



PhD position in ancient DNA and proteins

We are looking for a motivated PhD student for an exciting four-year funded project on metagenomic and proteomic studies on Stone Age anthropogenic birch tar samples. A good candidate has a master degree or equivalent in population genetics, evolutionary studies, proteomic studies, bioinformatics or other related fields. The general project description below can be tailored to reflect personal interests of a motivated candidate.

Deadline for applications: 24th March 2024. Please send your CV and motivation letter to mait.metspalu@ut.ee

Title of project in Estonian:

Kiviaegsete inimseoseliste kasetõrvaproovide metagenoomne ja proteoomiline analüüs

Title of project in English:

Metagenomic and proteomic studies of Stone Age anthropogenic birch tar samples

PhD Project plan

Recent advances in laboratory methods and DNA sequencing technology have fueled a surge in ancient DNA (aDNA) research. This has greatly enhanced our ability to accurately date and geolocate samples, significantly advancing the study of our evolutionary past. Unlike conventional genetic studies that focus on present populations, aDNA allows us to explore the full genetic diversity of past populations. Notably, our lab has made significant contributions to understanding the sequence of demographic changes in Northeast Europe.

A common challenge in aDNA research is the scarcity of human remains during certain periods and in specific regions. To address this, we are exploring an innovative and overlooked source of aDNA: birch tar. Birch tar was extensively used in the Stone Age as an adhesive material. It is derived from the dry distillation of birch bark (*Betula* sp.) and was processed through chewing. Amazingly, ancient birch tar chunks have been found to contain human and oral microbiome DNA, as well as biomolecular signatures of the local environment and diet.



Institute of Genomics

Task 1. Microbial ancient metagenomics

This project aims to utilize birch tar to shed light on the diverse population of commensal and pathogenic microbes, particularly the human oral microbiome (HOM). Understanding the composition and prevalence of certain species in the HOM is crucial as they have been linked to diet, oral and systemic diseases like diabetes, Alzheimer's, arthritis, and cancer. Additionally, analysis of birch tar could reveal the status of active oral infections in ancient individuals, providing insights into the evolutionary history of these pathogens. Saliva also contains diagnostic proteins for these diseases. For example, the Epstein-Barr virus (EBV) has been identified in birch tar, and similar infections have been found in ancient teeth. Uncovering the extent of herpes virus diversity in pre-Neolithic human populations and exploring potential viral exchanges with Neanderthals are key outstanding questions.

Task 2. ancient proteomics to reveal infection dynamics

Examining proteins preserved in the ancient birch tar through palaeoproteomic analysis could help us identify peptides associated with oral pathogens. This approach holds promise in uncovering RNA pathogens that were once present and prevalent in ancient oral cavities. This is significant because RNA preservation is limited over time, and thus the prevalence of various RNA viruses like coronaviruses, measles, influenza, or respiratory syncytial virus during prehistoric times remains unknown. By studying these peptides, we can gain insights into the evolutionary factors influencing the human immune response to viral infections and the evolution, dynamics, and dispersion of RNA pathogens in Europe.

Task3. Ancient biomolecules reveal ancient diet

Furthermore, the DNA and proteins extracted from the food particles trapped in birch tar offer direct evidence of ancient diets. Dental calculus, potsherds, and carbonized food crusts have already revealed proteins from dairy, plants, and meats. Our lab has developed a combined protocol to extract both ancient DNA and paleoproteins from the same sample, enabling a more comprehensive understanding of Baltic hunter-gatherer food consumption. This high-resolution shotgun proteomics approach could uncover the species-level details of consumed foods, including minor components usually undetectable by traditional lipidomics and stable isotope analysis methods. Additionally, by analyzing proteins trapped in chewing gums, we can reconstruct an individual's diet within a specific timeframe, including seasonal variations.

Overall, leveraging the potential of birch tar as a source of aDNA and proteins allows us to investigate the ancient human demographic past, the diversity of pathogens, and the intricacies of ancient diets with unprecedented precision.



Institute of Genomics

Supervisors:

Dr. Mait Metspalu (<https://scholar.google.com/citations?user=xOPd7N0AAAAJ&hl=en>) is an evolutionary geneticist, an expert of population genetics and ancient DNA. He is coordinating the Birch Tar project of which this PhD work plan is part of. He will be responsible for general scientific supervision.

Dr. Ester Oras (<https://scholar.google.com/citations?hl=en&user=h94m6lMAAAAJ>) is an expert in analytical chemistry and archaeology specializing on biomolecular archaeology, incl. protein studies. She will be instrumental in supervising proteome studies of food residues etc in the birch tar samples.

Dr. Antonio de Dios Martinez (<https://orcid.org/0000-0001-9260-8846>) is an expert in ancient metagenomic studies and will supervise pathogen and diet related metagenomic research.

Dr. Remi Philippe Barbieri (<https://scholar.google.com/citations?hl=en&user=Zj-mUQMAAAAJ>) is an expert in ancient proteomics and will supervise pathogen- related ancient proteomics study direction.

