, K. (2017). Mpn491, a secreted nuclease of Mycoplasma pneumoniae, plays a critical role in evading killing by neutrophil extracellular traps. *Cellular Microbiology*, *19*(3), e12666. https://doi.org/10.1111/cmi.12666

Yu, G., Smith, D. K., Zhu, H., Guan, Y., & Lam, T. T.-Y. (2017). ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods in Ecology and Evolution*, *8*(1), 28–36. https://doi.org/10.1111/2041-210X.12628

Yu, H., Jamieson, A., Hulme-Beaman, A., Conroy, C. J., Knight, B., Speller, C., Al-Jarah, H., Eager, H., Trinks, A., Adikari, G., Baron, H., Böhlendorf-Arslan, B., Bohingamuwa, W., Crowther, A., Cucchi, T., Esser, K., Fleisher, J., Gidney, L., Gladilina, E., … Orton, D. (2022). Palaeogenomic analysis of black rat (Rattus rattus) reveals multiple European introductions associated with human economic history. *Nature Communications*, *13*(1), 2399. https://doi.org/10.1038/s41467-022-30009-z

Zampirolo, G., H Ruter, A., Živaljević, I., Penezić, K., Thomsen, P. F., Orton, D., Grubin, M. K., Antić, N., & Pedersen, M. W. (2026). Ancient DNA reconstruction of late holocene ecosystems within the Carpathian basin from paleo-meanders and archaeological deposits. *Scientific Reports*, *16*(1), 4301. https://doi.org/10.1038/s41598-026-35509-2

Zavala, E. I., Jacobs, Z., Vernot, B., Shunkov, M. V., Kozlikin, M. B., Derevianko, A. P., Essel, E., de Fillipo, C., Nagel, S., Richter, J., Romagné, F., Schmidt, A., Li, B., O'Gorman, K., Slon, V., Kelso, J., Pääbo, S., Roberts, R. G., & Meyer, M. (2021). Pleistocene sediment DNA reveals hominin and faunal turnovers at Denisova Cave. *Nature*, *595*(7867), 399–403. https://doi.org/10.1038/s41586-021-03675-0

Zhang, D., Xia, H., Chen, F., Li, B., Slon, V., Cheng, T., Yang, R., Jacobs, Z., Dai, Q., Massilani, D., Shen, X., Wang, J., Feng, X., Cao, P., Yang, M. A., Yao, J., Yang, J., Madsen, D. B., Han, Y., … Fu, Q. (2020). Denisovan DNA in Late Pleistocene sediments from Baishiya Karst Cave on the Tibetan Plateau. *Science (New York, N.Y.)*, *370*(6516), 584–587. https://doi.org/10.1126/science.abb6320

Zhao, W. (2024). Reconstructing human history from ancient genomes: an interview with Nobel laureate Svante Pääbo. *National Science Review*, *11*(9), nwae120. https://doi.org/10.1093/nsr/nwae120

Ziesemer, K. A., Mann, A. E., Sankaranarayanan, K., Schroeder, H., Ozga, A. T., Brandt, B. W., Zaura, E., Waters-Rist, A., Hoogland, M., Salazar-García, D. C., Aldenderfer, M., Speller, C., Hendy, J., Weston, D. A., MacDonald, S. J., Thomas, G. H., Collins, M. J., Lewis, C. M., Hofman, C., & Warinner, C. (2015). Intrinsic challenges in ancient microbiome reconstruction using 16S rRNA gene amplification. *Scientific Reports*, *5*(1), 16498. https://doi.org/10.1038/srep16498

ACKNOWLEDGEMENTS

I cannot promise to be brief, because there are so many people I wish to acknowledge at the end of this journey. This thesis and PhD is not only the result of my own work, but also of the support of many wonderful people along the way. I believe all the people we encounter along the way shape who we are, but there are some in my life that I would like to acknowledge further.

I would like to start by thanking my supervisors, Christiana Scheib ("Freddi"), Kristiina Tambets and Toni de-Dios. Freddi, by accepting me as a trainee before and a PhD student later, you have led me to where I am now. I am grateful for all the opportunities to network, discuss ideas, and explore my own research questions. Thank you Kristiina for trusting me, supporting me in these years in Tartu and encouraging me to ask myself questions. Thank you also for the help with all the translations needed for this thesis. Toni, any word I could write here wouldn't be enough to express the gratitude I have towards you. In these 5 years, you have been a supervisor, a colleague, a friend. You are one of the most brilliant people I know, and I am so glad I could listen to you talk about biology, history, and science in general. You have not only taught me everything you know about bioinformatics, but have always pushed me to learn more, to improve my skills, to read more, to try new software and tools. You have involved me in projects and encouraged me every day. This thesis is a bit yours, too.

A huge thanks to Jaak Truu, for being my internal reviewer, for the useful comments on my thesis and his patience, despite the time constraints. Thanks to Lota Vana for the help with the popular science article.

However, this journey started long before the PhD. For this, I want to thank my former laboratory in Rome, and in particular Prof. Fulvio Cruciani, Prof. Beniamino Trombetta (Ben), and Eugenia D'Atanasio, for being among the first people to believe in me. Eugenia, a big thank you goes to you, with all my heart. You were the person who encouraged me to apply for the traineeship in Tartu. You have supported me since day one, and you have always been there. You were my co-supervisor, and now you are also a friend. We can discuss research, send each other memes, and be there when the other is in need. I hope one day we will be able to work together again (our real dream).

These last five years have given me the opportunity to work together with inspiring researchers across disciplines and countries. I would like to thank Dr. Sarah Inskip and Dr. Anna-Davies Barrett from the Tobacco, Health and History Project in particular for the trust they put in me with their dental calculus, for giving me the possibility to participate in engaging discussions on tobacco usage and health and how to study it from different perspectives.

I wouldn't be the researcher I am now if I hadn't been part of the "Ancestors project". This project changed my life not only for the perspectives it gave me on the importance of interdisciplinary research, but also for the people I met thanks to it. Mary-Anne, Silvia, Sofia, Martina, Harriett, Sue, Toomas, John, Jess, it has been a pleasure to work with you. John, thank you for your guidance, for your patience, for the trust and the care you showed in these years. Jess, I do not have

enough words to thank you properly…You are an example of determination and passion, you are brilliant and you have shown me how important it is to go beyond your own discipline. Thank you for your help with applications and abstracts, thank you for seeing in me what I can't. I wish one day I can be like you. I look forward to continuing to discuss ethics, human remains, theories with you, but also I can't wait for our little runs, chats, laughs and cries together.

These years have allowed me to get to know young researchers who, like me, have tried to make sense of complex metagenomic data. Thanks to the SPAAM community for existing, for having given me the space to discuss metagenomics, pathogens, databases, tools. Between the summer school, the SPAAM meetings, the community projects, I have learnt a lot and found incredible, brilliant young researchers. I am grateful I met and I could work with many of you, like James, Aida, Alex, Luis, Minna, Taru, Yuejiao, Maxime, Andrea, Betsy, and many others, but especially Maria. You are a strong and incredibly smart woman and researcher, and I am so honored I can call you a friend. Thank you for pushing me and believing in me when I don't.

Last year, my research brought me to the microARCH lab at Penn State, where, in just three months, I found a second home. I learnt so much about the oral microbiome in that short time, and I am grateful for the opportunity to discuss my research and be part of such a vibrant and stimulating research environment. Thank you to Professor Laura Weyrich for the opportunity, the discussions, the enthusiasm and the encouragement. I will never forget how welcome I felt in your group. I hope this is just the beginning. Thanks to everyone I met in those days, but especially Christine (T), Iyun, Will, Fred, Cameron, Vaishnavi, Melina, Grace, Nazifa and everyone I met in that little town in Pennsylvania for the time we shared.

Tartu has been my home for the past 5 years, there are so many people that need to be acknowledged. First and foremost, I would like to thank the archaeo-genomics group, for having been the group I could be part of and develop as a young researcher for the last years. Thank you to Kristiina, Lehti, Lena, Anu, Toni, Stefania, Madeleine, Marcel, Meriam, Remi, Kadri, Tina, Erkin, Roberto, Olga, Helja, Stella. Thank you for the meetings, the group activities, thank you all for having created this environment. A shoutout to Helja for the guidance she gave me in my first steps in an ancient DNA lab, to Anu for being an example of patience and care, to Lehti for the trust and fruitful discussions. It's been great to develop as a researcher in this environment.

Nothing would have been the same in these years if I hadn't found other fellow PhD students I could share the journey with. Thank you Mathilde and Stefania, for being my first friends in the Institute. Thank you Danat and Ivan. You are two of the most brilliant people I have known, it's been an honor not only to become your friend, but to see you involved in JC and WS because of your interest in discussing science. You are my examples of how a curious, smart scientist should be. Thank you for the care you have always shown towards me, for the chats, the saunas (you are the ones that really taught me what a real sauna is) and the hikes we shared. Erkin, Roberto, Rodrigo, Elia, Madeleine, Kai, Rutvi, Ludovica, I am

grateful for having crossed paths and shared moments with you, because a PhD journey seems a little bit lighter if shared with friends.

Beyond the PhD students, the third floor has also been home to other incredible people who made these years amazing. Thank you Ajai, Anne-Mai, Hovik, Monika and Linda for the time spent together, the drinks, the parties, especially those organized by Ajai, and the chats shared inside and outside the Institute. Thanks to you, I always felt I had a safety net of people around me. Thank you Tuuli for your help with everything sequencing-related. Thanks to Merilin, Mariza, Merit and Kadri, because an Institute works well when there is incredible administrative staff behind it.

Thanks to the amazing Institute writing retreats, summer days, conferences, I could not only write my articles and speak about science, but also meet some people outside of my floor, with whom I could spend time and collaborate on Institute's projects. I am grateful I have met wonderful and inspiring people and researchers such as Valentina, Jelisaveta, Gala, Natalia, Kertu, Annabel, Kanwal, Triin, Kelli, Kateryna, Erik. Overall, thanks to all the PhD students I met in these years of studies, and to the ones that are still going through the process I say..you have it, you can do it, and it was a pleasure to share a bit of this path with you. To Mait, thank you for creating, together with the others PI in the Institute, a collaborative, fun, interesting environment such as the Institute of Genomics, my home for the last 5 years.

Much of my life has been spent in the Institute, but these years were also shaped by life outside it. In Tartu, I met people who, little by little or almost immediately, became part of my daily life. Thanks to Tartu, I found friends and family, some of whom are still here and some of whom I can call friends even from the other side of the globe. People such as Miriam, Luis, Ayisha, Raul, Lenards, Mery, Sam, Sevanna, Kate, Connor, Avi, Ninad, Vitto, Chiara, Maria Rosa, Adri, Merlyn, Simone, Carmen, Ilaria, Carlos, Papuna, Valeria, Pepe. Grazie Ilaria, perché mi capisci, senza mai giudicarmi e giudicare. Grazie Carmen, perché in poco tempo sei arrivata e sei rimasta, grazie perché sei genuina.

Grazie Simone per aver creato uno spazio magico come il Barlova. Grazie perché è diventato una seconda casa a Tartu, un rifugio per i momenti di gioia e sconforto. Grazie Joshua per le pizze che in questi ultimi due anni sono state contorno delle mie giornate lavorative. Barlova was the place where I met some of the most important people in this crazy journey. Thank you Carlos and Papuna, for having been, together with Pepe, my little family in the last two years. Thank you for encouraging me in every step and cheering me up. Valeria (BiancaValeria), Tartu ti ha portata a me e la vita ti ha fatta rimanere. Grazie per esserci, perché sai incoraggiarmi, sai quando spingermi e farmi razionalizzare. Grazie per l'amore, le risate, gli abbraccini. Che bello saperci nello stesso ambito, sapere che ci saremo sempre come amiche e come colleghe, giorno dopo giorno.

Decidere di partire non è stato per niente facile. Ho lasciato un pezzo di cuore e un pezzo di me in Italia. Ma ci sono persone che, dall'Italia, non hanno mai smesso di supportarmi. A Fra e le chiacchiere sulla vita a ogni mio ritorno. Allo scautismo, che mi ha insegnato a guardare lontano. Alla mia Associazione Scout, che mi ha formata e ha continuato a darmi fiducia nonostante la distanza. Grazie

al mio Gruppo Scout per esserci stato. Un ringraziamento speciale va a Cecilia, per avermi spinta quando non avevo coraggio. Alla comunità RS, per aver sempre fatto il tifo per me. Ai miei amici, "quelli di scout", a Marzia, Federica (Beli), Giorgio, Fede, Sara, Elisa, Giordana, Arianna, Yuri, Ilaria, Yuppi, per aver creduto in me senza capire fino in fondo cosa stessi facendo, per esserci stati ogni volta che tornavo in Italia. Grazie Giorgio per le chiamate e le risate, per le colazioni al ritorno a Roma, per avermi accettata anche se 'prendevo le ossa dei bambini', grazie Fe per l'amore, per essere fiera di me anche se ti sono lontana, per la fiducia.

