Scientific abstracts from the 8th International Barcode of Life Conference

The NTNU University Museum and the Norwegian Biodiversity Information Centre in collaboration with NorBOL and the Norwegian Environment Agency

Trondheim, Norway, 17–20 June 2019

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Use of emerging complete organelle DNA reference databases in the diet analyses of the herbivorous bird, western capercaillies (*Tetrao urogallus*)

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Background: Diet analysis is an important tool used in conservation biology, providing information such as resource use and habitat requirements of the studied species. Traditionally, methods used to study animals' diet have relied on either direct observation or on morphological identification of undigested remains in the faeces. However, such methods are usually fraught with identification errors and are both labour and time intensive. Advances in metabarcoding have enabled the use of environmental DNA (eDNA) to reconstruct diets, but the use of reference databases comprising short DNA sequence markers limits the resolution for accurate species identification. To address this issue, we explore how the optimal use of emerging national DNA reference databases, such as NorBOL and PhyloAlps, may lead to a more accurate species-level identification of plants in animals' diet. These comprehensive reference databases comprise complete organelle genomes of the Norwegian and Alpine flora, respectively. **Results:** As our study is still in the preliminary phase, data analysis is currently ongoing to document the potential of using localised DNA reference databases. We collected faecal samples from western capercaillies (*Tetrao urogallus*) located in Norway and France. The selected study sites represented a huge variation in vegetation types and, thus, the potential variation in the capercaillies' diet. We will use the primers trnl P6 loop and 16S rRNA for diet analysis, and additional 18S rRNA and COI primers to detect intestinal parasites. Taxonomic inference of sequences will be realised using both localised DNA reference databases such as NorBOL and PhyloAlps, and traditional general databases such as the Barcode of Life Data Systems (BOLD). **Significance:** We predict that the use of localised DNA reference databases comprising complete organelle genomes of the local flora may improve the identification of plant species found in faecal samples, possibly giving rise to new knowledge of the studied species.

True story of the riffle beetles diversity in Latin America — revealed by DNA barcoding

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Background: The riffle beetles of the family Elmidae are an important part of the stream and river biota. They are very sensitive to the quality and changes of the environment, and so they are often included in the monitoring of ecological status and water quality assessments. Research on the Elmidae fauna of Latin America has a long history, but so far all taxa were defined only based on morphological features. From Central and South America, about 500 species were described in 46 genera (http://elmidae.myspecies.info), representing about one third of the world's diversity of the family. A recent analysis of the material collected since 2011 suggests that this state may differ greatly from reality, and DNA barcoding confirms these assumptions. **Results:** More than 600 specimens have been barcoded so far; data on BOLD include around 150 BINs. The samples cover more than 80% of known Latin American genera. We have found out that (*i*) the use of barcodes enables discovering hidden diversity, which can be subsequently supported by morphology; (*ii*) existing morphological diagnoses are often unreliable for identification of taxa; (*iii*) some known genera need to be split and new genera have to be described; and (*iv*) some genera should be synonymised. **Significance:** DNA barcoding seems to be a very powerful tool for stabilizing Elmidae taxonomy. Our data clearly demonstrate that many of the currently valid taxa must be revised. Our results show that true diversity, not only at the species but also at the genus level, is significantly higher than present knowledge. Many areas in Latin America have never been studied. The recent data suggest that the large and still-unexplored regions must harbour a huge amount of unknown diversity, which would be good to describe before it is lost.

