

## **Illuminating the evolutionary history of the stomach pathogen *Helicobacter pylori***

### **Keywords:**

1. Biological function: *Helicobacter pylori*, stomach pathogen, evolution
2. Biological material: ancient human remains, ancient DNA
3. Methods and instruments: anthropology, paleopathology, DNA capture enrichment, ancient genome sequencing, molecular strain typing, population genetics

### **Disciplines:**

anthropology, paleopathology, history, evolutionary biology, microbiology, molecular biology, bioinformatics, population genetics

### **Project team:**

#### **Project manager**

Frank Maixner (Coordinator), EURAC research, Institute for Mummies and the Iceman

#### **Project vice manager**

Albert Zink (Head of Institute), EURAC research, Institute for Mummies and the Iceman

#### **External project partner 1**

Prof. Thomas Rattai, University of Vienna, CUBE - Division of Computational Systems Biology, Department of Microbiology and Ecosystem Science, Austria

#### **External project partner 2**

Prof. Yoshan Moodley, University of Venda, Department of Zoology, South Africa

### **Duration of the project**

24 months, Start date 01.01.2017, End date 31.12.2018

### **Fundings:**

Total budget: 200.000; EURAC contribution: 60.000, Third party (Province) contribution: 140.000

## Summary of the overall project:

The bacterium *Helicobacter pylori* infects the stomachs of approximately 50% of all humans. With its universal occurrence, high infectivity and virulence properties it is considered as one of the most severe global burdens of modern humankind. It has accompanied humans for many thousands of years, and due to its high genetic variability and vertical transmission, its population genetics reflects the history of human migrations.

In the proposed project, we will study ancient *H. pylori* strains from all over the world to learn more about its genome evolution, changes in virulence and population history. We will combine a novel molecular approach that enriches *Helicobacter* genomic DNA for next generation sequencing (NGS) with innovative tools in computational biology to generate ancient genome sequences for further comparative sequence analysis. Our recent study of *H. pylori* in the Iceman, a 5,300 year-old Copper age individual, provided important details on a possible disease manifestation in the mummy and the origin of the stomach pathogen in Europe. The scientific expertise acquired in the Iceman study will be valuable for the present interdisciplinary project aiming to reconstruct ancient *H. pylori* genomes of different times from various parts of the world. Our ancient genomic data will provide important details on the onset and spread of the *H. pylori* populations in Europe, Africa, America and Asia offering insights into even slight evolutionary changes in the pathogen genome on a continental scale.

## Description of the overall project:

The stomach bacterium *Helicobacter pylori* is one of the most prevalent human pathogens. It has dispersed globally with its human host, resulting in a distinct phylogeographic pattern that can be used to reconstruct both recent and ancient human migrations (Falush *et al.* 2003, Linz *et al.* 2007, Moodley *et al.* 2012). The extant European population of *H. pylori* (hpEurope) is known to be a hybrid between Asian and African bacteria. However, the precise hybridization zone of the parental populations and the true origin of hpEurope are currently discussed controversially. Ancient DNA analysis can help to resolve such difficult demographic questions. Recently, we reconstructed the 5,300 year old *H. pylori* genome of the Iceman, a European Copper age individual. Our results provided important details on a possible disease manifestation in the mummy and the origin of the stomach pathogen in Europe (Maixner *et al.*, 2016). The application of technological and conceptual advances in the Iceman study has paved the way for future studies on *H. pylori* in ancient human remains and provides a solid methodological platform for this proposal.

The present project aims to bring together different scientific expertise such as anthropology, molecular biology, and bioinformatics and populations genetics to illuminate the evolutionary history of *H. pylori* on the European continent and in various other parts of the world. The methodological approach will follow our protocols that were established in the framework of the Iceman stomach project (legge 14 project no. 1/40.3 of the 23<sup>rd</sup> of November 2012), including DNA capture enrichment, NGS sequencing and bioinformatics analysis (genome reconstruction, population genetics). In the first part of the project, we intend to reconstruct in more detail the onset and spread of the *H. pylori* population found in most Europeans today by analyzing ancient coprolite and

dental calculus that were identified as *H. pylori* reservoirs. Especially our diachronic approach with sample material starting from the Neolithic onwards from various sites (Italy, Austria, Switzerland, Hungary, and Germany) will allow us to identify crucial points in the complex demographic history of Europe that shaped modern European *H. pylori* genomes. In the second part of this project we will focus on the analysis of selected mummified human gut contents from various sites outside the European continent. Based on our publication of the Iceman's *H. pylori* in Science early this year, we have contacted several colleagues and got access to intestinal contents of precious mummies from different parts of the world. Thereby, we were able to retrieve sample material that include gut contents of pre-Columbian mummies from Middle and South America, Egyptian mummies from different dynasties and very well preserved gut content of Korean mummies. Our analysis of ancient *H. pylori* strains in these mummies will provide important insights into the presence of the stomach pathogen in different geographic regions and time periods and elucidate the worldwide spread and evolution using *H. pylori* genomics. Moreover, it has the potential to gain additional information on the effects of prehistoric peopling events, such as from Polynesia and Africa on the New World before European contacts, the reconstruction of the demographic history of ancient Egypt and the Korean peninsula and the Japanese archipelago using a preexisting reference database of strains from these regions.