Ari, amica mia. Ci sei da quando studiavamo biologia insieme e, nonostante la vita ci abbia portato su strade diverse, sei sempre pronta a farmi il tifo, pronta a incoraggiarmi e sostenermi. Sei una delle donne più forti che conosca, sei un'amica e una mamma meravigliosa e sono onorata di averti al mio fianco da così tanti anni. Vero, soprattutto negli ultimi anni ci sei stata, mi hai accompagnata nei momenti più difficili e bui. Sei stata sempre una mia 'fan', mi hai sostenuta e accompagnata. Mi hai ripresa quando non credevo in me, mi hai spinta quando non avevo coraggio. Mi hai insegnato ad alzare la testa e ti sarò sempre grata per non avermi mai abbandonata.

Alla mia famiglia, biologica ma anche acquisita, per avermi dato esempi di coraggio e avermi dato amore ogni volta che tornavo a casa. A tutti miei zii e cugini, che mi hanno fatto sentire di nuovo a casa ogni volta che tornavo. A zia Daniela, per avermi insegnato il coraggio di rialzarsi nonostante tutto. A zia Ross, per aver creduto in me da quando mi ha conosciuta, piccola piccola, per aver gioito a ogni mio successo, piccolo o grande che fosse. A Maria e Sabatino, i miei 'nonni acquisiti' per averci amati incondizionatamente, per avermi sostenuta nonostante il dispiacere di avermi lontana, a Maria per ogni 'mi raccomando sempre attenta'.

Alle mie fate, a Cami, Viky, Vivi e Fra. Ci siete state dal giorno 1, ci siete ogni giorno, dal buongiorno alla buonanotte. Ci siete nelle tempeste e nei giorni calmi. Sono così fortunata a poter chiamare voi le mie sorelle. Mi avete dato forza in questi cinque anni, mi avete presentata come la vostra amica dall'Estonia e fatta sentire amata, incoraggiata, forte. Mi avete insegnato a non farmi spaventare, a credere nei miei sogni, ma anche a prendere una pausa quando serve, per stare con voi. Grazie per avermi sempre festeggiata, grazie per tutto l'amore.

And if I speak about love, I cannot avoid speaking about you, Pepe. We both know that this thesis is a bit yours, too. You have been there since the first abstract I had to send to a conference. Over these years, you have been my compass, my companion and my friend. You encouraged me and pushed me with love and patience. You celebrated my small achievements and picked me up in my lowest moments. I am so grateful to have you by my side. You are an incredible researcher and human being: humble, brave, caring, dedicated and a dreamer. Every day, I learn so much from your approach to science and life, you push me to be the best version of myself. Thank you for being with me throughout these PhD years.

Le ultime righe di questi ringraziamenti le voglio dedicare alle tre persone più speciali della mia vita. Nicco, fratello mio. Sei la persona più determinata, divertente, intelligente che conosca. Sei un esempio e un compagno di vita. Per quanto

diversi, so che posso contare su di te in ogni momento, perché sarai sempre lì pronto a incoraggiarmi, a dirmi di non abbattermi, a dirmi di 'spingere come capre'. E so che se ho te non ho paura. Sono fiera di te e del tuo dottorato, spero di poter essere per te una roccia come tu lo sei stato per me in questi anni.

Mamma, Papà, le ultime parole di questa tesi sono per voi. Dico sempre di essere una persona privilegiata, perché ho avuto due genitori che da sempre mi hanno spinta a inseguire i miei sogni. Grazie per essere stati esempi di forza e coraggio, grazie per avermi spinta a essere la versione migliore di me stessa. Grazie per le opportunità che mi avete dato fin da quando ero piccola, per avermi fatto studiare inglese, per avermi fatto conoscere il mondo. Non potrò mai ripagarvi. Grazie perché mi avete lasciata andare, per quanto difficile potesse essere per voi. Il vostro amore mi dà forza ogni giorno, il vostro esempio mi guida. Mi avete insegnato l'importanza del coraggio, della curiosità, dell'attenzione all'altro. Questa tesi la dedico a voi.

PUBLICATIONS

CURRICULUM VITAE

Name: Biancamaria Bonucci
Date of birth: May 15, 1996
Nationality: Italian
E-mail: biancamaria.bonucci@ut.ee

Education

2021–2026	Doctoral studies, Gene Technology Curriculum. Institute of Genomics, Faculty of Science and Technology, University of Tartu
2025–2025	Visiting scholar, microARCH Lab, Department of Anthropology, The Pennsylvania State University, University Park, PA (USA)
2018–2021	MSc, Genetics and Molecular Biology (cum laude), Sapienza University of Rome, Rome (Italy)
2015–2018	BSc, Biological Sciences (cum laude), Sapienza University of Rome, Rome (Italy)

Professional employment

2021–	Junior Research Fellow of Ancient DNA, Institute of Genomics, University of Tartu
2021–2021	Research Assistant, Sapienza University of Rome, Rome (Italy)
2019–2019	Paid intern at Sapienza Museum Network (Polo Museale), Sapienza University of Rome, Rome (Italy)
2017–2018	Paid intern at the Anthropology Museum, Sapienza University of Rome, Rome (Italy)

Teaching and tutoring activity:

2024	Tutor, University of Tartu. Archaeogenetics Workshop, OCSEAN Summer School.
2022–2023	Teaching assistant, University of Tartu, Bachelor in Science and Technology, Chair of Evolutionary Biology, Evolution and the Natural World course.
2022	Tutor, University of Tartu. Workshop “Building bridges between disciplines: ancient DNA & archaeology”

Supervised dissertations:

Sandor-Hannes Soosaar. Bachelor’s Degree, Institute of Molecular and Cell Biology, University of Tartu, Estonia, 2026, “Metagenomic analysis of medieval and early modern period dental calculus samples from Estonia”

International conference presentations and courses

2026 American Association of Biological Anthropologists (AABA) annual meeting. Denver, Colorado, USA. Conference talk: *Earliest evidence of non-flea-adapted* Yersinia pestis *in Southern Europe.*

2025 Svante Pääbo's masterclass for early-career researchers. Tartu, Estonia.

2025 International Society for Biomolecular Archaeology (ISBA) 11th Meeting. Turin, Italy. Poster: *Ancient DNA and protein preservation and transfer in concretions covering human remains.*

2024 Practical Palaeoproteomics Summer School. Organised by the University of Copenhagen, Copenhagen, Denmark.

2024 Hands-on workshop: Current developments in bioinformatic workflows applied to ancient environmental DNA data. Organised by the Australian Centre for Ancient DNA (ACAD), Adelaide, Australia.

2023 AHEAD conference. Tarragona, Spain. Conference talk: *Metagenomics analysis of ancient dental calculus provide an insight into oral microbial ecology in an Anglo-Saxon population.*

2023 International Society for Biomolecular Archaeology (ISBA) 10th Meeting. Tartu, Estonia. Poster: *Metagenomics analysis of ancient dental calculus provide an insight into oral microbial ecology in an Anglo-Saxon population.*

2023 Introduction to Ancient Metagenomics summer school. Organised by the SPAAM (Standards, Precautions & Advances in Ancient Metagenomics) Community, online.

2023 Introduction to Palaeogenomics, course held by Transmitting Science, online

2022 EMBO-EMBL Symposium – Reconstructing the Human past: using ancient and modern genomics. Heidelberg, Germany. Poster: *Concretion-covered tissues as potential sources of proteins and aDNA.*

Conference organization

2025 SPAAM (Standards, Precautions & Advances in Ancient Metagenomics) 7th Meeting. Turin, Italy.

2024 EAA Annual Meeting. Rome, Italy. *Session 854:"Building bridges: an open forum for archaeology and metagenomics"*.

2024 EAA Annual Meeting. Rome, Italy. *Session 834: "Women through the Ages: Roles, Rituals, and Research through the Lenses of Archaeological Sciences".*

2023 International Society for Biomolecular Archaeology (ISBA) 10th Meeting. Tartu, Estonia.

Awards

2026 CWT Estonia scholarship. University of Tartu, Estonia.

2025 Erasmus+ Traineeship Grant. University of Tartu, Estonia.

2024 Three Minutes Thesis Competition Award (First place, English competition). University of Tartu, Estonia.

2024 Outstanding Poster Award. Institute of Molecular and Cell Biology and Institute of Genomics Annual Conference, University of Tartu, Estonia.

2024 Erasmus+ Short Term Mobility Grant. University of Tartu, Estonia.

2021 Erasmus+ Traineeship Grant. Sapienza University of Rome, Italy.

Volunteer activity:

2026 Volunteer, American Association of Biological Anthropologists (AABA) Annual Meeting. Denver, CO, USA.

2025– Core Team Member, AncientMetagenomeDir. SPAAM community.

2023– Co-organiser, SPAAMtisch seminars. SPAAM Community.

2023–2025 PhD students representative in the council of the Institute of Genomics.

Publications

2026 de-Dios T., **Bonucci B.**, Thompson J. E., Panella S., Barbieri R., Keller M., Saupe T., Bernardini S., Sasso S., Thirolle M., Solnik A., Kabral H., Aranguren B., Sanz M., Daura J., Lalueza-Fox C., Kivisild T., Metspalu M., Guellil M., Tafuri M. A., Robb J., Scheib C. L. Co-occurrence of *Yersinia pestis* and other zoonoses during European prehistory. bioRxiv, [preprint], 2026.02.20.707102; doi: https://doi.org/10.64898/2026.02.20.707102.

2026 Hearne K., Spurīte D., Hübner A., **Bonucci B**., Huang Y., Bartholdy B. P., Rozwalak P., Becerra-Valdivia L., Borry M., Bozzi D., Brativnyk A., Brunt J., Dagtas N. D., de-Dios T., Ferguson C., Frangenberg J., Guinet B., Haller-Caskie M., Harder R., Jackson I., Jin C., Keller M., Kocher A., Light-Maka I., Lopopolo M., Lutz F., Michel M., Nunes Yamunaque D. J., Özdogan K., Pochon Z., Ramirez D. A., Schumacher M. L., Ponce-Soto G. Y., Psonis N., Richtermeier T., Schreiber L., Swali P., Trần C. N. H., Velsko I. M., Warinner C., White A. E., Zampirolo G., Fellows Yates J. A., Andrades Valtueña A. Ancientmetagenomedir dating metadataset highlights need for standardised radiocarbon reporting in ancient dna. bioRxiv, [preprint], 2026.02.06.704039; doi: https://doi.org/10.64898/2026.02.06.704039.

2026 Guellil M., van Dorp L., Saag L., Beneker O., **Bonucci B**., Sasso S., Saupe T., Solnik A., Kabral H., Allmäe R., Bates J., Dittmar J. M., Ge X. J., Inskip S., Jonuks T., Karmanov V. N., Khartanovich V. I., Larmuseau M.

H. D., Aneli S., Cessford C., Kriiska A., Mägi M., Malve M., De Winter N., Metspalu M., Pagani L., Robb J. E., Kivisild T., Houldcroft C. J., Scheib C. L., Tambets K. (2026). Tracing 2500 years of human betaherpesvirus 6A and 6B diversity through ancient DNA. Science Advances, 12(1), eadx5460. https://doi.org/10.1126/sciadv.adx5460

2025 Thompson, J. E., Recchia, G., Cazzella, A., Soncin, S., Panella, S., Farese, M., Saupe, T., de-Dios, T., **Bonucci, B**., Scheib, C. L., Tafuri, M. A., & Robb, J. (2025, published online 2 Dec 2025). Inserting the dead in living spaces in Bronze Age Southern Italy: the case of Coppa Nevigata. World Archaeology. https://doi.org/10.1080/00438243.2025.2594201

Bonucci, B., de-Dios, T., Barbieri, R., Thompson, J. E., Panella, S., Radina, F., Sivilli, S., Kabral, H., Solnik, A., Tafuri, M. A., Robb, J., & Scheib, C. L. (2025). Ancient microbial DNA and proteins preserve in concretions covering human remains. iScience, 28(8), 113182. https://doi.org/10.1016/j.isci.2025.113182

de-Dios, T., **Bonucci, B**., Barbieri, R., Kushniarevich, A., D'Atanasio, E., Dittmar, J., Cessford, C., Solnik, A., Robb, J. E., Warinner, C., Oras, E., & Scheib, C. L. (2025). Bone adhered sediments as a source of target and environmental DNA and proteins. Molecular Biology and Evolution, 42(9), msaf202. https://doi.org/10.1093/molbev/msaf202

Beneker, O., Molinaro, L., Guellil, M., Sasso, S., Kabral, H., **Bonucci, B.**, Gaens, N., D'Atanasio, E., Mezzavilla, M., Delbrassine, H., Braet, L., Lambert, B., Deckers, P., Biagini, S. A., Hui, R., Becelaere, S., Geypen, J., Hoebreckx, M., Berk, B., Driesen, P., Pijpelink, A., van Damme, P., Vanhoutte, S., De Winter, N., Saag, L., Pagani, L., Tambets, K., Scheib, C. L., Larmuseau, M. H. D., & Kivisild, T. (2025). Urbanization and genetic homogenization in the medieval Low Countries revealed through a ten-century paleogenomic study of the city of Sint-Truiden. Genome Biology, 26(1), 127. https://doi.org/10.1186/s13059-025-03580-z

