Integrative approach to adaptive radiation in ancient lakes - a case study of Lake Ohrid *Gammarus* species flock

The study aims to unravel the evolutionary process behind evolution of the endemic *Gammarus* species flock in the ancient Lake Ohrid using an integrative approach. The main hypothesis of the research is that the observed species diversity resulted from adaptive radiation – a rapid diversification of multiple species from a common ancestor as a consequence of adaptation to new ecological niches.

Ancient lakes, i.e. water bodies that last for more than hundred thousand years, are well known as "natural laboratories", in which speciation processes can be studied in details. Lake Ohrid, located in the Balkan Peninsula at the Albanian/Macedonian border, is the oldest European lake (1.3-1.9 My old) and one of the World's smallest ancient lake. Its geological history is interesting and well recognized. At the same time, taking into account size of the Lake and its biodiversity, it harbors the highest level of endemism, especially within amphipod crustaceans (ca. 90%) with endemic *Gammarus* species flock. Gammarids are often used as model taxa in ecological studies in freshwaters, but recently also in phylogeographical researches, showing extreme degree of cryptic diversity demonstrated as a substantial decoupling between molecular and morphological diversity. Nevertheless, in general, little is known about evolutionary processes underlying speciation within this group in ancient lakes. Therefore, the Ohridian *Gammarus* species flock is an exceptional model for testing adaptive radiation compared to other such systems. To date, spatial structure of ecological niches inhabited by different species has already been recognized but not fully explored. Phylogenetic studies though revealed significant level of incongruence between molecular and morphological characters between species. Moreover, preliminary karyological studies showed variable number of chromosomes amongst species within the flock. All previous studies concluded that only an integrative strategy combining genomic information with data based on ecology and morphology could disentangle the Ohridian *Gammarus* puzzle.

We propose incorporation of molecular study through transcriptome sequencing, assessment of diet preferences using DNA metabarcoding and genome size analysis by flow cytometry combined with detailed examination of functional morphology (incorporating geometric morphometrics) of *Gammarus* species from the Lake Ohrid and their relatives from neighboring springs and rivers. The project would result in the first ever in-depth analysis of the complete ancient lake endemic species flock of crustaceans.