AquaBOL.SK — barcoding of Slovak aquatic biota launched in the mountains

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Background: Recent detailed survey, arising from the DNAqua-Net EU COST Action (CA15219), has revealed significant gaps in the availability of molecular data on aquatic biota usable, inter alia, for future monitoring and water quality assessment of various water bodies. The differences in DNA barcode coverage are significant at the level of taxonomic groups as well as geographical location, with the least data being available from central or eastern European countries and the Balkans, respectively. This led us to launch the AquaBOL.SK campaign, aiming to build a barcode reference library for Slovak aquatic biota. Since many species commonly occurring in Slovakia are covered by data from other countries, at this stage we have focused on one of the least explored habitat types within Europe—the alpine glacial lakes of Tatra Mts. (western Carpathians). The Tatras are the highest mountain range within the Carpathian Arc and also one of Europe's biodiversity hotspots. There are about 120 permanent lakes and dozens of ponds inhabited by specific and still poorly known biota. **Results:** The barcodes obtained within AquaBOL.SK currently represent benthic invertebrates, especially water insects. Among 1000+ samples already uploaded in BOLD, the most thoroughly covered are water beetles from the Tatra region. Based on an analysis of 11 annual samplings from multiple locations, we obtained an exhaustive set of around 400 samples representing up to 70 OTUs (species), which is reasonably higher diversity than ever reported for this area using classical morphology-based identification. Other groups of alpine lakes invertebrates are still processed. **Significance:** Our results, although initial, improved the information on the alpine aquatic biota, confirm the importance of molecular data in biodiversity research, and are a prerequisite for effective and nondestructive monitoring of vulnerable, threatened, and protected biotopes such as glacial lakes.

Resolving the *Nitzschia palea* species complex using transcriptomics to improve fresh water quality assessment

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More than 1000 diatom taxa are used in the biological assessment of the water quality of rivers and lakes in the Netherlands and Belgium. Those include several varieties that can be hard to tell apart with light microscopy and more importantly have contrasting environmental tolerances and preferences. *Nitzschia palea* and *Nitzschia palea* var. *debilis*

of commercially available fishmeals. **Results:** DNA metabarcoding of mixed DNA samples detected all species, even in highly complex samples containing up to 30 species of equal DNA concentration. However, the percentage reads did not correspond well to the known DNA concentration in each sample, and was heavily biased towards a handful of species, especially *Salmo salar* and *Sardina pilchardus*. Other species, such as *Sardinella aurita* and *Brevoortia patronus*, were underrepresented. DNA metabarcoding of mixed fishmeals performed better, and the number of reads corresponded closely to the known mass composition. In addition, DNA metabarcoding proved useful in revealing undocumented species in presumably species-pure fishmeals, and in detecting traces of pests. **Significance:** Because fishmeals of different species trade at different prices in a competitive market, there is a risk of fraud. We conclude that while DNA metabarcoding using COI is subjected to primer and other biases, it is still effective in detecting unwanted species in mixed samples, even at low concentrations. DNA metabarcoding using general COI primers is a useful tool in quality control of fishmeal and detection of fraud.

The effect of rotenone on protist community composition in freshwater lakes

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Background: Rotenone is a plant-derived toxin, which inhibits the mitochondrial electron transport chain in the mitochondria. This is why rotenone is lethal for a broad spectrum of animals, and why it is extensively used as a pesticide. In freshwater management, rotenone is used to kill invasive or otherwise unwanted fish. However, rotenone treatment affects directly (by killing) and indirectly (by changing food web interactions) other organisms present in the waterbodies. In autumn 2016, the city of Trondheim treated several lakes with rotenone to remove common roach, an invasive species in the area, and to recreate the local trout populations. The treated lakes and adjacent nontreated lakes were intensively investigated before and after the treatment to follow the effects of the treatment on fish, invertebrate, and protist communities. We sampled water from three rotenone-treated lakes and three nontreated lakes once before rotenone addition and once after the addition, as well as three times the following year 2017. **Results:** We amplified environmental DNA (eDNA) present in the samples, using one universal primer pair for the mitochondrial COI gene and one universal primer pair for the 18S rRNA gene to target the whole eukaryotic tree of life. Here, we report on the observed (dis)similarities among the lakes, and consider whether rotenone treatment affected protist communities either directly or indirectly via food web interactions. **Significance:** While invasive species are a severe threat to native ecosystems, the ways to remove them need thorough consideration. Rotenone has proven effective to eliminate fish, but it is not a selective toxin without effects on other organisms. Our results will add valuable information on the detrimental effects of rotenone on protistan communities in freshwater lakes.