2023 D'Atanasio, E., Risi, F., Ravasini, F., Montinaro, F., Hajiesmaeil, M., **Bonucci, B.**, Pistacchia, L., Amoako-Sakyi, D., Bonito, M., Onidi, S., Colombo, G., Semino, O., Destro Bisol, G., Anagnostou, P., Metspalu, M., Tambets, K., Trombetta, B., & Cruciani, F. (2023). The genomic echoes of the last Green Sahara on the Fulani and Sahelian people. Current Biology, 33(24), 5495–5504.e4. https://doi.org/10.1016/j.cub.2023.10.075

Keller, M., Scheib, C. L., & **Bonucci, B.** (2023). Indexing PCR and purification of dsDNA libraries. protocols.io. https://dx.doi.org/10.17504/protocols.io.rm7vzboy4vx1/v1

Keller, M., Scheib, C. L., & **Bonucci, B.** (2023). Library preparation (dsDNA double indexing, non-UDG, 2× split). protocols.io. https://dx.doi.org/10.17504/protocols.io.n2bvj6xqxlk5/v1

2021 Ravasini, F., D'Atanasio, E., Bonito, M., **Bonucci, B**., Della Rocca, C., Berti, A., Trombetta, B., & Cruciani, F. (2021). Sequence read depth analysis of a monophyletic cluster of Y chromosomes characterized by structural rearrangements in the AZFc region resulting in DYS448 deletion and DYF387S1 duplication. Frontiers in Genetics, 12, 669405. https://doi.org/10.3389/fgene.2021.669405

Languages: Italian – native; English – fluent; Spanish – Elementary

Hobbies: Hiking, running, climbing and anything outdoor; being part of a Scout Association; spending time with friends (outdoor, indoor, at parties and festivals); organising events (more and less serious ones).

ELULOOKIRJELDUS

Nimi: Biancamaria Bonucci
Sünniaeg: 15. mai 1996
Rahvus: itaallane
E-post: biancamaria.bonucci@ut.ee

Haridustee

2021–2026 Doktoriõpingud, geenitehnoloogia õppekava. Genoomika instituut, loodus- ja täppisteaduste valdkond, Tartu Ülikool

2025–2025 Külalisteadur, microARCH labor, antropoloogia osakond, Pennsylvania osariigi ülikool, University Park, PA (USA)

2018–2021 Magistriõpe, geneetika ja molekulaarbioloogia (cum laude), Rooma Sapienza Ülikool, Rooma (Itaalia)

2015–2018 Bakalaureuseõpe, bioloogiateadused (cum laude), Rooma Sapienza Ülikool, Rooma (Itaalia)

Erialane tööhõive

2021– Vana DNA nooremteadur, genoomika instituut, Tartu Ülikool

2021–2021 Teadusassistent, Rooma Sapienza Ülikool, Rooma (Itaalia)

2019–2019 Tasustatud praktikant Sapienza muuseumivõrgustikus (Polo Museale), Rooma Sapienza Ülikool, Rooma (Itaalia)

2017–2018 Tasustatud praktikant antropoloogiamuuseumis, Rooma Sapienza Ülikool, Rooma (Itaalia)

Õpetamis- ja juhendamistegevus:

2024 Tuutor, Tartu Ülikool. Arheogeneetika töötuba, OCSEAN-i suvekool.

2022–2023 Õppeassistent, Tartu Ülikool, loodus- ja täppisteaduste bakalaureuseõpe, evolutsioonibioloogia õppetool, kursus „Evolutsioon ja elusloodus“.

2022 Tuutor, Tartu Ülikool. Töötuba „Sildade loomine erialade vahel: vana DNA ja arheoloogia“.

Juhendatud lõputööd:

Sandor-Hannes Soosaar. Bakalaureusetöö, molekulaar- ja rakubioloogia instituut, Tartu Ülikool, Eesti, 2026, „Keskaegsete ja varauusaegsete Eesti hambakivi proovide metagenoomiline analüüs“.

Rahvusvahelised konverentsiettekanded ja kursused

2026 Ameerika Bioloogiliste Antropoloogide Assotsiatsiooni (AABA) aastakoosolek. Denver, Colorado, USA. Konverentsiettekanne: varaseim tõend kirpudega kohastumata *Yersinia pestis*'e kohta Lõuna-Euroopas.

2025 Svante Pääbo meistriklass karjääri alguses olevatele teadlastele. Tartu, Eesti.

2025 Rahvusvahelise Biomolekulaarse Arheoloogia Seltsi (ISBA) 11. kohtumine. Torino, Itaalia. Postriettekanne: vana DNA ja valkude säilimine ning ülekandumine inimjäänuseid katvates konkretsioonides.

2024 Praktilise paleoproteoomika suvekool. Korraldaja: Kopenhaageni Ülikool, Kopenhaagen, Taani.

2024 Praktiline töötuba: praegused arengud vanale keskkonna-DNA andmestikule rakendatavates bioinformaatilistes töövoogudes. Korraldaja: Austraalia Vana DNA Keskus (ACAD), Adelaide, Austraalia.

2023 AHEAD-i konverents. Tarragona, Hispaania. Konverentsiettekanne: vana hambakivi metagenoomiline analüüs annab ülevaate anglosaksi populatsiooni suuõõne mikroobsest ökoloogiast.

2023 Rahvusvahelise Biomolekulaarse Arheoloogia Seltsi (ISBA) 10. kohtumine. Tartu, Eesti. Postriettekanne: vana hambakivi metagenoomiline analüüs annab ülevaate anglosaksi populatsiooni suuõõne mikroobsest ökoloogiast.

2023 Vana metagenoomika sissejuhatav suvekool. Korraldaja: SPAAM-i kogukond (Standardid, ettevaatusabinõud ja edusammud vanas metagenoomikas), veebis.

2023 Sissejuhatus paleogenoomikasse, kursus korraldajalt Transmitting Science, veebis.

2022 EMBO-EMBL-i sümpoosion – inimese mineviku rekonstrueerimine vana ja tänapäevase genoomika abil. Heidelberg, Saksamaa. Postriettekanne: konkretsioonidega kaetud koed kui potentsiaalsed valkude ja vana DNA allikad.

Konverentside korraldamine

2025 SPAAM-i 7. kohtumine (Standardid, ettevaatusabinõud ja edusammud vanas metagenoomikas). Torino, Itaalia.

2024 Euroopa Arheoloogide Assotsiatsiooni (EAA) aastakoosolek. Rooma, Itaalia. Sessioon 854: „Sildade loomine: arheoloogia ja metagenoomika avatud foorum".

2024 Euroopa Arheoloogide Assotsiatsiooni (EAA) aastakoosolek. Rooma, Itaalia. Sessioon 834: „Naised läbi aegade: rollid, rituaalid ja teadusuuringud arheoloogiateaduste vaatenurgast".

2023 Rahvusvahelise Biomolekulaarse Arheoloogia Seltsi (ISBA) 10. kohtumine. Tartu, Eesti.

Auhinnad

2026 CWT Estonia stipendium. Tartu Ülikool, Eesti.

2025 Erasmus+ praktika stipendium. Tartu Ülikool, Eesti.

2024 Kolme minuti doktoritöö konkursi auhind (esimene koht ingliskeelses konkursis). Tartu Ülikool, Eesti.

2024 Parima postri auhind. Molekulaar- ja rakubioloogia instituudi ning genoomika instituudi aastakonverents, Tartu Ülikool, Eesti.

2024 Erasmus+ lühiajalise õpirände stipendium. Tartu Ülikool, Eesti.

2021 Erasmus+ praktika stipendium. Rooma Sapienza Ülikool, Itaalia.

Vabatahtlik tegevus:

2026 Vabatahtlik, Ameerika Bioloogiliste Antropoloogide Assotsiatsiooni (AABA) aastakoosolek. Denver, CO, USA.

2025– AncientMetagenomeDiri tuumikmeeskonna liige. SPAAM-i kogukond.

2023– SPAAMtischi seminaride kaaskorraldaja. SPAAM-i kogukond.

2023–2025 Doktorantide esindaja genoomika instituudi nõukogus.

Publikatsioonid: Loetletud inglisekeelse CV rubriigis publikatsioonid (‘Publications’)

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

1. **Toivo Maimets**. Studies of human oncoprotein p53. Tartu, 1991, 96 p.
2. **Enn K. Seppet**. Thyroid state control over energy metabolism, ion transport and contractile functions in rat heart. Tartu, 1991, 135 p.
3. **Kristjan Zobel**. Epifüütsete makrosamblike väärtus õhu saastuse indikaatoritena Hamar-Dobani boreaalsetes mägimetsades. Tartu, 1992, 131 lk.
4. **Andres Mäe**. Conjugal mobilization of catabolic plasmids by transposable elements in helper plasmids. Tartu, 1992, 91 p.
5. **Maia Kivisaar**. Studies on phenol degradation genes of *Pseudomonas* sp. strain EST 1001. Tartu, 1992, 61 p.
6. **Allan Nurk**. Nucleotide sequences of phenol degradative genes from *Pseudomonas sp.* strain EST 1001 and their transcriptional activation in *Pseudomonas putida.* Tartu, 1992, 72 p.
7. **Ülo Tamm**. The genus *Populus* L. in Estonia: variation of the species biology and introduction. Tartu, 1993, 91 p.
8. **Jaanus Remme**. Studies on the peptidyltransferase centre of the *E.coli* ribosome. Tartu, 1993, 68 p.
9. **Ülo Langel**. Galanin and galanin antagonists. Tartu, 1993, 97 p.
10. **Arvo Käärd**. The development of an automatic online dynamic fluorescense-based pH-dependent fiber optic penicillin flowthrought biosensor for the control of the benzylpenicillin hydrolysis. Tartu, 1993, 117 p.
11. **Lilian Järvekülg**. Antigenic analysis and development of sensitive immunoassay for potato viruses. Tartu, 1993, 147 p.
12. **Jaak Palumets**. Analysis of phytomass partition in Norway spruce. Tartu, 1993, 47 p.
13. **Arne Sellin**. Variation in hydraulic architecture of *Picea abies* (L.) Karst. trees grown under different enviromental conditions. Tartu, 1994, 119 p.
13. **Mati Reeben**. Regulation of light neurofilament gene expression. Tartu, 1994, 108 p.
14. **Urmas Tartes**. Respiration rhytms in insects. Tartu, 1995, 109 p.
15. **Ülo Puurand**. The complete nucleotide sequence and infections *in vitro* transcripts from cloned cDNA of a potato A potyvirus. Tartu, 1995, 96 p.
16. **Peeter Hõrak**. Pathways of selection in avian reproduction: a functional framework and its application in the population study of the great tit (*Parus major*). Tartu, 1995, 118 p.
17. **Erkki Truve**. Studies on specific and broad spectrum virus resistance in transgenic plants. Tartu, 1996, 158 p.
18. **Illar Pata**. Cloning and characterization of human and mouse ribosomal protein S6-encoding genes. Tartu, 1996, 60 p.
19. **Ülo Niinemets**. Importance of structural features of leaves and canopy in determining species shade-tolerance in temperature deciduous woody taxa. Tartu, 1996, 150 p.

