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BOOK OF ABSTRACTS

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ABSTRACTS

ORAL PRESENTATIONS

Genetic diversity of aquatic invertebrates in the Western Carpathians

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The extensive mountain systems of the Carpathian Arch extend *across* the Central and Eastern Europe and is recognised among the most valuable biodiversity hotspots in Europe. Particularly interesting part of the Carpathian Arch are the Western Carpathians, where the existence of glacial microrefugia for cold-adapted species are supported by recent molecular data. These refugia were localised in relatively high altitudes and proximity to Pleistocene glaciers. Possibly, their presence was enabled by the vast systems of karst springs, characterized by relatively stable thermal and chemical stability throughout seasons. Due to their specific environmental characteristics, karst springs are considered as refugia for many rare species of fauna and flora. Nevertheless, the molecular diversity and phylogeography of biota of the karst springs in the Western Carpathians have not been examined so far. The main aim of our study is to reveal and compare the population genetic diversity patterns in benthic invertebrates of different life strategies in the spring ecosystems of the Western Carpathians based on the barcoding fragment of mtDNA (COI). All the generated barcodes will be uploaded to BOLD (Barcode of Life Data Systems) in order to create a reference library. So far, we have focused on the reophilic beetles, *Elmis aenea* and *Limnius perrisi* (Elmidae), and the amphipod *Gammarus fossarum*-complex (Gammaridae), inhabits majority of small streams and springs in the Western Carpathians. The material was collected from more than 100 localities from Slovakia, Czech Republic and Poland. The populations of *Elmis aenea* and *Limnius perrisi* are genetically homogeneous with one dominant haplotype. Additionally, the Mismatch Distribution and Bayesian Skyline Plot show a recent demographic and spatial population expansion in both species, suggesting post-glacial colonisation, although of different dynamics. A completely different pattern of genetic variability was revealed in the *Gammarus fossarum*-complex, with at least 146 haplotypes recorded in the karst springs of Western Carpathians. Our results confirmed presence of 7 MOTU's (molecular operational taxonomical units) of this amphipod recorded in earlier studies and 1 new MOTU for Slovakia that was previously known only from the Apușeni Mountains in Romania. Additionally, our data support the hypothesis that this species complex survived in the Carpathian microrefugia.

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Population genetics of diving beetles populations in Tatra Mountains alpine lakes

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Europe's alpine ecosystems, such as Alps or Carpathians, represent an important source of the European biodiversity, yet the composition of their fauna, the structure and diversity of the populations of alpine species, or the trends of the biota evolution in these areas are still poorly known. It is however exactly such data that is crucial for their effective conservation. In this work, we focused on the populations analysis of two diving beetle species of the genus *Agabus* in alpine lakes of Tatra Mountains, the northernmost and the highest mountain system in Carpathians. Both species are typical representatives of alpine aquatic fauna, they are evolutionarily closely related, but have different habitat preferences and dispersal abilities. In the recent years, we collected around 1.500 specimens from more than 130 alpine lakes and ponds and adjacent streams from Tatra Mts and the surrounding area. In the study, two mtDNA fragments (cox, cytb) and twenty one newly developed polymorphic microsatellites were used, compared with the mtDNA data from BOLD and GenBank. The results suggest that the Tatra populations of both species are highly genetically diversified, despite the young age of alpine lakes (ca 10.000 years). Most of the recorded haplotypes were private to the Tatras. Close and unresolved relationships between haplotypes confirm recent origin of the studied species' populations, molecular data also suggest contact (gene flow) with localities in the vicinity. The results also indicate that at least part of the recorded diversity could develop in the Tatra Mountains' environment, suggesting that alpine regions could function as a source of local biodiversity. This is a very strong argument supporting the need for their protection. Nuclear markers (microsatellites) analysis suggested recent history of the populations of both species, possible sources of the Tatra populations, and confirmed recent contact with populations from the regions in the neighbourhood of Tatra Mts, which may indicate the shift of individuals from lower altitudes to higher elevated Tatra lakes due to climate change. On the other hand, microsatellites support the role of the mountain ridges as barriers in the distribution of these beetles.

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Slovak aquatic macroinvertebrate fauna DNA barcoding – status quo

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Long year praxis of monitoring water quality and assessing the biodiversity of freshwater ecosystems in Europe (according to EU Water Framework Directive 2000/60/EC) still brings more and more evidence that the traditionally used morphological identification of monitored and evaluated taxa has several reasonable shortcomings (inaccurate identification, absence of experts, time-consuming). This is also why initiatives, like DNAqua-Net project, were launched in the EU, which aim to support opportunities for the utilizing modern genomic tools in the biological assessment of European waters. One of the activities that the initiative seeks to promote is to improve the status of DNA barcodes reference databases for freshwater taxa. Barcode reference libraries of all major freshwater groups of organisms are being produced within several national campaigns. Unfortunately, in Slovakia the level of available DNA barcode data is still very low.

In connection with the WFD implementation, a first detailed checklist of all macroinvertebrate taxa recorded from Slovak fresh waters, was published in 2003. This list contains 1.700 species of aquatic macroinvertebrates and after several years of the surface waters monitoring, about 50 other species were included. Nowadays, along with sporadic new records and unknown cryptic species, it is estimated that around 2.000 macroinvertebrate taxa can be present in Slovak fresh waters. Within the BOLD (The Barcode of Life Data System) database however, there are only 165 public records of aquatic invertebrate taxa collected in Slovakia covering 60 OTUs (end of 2017) and most of them (95%) are older records extracted from GenBank (NCBI) that cannot be considered entirely reliable. Due to the present gaps and following the goals of the COST DNAqua-Net project's Working Group 1 ("DNA Barcode Database"), our laboratory has begun to create a reference barcode library of the Slovak aquatic fauna in 2016. At present, we focus on the fauna of aquatic beetles, stoneflies and caddisflies. Numerous aquatic macroinvertebrate species from commonly occurring habitats are covered by barcodes produced in other European countries, so we primarily focused on data collection from specific habitats such as alpine lakes and karst springs, as well as less explored areas of Europe, such as the Western and Slovak part of Eastern Carpathians, and here we provide preliminary data.

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In search for refugia in Western Carpathians through DNA barcoding. The case of stoneflies (Plecoptera)

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Western Carpathians are one of the key “biodiversity hotspots” in Europe. The mountain chain uplifted during Alpine orogenesis and is characterized by a complicated geological history. Their current biodiversity was highly influenced by Pleistocene glaciations. Recent studies have shown that cold adapted gammarids (crustaceans), survived the Ice Age in cryptic refugia of the Western Carpathians. Other cold adapted and widely distributed in mountains taxa are stoneflies (Plecoptera). Stoneflies are characterized by limited dispersal capabilities (relatively short living imago) and weak or medium flying abilities, making them a good model in phylogeography studies. Moreover, they are used as bioindicators in the assessment of the aquatic ecosystems quality.

The main goal of the presented studies is the identification of putative glacial refugia using selected Plecoptera taxa in Western Carpathian through DNA barcoding.

The material was collected during joint expeditions with researchers from Slovak Academy of Science in 2015 and 2016 year. Samples were taken from 76 stations, streams and springs, using benthic kick net. Morphological diagnosis of the collected larvae using the available keys was performed. Three genera which were most abundant among samples were selected for the barcoding studies: *Isoperla*, *Brachyptera* and *Leuctra*. Over 200 individuals were barcoded using mtDNA COI marker. In the first step barcode library was created in Barcode of Life Data System (BOLD). Next steps will focus on mapping of the geographical and historical (molecular clock) patterns of diversity.

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