

Voles are small ground rodents grouped in the genus *Microtus*. The genus consists of more than 90 species distributed over most of the Northern Hemisphere and includes such species as the common vole (*Microtus arvalis*) – a pest of crops abundant in Europe. Voles inhabit a range of different habitats, however, mainly prefer open areas like grasslands and meadows. The history of the genus was intensively studied using both paleontological and molecular approaches. It revealed that the early representatives of the genus evolved probably between 1.2 and 0.8 million years ago as a result of rapid radiation and that extant species are grouped in seven subgenera. However, in spite of numerous studies, phylogenetic relationships within *Microtus* are not fully resolved, and the divergence times of the main lineages are uncertain and based only on imprecise paleontological calibrations. These relationships should be resolved with higher confidence using sequences of nuclear genomes while the direct molecular clock calibration may be provided by the DNA sequences from paleontological specimens.

The main aim of this project is to reconstruct phylogenetic relationships between species from *Microtus* genus using partial sequences of nuclear genomes and to estimate the divergence times of main branches using the genomes of paleontological specimens to calibrate the molecular clock. The information about the relation between species and the age of the main splits in the phylogeny will allow to link it with the specific events, either climatic, environmental or biogeographic. This will help to identify factors which drive such rapid radiations and to understand the process of species formation. To achieve this goal, we will generate genome sequences of 15-20 modern specimens from various subgenera of *Microtus*. Moreover, we will obtain vole remains from two key paleontological sites: Denisova cave located in Altai, Russia and Geißenklösterle located in South-Western Germany. In the Denisova cave the remains of mysterious relatives of humans, Denisovans, were discovered. Geißenklösterle, in turn, yielded a rich assemblage of flint and stone tools associated with Neanderthals and Early Modern Humans. Because of these discoveries, the chronology of both sites is very well recognized, and they contain deposits ranging in age from ca. 250 to 40 ka and 90 to 30 ka, respectively. In addition, we will also obtain specimens from Oblazowa 2 site in Poland dated to between 40 and 30 ka and from Emine Bair Khosar cave in Crimea, Ukraine. Specimens from these caves will allow for simultaneous genome sequencing and direct radiocarbon dating of vole mandibles. Sequencing of the genomes of paleontological specimens is very challenging. DNA in such remains is usually present in the minute amounts, is very fragmented and modified. DNA extracts are also highly contaminated with microbial DNA. A pilot study and the previous research of the PI suggests, however, that the DNA preservation in the vole's molar teeth is exceptional and of quality high enough to reconstruct partial genomes even from very old specimens. The state-of-art DNA extraction and library preparation methods combined with high-throughput sequencing will be used to generate low coverage genomes from multiple paleontological specimens with the best-preserved DNA. This will result in a dataset comprising modern and ancient genomes of multiple *Microtus* species. It will be used to infer and calibrate the phylogeny of the *Microtus* genus. The amount and nature of the genome-wide data gives a promise of highly supported reconstruction while the age of the sequenced paleogenomes (up to 200 ka) of the reliability of the calibration. Recent studies showed that many species hybridized in the past. This may be read from their genome sequences. The best examples are humans and Neanderthals or polar and brown bears. In case of *Microtus* this may have been common as the ranges of many species overlap substantially. Many vole species occupy different habitats and differs in their adaptations and genetic signature of these adaptations and selection may be seen in their genomes. The genome sequences generated in this project will allow a search for both the signatures of admixture between species and of selection acting on species or groups.

Previous studies of equine, woolly mammoth and cave bear paleogenomes substantially influenced the understanding of the evolution of these taxa, thus we believe this project will provide valuable contribution to the reconstruction of the evolutionary history of *Microtus* voles and to understanding speciation and species formation processes.