20. **Ants Kurg**. Bovine leukemia virus: molecular studies on the packaging region and DNA diagnostics in cattle. Tartu, 1996, 104 p.
21. **Ene Ustav**. E2 as the modulator of the BPV1 DNA replication. Tartu, 1996, 100 p.
22. **Aksel Soosaar**. Role of helix-loop-helix and nuclear hormone receptor transcription factors in neurogenesis. Tartu, 1996, 109 p.
23. **Maido Remm**. Human papillomavirus type 18: replication, transformation and gene expression. Tartu, 1997, 117 p.
24. **Tiiu Kull**. Population dynamics in *Cypripedium calceolus* L. Tartu, 1997, 124 p.
25. **Kalle Olli**. Evolutionary life-strategies of autotrophic planktonic microorganisms in the Baltic Sea. Tartu, 1997, 180 p.
26. **Meelis Pärtel**. Species diversity and community dynamics in calcareous grassland communities in Western Estonia. Tartu, 1997, 124 p.
27. **Malle Leht**. The Genus *Potentilla* L. in Estonia, Latvia and Lithuania: distribution, morphology and taxonomy. Tartu, 1997, 186 p.
28. **Tanel Tenson**. Ribosomes, peptides and antibiotic resistance. Tartu, 1997, 80 p.
29. **Arvo Tuvikene**. Assessment of inland water pollution using biomarker responses in fish *in vivo* and *in vitro.* Tartu, 1997, 160 p.
30. **Urmas Saarma**. Tuning ribosomal elongation cycle by mutagenesis of 23S rRNA. Tartu, 1997, 134 p.
31. **Henn Ojaveer**. Composition and dynamics of fish stocks in the gulf of Riga ecosystem. Tartu, 1997, 138 p.
32. **Lembi Lõugas**. Post-glacial development of vertebrate fauna in Estonian water bodies. Tartu, 1997, 138 p.
33. **Margus Pooga**. Cell penetrating peptide, transportan, and its predecessors, galanin-based chimeric peptides. Tartu, 1998, 110 p.
34. **Andres Saag**. Evolutionary relationships in some cetrarioid genera (Lichenized Ascomycota). Tartu, 1998, 196 p.
35. **Aivar Liiv**. Ribosomal large subunit assembly *in vivo*. Tartu, 1998, 158 p.
36. **Tatjana Oja**. Isoenzyme diversity and phylogenetic affinities among the eurasian annual bromes (*Bromus* L., Poaceae). Tartu, 1998, 92 p.
37. **Mari Moora**. The influence of arbuscular mycorrhizal (AM) symbiosis on the competition and coexistence of calcareous grassland plant species. Tartu, 1998, 78 p.
38. **Olavi Kurina**. Fungus gnats in Estonia (*Diptera: Bolitophilidae, Keroplatidae, Macroceridae, Ditomyiidae, Diadocidiidae, Mycetophilidae*). Tartu, 1998, 200 p.
39. **Andrus Tasa**. Biological leaching of shales: black shale and oil shale. Tartu, 1998, 98 p.
40. **Arnold Kristjuhan**. Studies on transcriptional activator properties of tumor suppressor protein p53. Tartu, 1998, 86 p.
41. **Sulev Ingerpuu**. Characterization of some human myeloid cell surface and nuclear differentiation antigens. Tartu, 1998, 163 p.

42. **Veljo Kisand**. Responses of planktonic bacteria to the abiotic and biotic factors in the shallow lake Võrtsjärv. Tartu, 1998, 118 p.
43. **Kadri Põldmaa**. Studies in the systematics of hypomyces and allied genera (Hypocreales, Ascomycota). Tartu, 1998, 178 p.
44. **Markus Vetemaa**. Reproduction parameters of fish as indicators in environmental monitoring. Tartu, 1998, 117 p.
45. **Heli Talvik**. Prepatent periods and species composition of different *Oesophagostomum* spp. populations in Estonia and Denmark. Tartu, 1998, 104 p.
46. **Katrin Heinsoo**. Cuticular and stomatal antechamber conductance to water vapour diffusion in *Picea abies* (L.) karst. Tartu, 1999, 133 p.
47. **Tarmo Annilo**. Studies on mammalian ribosomal protein S7. Tartu, 1998, 77 p.
48. **Indrek Ots**. Health state indicies of reproducing great tits (*Parus major*): sources of variation and connections with life-history traits. Tartu, 1999, 117 p.
49. **Juan Jose Cantero**. Plant community diversity and habitat relationships in central Argentina grasslands. Tartu, 1999, 161 p.
50. **Rein Kalamees**. Seed bank, seed rain and community regeneration in Estonian calcareous grasslands. Tartu, 1999, 107 p.
51. **Sulev Kõks**. Cholecystokinin (CCK) – induced anxiety in rats: influence of environmental stimuli and involvement of endopioid mechanisms and serotonin. Tartu, 1999, 123 p.
52. **Ebe Sild**. Impact of increasing concentrations of O_3 and CO_2 on wheat, clover and pasture. Tartu, 1999, 123 p.
53. **Ljudmilla Timofejeva**. Electron microscopical analysis of the synaptonemal complex formation in cereals. Tartu, 1999, 99 p.
54. **Andres Valkna**. Interactions of galanin receptor with ligands and G-proteins: studies with synthetic peptides. Tartu, 1999, 103 p.
55. **Taavi Virro**. Life cycles of planktonic rotifers in lake Peipsi. Tartu, 1999, 101 p.
56. **Ana Rebane**. Mammalian ribosomal protein S3a genes and intron-encoded small nucleolar RNAs U73 and U82. Tartu, 1999, 85 p.
57. **Tiina Tamm**. Cocksfoot mottle virus: the genome organisation and translational strategies. Tartu, 2000, 101 p.
58. **Reet Kurg**. Structure-function relationship of the bovine papilloma virus E2 protein. Tartu, 2000, 89 p.
59. **Toomas Kivisild**. The origins of Southern and Western Eurasian populations: an mtDNA study. Tartu, 2000, 121 p.
60. **Niilo Kaldalu**. Studies of the TOL plasmid transcription factor XylS. Tartu, 2000, 88 p.
61. **Dina Lepik**. Modulation of viral DNA replication by tumor suppressor protein p53. Tartu, 2000, 106 p.
62. **Kai Vellak**. Influence of different factors on the diversity of the bryophyte vegetation in forest and wooded meadow communities. Tartu, 2000, 122 p.

63. **Jonne Kotta**. Impact of eutrophication and biological invasionas on the structure and functions of benthic macrofauna. Tartu, 2000, 160 p.
64. **Georg Martin**. Phytobenthic communities of the Gulf of Riga and the inner sea the West-Estonian archipelago. Tartu, 2000, 139 p.
65. **Silvia Sepp**. Morphological and genetical variation of *Alchemilla L.* in Estonia. Tartu, 2000. 124 p.
66. **Jaan Liira**. On the determinants of structure and diversity in herbaceous plant communities. Tartu, 2000, 96 p.
67. **Priit Zingel**. The role of planktonic ciliates in lake ecosystems. Tartu, 2001, 111 p.
68. **Tiit Teder**. Direct and indirect effects in Host-parasitoid interactions: ecological and evolutionary consequences. Tartu, 2001, 122 p.
69. **Hannes Kollist**. Leaf apoplastic ascorbate as ozone scavenger and its transport across the plasma membrane. Tartu, 2001, 80 p.
70. **Reet Marits**. Role of two-component regulator system PehR-PehS and extracellular protease PrtW in virulence of *Erwinia Carotovora* subsp. *Carotovora*. Tartu, 2001, 112 p.
71. **Vallo Tilgar**. Effect of calcium supplementation on reproductive performance of the pied flycatcher *Ficedula hypoleuca* and the great tit *Parus major,* breeding in Nothern temperate forests. Tartu, 2002, 126 p.
72. **Rita Hõrak**. Regulation of transposition of transposon Tn*4652* in *Pseudomonas putida*. Tartu, 2002, 108 p.
73. **Liina Eek-Piirsoo**. The effect of fertilization, mowing and additional illumination on the structure of a species-rich grassland community. Tartu, 2002, 74 p.
74. **Krõõt Aasamaa**. Shoot hydraulic conductance and stomatal conductance of six temperate deciduous tree species. Tartu, 2002, 110 p.
75. **Nele Ingerpuu**. Bryophyte diversity and vascular plants. Tartu, 2002, 112 p.
76. **Neeme Tõnisson**. Mutation detection by primer extension on oligonucleotide microarrays. Tartu, 2002, 124 p.
77. **Margus Pensa**. Variation in needle retention of Scots pine in relation to leaf morphology, nitrogen conservation and tree age. Tartu, 2003, 110 p.
78. **Asko Lõhmus**. Habitat preferences and quality for birds of prey: from principles to applications. Tartu, 2003, 168 p.
79. **Viljar Jaks**. p53 – a switch in cellular circuit. Tartu, 2003, 160 p.
80. **Jaana Männik**. Characterization and genetic studies of four ATP-binding cassette (ABC) transporters. Tartu, 2003, 140 p.
81. **Marek Sammul**. Competition and coexistence of clonal plants in relation to productivity. Tartu, 2003, 159 p
82. **Ivar Ilves**. Virus-cell interactions in the replication cycle of bovine papillomavirus type 1. Tartu, 2003, 89 p.
83. **Andres Männik**. Design and characterization of a novel vector system based on the stable replicator of bovine papillomavirus type 1. Tartu, 2003, 109 p.

84. **Ivika Ostonen**. Fine root structure, dynamics and proportion in net primary production of Norway spruce forest ecosystem in relation to site conditions. Tartu, 2003, 158 p.
85. **Gudrun Veldre**. Somatic status of 12–15-year-old Tartu schoolchildren. Tartu, 2003, 199 p.
86. **Ülo Väli.** The greater spotted eagle *Aquila clanga* and the lesser spotted eagle *A. pomarina*: taxonomy, phylogeography and ecology. Tartu, 2004, 159 p.
87. **Aare Abroi**. The determinants for the native activities of the bovine papillomavirus type 1 E2 protein are separable. Tartu, 2004, 135 p.
88. **Tiina Kahre**. Cystic fibrosis in Estonia. Tartu, 2004, 116 p.
89. **Helen Orav-Kotta**. Habitat choice and feeding activity of benthic suspension feeders and mesograzers in the northern Baltic Sea. Tartu, 2004, 117 p.
90. **Maarja Öpik**. Diversity of arbuscular mycorrhizal fungi in the roots of perennial plants and their effect on plant performance. Tartu, 2004, 175 p.
91. **Kadri Tali**. Species structure of *Neotinea ustulata*. Tartu, 2004, 109 p.
92. **Kristiina Tambets**. Towards the understanding of post-glacial spread of human mitochondrial DNA haplogroups in Europe and beyond: a phylogeographic approach. Tartu, 2004, 163 p.
93. **Arvi Jõers**. Regulation of p53-dependent transcription. Tartu, 2004, 103 p.
94. **Lilian Kadaja**. Studies on modulation of the activity of tumor suppressor protein p53. Tartu, 2004, 103 p.
95. **Jaak Truu**. Oil shale industry wastewater: impact on river microbial community and possibilities for bioremediation. Tartu, 2004, 128 p.
96. **Maire Peters**. Natural horizontal transfer of the *pheBA* operon. Tartu, 2004, 105 p.
97. **Ülo Maiväli**. Studies on the structure-function relationship of the bacterial ribosome. Tartu, 2004, 130 p.
98. **Merit Otsus**. Plant community regeneration and species diversity in dry calcareous grasslands. Tartu, 2004, 103 p.
99. **Mikk Heidemaa**. Systematic studies on sawflies of the genera *Dolerus, Empria,* and *Caliroa* (Hymenoptera: Tenthredinidae). Tartu, 2004, 167 p.
100. **Ilmar Tõnno**. The impact of nitrogen and phosphorus concentration and N/P ratio on cyanobacterial dominance and N_2 fixation in some Estonian lakes. Tartu, 2004, 111 p.
101. **Lauri Saks**. Immune function, parasites, and carotenoid-based ornaments in greenfinches. Tartu, 2004, 144 p.
102. **Siiri Rootsi**. Human Y-chromosomal variation in European populations. Tartu, 2004, 142 p.
103. **Eve Vedler**. Structure of the 2,4-dichloro-phenoxyacetic acid-degradative plasmid pEST4011. Tartu, 2005. 106 p.
104. **Andres Tover**. Regulation of transcription of the phenol degradation *pheBA* operon in *Pseudomonas putida*. Tartu, 2005, 126 p.
105. **Helen Udras**. Hexose kinases and glucose transport in the yeast *Hansenula polymorpha*. Tartu, 2005, 100 p.

106. **Ave Suija**. Lichens and lichenicolous fungi in Estonia: diversity, distribution patterns, taxonomy. Tartu, 2005, 162 p.
107. **Piret Lõhmus**. Forest lichens and their substrata in Estonia. Tartu, 2005, 162 p.
108. **Inga Lips**. Abiotic factors controlling the cyanobacterial bloom occurrence in the Gulf of Finland. Tartu, 2005, 156 p.
109. **Krista Kaasik**. Circadian clock genes in mammalian clockwork, metabolism and behaviour. Tartu, 2005, 121 p.
110. **Juhan Javoiš**. The effects of experience on host acceptance in ovipositing moths. Tartu, 2005, 112 p.
111. **Tiina Sedman**. Characterization of the yeast *Saccharomyces cerevisiae* mitochondrial DNA helicase Hmi1. Tartu, 2005, 103 p.
112. **Ruth Aguraiuja**. Hawaiian endemic fern lineage *Diellia* (Aspleniaceae): distribution, population structure and ecology. Tartu, 2005, 112 p.
113. **Riho Teras**. Regulation of transcription from the fusion promoters generated by transposition of Tn*4652* into the upstream region of *pheBA* operon in *Pseudomonas putida*. Tartu, 2005, 106 p.
114. **Mait Metspalu**. Through the course of prehistory in India: tracing the mtDNA trail. Tartu, 2005, 138 p.
115. **Elin Lõhmussaar**. The comparative patterns of linkage disequilibrium in European populations and its implication for genetic association studies. Tartu, 2006, 124 p.
116. **Priit Kupper**. Hydraulic and environmental limitations to leaf water relations in trees with respect to canopy position. Tartu, 2006, 126 p.
117. **Heili Ilves**. Stress-induced transposition of Tn*4652* in *Pseudomonas Putida.* Tartu, 2006, 120 p.
118. **Silja Kuusk**. Biochemical properties of Hmi1p, a DNA helicase from *Saccharomyces cerevisiae* mitochondria. Tartu, 2006, 126 p.
119. **Kersti Püssa**. Forest edges on medium resolution landsat thematic mapper satellite images. Tartu, 2006, 90 p.
120. **Lea Tummeleht**. Physiological condition and immune function in great tits (*Parus major* l.): Sources of variation and trade-offs in relation to growth. Tartu, 2006, 94 p.
121. **Toomas Esperk**. Larval instar as a key element of insect growth schedules. Tartu, 2006, 186 p.
122. **Harri Valdmann**. Lynx (*Lynx lynx*) and wolf (*Canis lupus*) in the Baltic region: Diets, helminth parasites and genetic variation. Tartu, 2006. 102 p.
123. **Priit Jõers**. Studies of the mitochondrial helicase Hmi1p in *Candida albicans* and *Saccharomyces cerevisia*. Tartu, 2006. 113 p.
124. **Kersti Lilleväli**. Gata3 and Gata2 in inner ear development. Tartu, 2007, 123 p.
125. **Kai Rünk**. Comparative ecology of three fern species: *Dryopteris carthusiana* (Vill.) H.P. Fuchs, *D. expansa* (C. Presl) Fraser-Jenkins & Jermy and *D. dilatata* (Hoffm.) A. Gray (Dryopteridaceae). Tartu, 2007, 143 p.