Overall, our ancient *Helicobacter* genomes sequence data from all over the world offers the opportunity to significantly contribute to our understanding of the basic biology and evolution of this important stomach pathogen and will add subsequently an additional chapter to the human demographic history from a bacterial point of view.

### **Description of the subproject – Principal Applicants**

The ancient DNA laboratory at the EURAC Institute for Mummies and the Iceman in Bolzano represents the intersection between paleopathological and modern molecular analyses and thus provides an excellent basis to perform cutting-edge paleomicrobiology research. All sample preparations and DNA-extractions will be performed in a dedicated pre-PCR area following strict procedures required for studies of ancient DNA. The anthropological section of the Iceman institute and their worldwide cooperation partners will support the study with selected ancient gut contents, coprolite and dental calculus material from the Neolithic onwards. One focus of our study will be on specimens from periods of major cultural changes in Europe such as the Bronze Age and the medieval time (Allentoft *et al.*, 2015). Furthermore, we will analyze intestinal contents from precious historical mummies from the American, African and Asian continent. During this project, we will conduct selected, well-preserved material to a novel innovative molecular approach aiming to reconstruct ancient *H. pylori* genomes. Therefore, we are planning to first enrich *Helicobacter* genomic DNA using a hybrid selection technique followed by a massively-parallel sequencing of the sample. Thereby generated ancient genome sequences will be unique milestones in the prediction of the evolutionary history of *H. pylori* in Europe and other parts of the world.

### **Description of the subproject – External Partner 1**

The power of modern molecular biology methods for studying ancient remains, coupled with the increasing repertoire of advanced data analysis approaches in computational biology, has triggered novel interdisciplinary research linking these two areas. The DNA-based methodologies will produce enormous amounts of data, which have to be sorted, processed and analyzed by various bioinformatics means. Throughout the project, there will be an intense collaboration with our cooperation partner Prof. Thomas Rattei (University of Vienna, Austria). His institute will provide the necessary bioinformatics support and specialized software to carry out ancient metagenome analysis, comparative genomics, high performance computing, automated large-scale sequence analysis and annotation tools. In a first phase, the read quality will be assessed and after trimming, filtering and merging of reads the sequences will be taxonomically and functionally assigned using various well-established software tools. In particular, Rattei's group will characterize the virulence factors present in the ancient *H. pylori* and they will check for antibiotic resistance mutations in strains predating the antibiotic era.

### **Description of the subproject – External Partner 2**

To reconstruct the population genetic history of *H. pylori*, we will assign ancient strains to modern *H. pylori* populations using population genetics methods. Prof. Yohan Moodley (University of Venda, South Africa) will perform the analysis using both multilocus sequence typing (MLST) and whole genome population genetics, analyze both ancient and modern strains for signals of admixture and pinpoint the contributing populations using an available reference data set of over 1000 *H. pylori* strains from across the world. Our diachronic approach that makes use of several strains from slightly different times will allow the reconstruction of demographic processes using coalescent modeling and a simulated applied Bayesian computation (ABC) approach. To further increase the power of resolution of our whole-genome comparative analysis additional 500 extant *H. pylori* strains from Europe, Africa, Asia and America will be subjected to whole-genome sequences. Comparing ancient *H. pylori* genomes of different times with this extended modern dataset can provide important insights into even slight evolutionary changes in the pathogen genome on a continental scale.

### **Innovative aspects of the proposed project**

The two following aspects make this project unique in the field of life science and will give us the opportunity to study fundamental evolutionary questions in *Helicobacter* research. For the first time in paleomicrobiology such a large worldwide collection of ancient human remains from different time periods will be screened for *H. pylori* cases. In an unprecedented extend *Helicobacter* strain typing data will be linked to the anthropological result to infer occurrence and epidemic spread of the stomach pathogen. Highlight of this project will be the reconstruction of *H. pylori* genomes by using an innovative sequence-capture sequencing approach. Ancient *Helicobacter* genomes sequence data will highly contribute to our understanding of the basic biology of the stomach pathogen and will herald a new era in paleomicrobiology.

## Essential collaborative elements for the project

Our intercontinental multidisciplinary team including a microbiologist, a bioinformatics and an evolutionary biologist displays a unique combination of different scientific expertise in the field of life sciences. There will be a close cooperation between the principal applicant and the other two applicants to adapt all molecular assays to the special demands of ancient DNA. Thereby obtained data will be continuously linked to the anthropological factors provided by the principal applicant's group such as the nutritional state, signs for urbanization and the family context in order to capture the ancient social and epidemiological aspects of *Helicobacter pylori* infection. Thus, there will be frequent exchange between all partners and only the sum of all obtained results will make it possible to infer evolutionary scenarios.

## Key references related to the project

Falush, D. *et al.* (2003). "Traces of human migrations in *Helicobacter pylori* populations." *Science* 299(5612): 1582-1585.

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