1. Description of available resources required for work (equipment, finances):

The research will be carried out in the facilities of UT IG Estonian Biocentre in collaboration with the archaeologists of the Collegium of Transdisciplinary Studies of Archaeology, Genetics and Linguistics of UT, and the newly established Centre of Excellence “Estonian Roots: Centre of Excellence for transdisciplinary studies on ethnogenesis and cultural diversity”, PI K. Tambets. IG runs an ancient DNA cleanroom that allows us to process sensitive bioarchaeological materials and optimize the extraction protocols. Both metagenomic and proteomic analysis will take in the DNA facilities, the latter in collaboration with the dedicated lab for archaeological chemistry <https://www.archemy.ee/>

- **Samples:** Prof. A. Kriiska from the Institute of History and Archaeology of UT has over the years collected a sizeable sample of birch tar chunks and archaeological material where birch tar has been used as adhesive. These Stone Age samples come from the Baltic states and Russia. Due to Russia’s aggression war against Ukraine, we are currently using only the samples from the Baltic countries. In another PhD project we have already shown that these samples do contain DNA and are doing human population genetics studies. This PhD project is complementary to that.



Institute of Genomics

- **DNA work:** DNA wet lab work of the project will be carried out in specially designed cleanroom facilities of IG which have been solely dedicated to work with sensitive ancient materials and have been in use since May 2018. These are fully equipped facilities to store, record, extract and process hundreds of human bone and tooth samples per week.
- **Protein work:** Proteomics wet lab work will be also carried out in specially designed cleanroom facilities of IG to minimise contamination issues. High-resolution mass-spectrometry analysis using LC-MS/MS (Q-Exactive) will be carried out at the Proteomics Core Facility of the UT. Pipeline and procedures for palaeoproteomics analysis from optimal sample preparation methods, instrumental runs and bioinformatic data analysis have been developed since May 2020.
- **DNA Sequencing:** The core facility of the IG operates a modern Sanger sequencing platform, Illumina NextSeq500 and NextSeq2000 next generation sequencing platforms and Illumina genotyping chip scanners.
- **Databases:** We have access to comprehensive genomic databases of modern and ancient human populations and microorganisms that can be used for the comparison of the data generated during the project. We have optimized pipelines for in silico analysis of large genomic datasets and competence in analysing these. For proteins the combination of universal protein search databases (e.g. Swiss-Prot), but also dedicated self-built databases will be employed
- **Computing:** We have access to the core lab of UT IG equipped with next generation sequencers and to the High-Performance Computing Cluster of UT
<https://hpc.ut.ee/en/home/>



2. Summary in Estonian

Title: Kiviaegsete inimseoseliste kasetõrvaproovide metagenoomne ja proteoomiline analüüs

Supervisor(s):

Mait Metspalu

Ester Oras

Antonio de Dios Martinez

Remi Philippe Barbieri

Hiljutised edusammud vana DNA (aDNA) uurimises on pööranud pea peale meie arusaamise eelajaloost. Aja- ja geograafilise asukoha täpne määramine võimaldab aDNA-l põhinevat põhjalikku minevikugenoomide uurimist, lahendades olulisi küsimusi meie demograafilise ajaloo kohta. Samas napib osade eelajaloo perioodide ja piirkondade puhul inimjäänuseid, mis raskendab oluliselt DNA-l põhinevat eelajaloo uurimist. Selle probleemi lahendamiseks uurime uudse proovimaterjalina kasetõrva, mida kiviajal kasutati liimainena ja mida kasutuskõlblikuks töödeldi osaliselt ka suus nätsutades. Seega sisaldavad sellised kasetõrva tükid inimese ja mikroobide DNA-d ning valke pakkudes ülevaadet muistsest toidust ja patogeenidest. Inimese näritud kasetõrvast saadud metagenoomsed analüüsid paljastavad teavet inimese suuõõne mikrobioloogia ja võimalike nakkuste kohta. Lisaks pakuvad toiduosakeste DNA ja valgud kasetõrvas otseseid tõendeid muistsete dieetide kohta, täiendades traditsioonilisi meetodeid kõrgema resolutsiooniga proteoomika abil. See interdistsiplinaarne lähenemine lubab täiendada meie arusaama muistsetest populatsioonidest ja nende elustiilidest.



3. Summary in English

Title: Metagenomic and proteomic studies of Stone Age anthropogenic birch tar samples

Supervisor(s):

Mait Metspalu

Ester Oras

Antonio de Dios Martinez

Remi Philippe Barbieri

Recent advancements in ancient DNA (aDNA) research, facilitated by improved laboratory methods and sequencing technology, have revolutionized our understanding of prehistory. By accurately dating and geolocating samples, aDNA enables comprehensive studies of past genetic diversity, addressing key questions in human demography. However, the scarcity of human remains in certain periods and regions poses challenges. To overcome this, birch tar, a novel source of aDNA and proteins, is being explored. Birch tar, commonly used in the Stone Age, contains human and microbial DNA, providing insights into ancient diets and pathogens. Metagenomic analyses of chewed birch tars reveal information about the human oral microbiome and potential infections. Additionally, DNA and proteins from food particles in birch tar offer direct evidence of ancient diets, complementing traditional methods with higher resolution proteomics. This interdisciplinary approach promises to enhance our understanding of ancient populations and their lifestyles.