126. **Aveliina Helm**. Formation and persistence of dry grassland diversity: role of human history and landscape structure. Tartu, 2007, 89 p.
127. **Leho Tedersoo**. Ectomycorrhizal fungi: diversity and community structure in Estonia, Seychelles and Australia. Tartu, 2007, 233 p.
128. **Marko Mägi**. The habitat-related variation of reproductive performance of great tits in a deciduous-coniferous forest mosaic: looking for causes and consequences. Tartu, 2007, 135 p.
129. **Valeria Lulla**. Replication strategies and applications of Semliki Forest virus. Tartu, 2007, 109 p.
130. **Ülle Reier**. Estonian threatened vascular plant species: causes of rarity and conservation. Tartu, 2007, 79 p.
131. **Inga Jüriado**. Diversity of lichen species in Estonia: influence of regional and local factors. Tartu, 2007, 171 p.
132. **Tatjana Krama**. Mobbing behaviour in birds: costs and reciprocity based cooperation. Tartu, 2007, 112 p.
133. **Signe Saumaa**. The role of DNA mismatch repair and oxidative DNA damage defense systems in avoidance of stationary phase mutations in *Pseudomonas putida.* Tartu, 2007, 172 p.
134. **Reedik Mägi**. The linkage disequilibrium and the selection of genetic markers for association studies in european populations. Tartu, 2007, 96 p.
135. **Priit Kilgas**. Blood parameters as indicators of physiological condition and skeletal development in great tits (*Parus major*): natural variation and application in the reproductive ecology of birds. Tartu, 2007, 129 p.
136. **Anu Albert**. The role of water salinity in structuring eastern Baltic coastal fish communities. Tartu, 2007, 95 p.
137. **Kärt Padari**. Protein transduction mechanisms of transportans. Tartu, 2008, 128 p.
138. **Siiri-Lii Sandre**. Selective forces on larval colouration in a moth. Tartu, 2008, 125 p.
139. **Ülle Jõgar**. Conservation and restoration of semi-natural floodplain meadows and their rare plant species. Tartu, 2008, 99 p.
140. **Lauri Laanisto**. Macroecological approach in vegetation science: generality of ecological relationships at the global scale. Tartu, 2008, 133 p.
141. **Reidar Andreson**. Methods and software for predicting PCR failure rate in large genomes. Tartu, 2008, 105 p.
142. **Birgot Paavel**. Bio-optical properties of turbid lakes. Tartu, 2008, 175 p.
143. **Kaire Torn**. Distribution and ecology of charophytes in the Baltic Sea. Tartu, 2008, 98 p.
144. **Vladimir Vimberg**. Peptide mediated macrolide resistance. Tartu, 2008, 190 p.
145. **Daima Örd**. Studies on the stress-inducible pseudokinase TRB3, a novel inhibitor of transcription factor ATF4. Tartu, 2008, 108 p.
146. **Lauri Saag**. Taxonomic and ecologic problems in the genus *Lepraria* (*Stereocaulaceae*, lichenised *Ascomycota*). Tartu, 2008, 175 p.

147. **Ulvi Karu**. Antioxidant protection, carotenoids and coccidians in greenfinches – assessment of the costs of immune activation and mechanisms of parasite resistance in a passerine with carotenoid-based ornaments. Tartu, 2008, 124 p.
148. **Jaanus Remm**. Tree-cavities in forests: density, characteristics and occupancy by animals. Tartu, 2008, 128 p.
149. **Epp Moks**. Tapeworm parasites *Echinococcus multilocularis* and *E. granulosus* in Estonia: phylogenetic relationships and occurrence in wild carnivores and ungulates. Tartu, 2008, 82 p.
150. **Eve Eensalu**. Acclimation of stomatal structure and function in tree canopy: effect of light and CO_2 concentration. Tartu, 2008, 108 p.
151. **Janne Pullat**. Design, functionlization and application of an *in situ* synthesized oligonucleotide microarray. Tartu, 2008, 108 p.
152. **Marta Putrinš**. Responses of *Pseudomonas putida* to phenol-induced metabolic and stress signals. Tartu, 2008, 142 p.
153. **Marina Semtšenko**. Plant root behaviour: responses to neighbours and physical obstructions. Tartu, 2008, 106 p.
154. **Marge Starast**. Influence of cultivation techniques on productivity and fruit quality of some *Vaccinium* and *Rubus* taxa. Tartu, 2008, 154 p.
155. **Age Tats**. Sequence motifs influencing the efficiency of translation. Tartu, 2009, 104 p.
156. **Radi Tegova**. The role of specialized DNA polymerases in mutagenesis in *Pseudomonas putida.* Tartu, 2009, 124 p.
157. **Tsipe Aavik**. Plant species richness, composition and functional trait pattern in agricultural landscapes – the role of land use intensity and landscape structure. Tartu, 2009, 112 p.
158. **Kaja Kiiver**. Semliki forest virus based vectors and cell lines for studying the replication and interactions of alphaviruses and hepaciviruses. Tartu, 2009, 104 p.
159. **Meelis Kadaja**. Papillomavirus Replication Machinery Induces Genomic Instability in its Host Cell. Tartu, 2009, 126 p.
160. **Pille Hallast**. Human and chimpanzee Luteinizing hormone/Chorionic Gonadotropin beta (*LHB/CGB*) gene clusters: diversity and divergence of young duplicated genes. Tartu, 2009, 168 p.
161. **Ain Vellak**. Spatial and temporal aspects of plant species conservation. Tartu, 2009, 86 p.
162. **Triinu Remmel**. Body size evolution in insects with different colouration strategies: the role of predation risk. Tartu, 2009, 168 p.
163. **Jaana Salujõe**. Zooplankton as the indicator of ecological quality and fish predation in lake ecosystems. Tartu, 2009, 129 p.
164. **Ele Vahtmäe**. Mapping benthic habitat with remote sensing in optically complex coastal environments. Tartu, 2009, 109 p.
165. **Liisa Metsamaa**. Model-based assessment to improve the use of remote sensing in recognition and quantitative mapping of cyanobacteria. Tartu, 2009, 114 p.

166. **Pille Säälik**. The role of endocytosis in the protein transduction by cell-penetrating peptides. Tartu, 2009, 155 p.
167. **Lauri Peil**. Ribosome assembly factors in *Escherichia coli.* Tartu, 2009, 147 p.
168. **Lea Hallik**. Generality and specificity in light harvesting, carbon gain capacity and shade tolerance among plant functional groups. Tartu, 2009, 99 p.
169. **Mariliis Tark**. Mutagenic potential of DNA damage repair and tolerance mechanisms under starvation stress. Tartu, 2009, 191 p.
170. **Riinu Rannap**. Impacts of habitat loss and restoration on amphibian populations. Tartu, 2009, 117 p.
171. **Maarja Adojaan**. Molecular variation of HIV-1 and the use of this knowledge in vaccine development. Tartu, 2009, 95 p.
172. **Signe Altmäe**. Genomics and transcriptomics of human induced ovarian folliculogenesis. Tartu, 2010, 179 p.
173. **Triin Suvi**. Mycorrhizal fungi of native and introduced trees in the Seychelles Islands. Tartu, 2010, 107 p.
174. **Velda Lauringson**. Role of suspension feeding in a brackish-water coastal sea. Tartu, 2010, 123 p.
175. **Eero Talts**. Photosynthetic cyclic electron transport – measurement and variably proton-coupled mechanism. Tartu, 2010, 121 p.
176. **Mari Nelis**. Genetic structure of the Estonian population and genetic distance from other populations of European descent. Tartu, 2010, 97 p.
177. **Kaarel Krjutškov**. Arrayed Primer Extension-2 as a multiplex PCR-based method for nucleic acid variation analysis: method and applications. Tartu, 2010, 129 p.
178. **Egle Köster**. Morphological and genetical variation within species complexes: *Anthyllis vulneraria* s. l. and *Alchemilla vulgaris* (coll.). Tartu, 2010, 101 p.
179. **Erki Õunap**. Systematic studies on the subfamily Sterrhinae (Lepidoptera: Geometridae). Tartu, 2010, 111 p.
180. **Merike Jõesaar**. Diversity of key catabolic genes at degradation of phenol and *p*-cresol in pseudomonads. Tartu, 2010, 125 p.
181. **Kristjan Herkül**. Effects of physical disturbance and habitat-modifying species on sediment properties and benthic communities in the northern Baltic Sea. Tartu, 2010, 123 p.
182. **Arto Pulk**. Studies on bacterial ribosomes by chemical modification approaches. Tartu, 2010, 161 p.
183. **Maria Põllupüü**. Ecological relations of cladocerans in a brackish-water ecosystem. Tartu, 2010, 126 p.
184. **Toomas Silla**. Study of the segregation mechanism of the Bovine Papillomavirus Type 1. Tartu, 2010, 188 p.
185. **Gyaneshwer Chaubey**. The demographic history of India: A perspective based on genetic evidence. Tartu, 2010, 184 p.

186. **Katrin Kepp**. Genes involved in cardiovascular traits: detection of genetic variation in Estonian and Czech populations. Tartu, 2010, 164 p.
187. **Virve Sõber**. The role of biotic interactions in plant reproductive performance. Tartu, 2010, 92 p.
188. **Kersti Kangro**. The response of phytoplankton community to the changes in nutrient loading. Tartu, 2010, 144 p.
189. **Joachim M. Gerhold**. Replication and Recombination of mitochondrial DNA in Yeast. Tartu, 2010, 120 p.
190. **Helen Tammert**. Ecological role of physiological and phylogenetic diversity in aquatic bacterial communities. Tartu, 2010, 140 p.
191. **Elle Rajandu**. Factors determining plant and lichen species diversity and composition in Estonian *Calamagrostis* and *Hepatica* site type forests. Tartu, 2010, 123 p.
192. **Paula Ann Kivistik**. ColR-ColS signalling system and transposition of Tn*4652* in the adaptation of *Pseudomonas putida.* Tartu, 2010, 118 p.
193. **Siim Sõber**. Blood pressure genetics: from candidate genes to genome-wide association studies. Tartu, 2011, 120 p.
194. **Kalle Kipper**. Studies on the role of helix 69 of 23S rRNA in the factor-dependent stages of translation initiation, elongation, and termination. Tartu, 2011, 178 p.
195. **Triinu Siibak**. Effect of antibiotics on ribosome assembly is indirect. Tartu, 2011, 134 p.
196. **Tambet Tõnissoo**. Identification and molecular analysis of the role of guanine nucleotide exchange factor RIC-8 in mouse development and neural function. Tartu, 2011, 110 p.
197. **Helin Räägel**. Multiple faces of cell-penetrating peptides – their intracellular trafficking, stability and endosomal escape during protein transduction. Tartu, 2011, 161 p.
198. **Andres Jaanus**. Phytoplankton in Estonian coastal waters – variability, trends and response to environmental pressures. Tartu, 2011, 157 p.
199. **Tiit Nikopensius**. Genetic predisposition to nonsyndromic orofacial clefts. Tartu, 2011, 152 p.
200. **Signe Värv**. Studies on the mechanisms of RNA polymerase II-dependent transcription elongation. Tartu, 2011, 108 p.
201. **Kristjan Välk**. Gene expression profiling and genome-wide association studies of non-small cell lung cancer. Tartu, 2011, 98 p.
202. **Arno Põllumäe**. Spatio-temporal patterns of native and invasive zooplankton species under changing climate and eutrophication conditions. Tartu, 2011, 153 p.
203. **Egle Tammeleht**. Brown bear (*Ursus arctos*) population structure, demographic processes and variations in diet in northern Eurasia. Tartu, 2011, 143 p.
205. **Teele Jairus**. Species composition and host preference among ectomycorrhizal fungi in Australian and African ecosystems. Tartu, 2011, 106 p.