Comparative phylogeography of freshwater invertebrates with different dispersal potential in northern part of Carpathians

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Background: The Carpathians, uplifted during Alpine orogeny, represent an ancient archipelago present in the Neogene on the Paratethys Sea. Their northern part, the western and eastern Carpathians, are characterized by a complex geological and climatic history, largely influenced by Pleistocene glaciations. This history shaped the diversity of local fauna. The northern Carpathians are considered as one of the European biodiversity hotspots. However, the diversity of freshwater invertebrates from that region has still been understudied, particularly at the molecular level. The aim of our study was to trace the evolutionary history of model freshwater invertebrates in relation to the geological history of the northern Carpathians. Gammarid crustaceans of the genus *Gammarus* (Amphipoda), beetles of the family Elmidae, and three genera of stoneflies (Plecoptera) were chosen as model organisms, due to their different dispersal capabilities. Material for the study was collected from almost 150 sampling stations: streams, springs, and small rivers. DNA (COI, 16S, 28S) was isolated from over 500 specimens and analyzed using most up-to-date phylogeographical methods incorporating molecular clock dating. **Results:** Results of the study revealed contrasting pattern of molecular diversity between gammarids, stoneflies, and beetles. Gammaridae from the northern Carpathians are characterized by a deep divergence that reaches the Miocene. Particular phylogenetic lineages survived Pleistocene glaciations in local microrefugia. After the Last Glacial Maximum, their populations expanded both in spatial and demographic terms. Stoneflies also show notable diversity and patterns of recent expansion, but their divergence is much younger. In contrast, the elmid beetles show rather low molecular diversity but also support a general pattern of postglacial expansion. **Significance:** Our results support the thesis about the presence of glacial refugia in the northern Carpathians and document postglacial colonization processes in the region for aquatic invertebrates. The obtained barcodes will serve as a reference library for future research.

Biodiversity in complex marketed herbal products — species identification and authentication for safety and efficacy

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Background: Herbal products play an important role in meeting primary healthcare needs around the world, and have gained increasing popularity in the industrialised countries as complementary and alternative therapies to synthetic pharmaceuticals. However, the deliberate or accidental use of undeclared ingredients may occur throughout the entire value chain of the herbal product. Currently, the identification and quality inspection encompasses tests to establish the identity, purity, and constituents of the herbal products, by employing sensory and phytochemical inspection to detect species-specific characters or compounds, alongside with assays for toxic constituents. However, as the outcome of various manufacture procedures, herbal products are complex natural chemical formulations, often highly processed and with numerous ingredients, and these factors limit the accuracy of classical analytical methods to identify the targeted plant species, and even more to detect nontargeted species. Here, we propose new analytical approaches for molecular identification and quality control of herbal products by involving a complex multidisciplinary approach, comprising DNA metabarcoding and phytochemistry-based analytical methods. **Results:** The results showed that the phytochemistry-based analytical methods are accurate methods for detecting the presence of targeted chemical compounds, but have limited efficacy when it comes to identifying the targeted species, and they cannot be used to detect other plant ingredients within the product. Instead, DNA metabarcoding can be used to detect the presence of targeted plant species and simultaneously to detect discrepancies between constituent plant species and the plant species listed on the label of the products. **Significance:** Different analytical methods of quality control and authentication have varying resolution and usefulness along the value chain of herbal products. DNA metabarcoding can be used for authenticating products in processed products, but should, however, be used in combination with appropriate hyphenated chemical methods for quality control.

filtering steps, suggesting that the natural haplotype sets can be retrieved. When we compared haplotype networks of two species inferred independently by metaphylogeography and classical phylogeography, the results were also reassuringly similar. **Significance:** Our study shows that COI metabarcoding data can be used to infer intra- and interpopulation variability of hundreds of species at one time, providing a new tool with great potential for biogeography, conservation genetics, and invasion genetics.