206. **Kessy Abarenkov**. PlutoF – cloud database and computing services supporting biological research. Tartu, 2011, 125 p.
207. **Marina Grigorova**. Fine-scale genetic variation of follicle-stimulating hormone beta-subunit coding gene (*FSHB*) and its association with reproductive health. Tartu, 2011, 184 p.
208. **Anu Tiitsaar**. The effects of predation risk and habitat history on butterfly communities. Tartu, 2011, 97 p.
209. **Elin Sild**. Oxidative defences in immunoecological context: validation and application of assays for nitric oxide production and oxidative burst in a wild passerine. Tartu, 2011, 105 p.
210. **Irja Saar**. The taxonomy and phylogeny of the genera *Cystoderma* and *Cystodermella* (Agaricales, Fungi). Tartu, 2012, 167 p.
211. **Pauli Saag**. Natural variation in plumage bacterial assemblages in two wild breeding passerines. Tartu, 2012, 113 p.
212. **Aleksei Lulla**. Alphaviral nonstructural protease and its polyprotein substrate: arrangements for the perfect marriage. Tartu, 2012, 143 p.
213. **Mari Järve**. Different genetic perspectives on human history in Europe and the Caucasus: the stories told by uniparental and autosomal markers. Tartu, 2012, 119 p.
214. **Ott Scheler**. The application of tmRNA as a marker molecule in bacterial diagnostics using microarray and biosensor technology. Tartu, 2012, 93 p.
215. **Anna Balikova**. Studies on the functions of tumor-associated mucin-like leukosialin (CD43) in human cancer cells. Tartu, 2012, 129 p.
216. **Triinu Kõressaar**. Improvement of PCR primer design for detection of prokaryotic species. Tartu, 2012, 83 p.
217. **Tuul Sepp**. Hematological health state indices of greenfinches: sources of individual variation and responses to immune system manipulation. Tartu, 2012, 117 p.
218. **Rya Ero**. Modifier view of the bacterial ribosome. Tartu, 2012, 146 p.
219. **Mohammad Bahram**. Biogeography of ectomycorrhizal fungi across different spatial scales. Tartu, 2012, 165 p.
220. **Annely Lorents**. Overcoming the plasma membrane barrier: uptake of amphipathic cell-penetrating peptides induces influx of calcium ions and downstream responses. Tartu, 2012, 113 p.
221. **Katrin Männik**. Exploring the genomics of cognitive impairment: whole-genome SNP genotyping experience in Estonian patients and general population. Tartu, 2012, 171 p.
222. **Marko Prous**. Taxonomy and phylogeny of the sawfly genus *Empria* (Hymenoptera, Tenthredinidae). Tartu, 2012, 192 p.
223. **Triinu Visnapuu**. Levansucrases encoded in the genome of *Pseudomonas syringae* pv. tomato DC3000: heterologous expression, biochemical characterization, mutational analysis and spectrum of polymerization products. Tartu, 2012, 160 p.
224. **Nele Tamberg**. Studies on Semliki Forest virus replication and pathogenesis. Tartu, 2012, 109 p.

225. **Tõnu Esko**. Novel applications of SNP array data in the analysis of the genetic structure of Europeans and in genetic association studies. Tartu, 2012, 149 p.
226. **Timo Arula**. Ecology of early life-history stages of herring *Clupea harengus membras* in the northeastern Baltic Sea. Tartu, 2012, 143 p.
227. **Inga Hiiesalu**. Belowground plant diversity and coexistence patterns in grassland ecosystems. Tartu, 2012, 130 p.
228. **Kadri Koorem**. The influence of abiotic and biotic factors on small-scale plant community patterns and regeneration in boreonemoral forest. Tartu, 2012, 114 p.
229. **Liis Andresen**. Regulation of virulence in plant-pathogenic pectobacteria. Tartu, 2012, 122 p.
230. **Kaupo Kohv**. The direct and indirect effects of management on boreal forest structure and field layer vegetation. Tartu, 2012, 124 p.
231. **Mart Jüssi**. Living on an edge: landlocked seals in changing climate. Tartu, 2012, 114 p.
232. **Riina Klais**. Phytoplankton trends in the Baltic Sea. Tartu, 2012, 136 p.
233. **Rauno Veeroja**. Effects of winter weather, population density and timing of reproduction on life-history traits and population dynamics of moose (*Alces alces*) in Estonia. Tartu, 2012, 92 p.
234. **Marju Keis**. Brown bear (*Ursus arctos*) phylogeography in northern Eurasia. Tartu, 2013, 142 p.
235. **Sergei Põlme**. Biogeography and ecology of *alnus*- associated ectomycorrhizal fungi – from regional to global scale. Tartu, 2013, 90 p.
236. **Liis Uusküla**. Placental gene expression in normal and complicated pregnancy. Tartu, 2013, 173 p.
237. **Marko Lõoke**. Studies on DNA replication initiation in *Saccharomyces cerevisiae.* Tartu, 2013, 112 p.
238. **Anne Aan**. Light- and nitrogen-use and biomass allocation along productivity gradients in multilayer plant communities. Tartu, 2013, 127 p.
239. **Heidi Tamm**. Comprehending phylogenetic diversity – case studies in three groups of ascomycetes. Tartu, 2013, 136 p.
240. **Liina Kangur**. High-Pressure Spectroscopy Study of Chromophore-Binding Hydrogen Bonds in Light-Harvesting Complexes of Photosynthetic Bacteria. Tartu, 2013, 150 p.
241. **Margus Leppik**. Substrate specificity of the multisite specific pseudouridine synthase RluD. Tartu, 2013, 111 p.
242. **Lauris Kaplinski**. The application of oligonucleotide hybridization model for PCR and microarray optimization. Tartu, 2013, 103 p.
243. **Merli Pärnoja**. Patterns of macrophyte distribution and productivity in coastal ecosystems: effect of abiotic and biotic forcing. Tartu, 2013, 155 p.
244. **Tõnu Margus**. Distribution and phylogeny of the bacterial translational GTPases and the Mqsr/YgiT regulatory system. Tartu, 2013, 126 p.
245. **Pille Mänd**. Light use capacity and carbon and nitrogen budget of plants: remote assessment and physiological determinants. Tartu, 2013, 128 p.

246. **Mario Plaas**. Animal model of Wolfram Syndrome in mice: behavioural, biochemical and psychopharmacological characterization. Tartu, 2013, 144 p.
247. **Georgi Hudjašov**. Maps of mitochondrial DNA, Y-chromosome and tyrosinase variation in Eurasian and Oceanian populations. Tartu, 2013, 115 p.
248. **Mari Lepik**. Plasticity to light in herbaceous plants and its importance for community structure and diversity. Tartu, 2013, 102 p.
249. **Ede Leppik**. Diversity of lichens in semi-natural habitats of Estonia. Tartu, 2013, 151 p.
250. **Ülle Saks**. Arbuscular mycorrhizal fungal diversity patterns in boreonemoral forest ecosystems. Tartu, 2013, 151 p.
251. **Eneli Oitmaa**. Development of arrayed primer extension microarray assays for molecular diagnostic applications. Tartu, 2013, 147 p.
252. **Jekaterina Jutkina**. The horizontal gene pool for aromatics degradation: bacterial catabolic plasmids of the Baltic Sea aquatic system. Tartu, 2013, 121 p.
253. **Helen Vellau**. Reaction norms for size and age at maturity in insects: rules and exceptions. Tartu, 2014, 132 p.
254. **Randel Kreitsberg**. Using biomarkers in assessment of environmental contamination in fish – new perspectives. Tartu, 2014, 107 p.
255. **Krista Takkis**. Changes in plant species richness and population performance in response to habitat loss and fragmentation.Tartu, 2014, 141 p.
256. **Liina Nagirnaja**. Global and fine-scale genetic determinants of recurrent pregnancy loss. Tartu, 2014, 211 p.
257. **Triin Triisberg**. Factors influencing the re-vegetation of abandoned extracted peatlands in Estonia. Tartu, 2014, 133 p.
258. **Villu Soon**. A phylogenetic revision of the *Chrysis ignita* species group (Hymenoptera: Chrysididae) with emphasis on the northern European fauna. Tartu, 2014, 211 p.
259. **Andrei Nikonov**. RNA-Dependent RNA Polymerase Activity as a Basis for the Detection of Positive-Strand RNA Viruses by Vertebrate Host Cells. Tartu, 2014, 207 p.
260. **Eele Õunapuu-Pikas**. Spatio-temporal variability of leaf hydraulic conductance in woody plants: ecophysiological consequences. Tartu, 2014, 135 p.
261. **Marju Männiste**. Physiological ecology of greenfinches: information content of feathers in relation to immune function and behavior. Tartu, 2014, 121 p.
262. **Katre Kets**. Effects of elevated concentrations of CO_2 and O_3 on leaf photosynthetic parameters in *Populus tremuloides*: diurnal, seasonal and interannual patterns. Tartu, 2014, 115 p.
263. **Külli Lokko**. Seasonal and spatial variability of zoopsammon communities in relation to environmental parameters. Tartu, 2014, 129 p.
264. **Olga Žilina**. Chromosomal microarray analysis as diagnostic tool: Estonian experience. Tartu, 2014, 152 p.

265. **Kertu Lõhmus**. Colonisation ecology of forest-dwelling vascular plants and the conservation value of rural manor parks. Tartu, 2014, 111 p.
266. **Anu Aun**. Mitochondria as integral modulators of cellular signaling. Tartu, 2014, 167 p.
267. **Chandana Basu Mallick**. Genetics of adaptive traits and gender-specific demographic processes in South Asian populations. Tartu, 2014, 160 p.
268. **Riin Tamme**. The relationship between small-scale environmental heterogeneity and plant species diversity. Tartu, 2014, 130 p.
269. **Liina Remm**. Impacts of forest drainage on biodiversity and habitat quality: implications for sustainable management and conservation. Tartu, 2015, 126 p.
270. **Tiina Talve**. Genetic diversity and taxonomy within the genus *Rhinanthus.* Tartu, 2015, 106 p.
271. **Mehis Rohtla**. Otolith sclerochronological studies on migrations, spawning habitat preferences and age of freshwater fishes inhabiting the Baltic Sea. Tartu, 2015, 137 p.
272. **Alexey Reshchikov**. The world fauna of the genus *Lathrolestes* (Hymenoptera, Ichneumonidae). Tartu, 2015, 247 p.
273. **Martin Pook**. Studies on artificial and extracellular matrix protein-rich surfaces as regulators of cell growth and differentiation. Tartu, 2015, 142 p.
274. **Mai Kukumägi**. Factors affecting soil respiration and its components in silver birch and Norway spruce stands. Tartu, 2015, 155 p.
275. **Helen Karu**. Development of ecosystems under human activity in the North-East Estonian industrial region: forests on post-mining sites and bogs. Tartu, 2015, 152 p.
276. **Hedi Peterson**. Exploiting high-throughput data for establishing relationships between genes. Tartu, 2015, 186 p.
277. **Priit Adler**. Analysis and visualisation of large scale microarray data, Tartu, 2015, 126 p.
278. **Aigar Niglas**. Effects of environmental factors on gas exchange in deciduous trees: focus on photosynthetic water-use efficiency. Tartu, 2015, 152 p.
279. **Silja Laht**. Classification and identification of conopeptides using profile hidden Markov models and position-specific scoring matrices. Tartu, 2015, 100 p.
280. **Martin Kesler**. Biological characteristics and restoration of Atlantic salmon *Salmo salar* populations in the Rivers of Northern Estonia. Tartu, 2015, 97 p.
281. **Pratyush Kumar Das**. Biochemical perspective on alphaviral nonstructural protein 2: a tale from multiple domains to enzymatic profiling. Tartu, 2015, 205 p
282. **Priit Palta**. Computational methods for DNA copy number detection. Tartu, 2015, 130 p.
283. **Julia Sidorenko**. Combating DNA damage and maintenance of genome integrity in pseudomonads. Tartu, 2015, 174 p.

284. **Anastasiia Kovtun-Kante**. Charophytes of Estonian inland and coastal waters: distribution and environmental preferences. Tartu, 2015, 97 p.
285. **Ly Lindman**. The ecology of protected butterfly species in Estonia. Tartu, 2015, 171 p.
286. **Jaanis Lodjak**. Association of Insulin-like Growth Factor I and Corticosterone with Nestling Growth and Fledging Success in Wild Passerines. Tartu, 2016, 113 p.
287. **Ann Kraut**. Conservation of Wood-Inhabiting Biodiversity – Semi-Natural Forests as an Opportunity. Tartu, 2016, 141 p.
288. **Tiit Örd**. Functions and regulation of the mammalian pseudokinase TRIB3. Tartu, 2016, 182. p.
289. **Kairi Käiro**. Biological Quality According to Macroinvertebrates in Streams of Estonia (Baltic Ecoregion of Europe): Effects of Human-induced Hydromorphological Changes. Tartu, 2016, 126 p.
290. **Leidi Laurimaa**. *Echinococcus multilocularis* and other zoonotic parasites in Estonian canids. Tartu, 2016, 144 p.
291. **Helerin Margus**. Characterization of cell-penetrating peptide/nucleic acid nanocomplexes and their cell-entry mechanisms. Tartu, 2016, 173 p.
292. **Kadri Runnel**. Fungal targets and tools for forest conservation. Tartu, 2016, 157 p.
293. **Urmo Võsa**. MicroRNAs in disease and health: aberrant regulation in lung cancer and association with genomic variation. Tartu, 2016, 163 p.
294. **Kristina Mäemets-Allas**. Studies on cell growth promoting AKT signaling pathway – a promising anti-cancer drug target. Tartu, 2016, 146 p.
295. **Janeli Viil**. Studies on cellular and molecular mechanisms that drive normal and regenerative processes in the liver and pathological processes in Dupuytren's contracture. Tartu, 2016, 175 p.
296. **Ene Kook**. Genetic diversity and evolution of *Pulmonaria angustifolia* L. and *Myosotis laxa sensu lato* (Boraginaceae). Tartu, 2016, 106 p.
297. **Kadri Peil**. RNA polymerase II-dependent transcription elongation in *Saccharomyces cerevisiae*. Tartu, 2016, 113 p.
298. **Katrin Ruisu**. The role of RIC8A in mouse development and its function in cell-matrix adhesion and actin cytoskeletal organisation. Tartu, 2016, 129 p.
299. **Janely Pae**. Translocation of cell-penetrating peptides across biological membranes and interactions with plasma membrane constituents. Tartu, 2016, 126 p.
300. **Argo Ronk**. Plant diversity patterns across Europe: observed and dark diversity. Tartu, 2016, 153 p.
301. **Kristiina Mark**. Diversification and species delimitation of lichenized fungi in selected groups of the family Parmeliaceae (Ascomycota). Tartu, 2016, 181 p.
302. **Jaak-Albert Metsoja**. Vegetation dynamics in floodplain meadows: influence of mowing and sediment application. Tartu, 2016, 140 p.

303. **Hedvig Tamman**. The GraTA toxin-antitoxin system of *Pseudomonas putida*: regulation and role in stress tolerance. Tartu, 2016, 154 p.
304. **Kadri Pärtel**. Application of ultrastructural and molecular data in the taxonomy of helotialean fungi. Tartu, 2016, 183 p.
305. **Maris Hindrikson**. Grey wolf (*Canis lupus*) populations in Estonia and Europe: genetic diversity, population structure and -processes, and hybridization between wolves and dogs. Tartu, 2016, 121 p.
306. **Polina Degtjarenko**. Impacts of alkaline dust pollution on biodiversity of plants and lichens: from communities to genetic diversity. Tartu, 2016, 126 p.
307. **Liina Pajusalu**. The effect of CO_2 enrichment on net photosynthesis of macrophytes in a brackish water environment. Tartu, 2016, 126 p.
308. **Stoyan Tankov**. Random walks in the stringent response. Tartu, 2016, 94 p.
309. **Liis Leitsalu**. Communicating genomic research results to population-based biobank participants. Tartu, 2016, 158 p.
310. **Richard Meitern**. Redox physiology of wild birds: validation and application of techniques for detecting oxidative stress. Tartu, 2016, 134 p.
311. **Kaie Lokk**. Comparative genome-wide DNA methylation studies of healthy human tissues and non-small cell lung cancer tissue. Tartu, 2016, 127 p.
312. **Mihhail Kurašin**. Processivity of cellulases and chitinases. Tartu, 2017, 132 p.
313. **Carmen Tali**. Scavenger receptors as a target for nucleic acid delivery with peptide vectors. Tartu, 2017, 155 p.
314. **Katarina Oganjan**. Distribution, feeding and habitat of benthic suspension feeders in a shallow coastal sea. Tartu, 2017, 132 p.
315. **Taavi Paal**. Immigration limitation of forest plants into wooded landscape corridors. Tartu, 2017, 145 p.
316. **Kadri Õunap**. The Williams-Beuren syndrome chromosome region protein WBSCR22 is a ribosome biogenesis factor. Tartu, 2017, 135 p.
317. **Riin Tamm**. In-depth analysis of factors affecting variability in thiopurine methyltransferase activity. Tartu, 2017, 170 p.
318. **Keiu Kask**. The role of RIC8A in the development and regulation of mouse nervous system. Tartu, 2017, 184 p.
319. **Tiia Möller**. Mapping and modelling of the spatial distribution of benthic macrovegetation in the NE Baltic Sea with a special focus on the eelgrass *Zostera marina* Linnaeus, 1753. Tartu, 2017, 162 p.
320. **Silva Kasela**. Genetic regulation of gene expression: detection of tissue- and cell type-specific effects. Tartu, 2017, 150 p.
321. **Karmen Süld**. Food habits, parasites and space use of the raccoon dog *Nyctereutes procyonoides*: the role of an alien species as a predator and vector of zoonotic diseases in Estonia. Tartu, 2017, p.
322. **Ragne Oja**. Consequences of supplementary feeding of wild boar – concern for ground-nesting birds and endoparasite infection. Tartu, 2017, 141 p.
323. **Riin Kont**. The acquisition of cellulose chain by a processive cellobiohydrolase. Tartu, 2017, 117 p.

324. **Liis Kasari**. Plant diversity of semi-natural grasslands: drivers, current status and conservation challenges. Tartu, 2017, 141 p.
325. **Sirgi Saar**. Belowground interactions: the roles of plant genetic relatedness, root exudation and soil legacies. Tartu, 2017, 113 p.
326. **Sten Anslan**. Molecular identification of Collembola and their fungal associates. Tartu, 2017, 125 p.
327. **Imre Taal**. Causes of variation in littoral fish communities of the Eastern Baltic Sea: from community structure to individual life histories. Tartu, 2017, 118 p.
328. **Jürgen Jalak**. Dissecting the Mechanism of Enzymatic Degradation of Cellulose Using Low Molecular Weight Model Substrates. Tartu, 2017, 137 p.
329. **Kairi Kiik**. Reproduction and behaviour of the endangered European mink (*Mustela lutreola*) in captivity. Tartu, 2018, 112 p.
330. **Ivan Kuprijanov**. Habitat use and trophic interactions of native and invasive predatory macroinvertebrates in the northern Baltic Sea. Tartu, 2018, 117 p.
331. **Hendrik Meister**. Evolutionary ecology of insect growth: from geographic patterns to biochemical trade-offs. Tartu, 2018, 147 p.
332. **Ilja Gaidutšik**. Irc3 is a mitochondrial branch migration enzyme in *Saccharomyces cerevisiae.* Tartu, 2018, 161 p.
333. **Lena Neuenkamp**. The dynamics of plant and arbuscular mycorrhizal fungal communities in grasslands under changing land use. Tartu, 2018, 241 p.
334. **Laura Kasak**. Genome structural variation modulating the placenta and pregnancy maintenance. Tartu, 2018, 181 p.
335. **Kersti Riibak**. Importance of dispersal limitation in determining dark diversity of plants across spatial scales. Tartu, 2018, 133 p.
336. **Liina Saar**. Dynamics of grassland plant diversity in changing landscapes. Tartu, 2018, 206 p.
337. **Hanna Ainelo**. Fis regulates *Pseudomonas putida* biofilm formation by controlling the expression of *lapA*. Tartu, 2018, 143 p.
338. **Natalia Pervjakova**. Genomic imprinting in complex traits. Tartu, 2018, 176 p.
339. **Andrio Lahesaare**. The role of global regulator Fis in regulating the expression of *lapF* and the hydrophobicity of soil bacterium *Pseudomonas putida*. Tartu, 2018, 124 p.
340. **Märt Roosaare**. *K*-mer based methods for the identification of bacteria and plasmids. Tartu, 2018, 117 p.
341. **Maria Abakumova**. The relationship between competitive behaviour and the frequency and identity of neighbours in temperate grassland plants. Tartu, 2018, 104 p.
342. **Margus Vilbas**. Biotic interactions affecting habitat use of myrmecophilous butterflies in Northern Europe. Tartu, 2018, 142 p.

343. **Liina Kinkar**. Global patterns of genetic diversity and phylogeography of *Echinococcus granulosus* sensu stricto – a tapeworm species of significant public health concern. Tartu, 2018, 147 p.
344. **Teivi Laurimäe**. Taxonomy and genetic diversity of zoonotic tapeworms in the species complex of *Echinococcus granulosus* sensu lato. Tartu, 2018, 143 p.
345. **Tatjana Jatsenko**. Role of translesion DNA polymerases in mutagenesis and DNA damage tolerance in Pseudomonads. Tartu, 2018, 216 p.
346. **Katrin Viigand**. Utilization of α-glucosidic sugars by *Ogataea* (*Hansenula*) *polymorpha*. Tartu, 2018, 148 p.
347. **Andres Ainelo**. Physiological effects of the *Pseudomonas putida* toxin grat. Tartu, 2018, 146 p.
348. **Killu Timm**. Effects of two genes (DRD4 and SERT) on great tit (*Parus major*) behaviour and reproductive traits. Tartu, 2018, 117 p.
349. **Petr Kohout**. Ecology of ericoid mycorrhizal fungi. Tartu, 2018, 184 p.
350. **Gristin Rohula-Okunev**. Effects of endogenous and environmental factors on night-time water flux in deciduous woody tree species. Tartu, 2018, 184 p.
351. **Jane Oja**. Temporal and spatial patterns of orchid mycorrhizal fungi in forest and grassland ecosystems. Tartu, 2018, 102 p.
352. **Janek Urvik**. Multidimensionality of aging in a long-lived seabird. Tartu, 2018, 135 p.
353. **Lisanna Schmidt**. Phenotypic and genetic differentiation in the hybridizing species pair *Carex flava* and *C. viridula* in geographically different regions. Tartu, 2018, 133 p.
354. **Monika Karmin**. Perspectives from human Y chromosome – phylogeny, population dynamics and founder events. Tartu, 2018, 168 p.
355. **Maris Alver**. Value of genomics for atherosclerotic cardiovascular disease risk prediction. Tartu, 2019, 148 p.
356. **Lehti Saag**. The prehistory of Estonia from a genetic perspective: new insights from ancient DNA. Tartu, 2019, 171 p.
357. **Mari-Liis Viljur**. Local and landscape effects on butterfly assemblages in managed forests. Tartu, 2019, 115 p.
358. **Ivan Kisly**. The pleiotropic functions of ribosomal proteins eL19 and eL24 in the budding yeast ribosome. Tartu, 2019, 170 p.
359. **Mikk Puustusmaa**. On the origin of papillomavirus proteins. Tartu, 2019, 152 p.
360. **Anneliis Peterson**. Benthic biodiversity in the north-eastern Baltic Sea: mapping methods, spatial patterns, and relations to environmental gradients. Tartu, 2019, 159 p.
361. **Erwan Pennarun**. Meandering along the mtDNA phylogeny; causerie and digression about what it can tell us about human migrations. Tartu, 2019, 162 p.

362. **Karin Ernits**. Levansucrase Lsc3 and endo-levanase BT1760: characterization and application for the synthesis of novel prebiotics. Tartu, 2019, 217 p.
363. **Sille Holm**. Comparative ecology of geometrid moths: in search of contrasts between a temperate and a tropical forest. Tartu, 2019, 135 p.
364. **Anne-Mai Ilumäe**. Genetic history of the Uralic-speaking peoples as seen through the paternal haplogroup N and autosomal variation of northern Eurasians. Tartu, 2019, 172 p.
365. **Anu Lepik**. Plant competitive behaviour: relationships with functional traits and soil processes. Tartu, 2019, 152 p.
366. **Kunter Tätte**. Towards an integrated view of escape decisions in birds under variable levels of predation risk. Tartu, 2020, 172 p.
367. **Kaarin Parts**. The impact of climate change on fine roots and root-associated microbial communities in birch and spruce forests. Tartu, 2020, 143 p.
368. **Viktorija Kukuškina**. Understanding the mechanisms of endometrial receptivity through integration of 'omics' data layers. Tartu, 2020, 169 p.
369. **Martti Vasar**. Developing a bioinformatics pipeline gDAT to analyse arbuscular mycorrhizal fungal communities using sequence data from different marker regions. Tartu, 2020, 193 p.
370. **Ott Kangur**. Nocturnal water relations and predawn water potential disequilibrium in temperate deciduous tree species. Tartu, 2020, 126 p.
371. **Helen Post**. Overview of the phylogeny and phylogeography of the Y-chromosomal haplogroup N in northern Eurasia and case studies of two linguistically exceptional populations of Europe – Hungarians and Kalmyks. Tartu, 2020, 143 p.
372. **Kristi Krebs**. Exploring the genetics of adverse events in pharmacotherapy using Biobanks and Electronic Health Records. Tartu, 2020, 151 p.
373. **Kärt Ukkivi**. Mutagenic effect of transcription and transcription-coupled repair factors in *Pseudomonas putida.* Tartu, 2020, 154 p.
374. **Elin Soomets**. Focal species in wetland restoration. Tartu, 2020, 137 p.
375. **Kadi Tilk**. Signals and responses of ColRS two-component system in *Pseudomonas putida.* Tartu, 2020, 133 p.
376. **Indrek Teino**. Studies on aryl hydrocarbon receptor in the mouse granulosa cell model. Tartu, 2020, 139 p.
377. **Maarja Vaikre**. The impact of forest drainage on macroinvertebrates and amphibians in small waterbodies and opportunities for cost-effective mitigation. Tartu, 2020, 132 p.
378. **Siim-Kaarel Sepp**. Soil eukaryotic community responses to land use and host identity. Tartu, 2020, 222 p.
379. **Eveli Otsing**. Tree species effects on fungal richness and community structure. Tartu, 2020, 152 p.
380. **Mari Pent**. Bacterial communities associated with fungal fruitbodies. Tartu, 2020, 144 p.