Developing a collaborative eDNA monitoring program in estuarine systems

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Environmental DNA (eDNA) monitoring in estuarine systems is a potentially powerful tool for assessing fish communities and detecting invasive species. As eDNA methods become more developed, resource managers are interested in understanding and applying these new methods, but lack of standardization, limited laboratory capacity, and unfamiliarity with the science present barriers to implementation. The U.S. National Estuarine Research Reserve System (NERRS) is a network of 29 coastal sites designated to protect and study estuarine systems across the United States. The NERRS supports long-term monitoring of physical, chemical, and biological characteristics, and could be a platform for implementing a standardized eDNA coastal program. We will present a two-year pilot environmental eDNA monitoring program being implemented at several NERRs. Metabarcoding and digital PCR methods are applied to species detection with a focus on fish and crabs in both water and sediment. Sampling is conducted in coordination with several traditional monitoring programs, including seine surveys, fish ladder counts, crab trapping, and plankton tows. We collaborate closely with resource managers and other stakeholders to develop a sampling and analysis pipeline that is practical, reproducible, and accessible. This includes clearly communicating the strengths, limitations, and inherent uncertainties so that users are familiar with molecular-based monitoring programs. In turn, users provide feedback on practicality and usability of the project methods. We will discuss both the monitoring results and methods of presenting and discussing eDNA with stakeholders. This project will assess the value of eDNA monitoring to support management decisions at estuarine sites, and will provide end users with key training to support informed decisions regarding the implementation and use of eDNA monitoring in estuarine systems.

A barcode gap analysis for aquatic biomonitoring in Europe

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Background: The biotic composition is a key element when evaluating the ecological status of aquatic ecosystems under the European Water Framework Directive (WFD) and the European Marine Strategy Framework Directive (MSFD). Although many countries do not use species-level identification for all biological quality elements they monitor, several thousand marine and freshwater species are targeted throughout Europe. Thus, molecular species identification has the potential to accelerate, streamline, and standardise monitoring routines under the WFD and MSFD. In this regard, the extent and quality of DNA barcode reference libraries is essential for the implementation of metabarcoding in aquatic biomonitoring. As part of the EU COST-Action DNAqua-Net, we performed a pan-European gap-analysis of species barcodes relevant for aquatic biomonitoring. **Results:** The barcode coverage seen in BOLD and GenBank varied strongly between taxonomic groups and between geographical regions. In general, groups where there have been multiple active barcode projects (e.g., fish, mayflies, caddisflies, and vascular plants) are well represented in the barcode libraries, while others have fewer records (e.g., marine molluscs and ascidians, and freshwater diatoms). We also found that species monitored in several countries often are represented by barcodes in reference libraries, while species monitored by one country frequently lacked sequence records. A large number of species in several taxonomic groups are only represented by private data in BOLD. **Significance:** Our results have implications for the future strategy to fill existing gaps in barcode libraries, especially if DNA metabarcoding is to be used in monitoring of the European aquatic biota under the WFD and MSFD. For example, missing species relevant to monitoring in multiple countries should be prioritized. Also, a strategy for quality control and quality assurance of barcode reference libraries is needed to ensure the applicability of metabarcoding in aquatic biomonitoring.

DNA barcode identification of "magic mushrooms"

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Background: Barcoding of fungal species has been described for diverse applications. However, the ideal marker for forensic species identification of hallucinogenic fungi has been debated. As species or genus identification of *Psilocybe* mushrooms is required in many legal frameworks, a better understanding of the performance of different DNA markers is necessary. Apart from the molecular features, knowledge of relevant developments in fungal taxonomy and specific (local) legislation is required to feasibly apply DNA barcoding in forensics. **Results:** Sequences from authenticated samples, market samples, seized samples, as well as sequences retrieved from GenBank and UNITE were included in this study. The complete ITS region was shown to be the most informative for identification at the species level, outperforming the separate ITS1 and ITS2 regions and far superior to LSU. Intraspecies variation generally consisted of sequence variation, whilst interspecies variation consisted of both length and sequence variation. For genus identification, the marker LSU proved to be at least as suitable as marker ITS, due to ease of alignment of LSU sequences, thereby allowing unequivocal separation between *Psilocybe* and *Deconica* species. With all markers, groups of samples with different names were recognised that could not be distinguished based on their sequences. Different causes for these heterogeneous groups were identified, including groups of closely related fungi, misidentified or mislabelled samples, and samples with names not referring to scientifically accepted species names. **Significance:** This study illustrates that the marker ITS is suitable for the forensic identification of species of "magic mushrooms", whilst the LSU marker provides sufficient resolution when only genus identification is required. However, the presence of multiple incorrect or scientifically invalid labelled sequences in public data repositories may raise concerns about the validity of DNA barcoding, demonstrating the need to validate reference sequences or generate designated reference sequences for forensic applications.