381. **Einar Kärgenberg**. Movement patterns of lithophilous migratory fish in free-flowing and fragmented rivers. Tartu, 2020, 167 p.
382. **Antti Matvere**. The studies on aryl hydrocarbon receptor in murine granulosa cells and human embryonic stem cells. Tartu, 2021, 163 p.
383. **Jhonny Capichoni Massante**. Phylogenetic structure of plant communities along environmental gradients: a macroecological and evolutionary approach. Tartu, 2021, 144 p.
384. **Ajai Kumar Pathak**. Delineating genetic ancestries of people of the Indus Valley, Parsis, Indian Jews and Tharu tribe. Tartu, 2021, 197 p.
385. **Tanel Vahter**. Arbuscular mycorrhizal fungal biodiversity for sustainable agroecosystems. Tartu, 2021, 191 p.
386. **Burak Yelmen**. Characterization of ancient Eurasian influences within modern human genomes. Tartu, 2021, 134 p.
387. **Linda Ongaro**. A genomic portrait of American populations. Tartu, 2021, 182 p.
388. **Kairi Raime**. The identification of plant DNA in metagenomic samples. Tartu, 2021, 108 p.
389. **Heli Einberg**. Non-linear and non-stationary relationships in the pelagic ecosystem of the Gulf of Riga (Baltic Sea). Tartu, 2021, 119 p.
390. **Mickaël Mathieu Pihain**. The evolutionary effect of phylogenetic neighbourhoods of trees on their resistance to herbivores and climatic stress. Tartu, 2022, 145 p.
391. **Annika Joy Meitern**. Impact of potassium ion content of xylem sap and of light conditions on the hydraulic properties of trees. Tartu, 2022, 132 p.
392. **Elise Joonas**. Evaluation of metal contaminant hazard on microalgae with environmentally relevant testing strategies. Tartu, 2022, 118 p.
393. **Kreete Lüll**. Investigating the relationships between human microbiome, host factors and female health. Tartu, 2022, 141 p.
394. **Triin Kaasiku**. A wader perspective to Boreal Baltic coastal grasslands: from habitat availability to breeding site selection and nest survival. Tartu, 2022, 141 p.
395. **Meeli Alber**. Impact of elevated atmospheric humidity on the structure of the water transport pathway in deciduous trees. Tartu, 2022, 170 p.
396. **Ludovica Molinaro**. Ancestry deconvolution of Estonian, European and Worldwide genomic layers: a human population genomics excavation. Tartu, 2022, 138 p.
397. **Tina Saupe**. The genetic history of the Mediterranean before the common era: a focus on the Italian Peninsula. Tartu, 2022, 165 p.
398. **Mari-Ann Lind**. Internal constraints on energy processing and their consequences: an integrative study of behaviour, ornaments and digestive health in greenfinches. Tartu, 2022, 137 p.
399. **Markus Valge**. Testing the predictions of life history theory on anthropometric data. Tartu, 2022, 171 p.
400. **Ants Tull**. Domesticated and wild mammals as reservoirs for zoonotic helminth parasites in Estonia. Tartu, 2022, 152 p.

401. **Saleh Rahimlouye Barabi**. Investigation of diazotrophic bacteria association with plants. Tartu, 2022, 137 p.
402. **Farzad Aslani**. Towards revealing the biogeography of belowground diversity. Tartu, 2022, 124 p.
403. **Nele Taba**. Diet, blood metabolites, and health. Tartu, 2022, 163 p.
404. **Katri Pärna**. Improving the personalized prediction of complex traits and diseases: application to type 2 diabetes. Tartu, 2022, 190 p.
405. **Silva Lilleorg**. Bacterial ribosome heterogeneity on the example of bL31 paralogs in *Escherichia coli.* Tartu, 2022, 189 p.
406. **Oliver Aasmets**. The importance of microbiome in human health. Tartu, 2022, 123 p.
407. **Henel Jürgens**. Exploring post-translational modifications of histones in RNA polymerase II-dependent transcription. Tartu, 2022, 147 p.
408. **Mari Tagel**. Finding novel factors affecting the mutation frequency: a case study of tRNA modification enzymes TruA and RluA. Tartu, 2022, 176 p.
409. **Marili Sell**. The impact of environmental change on ecophysiology of hemiboreal tree species – acclimation mechanisms in belowground. Tartu, 2022, 163 p.
410. **Kaarin Hein**. The hissing behaviour of Great Tit (*Parus major*) females reflects behavioural phenotype and breeding success in a wild population. Tartu, 2022, 96 p.
411. **Maret Gerz**. The distribution and role of mycorrhizal symbiosis in plant communities. Tartu, 2022, 206 p.
412. **Kristiina Nõomaa**. Role of invasive species in brackish benthic community structure and biomass changes. Tartu, 2023, 151 p.
413. **Anton Savchenko**. Taxonomic studies in Dacrymycetes: *Cerinomyces* and allied taxa. Tartu, 2023, 181 p.
414. **Ahto Agan**. Interactions between invasive pathogens and resident mycobiome in the foliage of trees. Tartu, 2023, 155 p.
415. **Diego Pires Ferraz Trindade**. Dark diversity dynamics linked to global change: taxonomic and functional perspective. Tartu, 2023, 134 p.
416. **Madli Jõks**. Biodiversity drivers in oceanic archipelagos and habitat fragments, explored by agent-based simulation models. Tartu, 2023, 116 p.
417. **Ciara Baines**. Adaptation to oncogenic pollution and natural cancer defences in the aquatic environment. Tartu, 2023, 164 p.
418. **Rain Inno**. Placental transcriptome and miRNome in normal and complicated pregnancies. Tartu, 2023, 145 p.
419. **Daniyal Gohar**. Diversity, genomics, and potential functions of fungus-inhabiting bacteria. Tartu, 2023, 138 p.
420. **Sirli Rosendahl**. Fitness effects of chromosomal toxin-antitoxin systems in *Pseudomonas putida.* Tartu, 2023, 154 p.
421. **Mathilde Frédérique E. André**. New Guinea, a hotspot for Human evolution: settlement history and adaptation in northern Sahul. Tartu, 2023, 202 p.

422. **Vlad-Julian Piljukov**. Biochemical characterization of Irc3 helicase. Tartu, 2023, 137 p.
423. **Gerli Albert**. Carbon use strategies of macrophyte communities in the northeastern Baltic Sea: implications for a high CO_2 environment. Tartu, 2023, 128 p.
424. **Mariann Koel**. The molecular interactions between trophoblast and endometrial cells in embryo implantation. Tartu, 2023, 171 p.
425. **Robin Gielen**. Diversity and ecological role of pathogenic fungi in insect populations. Tartu, 2023, 139 p.
426. **Kaspar Reier**. Quantity, stability and disparity of ribosomal components in *Escherichia coli* stationary phase. Tartu, 2023, 151 p.
427. **Linda Rusalepp**. The impact of environmental drivers and competition on phenolic metabolite profiles in hybrid aspen and silver birch. Tartu, 2023, 153 p.
428. **Eliisa Pass**. The effect of managed forest-wetland landscapes on forest grouse and nest predation. Tartu, 2023, 115 p.
429. **Sanni Färkkilä**. Methods for studying plant-fungal interactions – reflecting on the old, the new and the upcoming. Tartu, 2024, 147 p.
430. **Maarja Jõeloo**. Advances in microarray-based copy number variation discovery and phenotypic associations. Tartu, 2024, 209 p.
431. **Natàlia Pujol Gualdo**. Decoding genetic associations of female reproductive health traits. Tartu, 2024, 205 p.
432. **Sirelin Sillamaa**. The role of helicases Hmi1 and Irc3 in yeast mitochondrial DNA maintenance. Tartu, 2024, 189 p.
433. **Iris Reinula**. Genetic variation of grassland plants in changing landscapes. Tartu, 2024, 201 p.
434. **Vi Ngan Tran**. The cellular dynamics and epithelial morphogenesis in *Drosophila* wing development. Tartu, 2024, 158 p.
435. **Slendy Julieth Rodríguez Alarcón**. Intraspecific trait diversity in plants: characterizing effects of trait variation on community assembly and ecosystem functioning. Tartu, 2024, 129 p.
436. **Arun Kumar Devarajan**. Microbes and climate change: insights from plant-microbe interactions in rice phyllosphere and soil microbiomes in subarctic grasslands. Tartu, 2024, 224 p.
437. **Leonard Owuraku Opare**. Rearing density effects on a commercially important insect species. Tartu, 2024, 145 p.
438. **Siqiao Liu**. The effect of anthropogenic disturbance on soil fungal communities. Tartu, 2024, 172 p.
439. **Kertu Liis Krigul**. The gut microbiome at the interface of human health and disease. Tartu, 2024, 158 p.
440. **Danat Yermakovich**. The evolutionary history of complex traits: implications of archaic admixture. Tartu, 2024, 153 p.
441. **Yiming Meng**. Plant mycorrhizal type and status in the global flora. Tartu, 2024, 200 p.

442. **Iryna Yatsiuk**. Evolution, species delimitation and diversity in myxomycetes: *Arcyria* and allied genera. Tartu, 2024, 193 p.
443. **Daniela León Velandia**. Mycorrhizal trait distribution and composition in plant communities under natural gradients. Tartu, 2024, 121 p.
444. **Bruno Paganeli**. Dark diversity methods for prioritization of areas and species in nature conservation. Tartu, 2024, 155 p.
445. **Mario Reiman**. Placental transcriptome in normal and complicated pregnancies. Tartu, 2025, 167 p.
446. **Maarja Kõrkjas**. Dynamics of tree-related microhabitats in live forest trees and its links with biodiversity. Tartu, 2025, 134 p.
447. **Eleonora Beccari**. Mapping and exploring trait spaces across the tree of life. Tartu, 2025, 190 p.
448. **Jack R. Hall**. Dissolved organic carbon dynamics of Baltic Sea macroalgae: production, bioavailability and ecosystem effects. Tartu, 2025, 135 p.
449. **Artjom Stepanjuk**. Function of adhesion molecules and signalling pathways in human endometrial and embryonic models. Tartu, 2025, 247 p.
450. **Marianne Kivastik**. Heterostylous plants in an era of global change: the role of local, landscape and climatic actors. Tartu, 2025, 167 p.
451. **Yehor Yatsiuk**. Large tree-cavities as key structures for forest biodiversity. Tartu, 2025, 215 p.
452. **Ovidiu Copoț**. Relevance of eDNA, citizen science, and species distribution modelling for fungal conservation. Tartu, 2025, 198 p.
453. **Tarmo Puurand**. Human genome studies with k-mer frequencies. Tartu, 2025, 184 p.
454. **Stênio Ítalo Araújo Foerster**. Phylogenetic comparative studies of body size in insects and arachnids: from predictions to applications. Tartu, 2025, 171 p.
455. **Hanna Maria Kariis**. Improving pharmacotherapy outcomes in psychiatric and cardiovascular conditions. Tartu, 2025, 193 p.
456. **Elisabeth Prangel**. The impact of land-use change and ecological restoration on biodiversity and ecosystem service supply in semi-natural grasslands. Tartu, 2025, 233 p.
457. **Nidal Fetnassi**. Determinants of moth assemblages across human-modified landscapes of Estonia and Morocco. Tartu, 2025, 162 p.
458. **Vineesh Nedumpally**. Assembling the phylogenetic tree of northern European macroheteroceran moths. Tartu, 2025, 171 p.
459. **Ali Hakimzadeh**. Long-read metabarcoding: from available tools to reference databases. Tartu, 2026, 124 p.
460. **John Yangyuoru Kupagme**. Biodiversity of African soil fungi. Tartu, 2026, 162 p.
461. **Bariş Yaşar**. Advanced chromosomal testing tools for embryo quality and fetal health. Tartu, 2026, 248 p.